PI: **Li, Fang**	Title: Receptor recognition mechanisms of coronaviruses	
Received: 09/29/2009	FOA: PA07-070	Council: 05/2010
Competition ID: ADOBE-FORMS-A	FOA Title: RESEARCH PROJECT GRANT (PARENT R01)	
1 R01 AI089728-01	Dual:	Accession Number: 3228034
IPF: 1450402	Organization: UNIVERSITY OF MINNESOTA	
Former Number:	Department: Pharmacology	
IRG/SRG: VIRA	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 250,000 Year 2: 250,000 Year 3: 250,000 Year 4: 250,000 Year 5: 250,000	Animals: N Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: Y Early Stage Investigator: Y
Senior/Key Personnel:	*Organization:*	*Role Category:*
Fang Li Ph.D.	University of Minnesota	PD/PI
Robert Geraghty PhD	University of Minnesota	Other (Specify)-Co-Investigator

Additions for Review

Supplemental Material Li supplement

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

2. DATE SUBMITTED	Applicant Identifier
09/29/2009	651709 Li NIH
3. DATE RECEIVED BY STATE	**State Application Identifier**

1. * TYPE OF SUBMISSION

☐ Pre-application ☒ Application ☐ Changed/Corrected Application

4. Federal Identifier

5. APPLICANT INFORMATION *** Organizational DUNS:** 5559179960000

* Legal Name: Regents of the University of Minnesota

Department: Sponsored Projects Admin Division:

* Street1: 450 McNamara Alumni Center

Street2: 200 Oak Street SE

* City: Minneapolis County: Hennepin

* State: MN: Minnesota Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 55455-2070

Person to be contacted on matters involving this application

Prefix: * First Name: Lorrie Middle Name:

* Last Name: Awoyinka Suffix:

* Phone Number: 612-624-5599 Fax Number: 612-624-4843

Email: awards@umn.edu

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN):* 41-6007513

7. * TYPE OF APPLICANT: H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:

☒ New ☐ Resubmission

☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).

☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration

☐ E. Other (specify):

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. * NAME OF FEDERAL AGENCY: National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Receptor recognition mechanisms of coronaviruses

12. * AREAS AFFECTED BY PROJECT *(cities, counties, states, etc.)*

N/A

13. PROPOSED PROJECT:

* Start Date: 07/01/2010 * Ending Date: 06/30/2015

14. CONGRESSIONAL DISTRICTS OF:

a. * Applicant: MN-005 b. * Project: MN-005

15. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: * First Name: Fang Middle Name:

* Last Name: Li Suffix: Ph.D.

Position/Title: Assistant Professor

* Organization Name: University of Minnesota

Department: Pharmacology Division: Medical School

* Street1: 6-120 Jackson Hall

Street2: 321 Church Street SE

* City: Minneapolis County: Hennepin

* State: MN: Minnesota Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 55455-0217

* Phone Number: 612-625-6149 Fax Number: 612-625-8408

* Email: lifang@umn.edu

OMB Number: 4040-0001
Expiration Date: 04/30/2008

16. ESTIMATED PROJECT FUNDING

a. * Total Estimated Project Funding	1,887,500.00
b. * Total Federal & Non-Federal Funds	1,887,500.00
c. * Estimated Program Income	0.00

17. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

18. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

☒ *** I agree**

**** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.***

19. Authorized Representative

Prefix: * First Name: David Middle Name:

* Last Name: Hagen Suffix:

* Position/Title: Associate Director

* Organization: University of Minnesota

Department: Sponsored Projects Admin Division:

* Street1: 450 McNamara Alumni Center

Street2: 200 Oak Street SE

* City: Minneapolis County: Hennepin

* State: MN: Minnesota Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 55455-2070

* Phone Number: 612-624-5599 Fax Number: 612-624-4843

* Email: awards@umn.edu

*** Signature of Authorized Representative**: David Hagen

*** Date Signed**: 09/29/2009

20. Pre-application Add Attachment | Delete Attachment | View Attachment

21. Attach an additional list of Project Congressional Districts if needed.

Add Attachment | Delete Attachment | View Attachment

OMB Number: 4040-0001
Expiration Date: 04/30/2008

424 R&R and PHS-398 Specific Table Of Contents

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RESEARCH & RELATED Project/Performance Site Location(s)

Project/Performance Site Primary Location

Organization Name: University of Minnesota
* Street1: 3-284 Nils Hasselmo Hall
Street2: 312 Church Street SE
* City: Minneapolis County: Hennepin
* State: MN: Minnesota Province:
* Country: USA: UNITED STATES * ZIP / Postal Code: 55455

Project/Performance Site Location 1

Organization Name: University of of Minnesota
* Street1: 7-227 Phillips Wangensteen Building
Street2: 516 Delaware Street SE
* City: Minneapolis County: Hennepin
* State: MN: Minnesota Province:
* Country: USA: UNITED STATES * ZIP / Postal Code: 55455

Additional Location(s) Add Attachment Delete Attachment View Attachment

OMB Number: 4040-0001
Expiration Date: 04/30/2008

Close Form | Print Page | About

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ☐ Yes ☒ No

1.a If YES to Human Subjects

Is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Exemption Number: ☐1 ☐2 ☐3 ☐4 ☐5 ☐6

Human Subject Assurance Number:

2. * Are Vertebrate Animals Used? ☐ Yes ☒ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☐ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. * Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. * Does this project have an actual or potential impact on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5.a. * Does this project involve activities outside the U.S. or partnership with International Collaborators? ☐ Yes ☒ No

5.b. If yes, identify countries:

5.c. Optional Explanation:

6. * Project Summary/Abstract 1236-Li Project Summary REV 4.pdf | Add Attachment | Delete Attachment | View Attachment

7. * Project Narrative 1240-Li Narrative REV 4.pdf | Add Attachment | Delete Attachment | View Attachment

8. Bibliography & References Cited 1239-Li Lit Cited REV 4.pdf | Add Attachment | Delete Attachment | View Attachment

9. Facilities & Other Resources 1237-Li Resources REV 4.pdf | Add Attachment | Delete Attachment | View Attachment

10. Equipment 1238-Li Equipment REV 4.pdf | Add Attachment | Delete Attachment | View Attachment

11. Other Attachments Add Attachments | Delete Attachments | View Attachments ☐

OMB Number: 4040-0001
Expiration Date: 04/30/2008

Project Summary

Receptor recognition by viruses is the first and essential step of viral infections. It is an important determinant of viral host ranges and cross-species infections, and a primary target for human intervention. Coronaviruses recognize a variety of receptors, infect many hosts, and are significant health threats to humans and other animals. NL63 coronavirus (NL63-CoV), a prevalent human respiratory virus, is the only group-I coronavirus known to use angiotensin-converting enzyme 2 (ACE2) as its receptor, whereas other group-I coronaviruses use aminopeptidase-N (APN). Curiously, ACE2 is also used by group-II SARS coronavirus (SARS-CoV), the agent for the fatal human severe acute respiratory syndrome (SARS). Defined receptor-binding domains (RBDs) on the spike proteins of NL63-CoV and SARS-CoV bind ACE2 with high affinity.

This research investigates the receptor recognition mechanisms and cross-species infections of coronaviruses. Our preliminary studies have determined the crystal structures of NL63-CoV RBD complexed with human ACE2 and of SARS-CoV RBD complexed with human ACE2. NL63-CoV and SARS-CoV RBDs have no structural homology in cores or receptor-binding motifs (RBMs) that directly contact ACE2, but recognize the same “virus-binding hotspot” on ACE2. Among group-I coronaviruses, RBD cores are conserved, but RBMs are variable, explaining how these viruses recognize different receptors. We have also determined crystal structures of the RBDs from various SARS-CoV strains complexed with ACE2 proteins from humans and palm civets, revealing mechanisms whereby SARS-CoV transmitted between the two hosts.

There are three specific aims in this project. Aim 1 focuses on the receptor recognition mechanisms of group-I NL63-CoV. It investigates specific functions of the virus-binding hotspot on human ACE2 in NL63-CoV binding, and examines interactions between the NL63-CoV spike protein and ACE2 proteins from non-human hosts. Aim 2 focuses on the receptor recognition mechanisms of other group-I coronaviruses. It investigates specific functions of the RBMs of other group-I coronavirus spike proteins, and studies whether modifying the RBMs changes the receptor specificities of the spike proteins. Aim 3 focuses on the receptor recognition mechanisms of group-II SARS-CoV. It investigates why mutations in the RBM region of past SARS-CoV strains were selected, whether and how these mutations affect receptor binding, and what combinations of these mutations may appear in potential future SARS-CoV strains. The research approaches of this proposal include crystallographic analysis of the virus-receptor interfaces, identification of structural elements important for virus-receptor interactions, and characterization of these elements using crystallographic, biochemical, molecular, and virological tools. Overall, these studies will provide the molecular and structural basis for understanding viral evolution, virus-receptor interactions, viral host ranges and cross-species infections. They will also guide the development of novel antiviral strategies against coronavirus infections.

Narrative

This research investigates how coronaviruses recognize their receptors and how they interact with receptors from different hosts. It explores novel principles governing viral evolution, virus-receptor interactions, viral host ranges and cross-species infections, and may lead to new approaches in the prevention and treatment of coronavirus infections in humans and other animals.

Resources

(1) Laboratory:

All bench work will be conducted at the University of Minnesota, primarily in Dr. Fang Li's laboratory. Some bench work will be conducted in Dr. Robert J. Geraghty's laboratory. Dr. Li's laboratory has four benches, each allowing two experimenters to work simultaneously. Dr. Geraghty's laboratory occupies 860 sq. ft. of laboratory space and operates at BL2 containment.

(2) Computers:

We have free access to computers in the Basic Sciences Computing Laboratory at the University of Minnesota.

(3) Other:

Crystallization screens will be performed using Rigaku's CrystalMation (crystal growth automation equipment).

In house X-ray data will be collected at the Kahlert Structural Biology Laboratory at the University of Minnesota.

Synchrotron X-ray data will be collected at national synchrotron facilities including the Advanced Photon Source near Chicago IL and Advanced Light Source at Berkeley CA.

We also have access to the University of Minnesota Masonic Cancer Center Flow Cytometry facility, which contains BD LSRII and 3 FacsCalibur flow cytometers as well as high-speed cell sorting capabilities up to 22,000 events/sec with the FACSDiVa and FACSAria cell sorters.

Major Equipments

The following major equipments are available at the PI's laboratory or other facilities at the University of Minnesota:

Protein purification facilities: AKTA prime protein purification system, Pall Minimate concentrator, protein purification chromatography columns. Protein binding measurement facilities: Biacore 3000, Isothermal Titration Calorimeter. Cell culture hoods: two 4 ft. Thermo Sci Class II A2 biosafety hoods. Dishwasher: Miele G7804 lab glass and plastic washer. Freezers: Thermo Sci Revco UltraPlus -80°C, Thermo Sci Isotemp –20°C. Refrigerator: Thermo Sci chromatography sliding double-door refrigerator. Incubators: Thermo Sci Excellar E25 incubator/shaker, Thermo Sci MaxQ 4000 incubator/shaker, three Thermo Sci Forma Steri-Cycle CO_2 incubators with gas cylinder monitor and two CO_2 regulators, and two Bellco Bench Top Incubators with Bellco Rocker Platform, Bellco Orbital Shaker, and Bellco Cell-Production Roller Apparatus. Microscopy: Leica DMIL inverted-stand microscope with 3.2, 10, 20, and 40X phase objectives and fluorescence capabilities. Microcentrifuges: Sorvall Biofuge Pico and Sorvall Biofuge Fresco. Table-top centrifuge: Sorvall Legend RT plus refrigerated centrifuge with swinging bucket rotor and adaptors for 50, 15, and 5ml tubes, and 96-well plates. Floor model centrifuge: Beckman Avanti JE with JA-17 and JLA 10.5 rotors. Sonicator: Fisher Scientific 60 Sonic Dismembrator with Sonabox Accoustic Enclosure. Thermal cycler: BioRad DNA engine. Plate reader: Molecular Devices SpectraMaxM5 Plate Reader with SOFTmax PRO Software. Power supplies: BioRad PowerPac Universal, BioRad Power Pac 200, BioRad Power Pac 1000, and EC Apparatus Corporation EC-150 units. Electrophoresis: Two BioRad Transblot Cells, two BioRad Mini PROTEAN 3 Cells, two Continental Lab Products 12 cm x 14 cm horizontal electrophoresis devices, 1 Continental Lab Products mini-gel horizontal electrophoresis device, two BioRad Protean II xi vertical electrophoresis rigs. Miscellaneous: Mettler-Toledo PB-S/FACT series balance, Denver Instrument PI series analytical balance, Fisher Scientific Accumet Basic AB15 pH Meter, and Panasonic Microwave. Bellco pipet plugger. Biomate 3 spectrophotometer. Thermo Sci drying oven.

Kahlert Structural Biology Lab: one RigakuMSC Micromax 007 X-ray generator with a Cr anode, VariMax Confocal Max-Flux Cr optics, R-axis IV++ image plate, helium cone, and X-stream low temperature device for *ab initio* structure solution by sulfur single wavelength anomalous diffraction (S-SAD); one RigakuMSC Micromax HF X-ray generator with a Cu anode, and two data collection systems; (1) VariMax Confocal Max-Flux optics, R-axis IV++ image plate, 2θ stage, and RigakuMSC XStream low temperature device for high resolution in-house X-ray data collection; (2) Confocal Blue optics, inverse phi, and Oxford Instruments low temperature Cryojet for crystal screening; one RigakuMSC Micromax 007 HFM X-ray generator with a Cu anode, VariMax-HF Confocal Max-Flux optics, an AFC11 goniometer, a RigakuMSC Saturn 944+ CCD detector, and an X-Stream 2000 low temperature system. Multiple crystal incubators, running at a range of temperatures, are available for crystallization trials, and multiple stereomicroscopes enable crystals to be visualized and photographed. Two data processing computers. Rigaku's CrystalMation (crystal growth automation equipment).

Basic Sciences Computing Laboratory: three shared memory systems (SGI Altix 3700, SGI Onyx4, Sun V880), sixteen workstations (linux, SGI, and PC), and all major software for structural biology.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: | * First Name: Fang | Middle Name:

* Last Name: Li | Suffix: Ph.D.

Position/Title: Assistant Professor | Department: Pharmacology

Organization Name: University of Minnesota | Division: Medical School

* Street1: 6-120 Jackson Hall

Street2: 321 Church Street SE

* City: Minneapolis | County: Hennepin

* State: MN: Minnesota | Province:

* Country: USA: UNITED STATES | * Zip / Postal Code: 55455-0217

* Phone Number: 612-625-6149 | Fax Number: 612-625-8408

* E-Mail: lifang@umn.edu

Credential, e.g., agency login: fangli

*** Project Role:** PD/PI | **Other Project Role Category:**

***Attach Biographical Sketch** 1234-Li biosketch 092509.pdf | Add Attachment | Delete Attachment | View Attachment

Attach Current & Pending Support | Add Attachment | Delete Attachment | View Attachment

PROFILE - Senior/Key Person 1

Prefix: | * First Name: Robert | Middle Name: J.

* Last Name: Geraghty | Suffix: PhD

Position/Title: Assoc. Professor/Assoc. Director - Virology | Department: Center for Drug Design

Organization Name: University of Minnesota | Division: Academic Health Center

* Street1: MMC204, 7222 PWB

Street2: 516 Delaware Street SE

* City: Minneapolis | County: Hennepin

* State: MN: Minnesota | Province:

* Country: USA: UNITED STATES | * Zip / Postal Code: 55455

* Phone Number: 612-625-3281 | Fax Number: 612-625-8154

* E-Mail: gerag012@umn.edu

Credential, e.g., agency login:

*** Project Role:** Other (Specify) | **Other Project Role Category:** Co-Investigator

***Attach Biographical Sketch** 1235-Geraghty biosketch 09.pd | Add Attachment | Delete Attachment | View Attachment

Attach Current & Pending Support | Add Attachment | Delete Attachment | View Attachment

ADDITIONAL SENIOR/KEY PERSON PROFILE(S) | Add Attachment | Delete Attachment | View Attachment

Additional Biographical Sketch(es) (Senior/Key Person) | Add Attachment | Delete Attachment | View Attachment

Additional Current and Pending Support(s) | Add Attachment | Delete Attachment | View Attachment

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Fang Li	Assistant Professor
eRA COMMONS USER NAME fangli	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Beijing University, Beijing, China	B.S.	1996	Biochemistry & Molecular Biology
Yale University, New Haven, CT	Ph.D.	2002	Molecular Biophysics & Biochemistry
Yale University, New Haven, CT	Postdoctoral	2002-2003	Structural Biology
Harvard Medical School, Children's Hospital, Boston, MA	Postdoctoral	2003-2006	Structural Biology

A. Positions and Employment

2002 – 2003	Postdoctoral Fellow, Department of Molecular Biophysics and Biochemistry, Yale University
2003 – 2006	Postdoctoral Fellow, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston Children's Hospital
2007 – present	Assistant Professor, Department of Pharmacology, University of Minnesota Twin Cities

B. Publications (in chronological order)

1. **Li, F.,** Wang, J., Steitz, T.A. (2000) Sulfolobus shibatae CCA-adding enzyme forms a tetramer upon binding two tRNA molecules: a scrunching-shuttling model of CCA specificity. *Journal of Molecular Biology, 304,* 483-492

2. **Li, F.,** Xiong, Y., Wang, J., Cho, H.D., Tomita, K., Weiner, A.M., Steitz, T.A. (2002) Crystal structures of the Bacillus stearothermophilus CCA-adding enzyme and its complexes with ATP or CTP. *Cell, 111,* 815-824

3. **Li, F.,** Steitz, T.A. (2003) A novel method of determining the number of macromolecules per asymmetric unit from accurate crystal-volume measurements. *Acta Crystallographica Section D: Biological Crystallography, 59,* 1793-1796

4. Xiong, Y., **Li, F.,** Wang, J., Weiner, A.M., Steitz, T.A. (2003) Crystal structures of an archaeal class I CCA-adding enzyme and its nucleotide complexes. *Molecular Cell, 12,* 1165-1172

5. **Li, F.,** Li, W., Farzan, M., Harrison S.C. (2005) Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science, 309,* 1864-1868

6. Huang, I.C., Bosch, B.J., **Li, F.,** Li, W., Lee, K.H., Ghiran, H., Vasilieva, N., Dermody, T.S., Harrison, S.C., Dormitzer, P.R., Farzan, M., Rottier, P.M., Choe, H. (2006) SARS coronavirus, but not human coronavirus NL63, utilizes cathepsin L to infect ACE2-expressing cells. *Journal of Biological Chemistry, 281,* 3198-3203

7. Li, W., Wong, S.K., **Li, F.,** Kuhn, J.H., Huang, I.C., Choe, H., Farzan, M. (2006) Animal origins of the severe acute respiratory syndrome coronavirus: insight from ACE2-S-protein interactions. *Journal of Virology, 80,* 4211-4219

8. **Li, F.,** Berardi, M., Li, W., Farzan, M., Dormitzer, P.R., Harrison, S.C. (2006) Conformational states of the severe acute respiratory syndrome coronavirus spike protein ectodomain. *Journal of Virology, 80,* 6794-6800

9. **Li, F.,** Li, W., Farzan, M., Harrison, S.C. (2006) Interactions between SARS coronavirus and its receptor. *Advances in Experimental Medicine and Biology, 581,* 229-234

10. **Li, F.** (2008) Structural analysis of major species barriers between humans and palm civets for SARS coronavirus infections. *Journal of Virology, 82,* 6984-6991

11. Wu, K., Li, W., Peng, G., **Li, F.** (2009) Crystal structure of NL63 respiratory coronavirus receptor-binding domain complexed with its human receptor. *Proceedings of the National Academy of Sciences USA* (direction submission) In press

C. Research Support

Active

AHC Faculty Research Development Grant, University of Minnesota **01/01/09 – 12/31/11**
Receptor recognition and cross-species infections by human coronaviruses
Role: Principal Investigator

Completed

Minnesota Medical Foundation Equipment Grant **06/28/07 – 06/30/09**
Structural studies of the SARS coronavirus spike protein
Role: Principal Investigator

Minnesota Medical Foundation Research Grant **07/09/07 – 07/08/09**
Structural studies of the SARS coronavirus spike protein
Role: Principal Investigator

Grant-in-Aid, Graduate School of the University of Minnesota **01/01/08 – 06/30/09**
Triggers for SARS coronavirus entry into host cells
Role: Principal Investigator

AHC Seed Grant, University of Minnesota **07/01/08 – 06/30/09**
Structural studies of the NL63 coronavirus spike protein by cryo-electron microscopy
Role: Principal Investigator

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Robert J. Geraghty	Associate Professor
eRA COMMONS USER NAME robert.geraghty	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Minnesota	B.S.	1983-1988	Genetics/Cell Biology
University of Wisconsin, Madison	Ph.D.	1988-1995	Oncology

A. Positions and Honors.

Positions and Employment

02/1995-8/2000 Postdoctoral fellow; Dr. Patricia G. Spear, Dept. of Microbiology-Immunology, Northwestern University Medical School

08/2000-06/2007 Assistant Professor; Dept. of Microbiology, Immunology, and Molecular Genetics, University of Kentucky College of Medicine.

06/2007-05/2008 Associate Professor; Dept. of Microbiology, Immunology, and Molecular Genetics, University of Kentucky College of Medicine.

05/2008-present Associate Professor, Associate Director of Virology; Center for Drug Design, University of Minnesota

B. Selected peer-reviewed publications (in chronological order).

Geraghty, R.J., Panganiban, A.T., (1993) Human Immunodeficiency Virus Type 1 Has A CD4- And Envelope Glycoprotein-Independent Function. *Journal of Virology* 67:4190-4194

Geraghty, R.J., Talbot, K.J., Callahan, M., Harper W., Panganiban, A.T., (1994) Cell Type Dependence For Vpu Function. *Journal of Medical Primatology* 23:146-150

Schwartz, M.D., **Geraghty, R.J.**, Panganiban, A.T., (1996) HIV-1 Particle Release Mediated By Vpu Is Distinct From That Mediated By p6. *Virology* 224:302-309

Geraghty, R.J., Krummenacher, C., Cohen, G.H., Eisenberg, R.J., Spear, P.G., (1998) Entry Of Alphaherpesviruses Mediated By Poliovirus Receptor-Related Protein 1 And Poliovirus Receptor. *Science* 280:1618-1620

Warner, M.S., **Geraghty, R.J.**, Martinez, W.M., Montgomery, R.I., Whitbeck, C., Xu, R., Eisenberg, R.J., Cohen, G.H., Spear, P.G., (1998) A Cell Surface Protein With Herpesvirus Entry Activity (HveB) Confers Susceptibility To Infection By Mutants Of Herpes Simplex Virus Type 1, Herpes Simplex Virus Type 2, And Pseudorabies Virus. *Virology* 246:179-189

Krummenacher, C., Nicola, A.V., Whitbeck, J.C., Lou, H., Hou, W., Lambris, J.D., **Geraghty, R.J.**, Spear, P.G., Cohen, G.H., Eisenberg, R.J., (1998) Herpes Simplex Virus Glycoprotein D Can Bind To Poliovirus Receptor Related Protein 1 (PRR1/HveC) Or Herpes Virus Entry Mediator (HVEM/HveA), Two Structurally Unrelated Mediators Of Virus Entry. *Journal of Virology* 72:7064-7074

Krummenacher, C., Rux, A.H., Whitbeck, J.C., Ponce de Leon, M., Lou, H., Baribaud, I., Hou, W., Zou, C., **Geraghty, R.J.**, Spear, P.G., Eisenberg, R.J., Cohen, G.H., (1999) The First Ig-Like Domain Of HveC Is Sufficient To Bind HSV gD With Full Affinity While The Third Domain Is Involved In Oligomerization Of HveC. *Journal of Virology* 73:8127-8137.

Geraghty, R.J., Jogger, C.R., Spear, P.G., (2000) Cellular Expression Of Alphaherpesvirus gD Interferes With Entry Of Homologous And Heterologous Alphaherpesviruses By Blocking Access To A Shared gD Receptor. *Virology* 268:147-158.

Terry-Allison, T., Montgomery, R.I., Warner, M.S., **Geraghty, R.J.**, Spear, P.G., (2001) Contributions Of gD Receptors And Glycosaminoglycans To Cell Fusion Mediated By Herpes Simplex Virus 1. *Virus Research* 74: 39-45.

Baury, B., **Geraghty, R.J.**, Masson, D., Lustenberger, P., Spear, P.G., and Denis, M.G. (2001). Rat Tage4 Gene: Similarities To The Human CD155 Gene In Organization And In Herpesvirus Entry Activities Of The Encoded Proteins. *Gene* 265:185-194.

Geraghty, R.J., Krummenacher, C., Cohen, G.H., Eisenberg, R.J., Spear, P.G., (2001) Use of Chimeric Nectin-1(HveC)-Related Receptors to Demonstrate That Ability to Bind gD Is Not Necessarily Sufficient for Alphaherpesvirus Entry. *Virology* 285:366-375.

Jones, N.A. and **R.J. Geraghty**. (2004). Fusion Activity Of Lipid-Anchored Envelope Glycoproteins Of Herpes Simplex Virus Type 1. *Virology* 324(1):213-228.

Subramanian, R.P., J.E. Dunn, and **R.J. Geraghty**, (2005) The Nectin-1α Transmembrane Domain, But Not The Cytoplasmic Tail, Influences Cell Fusion Induced by HSV-1 Glycoproteins. *Virology* 339:176-191.

Klyachkin, Y.M., K.D. Stoops, and **R.J. Geraghty**, (2005) Herpes Simplex Virus Type 1 Glycoprotein L Mutants That Fail To Promote Trafficking of Glycoprotein H And Fail To Function In Fusion Can Induce Binding Of Glycoprotein L-Dependent Anti-Glycoprotein H Antibodies. *Journal of General Virology* 87:759-767.

Even, D.L., Henley, A.M, and **R.J. Geraghty**, (2006) The Requirements For Herpes Simplex Virus Type 1 Cell-Cell Spread Via Nectin-1 Parallel Those For Virus Entry. *Virus Research*. 119:195-207.

Subramanian, R.P. and **R.J. Geraghty**, (2007) Herpes Simplex Virus Type-1 Mediates Fusion Through a Hemifusion Intermediate by Sequential Activity of Glycoproteins D, H, L, and B. *Proceedings of the National Academy of Sciences* 104(8):2903-2908.

Klyachkin, Y.M. and **R.J. Geraghty**, (2008) Mutational Analysis of Herpes Simplex Virus Type 1 Glycoprotein L Reveals The Importance Of An Arginine-Rich Region For Function. *Virology* 374(1): 23-32.

C. Research Support

Completed Research Support

1/02-12/02
American Cancer Society Institutional Research Grant #85-001-13-IRG.
Cellular Factors Involved in Cell-to-cell Spread of Herpesviruses.
This grant was awarded to facilitate pilot studies on cell proteins involved in cell-to-cell spread of herpesviruses.
Role: PI

9/30/02 - 2/28/08
R01 AI051476 NIH/NIAID
Spread of HSV-1 mediated by the gD receptor nectin-1.
This study seeks to identify regions and characteristics of the gD receptor nectin-1 that are important in all forms of HSV-1 spread in cell culture. (35% effort).
Role: PI

Ongoing

5/15/09 – 5/14/11
R03 AI078079 NIH/NIAID
Membrane fusion during HSV-1 entry.
This proposal details experiments to analyze HSV-1 membrane fusion and hemifusion occurring during receptor-mediated endocytosis. (10% effort).
Role: PI

Pending

R01 AI051476 NIH/NIAID
Spread of HSV-1 mediated by the gD receptor nectin-1.
This **competitive renewal** seeks to characterize the hemifusion intermediate occurring during viral spread and understand protein-protein interactions during membrane fusion.
Role: PI

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
* First Name: Fang
Middle Name:
* Last Name: Li
Suffix: Ph.D.

* New Investigator? ☐ No ☒ Yes

Degrees: PhD

2. Human Subjects

Clinical Trial? ☐ No ☐ Yes

* Agency-Defined Phase III Clinical Trial? ☐ No ☐ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix:
* First Name: Lorrie
Middle Name:
* Last Name: Awoyinka
Suffix:

* Phone Number: 612-624-5599 Fax Number: 612-624-4843
Email: awards@umn.edu

* Title: Principal Grants and Contracts Administrator

* Street1: 450 McNamara Alumni Center
Street2: 200 Oak Street SE
* City: Minneapolis
County: Hennepin
* State: MN: Minnesota
Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 55455-2070

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/registry/index.asp. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

PHS 398 Modular Budget, Periods 1 and 2

OMB Number: 0925-0001

Budget Period: 1

Reset Entries | Start Date: 07/01/2010 | End Date: 06/30/2011

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Costs	51	250,000.00	127,500.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): Department of Health and Human Services, Wanda Rayfield, (214) 767-5249

Indirect Cost Rate Agreement Date: 01/15/2009

Total Indirect Costs: 127,500.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 377,500.00

Budget Period: 2

Reset Entries | Start Date: 07/01/2011 | End Date: 06/30/2012

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Costs	51	250,000.00	127,500.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): Department of Health and Human Services, Wanda Rayfield, (214) 767-5249

Indirect Cost Rate Agreement Date: 01/15/2009

Total Indirect Costs: 127,500.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 377,500.00

PHS 398 Modular Budget, Periods 3 and 4

Budget Period: 3

Reset Entries | Start Date: 07/01/2012 | End Date: 06/30/2013

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Costs	51	250,000.00	127,500.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): Department of Health and Human Services, Wanda Rayfield, (214) 767-5249

Indirect Cost Rate Agreement Date: 01/15/2009

Total Indirect Costs: 127,500.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 377,500.00

Budget Period: 4

Reset Entries | Start Date: 07/01/2013 | End Date: 06/30/2014

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Costs	51	250,000.00	127,500.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): Department of Health and Human Services, Wanda Rayfield, (214) 767-5249

Indirect Cost Rate Agreement Date: 01/15/2009

Total Indirect Costs: 127,500.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 377,500.00

PHS 398 Modular Budget, Periods 5 and Cumulative

Budget Period: 5

Reset Entries | Start Date: 07/01/2014 | End Date: 06/30/2015

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Costs	51	250,000.00	127,500.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): Department of Health and Human Services, Wanda Rayfield, (214) 767-5249

Indirect Cost Rate Agreement Date: 01/15/2009

Total Indirect Costs: 127,500.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 377,500.00

Cumulative Budget Information

1. Total Costs, Entire Project Period

*Section A, Total Direct Cost less Consortium F&A for Entire Project Period	$ 1,250,000.00
Section A, Total Consortium F&A for Entire Project Period	$
*Section A, Total Direct Costs for Entire Project Period	$ 1,250,000.00
*Section B, Total Indirect Costs for Entire Project Period	$ 637,500.00
*Section C, Total Direct and Indirect Costs (A+B) for Entire Project Period	$ 1,887,500.00

2. Budget Justifications

Personnel Justification	1242-Li Personnel Justify REV	Add Attachment	Delete Attachment	View Attachment
Consortium Justification		Add Attachment	Delete Attachment	View Attachment
Additional Narrative Justification		Add Attachment	Delete Attachment	View Attachment

Tracking Number:GRANT10428706 Funding Opportunity Number:PA-07-070 Received Date:2009-09-29T14:48:27-04:00

Personnel Justification

Fang Li, PhD, Principal Investigator (6.00 calendar months), will be responsible for laboratory management and supervision of all projects, including experimental design, execution, data analysis, and write-up.

Robert J. Geraghty, PhD, Co-investigator (1.20 calendar months), effort during three summer months, will be responsible for supervision of the virology parts of the projects, including experimental design, execution, and data analysis.

One Postdoctoral Scientist (Kailang Wu, PhD) (12.00 calendar months), will be responsible for conducting experiments on the receptor recognition mechanisms of group-I NL63 coronavirus and other group-I coronaviruses.

One Postdoctoral Scientist (Guiqing Peng, PhD) (12.00 calendar months), will be responsible for conducting experiments on the receptor recognition mechanisms of group-II SARS coronavirus.

One Postdoctoral Scientist (Wenbo Zhou, PhD) (4.80 calendar months), will work with Drs. Geraghty and Li on the virology parts of the project.

Kathryn V. Holmes, PhD, Consultant, will be responsible for providing advice and discussions about experimental design, execution, and data analysis.

Close Form | Print Page | About

OMB Number: 0925-0001

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page and PHS398 Checklist. The responses provided on these pages, regarding the type of application being submitted, are repeated for your reference, as you attach the appropriate sections of the research plan.

*Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)		Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	1243-Li Specific Aims REV 4	Add Attachment	Delete Attachment	View Attachment
3. Background and Significance	1244-Li Background Sig REV	Add Attachment	Delete Attachment	View Attachment
4. Preliminary Studies / Progress Report	1245-Li Prelim Study REV 4.p	Add Attachment	Delete Attachment	View Attachment
5. Research Design and Methods	1246-Li Res Design REV 4.pd	Add Attachment	Delete Attachment	View Attachment
6. Inclusion Enrollment Report		Add Attachment	Delete Attachment	View Attachment
7. Progress Report Publication List		Add Attachment	Delete Attachment	View Attachment

Human Subjects Sections

Attachments 8-11 apply only when you have answered "yes" to the question "are human subjects involved" on the R&R Other Project Information Form. In this case, attachments 8-11 may be required, and you are encouraged to consult the Application guide instructions and/or the specific Funding Opportunity Announcement to determine which sections must be submitted with this application.

8. Protection of Human Subjects		Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
10. Targeted/Planned Enrollment		Add Attachment	Delete Attachment	View Attachment
11. Inclusion of Children		Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

12. Vertebrate Animals		Add Attachment	Delete Attachment	View Attachment
13. Select Agent Research		Add Attachment	Delete Attachment	View Attachment
14. Multiple PI Leadership Plan		Add Attachment	Delete Attachment	View Attachment
15. Consortium/Contractual Arrangements		Add Attachment	Delete Attachment	View Attachment
16. Letters of Support	1247-Li Holmes Support Ltr B	Add Attachment	Delete Attachment	View Attachment
17. Resource Sharing Plan(s)	1248-Li Resource Sharing REV	Add Attachment	Delete Attachment	View Attachment

18. Appendix: Add Attachments | Remove Attachments | View Attachments

A. Specific Aims

Coronaviruses, a significant health threat to humans and other animals, have perplexing receptor recognition patterns. NL63 coronavirus (NL63-CoV), a prevalent human respiratory virus, is the only group-I coronavirus known to use angiotensin-converting enzyme 2 (ACE2) as its receptor. The other group-I coronaviruses use aminopeptidase-N (APN) from their respective hosts. Curiously, ACE2 is also used by group-II SARS coronavirus (SARS-CoV), the agent for the fatal human severe acute respiratory syndrome (SARS). Defined receptor-binding domains (RBDs) on the spike proteins of NL63-CoV and SARS-CoV both bind ACE2 with high affinity. This research investigates how coronaviruses recognize their receptors and how they interact with receptors from multiple hosts. Specifically, we will determine crystal structures of viral RBDs complexed with their receptor, use surface plasmon resonance to examine interactions between viral RBDs and their receptors, and use pseudotyped viral infection and cell-cell fusion assays to examine interactions between viral spike proteins and their receptors. This research has significant scientific and medical implications. It will not only explore novel principles governing viral evolution, virus-receptor interactions, viral host ranges and cross-species infections, but also guide antiviral efforts. The proposal has three specific aims.

Specific Aim 1: Receptor recognition mechanisms of group-I NL63 coronavirus

How do NL63-CoV and SARS-CoV both recognize human ACE2, despite no obvious sequence homology in their RBDs? One hypothesis is that NL63-CoV and SARS-CoV evolved two independent ways to bind ACE2. An alternative hypothesis is that NL63-CoV and SARS-CoV RBDs share homologous receptor-binding motifs (RBMs) that bind ACE2 in similar ways. In preliminary studies, we have determined the crystal structures of NL63-CoV RBD complexed with human ACE2 and of SARS-CoV RBD complexed with human ACE2. The structures show that although the two viruses have no structural homology in RBD cores or RBMs, they bind to the same “virus-binding hotspot” on ACE2. To further investigate the receptor recognition mechanisms of NL63-CoV, we will (i) improve the structural model of NL63-CoV RBD complexed with human ACE2 by improving the crystals of the RBD-ACE2 complex, (ii) study detailed structures of the virus-binding hotspot on human ACE2 and its functions in NL63-CoV binding by structure-guided mutagenesis, and (iii) study the interactions between the NL63-CoV spike protein and ACE2 proteins from different hosts. These studies will provide the molecular and structural basis for understanding receptor recognition mechanisms and host ranges of NL63-CoV, virus-binding hotspots on viral receptors, and for controlling NL63-CoV infections.

Specific Aim 2: Receptor recognition mechanisms of other group-I coronaviruses

How do NL63-CoV and other group-I coronaviruses, such as feline infectious peritonitis virus (FIPV) and porcine transmissible gastroenteritis virus (TGEV), recognize different receptor proteins, despite obvious sequence homology in their spike proteins? Our preliminary structural studies suggest that among group-I coronaviruses, RBD cores are conserved, but three RBM loops are variable. Thus we hypothesize that the three RBMs determine the receptor specificities of FIPV and TGEV. To test the above hypothesis, we will (i) characterize the receptor specificity of the predicted FIPV RBD by receptor binding assays, (ii) study the functions of the predicted FIPV RBMs, by systematically changing the RBMs of FIPV to those of NL63-CoV and monitoring whether the FIPV spike protein loses affinity for feline APN and gains affinity for human ACE2, and (iii) systematically change the RBMs of FIPV to those of TGEV and monitor whether the FIPV spike protein gains binding affinity for porcine APN. These studies will elucidate receptor recognition mechanisms of these animal coronaviruses, help control their infections, and provide insight into coronavirus evolution.

Specific Aim 3: Receptor recognition mechanisms of group-II SARS coronavirus

During the course of the SARS epidemic, various mutations were found in the RBM region of different SARS-CoV strains isolated from humans or palm civets. Why was each of the mutations selected, and what RBM mutations may appear in potential future SARS epidemics? Our preliminary studies have determined more crystal structures of the RBDs from different SARS-CoV strains complexed with human and civet ACE2. Based on these comparative structural studies, we hypothesize that the RBM mutations are viral adaptations to two important virus-binding spots on ACE2, and that they increase viral binding affinity for either human or civet ACE2. To test this hypothesis, we will (i) study detailed structures of the important virus-binding spots on ACE2 and their functions in SARS-CoV binding by structure-guided mutagenesis, (ii) study how each of the RBM mutations affect the interactions between the SARS-CoV spike protein and human or civet ACE2, and (iii) generate predictions of the RBM sequences of potential future SARS-CoV strains that have higher affinity for human or civet ACE2 than those of the past viral strains. These studies and predictions will elucidate receptor recognition and cross-species infection mechanisms of SARS-CoV. They will also be important for guiding epidemic monitoring and for the prevention and treatment of potential future SARS epidemics.

B. Background and significance

Receptor recognition by viruses

A fundamental yet unresolved puzzle in virology is how viruses evolve to recognize their receptor proteins (*1*). Specifically, how do different viruses recognize the same receptor protein, and how do similar viruses recognize different receptor proteins? Do viruses select their receptor proteins by chance, or do they target specific virus-binding hotspots on these receptor proteins? Structural information of virus-receptor interfaces can potentially answer these questions. To date, although a few studies have obtained structural information for a single virus-receptor interface (*2-6*), no study has provided structural information for the interfaces between different viruses and their common receptor protein. To fill in this critical gap in virology, this proposal explores the molecular and structural basis for how non-homologous receptor-binding proteins of two coronaviruses bind to their common protein receptor and how homologous receptor-binding proteins of a group of coronaviruses bind to different receptor proteins. This research also investigates potential virus-binding hotspots on viral receptor proteins.

Coronaviruses

Coronaviruses are significant health threats to both humans and other animals. In 2002-2003, a new type of human coronavirus, SARS coronavirus (SARS-CoV), caused the worldwide epidemic of severe acute respiratory syndrome (SARS), with over 8,000 infections and a fatality rate of about 10% (*7, 8*). Another human coronavirus, NL63 coronavirus (NL63-CoV), was identified in 2005, spreads worldwide, and is associated with common colds, croup and other respiratory diseases (*9, 10*). Potent neutralizing antibodies against NL63-CoV are detected in sera from nearly all humans above age 8, suggesting that NL63-CoV infection is common in childhood (*9, 11*). Moreover, coronaviruses are among major pathogens in non-human animals and frequently cause life-threatening diseases in economically important animals. For example, porcine transmissible gastroenteritis virus (TGEV) causes severe diarrhea in piglets, and feline infectious peritonitis virus (FIPV) causes fatal enteric or systemic diseases in cats (*12*). Overall, coronaviruses infect many mammalian and avian species and cause upper respiratory, gastrointestinal, and central nervous system diseases (*13*).

Coronaviruses are large, enveloped, positive-stranded RNA viruses. They have the largest genomes (about 30 kb in length) among RNA viruses. Coronaviruses are classified into three serological and genetic groups: mammalian group I, mammalian group II, and avian group III (*13*). Group I includes, among other viruses, NL63-CoV, FIPV, and TGEV. Group II includes human SARS-CoV, mouse hepatitis coronavirus (MHV), and other viruses. Group III includes avian infectious bronchitis virus (IBV) and other viruses.

Coronaviruses encode proteins that play different roles in the viral life cycle (*13*). Upon infecting a cell, the 5'-most open reading frame is translated into a large polypeptide that is subsequently cleaved by viral-encoded proteases. Several nonstructural proteins are released, including an RNA-dependent RNA polymerase and a helicase, which produce new genomic RNAs and subgenomic mRNAs. The mRNAs are used for the synthesis of other viral proteins, including structural proteins S (spike), M (membrane), E (small envelope) and N (nucleocapsid). The new genomic RNA assembles with the structural proteins, and the resulting nucleocapsid complexes bud into the lumen of the ER to form immature virus particles, which migrate through the Golgi complex and mature during this process. New mature virus particles are released from the infected cell by exocytosis. As new viruses attach to their receptor on new host cells, a new life cycle starts.

Coronavirus receptors

Coronaviruses employ a variety of host receptors. NL63-CoV is the only group-I coronavirus known to use angiotensin-converting enzyme 2 (ACE2) as its receptor (*11*), whereas the other group-I coronaviruses use aminopeptidase N (APN) from their respective hosts (*13-15*). Curiously, ACE2 is also the receptor for group-II SARS-CoV (*16*). Moreover, group-II MHV uses carcinoembryonic-antigen-cell-adhesion-molecule 1a (CEACAM1a) as its receptor (*17*). The perplexing receptor recognition patterns of coronaviruses described above offer a unique opportunity for studying and understanding virus-receptor interactions.

Coronavirus receptor proteins all have important physiological functions in their hosts. For example, ACE2 is a type I integral membrane protein and a zinc peptidase. The crystal structure of human ACE2 shows that the enzyme contains a claw-like N-terminal peptidase domain, with two lobes harboring the active site in a deep groove (*18*). ACE2 cleaves a variety of regulatory peptides, including angiotensin II. Some of the products it generates are potent vasodilators (*19*). Hence ACE2 has direct effects on heart function and blood pressure regulation (*20*). It is expressed in the lung, the gastrointestinal tract, and the heart (*21, 22*). The first

two organs are the major sites of SARS-CoV replications (*21*), whilst the lung is the major site of NL63-CoV replications (*9*). APN is a type II integral membrane protein and also a zinc peptidase. It functions in peptide metabolism by degrading bioactive peptides. APN is expressed in many cells and tissues including the lung and the gastrointestinal tract, both of which are major sites for FIPV and TGEV replications (*13*). The enzymatic activities of ACE2 and APN as zinc peptidases are not associated with their activities as coronavirus receptors (*13, 23*), raising the interesting question as to why they were selected by coronaviruses as receptors.

Coronavirus spike proteins

A coronavirus spike protein on the viral envelope guides viral entry into host cells. It first binds to its receptor on the host cell surface and subsequently fuses viral and host membranes (*13*). Thus the coronavirus spike protein is one of the most important determinants of virus-host interactions, viral host range, tropism, and viral entry into cells. It also induces most of the host immune responses.

The coronavirus spike protein is a member of the "class 1" fusion proteins that also include those of HIV, influenza, and Ebola viruses (*24*). A class 1 fusion protein is trimeric, contains a receptor-binding subunit and a membrane-fusion subunit, and has two different states. The "pre-fusion state" is a metastable state and can be triggered to undergo dramatic structural rearrangement to achieve a "post-fusion state". In the post-fusion state, two "heptad-repeat segments" from the membrane-fusion subunit form a stable "trimers-of-hairpin" structure, a previously buried hydrophobic "fusion peptide" becomes exposed and inserts into host membranes, and the viral and host membranes are brought together. Like other class 1 fusion proteins, the coronavirus spike protein has three segments: an ectodomain, a transmembrane anchor, and a short intracellular tail (Fig.1) (*13*). The ectodomain contains a receptor-binding subunit S1 and a membrane-fusion subunit S2. The S2 subunits from group-I and group-II coronaviruses both contain homologous heptad-repeat fragments that fold into the conserved trimers-of-hairpin structure (*25, 26*). Thus group-I and group-II coronaviruses share both sequence and structural homology in their S2 subunits.

Surprisingly, the S1 subunits from group-I and group-II coronaviruses have no obvious sequence homology. Nevertheless, they can both be divided roughly into N-terminal region (NTR), central region, and C-terminal region (CTR) (Fig.1). The S1 central regions of both NL63-CoV and SARS-CoV are defined receptor-binding domains (RBDs) that are sufficient for high-affinity binding to their common receptor ACE2 (*27-31*), whereas the NTR of MHV S1 is a defined RBD that binds CEACAM1a (*32*). Understanding the structural relationships among coronavirus S1 subunits can provide critical insight into coronavirus evolution.

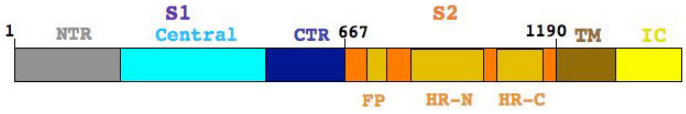


Figure 1. *Domain structure of the SARS-CoV spike protein*. It contains an ectodomain, a trans-membrane anchor (TM), and an intracellular tail (IC). The ectodomain contains a receptor-binding subunit S1 and a membrane-fusion subunit S2. S1 can be approximately divided into N-terminal region (NTR), Central region, and C-terminal region (CTR).

Receptor recognition by coronaviruses

How do NL63-CoV and SARS-CoV both use ACE2 as their receptor protein, despite no obvious sequence homology in their S1 subunits? One hypothesis is that the S1 subunits of NL63-CoV and SARS-CoV share homologous receptor-binding motifs (RBMs) that directly contact ACE2, and that through RNA recombination, SARS-CoV acquired its RBMs from NL63-CoV or an NL63-CoV-related group-I coronavirus, gaining its binding affinity for ACE2 and infectivity for human cells (*29, 33*). Extensive mutagenesis studies have been done to characterize the interactions between NL63-CoV and ACE2, but have failed to yield consistent results regarding the NL63-CoV binding site on ACE2 or the domain boundaries of NL63-CoV RBD (*28-30*). Structures of both the NL63-CoV-ACE2 and SARS-CoV-ACE2 interfaces will reveal the exact binding sites of the two viruses on ACE2 (Specific Aims 1 and 3). They will also elucidate how the non-homologous RBDs of two viruses can bind the same receptor protein, a long-standing puzzle in virology.

How do NL63-CoV and other group-I coronaviruses recognize different receptor proteins, despite obvious sequence homology in their S1 subunits? So far the receptor recognition mechanisms of FIPV and TGEV remain elusive. Although mutagenesis studies have identified some residues on feline and porcine APN that are important for viral binding (*34*), the viral elements required for receptor recognition are unknown. Given the sequence homology of their S1 subunits, structural information of NL63-CoV S1 will shed light on the receptor recognition mechanisms of not only NL63-CoV, but also FIPV and TGEV (Specific Aims 1 and 2).

The structures of virus-receptor interfaces are primary targets for human intervention. To date, no effective treatment exists for any coronavirus infections. Live attenuated coronavirus vaccines are prone to RNA recombination with circulating coronaviruses, making these vaccines ineffective and even dangerous (*12*). Coronavirus spike proteins, especially RBDs, are ideal candidates for subunit vaccines; they induce most of the host immune responses, are recognized by most of the neutralizing antibodies, and are safe (*13*). Structural information of coronavirus RBDs will guide the development of stable and effective subunit vaccines, whereas structural information of the virus-receptor interfaces can guide the design of drugs that block the virus-receptor interactions. Therefore, structural studies of coronavirus RBDs complexed with their receptors, as outlined in this proposal, will provide the structural basis for the development of subunit vaccines and antiviral drugs against human SARS, NL63-CoV-induced human respiratory diseases, and animal coronavirus infectious diseases.

Host ranges and cross-species infections of coronaviruses

SARS-CoV infects a wide variety of species, including humans, palm civets, monkeys, domestic cats, raccoon dogs, and bats (*33, 35*). Although the natural reservoir of SARS-CoV is still unknown, civets played a key role in the spread of SARS-CoV to humans (*33, 35, 36*). There have been at least two independent introductions of SARS-CoV into the human population: severe outbreaks in 2002-2003 (*37, 38*), and mild and sporadic infections in 2003-2004 (*39-41*). SARS has been absent in humans ever since. During the SARS outbreak, civets in Guangdong animal markets harbored viruses highly similar to human viral strains (*39*). In 2005 to 2006, the virus was found in civets in the wild (*42*). Given its animal origins, it is possible that SARS-CoV will cross species barriers between animals and humans to infect humans again, as it did twice before.

To study the cross-species transmission of SARS-CoV, we will first define the molecular and structural basis for the receptor-binding specificity of the viral spike protein. Although we recognize the importance of many other host factors that can also affect the efficiency of infection and replication of a virus in a specific host - for example host factors that affect viral membrane fusion, intracellular trafficking, genome replication, gene expression, virus assembly, and virus shedding - these host factors only come into play after virus has bound to a receptor on the cell membrane.

Several lines of study have established receptor recognition as the first of the major species barriers between humans and animals for SARS-CoV infection (*23, 43-46*). In other words, which host is susceptible to SARS-CoV is largely determined by the affinity between the viral spike protein and host ACE2 in the initial viral attachment step First, epidemic and biochemical studies show that there exist four prototypic SARS-CoV strains: hTor02 (human strain isolated in 2002-2003), cSz02 (civet strain isolated in 2002-2003), hcGd03 (human and civet strain isolated in 2003-2004), and cGd05 (civet strain isolated in 2005-2006) (see Fig.3D for amino acid variations in viral RBDs) (*39, 41, 42*). Human ACE2 has high affinity for hTor02 RBD, moderate affinity for hcGd03 RBD, and poor affinities for cSz02 and cGd05 RBDs. In contrast, civet ACE2 has high affinity for all of the four RBDs (*23, 42*). Correspondingly, humans are most susceptible to hTor02, whereas civets are susceptible to all four viral strains (*23, 42*). Second, cell biology studies show that SARS-CoV does not infect murine cells efficiently because of the poor binding affinity between murine ACE2 and SARS-CoV. However, murine cells that express human ACE2 on their surface are highly susceptible to SARS-CoV (*43*). Third, animal studies show that mice are not effective hosts for SARS-CoV, but transgenic mice that express human ACE2 become good hosts for SARS-CoV (*44, 47*). Fourth, reverse genetics studies show that synthetically reconstructed civet SARS-CoV strains are completely dependent on ACE2 for entry and infection. For example, the cSz02 strain only efficiently infects cell lines that express civet ACE2, but not the same cell lines that express human ACE2. However, when adapted to human airway epithelial (HAE) cells, one or a few mutations on the cSz02 RBD are sufficient to fully restore viral infectivity in human cells, generating a human strain hHAE08 (Fig.3D) (*48, 49*). Therefore, all evidence so far has identified receptor recognition as an important limiting step for SARS-CoV to transmit between humans and several animal hosts such as civets and mice. Structural studies of the SARS-CoV-ACE2 interactions (Specific Aim 3) can potentially elucidate the molecular and structural mechanisms of the viral cross-species infections pertinent to receptor recognition.

The above studies have established SARS-CoV as an excellent model system for understanding host ranges and cross-species infections of coronaviruses. Like SARS-CoV, the host ranges of FIPV and TGEV are also associated with the abilities of their spike proteins to recognize the receptor proteins from their hosts. Despite extremely high sequence homology in the FIPV and TGEV S1 subunits, FIPV only recognizes feline APN, not porcine APN, whereas TGEV recognizes both porcine and feline APN (*34*). Correspondingly,

although FIPV and TGEV cause transmissible diseases only in their natural hosts, TGEV can also replicate in cats without causing disease (*50*). To date, the host range of NL63-CoV is unknown. It has been shown that the NL63-CoV spike protein has high affinity for human and civet ACE2 but low affinity for murine ACE2 (*11, 29*). Structural studies of the interactions between the coronavirus spike proteins and their animal receptors, as outlined in this proposal, will have important implications for viral host ranges and cross-species infections.

C. Preliminary Studies

Electron microscopic studies of the SARS-CoV spike protein

In a previous study, we obtained a low-resolution view of the SARS-CoV spike protein in both pre-fusion and post-fusion states. We first prepared and characterized the spike protein ectodomain in the two states, and then collected negative-stain electron microscopic images of the molecules (Fig.2A) (*51*). The pre-fusion molecule is a clove-shaped trimer, with three individual S1 heads and a trimeric S2 stalk. The post-fusion molecule corresponds to structurally rearranged trimeric S2 that associates together through exposed hydrophobic fusion peptides to form rosettes. We further showed that the transition of the spike protein from pre-fusion to post-fusion states requires both proteolysis at the S1/S2 boundary and ensuing dissociation of S1. The states and transitions suggest conformational changes of the spike protein that mediate viral entry into cells (*51*). To locate the receptor-binding site on the spike protein, we prepared human ACE2 and collected negative-stain electron microscopic images of the pre-fusion spike protein with bound ACE2. ACE2 binds to the tip of the spike protein trimer, which we conclude contains the RBD (Fig.2B) (*51*).

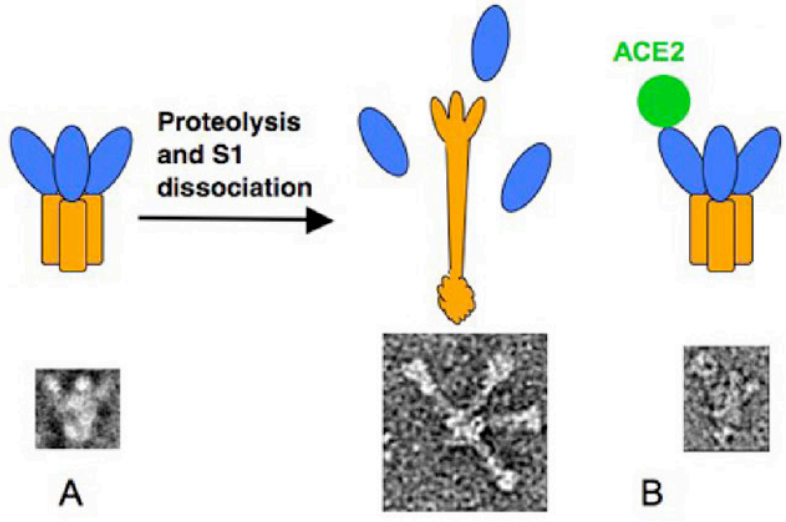


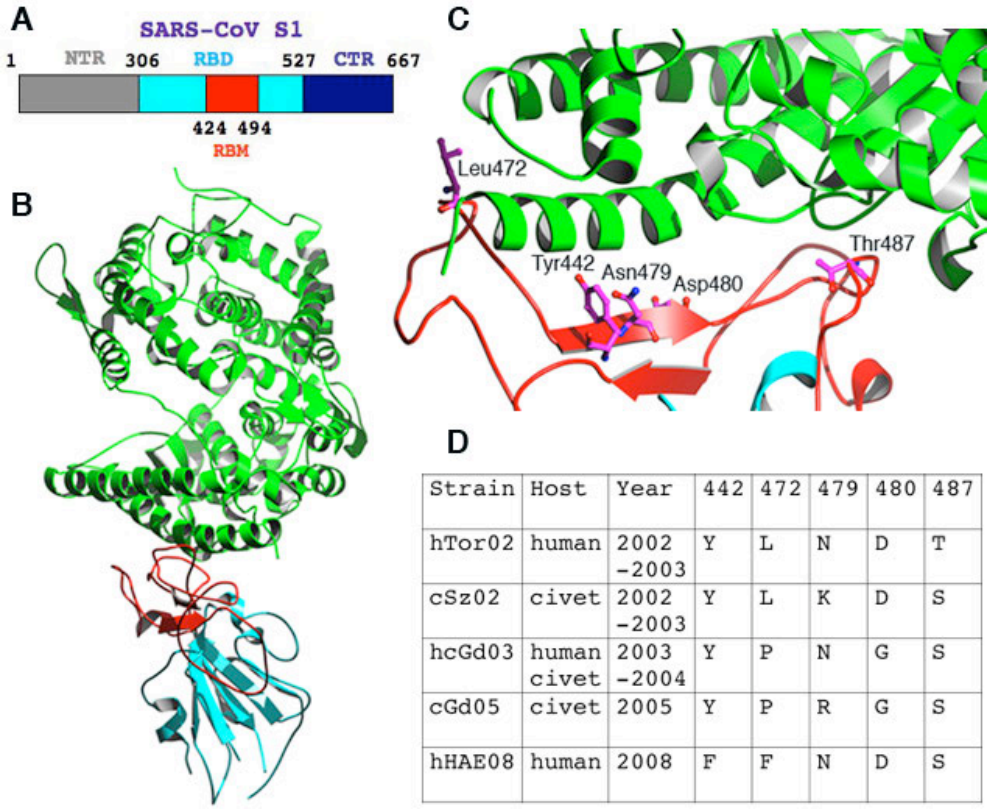
Figure 2. *Electron microscopic studies of the SARS-CoV spike protein (51).* (A) Schematic drawings of the spike protein in two different states and their corresponding negative-stain electron microscopic images. The molecule in the pre-fusion state (left) is a clove-shaped trimer, with three individual S1 heads (blue) and a trimeric S2 stalk (orange). The molecule in the post-fusion state (right) corresponds to structurally rearranged trimeric S2 and dissociated monomeric S1. Conformational changes of the molecule require both proteolysis at the S1/S2 boundary and ensuing dissociation of S1. (B) The spike protein in the pre-fusion state decorated by receptor ACE2 (in green).

Crystal structure of SARS-CoV RBD complexed with human ACE2

In another previous study, we determined the crystal structure of the hTor02 RBD complexed with human ACE2 (Fig.3) (*5*). We first identified the domain boundaries of the RBD by limited proteolysis of a longer S1 fragment (Fig.3A), prepared the corresponding RBD fragment, and crystallized it in complex with human ACE2. We determined the structure by molecular replacement using the ACE2 structure as the search model, and refined the structure at 2.9Å resolution. The SARS-CoV RBD has a novel fold. It contains two subdomains: a core structure and an extended loop. The core structure is a five-stranded antiparallel β-sheet, with three short connecting α-helices. The extended loop lies on one edge of the core structure and makes all of the contacts with the receptor, and thus we refer to it as receptor-binding motif (RBM). The structure of the RBD-ACE2 complex showed that the RBD presents a gently concave surface to cradle the outer surface of the N-terminal lobe of ACE2 (Fig.3B, 3C) (*5*). The atomic details at the RBD-ACE2 interface clarify the importance of viral mutations that facilitate efficient civet-to-human and human-to-human transmissions. These mutations have been elucidated further by our preliminary studies in this proposal.

Structural analysis of major species barriers between humans and civets for SARS-CoV infections

In preliminary studies, we determined two additional crystal structures of the hTor02 and cSz02 RBDs each complexed with a human-civet chimeric ACE2. The rationale and design of the chimeric ACE2 are discussed in detail in Section D.1. In brief, the chimeric ACE2 has the same receptor activity as civet ACE2 (*52*). Thus our preliminary structural studies have revealed detailed, bona fide interactions between the two SARS-CoV strains and civet ACE2 (*52*). These structures, combined with the structure of the hTor02 RBD complexed with human ACE2, have provided the structural basis for understanding some of the major species barriers between humans and civets for SARS-CoV infections. Specifically, viral RBD mutations at 479 and 487 positions are critical for the cross-species infections of SARS-CoV.



D

Strain	Host	Year	442	472	479	480	487
hTor02	human	2002 -2003	Y	L	N	D	T
cSz02	civet	2002 -2003	Y	L	K	D	S
hcGd03	human civet	2003 -2004	Y	P	N	G	S
cGd05	civet	2005	Y	P	R	G	S
hHAE08	human	2008	F	F	N	D	S

Figure 3. *Crystal structure of SARS-CoV RBD complexed with human ACE2 (5)*. (A) Domain structure of SARS-CoV S1. The boundaries of the RBD were determined by limited proteolysis of longer S1 fragments. The receptor-binding motif (RBM) was identified from the structure. (B) Overall structure of the complex. RBD core is in cyan, RBM in red, and ACE2 in green. (C) Details of the interface, showing residues on the RBM that underwent mutations in past viral strains. The view is derived by rotating the one in (B) 90° clockwise along a vertical axis. (D) Alignment of RBM residues that underwent mutations.

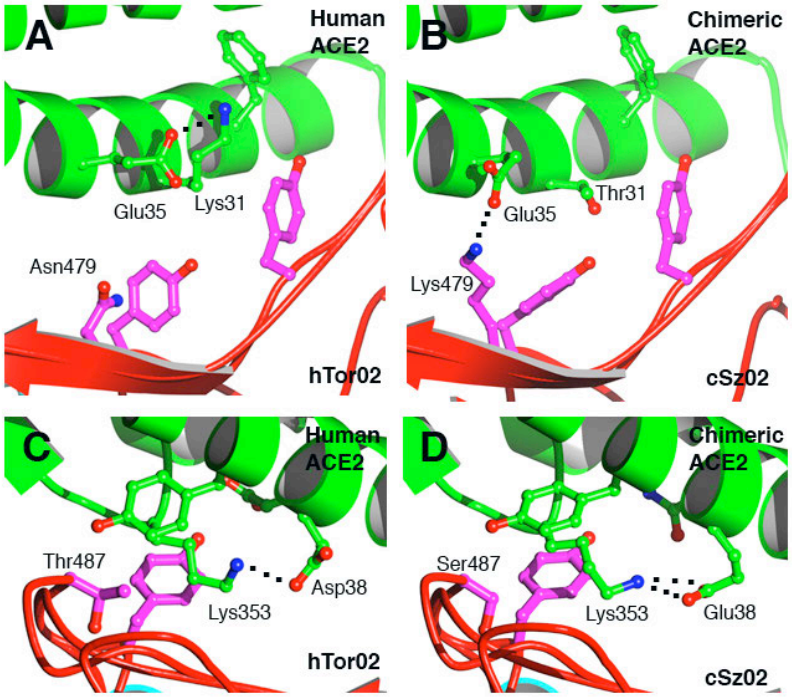


Figure 4. *Structural basis for some of the major species barriers between humans and civets for SARS-CoV infections.* (A) Structure of the interfaces between hTor02 strain and human ACE2. (B) Interface between cSz02 and chimeric ACE2. (C) Interface between hTor02 and human ACE2. (D) Interface between cSz02 and chimeric ACE2. Asn479, but not Lys479, on the RBD is compatible with human ACE2. Both Asn479 and Lys479 are compatible with civet ACE2. Thr487, but not Ser487, on the RBD is compatible with human ACE2. Both Thr487 and Ser487 are compatible with civet ACE2. Thus the K479N mutation is important for SARS-CoV to jump from civets to humans, whereas the S487T mutation is important for SARS-CoV to transmit from humans to humans.

Residue 479 is an asparagine in the hTor02 strain, but a lysine in the cSz02 strain (Fig.3D). Based on our structures, Lys31 on human ACE2 is embedded in a hydrophobic environment, and its charge is neutralized by ACE2 Glu35. Lys479 in the cSz02 strain competes with Lys31 for Glu35 as a salt bridge partner, leaving Lys31 as a free charge in a hydrophobic environment and destabilizing the binding interface (Fig.4A). Consistent with our structural analysis, the K479N mutation on cSz02 RBD increases its affinity for human ACE2 by over 30-fold (*23*), and thus is a critical step for SARS-CoV to jump from civets to humans.

An important question is why civet ACE2, but not human ACE2, can accommodate a lysine at the 479 position. On civet ACE2, residue 31 is a threonine, which cannot form a salt bridge with Glu35, making Glu35 available to form a salt bridge with Lys479 in the cSz02 strain (Fig.4B). Thus SARS-CoV strains with Lys479 have high affinity for civet ACE2, but not human ACE2.

Residue 487 is a threonine in the hTro02 strain, but a serine in the cSz02 strain (Fig.3D). Based on our structures, the methyl group of Thr487 interacts with ACE2 Lys353 (Fig.4C). Like Lys31, Lys353 is embedded in a hydrophobic environment, and its charge is neutralized by ACE2 Asp38. The salt bridge between Lys353 and Asp38 is not strong due to the positioning of the two side chains. The methyl group of Thr487 provides essential support for the formation of this salt bridge. Without this methyl group, Lys353 becomes a free charge in a highly hydrophobic environment, destabilizing the binding interface. Consistent with our structural analysis, the T487S viral mutation on the hTor02 RBD decreases its binding affinity for human ACE2 by over 20-fold (*23*). Indeed, residue 487 is a serine in the hcGd03 strain and there was no human-to-human transmission during the 2003-2004 SARS infections. Therefore, the S487T mutation is a critical step for SARS-CoV to transmit from humans to humans.

Why is civet ACE2, but not human ACE2, capable of accommodating a serine at the 487 position? On civet ACE2, residue 38 is a glutamate, whose side chain is longer than that of an aspartate. Hence Lys353 can form a strong bifurcated salt bridge with Glu38 with or without the support from residue 487 (Fig.4D). Thus SARS-CoV strains with Ser487 have high affinity for civet ACE2, but not human ACE2.

Overall, our preliminary studies suggest that early civet SARS-CoV isolates were prevented from infecting human cells because they were not adapted to two important virus-binding spots, one around Lys31 and the other around Lys353, on human ACE2, and that SARS-CoV has evolved to gain sustained infectivity for human cells through stepwise adaptation mutations at residues 479 and 487 on its RBD. The former likely determines whether SARS-CoV can infect humans, whereas the latter largely determines whether SARS-CoV can maintain sustained infections in humans. These results enhance our understanding of host adaptations and cross-species infections of SARS-CoV and potentially of other emerging animal viruses. The two important virus-binding spots on human ACE2 will be studied in more detail in this proposal (Specific Aims 1 and 3).

Structure determination of NL63-CoV RBD complexed with human ACE2

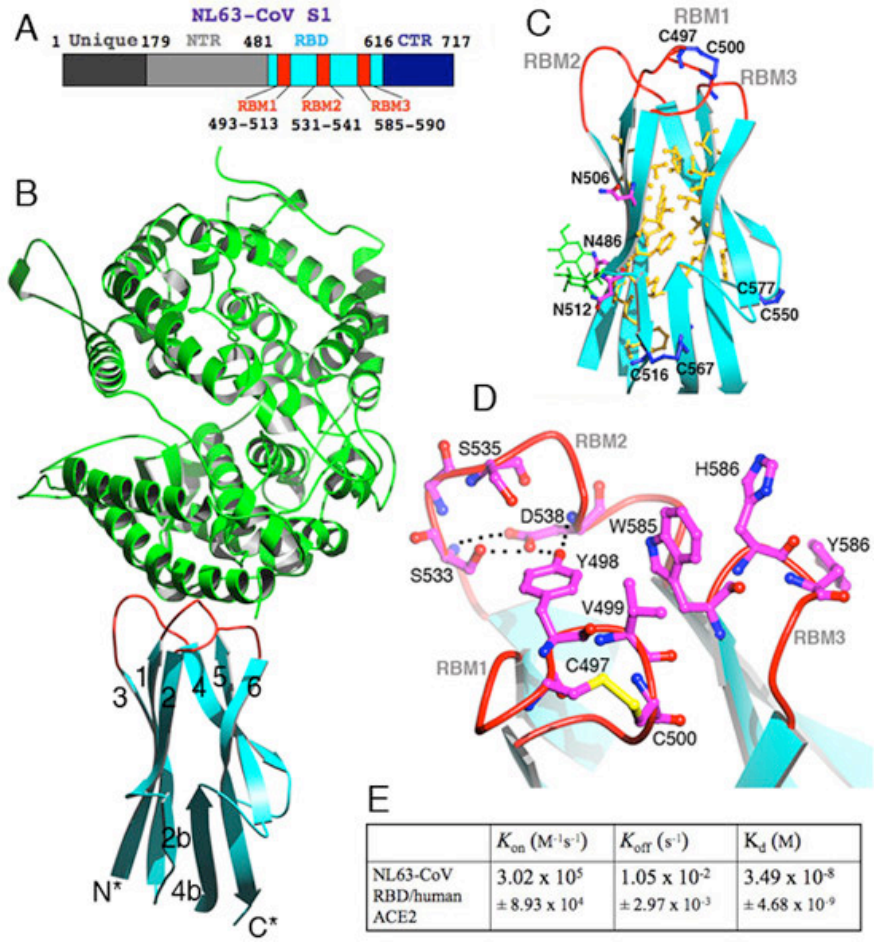
In other preliminary studies, we determined the crystal structure of NL63-CoV RBD complexed with human ACE2. To prepare NL63-CoV RBD for crystallization, we designed 36 RBD constructs with different N- and C-termini, based on biochemical studies (*28-30*) and secondary structure prediction of the S1 sequence. We expressed and purified each of these fragments in insect cells. None of the originally expressed fragments yielded crystals, either alone or in complex with ACE2. However, α-chymotrypsin treatment of one fragment (residues 461-616) yielded a smaller but more stable fragment (residues 481-616), which was subsequently crystallized in complex with the human ACE2 peptidase domain (Fig.5A). We determined the structure by molecular replacement using ACE2 as the search model (Fig.5B). We refined the structure at 3.3Å resolution.

Structure of NL63-CoV RBD

The core of NL63-CoV RBD can be best described as a β-sandwich. It consists of two layers of three-stranded β-sheets, one with mixed polarity (strands 3-1-2) and the other anti-parallel (strands 4-5-6) (Fig.5B). The two layers stack tightly against each other through extensive hydrophobic interactions (Fig.5C). A short three-stranded, anti-parallel β-sheet (strands 2b-4b-6) stabilizes the distal end of the RBD (opposite the receptor-binding interface); this end contains both the N- and C-termini of the RBD, and hence likely interacts with the rest of S1 and is not as ordered as the receptor-binding interface (Fig.5B). Three disulfide bonds are present in the RBD (Fig.5C). The Cys497-Cys500 disulfide bond strengthens a critical receptor-binding loop. Two other disulfide bonds, connecting Cys516-Cys567 and Cys550-Cys577, strengthen the region at the distal end. Alanine substitutions for Cys516, Cys550, Cys567 or Cys577 decrease protein stability and abolish protein expression (*30*). Overall, the β-sandwich structure of NL63-CoV RBD is a novel protein fold.

Three discontinuous receptor-binding sites on NL63-CoV RBD, which we term “receptor-binding motifs” (RBMs), are presented by the β-sandwich core to bind ACE2. Based on their order in the primary structure, we define the three RBMs as RBM1 (residues 493-513), RBM2 (residues 531-541), and RBM3 (residues 585-590) (Fig.5A). All three RBMs are connected to β-strands and are thus β-loops. RBM3 is more compact than RBM1 and RBM2. Nevertheless, RBM1 is strengthened by the Cys497-Cys500 disulfide bond, and RBM2 is reinforced by key hydrogen-bonds involving Asp538 (Fig.5D). Alanine substitutions for Cys497, Cys500 or

Asp538 abolish receptor binding without significantly affecting protein expression (*30*). At the top of the RBD, the three protruding RBMs surround a shallow bowl-shaped cavity, which is important for receptor binding.



	K_{on} ($M^{-1}s^{-1}$)	K_{off} (s^{-1})	K_d (M)
NL63-CoV RBD/human ACE2	3.02×10^5 $\pm 8.93 \times 10^4$	1.05×10^{-2} $\pm 2.97 \times 10^{-3}$	3.49×10^{-8} $\pm 4.68 \times 10^{-9}$

Figure 5. *Crystal structure of NL63-CoV RBD complexed with human ACE2.* (A) Domain structure of NL63-CoV S1. The N-terminal unique domain only exists in NL63-CoV, and is not involved in ACE2 binding (*28, 29*). The boundaries of the RBD were determined by limited proteolysis of longer S1 fragments. RBMs were identified from the structure. (B) Overall structure of the complex. The RBD core is in cyan, RBMs in red, and ACE2 in green. (C) β-sandwich core structure of NL63-CoV RBD. Hydrophobic residues between β-sheet layers are in yellow, cysteines in blue, glycans in green, and glycosylated asparagines in magenta. Two of the three predicted N-linked glycosylation sites in the RBD (Asn486, Asn506 and Asn512) are confirmed in the structure (Asn486 and Asn512). (D) NL63-CoV RBMs. (E) Kinetics and binding affinity of NL63-CoV RBD and human ACE2 by surface plasmon resonance using Biacore.

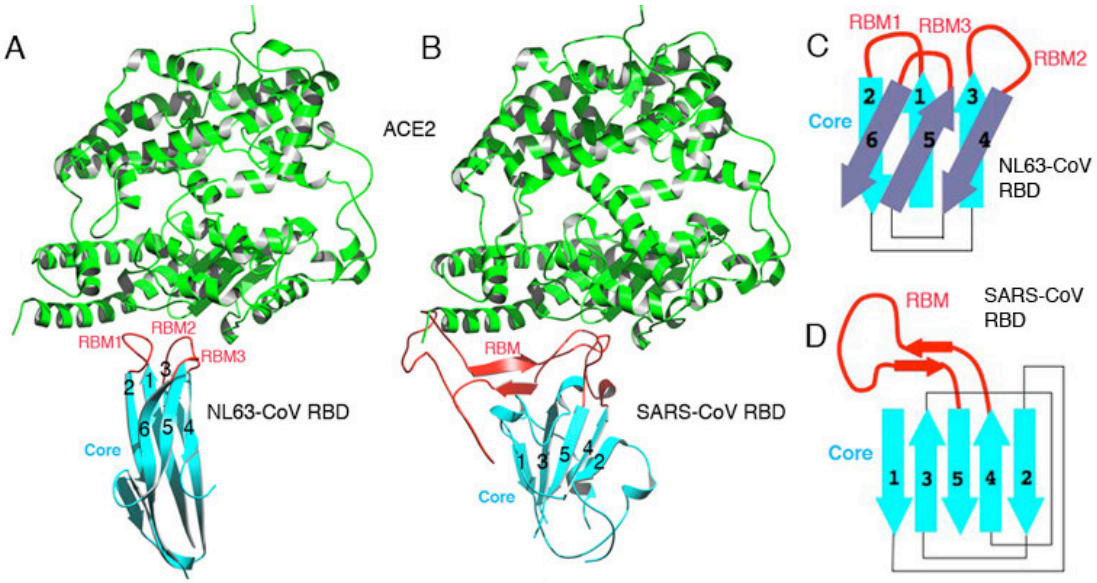


Figure 6. *Structural comparison of NL63-CoV and SARS-CoV RBDs*. (A) Another view of the structure of the NL63-CoV-RBD-ACE2 complex, which is derived by rotating the one in Fig.5B 90° clockwise along a vertical axis. (B) Structure of the SARS-CoV-RBD-ACE2 complex, from the same orientation as in (A). (C) Schematic illustration of the topology of NL63-CoV RBD. Strands are drawn as arrows. (D) Schematic illustration of the topology of SARS-CoV RBD.

Structural comparison of NL63-CoV and SARS-CoV RBDs

NL63-CoV and SARS-CoV RBDs have no structural homology in either cores or RBMs, although they are both located in the S1 central regions (Fig.3A, 5A). The core of SARS-CoV RBD is a single-layer, five-stranded anti-parallel β-sheet, stabilized by three short α-helices (Fig.6B, 6D); the core of NL63-CoV RBD is a β-sandwich with novel topology (Fig.6A, 6C). The number of β-sheet layers, the number of β-strands and most notably, the topologies of the β-sheets, all differ in the two RBD cores. The RBMs of SARS-CoV and NL63-CoV are also completely different. The SARS-CoV RBM is a continuous 70-residue-long subdomain that lies on one edge of the core (Fig.6B, 6D). The NL63-CoV RBMs are three discontinuous β-loops (Fig.6A, 6C). The lack of structural homology in RBD cores and more importantly, the lack of structural homology in RBMs, indicate two independent ways in which NL63-CoV and SARS-CoV recognize their common receptor protein.

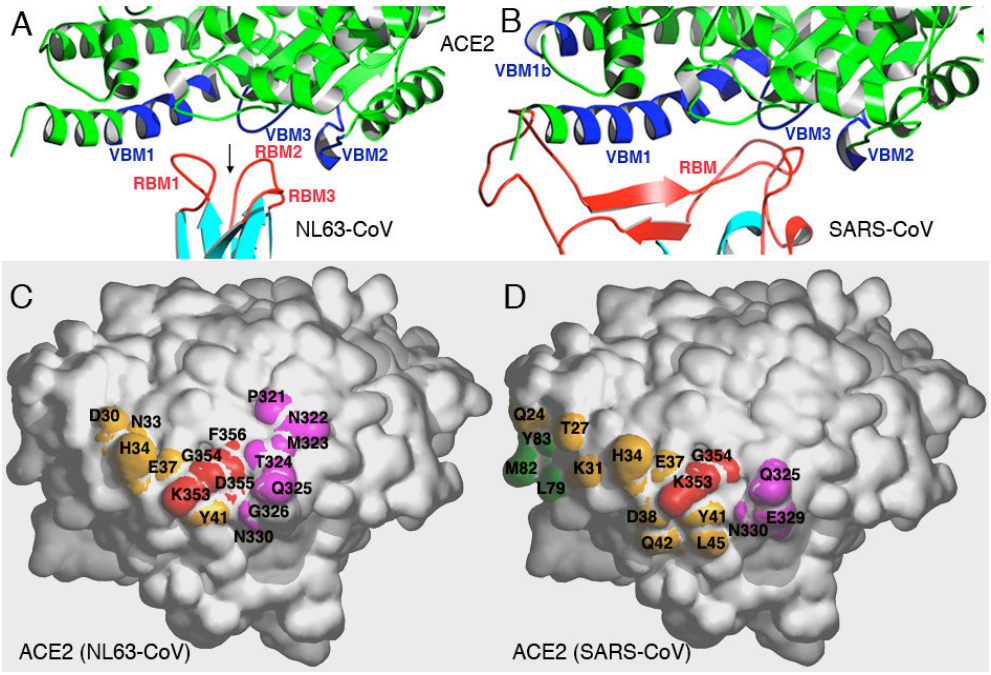


Figure 7. *Structural comparison of NL63-CoV-ACE2 and SARS-CoV-ACE2 interfaces*. (A) Enlarged view of the NL63-CoV-ACE2 interface, from the same orientation as in Fig.2C. Virus-binding motifs (VBMs) on ACE2 are in blue, and RBMs on NL63-CoV are in red. Arrow indicates the bowl-shaped cavity surrounded by three viral RBMs. (B) Enlarged view of the SARS-CoV-ACE2 interface, from the same orientation as in (A). (C) Footprint of NL63-CoV on the surface of ACE2. The view is derived from the one in (A) by rotating ACE2 by 90° along a horizontal axis, in such a way that the edge facing the viewer moves up. VBM1 residues are in orange, VBM2 residues in magenta, and VBM3 residues in red. (D) Footprint of SARS-CoV on the surface of ACE2, from the same orientation as in (C). VBM1b residues are in green.

Structure of NL63-CoV-receptor interface

NL63-CoV RBD binds to the outer surface of the N-terminal lobe of the ACE2 peptidase domain (Fig.5B, 6A). Three discontinuous virus-binding sites on ACE2, which we term “virus-binding motifs” (VBMs), are directly involved in virus binding (Fig.7A). Based on their order in the primary structure, we define the three VBMs as VBM1 (residues 30-41 on the N-terminal helix), VBM2 (loop containing residues 321-330), and VBM3 (loop containing residues 353-356). The binding surfaces of ACE2 and RBD are complementary, with receptor VBM3 inserting into the bowl-shaped cavity on RBD (Fig.7A). All of the predicted and identified glycosylation sites on RBD and ACE2 are located away from the virus-receptor interface (Fig.5C, (*18*)), and thus do not interfere with virus-receptor interactions. A total of 11 viral residues directly contact 16 receptor residues, and the binding buries about 1300Å^2 total surface area. This binding interface is slightly smaller than other virus-receptor binding interfaces that typically range between 1400-1800Å^2 (2-6). Nevertheless, the binding affinity between NL63-CoV RBD and ACE2 is high. By surface plasmon resonance using Biacore, we measured the dissociation constant between the two proteins as 34.9nM (Fig.5E).

NL63-CoV and SARS-CoV bind to common regions on ACE2, even though they have no structural homology in RBD cores or RBMs. Both viruses recognize the same three VBMs on the outer surface of the N-terminal lobe of ACE2 (SARS-CoV recognizes an additional short VBM1b) (Fig.7A, 7B). Compared with SARS-CoV RBD, NL63-CoV RBD has less extensive contact with VBM1, but more extensive contact with VBM2 and VBM3 (Fig.7C, 7D). In addition, nearly the same number of ACE2 residues directly contact each of the two

viruses. Among these ACE2 residues, seven directly contact both of the viruses (Fig.7C, 7D). Overall, although SARS-CoV forms a larger binding interface with ACE2 than NL63-CoV does, the two viral RBDs bind to ACE2 with similar affinity (Fig.5E, (*23*)).

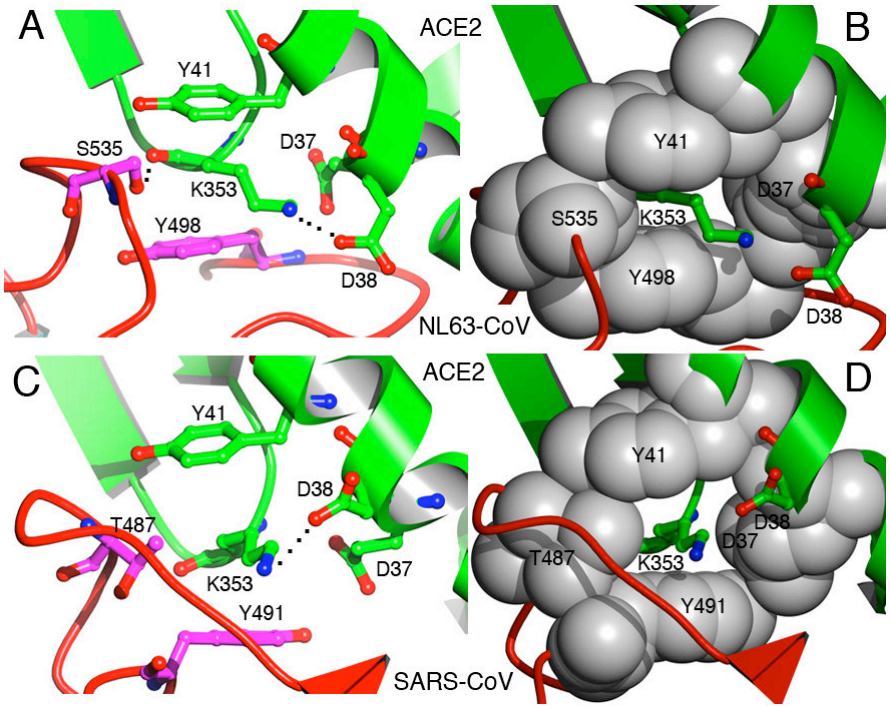


Figure 8. *A common virus-binding hotspot on ACE2 for the binding of both NL63-CoV and SARS-CoV.* (A) Stick-and-ball representation of the hotspot at the NL63-CoV-ACE2 interface. (B) Corey-Pauling-Koltun representation of the hotspot at the NL63-CoV-ACE2 interface. (C) Stick-and-ball representation of the hotspot at the SARS-CoV-ACE2 interface. (D) Corey-Pauling-Koltun representation of the hotspot at the SARS-CoV-ACE2 interface.

A common virus-binding hotspot on human ACE2

A virus-binding hotspot on ACE2 lies at the center of the NL63-CoV-receptor interface. Lys353 is embedded in a hydrophobic tunnel surrounded by two aromatic rings of ACE2 Tyr41 and NL63-CoV Tyr498 and by two alkyl chains of ACE2 Asp37 and NL63-CoV Ser535 (Fig.8A, 8B). At the end of the tunnel, ACE2 Asp38 forms a salt bridge with Lys353, neutralizing its charge. Because of the hydrophobic environment, this salt bridge is energetically stabilizing (*53*) and critical for virus-receptor interactions. Alanine substitution for Lys353 abolishes NL63-CoV binding (*29, 30*). The same virus-binding hotspot on ACE2 is also key to the binding of SARS-CoV. The structures of the hotspots at the two different virus-receptor interfaces are strikingly similar (Fig.8). The receptor parts are nearly identical, with subtle changes in protein side chain conformations. In the viral parts, Thr487 and Tyr491 on SARS-CoV replace Ser535 and Tyr498 on NL63-CoV, respectively, as two of the four tunnel walls. Adaptation to the hotspot on ACE2 is critical for SARS-CoV pathogenesis. A single T487S mutation that disturbs the hotspot decreases the binding affinity between SARS-CoV RBD and human ACE2 by over 20-fold, and may explain why the SARS infections in 2003-2004 were mild (*23, 33, 52*). Therefore, virus-binding hotspots on receptor proteins likely dictate virus-receptor interactions, viral pathogenesis and viral transmissibility, and thus are major binding targets for viruses.

What are the differences between a virus-binding hotspot on a receptor protein and a hotspot for host protein-protein interactions? Host protein partners have co-evolutionary relationships (*54, 55*). Correspondingly, hotspots for host protein-protein interactions are usually pre-existent and pre-organized in both of the unbound host protein structures (*56, 57*). Viruses and receptors, however, do not have co-evolutionary relationships; only viruses evolve to adapt to host receptors, but receptors do not evolve to adapt to viruses. Moreover, if a virus exerts large enough impacts on a host, the host receptor may evolve away from virus binding (*58*). So far the virus-binding hotspot on ACE2 is not known for its interaction with any other host proteins. In the unbound ACE2 structure where structural restrains from viruses are absent, Lys353 projects into solution; it does not form a salt bridge with Asp38 or stack with Tyr41 or Asp37 (*18, 52*). Thus the common virus-binding hotspot on ACE2 is not pre-existent or pre-organized; instead, it is induced to form by virus binding, consistent with the one-sided evolutionary relationships between viruses and their receptors.

Receptor-recognition mechanisms of other group-I coronaviruses

Despite their homologous spike proteins, NL63-CoV and other group-I coronaviruses recognize different receptors. The S1 central regions of porcine transmissible gastroenteritis virus (TGEV), feline infectious peritonitis virus (FIPV), porcine respiratory coronavirus (PRCV), and canine coronavirus (CCoV) are highly homologous to each other, and they all use APN from their respective hosts as receptors (*34*). Based on structural data for NL63-CoV RBD and sequence alignment of the S1 central domains of group-I coronaviruses (Fig.9), we make the following predictions. First, the β-sandwich core structure of NL63-CoV RBD is likely conserved among group-I coronaviruses. The sequences of all of the β-strands are conserved, so are all of the four cysteines in the core that form two disulfide bonds and strengthen the β-sandwich structure. Second, we predict that the three ACE2-binding RBMs in NL63-CoV are APN-binding sites in TGEV, FIPV, PRCV and CCoV; structural changes in these RBMs result in the recognition of different receptors by these homologous viruses. Compared with NL63-CoV RBMs, the predicted RBM1 of other group-I coronaviruses has a two-residue insertion and loses a critical disulfide bond, the predicted RBM2 has a nine-residue insertion and two additional cysteines that likely form a disulfide bond, and the predicted RBM3 contains a one-residue deletion. These structural changes are expected to abolish ACE2-binding capabilities of NL63-CoV and may help confer APN-binding capabilities of other group-I coronaviruses. Indeed, mutagenesis studies show that the predicted RBM2 regions of FIPV and TGEV are involved in binding feline and porcine APNs, respectively (Wennier, S.T. and Holmes, K.V., personal communication). The receptor-recognition mechanisms of these important animal coronaviruses will be further studied in this proposal (Specific Aim 2).



Figure 9. *Sequence alignment of NL63-CoV RBD with predicted RBDs of four other group-I coronaviruses*. The alignment was generated by ClustalW program. RBMs are in red, four conserved cysteines in the core structure are in magenta, two cysteines existent in RBM1 of NL63-CoV but missing in other group-I coronaviruses are in blue, and two cysteines existent in RBM2 of other group-I coronaviruses but missing in NL63-CoV are also in blue. β-strands are drawn as arrows. FIPV: feline infectious peritonitis virus; TGEV: porcine transmissible gastroenteritis virus; CCoV: canine coronavirus; PRCV: porcine respiratory coronavirus. The GenBank accession numbers are: AAY16375 (FIPV), DQ112226 (CCoV), AAB30949 (TGEV), CAA42686 (PRCV), and CAA42686 (NL63). "*" indicates positions that have a single, fully conserved residue. ":" indicates positions that have strongly conserved residues. "." indicates positions that have weakly conserved residues. FIPV sequence is numbered.

Evolution of coronaviruses

Coronaviruses are believed to have common ancestors because they share similar replication mechanisms, genomic structures, and overall gene sequences (*13*). Among all of the coronavirus genes, the one encoding the spike protein is the most variable. Between the spike protein subunits, S1 is more variable than S2. The hypervariation of S1 reveals the tremendous evolutionary pressure that coronaviruses face to adapt to different host receptors. The NL63-CoV and SARS-CoV RBDs both reside in the S1 central domain, as do the predicted RBDs of other group-I coronaviruses. The prominent role of the S1 central domain in receptor recognition is likely due to its location on the tip of the spike protein trimer, which is the most exposed

and accessible area on the spike protein (Fig.2B) (*51*). Importantly, the current structural divergences of the S1 central domains reflect on the evolutionary history of coronaviruses and their receptor selections.

Our study clarifies the baffling evolutionary relationships between NL63-CoV and SARS-CoV. First, due to lack of structural homology in RBD cores and more importantly, lack of structural homology in RBMs, NL63-CoV and SARS-CoV must have evolved their ACE2-binding capabilities independently, disproving the previous hypothesis that the newly emerged SARS-CoV acquired its RBM from the more ancient NL63-CoV or an NL63-CoV-related group-I coronavirus (*29, 33*). Second, binding to the same virus-binding hotspot on their common receptor protein, the two viruses present a remarkable case of functionally convergent evolution that occurred after divergent evolution. Whereas differences in ancient host environments likely caused the two viruses to initially diverge, the virus-binding hotspot on their common receptor protein was the probable driving force for the subsequent convergent evolution.

Antiviral strategies

This research has important medical implications. Based on our structures of the SARS-CoV-RBD-ACE2 and NL63-CoV-RBD-ACE2 complexes, four antiviral strategies may be developed to combat coronavirus infections. First, small molecule inhibitors may be designed to target the hydrophobic bowl-shaped cavity surrounded by the three protruding RBMs of NL63-CoV (Fig.7A). This cavity accommodates VBM3 from ACE2, and blocking it with bound compounds may prevent NL63-CoV from binding to ACE2. Second, small molecule inhibitors may be designed to target the cavity between VBM1 and VBM3 on ACE2 (to block the binding of NL63-CoV) or the cavity between VBM2 and VBM3 on ACE2 (to block the binding of both NL63-CoV and SARS-CoV) (Fig.7A, 7B). Third, the RBD of either SARS-CoV or NL63-CoV may be used to inhibit both SARS-CoV and NL63-CoV infections, due to potential competition with both viruses for the common virus-binding hotspot on ACE2 (Fig.7, 8). Finally, SARS-CoV and NL63-CoV RBDs may be used as subunit vaccines. Strengthening the regions at the distal end of both RBDs by protein engineering, such as shortening disordered loops and introducing additional disulfide bonds, may facilitate vaccine development (Fig.3B, 5B). The predicted RBDs of other group-I coronaviruses may also be used as subunit vaccines for animals. Overall, our research provides a structural framework for the development of antiviral strategies to combat human SARS, NL63-CoV-induced human respiratory diseases, and animal coronavirus infectious diseases.

D. Research Design and Methods

This research elucidates receptor recognition mechanisms of representative coronaviruses including group-I NL63-CoV, other group-I coronaviruses, and group-II SARS-CoV. This research provides the molecular and structural basis for understanding coronavirus evolution, coronavirus-receptor interactions, coronavirus host ranges and cross-species infections, and for the development of novel antiviral strategies against coronavirus infections. It also investigates a long-standing puzzle in virology, which is how different viruses recognize the same receptor protein and how similar viruses recognize different receptor proteins. Furthermore, this research explores novel principles of virology that govern virus-binding hotspots on viral receptor proteins, and host adaptations and cross-species infections of emerging animal viruses. Our preliminary studies have set up an initial structural framework within which these goals can be achieved. Detailed knowledge about the above mechanisms and principles, however, requires in-depth and systematic studies using multi-disciplinary approaches. In this section, we first lay out our research tools that include crystallography, biochemistry, molecular biology and virology, and then describe our research plans to address three specific aims.

D.1 General research tools

Cloning, protein expression and purification: Proteins (coronavirus RBDs and soluble receptors) are expressed in insect cells using the Bac-to-Bac expression system (Life Technologies Inc.). Expression constructs in pFastbac I vector (Invitrogen) contain an N-terminal honeybee melittin signal sequence and a C-terminal His tag sequence. Mutations are introduced by PCR site-directed mutagenesis to the expression constructs. Recombinant baculoviruses are generated and amplified in Sf9 insect cells. For large-scale protein production, the supernatant of 10L Sf9 cells is harvested 3 days after baculovirus infection and concentrated to 200 ml in a filtration system (Pall Corp.). The protein in the concentrated supernatant is sequentially purified on a Con A column (elution by 0.5M mannose buffer), an NiNTA column (elution by 0.5M imidazole solution), and a Superdex 200 gel filtration column (all columns are from GE healthcare). The protein is concentrated to 10 mg/ml. To prepare RBD-receptor complexes, the two components are mixed together in solution, and the complex is purified on the Superdex 200 gel filtration column.

Limited proteolysis of proteins and characterization of proteolysis fragments: Limited proteolysis is a tool to remove flexible termini of proteins and to identify stable protein fragments for crystallization studies. In a protease digestion reaction, protein samples are incubated with different concentrations of α-chymotrypsin or trypsin, and reactions are stopped by adding PMSF. The proteolysis products are analyzed by reducing SDS-PAGE and stained by Coomasie blue. As negative controls, no protease is added to the protein samples. The proteolysis products are purified by gel filtration chromatography, further analyzed by N-terminal peptide sequencing and mass spectrometry, and used for crystallization studies.

Crystallographic studies of SARS-CoV RBD complexed with human ACE2: Crystals of SARS-CoV-RBD-human-ACE2 complex are grown in sitting drops at room temperature, over wells containing 100mM Tris pH8.5, 22% PEG6000, 100mM NaCl. Crystals are harvested in a week, stabilized in 25% PEG6000, 100mM Tris pH8.5, and 30% ethylene glycol, and flash frozen in liquid nitrogen. X-ray diffraction data are collected at synchrotron beamlines and processed using HKL2000 (*59*). Using program CNS (*60*), the structure of the RBD-ACE2 complex was determined by molecular replacement using human ACE2 peptidase domain structure as the search model (*18*). The crystals belong to space group $P2_1$, a=82.3Å, b=119.4Å, c=113.2Å, b=91.2°, with two complexes per asymmetric unit. Two-fold non-crystallographic symmetry (NCS) averaging within the crystal and multiple cross-crystal averaging of the ACE2 region with two other structures (ACE2 by itself and in complex with an inhibitor) (*5, 18*) dramatically improved electron density in the SARS-CoV RBD region. The structure is refined using programs CNS (*60*) and CCP4 refmac (*61*).

Crystals of the mutant RBD-ACE2 complexes are prepared in the same way as the wild-type complex. If the crystals are isomorphous to those of the wild-type complex, difference Fourier and rigid body refinement will be used to determine the structures of the mutant complexes. Alternatively, each structure of the mutant complexes is determined by molecular replacement using the structure of the wild-type complex as the search model (*5*). Residue changes of models are done using program O (*62*).

Use of a human-civet chimeric ACE2 for crystallographic studies: To facilitate crystallographic studies of the interactions between SARS-CoV and civet ACE2, we have constructed a human-civet chimeric ACE2 that contains the SARS-CoV-binding residues from civet ACE2 and the rest of the protein from human ACE2 (Table 1). Using gel filtration chromatography, we have shown that the chimeric ACE2 has high affinity for all

civet viral RBDs and hence has the same receptor activity as civet ACE2 (*52*). Our pilot studies have also confirmed the above results using surface plasmon resonance assays (Specific Aim 3 in Section D.4). Crystallization and structure determination of the SARS-CoV-RBD-chimeric-ACE2 complexes are carried out in the same way as the SARS-CoV-RBD-human-ACE2 complexes, but the structures of the SARS-CoV-RBD-chimeric-ACE2 interfaces reflect those of the SARS-CoV-RBD-civet-ACE2 (Fig.4).

ACE2	24	31	34	37	38	45	82
Human	Q	K	H	E	D	L	M
Civet	L	T	Y	Q	E	V	T

Table 1. *Alignment of residues in ACE2 that directly contact SARS-CoV RBD but differ between human and civet ACE2.* The GenBank accession numbers are: AY623811 (human), AY881174 (civet).

Crystallographic studies of NL63-CoV RBD complexed with human ACE2: Crystals of the NL63-CoV-RBD-human-ACE2 complex are grown at room temperature in sitting drops, over wells containing 20% PEG6000 and 100mM Na citrate pH5.5. Crystals are harvested in two weeks, stabilized in 25% PEG6000, 100mM Na citrate pH5.5, and 30% ethylene glycol, and flash frozen in liquid nitrogen. The crystals belong to space group $P4_3$, a= 77.8Å, b=77.8Å, c=631.2Å, with four complexes per crystallographic asymmetric unit. The structure of RBD-ACE2 complex was determined by molecular replacement using the human ACE2 peptidase domain structure as the search model (*18*). Four-fold NCS averaging within the crystal and multiple cross-crystal averaging of the ACE2 region with three other structures (SARS-CoV-RBD/ACE2 complex, ACE2 by itself and in complex with an inhibitor) (*5, 18*) dramatically improved electron density in the NL63-CoV RBD region. Procedures for structure refinement are the same as above. Strategies for structure determination of the mutant NL63-CoV-RBD-ACE2 complexes are the same as those for the mutant SARS-CoV-RBD-ACE2 complexes.

Surface plasmon resonance: The Biacore is an optical biosensor that uses surface plasmon resonance (SPR) for real-time monitoring of macromolecular interactions. To measure the binding affinity between viral spike RBD and its receptor protein using a Biacore 3000, the receptor is immobilized on a C5 sensor chip through direct covalent coupling via amine groups. The surface of the sensor chip is first activated with N-hydroxysuccinimide (NHS); the receptor is then injected and immobilized to the surface of the chip; finally the remaining activated surface of the chip is blocked with ethanolamine. Soluble RBD is introduced at a flow rate of 20 ul/min at different concentrations. Kinetic parameters, k_{on}, k_{off}, and Kd, are determined for the RBD-receptor binding interactions. As negative controls, the RBD flows through an empty sensor chip.

Although direct covalent coupling is the easiest and most versatile and widely used immobilization method, it may place the receptor in orientations that are unfavorable for RBD binding. To overcome the potential orientation problem, we will make a receptor construct whose C terminus is fused to the Fc domain of human IgG1, and then couple the receptor-Ig-Fc chimeric protein to an anti-human-Ig-Fc antibody immobilized on a sensor chip. Subsequently the RBD is introduced to flow through the chip, and kinetic parameters are determined. As negative controls, the RBD flows through a chip immobilized only with the anti-human-Ig-Fc antibody but not the receptor-Ig chimeric protein.

Protein binding studies and functional studies: This proposal not only studies direct interactions between coronavirus RBDs and their receptors using recombinant proteins, but also examines the spike-receptor interactions by functional assays. The functional assays are essential to this proposal because it is important to investigate whether changes in receptor recognition by coronavirus RBDs can lead to corresponding changes in viral entry or membrane fusion in the context of the full-length coronavirus spike proteins or receptor proteins. Two alternative functional assays are used in this proposal, cell-cell fusion and pseudotyped viral infection assays, both of which have been widely used to study coronavirus-spike-receptor interactions (*23, 29, 45, 46, 63-66*).

All of the designed mutations in this proposal are located on coronavirus RBDs and their receptors, and likely do not interfere with the membrane fusion activities of S2. Therefore, in general it is reasonable to believe that mutations on the coronavirus RBD or the corresponding cellular receptor that increase binding affinity also increase cell-cell fusion and pseudotyped viral infectivity. Similarly, mutations that decrease binding affinity are likely to decrease cell-cell fusion and pseudotyped viral infectivity. Mutations that do not adhere to this general principle will point out important interactions occurring during coronavirus entry that are likely outside of simple spike-receptor binding. Such mutations will be of great interest for further investigation by crystallographic studies of the RBD-receptor complex.

Cell-cell fusion assays: Cell-cell fusion assays examine the interactions between a viral envelope protein and its receptor on the surface of contiguous cells. Cell-cell fusion results in syncytium (multi-nucleated cell) formation. The cells expressing the viral envelope protein are called effector and the cells expressing the viral receptor protein are called target cells. Cell-cell fusion is detected by mixing effector and target cells and monitoring the fusion-induced expression of a reporter gene.

Specifically, CHO-K1 effector cells are transfected with the plasmids expressing the coronavirus spike protein under study and β-gal under control of the T7 promoter (pG1NT7B-gal) (3:1 molar ratio). Target CHO-K1 cells are transfected with plasmids expressing the coronavirus receptor protein under study and T7 RNA polymerase (3:1 molar ratio). We use CHO-K1 cells because they are not normally susceptible to membrane fusion induced by coronavirus spike proteins. Therefore, only cells expressing receptor and T7 plasmids will fuse with cells expressing spike protein. 24 hours later, effector and target cells are mixed in a 1:1 ratio and co-cultivated for 18 hours. β-gal activity is quantified using the substrate CPRG (0.7 mg/ml in PBS with 0.5% NP40) and spectrometry. As negative controls, the plasmid expressing the spike protein or the receptor is replaced by a control plasmid expressing no protein. A positive control is a plasmid expressing the vesicular stomatitis virus (VSV) G envelope protein in place of the spike plasmid. VSV G is a highly fusogenic protein and fuses CHO-K1 cells at neutral pH.

Pseudotyped viral infection assays: Pseudotyping is complementation of a retrovirus with an envelope protein from another virus. The retrovirus does not express an envelope protein and thus requires a heterologous viral envelope protein to be provided in trans to be infectious. Pseudotyped viral infection assays make it possible to study the interactions between a viral glycoprotein and its receptor during virus entry without using replication competent virus.

Infection is assayed with a lentivirus expressing a green fluorescent protein (GFP) reporter gene and pseudotyped with a coronavirus spike protein. HEK293T cells are cotransfected with a plasmid encoding the spike protein and a plasmid (pNL43HIG) encoding an HIV isolate with mutated *env* and *nef* genes. The start codon for the env gene has been mutated to make the isolate *env*$^-$, and GFP was inserted into the nef open reading frame to allow quantification of infectivity. The *nef* gene product is not required for infection in cell culture. At 2 days post-transfection, viral supernatants are harvested and spike-protein-pseudotyped virus is inoculated onto HEK293T cells expressing the viral receptor protein under study. Infection efficiency is quantified 2-3 days later by measuring the percentage of infected (GFP$^+$) cells using flow cytometry. As a negative control, the plasmid expressing the spike protein is replaced by a plasmid that does not express a protein. A positive control is a plasmid expressing the VSV G envelope protein in place of the spike plasmid. VSV G is a commonly used envelope protein that produces high levels of infectivity in the pseudotype assays.

An advantage of cell-cell fusion and pseudotyped viral infection assays is that many spike and receptor mutants can be tested. However, it is important to control for cellular expression of any given mutant spike protein or receptor protein. To this end, all spike and receptor proteins have a C9 tag and a hemagglutinin (HA) tag fused to their C terminus, respectively. The levels of cellular expression of the mutant proteins are determined by Western blotting using anti-C9 or anti-HA antibodies and compared to wild-type spike or receptor proteins, respectively. It has been shown that these C-terminal tags do not interfere with coronavirus-spike-receptor interactions in the above functional assays (*23, 29*).

Feasibilities of the above research tools: Dr. Li has extensive experience in protein crystallography, molecular cloning, and protein biochemistry such as limited proteolysis and surface plasmon resonance using Biacore. Dr. Geraghty is an expert in cell-cell fusion assays and pseudotyped viral infection assays in the herpesvirus system (*67-70*). Dr. Holmes is an expert in pseudotyped viral infection assays in the coronavirus system (*63*). We have already applied these research tools to produce the preliminary and pilot data presented in this funding application. Therefore, the feasibility of the proposed research is high.

Mutations and structural models: In this proposal, both the designed mutations and generated hypotheses are based on the structural models of our preliminary studies. The likelihood that data will support the hypotheses is high. However, even if some of the mutations do not have the expected effects on virus-receptor interactions, we will still proceed to pursue structural information for these mutations by crystallographic studies of the RBD-receptor complex and find out why they do not have the expected effects, which will shed light on the receptor recognition mechanisms of the viruses.

D.2 Specific Aim 1: Receptor recognition mechanisms of group-I NL63 coronavirus

D.2.1 Overview

General question: How does NL63-CoV recognize human ACE2, and how does it interact with ACE2 proteins from other animals?

General hypothesis: A virus-binding hotspot on human ACE2 is critical for NL63-CoV binding, and variations of the hotspot structure on ACE2 proteins from other animals largely determine whether NL63-CoV recognizes these ACE2 proteins.

General background and significance: Our preliminary structural studies show that the two viruses have no structural homology in either RBDs cores or RBMs, but bind to the same "virus-binding hotspot" on ACE2. In this Specific Aim, we will study the receptor recognition mechanisms of NL63-CoV in depth. We will improve the structural model of the NL63-CoV-RBD-human-ACE2 complex, characterize detailed functions of the virus-binding hotspot on human ACE2 in NL63-CoV binding, and study how the NL63-CoV spike protein interacts with ACE2 proteins from different animals. These studies will provide the molecular and structural basis for understanding NL63-CoV-ACE2 interactions, the common virus-binding hotspot on ACE2, and the host ranges of NL63-CoV, and for developing novel antiviral strategies against NL63-CoV infections.

D.2.2 Improving the structural model of NL63-CoV RBD complexed with human ACE2

Rationale: Our preliminary structural studies are the first to show how two non-homologous receptor-binding proteins recognize their common receptor protein. Thus, the structure of the NL63-CoV-RBD-ACE2 complex, along with the structure of SARS-CoV-RBD-ACE2 complex, has become an excellent model system for studying virus-receptor interactions. The current resolution of the NL63-CoV-RBD-human-ACE2 complex structure is 3.3Å. At this resolution, aided by a complex crystal averaging strategy (Section D.1), we were able to identify key interactions between NL63-CoV RBD and human ACE2. However, the accurate geometries of some of these interactions wait to be better characterized by a higher-resolution structure. Better structural characterizations are especially necessary for understanding the interactions surrounding the common virus-binding hotspot due to their important roles in virus-receptor interactions. A higher-resolution structure will also help in the design of drugs that block virus-receptor interactions. Therefore, improving the structural model of NL63-CoV-RBD-ACE2 complex has both scientific and medical significance.

Experimental designs: We will apply several alternative approaches to improve the quality of crystals.

First, we will optimize existent rod-like crystals that are only about 30 micrometers in their shortest dimension. The crystal size can be increased using both macro- and micro-seeding strategies. To do macro-seeding, small existent crystals are transferred from an old crystallization drop to a new pre-equilibrated crystallization drop for further growth. To do micro-seeding, small existent crystals are crushed into small pieces and transferred to a new pre-equilibrated crystallization drop for further growth. We also plan to improve the quality of the crystals by adding different salt and detergent additives to crystallization drops. One or some of the additives may stabilize or engender conformity by specific interactions with the RBD-ACE2 complex, and thus improve the quality of crystals and hence diffraction limits of the crystals. In addition, existent crystals are grown at room temperature, and different temperatures will be screened for larger and higher-quality crystals.

Second, we will screen for new crystal forms of the complex under new crystallization conditions.

Third, we will modify the RBD constructs. So far the RBD fragment used for crystallization experiments is prepared in the following way: we first express and purify an S1 fragment containing residues 461 to 616, and then use α-chymotrypsin to trim it to a shorter and more stable fragment containing residues 481 to 616. Mass spectrometry shows that the proteolysis product is about 80% pure. The inhomogeneity of proteolysis may have adverse effects on the diffraction limits of the crystals. To solve the inhomogeneity problem, we will make a new RBD construct containing residues 481 to 616, express and purify it, and use it for crystallization experiments. Moreover, although the RBD fragment that crystallizes in complex with ACE2 contains residues 481 to 616, only the region covering residues 482 to 603 is ordered in the final refined structure. Removing the disordered regions from the RBD fragment will likely improve the quality of the crystals and hence their diffraction limit. Therefore, as an alternative approach, we will also make an even shorter RBD construct containing residues 482 to 603, express and purify it, and use it for crystallization experiments.

Expected results: Using one of the above alternative approaches, we will obtain crystals with improved diffraction limit, and refine the structure to better accuracy. If we cannot improve the quality of the crystals, we will focus on the four-fold NCS averaging and multiple cross-crystal averaging to improve the structural model.

D.2.3 Structure of the common virus-binding hotspot on human ACE2 and its functions in NL63-CoV binding

Rationale: Our preliminary studies are the first to identify a common virus-binding hotspot on a viral receptor protein. This proposal will further develop this novel concept of virus-binding hotspots by studying its structure and functions in virus binding. Our comparative structural studies of NL63-CoV-ACE2 and SARS-CoV-ACE2 interactions suggest that the virus-binding hotspot is mainly an intrinsic property of human ACE2, as it is exploited independently by both NL63-CoV and SARS-CoV with structurally non-homologous RBDs and RBMs. However, the hotspot structure is not pre-existent or pre-organized; instead, it is induced to form by virus binding (Section C). Therefore, the hotspot structure is dynamic and receives structural contributions from both the receptor and the virus, although the contribution from the receptor is more pronounced.

At both the NL63-CoV-human-ACE2 and SARS-CoV-human-ACE2 interfaces, the hotspot structure consists of a salt bridge embedded in a hydrophobic tunnel. The formation of the hotspot structure likely provides significant energy for the virus-receptor binding interactions, and thus plays an important role in virus-receptor interactions, viral pathogenesis and viral transmissibility. Indeed, alanine substitution for Lys353 on ACE2, a central component of the hotspot structure, abolishes the binding of both NL63-CoV and SARS-CoV. However, many other components of the hotspot structure have not been characterized by biochemical studies. Here we will study detailed structure and functions of the virus-binding hotspot, focusing on the roles of each component of the hotspot structure in virus-receptor interactions. Overall, this study will provide critical insight into virus-binding hotspots and virus-receptor interactions.

Hypothesis: The virus-binding hotspot on human ACE2 provides significant energy for the NL63-CoV-ACE2 binding interactions, and thus mutations that disturb the hotspot structure have significant effects on the spike-protein-ACE2 interactions.

Experimental designs: Based on our preliminary structural studies (Fig.8A, 8B), we will make mutations at the RBD-ACE2 interface that potentially disturb the hotspot structure. The mutations fall into two categories: those affecting the salt bridge (D38A, K353D, and K353D/D38K on ACE2), and those affecting the hydrophobic tunnel walls (Y41A, Y41F, D37A on ACE2, and Y498A, Y498F, S535A, S535T on NL63-CoV RBD). We will express and purify each of the mutant RBDs and mutant ACE2, and use Biacore to study the interactions between the mutant RBD and human ACE2 or between the mutant ACE2 and the wild-type RBD. We will make the same mutations on the full-length spike protein and human ACE2, and use pseudotyped viral infection and cell-cell fusion assays to study the spike-protein-ACE2 interactions. If a stable RBD-ACE2 complex can form in solution, we will proceed to determine its crystal structure to understand the structural and functional effects of the mutations.

The hypothesis predicts that: (see Fig.8A, 8B)

(1) The D38A mutation on ACE2 decreases its binding affinity for the spike protein. Asp38 on ACE2 is an essential component of the hotspot by forming a salt bridge with Lys353. The D38A mutation would leave the charge of Lys353 unneutralized, which destabilizes the binding interface.

(2) The K353D mutation on ACE2 decreases its binding affinity for the spike protein. It has been shown that the K353A mutation abolishes the binding of NL63-CoV (*29*), likely by not providing the essential energy to the virus-receptor interactions. The K353D mutation not only fails to provide the binding energy, but also introduces a negative charge in the hydrophobic tunnel, which destabilizes the binding interface.

(3) The K353D/D38K double mutation on ACE2 rescues the loss of virus-receptor binding affinity caused by the K353D mutation. The double mutation likely rebuilds the salt bridge in the hydrophobic tunnel, albeit in a reversed direction, and thus restores the binding affinity between the spike protein and ACE2.

(4) The Y41A mutation on ACE2 decreases its binding affinity for the spike protein. The Y41A mutation removes one of the hydrophobic tunnel walls surrounding the hotspot, which weakens the hotspot structure and destabilizes the binding interface.

(5) The Y41F mutation on ACE2 decreases its binding affinity for the spike protein. As one of the hydrophobic tunnel walls surrounding the hotspot, Tyr41 is positioned firmly through a hydrogen bond with Asp355 on ACE2. The Y41F mutation removes this hydrogen bond, which weakens the hotspot structure.

(6) The D37A mutation on ACE2 decreases its binding affinity for the spike protein. Like Y41A, the D37A mutation removes one of the tunnel walls.

(7) The Y498A mutation on the RBD decreases its binding affinity for ACE2. Like the Y41A mutation on ACE2, the Y498A mutation on the RBD removes one of the tunnel walls.

(8) The Y498F mutation on the RBD has no significant effect on its binding affinity for ACE2. Tyr498 on the RBD is positioned not only through a hydrogen bond with Asp538 on the RBD, but also through extensive

hydrophobic interactions with Trp541, Trp585 and Phe493 on the RBD. Thus the loss of the hydrogen bond by the Y498F mutation may not have significant effects on the spike-protein-ACE2 interactions.

(9) The S535A mutation on the RBD decreases its binding affinity for ACE2. Like the Y41A mutation on ACE2, the Y498A mutation on the RBD removes one of the tunnel walls.

(10) The S535T mutation on the RBD increases its binding affinity for ACE2. Despite lack of homology between SARS-CoV and NL63-CoV RBMs, Ser487 in the cSz02 SARS-CoV strain and Ser535 in the human NL63-CoV strain occupy identical positions in the common hotspot structure (Fig.8), a likely result of convergent evolution of the two viruses. This demonstrates a key role of this side of the tunnel walls in the hotspot structure. Hence, like the S487T mutation on the SARS-CoV RBD, the S535T mutation on the NL63-CoV RBD possibly provides additional support for the hotspot structure.

D.2.4 Interactions between the NL63-CoV spike protein and ACE2 proteins from different animals

Rationale: Receptor recognition is one of the most important determinants of coronavirus host ranges (*13*). One of the reasons why SARS-CoV has a wide host range is because ACE2 is highly conserved among mammals (*52*). Thus the common virus-binding hotspot on human ACE2 may also exist on ACE2 proteins from other mammals. Therefore, it is possible that like SARS-CoV, NL63-CoV can also infect many species. Based on the sequence alignment of ACE2 proteins from different mammals (*52*), most of the residues on human ACE2 that directly contact NL63-CoV are highly conserved, with several notable amino acid variations, all of which are around the common virus-binding hotspot on human ACE2 (Table 2). It has been shown by pseudotyped viral infection assays that that the NL63-CoV spike protein interacts with civet ACE2, not rat ACE2 (*29*), but the molecular and structural basis for these interactions is unknown. Also unknown is how the NL63-CoV spike protein interacts with ACE2 proteins from cats, mice, and raccoon dogs. Here we will study the interactions between the NL63-CoV spike protein and ACE2 proteins from civets, cats, raccoon dogs, mice and rats, all of which have close relationships with humans in daily life. These studies will not only elucidate the structures and functions of the potential virus-binding hotspots on ACE2 proteins from these animals, but also have implications for the host ranges of NL63-CoV.

ACE2	37	38	41	353
Human	E	D	Y	K
Civet	Q	E	Y	K
Cat	E	E	Y	K
Raccoon dog	E	E	Y	R
Rat	E	D	Y	H
Mouse	E	D	Y	H

Table 2. *Alignment of four hotspot residues of ACE2 proteins from six mammals.* The GenBank accession numbers are: NM_001039456 (cat), AB211998 (raccoon dog), AY881244 (rat), EF408740 (mouse).

Hypothesis: Amino acid variations around the virus-binding hotspot on ACE2 have significant effects on the binding affinity between the NL63-CoV spike protein and ACE2 proteins from these animals.

Experimental designs: We will use Biacore to measure the binding affinity between the NL63-CoV RBD and ACE2 proteins from civets, cats, raccoon dogs, mice and rats, and use pseudotyped viral infection and cell-cell fusion assays to study the interactions between the NL63-CoV spike protein and ACE2 proteins from these animals. In addition, we will identify and mutate critical amino acid variations on ACE2 proteins from these animals, and study the effects of these mutations on the hotspot structure and viral binding. These mutations include H353K on mouse and rat ACE2, E38D on civet and cat ACE2, and R353K and E38D/R353K on raccoon dog ACE2. If a stable complex of NL63-CoV RBD and animal ACE2 can form in solution, we will proceed to determine the crystal structure of the NL63-CoV-RBD-animal-ACE2 complex.

The hypothesis predicts that: (see Fig.8A, 8B)

(1) Mouse and rat ACE2 are less efficient receptors for the NL63-CoV spike protein than human ACE2 is. Both mouse and rat ACE2 have a histidine at the 353 position, instead of a lysine. His353 does not fit into the hotspot structure as well as a lysine does, and thus mouse and rat ACE2 likely have lower affinity for the NL63-CoV spike protein than human ACE2 does. An H353K mutation on mouse or rat ACE2 likely increases their affinity for the NL63-CoV spike protein.

(2) Civet and cat ACE2 are more efficient receptors for the NL63-CoV spike protein than human ACE2 is. Both civet and cat ACE2 have a glutamate at the 38 position, instead of an aspartate. At the SARS-CoV-civet-ACE2 interface, a strong bifurcated salt bridge between Glu38 and Lys353 has been observed, compared

with a weaker salt bridge between Asp38 and Lys353 (Fig.4C, 4D). Such a strong bifurcated salt bridge between Glu38 and Lys353 may also exist at the NL63-CoV-civet-ACE2 interface. Thus civet and cat ACE2 likely have higher affinity for the NL63-CoV spike protein than human ACE2 does. The E38D mutation on civet or cat ACE2 likely decreases their affinity for the NL63-CoV spike protein.

(3) Raccoon dog ACE2 is an even more efficient receptor for the NL63-CoV spike protein than civet and cat ACE2 are. Raccoon dog ACE2 contains not only Glu38, but also Arg353. Compared with Lys353, Arg353 has a longer side chain and likely forms an even stronger salt bridge with Glu38. Both the R353K and E38D mutations on raccoon dog ACE2 decrease its affinity for the NL63-CoV spike protein.

Potential pitfalls and other considerations: The complex of NL63-CoV RBD and animal ACE2 may not crystallize under the same condition as the complex of NL63-CoV RBD and human ACE2, despite very high sequence homology of these ACE2 proteins. If so, we will construct human-animal chimeric ACE2 and crystallize the NL63-CoV-chimeric-ACE2 complex, using the same strategy we have used to study the interactions between SARS-CoV RBD and the human-civet chimeric ACE2 (*52*) (Sections C and D.1).

D.3 Specific Aim 2: Receptor recognition mechanisms of other group-I coronaviruses

D.3.1 Overview

General question: Other group-I coronaviruses, such as FIPV and TGEV, use APN from their respective hosts as receptors. Interestingly, the TGEV spike protein can also use feline APN as its receptor, whereas FIPV cannot use porcine APN as its receptor (*34*). What are the receptor recognition mechanisms of these viruses? How do NL63-CoV and FIPV spike proteins recognize different receptor proteins, despite their obvious sequence homology? And how do FIPV and TGEV spike proteins have different receptor specificity, despite their extremely high sequence homology?

General hypothesis: Among group-I coronaviruses, the RBD cores are conserved, whereas the three RBM loops are variable; the latter determine receptor specificity of other group-I coronaviruses.

General background and significance: Animal coronaviruses, such as FIPV and TGEV, are frequently lethal in economically important animals (*12*). Despite decades of studies, their receptor recognition mechanisms remain elusive. Our preliminary structural studies of group-I human NL63-CoV offer a unique opportunity for studying the receptor recognition mechanisms of these animal coronaviruses. Specifically, using FIPV as the model system, we will characterize the receptor specificity of the predicted FIPV RBD and the functions of the predicted FIPV RBMs. These studies will elucidate the receptor recognition mechanisms of FIPV, and provide insight into evolution and host adaptations of group-I coronaviruses. The FIPV RBD may be used as a subunit vaccine candidate to combat FIPV infections in cats. Because the spike proteins of APN-binding group-I coronaviruses share extremely high sequence homology (Fig.9 in Section C), these viruses likely share similar receptor recognition mechanisms and may be controlled using similar antiviral strategies.

D.3.2 Identification, purification, and characterization of the predicted RBD of FIPV

Rationale: Based on the structural information for NL63-CoV RBD and sequence alignment between the NL63-CoV and FIPV spike proteins, we predict the RBD and three RBMs of FIPV (Fig.9). In this study, we will prepare the predicted FIPV RBD, and characterize its receptor specificity. This study will pave way for studying the receptor specificity of the FIPV spike protein. In addition, the FIPV RBD may be used as a subunit vaccine candidate to combat FIPV infections in cats.

Hypothesis: The FIPV RBD has high affinity for feline APN, but not porcine APN or human ACE2.

Experimental designs: The codon-optimized gene of the predicted FIPV RBD (residues 530-678) (Fig.9) will be fused to an N-terminal honeybee melittin signal peptide sequence and a C-terminal His tag sequence, and expressed in insect cells and purified as described in Section D.1. The ectodomains of feline APN, porcine APN and human ACE2 will also be expressed and purified using the same procedure. We will use Biacore to measure the binding affinity between FIPV RBD and the ectodomain of feline APN, porcine APN or human ACE2. We will also use pseudotyped viral infection and cell-cell fusion assays to study the interactions between the FIPV spike protein and feline APN, porcine APN or human ACE2.

The hypothesis predicts that: The predicted FIPV RBD is a stably folded fragment and has high binding affinity for feline APN, but not for porcine APN or human ACE2.

Potential pitfalls and other considerations: If the predicted FIPV RBD does not bind feline APN, we will prepare longer FIPV S1 fragments and use limited proteolysis to identify the FIPV RBD, as we have done to identify both of the SARS-CoV and NL63-CoV RBDs (Section C).

D.3.3 Functions of the predicted RBMs of FIPV

Rationale: The FIPV and NL63-CoV spike proteins are homologous to each other, but they recognize feline APN and human ACE2, respectively. Our preliminary studies have predicted three RBMs on the FIPV spike protein that likely determine the receptor specificity of FIPV. In this study, we will systematically change each or all of the three RBMs of the FIPV spike protein to those of NL63-CoV, and monitor possible changes in the receptor specificity of the FIPV spike protein. The primary goal of this study is to explore the functions of each of the FIPV RBMs in binding feline APN, and the secondary goal is to test the possibility of changing the receptor specificity of the FIPV spike protein from feline APN to human ACE2 by protein engineering.

Hypothesis: The three predicted FIPV RBMs determine the receptor specificity of the FIPV spike protein; changing these RBMs to those of NL63-CoV can switch the receptor specificity of the FIPV spike protein from feline APN to human ACE2.

Experimental designs: Based on the sequence alignment of the FIPV and NL63-CoV RBDs (Fig.9), we will use PCR to change each or all of the three RBMs of the FIPV spike protein (RBM1: residues 542-554; RBM2: residues 585-604; RBM3: residues 647-652) to those of NL63-CoV. We will express and purify each of the mutant FIPV RBDs, and use Biacore to measure their affinity for feline APN or human ACE2. We will also change the RBMs of the full-length FIPV spike protein, and use pseudotyped viral infection and cell-cell fusion assays to study the interactions between the FIPV spike protein and feline APN or human ACE2.

The hypothesis predicts that: Modification of the RBMs decreases the binding affinity between the FIPV spike protein and feline APN; after all of the three RBMs are changed, the receptor specificity of the FIPV spike protein switches from feline APN to human ACE2.

Potential pitfalls and other considerations: It is possible that the short regions flanking the three RBMs may impact the conformations of the RBM loops and hence indirectly contribute to the receptor specificity of the spike proteins. Thus, as an alternative approach, we will also mutate one or a few residues flanking each of the three RBMs on the FIPV spike protein to those on the NL63-CoV spike protein. The mutation targets will be the residues that have no or weak homology between the FIPV and NL63-CoV spike proteins (Fig.9).

D.3.4 Determinants of receptor specificities of FIPV and TGEV

Rationale: The FIPV and TGEV spike proteins are highly homologous to each other, with only six amino acid differences in the three predicted RBMs (Fig.9). Yet the TGEV spike protein recognizes both porcine and feline APN, whereas the FIPV spike protein only recognizes feline APN (*34*). In this study, we will systematically mutate each of the six amino acids on the three predicted FIPV RBMs, and study whether the mutant FIPV spike proteins gain affinity for porcine APN. This study will further elucidate the functions of the three FIPV RBMs, and identify the elements on the FIPV RBMs that prevent the FIPV spike protein from recognizing porcine APN. These elements are part of the species barriers between cats and pigs for FIPV infections, and hence this study will also have implications for the cross-species infections of FIPV and TGEV and for the evolutionary relationships of group-I coronaviruses.

Hypothesis: The FIPV spike protein with all of the three RBM loops from TGEV recognizes both porcine APN and feline APN.

Experimental designs: Based on the sequence alignment of the FIPV and TGEV RBDs (Fig.9), we will make the following single mutations on the FIPV RBMs: L544R, S588A, N595K, Q596R, D597N and E603D. We will express and purify each of the mutant FIPV RBDs, and use Biacore to measure their binding affinity for feline APN or porcine APN. We will also make the same mutations on the full-length FIPV spike protein, and use pseudotyped viral infection and cell-cell fusion assays to study the interactions between the FIPV spike protein and feline APN or porcine APN.

It is possible that more than one amino acid on the FIPV RBMs prevents the FIPV spike protein from recognizing porcine APN. Thus, if several mutations all increase the binding affinity between the FIPV spike protein and porcine APN, we will make multiple mutations on the FIPV spike protein and measure the cumulative effects of these mutations on receptor recognition.

The hypothesis predicts that: One or a few of the six RBM mutations increase the binding affinity between the FIPV spike protein and porcine APN. All of the six RBM mutations change the receptor specificity of the FIPV spike protein to that of the TGEV spike protein. In other words, the FIPV spike protein containing all of the six RBM mutations recognize both porcine APN and feline APN.

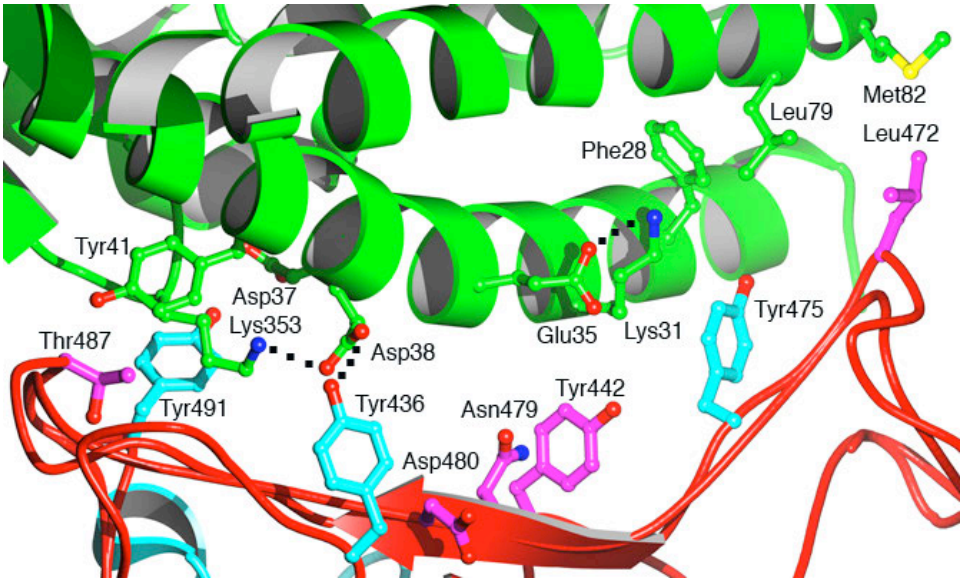


Figure 10. *Residues on the viral RBM that underwent mutations in past SARS-CoV strains, and their relationships with the two important virus-binding spots on human ACE2.* The RBM residues that underwent mutations are colored in magenta, other RBM residues are colored in cyan, and human ACE2 residues are colored in green. The view is derived by rotating the one in Fig.3B 180° along a vertical axis.

D.4 Specific Aim 3: Receptor recognition mechanisms of group-II SARS coronavirus

D.4.1 Overview

General questions: Receptor recognition is among the key elements of SARS-CoV pathogenesis and cross-species infections. During the course of the SARS epidemic, various mutations were found in the RBM region of different SARS-CoV strains isolated from humans or palm civets (Fig.3D, 10). Why was each of the mutations selected, and what RBM mutations may appear in potential future SARS epidemics?

General hypothesis: The RBM mutations are viral adaptations to two important virus-binding spots on ACE2, and they increase viral binding affinity for either human or civet ACE2.

General background and significance: Our preliminary studies show that NL63-CoV and SARS-CoV RBDs bind to common regions on human ACE2, largely because of a common virus-binding hotspot surrounding Lys353 on ACE2. Compared with NL63-CoV RBD, SARS-CoV RBD has additional interactions with human ACE2 (Fig.7C, 7D). The SARS-CoV-ACE2 interface contains a second important virus-binding spot surrounding Lys31 on ACE2 (Fig.4A, 4B, 10). In sum, we have identified two important virus-binding spots on human ACE2: one is a common virus-binding hotspot, is exploited by both NL63-CoV and SARS-CoV, and will be studied in Specific Aim 1; the other is exploited only by SARS-CoV and will be studied here.

Moreover, our preliminary studies have elucidated the molecular and structural basis for two viral RBM mutations, K479N and S487T, which are critical for the civet-to-human and human-to-human transmissions of SARS-CoV, respectively. However, the molecular and structural basis for the other RBM mutations is not known and will be investigated here. These studies will establish principles that govern the evolution, receptor recognition, host adaptations and cross-species infections of not only SARS-CoV, but also of a wide range of viruses such as other emerging animal viruses. Furthermore, based on our discoveries about the RBM mutations in the past viral strains, we will make predictions of the RBM sequences of potential future SARS-CoV strains that have higher affinity for human or civet ACE2 than those of the past viral strains. These predictions will be important for epidemic monitoring. The RBDs containing the predicted RBM sequences can be used as subunit vaccine candidates to combat potential future SARS epidemics. Overall, our studies have important implications for the virology, structural biology, epidemics and medical fields.

D.4.2 Characterization of a new important virus-binding spot on human ACE2 for SARS-CoV binding

Rationale: On human ACE2, the virus-binding spot surrounding Lys31 shares structural features with that surrounding Lys353. In the unbound ACE2, Lys31 points into solution; upon SARS-CoV binding, it forms a salt bridge with Glu35 in a hydrophobic environment (Fig.4, 10). The difference between the two lysines is that Lys353 is in a relatively extended conformation that is energetically favorable, whereas Lys31 is in a strained conformation that is energetically unfavorable. If energy gain from the salt bridge formation outweighs energy loss from the strained conformation, then Lys31 should be a new hotspot for virus binding. If vice versa, then it

should be a “cold spot”. If the energy gain and energy loss are equivalent, then it should be neutral. Alanine substitution for Lys353 on human ACE2 abolishes SARS-CoV binding (*23*), confirming that Lys353 is a hotspot. However, alanine substitution for Lys31 has not been done on human ACE2 and thus it is not clear what type of virus-binding spot Lys31 is. Nevertheless, civet ACE2 contains a threonine at position 31, which has a smaller side chain than a lysine, and civet ACE2 in general is a more efficient receptor for SARS-CoV binding than human ACE2 is (*5, 23, 52*). Thus Lys31 on human ACE2 may be a cold spot. Regardless of whether Lys31 is a cold spot or a hotspot, our preliminary studies have shown that viral adaptation to Lys31 on human ACE2 is critical for SARS-CoV to transmit from civets to humans (Fig.4A, 4B in Section C) (*5, 52*). In this study we will investigate the structure and functions of virus-binding spot surrounding Lys31. This study will provide critical structural insight into viral evolution and virus-receptor interactions.

Hypothesis: Lys31 and its surrounding residues on human ACE2 is a new important virus-binding spot; furthermore, it can be either a cold spot or a hotspot for virus binding, depending on whether it contributes negatively or positively to virus-receptor binding interactions.

Experimental designs: We will make single mutations, K31A, K31R and E35A, on human ACE2 (Fig.4A, 10), and express and purify each of the mutant ACE2 proteins. We will use Biacore to measure the binding affinity between each of the mutant ACE2 proteins and hTor02 RBD, and use pseudotyped viral infection and cell-cell fusion assays to study the interactions between each of the mutant ACE2 proteins and the hTor02 spike protein. If a stable RBD-ACE2 complex can form in solution, we will proceed to determine its crystal structure and explore structural details of the mutations.

The hypothesis predicts that: If Lys31 is a cold spot, both the K31A and E35A mutations increase the binding affinity between the spike protein and ACE2 by releasing the structural strains of Ly31. In addition, because Arg31 has a longer and more inflexible side chain than Lys31 does, the K31R mutation decreases the binding affinity by increasing the structural restrain. If Lys31 is a hotspot, the opposite results will be obtained.

Potential pitfalls and other considerations: Why do cold spots possibly exist on viral receptor proteins? Cold spots likely have been eliminated from host protein-protein binding interfaces through evolution because of the co-evolutionary relationships between host protein partners. However, a virus may be forced to adapt to a receptor region containing both hotspots and cold spots, if energy gain from binding the hotspots outweighs energy loss from binding the cold spots. In this case, viral adaptation to cold spots is to minimize the energy loss from binding the cold spots and to maximize the overall energy gain from binding the receptor. Thus, theoretically cold spots may exist at virus-receptor interfaces.

Pilot studies: We have performed pilot studies of the cell-cell fusion and pseudotyped viral infection mediated by the SARS-CoV spike protein (hTor02 strain) (Fig.11).

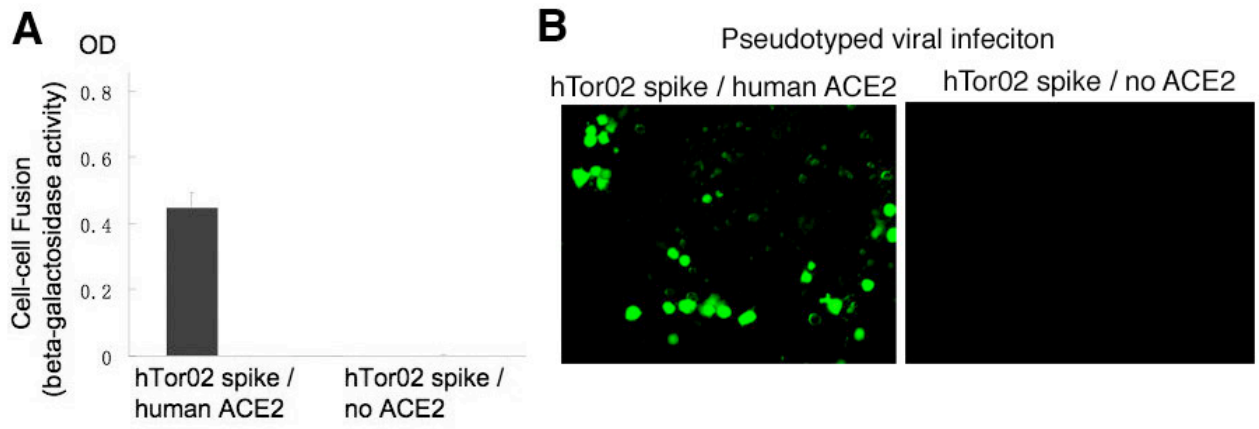


Figure 11. *Pilot studies of functional assays of the SARS-CoV spike protein.* (A) Cell-cell fusion assays. CHO-K1 cells expressing SARS-CoV spike protein (hTor02 strain) and a T7 polymerase-inducible β-gal plasmid are mixed with CHO-K1 cells expressing human ACE2 or an empty vector control plasmid and a T7 expression plasmid. The next day, cultures are monitored for β-gal activity as an indication of the extent of cell fusion. (B) Pseudotyped virus entry. HEK293T cells are transfected with plasmids encoding human ACE2 or an empty vector control plasmid, infected with GFP-expressing lentivirus pseudotyped with the SARS-CoV spike protein (strain hTor02), and imaged three days later. See Section D.1 for more details.

D.4.3 Molecular and structural basis for RBM mutations in past SARS-CoV strains

Rationale: Five RBM residues in past SARS-CoV strains underwent mutations. These residues and their amino acid variations are 442 (Y/F), 472 (P/L/F), 479 (N/K/R), 480 (G/D), and 487 (S/T) (Fig.3D). In preliminary studies, we have elucidated the molecular and structural basis for two of the mutations, K479N and S487T, both of which increase viral binding affinity for human ACE2. The molecular and structural basis for other mutations is not clear, and will be investigated here.

Hypothesis: The RBM mutations in past SARS-CoV strains are viral adaptations to two important virus-binding spots on ACE2; they increase viral binding affinity for either human or civet ACE2.

Experimental designs: We will make single mutations, Y442F, L472F, L472P, N479K, N479R, D480G and T487S, on the hTor02 RBD (RBM genotype: Y442/L472/N479/D480/T487) (Fig.10). We will express and purify each of the above mutant RBDs as well as each of the RBDs of the past viral strains: cSz02, hcGd03, cGd05, and hHAE08. We will use Biacore to measure the binding affinity between each of the RBDs and human or civet ACE2. We will also make the same mutations on the hTor02 spike protein, and use pseudotyped viral infection and cell-cell fusion assays to study the interactions between each of the mutant spike proteins and human or civet ACE2. If a stable RBD-ACE2 complex can form in solution, we will proceed to determine its crystal structure and explore structural details of the mutations.

The hypothesis predicts that: (see Fig.10)

(1) The Y442F mutation (Phe442 was found in hHAE08 strain) increases viral binding affinity for human ACE2. According to the hypothesis, the mutation is viral adaptation to human ACE2. Thus the effects of this mutation on viral binding affinity for human ACE2, not civet ACE2, are the focus of this study. Based on our preliminary structural studies, the hydroxyl group of Tyr442 has partial steric clash with the alky side chain of Lys31 on human ACE2; removing the hydroxyl group through the Y442F mutation may release some of the structural strains of Lys31.

(2) The L472F mutation (Phe472 was found in hHAE08 strain) increases viral binding affinity for human ACE2. Leu472 on the RBD forms a hydrophobic cluster with Met82, Leu79, and Phe28 from ACE2. Among these residues, Phe28 forms hydrophobic interactions with the alkyl side chain of Lys31. The L472F mutation may indirectly affect the conformation of Lys31 and release part of the structural strains of Lys31 that are exerted by Tyr442.

(3) The L472P mutation (Pro472 was found in both hcGd03 and cGd05 strains) may not have significant effect on viral binding affinity for civet ACE2. Civet ACE2 has a threonine at the 82 position, which has no contact with Pro472 on the RBD. Instead, the L472P mutation may increase protein solubility and decrease conformational entropy.

(4) The N479K mutation (Lys479 was found in cSz02 strain) increases viral binding affinity for civet ACE2. Lys479 can form a salt bridge with Glu35 on civet ACE2, and its alkyl side chain can also form hydrophobic interactions with several tyrosine residues at the interface. These favorable interactions may enhance RBD-civet-ACE2 interactions.

(5) The N479R mutation (Arg479 was found in cGd05 strain) increases viral binding affinity for civet ACE2. As a better salt bridge partner and hydrophobic stacker, Arg479 forms stronger favorable interactions with civet ACE2 than Lys479 does.

(6) The D480G mutation (Gly480 was found in both hcGd03 and cGd05 strains) increases viral binding affinity for civet ACE2. The alkyl side chain of Asp480 forms a hydrophobic interaction with Tyr436 on the RBD, which forms a critical hydrogen bond with Asp38 on human ACE2. Asp38 on human ACE2 is an important component of the common virus-binding hotspot. On civet ACE2, residue 38 is a glutamate that has a different conformation from Asp38 (Fig.4C, 4D). Hence, Tyr436 needs to change its conformation to keep the hydrogen bond with Glu38. The D480G mutation removes the hydrophobic interaction with Tyr436 and thus may facilitate the conformational change of Tyr436.

(7) The T487S mutation (Ser487 was found in cSz02, hcGd03, and cGd05 strains) decreases viral binding affinity for both human and civet ACE2. In other words, the S487T mutation increases viral binding affinity for both human and civet ACE2. The S487T mutation provides essential support for the salt bridge between Lys353 on the RBD and Asp38 on human ACE2. Whereas the salt bridge between Lys353 on the RBD and Glu38 on civet ACE2 is stable even when residue 487 on the RBD is a serine (Fig.4D), the stability of the salt bridge may further increase when residues 487 on the RBD is a threonine.

(9) The Sz02, hcGd03 and cGd05 RBDs all bind civet ACE2 with high affinity, whereas the hHAE08 RBD binds human ACE2 with high affinity. The dissociation constants for these interactions are not available, and will be determined in this study.

Potential pitfalls and other considerations: Some of the above mutations on the RBD may cause relatively small changes in its binding affinity for human or civet ACE2. Thus the Biacore assays need to be performed with great accuracy. To this end, we will prepare our proteins to crystallography-grade purity, and repeat each of the Biacore assays at least 10 times to minimize standard deviations of the measurements.

Pilot studies: We have prepared a few of the mutant RBDs, and have measured their binding affinity for human or civet ACE2 (Table 3). The results all support the hypothesis. However, before drawing any conclusions, we will need to collect more data for the following reasons. First, as discussed above, the Biacore data from the pilot studies need to be repeated more times to minimize experimental errors. Second, the majority of the mutant RBDs have not been prepared, and their binding affinity for human or civet ACE2 has not been characterized. Third, importantly, the mutations need to be understood in the context of the full-length spike protein, and hence pseudotyped viral infection and cell-cell fusion assays will be performed to complement Biacore assays. Last, we will investigate the structural basis for the mutations by crystallography.

RBM sequences	hACE2			cACE2			hcACE2
442 472 479 480 487	k_{on} ($10^6 M^{-1}s^{-1}$)	k_{off} ($10^{-2}s^{-1}$)	**Kd (nM)**	k_{on} ($10^6 M^{-1}s^{-1}$)	k_{off} ($10^{-2}s^{-1}$)	**Kd (nM)**	Kd (nM)
Y L N D T (hTor02 strain)	1.05 ±0.33	3.54 ±0.32	**35.7 ±7.2**	1.09 ±0.50	4.68 ±0.66	**50.1 ±18.1**	52.7 ±17.5
Y L K D T			**N.D.**	2.02 ±0.67	3.01 ±0.46	**16.4 ±5.3**	15.1 ±4.9
Y L N G T	1.13 ±0.35	5.49 ±0.26	**52.4 ±14.0**	0.73 ±0.19	1.05 ±0.05	**14.1 ±1.9**	18.2 ±3.4
Y P R G S (cGd05 strain)			**N.D.**	3.23 ±0.69	5.92 ±0.79	**18.2 ±4.7**	24.6 ±7.6
F F N D S (hHAE08 strain)	0.94 ±0.18	3.37 ±0.19	**36.9 ±5.2**			**N.D.**	N.D.
F F N D T (hPredict09 strain)	1.60 ±0.46	0.38 ±0.03	**2.34 ±0.37**			**N.D.**	N.D.
Y P R G T (cPredict09 strain)			**N.D.**	3.9 ±0.75	0.68 ±0.06	**1.74 ±0.30**	2.50 ±0.37

Table 3. *Kinetics and binding affinity of SARS-CoV RBDs and human ACE2, civet ACE2 or human-civet chimeric ACE2 by surface plasmon resonance using Biacore.* Direct covalent coupling methods are used. N.D. means not determined reliably due to low binding affinity.

D.4.4 Predictions of RBM sequences of potential future SARS-CoV strains

Rationale: Although SARS-CoV has not infected humans since 2003-2004, it is carried by animals including wild civets (*42*) and thus can potentially cross the species barriers between animals and humans to infect humans again, as it did twice before (*33*). Compared with the viral strains that newly infected humans or civets in the past, potential future SARS-CoV strains may be better adapted to their hosts with higher binding affinity for human or civet ACE2. Prediction of their RBM sequences based on our knowledge about the past viral strains will deepen our understanding of viral host adaptations and cross-species infections. It will also be important for epidemic monitoring. Furthermore, the RBDs containing the predicted RBM sequences can be used as subunit vaccine candidates to combat future SARS-CoV infections. Overall, this study has important scientific, epidemic, and medical implications.

Hypothesis: Compared with those of the past viral strains, a spike protein (hPredict09) with RBM sequence F442/F472/N479/D480/T487 has higher binding affinity for human ACE2; a spike protein (cPredict09) with RBM sequence Y442/P472/R479/G480/S487 has higher binding affinity for civet ACE2.

Experimental designs: We will construct hPredict09 and cPredict09 RBDs, express and purify them, and use Biacore to measure their binding affinity for human or civet ACE2. We will also construct the hPredict09 and cPredict09 spike proteins, and use pseudotyped viral infection and cell-cell fusion assays to study their interactions with human or civet ACE2.

The hypothesis predicts that:

(1) The hPredict09 spike protein has higher binding affinity for human ACE2 than those of the past viral strains. Compared with the hHAE08 spike protein, the hPredict09 spike protein contains an S487T mutation

that further increases its affinity for human ACE2. Compared with the hTor02 spike protein, the hPredict09 spike protein contains L442F/L472F double mutations that also increase its affinity for human ACE2.

(2) The cPredict09 spike protein has higher binding affinity for civet ACE2 than those of the past viral strains. Compared with the cGd05 spike protein, the cPredict09 spike protein contains an S487T mutation that increases its affinity for civet ACE2. Compared with the cSz02 spike protein, the cPredict09 spike protein contains K479R, D480G and S487T mutations all of which increase its affinity for civet ACE2.

Potential pitfalls and other considerations: Other genomic changes will be expected in potential future SARS-CoV strains. This study only focuses on RBM mutations that potentially affect receptor recognition, which has been shown to be one of the most important determinants of host infections and cross-species infections of SARS-CoV (see Section B for details).

Pilot studies: We have prepared hPredict09 and cPredict09 RBDs, and measured their binding affinity for human or civet ACE2 (Table 3). The results support the hypothesis. We will repeat the Biacore experiments, and also perform pseudotyped viral infection and cell-cell fusion assays.

D.5 Summary

This research studies coronavirus-receptor interactions using multi-disciplinary tools including crystallography, biochemistry, molecular biology and virology. It not only establishes novel principles in virology that govern viral evolution, virus-receptor interactions, viral host ranges and cross-species interactions, but also guides development of novel antiviral strategies against coronavirus infections.

More specifically, this proposal is the first to investigate how two different viruses recognize a common receptor protein. It is also the first to identify and study virus-binding hotspots on viral receptor proteins. These hotspots have important implications for viral evolution and virus-receptor interactions. In addition, this proposal determines and studies the structures of group-I NL63-CoV and group-II SARS-CoV RBDs, the only two such structures available for the coronavirus family. They provide critical information about coronavirus evolution. Furthermore, this research studies the cross-species infections of SARS-CoV and other coronaviruses, which is crucial not only for preventing future potential SARS epidemics but also for controlling emerging animal viruses in general. Results from the proposed studies will guide the development of subunit vaccines and the design of antiviral drugs that combat human SARS, NL63-CoV-induced human respiratory diseases, and animal coronavirus infections.

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University of Colorado Denver School of Medicine

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Department of Microbiology

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September 25, 2009

Dr. Fang Li
Department of Pharmacology
University of Minnesota
6-120 Jackson Hall
321 Church Street S.E.
Minneapolis, MN 55455-0217

Dear Fang,

I will be delighted to serve as a consultant on your NIH grant application entitled "Receptor Recognition Mechanisms of Coronaviruses." As you know, my lab discovered receptors for 4 coronaviruses, and we have worked on biochemical and biological aspects of coronavirus-receptor interactions for more than 19 years. We have published more than 75 papers on the role of receptors in determining host range and tissue tropism of coronavirus infections, receptor-induced conformational changes in coronavirus spike proteins that lead to membrane fusion and virus entry, peptide inhibitors of coronavirus entry, pathogenesis of human coronavirus 229E infection in hAPN receptor transgenic mice and of murine coronavirus MHV-A59 in mCEACAM1a receptor knockout mice, and effects of mutations in receptor and spike proteins that modify the host ranges of group 1 and group 2 coronaviruses.

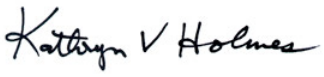
Coronaviruses are large, enveloped RNA viruses which cause respiratory and/or enteric disease in many species of mammals and birds. Coronavirus host range is primarily due to the specificity of binding of the large (200 kDa) coronavirus spike protein with a cellular membrane protein that serves as a receptor for the virus. Because each of these RNA viruses exists as a quasispecies of mutants and also has the unusual capacity to undergo high frequency recombination with RNA from coronaviruses, other viruses and even host mRNAs, coronaviruses with mutant spike proteins can rather frequently jump from one host species to another, and become established in the new host species. MInor species-specific differences between amino acids on the surface of a coronavirus receptor protein, such as aminopeptidase N or angiotensin-converting enzyme 2, can determine whether or not a coronavirus spike protein can use the receptor protein to infect a particular host species.

Your landmark structural analysis of the interactions of the receptor-binding domain of SARS-CoV spike protein with the virus-binding surface of its human ACE2 receptor protein vs. the palm civet ACE2 protein revealed the molecular basis for host range changes and receptor jumping that led to the SARS epidemic. Your recent analysis of the crystal structure of the receptor-binding domain of human coronavirus NL63 spike in complex with its human ACE2 receptor protein surprisingly showed that NL63 and SARS-CoV bind to the same hot spot on human ACE2, but by very different structures. Your analysis of the unique fold of the receptor-binding domain of NL63 spike protein and your structure-based predictions of the key receptor-binding motifs within the same domain in other group 1 coronaviruses of humans, cats and dogs has opened the way to understanding the host range determinants of these important coronaviruses. I am very impressed with your comprehensive plan to analyze and compare the receptor recognition mechanisms between NL63 spike and hACE2, and between SARS-CoV spike and hACE2, and to identify key receptor-binding motifs of other group 1 coronaviruses using biochemical, genetic and structural approaches.

I will be happy to continue to serve as a consultant for your ongoing research. We have many purified coronavirus spike and receptor proteins, polyclonal and monoclonal antibodies to these proteins, clones of codon-optimized spike proteins, and cell lines expressing coronavirus receptor proteins that we will provide to you for your research. We have had extensive experience with sensitive assays for coronavirus spike-induced, receptor-dependent cell fusion, in preparing retrovirus pseudotypes with coronavirus spikes for studies on spike-mediated, receptor-dependent virus entry, and on genetic manipulation of coronavirus genomes, and I will provide this expertise to facilitate your research. I will provide expert advice on the biology and pathogenesis of coronaviruses, and help with planning and analyzing your structural, biophysical, biochemical and genetic experiments designed to answer important biological questions about how coronaviruses jump from one host to another to cause emerging diseases. This is a very important area of research since coronaviruses will continue to jump from one host to another, and you have unique expertise to make terrific contributions to this field. Your work will serve not only to answer important questions about coronavirus emergence, but it will also serve as a model system for analysis of complexes of receptor-binding domains with receptors for other types of viruses.

I hope that you will receive an excellent score for your innovative grant application so that you can proceed with these studies immediately! Good luck!

Sincerely,



Kathryn V. Holmes, PhD
Professor

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Holmes, Kathryn Voelker	Professor of Microbiology
eRA COMMONS USER NAME HOLMES.KATHRYN	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Radcliffe College, Cambridge, MA	AB	1962	Biology
Rockefeller University, NY, NY	Ph.D.	1968	Virology/Cell Biology
Harvard University, Cambridge, MA	Post-doctoral	1968-70	Cell Biology

A. Positions and Honors

Positions and Employment

1970-1972 Assistant Professor, Dept. of Microbiology, Georgetown University Schools of Medicine and Dentistry, Washington, D.C.
1972-1976 Assistant Professor, Dept. of Microbiology, University of Texas Southwestern Medical School, Dallas, TX
1976-1982 Associate Professor, Dept. of Pathology, Uniformed Services Univ. of the Health Sciences, Bethesda, MD
1982-1995 Professor, Dept. of Pathology, Uniformed Services Univ. of the Health Sciences, Bethesda, MD
1995-present, Professor, Dept. of Microbiology, University of Colorado Denver School of Medicine, Aurora, CO

Honors

National Merit Scholar; Magna cum laude graduate of Radcliffe College; Phi Beta Kappa; Sigma Xi; NIH Postdoctoral Fellowship, 1968-70; NIH Virology Study Section 1980-84; President, Chesapeake Society for Electron Microscopy, 1983-4; Assoc. Editor Virology 1983-89; J. Virology, 1989-98; Assoc. Editor PLoS Pathogens, 2006-present; Editorial Board Cell Host and Microbe, 2007-present; American Society for Microbiology, Chairman, Division T, 1985, ASM Foundation for Microbiology Lecturer, 1988-89; ASM Division IV Group Representative,1991-93. American Society for Virology Council, 1989-91; ASV President, 1993-94; Fellow, American Association for the Advancement of Science, 1997; Panel, Howard Hughes Medical Institute Undergraduate Biological Sciences Education Initiative, 1994, 2002; Aaron Diamond Foundation Study Section for Postdoctoral Fellowships on AIDS; Co-chair, Gordon Research Conference on Animal Cells and Viruses, 1993; Chairman, VIII International Workshop on CEA/BGP/PSG Glycoproteins, 1997; Chairman, FASEB meeting on Microbial Pathogenesis: Mechanisms of Infectious Diseases, 1998; Co-founder Rocky Mountain Virology Club; Chair, Howard Hughes Medical Institute Review Panel for International Research Scholars in Infectious Diseases, 2000; Member, Infectious Diseases and Microbiology Working Group for NIH Center for Scientific Review, 2001; Ad hoc member Virology Study Section and Tropical Medicine Study Section, 2001; Ad hoc member NIAID Council subcommittee on Microbiology and Infectious Diseases, May, 2003; Founder and chair, new Gordon Research Conference on Immunoglobulin Superfamily Members in Infection, Immunity and Cancer, 2004; Co-Chair, Xth International Nidovirus Symposium:Toward Control of SARS and Other Nidovirus Diseases, 2005. American Academy of Microbiology, 2005-present; Chair, membership committee; NIH. member of MID-B study section, 2009-present.

B. Selected publications (from over 170)

Wang, G., Deering, C., Macke, M., Shao, J., Burns, R., Blau, D.M., Holmes, K.V., Davidson, B.L., Perlman, S., and McCray, P.B., Jr. Human coronavirus 229E infects polarized airway epithelia from the apical surface. J. Virol. 74:9234-9239 (2000)

Wentworth, D.E. and Holmes, K.V. Molecular determinants of species specificity in the coronavirus receptor aminopeptidase N (CD13): Influence of N-linked glycosylation J Virol.75:9741-52 (2001)

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Tan*, K, Zelus,* B.D., Meijers*, R., Liu, J.H., Bergelson, J.M., Duke, N., Zhang, R., Joachimiak, A., Holmes, K.V., and Wang, J.H. Crystal structure of murine sCEACAM1a[1,4]: A coronavirus receptor in the CEA family EMBO J. 21:2076-2086 (2002) (* indicates co-first authors**)**

Zelus, B.D., Schickli, J.H., Blau, D.M., Weiss, S.R. and Holmes, K.V. Conformational changes in the spike glycoprotein of murine coronavirus are induced at 37°C either by soluble murine CEACAM1 receptors or by pH 8. J. Virol. 77:830-840 (2003)

Tsai, J.C., Zelus, B.D., Holmes, K.V., and Weiss, S.R. The N-terminal domain of the murine coronavirus spike glycoprotein determines the CEACAM1 receptor specificity of the virus strain. J. Virol.77:841-850 (2003)

Bonavia, A, Zelus, B.D., Wentworth, D.E., Talbot, P.J., and Holmes, K.V. Identification of a receptor binding domain of the spike glycoprotein of human coronavirus HCoV-229E. J. Virol. 77:2530-8 (2003)

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Holmes, K.V., Editorial perspective: SARS-Associated Coronavirus, N. Eng. J. Med. 348:1948-1951. (2003)

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Turner BC, Hemmila EM, Beauchemin N, Holmes KV, Receptor-dependent coronavirus infection of dendritic cells. J Virol. May;78(10):5486-90 (2004).

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Thackray, L.B., and Holmes, K.V. Amino acid substitutions and an insertion in the spike glycoprotein extend the host range of the murine coronavirus MHV-A59. Virology, 324(2):510-24 (2004).

Hemmila, E., Turbide, C., Olson, M., Jothy, S., Holmes, K.V., and Beauchemin, N. C*eacam1a* -/- mice are completely resistant to infection by murine coronavirus MHV-A59 J. Virol. 78(18):10156-65 (2004)

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Jeffers SA, Tusell SM, Gillim-Ross L, Hemmila EM, Achenbach JE, Babcock GJ, Thomas WD Jr, Thackray LB, Young MD, Mason RJ, Ambrosino DM, Wentworth DE, Demartini JC, Holmes KV. CD209L (L-SIGN) is a receptor for severe acute respiratory syndrome coronavirus. Proc Natl Acad Sci U S A. 2;101(44):15748-53.(2004)

Thackray LB, Turner BC, Holmes KV Substitutions of conserved amino acids in the receptor-binding domain of the spike glycoprotein affect utilization of murine CEACAM1a by the murine coronavirus MHV-A59. Virology Mar 30;334(1):98-110 (2005)

Wentworth, D.E., Tresnan, D.B., Turner, B.C., Lerman, I.R., Bullis, B., Hemmila, E.M., Levis, R., Shapiro, L.H., Holmes, K.V. Cells of Human Aminopeptidase N (CD13) transgenic mice are infected by human coronavirus-229E in vitro, but not in vivo. Virology.335(2):185-97 (2005)

Holmes, K.V., Structural biology. Adaptation of SARS coronavirus to humans. (Perspective) Science 307:1822-3 (2005)

Calisher, C.H., Childs, J.E., Field H.E., Holmes, K.V., Schountz, T.. Bats: Important reservoir hosts of emerging viruses Clin Microbiol. Rev. 19:531-45 (2006)

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Nathanson N. and Holmes, K.V. Cellular Receptors and Viral Tropism, Chapter 3, pp27-40, In: Viral Pathogenesis and Immunity Second edition, Academic Press/Elsevier. San Diego, CA, (2007)

Dominguez, S.R., O'Shea, T.J., Oko, L.M., and Holmes, K.V. Detection of group 1 coronaviruses in bats in North America J Infect Dis. 194(12):1697-701 (2006).

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Miura, T., Travanty, E.A., Oko, L., Bielefeldt-Ohmann, H., Weiss, S.R., Beauchemin, N., and Holmes, K.V. The spike glycoprotein of murine coronavirus MHV-JHM mediates receptor-independent infection and spread in the central nervous system of Ceacam1a-/- mice. J Virol. 2008 Jan;82(2):755-63. Epub 2007 Nov 14.

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Mina-Osorio, P.,O'Conor, C.A., Winnicka, B., Vogel, L.K., Holmes, K.V., Rodriguez-Pinto, D., Ortega, E., and Shapiro. L.H. CD13 is a novel mediator of monocyte/endothelial cell adhesion. J. Leukocyte Biol., Aug;84(2):448-59. (2008) Epub 2008 May 21

Briese T, Renwick N, Venter M, Jarman RG, Ghosh D, Köndgen S, Shrestha SK, Hoegh AM, Casas I, Adjogoua EV, Akoua-Koffi C, Myint KS, Williams DT, Chidlow G, van den Berg R, Calvo C, Koch O, Palacios G, Kapoor V, Villari J, Dominguez SR, Holmes KV, Harnett G, Smith D, Mackenzie JS, Ellerbrok H, Schweiger B, Schønning K, Chadha MS, Leendertz FH, Mishra AC, Gibbons RV, Holmes EC, Lipkin WI. Global distribution of novel rhinovirus genotype. Emerg Infect Dis. 2008 Jun;14(6):944-7.

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Calisher, CH, Holmes, KV, Dominguez, SR, Schountz, T, and Cryan, P. Bats prove to be rich reservoirs for emerging viruses Microbe 3 (11) 522-528 (2008)

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Yan Z, Holmes KV, Hodges RS. Expression and characterization of recombinant S2 subunit of SARS-coronavirus S fusion protein. Adv Exp Med Biol. 611:153-4. (2009)

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Dominguez SR, Robinson, CC, aand Holmes, KV. Detection of four human coronaviruses in respiratory infections in children: a one-year study in Colorado. J Med Virol 81(9):1597-1604 (2009)

Miura, T, and Holmes, KV. Host-pathogen interactions during coronavirus infection of primary alveolar epithelial cells. J. Leuk. Biol. (epub ahead of print, July, 2009)

Ongoing Research Support

1 P01 AI059576 (KV Holmes, PI) 6/15/04-6/14/10 (No cost extension)
SARS Coronavirus: Inhibition of Entry
NIH/NIAID 4 projects (Holmes at UCHSC, Hodges at UCDSOM, Mason at NJH, Wentworth at NY State Dept of Health at Albany, Virus and Administrative Cores at UCDSOM)
The major goals of this program project grant are to identify receptors for SARS-CoV, characterize the molecular mechanisms of SARS-CoV binding specificity and entry, identify susceptible host cells from target tissues of humans, and develop inhibitors of SARS-CoV entry as lead compounds for anti-viral drug therapy.

Pending Research Support
1 R21 AI088531-01 (K.V. Holmes, PI) 4/1/2010-3/31/12
Novel Peptide Vaccines for Broad Spectrum Protection Against Influenza A Viruses
This grant is to optimize a novel conformation stabilized-synthetic peptide vaccine against influenza A H1N1 that we developed, and characterize its cross protection against heterotypic influenza A strains *in vitro* and in mouse model systems.

Completed Research Support in last 3 years
5 R01 AI252231-20 (KV Holmes, PI) 2/1/88-12/31/08
Molecular Genetics of the Receptor for MHV
NIH/NIAID
The goals of this grant were to study mutants of murine coronavirus MHV with altered receptor specificities and identify mutations in the viral spike glycoprotein that cause the mutant phenotypes; to examine the molecular mechanism of MHV neutralization by soluble CEACAM1a receptor; to identify new receptors used by extended host range mutants of MHV; to determine the key residues in CEA proteins that serve as coronavirus receptors, to analyze the crystal structures of coronavirus receptors, and to explore the pathogenesis of MHV infection in mice with genetically manipulated CEACAM1 receptors for MHV**.**

University of Colorado Technology Transfer Office (1/30/07-9/1/09)
Influenza virus: Inhibition of Entry
(R. Hodges and K.V. Holmes, Co-PIs)
This seed grant as to determine whether conformation-dependent antibody to the c-terminal heptad repeat of influenza HA glycoprotein inhibits virus entry in vitro and in vivo, and whether a conformationally constrained peptide antigen can be a useful addition to influenza vaccines.

Resources sharing

We will adhere to NIH's Grant Policy on Sharing of Unique Research Resources including the Sharing of Biomedical Research Resources Principles and Guidelines for Recipients of NIH Grants and Contracts issued in December, 1999: http://ott.od.nih.gov/policy/rt_guide_final.html.

All 'model organisms' generated by this project will be distributed freely or deposited into a repository/stock center, making them available to the broader research community, either before or immediately after publication. If we assume responsibility for distributing the newly generated model organisms, we will fill requests in a timely fashion. In addition, we will provide relevant protocols and published genetic and phenotypic data upon request.

Material transfers will be made with terms that are no more restrictive than in the Simple Letter Agreement (SLA) or the Uniform Biological Materials Transfer Agreement (UBMTA) and without reach through requirements. Should any intellectual property arise which requires a patent, we will ensure that the technology (materials and data) remains widely available to the research community in accordance with the NIH Principles and Guidelines document.

Publications derived from this proposal will be made freely available to the public through NIH's Publication Central.

PHS 398 Checklist

OMB Number: 0925-0001
Expiration Date: 9/30/2007

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier:

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☐

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ☐ No ☐

OMB Number. 0925-0001
Expiration Date: 9/30/2007

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. Assurances/Certifications (see instructions)

In agreeing to the assurances/certification section 18 on the SF424 (R&R) form, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the agency's application guide, when applicable. Descriptions of individual assurances/certifications are provided at: http://grants.nih.gov/grants/funding/424

If unable to certify compliance , where applicable, provide an explanation and attach below.

Explanation: Add Attachment | Delete Attachment | View Attachment

UNIVERSITY OF MINNESOTA

Notice of Grant or Contract Award

Sponsored Projects Administration

(Online Page Navigation: Main Menu > Grants >Awards > UM_NOGA Report)

Award: CON000000022345
Primary Project: 00013782
PRF #: 651709

AWARD INFORMATION (Online Page Navigation Main Menu > Grants > Awards >Award Profile)

Award Start Date:	05/15/2010	**Award End Date:**	04/30/2015
Funds Approved to Date:	$377,500 00		
Principal Investigator:	Li,Fang	**Award Type:**	Grant
Primary Project Dept:	PHCL Pharmacology Admin	**Purpose:**	Research
Sponsor:	NIH NIAID NATL INST OF ALLERGY		
Sponsor Award #:	1R01AI089728-01		
Award Title #:	Receptor recognition mechanisms of coronaviruses		

ASSOCIATED PROJECTS (Online Page Navigation: Main Menu > Grants >Awards > Award Profile – Funding Tab)

Project #: 00013782 **Department:** 11718 - PHCL Pharmacology Admin
Project PI: Li,Fang **Dates:** 05/15/2010 - 04/30/2015 **Project Title:** Receptor recognition mechanism
F&A: 51.00 MTDC **EFFDT:** 05/15/2010

AWARD HISTORY (Online Page Navigation Main Menu > Grants > Awards > Review Award Modifications)
(Displayed in action order ('Mod Issue Date' in descending order))

Reference Award#: 1R01AI089728-01 **Amount:** $377,500 00
Period: 1 **Begin Date:** 05/15/2010 **End Date:** 04/30/2011
Modification Seq: 1 **Modification Type:** Original **Mod Issue Date:** 05/27/2010
Comments:
An unobligated balance may be carried forward to the next budget period without prior agency approval.

NOTE: In additoin to the PI, Dr Robert Geraghty as been identified as key personnel (co-PI). Any absence, replacement or substantial reduction in effort by key personnel requires prior, written agency approval.

Modular award The significant rebudgeting provision does not apply. In addition, since costs were averaged over the project period, deficits may be carried forward from one budget period to the next within the same project period

ESTIMATED FUTURE FUNDING / ACTIONS (Online Page Navigation· Main Menu > Grants > Awards > Review Award Modifications)
(Displayed by 'Period' and 'Modification Sequence' in ascending order)

Reference Award#: 1R01AI089728-01 **Amount:** $377,500.00
Period: 2 **Begin Date:** 05/01/2011 **End Date:** 04/30/2012
Modification Seq: 2 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

Reference Award#: 1R01AI089728-01 **Amount:** $377,500 00
Period: 3 **Begin Date:** 05/01/2012 **End Date:** 04/30/2013
Modification Seq: 3 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

Reference Award#: 1R01AI089728-01 **Amount:** $377,500 00
Period: 4 **Begin Date:** 05/01/2013 **End Date:** 04/30/2014
Modification Seq: 4 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

Reference Award#: 1R01AI089728-01 **Amount:** $377,500 00

10
6 - 10
66

Period: 5 **Begin Date:** 05/01/2014 **End Date:** 04/30/2015
Modification Seq: 5 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

COMMENTS

Description: **Entered by:** HOWI0016 **Date & Time:** 5/27/2010 10:00 AM
Date and Time Stamp indicates Proposal-to-Award Process

Description: **Entered by:** HOWI0016 **Date & Time:** 5/27/2010 10:02 AM
Sponsor Contacts:
Grants Management
Cheryl Wall
Phone 301-451-2696
Fax: 301-493-0597
Email wallcy@niaid nih.gov

Program:
Frederick J. Cassels
Phone: 301-402-8601
Fax. 301-496-8030
Email: casselsf@niaid.nih gov

SPECIAL REVIEWS (Online Page Navigation: Main Menu > Grants >Awards > Award Profile – Certifications Tab)

Project #: 00013782

Description: rDNA, InfectAgents, BioToxins
Approval Date: 03/11/2010 **Expiration Date:** 03/10/2011 **AssuranceNumber:** 1002H77733
Comments:

BUDGET INFORMATION (Online Page Navigation Main Menu > Grants>Awards>Project Budget)

Please review online budget for valid account string combinations (ex PCBU, Activity, Fund, Dept_Id, Project, Account)

Project #: 00013782
Project Start Date: 05/15/2010
ICR Sharing **Department:** Drug Design, Center for
Cost Share Budget Total:
Project End Date: 04/30/2015
Percentage: 11 50
Percentage: 88 50

Period	Start Date	End Date	Direct Cost (Approved)	F & A Cost (Approved)	Direct Cost (Pending)	F & A Cost (Pending)	Total Period Cost
1	05/15/2010	04/30/2011	$250,000.00	$127,500 00	$0 00	$0.00	$377,500.00
2	05/01/2011	04/30/2012	$0.00	$0 00	$250,000 00	$127,500 00	$377,500 00
3	05/01/2012	04/30/2013	$0 00	$0 00	$250,000.00	$127,500.00	$377,500 00
4	05/01/2013	04/30/2014	$0 00	$0.00	$250,000 00	$127,500 00	$377,500 00
5	05/01/2014	04/30/2015	$0 00	$0 00	$250,000 00	$127,500 00	$377,500 00
TOTAL			$250,000 00	$127,500 00	$1,000,000.00	$510,000 00	$1,887,500 00

Budget Detail (all periods)

Fund Code	DeptID	Program Code	Chartfield 1	Chartfield 2	Fin EmplID	Activity	Analysis Type	Account	Cost Share	Budget Item	Amount	Hold
3002	11718					1	BUD	700100		FACULTY_SALARY	$52,850 00	
3002	11718					1	BUD	700300		GRAD_STU_SALARY	$81,400 00	
3002	11718					1	BUD	710100		FACULTY_FRINGE	$17,071 00	

Fund Code	DeptID	Program Code	Chartfield 1	Chartfield 2	Fin EmplID	Activity	Analysis Type	Account	Cost Share	Budget Item	Amount	Hold
3002	11718					1	BUD	710300		GRAD_STU_FRINGE	$16,117 00	
3002	11718					1	BUD	720200		LABMED_SUPPLIES	$66,762 00	
3002	11718					1	BUD	720300		GEN_OP_SERVICES	$1,900 00	
3002	11718					1	BUD	720500		TELECOMM	$400 00	
3002	11718					1	BUD	720600		TRAVL_MILE_MOVE	$2,000 00	
3002	11718					1	BUD	730100		CONSULTING_SVCS	$10,000 00	
3002	11718					1	BUD	780100		REPAIRS_MAINT	$1,500 00	
3002	11718					1	BUD	810500		FACADM	$127,500 00	

COST SHARE/MATCH/IN-KIND
CSM - Cost Share, Matching and InKind

CSM05 - Departments must document all cost share that is shown in the proposal.

PROGRAM INCOME INFORMATION
PIN - Program Income

PIN03 - Reportable Program Income must be deposited by the department into the Sponsored Program Income Unapplied Cash account

PIN04 - Additional - Program Income can be used in addition to sponsor funds to cover project costs that will further the objectives of the legislation under which the grant was made

TERMS & CONDITIONS (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Terms Tab)

APV - Additional Approval

APV01 - Prior sponsor approval or notification required for reduction of PI's effort by 25% or more

APV04 - Prior sponsor approval required to replace PI

APV07 - Prior sponsor approval required for changes to key personnel that would impact the scope of work.

APV10 - Prior sponsor approval required for changes to work scope

APV14 - Prior sponsor approval required for the absence of the PI for 3 months or more.

APV15 - Prior sponsor approval required for a second no cost extension or a first no cost extension that is submitted after the agency notification deadline

APV20 - Prior sponsor approval required for the addition of foreign sites or foreign subcontracts

FIS - Fiscal

FIS03 - Reversion of funds at termination / final

FIS07 - Deficits may be carried forward.

FIS10 - Grant/contract funds may not be used to pay the salary of an individual at a rate in excess of Executive Level I of the Federal Executive Pay Scale

FIS11 - Modular award - Expenses incurred must be allocated and accounted for in compliance with all applicable cost principles

FIS13 - Deficits may be carried forward within non-competing segments, but may not be carried forward between competing segments

IPR - IP Rights

IPR02 - Sponsor support must be acknowledged in patent applications and patent issuing

IPR04 - Title to U of M, non-exclusive, non-transferable, irrevocable, paid-up license to Federal Government.

IPR12 - PI obligation to report inventions (and other IP) to the sponsor (and to PTM per policy)

PRP - Property

PRP02 - U of M receives title to equipment purchased or fabricated under this award.

PRP03 - Sponsor may require transfer of equipment and its title upon award completion for those items costing in excess of $5000.

PUB - Publications

PUB02 - U of M will place an acknowledgement of sponsor support and a disclaimer, as appropriate, on any publication generated by this award or reporting on the award results and/or activity.

PUB07 - Peer-reviewed articles must be submitted to NIH Pubmed Central (PMC) upon acceptance for publication and made publicly available within 12 months Cite PMC ID number in progress reports/proposals

RBD - Rebudgeting

RBD10 - Rebudgeting is allowed as long as there is no change in work scope

REG - Regulatory

REG01 - 45 CFR Part 74 - Code of Federal Regulations as it pertains to institutions of higher education

REG03 - OMB Circular A-21 (www whitehouse gov/omb/circulars/a021/a021.html)

REG04 - OMB Circular A-110 (www.whitehouse.gov/omb/circulars/a110/a110 html)

REG05 - OMB Circular A-133 (www whitehouse gov/omb/circulars/a133/a133 html)

REG09 - Subject to Streamlined Noncompeting Award Procedures (SNAP)

REG10 - Federal Demonstration Partnership (FDP) guidelines (www thefdp.org)

REG19 - NIH Grants Policy Statement (grants.nih gov/grants/policy/nihgps_2003)

UMN - U of M Requirements

UMN01 - The U of M Effort Certification system must be used to reflect all U of M staff committed effort on this award, whether or not any salary is charged to the award

UMN02 - Salary cost transfers cannot be made after effort is submitted

UMN03 - All U of M Policy and Guidelines relating to research must be followed and can be read in detail on the web at http //www.ospa umn edu/policiesandprocedures/index.htm

UMN04 - U of M Cost Transfer guidelines state transfers of non-salary expenses on accounts be clearly documented & made within 30 days after the end of the accounting period the charge was processed.

NON-FINANCIAL REPORTING (Online Page Navigation. Main Menu > Grants >Awards > Award Profile – Milestones Tab)
Type: Report **Description:** Progress Report **Due Date:**
Comments: PHS 2590 - noncompeting continuation. Must be submitted via the eRA Commons Due 45 days prior to the end of the budget period.

INVOICE/BILLING INFORMATION

Basis of Payment: COST_REIMBURSE
Method of Payment: Electronic Fund Transfer
Invoice Form: GPC - GM_LOC
Interim Invoice Deadline: 0
Final Invoice Deadline: 0

Contact Name: Jacqueline Jones
Contact Phone:
Contact Fax:
Contact Email:
Contact Address: PSC-FMS (301) 443-3020
PARKLAWN BUILDING ROOM 16A-12
5600 FISHERS LANE
ROCKVILLE MD 20857

FINANCIAL REPORTING

Report Form ID: R_FFR
Reporting Frequency: Termination/Final
Interim Reporting Deadline: 90
Final Reporting Deadline: 90

GENERAL PROVISIONS

This document is intended to provide guidance in managing this award, to the Principal Investigator, Academic Unit, and to those who are noted as receiving this document Its intent is to provide general information regarding University and sponsor policies when given the award. In addition, it is intended to reference specific conditions related to this award. Specific questions should be addressed to the grant administrator from SPA noted below By accepting this award, the Principal Investigator acknowledges and agrees to abide by all provisions of the sponsoring agency and the University of Minnesota policies related to sponsored projects management, including the Code of Conduct

U OF M CONTACTS

Administrative questions to SPA staff member: Lorraine Awoyinka 612/626-2265 lorrie@umn.edu
Billing and financial questions to SFR: Tonya Knutson 612/624-7850 knuts055@umn.edu

Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Issue Date: 05/14/2010

Grant Number: 1R01AI089728-01

Principal Investigator(s):
Fang Li, PHD

Project Title: Receptor recognition mechanisms of coronaviruses

David Hagen
Principal Grants and Contracts Administrator
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 554552070

Award e-mailed to: awards@umn edu

Budget Period: 05/15/2010 – 04/30/2011
Project Period: 05/15/2010 – 04/30/2015

Dear Business Official

The National Institutes of Health hereby awards a grant in the amount of $377,500 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MINNESOTA TWIN CITIES in support of the above referenced project This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system

Each publication, press release or other document that cites results from NIH grant-supported research must include an acknowledgment of NIH grant support and disclaimer such as "The project described was supported by Award Number R01AI089728 from the National Institute Of Allergy And Infectious Diseases The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Allergy And Infectious Diseases or the National Institutes of Health "

Award recipients are required to comply with the NIH Public Access Policy This includes submission to PubMed Central (PMC), upon acceptance for publication, an electronic version of a final peer-reviewed, manuscript resulting from research supported in whole or in part, with direct costs from National Institutes of Health The author's final peer-reviewed manuscript is defined as the final version accepted for journal publication, and includes all modifications from the publishing peer review process. For additional information, please visit http //publicaccess nih gov/.

Award recipients must promote objectivity in research by establishing standards to ensure that the design, conduct and reporting of research funded under NIH-funded awards are not biased by a conflicting financial interest of an Investigator Investigator is defined as the Principal Investigator and any other person who is responsible for the design, conduct, or reporting of NIH-funded research or proposed research, including the Investigator's spouse and dependent children Awardees must have a written administrative process to identify and manage financial conflict of interest and must inform Investigators of the conflict of interest policy and of the Investigators' responsibilities Prior to expenditure of these awarded funds, the Awardee must report to the NIH Awarding Component the existence of a conflicting interest and within 60 days of any new conflicting interests identified after the initial report Awardees must comply with these and all other aspects of 42 CFR Part 50, Subpart F These requirements also apply to subgrantees. contractors. or collaborators engaged by the Awardee under this award The NIH website http.//grants nih gov/grants/policy/coi/index htm provides additional information

If you have any questions about this award, please contact the individual(s) referenced in Section IV

Sincerely yours,

Dawn M Mitchum
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 1R01AI089728-01

Award Calculation (U.S. Dollars)

Federal Direct Costs	$250,000
Federal F&A Costs	$127,500
Approved Budget	$377,500
Federal Share	$377,500
TOTAL FEDERAL AWARD AMOUNT	$377,500
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$377,500

SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
1	$377,500	$377,500
2	$377,500	$377,500
3	$377,500	$377,500
4	$377,500	$377,500
5	$377,500	$377,500

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93 855
EIN:	1416007513A1
Document Number:	RAI089728A
Fiscal Year:	2010

IC	*CAN*	*2010*	*2011*	*2012*	*2013*	*2014*
AI	8472364	$377,500	$377,500	$377,500	$377,500	$377,500

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC M51C S / **OC** 414A / **Processed** MITCHUMD 05/10/2010

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01AI089728-01

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http //grants nih gov/grants/policy/awardconditions htm

SECTION III – TERMS AND CONDITIONS – 1R01AI089728-01

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following

a. The grant program legislation and program regulation cited in this Notice of Award
b Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW

(See NIH Home Page at 'http //grants nih gov/grants/policy/awardconditions htm' for certain references cited above)

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase V Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP)

In accordance with P L 110-161, compliance with the NIH Public Access Policy is now mandatory For more information, see NOT-OD-08-033 and the Public Access website http //publicaccess nih gov/

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 1R01AI089728-01

The budget period anniversary start date for future year(s) will be May 1
******
This is a Modular Award without direct cost categorical breakdowns in accordance with the guidelines published in the NIH Grants Policy Statement, December 2003, see (http //grants2 nih gov/grants/policy/nihgps_2003/NIHGPS_Part12.htm#_Toc54600227), pages 217-219 Recipients are required to allocate and account for costs related to this award by category within their institutional accounting system in accordance with applicable cost principles
****
In addition to the PI, any absence, replacement, or substantial reduction in effort of the following individual(s) below, requires the written prior approval of the National Institutes of Health awarding component

Dr. Robert Geraghty,Associate Professor and co-PI

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions The Program Official is responsible for the scientific, programmatic and technical aspects of this project These individuals work together in overall project administration Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail

Grants Management Specialist Cheryl Wall
Email wallcy@niaid.nih gov **Phone** 301-451-2696 **Fax** 301-493-0597

Program Official: Frederick J Cassels
Email casselsf@niaid nih gov **Phone** 301-402-8601 **Fax** 301-496-8030

SPREADSHEET SUMMARY
GRANT NUMBER: 1R01AI089728-01

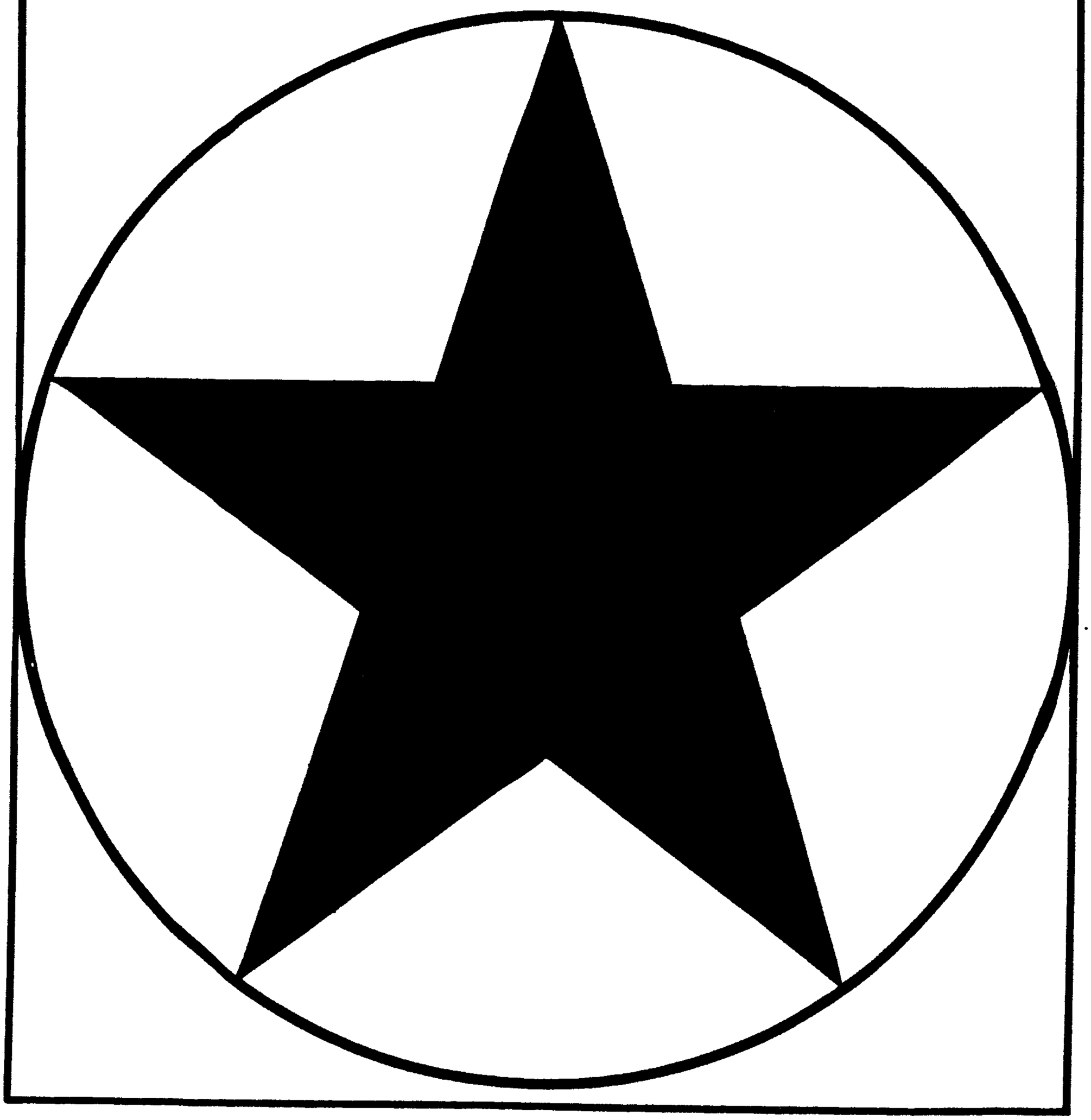
INSTITUTION: UNIVERSITY OF MINNESOTA TWIN CITIES

Budget	*Year 1*	*Year 2*	*Year 3*	*Year 4*	*Year 5*
TOTAL FEDERAL DC	$250,000	$250,000	$250,000	$250,000	$250,000
TOTAL FEDERAL F&A	$127,500	$127,500	$127,500	$127,500	$127,500
TOTAL COST	$377,500	$377,500	$377,500	$377,500	$377,500

Facilities and Administrative Costs	*Year 1*	*Year 2*	*Year 3*	*Year 4*	*Year 5*

F&A Cost Rate 1	51%	51%	51%	51%	51%
F&A Cost Base 1	$250,000	$250,000	$250,000	$250.000	$250.000
F&A Costs 1	$127,500	$127,500	$127,500	$127,500	$127.500

Please Mail All Pages Prior To Page Separator



AWARD SETUP INFORMATION PAGE GA LA DATE 5/27

CON 22345 PROJECT 13782

X New

____ PreAward – see bottom of page for information eTool Completed X No info in eTool ____

____Modification, original Award = CON ____(this is the award that will be updated)

____Stimulus Funding

INVOICING AND FINANCIAL REPORTING INFORMATION CAN BE FOUND ON THE FOLLOWING PAGE(S) IN THE AWARD DOCUMENT ____

TERMS AND CONDITIONS

X are attached ____ are in eTool

____ are saved in MyNetworkPlaces\Award Setup\Award Setup-submissions TAB ____

(\\ovpr.ad.umn.edu\ovpr$\Shared\Groups\Award Setup)

BUDGET INFORMATION FOR FUNDS PROVIDED BY SPONSOR – for new awards only

3002 Fund 11718 DeptID

____ is in eTool

____See attached PS-friendly budget

COST SHARE INFORMATION – for new awards only (GA updates the budgets for modifications)

____Fund ____ DeptID ____ Program ____ Chartfield 1 ____ Chartfield 2 ____Emplid

____ is in eTool

____See attached PS-friendly budget

____**NEW PROJECT NEEDED** - For new projects on existing awards, please enter relevant information in the eTool or on separate page and include a completed PS-friendly budget.

ANY ADDITIONAL INFORMATION NEEDED FOR SET UP:

Item in grad fringe is for a post-doc. Therefore F+A needs to be adjusted to $127,500

F+A split 88.5/11.5

PUT AWARD FILE IN THE FOLLOWING ORDER:

Agency award documents

Guidance documents (to be included with award emailed to PI/Dept)

Award Imaging Divider page (yellow star sheet)

Award Setup Information page (this page)

PS-friendly budget (if any)

Terms and Conditions spreadsheet (if any)

Negotiation notes, proposal correspondence, proposal, etc.

SET ALL NEGOTIATE AWARD NEGOTIATION TYPES TO COMPLETED EXCEPT FOR THE ONE FOR CONTRACT ENTRY AND THE ORIGINAL ONE CREATED BY FILES

PREAWARD INFORMATION REQUIRED - Provide information for each project

Note: No cost share information is required for PreAwards.

____Fund Budget accounts to be set up at $0 are listed below - OR - ____See Attached

____DeptID Remember to include 810500 if F&A should be charged

____Project

____Function ____ ____ ____ ____ ____

____F&A rate & base ____ ____ ____ ____ ____

____Rate Type *(Research, Other, Training, Off Campus, ClinTrial-nonfed, CREE2, CREES, DOD contract, Equip, Foundation, Hormel, Instruction, SBIR/STTR Phase I, State)*

PS / SPONSOR PROPOSAL BUDGET WORKSHEET

Please complete the cost share and F&A distribution information at the bottom of the page, if applicable

Award CON - 22345 Project #, if known 13782 Should this be the Primary Project? ____Yes ___ No

Project DeptID 11718 Project PI **Fang Li**

		Total of all worksheet tabs:	**Funding**	**Cost Share**	Total Projects
Fund	**3002**		$377,500	$0	$377,500
Dept ID	**11718**				

ITEM		**Sponsor Funding**	**Sponsor Funding**	**TOTAL PROJECT BUDGET**
Personnel				
Salary				
700100	Salary - Faculty	52,850		
700200	Salary - Professional and Admin			
700300	Salary - Post Doc / Professional Student	81,400		
700300	Salary - Grad Research Asst (expense account 700301)			
700400	Salary - Undergrad Student			
700500	Salary - Civil Service Bargaining Unit			
Total Salary		134,250	0	134,250
Fringe				
710100	Fringe - Faculty	17,071		
710200	Fringe - Professional and Admin			
710300	Fringe - Post Doc / Professional Student	16,117		
710300	Fringe - Grad Research Asst (expense account 710301)			
710400	Fringe - Undergrad Student			
710500	Fringe - Civil Service Bargaining Unit			
710600	Fringe - Direct Charge			
Total Fringe		33,188	0	33,188
Total Personnel		167,438	0	167,438
Consulting / Professional Services				
730100	Consulting Services	10,000		
730200	Professional Services			
Total Consulting / Professional Services		10,000	0	10,000
Equipment				
168100	Equipment			0
Supplies				
720100	General Operating Supplies			
720200	Lab & Medical Supplies	66,762		
750100	Non-Capitalized Equipment			
Total Supplies		66,762	0	66,762
Travel				
720600	Travel / Mileage / Moving	2,000		2,000
Other Direct Costs				
720300	General Operating Services	1,900		
720400	Lab & Medical Services			
720500	Telecommunications	400		
770100	Long Term Rents & Leases			
770150	Long Term Rents & Leases (bldg/facility)			
770200	Short Term Rents & Leases			
770250	Short Term Rents & Leases (bldg/facility)			
780100	Repairs & Maintenance	1,500		
810100	Research Subject Payments			
Total Other Direct Costs		3,800	0	3,800
Subawards / Subcontracts				
810300	Subcontracts not subject to F&A			
810400	Subcontracts subject to F&A			
Total Subawards / Subcontracts		0	0	0
Additional Accounts (show budget account and a description, please)				
				0
				0
				0
TOTAL DIRECT COSTS		250,000	0	250,000
810500	Facilities and Administrative Expenses	127,500	0	127,500
TOTAL COSTS		377,500	0	377,500

F&A Split 88.5% Pharmacology 11.5% Ctr for Drug Design

Subject: Dr Fang Li's Grant
From: Jim Burak <burak001@umn edu>
Date: Wed, 26 May 2010 10 21·07 -0500
To: Lorrie Awoyinka <lorrie@umn edu>
CC: Elizabeth Wolfson <ravdi002@umn edu>, Fang Li <lifang@umn edu>, Carrie DeJarlais <dejar001@umn edu>, Debi Marsh <mars0061@umn edu>, "Robert J Geraghty" <gerag012@umn edu>

On Dr. Fang Li's grant application to NIH (PRF ID: 651709) cost-sharing of IDC with the Center for Drug Design (CDD) was indicated in question 17.

Dr. Li has now received grant award 1 R01 AI089728-01 for this application. Please set up the award in the Department of Pharmacology, bu[redacted] 11.5% of the IDC (% that R. Geraghty and W. Zhou's compensation is of total award) [redacted] to the Center for Drug Design.

If you have questions on what CDD chart string to use for the IDC sharing, contact Elizabeth Wolfson (624-2227) in the CDD.

DeptID 11345

Jim Burak
MNPI Center &
Department of Pharmacology
University of Minnesota
6-120 Jackson Hall
321 Church Street SE
Minneapolis, MN 55455
612 625-8660

NIH
Modular
Not final year

TERMS AND CONDITIONS - NIH EXPANDED AUTHORITIES

Please check the terms that apply. An explanation of a term may be added at the end of the section (up to 150 char)
You may add up to 5 terms at the end of each section There is a 150 character limit for each term
You may add up to 10 terms under the OTHER section at the end of the list (150 character limit per term)

AGENCY PRIOR APPROVAL			
	GUIDELINE ID	DESCRIPTION	FULL DESCRIPTION
X	APV01	CHANGE IN PI EFFORT OF 25%	Prior sponsor approval or notification required for reduction of PI's effort by 25% or more
	APV02	TRNSFER WORKSCOPE TO 3RD PARTY	Prior sponsor approval required to transfer the actual performance of substantive work to a third party
	APV03	REQUIRED FOR NOCOST EXTENSIONS	Prior sponsor approval required for no-cost extensions
X	APV04	CHANGE OF PI	Prior sponsor approval required to replace PI
	APV05	AV/PUBLICATION COST > $25,000	Prior sponsor approval required for production or acquisition of a single audiovisual or publication costing in excess of $25,000
	APV06	REQUIRED FOR REBUDGETING	Prior sponsor approval required for rebudgeting - See Explanation
X	APV07	CHANGE IN KEY PERSONNEL	Prior sponsor approval required for changes to key personnel that would impact the scope of work
	APV08	CARRYFORWARD END OF BUDGET PD	Prior sponsor approval required to carryforward unobligated balances at end of budget period
	APV09	CARRYFORWARD END OF PROJECT PD	Prior sponsor approval required to carryforward unobligated balances at end of project period
X	APV10	CHANGE IN SCOPE OF WORK	Prior sponsor approval required for changes to work scope
	APV11	CONSULTANTS OR SUBCONTRACTS	Prior sponsor approval required for consultants and/or subcontracts.
	APV12	REBUDGET PARTICIPANT COSTS	Prior sponsor approval required for rebudgeting from Participant Costs.
	APV13	NOCOST EXTENSION VIA FAST LANE	Prior sponsor approval required for no-cost extensions, via Fast Lane
X	APV14	ABSENCE OF PI FOR 3+ MONTHS	Prior sponsor approval required for the absence of the PI for 3 months or more
X	APV15	2ND OR LATE NCE	Prior sponsor approval required for a second no cost extension or a first no cost extension that is submitted after the agency notification deadline
	APV16	SIGNIF REBUDG >25% TOTAL COST	Prior sponsor approval required for changes to workscope resulting from significant rebudgeting of 25% or more of total cost awarded
	APV17	REBUDGET PATIENT CARE	Prior sponsor approval required for patient care costs or rebudgeting in or out of this category
	APV18	USE OF TIP INCOME	Prior sponsor approval required before use of TIP income
	APV19	PUBLIC ANNOUNCEMENT	Prior sponsor approval required for public announcement
X	APV20	FOREIGN SITES OR SUBCONTRACTS	Prior sponsor approval required for the addition of foreign sites or foreign subcontracts
	APV21	REBUDGET 25% OF TOTAL AMOUNT	Prior sponsor approval required to rebudget 25% or more of total amount awarded
	APV22	CARRYOVER 25% OR $250,000	Prior sponsor approval required to carry forward unobligated balance in excess of 25% of the total amount awarded or $250,000, whichever is less
	APV23	SECOND NO COST EXTENSION	Prior sponsor approval required for a second no cost extension
	APV24	LATE NO COST EXTENSION REQUEST	Prior sponsor approval required for no cost extensions that are submitted after the agency notification deadline
	APV25	ALL SUBAWARDS	Prior sponsor approval required for subawards
	APV95	AWARD SPECIFIC - AGENCY APPROVAL	
	APV96	AWARD SPECIFIC - AGENCY APPROVAL	
	APV97	AWARD SPECIFIC - AGENCY APPROVAL	
	APV98	AWARD SPECIFIC - AGENCY APPROVAL	
	APV99	AWARD SPECIFIC - AGENCY APPROVAL	

Additional Explanations for selected Terms above. Please identify the Guideline ID and any text needed for clarification of the term. The explanation is limited to 150 characters			
	APV__		
	APV__		
	APV__		
	APV__		
	APV__		
COST SHARE			
	CSM01	REPORT COST SHARE BY COA	Cost share report based on U of M Chart of Accounts
	CSM02	REPORT COST SHARE BY TOTAL	Cost share report based on total
	CSM03	COST SHARE BUDGET REQUIRED	Cost share budget required
	CSM04	REPORT COST SHARE BY SPONS CAT	Cost share report based on agency category
X	CSM05	DEPT DOCUMENTS ALL COST SHARE	Departments must document all cost share that is shown in the proposal
	CSM06	COST SHARE REPORTING REQUIRED	Cost share reporting to sponsor is required
	CSM95	AWARD SPECIFIC - COST SHARE	
	CSM96	AWARD SPECIFIC - COST SHARE	
	CSM97	AWARD SPECIFIC - COST SHARE	
	CSM98	AWARD SPECIFIC - COST SHARE	
	CSM99	AWARD SPECIFIC - COST SHARE	
Additional Explanations for selected Terms above. Please identify the Guideline ID and any text needed for clarification of the term. The explanation is limited to 150 characters			
	CSM___		
	CSM___		
	CSM___		
EQUIPMENT			
	EQP01	GENERAL PURPOSE ITEMS REQ APRV	Prior sponsor approval required for the purchase of general purpose equipment
	EQP02	EQUIP COST >$25,000 REQS APRVL	Prior sponsor approval is required for all equipment acquisition greater than $25,000
	EQP03	EQUIP COST >$500 REQS APRVL	Prior sponsor approval required for all equipment purchases greater than $500
	EQP04	NO EQUIPMENT IS ALLOWED	No equipment is allowed
	EQP05	UNBUDGETED EQUIPMENT REQ APRV	Prior sponsor approval required for purchase of equipment items not included in the approved budget
	EQP06	EQP > SPON DEFIND LMT REQ APRV	Prior sponsor approval required for all equipment purchases greater than sponsor defined limit
	EQP07	UNBDGD OVER $5,000 REQ APRVL	Prior sponsor approval required for purchase of equipment costing $5,000 or more that was not included in the approved budget
	EQP95	AWARD SPECIFIC - EQUIPMENT TERM	
	EQP96	AWARD SPECIFIC - EQUIPMENT TERM	
	EQP97	AWARD SPECIFIC - EQUIPMENT TERM	

	EQP98	AWARD SPECIFIC - EQUIPMENT TERM	
	EQP99	AWARD SPECIFIC - EQUIPMENT TERM	
Additional Explanations for selected Terms above Please identify the Guideline ID and any text needed for clarification of the term. The explanation is limited to 150 characters			
	EQP___		
	EQP___		
	EQP___		
FISCAL			
	FIS01	FUNDS REVERT AT BUDGET PD END	Reversion of funds at end of budget period
	FIS02	FUNDS REVERT AT PROJECT PD END	Reversion of funds at end of project period
X	FIS03	FUNDS REVERT AT TERMINATION	Reversion of funds at termination / final
	FIS04	NO REVERSION OF FUNDS	No reversion of funds
	FIS05	SBE REPORTING REQUIRED	Small Business Enterprises (SBE) reporting required
	FIS06	MBE/WBE REPORTING REQUIRED	Minority Business Enterprises (MBE)/Women Business Enterprises (WBE) reporting required
X	FIS07	DEFICITS MAY CARRY FORWARD	Deficits may be carried forward
	FIS08	DEFICITS MAY NOT CARRY FORWARD	Deficits may not be carried forward
	FIS09	FIXED PRICE CLOSEOUT PROCESS	When a study is completed, all expectations met and revenue received, the PI should request this account be inactivated via the process for closing out fixed price projects
X	FIS10	NIH/DHHS SALARY CAP	Grant/contract funds may not be used to pay the salary of an individual at a rate in excess of Executive Level I of the Federal Executive Pay Scale
X	FIS11	MODULAR AWARD	Modular award - Expenses incurred must be allocated and accounted for in compliance with all applicable cost principles
	FIS12	PRIOR PD BAL USE PRIOR PD FA RATE	Any balance carried forward from the previous project period should be charged at the F&A rate associated with that period SFR will perform an F&A analysis and adjust F&A charged as necessary
X	FIS13	C/F DEFICIT BTWN BUD PDS ONLY	Deficits may be carried forward within non-competing segments, but may not be carried forward between competing segments
	FIS95	AWARD SPECIFIC - FISCAL TERMS	
	FIS96	AWARD SPECIFIC - FISCAL TERMS	
	FIS97	AWARD SPECIFIC - FISCAL TERMS	
	FIS98	AWARD SPECIFIC - FISCAL TERMS	
	FIS99	AWARD SPECIFIC - FISCAL TERMS	
Additional Explanations for selected Terms above Please identify the Guideline ID and any text needed for clarification of the term. The explanation is limited to 150 characters			
	FIS___		
	FIS___		
	FIS___		
INTELLECTUAL PROPERTY			
	IPR01	TITLE TO UNIV NONEX ROYFR SPON	Title to U of M, non-exclusive, royalty-free license to sponsor
X	IPR02	ACKNOWLEDGE SPONSOR ON PATENTS	Sponsor support must be acknowledged in patent applications and patent issuing
	IPR03	TITLE TO UNIV FOR SOLE DEVELOP	Title to U of M for inventions developed solely by U of M employees.

X	IPR04	TITLE TO UNIV NONEX ROYFR GOVT	Title to U of M, non-exclusive, non-transferable, irrevocable, paid-up license to Federal Government.
	IPR05	UNIV TITLE AGNCY OPTION LICENS	Title to U of M, option to sponsor for exclusive, royalty bearing license
	IPR06	TITLE TO UNIV INTRNAL LIC SPON	Computer software Title to U of M, non-exclusive, internal license to sponsor for software specified to be developed in scope of work
	IPR07	TITLE TO UNIV SOLE AND JOINT	Title to U of M for inventions developed solely by U of M employees and jointly by U of M and sponsor employees
	IPR08	TITLE TO SPONSOR FOR SPONS DEVS	Title to sponsor for inventions developed solely by sponsor employees
	IPR09	JOINT TITLE FOR COLLABORATIONS	Joint title to U of M and sponsor for inventions made by staff collaboration
	IPR10	AGENCY TITLE IF AGNCY PROTOCOL	Title to sponsor for all developments made from work done in accordance with the protocol
	IPR11	AGENCY TITLE IF AGNCY MATERIAL	Title to sponsor for all developments made from the use of sponsor's materials
X	IPR12	PI REPORT IP TO AGENCY AND OTC	PI obligation to report inventions (and other IP) to the sponsor (and to OTC per policy)
	IPR95	AWARD SPECIFIC INTELLECTUAL PROP	
	IPR96	AWARD SPECIFIC INTELLECTUAL PROP	
	IPR97	AWARD SPECIFIC INTELLECTUAL PROP	
	IPR98	AWARD SPECIFIC INTELLECTUAL PROP	
	IPR99	AWARD SPECIFIC INTELLECTUAL PROP	
Additional Explanations for selected Terms above Please identify the Guideline ID and any text needed for clarification of the term. The explanation is limited to 150 characters			
	IPR___		
	IPR___		
	IPR___		
PROGRAM INCOME			
	PIN01	PROGRAM INCOME - ADD/DEDUCT	Combination (Add/Deduct) - Program income generated by this project in excess of $25,000 will reduce the amount of funding provided by the sponsor by the amount in excess of $25,000
	PIN02	PROGRAM INCOME - DEDUCTION	Deduction - Program income generated by this project will reduce the funding provided by the sponsor by the same amount
X	PIN03	PROGRAM INCOME REPORTABLE	Reportable Program Income must be deposited by the department into the Sponsored Program Income Unapplied Cash account
X	PIN04	PROG INC-ADDITION W/IN PROJ PD	Additional - Program Income can be used in addition to sponsor funds to cover project costs that will further the objectives of the legislation under which the grant was made
	PIN05	PROG INC-ADDITION AFTER PROJ PD	Additional - Added to funds committed to the project or program and used to further eligible project or program objectives beyond the project period
	PIN06	PROGRAM INCOME NONREPORTABLE	Program Income is nonreportable
	PIN95	AWARD SPECIFIC PROGRAM INCOME	
	PIN96	AWARD SPECIFIC PROGRAM INCOME	
	PIN97	AWARD SPECIFIC PROGRAM INCOME	
	PIN98	AWARD SPECIFIC PROGRAM INCOME	
	PIN99	AWARD SPECIFIC PROGRAM INCOME	
Additional Explanations for selected Terms above. Please identify the Guideline ID and any text needed for clarification of the term. The explanation is limited to 150 characters			

	PIN___		
	PIN___		
PROPERTY			
	PRP01	AGNCY MAY TRANSFR EQUIP AT END	Sponsor may require transfer of equipment and its title upon project completion
X	PRP02	TITLE ASSIGNED TO UNIVERSITY	U of M receives title to equipment purchased or fabricated under this project
X	PRP03	SPON MAY REQ EQP TRANS >$5,000	Sponsor may require transfer of equipment and its title upon project completion for those items costing in excess of $5000
	PRP04	TITLE ASSIGNED TO SPONSOR	Sponsor receives title to equipment purchased or fabricated under this project
	PRP95	AWARD SPECIFIC PROPERTY TERM	
	PRP96	AWARD SPECIFIC PROPERTY TERM	
	PRP97	AWARD SPECIFIC PROPERTY TERM	
	PRP98	AWARD SPECIFIC PROPERTY TERM	
	PRP99	AWARD SPECIFIC PROPERTY TERM	
Additional Explanations for selected Terms above Please identify the Guideline ID and any text needed for clarification of the term. The explanation is limited to 150 characters			
	PRP___		
	PRP___		
PUBLICATIONS			
	PUB01	UNIV MAY PUBL ALL DATA PRODUCD	U of M may publish all data first produced in performance of project
X	PUB02	ACKNOWLEDGE/DISCLAIMER ON PUBL	U of M will place an acknowledgement of sponsor support and a disclaimer, as appropriate, on any publication generated by this project or reporting on the project results and/or activity
	PUB03	UNRESTRICTED W/SPONSOR REVIEW	Unrestricted right to publish with advance copy to sponsor for review
	PUB04	UNRESTRICTED W/COPY TO SPONSOR	Unrestricted right to publish, copy to sponsor
	PUB05	AGENCY REVIEW FOR CONFID INFO	Publications require prior review by sponsor for sponsor Confidential Information and patentable subject matter
	PUB06	SPON INSTRUCTN FOR REVIEW/PUBL	Sponsor instructions for review and dissemination - See Explanation
X	PUB07	PUBLICATNS-NIH PUBMED CENTRAL	Peer-reviewed articles must be submitted to NIH Pubmed Central (PMC) upon acceptance for publication and made publicly available within 12 months Cite PMC ID number in progress reports/proposals
	PUB95	AWARD SPECIFIC PUBLICATION TERM	
	PUB96	AWARD SPECIFIC PUBLICATION TERM	
	PUB97	AWARD SPECIFIC PUBLICATION TERM	
	PUB98	AWARD SPECIFIC PUBLICATION TERM	
	PUB99	AWARD SPECIFIC PUBLICATION TERM	
Additional Explanations for selected Terms above. Please identify the Guideline ID and any text needed for clarification of the term The explanation is limited to 150 characters			
	PUB___		
	PUB___		

REBUDGETING			
	RBD01	REBUDGETING IS UNRESTRICTED	Rebudgeting is unrestricted
	RBD02	REBUDGETING IS RESTRICTED	Rebudgeting restrictions - See Explanation
	RBD03	REBUDGET RESTRICTED - AMT OR %	Rebudgeting amount or percent - See Explanation
	RBD04	REBUDGET TERM IS BUDGET PD	Rebudgeting restrictions term is equal to the budget period
	RBD05	REBUDGET TERM IS ANNUAL	Rebudgeting restrictions term is equal to annually
	RBD06	REBUDGET TERM IS PROJECT PD	Rebudgeting restrictions term is equal to the project period
	RBD07	REBUDGET RESTRICTED BY CATEGORY	Rebudgeting restrictions are per category
	RBD08	REBUDGET RESTRICTED BY ACCOUNT	Rebudgeting restrictions are per account
	RBD09	REBUDGET RESTRICTED BY TOTAL	Rebudgeting restrictions are per total
X	RBD10	REBUDGET IF NO CHANGE TO SOW	Rebudgeting is allowed as long as there is no change in work scope
	RBD11	REBUDGET BTWN NOT OUT STPND/TUIT	Rebudgeting of stipend and tuition/fees categories is allowed between, but not out of these categories
	RBD95	AWARD SPECIFIC REBUDGETING TERM	
	RBD96	AWARD SPECIFIC REBUDGETING TERM	
	RBD97	AWARD SPECIFIC REBUDGETING TERM	
	RBD98	AWARD SPECIFIC REBUDGETING TERM	
	RBD99	AWARD SPECIFIC REBUDGETING TERM	
Additional Explanations for selected Terms above. Please identify the Guideline ID and any text needed for clarification of the term The explanation is limited to 150 characters			
	RBD___		
	RBD___		
REGULATORY			
X	REG01	SUBJECT TO 45 CFR 74	45 CFR Part 74 - Code of Federal Regulations as it pertains to institutions of higher education
	REG02	PHS POLICY STATEMENT	PHS Policy Statement (grants nih gov/grants/policy/gps/)
X	REG03	OMB CIRCULAR A-21	OMB Circular A-21 (www whitehouse gov/omb/circulars/a021/a021 html)
X	REG04	OMB CIRCULAR A-110	OMB Circular A-110 (www whitehouse gov/omb/circulars/a110/a110.html)
X	REG05	OMB CIRCULAR A-133	OMB Circular A-133 (www whitehouse.gov/omb/circulars/a133/a133 html)
	REG06	NSF GC-1, GPG, prog annc	NSF GC-1, current GPG, and program announcement/solicitation if applicable
	REG07	EDUCATION DEPT GEN ADMIN REGS	EDGAR (http //www ed.gov/policy/fund/reg/edgarReg/edgar html)
	REG08	SPONSOR GUIDELINES	Sponsor Guidelines
X	REG09	SUBJECT TO SNAP	Subject to Streamlined Noncompeting Award Procedures (SNAP)
X	REG10	FDP GEN TERMS CONDITIONS	Federal Demonstration Partnership (FDP) guidelines (www.thefdp.org)
	REG11	DODGAR (32 CFR)	DoDGAR (32 CFR)
	REG12	14 CFR PART 1260 21-1260 38	The following provisions are incorporated by reference· 14 CFR Part 1260 21-1260 38
	REG13	34 CFR PART 74	34 CFR Part 74 (www access.gpo gov/nara/cfr/cfr-table-search.html)
	REG14	FEDERL ACQUISITION REGULATIONS	Federal Acquisition Regulation (FAR)(www.arnet gov/far/)
	REG15	EXPANDED AUTHORITIES	Expanded Authorities (HRSA, CDC, USDE not under FDP)
	REG16	IRB APPROVAL PRIOR TO ACTIVITY	No funds may be expended for human subject related activities until all appropriate human subject related approvals are in place
	REG17	IACUC APPROVAL PRIOR TO ACTIVITY	No funds may be expended for animal related activities until all appropriate animal related approvals are in place
	REG18	HUMAN SUBJECT TRAINING REQUIRED	Any personnel involved in the design or conduct of human subject activity must satisfy the requirement for education on the protection of human subjects prior to participating in the study

X	REG19	NIH GRANTS POLICY STATEMENT	NIH Grants Policy Statement (grants nih gov/grants/policy/nihgps_2003)
	REG20	USDA GENL TERMS & CONDITNS - A	USDA General Terms and Conditions - A (http //www csrees usda gov/business/awards/awardterms html)
	REG 21	NEED IBC OK PRIOR TO ACTIVITY	No funds may be expended for activities involving recombinant DNA, Infectious Agents or Biological Toxins until Institutional Biosafety Committee (IBC) approval has been obtained
	REG95	AWARD SPECIFIC REGULATORY TERM	
	REG96	AWARD SPECIFIC REGULATORY TERM	
	REG97	AWARD SPECIFIC REGULATORY TERM	
	REG98	AWARD SPECIFIC REGULATORY TERM	
	REG99	AWARD SPECIFIC REGULATORY TERM	
Additional Explanations for selected Terms above. Please identify the Guideline ID and any text needed for clarification of the term. The explanation is limited to 150 characters			
	REG___		
	REG___		
	REG___		
	REG___		
	REG___		
TIP INCOME			
	TIP01	TIP INCOME REVERTS TO SPONSOR	TIP Income reverts to sponsor
	TIP02	TIP INCOME USED TOWARD PROJECT	TIP Income is used toward project
	TIP03	TIP INCOME RETAINED BY UNIV	TIP Income retained by U of M
	TIP04	TIP INCOME REPORTING REQUIRED	TIP Income reporting required
	TIP95	AWARD SPECIFIC TIP INCOME TERM	
	TIP96	AWARD SPECIFIC TIP INCOME TERM	
	TIP97	AWARD SPECIFIC TIP INCOME TERM	
	TIP98	AWARD SPECIFIC TIP INCOME TERM	
	TIP99	AWARD SPECIFIC TIP INCOME TERM	
Additional Explanations for selected Terms above Please identify the Guideline ID and any text needed for clarification of the term The explanation is limited to 150 characters			
	TIP___		
	TIP___		
TRAINING			
	TRN01	# SLOTS MAY NOT BE EXCEEDED	The number of trainee slots may not be exceeded
	TRN02	TYPE OF SLOTS MAY NOT CHANGE	The type of trainee slots awarded may not be changed
	TRN03	ONLY 1 TRAINEE PER SLOT	Only one trainee per awarded slot is allowed
	TRN04	MAY NOT EXCEED STIPEND LEVELS	Established stipend levels may not be exceeded
	TRN05	NO REBUDGET STIPEND/TUITION	Funds awarded for stipends or tuition may not be used for any other purpose.

	TRN06	UNSPENT TUITION REVERT BUDGET PD	Unspent tuition funds revert at the end of each budget period.
	TRN07	NOTIFY AGENCY OF ANY CHANGE	Fellow must notify agency of any change of plans regarding fellowship
	TRN08	APPT STMT/PAYBACK AGRMNT REQRD	A Statement of Appointment form and a Payback Agreement (if applicable) must be submitted for all trainees appointed to the grant
	TRN09	TERM NOTICE REQUIRED AT END	A termination notice must be submitted to NIH at the conclusion of the fellowship
	TRN10	2ND HALF INSTITUTIONAL ALLOW	Second half of Institutional Allowance will be released after fellow completes six months of training
	TRN11	INST ALLOW SET UP IN PROGRAM	Institutional Allowance Funds have been set up in a departmental accounts
	TRN95	AWARD SPECIFIC TRAINING TERM	
	TRN96	AWARD SPECIFIC TRAINING TERM	
	TRN97	AWARD SPECIFIC TRAINING TERM	
	TRN98	AWARD SPECIFIC TRAINING TERM	
	TRN99	AWARD SPECIFIC TRAINING TERM	
Additional Explanations for selected Terms above. Please identify the Guideline ID and any text needed for clarification of the term. The explanation is limited to 150 characters			
	TRN___		
	TRN___		
TRAVEL			
	TRV01	UNBUDGETED TRAVEL REQ APPROVAL	Prior sponsor approval required for travel not included in the approved budget.
	TRV02	OUT OF STATE TRAVEL REQ APPRVL	Prior sponsor approval required for out of state travel
	TRV03	TRAVEL FUNDS RESTRICTED	Travel funds cannot be used for any other purpose
	TRV04	TRAVEL LIMITED TO AMT AWARDED	Travel expenditures are limited to the amount in the approved budget
	TRV05	NO TRAVEL EXPENDITURES ALLOWED	No travel expenditures allowed
	TRV06	FOREIGN TRAVEL REQS APPROVAL	Prior sponsor approval required for all foreign travel, whether or not included in the approved budget
	TRV07	GENERAL SCIENTIFIC MEETINGS	Prior sponsor approval required for travel to general scientific meetings.
	TRV95	AWARD SPECIFIC TRAVEL TERM	
	TRV96	AWARD SPECIFIC TRAVEL TERM	
	TRV97	AWARD SPECIFIC TRAVEL TERM	
	TRV98	AWARD SPECIFIC TRAVEL TERM	
	TRV99	AWARD SPECIFIC TRAVEL TERM	
Additional Explanations for selected Terms above Please identify the Guideline ID and any text needed for clarification of the term. The explanation is limited to 150 characters			
	TRV___		
	TRV___		
UNIVERSITY OF MINNESOTA			
X	UMN01	EFFORT MUST BE CERTIFIED	The U of M Effort Certification system must be used to reflect all U of M staff committed effort on this project, whether or not any salary is charged to the project
X	UMN02	NO HAS AFTER EFFROT CERTIFIED	Salary cost transfers cannot be made after effort is submitted

X	UMN03	U OF M RESEARCH POLICIES	All U of M Policy and Guidelines relating to research must be followed and can be read in detail on the web at http //www ospa umn edu/policiesmanuals&education/policies/respolcy.htm
X	UMN04	U OF M COST TRANSFER GUIDELINE	U of M Cost Transfer guidelines state transfers of non-salary expenses on accounts be clearly documented & made within 30 days after the end of the accounting period the charge was processed
OTHER			
	OTH01	OTHER AWARD SPECIFIC TERM	
	OTH02	OTHER AWARD SPECIFIC TERM	
	OTH03	OTHER AWARD SPECIFIC TERM	
	OTH04	OTHER AWARD SPECIFIC TERM	
	OTH05	OTHER AWARD SPECIFIC TERM	
	OTH06	OTHER AWARD SPECIFIC TERM	
	OTH07	OTHER AWARD SPECIFIC TERM	
	OTH08	OTHER AWARD SPECIFIC TERM	
	OTH09	OTHER AWARD SPECIFIC TERM	
	OTH10	OTHER AWARD SPECIFIC TERM	

Subject: Re: Dr Fang Li's Grant
From: "Robert J Geraghty" <gerag012@umn edu>
Date: Wed, 26 May 2010 12 52 12 -0500
To: Lorrie Awoyinka <lorrie@umn edu>, Elizabeth Wolfson <ravdi002@umn edu>, Liz Hinske <hinsk001@umn edu>

Hello Lorrie,

I believe the Center For Drug Design DeptID is 11345.

11345 confirmed with Liz Hinske

Bob G

Robert J. Geraghty, PhD
Associate Professor & Associate Director
Center for Drug Design
University of Minnesota
516 Delaware Street SE
7-222 PWB, MMC 204
Minneapolis, MN 55455
Fax: 612-625-8154
Tel: 612-625-3281
http://www.cdd.umn.edu/directory/geraghty/home.html

Lorrie Awoyinka wrote:
> What is the Dept ID for the Center for Drug Design that is to receive the F&A?
> Lorrie Awoyinka
> Principal Grant & Contract Administrator
> University of Minnesota
> Sponsored Projects Administration
> 450 McNamara Alumni Center
> 200 Oak St. SE
> Minneapolis, MN 55455
>
> Phone: (612) 626-2265
> Fax: (612) 624-4843
> E-mail: lorrie@umn.edu
>
> Jim Burak wrote:
> > On Dr. Fang Li's grant application to NIH (PRF ID: 651709) cost-sharing of IDC with the Center for Drug Design (CDD) was indicated in question 17.
> >
> > Dr. Li has now received grant award 1 R01 AI089728-01 for this application. Please set up the award in the Department of Pharmacology, but have 11.5% of the IDC (% that R. Geraghty and W. Zhou's compensation is of total award) directed to the Center for Drug Design.
> >
> > If you have questions on what CDD chart string to use for the IDC sharing, contact Elizabeth Wolfson (624-2227) in the CDD.
> >
> > Jim Burak
> > MNPI Center &
> > Department of Pharmacology
> > University of Minnesota
> > 6-120 Jackson Hall
> > 321 Church Street SE
> > Minneapolis, MN 55455
> > 612 625-8660

Subject: L1 - ICR sharing
From: Lorrie Awoyinka <lorrie@umn edu>
Date: Thu, 20 May 2010 13:48 14 -0500
To: James L Burak <burak001@umn edu>

I talked to Ed and he said that before I can assign 100% of the ICR to Pharmacology, you need to get me an e-mail or something in writing from the Center for Drug Design that indicates they agree all the ICR received under the award will be given to Pharmacology.

--
Lorrie Awoyinka
Principal Grant & Contract Administrator
University of Minnesota
Sponsored Projects Administration
450 McNamara Alumni Center
200 Oak St. SE
Minneapolis, MN 55455

Phone: (612) 626-2265
Fax: (612) 624-4843
E-mail: lorrie@umn.edu

Subject: Re No Co-PI on Grant 1 R01 AI089728
From: Fang Li <lifang@umn edu>
Date: Fri, 21 May 2010 15.13.42 -0500
To: Jim Burak <burak001@umn edu>
CC: wallcy@niaid nih gov, Lorrie Awoyinka <lorrie@umn edu>

I confirm that Dr. Robert Geraghty is a Co-Investigator on the grant

On Fri, May 21, 2010 at 9 18 AM, Jim Burak <burak001@umn edu> wrote

> We recently received a NOGA for grant 1 R01 AI089728-01 for Dr. Fang Li, that started 05/15/10. On page 4 of the NOGA, Dr. Robert Geraghty is listed as a Co-PI. The grant application was submitted listing Dr. Geraghty as a Co-Investigator, not a Co-Principal Investigator. His role on the project will be that of a Co-Investigator.
>
> I am not sure if you need to issue a revised NOGA for this correction, but I thought I would call it to your attention. Please let me know if you have any questions regarding this.
> --
> Jim Burak
> MNPI Center &
> Department of Pharmacology
> University of Minnesota
> 6-120 Jackson Hall
> 321 Church Street SE
> Minneapolis, MN 55455
> 612 625-8660

--
"How I wish to be a happy tree,
smiling in the wind of sunny May "
****
Fang Li, Ph.D.
Assistant Professor, Pharmacology
University of Minnesota Twin Cities
612-625-6149, lifang@umn edu
http //www msi umn.edu/~lifang
****

Subject: No Co-PI on Grant 1 R01 AI089728
From: Jim Burak <burak001@umn edu>
Date: Fri, 21 May 2010 09.18:53 -0500
To: <wallcy@niaid.nih gov>
CC: Lorrie Awoyinka <lorrie@umn edu>, Fang Li <lifang@umn edu>

We recently received a NOGA for grant 1 R01 AI089728-01 for Dr. Fang Li, that started 05/15/10. On page 4 of the NOGA, Dr. Robert Geraghty is listed as a Co-PI. The grant application was submitted listing Dr. Geraghty as a Co-Investigator, not a Co-Principal Investigator. His role on the project will be that of a Co-Investigator.

I am not sure if you need to issue a revised NOGA for this correction, but I thought I would call it to your attention. Please let me know if you have any questions regarding this.

--

Jim Burak
MNPI Center &
Department of Pharmacology
University of Minnesota
6-120 Jackson Hall
321 Church Street SE
Minneapolis, MN 55455
612 625-8660

UNIVERSITY OF MINNESOTA

ReportID: UMFGM017F

PROPOSAL SUMMARY

Run Date 05/17/2010
Run Time 04:06:27 PM

Status **4 AwdB Award Sent to GA**

Grant Administrator	Awoyinka,Lorraine L		
PRF #	651709	Proposal #/Version	CON000000022345/A101
EGMS Proposal #		Sponsor Award #	1R01-AI-089728-01
Proposal Type	New	Concurrent	N
Previous Award		SubContract	N
Purpose			
Title	Receptor recognition mechanisms of coronaviruses		
Principal Investigator	Li,Fang	lifang@umn edu	

Budget Summary

Start Date	07/01/2010	End Date	06/30/2015	
Direct Costs	$1,250,000 00	Indirect Costs	$637,500 00	Total Costs $1,887,500 00

Sponsor: National Institutes of Health (NIH)

Submission Information

Due Date	10/05/2009	Date Type	Receipt Date
Submission Method	Electronically Only	# of Copies Submitted	_____

Mailing Address

Comments/Corrections

REPA	__verified	__email sent for _____		
IRB	__verified	__pending	__email sent	__Not Applicable
IACUC	__verified	__pending	__email sent	__Not Applicable
IBC	__verified	__pending	__email sent	__Not Applicable
F&A rate	__at full or agency limit	__waiver on file	__waiver requested	
Cost share documented?		__yes	__no	__Not Applicable
ICR sharing?		__yes	__no	

_____
_____
_____
_____

CON-22345
651709
LLA
RCR-Met

Subject: NoA 1 R01 AI089728-01 PI Li, Fang
From: era-notify@mail nih gov
Date: Fri, 14 May 2010 00 03 51 -0400 (EDT)
To: awards@umn edu

Grant Number 1 R01 AI089728-01
Principal Investigator Li, Fang
Project Title Receptor recognition mechanisms of coronaviruses
Institution UNIVERSITY OF MINNESOTA
CFDA 93 855
OC 414A
PCC M51C S
Award Issue Date 05/14/2010
Grants Management Officer Mitchum, Dawn
Program Official Cassels, Frederick
Grants Specialist Wall, Cheryl

*** This is an automated notification - Please do not reply to this message ***

temp48548.pdf	**Content-Type:**	application/octet-stream
	Content-Encoding:	base64

* * * COMMUNICATION RESULT REPORT (APR. 9. 2010 9:54AM) * * *

TTI SPONSORED PROJECTS ADMIN

TRANSMITTED/STORED APR. 9 2010 9:53AM

FILE MODE	OPTION	ADDRESS	RESULT	PAGE
0118 MEMORY TX		G3-AT:83014930597	OK	3/3

files attach to proposal

Li, NIH

CON-22345, 651709

REASON FOR ERROR
E-1) HANG UP OR LINE FAIL
E-2) BUSY
E-3) NO ANSWER
E-4) NO FACSIMILE CONNECTION

UNIVERSITY OF MINNESOTA

Sponsored Projects Administration

University Gateway
200 Oak St. SE, Suite 450
Minneapolis, MN 55443-2070
Phone: 612/624-0327
Fax: 612/624-4843

FAX

Date: April 9, 2010

To: Cheryl Wall
Fax Number: 301-493-0597
Agency/Organization: NIAID
Telephone: 301-451-2696

FROM: Lorrie Awoyinka
Fax Number: 612-624-4843
Telephone Number: 612-626-2265

Number of Pages 3 (Including Cover)

☐ Please Comment

Ms Wall,

As requested in your April 7th e-mail, following you will find the Current Other Support information for key personnel under Dr. [redacted]s pending gra[redacted].

Administrative endorsement for these materials is provided below:

Edward F. Wink
Associate Director
Sponsored Projects Administration

If you have questions or need any additional information, please let me know.

Thanks!

Lorrie Awoyinka

CONFIDENTIALITY NOTE: The documents accompanying this telecopy transmission contain information belonging to the University of Minnesota and are intended only for the use of the addressee. If you are not the intended recipient, you are hereby notified that any disclosure, copying, distribution or the taking of any action in reliance on the contents of this telecopied information is strictly prohibited. If you have received this telecopy in error, please immediately notify us by telephone to arrange for the return of the original documents.

If you do not receive all pages, please call the number provided above.

UNIVERSITY OF MINNESOTA

Sponsored Projects Administration

University Gateway
200 Oak St. SE, Suite 450
Minneapolis, MN 55443-2070
Phone: 612/624-0327
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FAX

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Telephone: 301-451-2696

FROM: Lorrie Awoyinka
Fax Number: 612-624-4843
Telephone Number: 612-626-2265

Number of Pages. 3 (Including Cover)

☐ Please Comment

Ms. Wall,

As requested in your April 7th e-mail, following you will find the Current Other Support information for key personnel under Dr. Fang Li's pending grant 1R01-AI089728-01.

Administrative endorsement for these materials is provided below:

Edward F. Wink
Associate Director
Sponsored Projects Administration

If you have questions or need any additional information, please let me know.

Thanks!

Lorrie Awoyinka

CONFIDENTIALITY NOTE: The documents accompanying this telecopy transmission contain information belonging to the University of Minnesota and are intended only for the use of the addressee. If you are not the intended recipient, you are hereby notified that any disclosure, copying, distribution or the taking of any action in reliance on the contents of this telecopied information is strictly prohibited. If you have received this telecopy in error, please immediately notify us by telephone to arrange for the return of the original documents.

If you do not receive all pages, please call the number provided above.

For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED
For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel

PHS 398/2590 OTHER SUPPORT

Provide active support for all key personnel **Other Support includes all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors, including but not limited to research grants, cooperative agreements, contracts, and/or institutional awards.** Training awards, prizes, or gifts do not need to be included

There is no "form page" for other support Information on other support should be provided in the *format* shown below, using continuation pages as necessary ***Include the principal investigator's name at the top and number consecutively with the rest of the application.*** The sample below is intended to provide guidance regarding the type and extent of information requested
For instructions and information pertaining to the use of and policy for other support, see Other Support in the PHS 398 Part III, Policies, Assurances, Definitions, and Other Information
Note effort devoted to projects must now be measured using person months Indicate calendar, academic, and/or summer months associated with each project.

FANG LI

Active

FRD 08-07 01/01/09 – 12/31/11 1.2 cal.
AHC Faculty Research Development Grant $50,000 Annual Direct Costs (no PI salary support)
University of Minnesota
"Receptor recognition and cross-species infections by human coronaviruses"
Role: Principal Investigator
Major Goal: The major goals of this project are to (1) identify and characterize the receptor-binding domain (RBD) of the NL63 coronavirus (NL63-CoV) spike protein; (2) crystallize and determine the structure of NL63-CoV RBD in complex with its human receptor angiotensin-converting enzyme 2 (ACE2); (3) crystallize and determine the structure of the SARS coronavirus (SARS-CoV) RBD complexed with animal ACE2.

Overlap: There is conceptual overlap, but no experimental overlap, between this grant and the pending NIH R01 grant (1R01AI089728 – 01).

Pending

Dana Farber Cancer Institute (NIH P01 Prime - PI: Marasco) 12/01/11 – 11/30/16
"Global Health and the Threat of Emerging Human Coronavirus Pathogens"
Subcontract – Project 3 12/01/11 – 11/30/16 3.0 cal.
"Structural studies of bat coronavirus spike proteins and the interactions with host receptors"
$124,936 Annual Direct Costs
Role: Subcontract Principal Investigator - Project 3
Major Goal: To investigate the structures and functions of bat coronavirus spike proteins, and their interactions with host receptors.

Overlap: There is no overlap between this grant and the pending NIH R01 grant (1R01AI089728 – 01).

Grant-in-Aid of Research 07/01/10 – 01/15/2012 **0.5** cal
University of Minnesota $19,304 Annual Direct Costs (no PI salary support)
"Structural studies of group-III coronavirus spike protein"
Role: Principal Investigator
Major Goal: To explore the crystal structures of the S1 subunits of group-III avian coronavirus spike proteins.

Overlap: There is no overlap between this grant and the pending NIH R01 grant (1R01AI089728 – 01).

For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED
For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel

PHS 398/2590 OTHER SUPPORT

Provide active support for all key personnel **Other Support includes all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors, including but not limited to research grants, cooperative agreements, contracts, and/or institutional awards.** Training awards, prizes, or gifts do not need to be included

There is no "form page" for other support Information on other support should be provided in the *format* shown below, using continuation pages as necessary ***Include the principal investigator's name at the top and number consecutively with the rest of the application.*** The sample below is intended to provide guidance regarding the type and extent of information requested
For instructions and information pertaining to the use of and policy for other support, see Other Support in the PHS 398 Part III, Policies, Assurances, Definitions, and Other Information
Note effort devoted to projects must now be measured using person months Indicate calendar, academic, and/or summer months associated with each project.

ROBERT J. GERAGHTY

Ongoing

R03 AI078079 5/15/09 – 5/14/11 1.2 calendar
NIH/NIAID $50,000
"Membrane fusion during HSV-1 entry"
Role: Principal Investigator
The major goal of this proposal is to elucidate molecular events involved in HSV-1 membrane fusion and hemifusion occurring during receptor-mediated endocytosis.

Subject. FW [Fwd. [Fwd. Grant Number 1R01AI089728 - 01 PI Name Li , Fang]]
From Lynn Weber <weber117@umn.edu>
Date Thu, 08 Apr 2010 16 00 24 -0500
To Lorrie Awoyinka <lorrie@umn.edu>
CC Tanya Walton <twalton@umn.edu>, Sowolo Abdullai <abdu0092@umn.edu>

Here is the Other Support information for Fang Li and Robert Geraghty as requested for Fang Li's 1R01AI089728 NIH grant application

--

Lynn Weber
Department of Pharmacology
University of Minnesota
Medical School
6-120 Jackson Hall
321 Church Street SE
Minneapolis, MN 55455
Phone 612-626-6902
Fax 612-625-8408
Email weber117@umn edu
http //www pharmacology med umn edu/

------ Forwarded Message
From Jim Burak <burak001@umn edu>
Date Wed, 07 Apr 2010 11 57 02 -0500
To Debi Marsh <mars0061@umn edu>, Lynn Weber <weber117@umn edu>
Conversation [Fwd [Fwd Grant Number 1R01AI089728 - 01 PI Name Li , Fang]]
Subject FW [Fwd [Fwd Grant Number 1R01AI089728 - 01 PI Name Li , Fang]]

You may have already received this from Fang Li
--
Jim Burak
MNPI Center &
Department of Pharmacology
University of Minnesota
6-120 Jackson Hall
321 Church Street SE
Minneapolis, MN 55455
612 625-8660

------ Forwarded Message
From: Lorrie Awoyinka <lorrie@umn edu>
Organization: University of Minnesota
Date: Wed, 07 Apr 2010 11 53 32 -0500
To: Carrie DeJarlais <dejar001@umn edu>, Jim Burak <burak001@umn edu>
Subject: [Fwd [Fwd Grant Number 1R01AI089728 - 01 PI Name Li , Fang]]

FYI and attention

-------- Original Message --------
Subject [Fwd Grant Number 1R01AI089728 - 01 PI Name Li , Fang]
Date Wed, 07 Apr 2010 11 24 19 -0500
From Sponsored Projects Award Monitoring <spamon@umn edu> <mailto spamon@umn edu>
To Lorrie Awoyinka <lorrie@umn edu> <mailto lorrie@umn edu> , Sowolo Abdullai <abdu0092@umn edu> <mailto abdu0092@umn edu> , tanya Walton <twalton@umn edu> <mailto twalton@umn edu>

-------- Original Message --------
Subject Grant Number 1R01AI089728 - 01 PI Name Li , Fang
Date Wed, 7 Apr 2010 11 01 01 -0400
From Wall, Cheryl (NIH/NIAID) [E] <wallcy@niaid.nih gov> <mailto wallcy@niaid nih gov>
To 'lifang@umn edu' <lifang@umn edu> <mailto lifang@umn edu> , 'awards@umn edu' <awards@umn edu> <mailto awards@umn edu>
CC Wall, Cheryl (NIH/NIAID) [E] <wallcy@niaid.nih gov> <mailto wallcy@niaid nih gov>

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Healt
National Institute of Allerg
and Infectious Diseases
Bethesda, Maryland 20892

Good Morning,

RE 1R01AI089728 - 01

For applications well received by study section during peer review, we attempt to obtain documentation that must be submitted to the National Institute of Allergy and Infectious Diseases should an application subsequently be identified for funding Since your application is among those favorably received, we request that you submit the information checked below

[redacted] Updated Other Support for ALL key personnel - Use Other Support Format Page - PHS 398 rev 05/01 ***Please identify percentage of effort on both awarded and pending applications.***
http //grants nih gov/grants/funding/phs398/phs398 html#forms <http //grants nih gov/grants/funding/phs398/phs398 html#forms%20>

_____ * Human Subjects Assurance number Information regarding the Federal Wide Assurance can be found at the following website
http //ohrp osophs dhhs gov/irbasur htm
< /Local%20Settings/Temporary%20Internet%20Files/Local%20Settings/Temporary%20Internet%20Files/Content Outlook/JLYMW26N/%20http /ohrp osophs dhhs gov/irbasu

_____ Grant Specific IRB approval letter with date For additional information please see the following link http //www hhs gov/ohrp/humansubjects/guidance/aplrev htm
<http //www hhs gov/ohrp/humansubjects/guidance/aplrev htm>

_____ * Documentation of the Required Education in the Protection of Human Subject Research Participants for all personnel involved.

_____ * IACUC verification statement/letter with approval date.

_____ Completed Checklist http //grants nih gov/grants/funding/phs398/phs398 html#forms <http //grants nih gov/grants/funding/phs398/phs398 html#forms%20>

_____ Categorical Budget for the Initial Budget Period - Please use PHS Form 398, page 4

_____ Composite Budget for the Entire Period of Support - Please use PHS Form 398, page 5

_____ Other (i e , Current Negotiated F & A Rate, IRG concerns found on the Summary Statement)

May be submitted using the Just-In-Time feature of the NIH Commons found in the Commons **Status section If using the Commons, ALL information must currently be submitted at one time For information on the Commons see https //commons era nih gov/commons/index jsp*

If not submitting via the Commons **and** for information unable to be submitted via the Commons[redacted] **countersigned by an authorized institutional business official by 4/13/2010.** My FAX number is (301) 493-0597 Timely submission of the above, checked, information will enable us to expedite the issuance of an award should an application be identified for funding

Sincerely,
Cheryl Wall
Cheryl Wall
Grants Management Specialist
National Institute of Allergy and Infectious Diseases, NIH
6700B Rockledge Drive, Room 2231, MSC 7614
Bethesda, MD 20892-7614
(Express Mail Bethesda, MD 20817)
Phone 301-451-2696
Fax 301-493-0597
Email wallcy@niaid nih gov <mailto wallcy@niaid nih gov>

The information in this e-mail and any of its attachments is confidential and may contain sensitive information It should not be used by anyone who is not the original intended recipient If you have received this e-mail in error please inform the sender and delete it from your mailbox or any other storage devices National Institute of Allergy and Infectious Diseases shall not accept liability for any statements made that are sender's own and not expressly made on behalf of the NIAID by one of its representatives

Before printing this e-mail please consider if it is necessary to do so The environment concerns us all

--
Lorrie Awoyinka
Principal Grant & Contract Administrator
University of Minnesota
Sponsored Projects Administration
450 McNamara Alumni Center
200 Oak St SE
Minneapolis, MN 55455

Phone (612) 626-2265
Fax (612) 624-4843
E-mail lorrie@umn edu

------ End of Forwarded Message

—— End of Forwarded Message

Fang Li Other Support 4-8-10 doc	**Content-Type**	application/msword
	Content-Encoding	base64
Geraghty Other Support 4-8-10 doc	**Content-Type**	application/msword
	Content-Encoding	base64

file
attach to
proposal
CON-22345
F. Li /NIH

Subject: Supplemental materials for VIRA study section review for 1R01AI089728-01
From: David Hagen <hagen027@umn edu>
Date: Tue, 12 Jan 2010 12·48·26 -0600
To: "Dr Joanna Pyper" <pyperj@csr nih gov>
CC: lifang@umn edu, Lorrie Awoyinka <lorrie@umn edu>, Ed Wink <ewink@umn edu>

Dr Pyper,

Administrative endorsement is being provided for the attached supplemental materials from Dr. Li Fang for his pending grant application 1R01-AI089728-01 These materials have been administratively reviewed and endorsed on behalf of the Regents of the University of Minnesota Questions regarding the programmatic aspect of this application should be directed to Dr Li Any other questions should be directed to Lorrie Awoyinka of my staff at (612) 626-2265 or via e-mail at lorrie@umn edu

651709

Sincerely yours,

David Hagen

--
David Hagen
Associate Director
Office of Sponsored Projects Administration
University of Minnesota
(612) 626-9895

-------- Original Message --------
Subject: [Fwd Supplemental materials for VIRA study section review for 1R01AI089728-01]
Date: Thu, 07 Jan 2010 14 26 43 -0600
From: spamon <spamon@umn edu>
Organization: University of Minnesota
To: Lorrie L Awoyinka-1 <lorrie@umn edu>

-------- Original Message --------
Subject: Supplemental materials for VIRA study section review for 1R01AI089728-01
Date: Thu, 7 Jan 2010 12.54 32 -0600
From: Fang Li <lifang@umn edu>
To: Pyper, Joanna (NIH/CSR) [E] <pyperj@csr nih gov>
CC: AWARDS@umn edu

Dear Dr Pyper,

Please find attached the supplemental materials I would like to submit for my NIH proposal 1R01AI089728-01, entitled "receptor recognition mechanisms of coronaviruses" We believe that the supplemental materials will strengthen our application by establishing new research methods and by supporting hypotheses Thank you for considering our request

I have cc-ed this message to U of Minnesota's grant signing official

Best,
Fang Li

On Fri, Dec 4, 2009 at 9 03 AM, Pyper, Joanna (NIH/CSR) [E] <pyperj@csr nih gov> wrote

DATE: December 4, 2009

FROM: Joanna Pyper, Ph D

Scientific Review Officer

Virology A Study Section

TO: Applicants to be reviewed at the February meeting of VIRA

SUBJECT: Important Information about the Review of Your Application

Please read this email completely because it contains some important and new information regarding the review of your application.

TOPICS IN THIS E-MAIL:

DEADLINE FOR ALL SUPPLEMENTS/CORRECTIONS (30 DAYS BEFORE THE MEETING)

Appendix requirements

Dates of meeting, score release, summary statement release

Enhancing Peer Review

Vertebrate animals and human subjects

New Investigators and Early Stage Investigators

Confidentiality of review process

Your grant application has been assigned to the **Virology A (VIRA) Study Section** for review The study section will meet to review the submitted applications on February 11, 2010 Between now and the time of review I am your NIH contact for your application If you have any questions you are welcome to contact me

The purpose of this e-mail is to provide you with some important and new information concerning the review process Please note the new guidelines on submission of additional grant application material—see below and the NIH Best Practice Guidelines for details **The Principal Investigator may submit corrections and/or supplemental material only with the concurrence of the Authorized Organizational Representative and the additional material will only be accepted prior to 30 calendar days before the study section meeting. The last day I will accept materials is January 12th.**

Please be sure that you have checked the electronic form of your application that is in your Grant Folder. I cannot accept any corrections after January 12th.

Additional information about the VIRA study section is available via the Web From the CSR home page (http //www csr nih gov/), choose "Study Section Information", then choose "Study Section Rosters " Choose **CSR Regular Standing Study Section Rosters**, from there you can reach the VIRA information links You can see the topics we review (guidelines) and the rosters, both the standing members and the panels from the last 3 meetings Please remember that not all members will attend the meeting and that this roster does not include the temporary members that will be recruited for the meeting The final roster for this meeting will be posted some time in the beginning of January

ENHANCED PEER REVIEW: NIH has implemented enhancements to peer review (http://enhancing-peer-review.nih.gov/) Reviewers will score applications on a scale of 1 to 9 (using integers only), scores will be averaged and multiplied by 10, so the final impact (rather than priority) scores will range from 10 (the best possible score) to 99 Applications judged to be less meritorious (roughly the lower half) will not be discussed at the meeting However, for all applications criteria scores (1 to 9) will be provided by the assigned reviewers for significance, investigators, innovation, approach, and environment These scores will be reported in summary statements A bulleted format designed to point out the strengths and weaknesses will be used to assess each criterion The expectation is that summary statements will be shorter, clearer, and more focused

VERTEBRATE ANIMALS RESEARCH: If you are using vertebrate animals or having someone else conduct animal experiments for you, please check to see if you have addressed the **5 points** as described in the instructions for the SF424 form Please note that when antibodies are made, and this is true even if you contract with a company to have them made, it is considered use of vertebrate animals

HUMAN SUBJECTS RESEARCH: Applicants must address whether human subjects research is involved in the application as outlined in the instructions on Human Subjects Research The definition of human subjects research has been recently clarified Under certain conditions, research involving unidentified coded private information or specimens is no longer considered human subjects research This affects human subject research previously designated exemption #4 Please check to see if your research is now designated as "no" human subjects research and what is required in section 8 of your application If doing human subjects research, you must address protection from research risk (**4 evaluation criteria**) and plans for **inclusion of women, minorities, and children** or justification where representation is limited or absent (Sections 9-11)

If you discover that you are missing information regarding use of human subjects or vertebrate animals, please send me the corrected information

APPENDIX MATERIAL: Published manuscripts that are publicly available in an online format may be referenced in the application and may not be included in the appendix URLs or NIH PubMed submission identification numbers may be included with the full reference in the References Cited Section, Progress Report Publication List, and/or the Biographical Sketch section Graphic images, micrographs, and/or photographs may be included in the Research Plan, but may not be included in the Appendix Applicants may submit up to 3 accepted manuscripts that are not yet published or published manuscripts that are not available in a publicly available free online journal link Surveys, questionnaires, clinical protocols and informed consent documents may be submitted in the Appendix as necessary

Do not submit any more than 3 published (not publicly available) or accepted manuscripts and do not include or submit any manuscripts that have not been accepted for publication

If you have included more than 3 appendix items, please let me know which 3 you wish the reviewers to consider If you do not inform me, I will instruct the reviewers to consider only the first 3 listed I will also instruct them not to consider any manuscripts included in the appendices that have not been accepted for publication

ADDITIONAL / SUPPLEMENTAL INFORMATION: NIH allows applicants to submit materials for peer review that are an addition to the original application The opportunity to submit additional materials should not be a means of circumventing submission deadlines, page limitations, or content requirements Additional materials should not be a means of substantially enhancing, altering, or adding to the specific aims, research plan, or overall strategy of the application

Additional materials will be accepted from the Principal Investigator only with the concurrence of the Authorized Organizational Representative (signing official) The best way to accomplish this is either 1) the PI sends the electronic materials to the institutional official who can then forward the email to the SRO or 2) the Principal Investigator transmits

the material to the SRO with cc to the signing official

Acceptable additional material include corrections to the application (including information about the use of animals and human subject research, missing letters of collaboration, revised budget pages, and missing biosketches), update of publications (indicating that a submitted manuscript has been accepted for publication) and supplemental data

You may submit supplementary data to be considered along with your application Limit submissions of new preliminary data to two pages and less than 25 Mb (only 1 page for applications with research plans of 12 pages or less) , including figures and tables, and **only one submission per application between now and the submission deadline** Please report only significant findings (e g , demonstration of a new technique), don't report five new data points that confirm the 20 already in the application.

If you are concerned that productivity is (or was, if your application is a resubmission) an issue, you may send a brief update to inform reviewers of your progress (i e , new *in press* manuscripts). Do not send new appendix material (accepted manuscripts) if you have already reached the limit (3) *Do not send manuscripts that have not been accepted yet*

To send additional material, send it as an email attachment of a PDF file or MS word document **You must cc your organization's signing official or it must be submitted through that office. Please label the files with your name and application number** *I will upload your email attachments to the Additions for Review in your Grant Folder* You can check that I have received it and the reviewers can see it if it is in your Grant Folder

I will accept additional material up to 30 calendar days before the meeting; that is up to and including Tuesday, January 12, 2009.

NEW INVESTIGATOR / EARLY STAGE INVESTIGATOR STATUS: The definition of a new investigator is one who has not previously competed successfully for a PHS-supported independent research award other than an R03, R15, or R21 or a beginning research career award (K01, K08, K22, and K23) It is important both in the review of applications and in the awarding of funding that this designation be correct Please let me know if there is an error in the listing of your status Early Stage Investigator status applies to New Investigators who have less than 10 years of research or research training experience after the completion of their terminal research degree or medical residency Eligibility is calculated by eRA Commons based on the investigator's entry of the date of their terminal research degree (http //grants1.nih gov/grants/guide/notice-files/not-od-09-034 html)

WHEN WILL SCORES / SUMMARY STATEMENTS BE AVAILABLE? Scores will be released to the NIH computer within three working days after the meeting NIH policy states that I cannot give out your score, please do not call me for this information Summary statements will be available within 30 days after the meeting The new NIH policy states that summary statements of new investigators will be prepared first followed by all other applications in priority score order - best score first If you have access to the NIH Commons system, you will be able to see your score / summary statement immediately after release Please refer to the URL for more information. https.//commons era nih gov/commons/ If necessary you may contact your Institute program representative for score/summary statement information and advice after the meeting

CONFIDENTIALITY OF THE REVIEW PROCESS: It is inappropriate for you, or anyone on your behalf, to contact a study section member (or potential member) regarding the review of your grant application either before or after the meeting of the study section Reviewers are instructed not to discuss *anything* about the review with anyone not at the study section meeting Questions and comments should be addressed to the SRO prior to the meeting and the Program Official after the meeting

If you have any other questions please call or e-mail me

Supplemental materials for VIRA study section review for 1R01AI08 ..

Jody

Joanna M Pyper, Ph D

Deputy Chief, Infectious Diseases and Microbiology IRG

Scientific Review Officer, Virology A (VIRA)

6701 Rockledge Drive, Room 3208, MSC 7808

Bethesda, MD 20892

(For delivery services, use 20817)

Telephone 301-435-1151

FAX 301-480-0940

e-mail pyperj@csr nih gov

--

"How I wish to be a happy tree,
smiling in the wind of sunny May "

****

Fang Li, Ph D
Assistant Professor, Pharmacology
University of Minnesota Twin Cities
612-625-6149, lifang@umn.edu
http //www msi umn edu/~lifang

****

Fang_Li_R01_supplements_010910.pdf **Content-Type:** application/pdf
Content-Encoding: base64

CON - 22345
PRF 651709

Subject: [Fwd [Fwd Supplemental materials for VIRA study section review for 1R01AI089728-01]]
From: Lorrie Awoyinka <lorrie@umn edu>
Date: Tue, 12 Jan 2010 11 31 05 -0600
To: david hagen <hagen027@umn edu>

David,

I have reviewed and recommend submission of the attached additional information Dr Fang Li's is submitting for his pending NIH grant 1R01-AI089728-01 If you concur, please provided administrative endorsement and forward these materials to Dr Joanna Pyper at pyperj@csr nih gov

Copies should be sent to

Dr Li at lifang@umn edu
Lorrie at lorrie@umn edu

Thanks,

Lorrie

==========

Dr Pyper,

Administrative endorsement is being provided for the attached supplemental materials from Dr Li Fang for his pending grant application 1R01-AI089728-01 These materials have been administratively reviewed and endorsed on behalf of the Regents of the University of Minnesota Questions regarding the programmatic aspect of this application should be directed to Dr Li Any other questions should be directed to Lorrie Awoyinka of my staff at (612) 626-2265 or via e-mail at lorrie@umn edu

Sincerely yours,

David Hagen
Associate Director

-------- Original Message --------
Subject:[Fwd Supplemental materials for VIRA study section review for 1R01AI089728-01]
Date: Thu, 07 Jan 2010 14.26.43 -0600
From:spamon <spamon@umn edu>
Organization: University of Minnesota
To: Lorrie L Awoyinka-1 <lorrie@umn edu>

-------- Original Message --------
Subject: Supplemental materials for VIRA study section review for 1R01AI089728-01
Date: Thu, 7 Jan 2010 12.54 32 -0600
From: Fang Li <lifang@umn edu>
To: Pyper, Joanna (NIH/CSR) [E] <pyperj@csr nih gov>
CC:AWARDS@umn.edu

Dear Dr Pyper,

Please find attached the supplemental materials I would like to submit for my NIH proposal 1R01AI089728-01, entitled "receptor recognition mechanisms of coronaviruses". We believe that the supplemental materials will strengthen our application by establishing new research methods and by supporting hypotheses Thank you for considering our request

[redacted]

Best,
Fang Li

On Fri, Dec 4, 2009 at 9 03 AM, Pyper, Joanna (NIH/CSR) [E] <pyperj@csr nih gov> wrote.

DATE: December 4, 2009

FROM: Joanna Pyper, Ph D

Scientific Review Officer

Virology A Study Section

TO: Applicants to be reviewed at the February meeting of VIRA

SUBJECT: Important Information about the Review of Your Application

Please read this email completely because it contains some important and new information regarding the review of your application.

TOPICS IN THIS E-MAIL:

-

DEADLINE FOR ALL SUPPLEMENTS/CORRECTIONS (30 DAYS BEFORE THE MEETING)

Appendix requirements

Dates of meeting, score release, summary statement release

Enhancing Peer Review

Vertebrate animals and human subjects

New Investigators and Early Stage Investigators

Confidentiality of review process

Your grant application has been assigned to the **Virology A (VIRA) Study Section** for review The study section will meet to review the submitted applications on February 11, 2010 Between now and the time of review I am your NIH contact for your application If you have any questions you are welcome to contact me

The purpose of this e-mail is to provide you with some important and new information concerning the review process Please note the new guidelines on submission of additional grant application material—see below and the NIH Best Practice Guidelines for details **The Principal Investigator may submit corrections and/or supplemental material only with the concurrence of the Authorized Organizational Representative and the additional material will only be accepted prior to 30 calendar days before the study section meeting. The last day I will accept materials is January 12th.**

Please be sure that you have checked the electronic form of your application that is in your Grant Folder. I cannot accept any corrections after January 12th.

Additional information about the VIRA study section is available via the Web From the CSR home page (http //www csr nih gov/), choose "Study Section Information", then choose "Study Section Rosters " Choose CSR Regular Standing Study Section Rosters, from there you can reach the VIRA information links You can see the topics we review (guidelines) and the rosters, both the standing

members and the panels from the last 3 meetings. Please remember that not all members will attend the meeting and that this roster does not include the temporary members that will be recruited for the meeting The final roster for this meeting will be posted some time in the beginning of January

ENHANCED PEER REVIEW: NIH has implemented enhancements to peer review (http://enhancing-peer-review nih gov/) Reviewers will score applications on a scale of 1 to 9 (using integers only), scores will be averaged and multiplied by 10, so the final impact (rather than priority) scores will range from 10 (the best possible score) to 99 Applications judged to be less meritorious (roughly the lower half) will not be discussed at the meeting However, for all applications criteria scores (1 to 9) will be provided by the assigned reviewers for significance, investigators, innovation, approach, and environment These scores will be reported in summary statements A bulleted format designed to point out the strengths and weaknesses will be used to assess each criterion The expectation is that summary statements will be shorter, clearer, and more focused

VERTEBRATE ANIMALS RESEARCH: If you are using vertebrate animals or having someone else conduct animal experiments for you, please check to see if you have addressed the **5 points** as described in the instructions for the SF424 form Please note that when antibodies are made, and this is true even if you contract with a company to have them made, it is considered use of vertebrate animals

HUMAN SUBJECTS RESEARCH: Applicants must address whether human subjects research is involved in the application as outlined in the instructions on Human Subjects Research The definition of human subjects research has been recently clarified Under certain conditions, research involving unidentified coded private information or specimens is no longer considered human subjects research This affects human subject research previously designated exemption #4 Please check to see if your research is now designated as "no" human subjects research and what is required in section 8 of your application If doing human subjects research, you must address protection from research risk (**4 evaluation criteria**) and plans for **inclusion of women, minorities, and children** or justification where representation is limited or absent (Sections 9-11)

If you discover that you are missing information regarding use of human subjects or vertebrate animals, please send me the corrected information

-

APPENDIX MATERIAL: Published manuscripts that are publicly available in an online format may be referenced in the application and may not be included in the appendix URLs or NIH PubMed submission identification numbers may be included with the full reference in the References Cited Section, Progress Report Publication List, and/or the Biographical Sketch section Graphic images, micrographs, and/or photographs may be included in the Research Plan, but may not be included in the Appendix Applicants may submit up to 3 accepted manuscripts that are not yet published or published manuscripts that are not available in a publicly available free online journal link Surveys, questionnaires, clinical protocols and informed consent documents may be submitted in the Appendix as necessary

Do not submit any more than 3 published (not publicly available) or accepted manuscripts and do not include or submit any manuscripts that have not been accepted for publication.

If you have included more than 3 appendix items, please let me know which 3 you wish the reviewers to consider If you do not inform me, I will instruct the reviewers to consider only the first 3 listed I will also instruct them not to consider any manuscripts included in the appendices that have not been accepted for publication

-

ADDITIONAL / SUPPLEMENTAL INFORMATION: NIH allows applicants to submit materials for peer review that are an addition to the original application The opportunity to submit additional materials should not be a means of circumventing submission deadlines, page limitations, or content requirements Additional materials should not be a means of substantially enhancing, altering, or adding to the specific aims, research plan, or overall strategy of the application

Additional materials will be accepted from the Principal Investigator only with the concurrence of the Authorized Organizational Representative (signing official) The best way to accomplish this is either 1) the PI sends the electronic materials to the institutional

official who can then forward the email to the SRO or 2) the Principal Investigator transmits the material to the SRO with cc to the signing official

Acceptable additional material include corrections to the application (including information about the use of animals and human subject research, missing letters of collaboration, revised budget pages, and missing biosketches), update of publications (indicating that a submitted manuscript has been accepted for publication) and supplemental data

You may submit supplementary data to be considered along with your application Limit submissions of new preliminary data to two pages and less than 25 Mb (only 1 page for applications with research plans of 12 pages or less), including figures and tables, and **only one submission per application between now and the submission deadline** Please report only significant findings (e g , demonstration of a new technique); don't report five new data points that confirm the 20 already in the application.

If you are concerned that productivity is (or was, if your application is a resubmission) an issue, you may send a brief update to inform reviewers of your progress (i e , new *in press* manuscripts) Do not send new appendix material (accepted manuscripts) if you have already reached the limit (3) *Do not send manuscripts that have not been accepted yet*

To send additional material, send it as an email attachment of a PDF file or MS word document **You must cc your organization's signing official or it must be submitted through that office. Please label the files with your name and application number** *I will upload your email attachments to the Additions for Review in your Grant Folder* You can check that I have received it and the reviewers can see it if it is in your Grant Folder

I will accept additional material up to 30 calendar days before the meeting; that is up to and including Tuesday, January 12, 2009.

NEW INVESTIGATOR / EARLY STAGE INVESTIGATOR STATUS: The definition of a new investigator is one who has not previously competed successfully for a PHS-supported independent research award other than an R03, R15, or R21 or a beginning research career award (K01, K08, K22, and K23) It is important both in the review of applications and in the awarding of funding that this designation be correct Please let me know if there is an error in the listing of your status Early Stage Investigator status applies to New Investigators who have less than 10 years of research or research training experience after the completion of their terminal research degree or medical residency. Eligibility is calculated by eRA Commons based on the investigator's entry of the date of their terminal research degree (http //grants1 nih gov/grants/guide/notice-files/not-od-09-034 html)

WHEN WILL SCORES / SUMMARY STATEMENTS BE AVAILABLE? Scores will be released to the NIH computer within three working days after the meeting NIH policy states that I cannot give out your score, please do not call me for this information Summary statements will be available within 30 days after the meeting The new NIH policy states that summary statements of new investigators will be prepared first followed by all other applications in priority score order - best score first If you have access to the NIH Commons system, you will be able to see your score / summary statement immediately after release Please refer to the URL for more information https //commons era nih gov/commons/ If necessary you may contact your Institute program representative for score/summary statement information and advice after the meeting

CONFIDENTIALITY OF THE REVIEW PROCESS: It is inappropriate for you, or anyone on your behalf, to contact a study section member (or potential member) regarding the review of your grant application either before or after the meeting of the study section Reviewers are instructed not to discuss *anything* about the review with anyone not at the study section meeting Questions and comments should be addressed to the SRO prior to the meeting and the Program Official after the meeting

If you have any other questions please call or e-mail me

Jody

Joanna M Pyper, Ph D

Deputy Chief, Infectious Diseases and Microbiology IRG

Scientific Review Officer, Virology A (VIRA)

6701 Rockledge Drive, Room 3208, MSC 7808

Bethesda, MD 20892

(For delivery services, use 20817)

Telephone 301-435-1151

FAX 301-480-0940

e-mail pyperj@csr nih gov

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"How I wish to be a happy tree,
smiling in the wind of sunny May "
****

Fang Li, Ph D
Assistant Professor, Pharmacology
University of Minnesota Twin Cities
612-625-6149, lifang@umn edu
http //www msi umn edu/~lifang
****

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Lorrie Awoyinka
Principal Grant & Contract Administrator
University of Minnesota
Sponsored Projects Administration
450 McNamara Alumni Center
200 Oak St. SE
Minneapolis, MN 55455

Phone: (612) 626-2265
Fax: (612) 624-4843
E-mail: lorrie@umn.edu

Fang_Li_R01_supplements_010910.pdf **Content-Type:** application/pdf
Content-Encoding: base64

BUDGET JUSTIFICATION

Applicant's Name Jennifer L. Hall, Ph.D.

PERSONNEL:

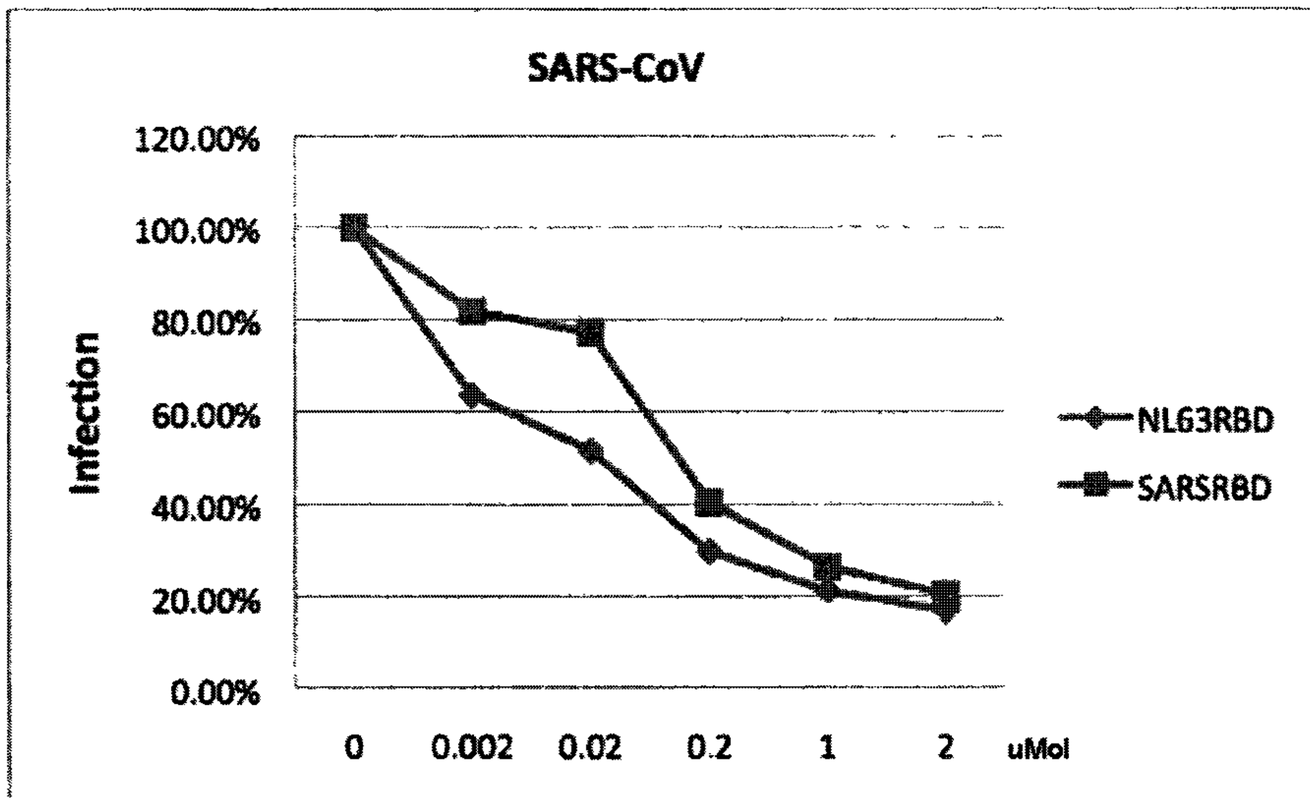
Dr. Jennifer L. Hall, Ph.D., Principal Investigator (5% effort) will be responsible for the overall supervision of the project including planning and execution of the studies, data interpretation and analysis. Dr. Hall will analyze genotyping data and mapping of SNPs. She will work closely with Dr. Sarver to analyze mRNA and miRNA data. Dr. Hall will oversee manuscript preparation and presentation of data at national meetings with data generated from the experiments in these aims. Dr. Hall directs the Program in Cardiovascular Translational Genomics. Her lab is the Central Core Lab for Clinical Trials connected to Cardiovascular Diseases focused on identifying how FDA approved devices or therapy alters DNA, RNA and protein expression. Dr. Hall has spent part of her time each month at the Broad Institute in the lab of David Altshuler for the past 2 years in a joint collaboration to identify the biological function of a recently identified genetic variant in a complex disease.

Marjorie Carlson, B.S., Assistant Scientist (32% effort) will also be responsible for generating RNA libraries for RNA sequencing, RNA and protein analysis for validation experiments and collecting and reconfirming all information in a separate database including DNA yields and ODs, mRNA content and ODs, and miRNA yields and ODs. Marjorie will also be responsible for all coordination with sample prep, delivery, data storage at the Biomedical Genomics Core for DNA, RNA and miRNA processing.

Dr. Aaron Sarver, Ph.D., Research Associate (10% effort) will be responsible for overseeing the sequence analysis data as well as miRNA and mRNA data analysis. This involves quality control metrics, pathways analysis, and statistical analysis. Dr. Sarver will develop a MYSQL database keyed by genomic location and queried using SQL statements to determine SNP's in the discrete 3' UTR region of differentially expressed genes. Dr. Sarver will work with our staff at the Supercomputing Institute to make this data publicly available per Hall lab policy and in line with Data Sharing Requirements by NIH despite this is not a GWAS study.

Fang Li, Department of Pharmacology, University of Minnesota

Re: Supplements for R01 application "1R01AI089728-01", entitled "Receptor recognition mechanisms of coronaviruses".



<u>Supplementary Figure 1</u>: NL63-CoV RBD inhibits SARS-CoV-spike-protein-mediated infection - pseudotyped viral infection data.

We prepared retroviral MLVs in this study. These MLVs express β-galactosidase and are pseudotyped with the SARS-CoV spike protein. These retroviral MLVs were incubated with human-ACE2-expressing HEK293 cell in the presence of the indicated concentrations of purified NL63-CoV RBD or SARS-CoV RBD. Infection is shown as a percentage of β-galactosidase activity observed in the absence of inhibitor.

This study shows that both the NL63-CoV and SARS-CoV RBDs compete with the SARS-CoV spike protein for a common binding site on human ACE2. <u>Through this study, we have established the pseudotyped viral infection assays. Further, the results support the hypothesis in Specific Aim 1 (section D.2) that a common virus-binding hotspot on human ACE2 is critical for the binding of both NL63-CoV and SARS-CoV. They also suggest that the NL63-CoV RBD may be used as a novel antiviral inhibitor against SARS-CoV infection.</u>

Supplementary Table 1: Roles of three predicted FIPV RBMs in binding to feline APN - flow cytometry data.

RBD of Coronavirus Spike or chimeric RBD	% of fAPN/BHK cells binding RBD*	% of RBD binding relative to FIPV RBD bound to fAPN/BHK cells	Mean fluorescence intensity
FIPV	26	100	318
NL63	1.7	6.5	132
NL63 RBM1 in FIPV RBD	2.4	9.2	131
NL63 RBM2 in FIPV RBD	0.8	3	120
NL63 RBM3 in FIPV RBD	19	74	259
NL63 RBM1,2,3 in FIPV RBD	0.7	2.7	122

*minus the control binding to BHK cells with no fAPN

In this study, we prepared BHK cells that were stably transfected with feline aminopeptidase N (fAPN), the receptor for feline coronavirus FIPV These cells and non-transfected BHK cells were incubated with purified 6-his tagged RBDs from spike proteins of FIPV or NL63 or with chimeric RBDs. Bound RBDs were detected with rabbit anti-6his antibody followed by fluorescein-conjugated goat anti-rabbit IgG. These data show that substitution in the FIPV RBD with NL63 RBMs 1 or 2, or 1,2, plus 3 for the corresponding FIPV RBMs blocked binding to fAPN. In addition, substitution of NL63 RBM3 alone for FIPV RBM3 still permitted substantial binding to fAPN. Thus, RBMs 1 and 2 of FIPV are required for strong fAPN binding, and RBM 3 plays only a minor role in fAPN binding.

<u>These preliminary experiments support the hypothesis in Specific Aim 2 (sections D.3.3 and D.3.4) that our predicted RBMs in the predicted RBD of the FIPV spike protein are directly involved in binding to the fAPN receptor.</u>

Status Information

eSubmission Errors/Warning

The Research Plan is limited to 25 pages. This may span 28 pages due to page breaks. If the total space occupied by text does not exceed 25 pages then no action is needed. (15.3.1d) - Warning

General Grant Information	
Status:	Scientific Review Group review pending
Institution Name:	UNIVERSITY OF MINNESOTA
School Name:	
School Category:	
Division Name:	
Department Name:	
PI Name:	Li, Fang
Application ID:	1R01AI089728-01
Proposal Title:	Receptor recognition mechanisms of coronaviruses
Proposal Receipt Date:	09/29/2009
Last Status Update Date:	10/07/2009
Current Award Notice Date:	
Application Source:	Grants gov
Project Period Begin Date:	07/01/2010
Project Period End Date:	06/30/2015
eApplication Status:	Submission Complete
FOA:	[PA07-070] - RESEARCH PROJECT GRANT (PARENT R01)
NIH Appl. ID:	7943661

Other Relevant Documents

e-Application

eSubmission Cover Letter

Additions for Review (0 documents)

Status History

Effective Date	Status Message
09/29/2009	Application entered into system

Institute or Center Assignment

Institute or Center	Assignment Date
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES **(Primary)**	10/02/2009

Application Information

Award Document Number	RAI089728A
FSR Accepted Code	N
Snap Indicator Code	
Early Stage Investigator Eligible	Y
New Investigator Eligible	Y

Study Section

Scientific Review Group	IDM
Council Meeting Date(YYYY/MM)	2010/05

Advisory Council(AC) Information

Reference Letter(s)

This list shows Reference Letters associated with this particular Grant Application Principal Investigator can see a list of all Reference Letters within Personal Profile - Reference Letters section on eRA Commons

Contacts

Administration	Name	Phone	Email
Grants Management Specialist(GMS)	Carlisle, Tina M	301-402-6579	carlislt@niaid nih gov

Close

Subject: Electronic Application assigned to study section
From: eRA_Notification@od nih gov
Date: Wed, 7 Oct 2009 18 32 47 -0500 (CDT)
To: lifang@UMN EDU
CC: awards@UMN EDU
Return-Path: <awards@tc umn edu>
X-Original-To: jean-d@amethyst tc umn edu
Delivered-To: jean-d@amethyst tc umn edu
Received: from mta-a3 tc umn edu (mta-a3 tc umn edu [134 84 119 232]) by amethyst tc umn edu (Postfix) with ESMTP id 6779A58C0 for <jean-d@amethyst tc umn edu>, Wed. 7 Oct 2009 18 32 48 -0500 (CDT)
Received: from garnet tc umn edu (garnet tc umn edu [134 84 119 141]) by mta-a3 tc umn edu (UMN smtpd) with ESMTP for <jean-d@UMN EDU>, Wed, 7 Oct 2009 18 32 48 -0500 (CDT)
X-Umn-Remote-Mta: [N] garnet tc umn edu [134 84 119 141] #+LO+NM+TR
X-Umn-Classification: local
Received: by garnet tc umn edu for jean-d@umn edu, Wed, 7 Oct 2009 18 32 48 -0500
Received: from mta-m2 tc umn edu (mta-m2 tc umn edu [134 84 135 96]) by garnet tc umn edu (UMN smtpd) with ESMTP for <awards@garnet tc umn edu>, Wed, 7 Oct 2009 18 32 48 -0500 (CDT)
X-Umn-Remote-Mta: [N] mta-m2 tc umn edu [134 84 135 96] #+LO+NM+TR
Received: from nihrelayxway2 hub nih gov (mailfwd nih gov [128 231 90 107]) by mta-m2 tc umn edu (UMN smtpd) with ESMTP Wed, 7 Oct 2009 18 32 47 -0500 (CDT)
X-Umn-Remote-Mta: [N] mailfwd nih gov [128 231 90 107] #+NM+NR+UF+CU (A,-)
X-Umn-Report-As-Spam: <http //umn edu/mc/s?DSW5wHWtDNEMzoPAQXA2YD9amW2JYCYx50VIFWxMWmZQ>
Message-ID: <7n819t$4o0f81@nihrelayxway2 hub nih gov>
X-IronPortListener: NIH_Relay
X-SBRS: None
X-IronPort-AV: E=Sophos,i="4 44,521,1249272000", d="scan'208",a="159399169"
Received: from cary cit nih gov (HELO mailfwd nih gov) ([165 112 231 135]) by nihrelayxway2 hub nih gov with SMTP, 07 Oct 2009 19 32 46 -0400
Sender: awards@tc umn edu

October 07, 2009

Dear Dr.Li:

Your grant application entitled "Receptor recognition mechanisms of coronaviruses" has been received by the National Institutes of Health
and assigned to a Scientific Review Group (SRG) for scientific merit evaluation and to an Institute/Center
for funding consideration. Specific information about your assignment is available on the NIH eRA Commons website
at https //commons.era.nih.gov/commons/
Log in with your username and password Then select the "Status" menu item, retrieve the grant application,
and click on the Application Id link available in the result set

A roster of the membership of the Scientific Review Group is located at the following website.
http://era.nih.gov/roster/index.cfm
The initial peer review should be completed by the end of March 2010 and a funding decision made
shortly after the appropriate National Advisory Group meets in May 2010
Questions about the assignment should be directed to the Scientific Review Administrator (SRA)
or the Division of Receipt and Referral, Center for Scientific Review at (301) 435-0715
Other questions prior to review should be directed to the Scientific Review Administrator and questions
after the review to the program staff in the Institute/Center.

IMPORTANT NOTICE: Please review the information on human and animal subjects research located at:
http://grants.nih.gov/grants/peer/hum_anim_notice.pdf as these requirements will affect the priority score
on your application.

[Contact Us/Help Desk | Privacy Notice | Disclaimer | Accessibility]

National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland
20892

Department of Health and Human Services

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Status Information

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Current Award Notice Date:	
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Project Period End Date:	06/30/2015
eApplication Status:	Submission Complete
FOA:	[PA07-070] - RESEARCH PROJECT GRANT (PARENT R01)
NIH Appl. ID:	7943661

Other Relevant Documents
e-Application
eSubmission Cover Letter
Additions for Review (0 documents)

Status History

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Institute or Center	Assignment Date
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES **(Primary)**	10/02/2009

Application Information

Award Document Number	
FSR Accepted Code	N
Snap Indicator Code	
Early Stage Investigator Eligible	Y
New Investigator Eligible	Y

Study Section

Scientific Review Group	
Council Meeting Date(YYYY/MM)	2010/05

Advisory Council(AC) Information

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Contacts

Administration	Name	Phone	Email

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National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland
20892

Department of Health and Human Services

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NOT ARRA

Grants.gov due Oct 5

UNIVERSITY OF MINNESOTA

PROPOSAL SUMMARY

ReportID: UMFGM017F

Page No: 1
Run Date: 09/29/2009
Run Time: 09:00:06 AM

Status 2 PropB Elect Received at SPA

Grant Administrator: [redacted]

PRF # [redacted]
EGMS Proposal #

Proposal #/Version [redacted]
Sponsor Award #

[redacted] FW

Proposal Type [redacted]
Previous Award
Purpose

Concurrent N
SubContract N

Title [redacted]

Principal Investigator [redacted] lifang@umn.edu

Budget Summary

Start Date 07/01/2010
Direct Costs ~~$0.00~~ 1,250,000

End Date 06/30/2015
Indirect Costs ~~$0.00~~ 637,500

Total Costs ~~$0.00~~ 1,887,500

Sponsor National Institutes of Health
[redacted]

Submission Information

Due Date 10/05/2009
Submission Method

Date Type
of Copies Submitted

Receipt Date _____

Mailing Address Grants.gov

Comments/Corrections

REPA	X verified	__email sent for _____		
IRB	__verified	__pending	__email sent	X Not Applicable
IACUC	__verified	__pending	__email sent	X Not Applicable
IBC	__verified	X pending	X email sent	__Not Applicable
F&A rate	X at full or agency limit	__waiver on file	__waiver requested	
Cost share documented?		__yes	__no	X Not Applicable
[redacted]		X yes	__no	

Grants.gov submission submitted 9/29/09 @ 2:48pm EDT
~ David Hagen

Commons ID. FANGLI

ELECTRONIC PROPOSAL
_ESNAP
_FEDERAL
_NON-FED
X G-GOV.

NO ATTACHMENT

SUBAWARDS

0

_____ #

UNIVERSITY OF MINNESOTA

EGMS ID: PRF ID: 651709

Proposal Routing Form for External Research, Training or Public Service Support

(1) Sponsor Name: National Institutes of Health

a) Is this a subcontract? [ ] Yes [X] No

If yes, indicate the Prime Funding Source:

(2) Proposal Title: Receptor recognition mechanisms of coronaviruses

Short Title: Li/NIH R01/Receptor recognition/10-05-09

(3) Sponsor Due Date: 10/05/2009 [X] Receipt [ ] Postmark [ ] Target

(4) Funding Opportunity RFA/RFP/CFDA #: PA-07-070

(5) Submission Medium

[X] Grants.gov

[ ] Electronic

Email Address:

Instructions:

[ ] Paper or Paper/Electronic (combination)

Number of Copies for sponsor plus one copy for SPA: Original + [ ] copies.

Mailing Information. (street address required for courier delivery)

Instructions:

Country:
Address 1
Address 2.
City:
State/Province
Zip/Postal Code.
Email Address:
Phone:

(6) University Contact Name. Weber, Lynn Phone: 6-6902

(7) Principal Investigator Name: Li, Fang

Title. ASSISTANT PROFESSOR [9403] X500 Username: lifang

Phone. (612) 625-6149 % Paid 50 Unpaid: 0 Total 50

Dept : PHCL PHARMACOLOGY ADMIN Deptid 11718

(8) Co-Investigator [ ] is also PI Name: Geraghty, Robert J

Title: RESEARCH ASSOCIATE [9702] X500 Username: gerag012

Phone: (612) 625-3281 % Paid 10 Unpaid: 0 Total: 10

Dept : DRUG DESIGN, CENTER FOR Deptid. 11345

RECEIVED TIME SEP. 29. 8:28AM

UNIVERSITY OF MINNESOTA EGMS ID PRF ID: 651709

Proposal Routing Form for External Research, Training or Public Service Support

(9) Proposal is for:

[X] Research [ ] Instruction [ ] Other sponsored activity [ ] Equipment only [ ] Clinical Trial

	Basic%	Applied%	Development%
If for Research:	100	0	0

If for Clinical Trial: [ ] PI Initiated [ ] Sponsor Initiated

(10) Proposal is: [X] New [ ] Renewal [ ] Revision to previous Proposal [ ] Supplement [ ] Continuation

If other than new or revision, provide University account number. [ ] Fund [ ] Area [ ] Org

(11) Proposed duration and amount requested.

	Start	End	Direct$	Indirect$	Total$
Budget	07/01/2010	06/30/2011	250000	127500	377500
Project	07/01/2010	06/30/2015	1250000	637500	1887500

Have you requested a reduced F&A rate (IDC)?

[X] No

[ ] Yes

Please enter the Request Number and date approved

Request Number:

Date Approved:

[ ] Pending

Please enter the date the reduced F&A rate was requested

Date Requested.

If using an F&A other than the negotiated F&A rate or sponsor mandated rate, provide Justification below

If you will be requesting a waiver or reduction of F&A, contact the Office of Sponsored Projects Administration at 612-624-1648.

(12) Does this project involve.

[ ] Yes [X] No a) Human Subjects (Research Subjects' Protection Program 612-626-5654)

If yes what is the status of the Human Subjects Application? [ ] Approved [ ] Exempt [ ] Pending

If Approved or Exempt Study Code Number

If Approved Approval Date

[ ] Yes [X] No [ ] Pending b) Animal Subjects (Research Subjects' Protection Program 612-626-5654)

If yes [ ] Approved Approval Date: Study Code Number:

[ ] Yes [X] No c) Purchase/use of custom antibodies that have been or will be produced in animals housed outside the University?
(Research Subjects' Protection Programs 612-626-5654)

[ ] Yes [X] No d) Human Blood, Body Fluids, or Other Potentially Infectious Materials

If yes do you have Bloodborne Pathogen training and immunization? [ ] Yes [ ] No
(Dept. of Environmental Health and Safety - 626-5892)

[X] Yes [ ] No e) Recombinant DNA, Infectious Agents or Biological Toxins

If yes have you filed a registration form concerning this project with the Institutional Biosafety Committee?
(Research Subjects' Protection Programs 612-626-5654)

[ ] Yes

Study Code Number.

Approval Date.

[ ] No

[X] Pending

[ ] Yes [X] No f) Radioactive Materials and/or Ionizing or Nonionizing Radiation Producing Equipment

If yes do you have the appropriate permits and adequate radiation safety information?
(Dept. of Environmental Health and Safety - 626-6764) [ ] Yes [ ] No

RECEIVED TIME SEP. 29. 8:28AM

UNIVERSITY OF MINNESOTA EGMS ID. PRF ID: 651709

Proposal Routing Form for External Research, Training or Public Service Support

[X] Yes [ ] No g) Chemicals
If yes do you have the appropriate chemical safety training (626-2330) and hazardous waste training (Dept. of Environmental Health and Safety - 626-7744) records? [X] Yes [ ] No

(13) [ ] Yes [X] No Does a potential conflict of interest exist?
If yes, please indicate REPA (Report of External Professional Activities), Part III and Part IV
Number: Approval Date:
Comments:

(14) [ ] Yes [X] No Is it likely that anything patentable (i e new, useful, or improved) will result from this research?
[ ] Yes [X] No If this is a renewal or continuing project, have any inventions been conceived or reduced to practice under prior research on this project?
If yes, [ ] not previously reported [ ] previously reported
[ ] Yes [X] No Does this proposal contain private commercial or trade secret information ? If yes, clearly identify the private commercial information in the text of the proposal

(15) [X] Yes [ ] No Is sufficient and suitable space to house this project presently assigned to the principal investigator's department or college? If **yes,** this space is located in:
Room Number(s) and Building Name(s)
3-284, Nils Hasselmo Hall
If **no,** this problem must be reviewed with the department head. A tentative solution must be identified and documented prior to submission of this proposal. If alterations are necessary, funds must be requested from the sponsor, or a University source

(16) [X] Yes [ ] No Does this project involve University resources, space or staff from more than one department or college?
If yes, this form must be approved by all department heads and deans involved.
1. Center for Drug Design
2
3.
4
5.

(17) [X] Yes [ ] No Is this proposal over $100,000 per year with indirect of at least $1000, AND does it involve an intercollegiate center or more than one college?
If yes, proposals involving an intercollegiate center or more than one college require sharing of Indirect Costs Recovery (ICR) unless waived by the dean
http //www.fpd finop umn edu/groups/ppd/documents/procedure/cost_proc4 cfm
How will ICR be shared?
[ ] By separate budgets
[ ] Budgets are attached to proposal
[ ] Budgets will be provided prior to grant award
[X] Based on contribution
[X] Will be shared but percentages have not been negotiated
[ ] Separate agreement has been negotiated and is attached to the proposal
[ ] Enter the tentative percentage, contingent upon receipt of grant and final negotiations, for each college or intercollegiate center
1
OR Intercollegiate Center (please, type in field).
%
2.
OR Intercollegiate Center (please, type in field):
%
3.

RECEIVED TIME SEP. 29. 8:28AM

UNIVERSITY OF MINNESOTA EGMS ID: PRF ID: 651709

Proposal Routing Form for External Research, Training or Public Service Support

OR Intercollegiate Center (please, type in field):

[ ] %

4 [ ]

OR Intercollegiate Center (please, type in field):

[ ] %

5 [ ]

OR Intercollegiate Center (please, type in field)

[ ] %

(18) [ ] Yes [X] No Is cost sharing, matching, or in-kind proposed and required by the sponsor for this submission?
[ ] Yes [X] No Is cost sharing, matching, or in-kind proposed but not required by the sponsor?

If yes to either question, indicate the funding source(s) and amount(s).

(19) Program Income

[ ] Yes [X] No Is program income anticipated on this project?

If yes, indicate specific type(s) of program income by selecting one or more items from questions a through d below.

- [ ] **a)** From fees for services performed?
- [ ] **b)** From the use or rental of real or personal property acquired under this project?
- [ ] **c)** From the sale of commodities or items fabricated under the award?
- [ ] **d)** From license fees and royalties on patents and copyrights that may develop from this project?

If yes to any of the above, agency rules, regulations, and University procedures regarding accounting for and reporting program income must be followed

(20) [ ] Yes [X] No Does this proposal make use of Fairview Health Services Resources?

(A) Executive Summary.

RECEIVED TIME SEP. 29. 8:28AM

UNIVERSITY OF MINNESOTA EGMS ID: PRF ID: 651709

Proposal Routing Form for External Research, Training or Public Service Support

APPROVALS and CERTIFICATIONS

Principal Investigator:

(1) I certify the information submitted within the application is true, complete and accurate to the best of the PI's knowledge (2) that any false, fictitious, or fraudulent statements or claims may subject the PI to criminal civil or administrative penalties, and (3) that the PI agrees to accept the responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of the application

Investigator:

The information provided on this form and in the accompanying proposal is correct to the best of my knowledge Equipment budgeted in this application is not otherwise available for use on this project from existing departmental or collegiate inventories In the event this application is awarded, I (we) agree to abide by all applicable institutional and sponsoring agency policies and procedures including the Intellectual Property Policy of the University of Minnesota and to follow commonly accepted scientific practices in recording and maintaining records of research I(we) certify that I (we) have read the University of Minnesota Code of Conduct policy and agree to abide by the rights and responsibilities as identified therein

lifang

Signature: _____ _____

Investigator(s) Date

Department Head(s) and/or Division Head(s): Academic Dean(s), Chancellor or Provost:

We certify that the above statements are correct to the best of our knowledge. The commitment of departmental and collegiate resources, if any, has been noted and approved. We agree that the scientific objectives of this application are in keeping with departmental and collegiate goals. When question 13 regarding conflict of interest is marked yes, we acknowledge that the INTEREST has been reviewed with the principal investigator and will not comprise a conflict.

weber117, jense109 for tmeddean, lohxx001, richa016 for moldo002, palle001

Signature: _____ _____

Department Head(s), Division Head(s), Academic Dean(s), Chancellor or Provost Date

burak001, vince001, hinsk001

Signature: _____ _____

Department Head(s) Division Head(s) Academic Dean(s) Chancellor or Provost Date

The University of Minnesota is an equal opportunity educator and employer

Date Printed
09/29/09 08 12

RECEIVED TIME SEP. 29. 8:28AM

Subject: PRF #651709 Institutional Biosafety Committee
From: Lorrie Awoyinka <lorrie@umn.edu>
Date: Tue, 29 Sep 2009 10:43:20 -0500
To: Fang Li-5 <lifang@umn.edu>, Institutional Biosafety Committee <ibc@umn.edu>

Title: Receptor recognition mechanisms of coronaviruses
PI: Fang Li
Sponsor: NIH
PRF: 651709

This email is to advise you that SPA was unable to locate an approved IBC protocol for this project during the proposal review process. Should this proposal be funded, no funds may be expended for activities involving recombinant DNA, infectious agents or biological toxins until Institutional Biosafety Committee (IBC) approval has been obtained.

The Principal Investigator should contact the Institutional Biosafety Committee at ibc@umn.edu or call the RSPP office at (612) 626-5654 with any questions or concerns regarding the status of the IBC protocol for this proposal.

--
Lorrie Awoyinka
Principal Grant & Contract Administrator
University of Minnesota
Sponsored Projects Administration
450 McNamara Alumni Center
200 Oak St. SE
Minneapolis, MN 55455

Phone: (612) 626-2265
Fax: (612) 624-4843
E-mail: lorrie@umn.edu

Errors/Warnings for Prior Failed eSubmissions

GRANT10428706 2009-09-29

WARNING The Research Plan is limited to 25 pages This may span 28 pages due to page breaks. If the total space occupied by text does not exceed 25 pages then no action is needed (15 3 1d)

Close

Lorrie

GRANTS.GOV™

Home > Apply for Grants > Confirmation

Confirmation

Thank you for submitting your grant application package via Grants.gov. Your application is currently being processed by the Grants.gov system. Once your submission has been processed, Grants.gov will send email messages to advise you of the progress of your application through the system. Over the next 24 to 48 hours, you should receive two emails. The first will confirm receipt of your application by the Grants.gov system, and the second will indicate that the application has either been successfully validated by the system prior to transmission to the grantor agency or has been rejected due to errors.

Please do not hit the back button on your browser.

If your application is successfully validated and subsequently retrieved by the grantor agency from the Grants.gov system, you will receive an additional email. This email may be delivered several days or weeks from the date of submission, depending on when the grantor agency retrieves it.

You may also monitor the processing status of your submission within the Grants.gov system by clicking on the "Track My Application" link listed at the end of this form.

Note: Once the grantor agency has retrieved your application from Grants.gov, you will need to contact them directly for any subsequent status updates. Grants.gov does not participate in making any award decisions.

IMPORTANT NOTICE: If you do not receive a receipt confirmation and either a validation confirmation or a rejection email message within 48 hours, please contact us. The Grants.gov Contact Center can be reached by email at support@grants.gov, or by telephone at 1-800-518-4726. Always include your Grants.gov tracking number in all correspondence. The tracking numbers issued by Grants.gov look like GRANTXXXXXXXXX.

Contact Center hours of operation are Monday-Friday from 7:00 A.M. to 9:00 P.M. Eastern Time.

The following application tracking information was generated by the system:

Grants.gov Tracking Number :	GRANT10428706
Applicant DUNS:	55-591-7996
Submitter's Name:	David Hagen
CFDA Number:	
CFDA Description:	
Funding Opportunity Number :	PA-07-070
Funding Opportunity Description :	Research Project Grant (Parent R01)
Agency Name :	National Institutes of Health
Application Name of this Submission :	651709 Li NIH
Date/Time of Receipt :	2009.09.29 2:48 PM, EDT

TRACK MY APPLICATION – To check the status of this application, please click the link below:

https://apply07.grants.gov/apply/checkSingleApplStatus.faces?tracking_num=GRANT10428706

It is suggested you Save and/or Print this response for your records.

Principal Investigator/Program Director (Last, First, Middle: Li, Fang

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM: 07/01/10 THROUGH: 6/30/11

PERSONNEL (*Applicant organization only*)		Months Devoted to Project			INST BASE SALARY	DOLLAR AMOUNT REQUESTED (*omit cents*)		
NAME	ROLE ON PROJECT	Cal Mnths	Acad Mnths	Summer Mnths		SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Fang Li	Principal Investigator	6 00			$84,500	$42,250	$13,647	$55,897
Robert Geraghty	Co-Invest	1 20			$106,000	$10,600	$3,424	$14,024
Kailang Wu	Postdoctoral	12 00			$34,000	$34,000	$6,732	$40,732
Guiqing Peng	Postdoctoral	12 00			$33,000	$33,000	$6,534	$39,534
Wenbo Zhou	Postdoctoral	4.80			$36,000	$14,400	$2,851	$17,251
					SUBTOTALS →	$134,250	$33,188	$167,438

CONSULTANT COSTS
Dr. Kathryn Holmes — $10,000

EQUIPMENT (*Itemize*) — $0

SUPPLIES (*Itemize by category*)

Molecular biology supplies	$12,000
Insect cell culture supplies	$8,000
Protein purification supplies	$14,000
Protein crystallization supplies	$12,000

$46,000

TRAVEL
PI and/or postdoctoral to attend one meeting per year — $2,000

PATIENT CARE COSTS — INPATIENT

OUTPATIENT

ALTERATIONS AND RENOVATIONS (*Itemize by category*)

OTHER EXPENSES (*Itemize by category*)

Publication costs/reprints	$1,500
Equipment maintenance	$1,500
Communication costs	$800

$3,800

CONSORTIUM/CONTRACTUAL COSTS — DIRECT COSTS — $0

SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD (*Item 7a, Face Page*) — $ 229,238

CONSORTIUM/CONTRACTUAL COSTS — FACILITIES AND ADMINISTRATIVE COSTS

TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD — $ 229,238

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Principal Investigator/Program Director (Last, First, Middle)

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD (from Form Page 4)	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL *Salary and fringe benefits Applicant organization only*		$167,438	$172,461	$177,635	$182,964	$188,453
CONSULTANT COSTS		$10,000	$10,000	$10,000	$10,000	$10,000
EQUIPMENT		$0				
SUPPLIES		$46,000	$47,380	$48,801	$50,265	$51,773
TRAVEL		$2,000	$2,060	$2,122	$2,186	$2,252
PATIENT CARE COSTS	INPATIENT	$0				
	OUTPATIENT	$0				
ALTERATIONS AND RENOVATIONS		$0				
OTHER EXPENSES		$3,800	$3,914	$4,031	$4,152	$4,277
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	$0				
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*		$229,238	$235,815	$242,589	$249,567	$256,755
CONSORTIUM/ CONTRACTUAL COSTS	F&A	$0				
TOTAL DIRECT COSTS		$229,238	$235,815	$242,589	$249,567	$256,755
TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD						$1,213,964

JUSTIFICATION Follow the budget justification instructions exactly Use continuation pages as needed

	Year 1	Year 2	Year 3	Year 4	Year 5
Indirect Cost Base	$250,000	$250,000	$250,000	$250,000	$250,000
IDC @ 51%	$127,500	$127,500	$127,500	$127,500	$127,500
Total Direct Costs	$250,000	$250,000	$250,000	$250,000	$250,000
IDC	$127,500	$127,500	$127,500	$127,500	$127,500
Total Costs	$377,500	$377,500	$377,500	$377,500	$377,500

DC 5 YEAR TOTAL: $1,250,000
IDC 5 YEAR TOTAL: $637,500
DC & IDC 5 YEAR TOTAL $1,887,500

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Personnel Justification

Fang Li, PhD, Principal Investigator (6 00 calendar months), will be responsible for laboratory management and supervision of all projects, including experimental design, execution, data analysis, and write-up.

Robert J. Geraghty, PhD, Co-investigator (1.20 calendar months), effort during three summer months, will be responsible for supervision of the virology parts of the projects, including experimental design, execution, and data analysis.

One Postdoctoral Scientist (Kailang Wu, PhD) (12.00 calendar months), will be responsible for conducting experiments on the receptor recognition mechanisms of group-I NL63 coronavirus and other group-I coronaviruses.

One Postdoctoral Scientist (Guiqing Peng, PhD) (12.00 calendar months), will be responsible for conducting experiments on the receptor recognition mechanisms of group-II SARS coronavirus

One Postdoctoral Scientist (Wenbo Zhou, PhD) (4.80 calendar months), will work with Drs. Geraghty and Li on the virology parts of the project.

Kathryn V. Holmes, PhD, Consultant, will be responsible for providing advice and discussions about experimental design, execution, and data analysis.

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UNIVERSITY OF MINNESOTA

Twin Cities Campus

Department of Pharmacology

Medical School
Academic Health Center

6-120 Jackson Hall
321 Church Street S.E.
Minneapolis, MN 55455 U.S.A.

612 625-9997
FAX. 612 625-8408

FAX COVER PAGE

Date: 9-29-09 Number of Pages (*including cover page*): 2

TO: **PROPOSALS - SPA**

FAX #: 4-4843

FROM: PHARMACOLOGY

Message:

RE: ELECTRONIC GRANT SUBMISSION

PRF #: 651709

PI NAME: Fang Li

Grant Type: NIH R01 (modular)

Detailed Budget Attached: Yes X No ____

Budget Justification Attached: Yes ____ No ____

Personnel Justification only X

RECEIVED TIME SEP. 29. 8:28AM

Subject: PRF # 651709 Fang Li NIH R01
From: Lynn Weber <weber117@umn edu>
Date: Tue, 29 Sep 2009 08:23:11 -0500
To: "proposal@umn.edu" <proposal@umn.edu>

--

Lynn Weber
Department of Pharmacology
University of Minnesota
Medical School
6-120 Jackson Hall
321 Church Street SE
Minneapolis, MN 55455
Phone. 612-626-6902
Fax: 612-625-8408
Email: weber117@umn.edu
http://www pharmacology.med umn.edu/

Li NIH R01 Receptor Recog 10-5-09.pdf	**Content-Type:** application/octet-stream **Content-Encoding:** base64

Files
CON-22345

2) Rebudget funds into new Accounts:

Sponsored Chartfields:

(4) FUND	(5) DeptID	(5) PCBU	(8) Project	(1) Activity
3002	11718	UMSPR	00013782	1

Please remember to include any necessary adjustments to F&A

Move From:

$	8798	*from Account*	700100
$	2929	*from Account*	710100
$		*from Account*	
$		*from Account*	
$		*from Account*	
$		*from Account*	
$		*from Account*	

Move To:

$	8798	*to Account*	700200
$	2929	*to Account*	710200
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	

Done 6/18/10

NIAID

JUSTIFICATION:

This is a new project. The original budget was setup with salary for Dr. Geraghty, co-I, in 700100. However, his paid job code is in P&A and he has a non-salary faculty appointment. So combo codes are needed for P&A salary and fringe.

By submission of this information, I certify that all parties have agreed to these budget reallocations.	
Name (CA)	Carrie Dejarlais
Dept ID #	11718
Phone #	5-4982
Email	dejar001@umn.edu

Subject: Fwd: Rebudget - Li 00013782
From: Carrie DeJarlais <dejar001@umn.edu>
Date: Fri, 4 Jun 2010 11:10:41 -0500
To: Lorrie Awoyinka <lorrie@umn.edu>
CC: Debi Marsh <mars0061@umn.edu>

Lorrie,

Please process this rebudget at your earliest convenient. This is Dept/Ca approved.

Thanks.

Begin forwarded message:

> **From:** Debi Marsh <mars0061@umn.edu>
> **Date:** June 4, 2010 11:06:59 AM CDT
> **To:** Carrie DeJarlais <dejar001@umn.edu>
> **Subject: Rebudget - Li 00013782**
>
> Carrie,
>
> Please review and send to SPA.
>
> Thanks,
> Debi
>
> =
>
> --
> Debi Marsh
>
> Department of Pharmacology
> University of Minnesota
> 6-120 Jackson Hall
> 321 Church St SE
> Minneapolis, MN 55455
>
> 612-625-0465 phone
> 612-625-8408 fax
>
> http://www.pharmacology.med.umn.edu/

Carrie DeJarlais
Dept. of Pharmacology
MNPI Center, U of MN
6-120 Jackson Hall
Minneapolis, MN 55455
612-625-4982

=

Li 00013782 6:10.xls	**Content-Type:**	application/vnd.ms-excel
	Content-Encoding:	base64

Part 1.1.3	**Content-Type:**	text/html
	Content-Encoding:	quoted-printable

Files | CON-22345

2) Rebudget funds into new Accounts:

Sponsored Chartfields:

(4) FUND	(5) DeptID	(5) PCBU	(8) Project	(1) Activity
3002	11718	UMSPR	00013782	1

Please remember to include any necessary adjustments to F&A

Move From:

$	571	*from Account*	720200
$		*from Account*	
$		*from Account*	
$		*from Account*	
$		*from Account*	
$		*from Account*	
$		*from Account*	
$		*from Account*	
$		*from Account*	

Move To:

$	571	*to Account*	750100
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	

Done 9/30/10

JUSTIFICATION:

Account code/rebudget needed to purchase non-capital lab equipment. A freezer is needed for conserving protein for expression and purification of the NL63 coronavirus spike protein as part of Dr. Fang Li's research on receptor recognition and cross-species infections by human coronaviruses. The freezer will beused exclusively by this project and is his only project and is necessary to complete the aim

By submission of this information, I certify that all parties have agreed to these budget reallocations.	
Name (CA)	Carrie Dejarlais
Dept ID #	11718
Phone #	5-4982
Email	dejar001@umn.edu

s of the project.

Subject: Rebudget request
From: Carrie DeJarlais <dejar001@umn.edu>
Date: Tue, 28 Sep 2010 09:48:57 -0500
To: Lorrie Awoyinka <lorrie@umn.edu>

Lorrie,

Please process this rebudget request at your earliest convenience. This is Dept & CA approved.

Thanks.

Carrie

--
Carrie DeJarlais
MNPI Center &
Department of Pharmacology
6-120 Jackson Hall
Minneapolis, MN 55455
612-625-4982
612-625-8408 fax

Li00013782rebudget9-10.xls	**Content-Type:** application/x-msexcel **Content-Encoding:** base64

Files
CON-22345

2) Rebudget funds into new Accounts:

Sponsored Chartfields:

(4) FUND	(5) DeptID	(5) PCBU	(8) Project
3002	11718	UMSPR	00013782

Please remember to include any necessary adjustments to F&A

Move From:

$	5,298	*from Account*	720200
$	2,702	*from Account*	810500
$		*from Account*	
$		*from Account*	
$		*from Account*	
$		*from Account*	
$		*from Account*	
$		*from Account*	
	8,000		

Done
9/30/10

Move To:

$	8,000	*to Account*	168000
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
	8,000		

JUSTIFICATION:

Dr. Fang Li requested to rebudget funds to purchase a new Shaker Incubator from Fisher Scientic on this proje incubator will be used for insect cell culture which is essential for expression and purification of viral spike prot receptors as proposed in the R01 proposal.The only other shaker incubator available is an old one in his lab. H incubator has a capacity of holding 6 2-litter cell culture flasks, which used to be sufficient for my lab when the postdoc working in the lab. Right now there are two postdocs, one technician, one rotation student, and one ur researcher, a total of five people in my lab. Therefore, we are in urgent need of another shaker incubator. Real cover the cost of this item is essential for the program. Without this item, insect cell culture work in my lab wil hampered due to lack to space to grown insect cell, which will subsequently hamper protein expression, purific structural studies. The Item will be used exclusively on this sponsored project.

(1)

Activity 1

ct. The shaker
eins and their
owever, each
ere was only one
ndergraduate
locating funds to
l be severely
ation and related

By submission of this information, I certify that all parties have agreed to these budget reallocations.	
Name (CA)	Carrie DeJarlais
Dept ID #	11718
Phone #	625-4982
Email	kaatz003

Subject: Fwd: Re: purchase of a shaker incubator for insect cell culture on project 3002-11718-00013782
From: Yang Chong <chong001@umn.edu>
Date: Tue, 28 Sep 2010 15:06:25 -0500
To: lorrie@umn.edu
CC: Carrie DeJarlais <dejar001@umn.edu>

Hi, Lorrie:

Dr. Fang Li, PI on project 3002-11718-00013782 has requested to purchase a Shaker incubator to complete the aims of this project. Carrie DeJarlais, CA for Dept ID 11718 has approved this request. See detail in the rebudget request attached. Please process the rebudget request ASAP. Let me know if you have any questions. Thanks.

Yang

X-Original-To: chong001@topaz.tc.umn.edu
Delivered-To: chong001@topaz.tc.umn.edu
X-Umn-Remote-Mta: [N] x-160-94-109-57.ahc.umn.edu [160.94.109.57] #+LO+TS+AU+HN
X-Umn-Classification: local
User-Agent: Microsoft-Entourage/12.26.0.100708
Date: Tue, 28 Sep 2010 14:17:53 -0500
Subject: Re: purchase of a shaker incubator for insect cell culture
From: Carrie DeJarlais <dejar001@umn.edu>
To: Fang Li <lifang@umn.edu>
CC: Yang Chong <chong001@umn.edu>
Thread-Topic: purchase of a shaker incubator for insect cell culture
Thread-Index: ActfQdkZUg8OfwhPgEqhS56oTF/A+A==

Rebudget request approved.

Yang – please submit a rebudget form to send to SPA.

Thanks.

Carrie

--
Carrie DeJarlais
MNPI Center &
Department of Pharmacology
6-120 Jackson Hall
Minneapolis, MN 55455
612-625-4982
612-625-8408 fax

From: Fang Li <lifang@umn.edu>
Date: Tue, 28 Sep 2010 14:03:11 -0500
To: Carrie DeJarlais <dejar001@umn.edu>
Cc: Yang Chong <chong001@umn.edu>
Subject: purchase of a shaker incubator for insect cell culture

Hi Carrie,

I would like to purchase an equipment using my NIH grant. Justification follows.

Thanks,
Fang

1. Items to be purchased
Shaker incubator from Thermofisher (item #1 in the attached quote)

2. Estimated cost of each item
$7790.23

3. Indication of project need (benefit) of each item
The shaker incubator will be used for insect cell culture which is essential for expression and purification of viral spike proteins and their receptors as proposed in the R01 proposal.

4. Statement indicating the need can not be met w/existing equipment in the department
The only other shaker incubator available is an old one in my lab. However, each incubator has a capacity of holding 6 2-litter cell culture flasks, which used to be sufficient for my lab when there was only one postdoc working in the lab. Right now there are two postdocs, one technician, one rotation student, and one undergraduate researcher, a total of five people in my lab. Therefore, we are in urgent need of another shaker incubator.

5. If reallocating funds to cover cost of item statement addressing the impact this rebudget will have on meeting the program's objectives
Reallocating funds to cover the cost of this item is essential for the program. Without this item, insect cell culture work in my lab will be severely hampered due to lack to space to grown insect cell, which will subsequently hamper protein expression, purification and related structural studies.

6. Statement of items percent usage on project.
The Item will be used 100% on project.

---------- Forwarded message ----------
From: **guiqing peng** <penggq2009@gmail.com>
Date: Mon, Sep 27, 2010 at 11:58 AM
Subject: Fwd: Your requested quote # 0270-7947-62
To: LIFANG <smiling.tree@gmail.com>

---------- Forwarded message ----------
From: <JO.OBRIEN@thermofisher.com >
Date: Mon, Sep 27, 2010 at 11:30 AM
Subject: Your requested quote # 0270-7947-62
To: PENGGQ2009@gmail.com, JO.OBRIEN@thermofisher.com

Thank you for your inquiry. Attached is the quote you requested.

--
"How I wish to be a happy tree,
smiling in the wind of sunny May."
****
Li, Fang Frank Ph.D.
Assistant Professor, Pharmacology
University of Minnesota Twin Cities
612-625-6149, lifang@umn.edu
http://www.msi.umn.edu/~lifang < http://www.msi.umn.edu/%7Elifang>
****

Yang Chong
Department of Integrative Biology &
Physiology, University of Minnesota
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Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** FWA00000312 **Phase III Clinical Trial:** ☒ No ☐ Yes	**Vertebrate Animals:** ☒ No ☐ Yes **Inventions and Patents:** ☒ No ☐ Yes ☐ **Previously Reported** ☐ **Not Previously Reported**
Program Income: ☒ No ☐ Yes	

Budget Period	**Anticipated Amount**	**Source**
F&A Changes:		

Primary Project/Performance Site Location		
Organizational Name: University of Minnesota		
DUNS: 5559179960000		
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City: Minneapolis	County: Hennepin	State: MN
Province:	Country: UNITED STATES	Zip/Postal Code: 554550215
Congressional Districts: MN-005		

Additional Project/Performance Site Location		
Organizational Name: University of of Minnesota		
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Project/Performance Site Congressional Districts: MN-005		

Program Director/Principal Investigator:	**Grant Number**
Fang Li	5R01AI89728-2
Applicant Organization:	**Period Covered by this Report:**
UNIVERSITY OF MINNESOTA TWIN CITIES	05/15/2010 - 04/30/2011

Title of Project:

Receptor recognition mechanisms of coronaviruses

SNAP Questions:

Has there been a change in the other support of Senior/Key Personnel since the last reporting period?

☒ **No** ☐ **Yes**

Justification:

Will there be, in the next budget period, a significant change in the level of effort for the PD/PI or other Senior/Key Personnel designated on the Notice of Award from what was approved for this project?

☒ **No** ☐ **Yes**

Justification:

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☒ **No** ☐ **Yes**

Justification:

Changes in Select Agent Research? ☒ **No** ☐ **Yes**

Changes in Multiple PD/PI Leadership plan? ☒ **No** ☐ **Yes**

Change in human embryonic stem cell (hESC) line(s) used? ☒ **No** ☐ **Yes**

Justification:

Human Subject Education Requirement:

Has the Involvement of Human Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Has the Involvement of Animal Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Publications:

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All Personnel Report

Program Director/Principal Investigator:	**Grant Number**
Fang Li	5R01AI89728-2

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Fang Li	FANGLI	PHD	XXX-XX[redacted]		**Months Devoted to Project**	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
PD/PI			[redacted]	6.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Robert James Geraghty	ROBERT.GERAGHTY	PhD	XXX-XX[redacted]		**Months Devoted to Project**	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-Investigator			[redacted]	1.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Guiqing Peng	GUIQINGPENG	PhD	XXX-XX[redacted]		**Months Devoted to Project**	
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Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			[redacted]	12.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Kailang Wu	KAILANGWU	PhD	XXX-XX[redacted]		**Months Devoted to Project**	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			[redacted]	12.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
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Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			[redacted]	4.8		

Progress report

A. Specific aims

Coronaviruses use a variety of cellular receptors and co-receptors including proteins and sugars. The diverse usage of receptors has allowed coronaviruses to infect a wide range of mammalian and avian species, and cause respiratory, enteric, systemic and neurological diseases. How coronaviruses have evolved to do so has been a major puzzle in virology. To solve this puzzle, this NIH-funded project "receptor recognition mechanisms of coronaviruses" investigates the molecular and structural basis for the complex receptor-recognition mechanisms of coronaviruses.

There are three specific aims in this project. Aim 1 focuses on the receptor recognition mechanisms of group-I NL63 coronavirus (NL63-CoV). Aim 2 focuses on the receptor recognition mechanisms of other group-1 coronaviruses such as feline infectious peritonitis (FIPV) and porcine transmissible gastroenteritis virus (TGEV). Aim 3 focuses on the receptor recognition mechanisms of group-II SARS coronavirus (SARS-CoV). The research approaches of this proposal include crystallographic analysis of the virus-receptor interfaces, identification of structural elements important for virus-receptor interactions, and characterization of these elements using crystallographic, biochemical, molecular, and virological tools.

B. Studies and Results

Our research in the past funding year has tapped into all of the three aims. Wu et al. (2011, *Journal of Virology*) addresses specific aim 1. In this study, we investigated the function of a virus-binding hotspot on human angiotensin-conversing enzyme2 (ACE2) that is critical for the binding of both NL63-CoV and SARS-CoV. The hotspot consists of a salt bridge surrounded by hydrophobic tunnel walls. Mutations that disturb the hotspot structure have significant effects on virus/receptor interactions, revealing critical energy contributions from the hotspot structure. The tunnel structure at the NL63-CoV/ACE2 interface is more compact than that at the SARS-CoV/ACE2 interface, and hence virus/ACE2 binding affinities are decreased either by NL63-CoV mutations that decrease the tunnel space or by SARS-CoV mutations that increase the tunnel space. Furthermore, NL63-CoV receptor-binding domain (RBD) inhibits ACE2-dependent infection by SARS-CoV, a successful application of our hotspot theory that has the potential to become a novel antiviral strategy against SARS-CoV infections. These results suggest that the structural features of the hotspot on ACE2 were among the driving forces for the convergent evolution of NL63-CoV and SARS-CoV.

Specific aim 2 has been partially addressed by Peng et al. (2011, in preparation). From our previous crystal structure of NL63-CoV RBD complexed with ACE2, we hypothesized that FIPV RBD has the same core structure as NL63-CoV RBD, but changes in three receptor-binding loops switch the receptor specificity from ACE2 in NL63-CoV to aminopeptidase-N (APN) in FIPV. In this study, we mutated the receptor-binding loops in FIPV RBD, and found that FIPV lost its APN-binding specificity, in agreement with our hypothesis. Moreover, because TGEV, but not FIPV, recognizes sugars in addition to APN, we have isolated and characterized the sugar-binding domain of TGEV spike protein that is separate from the APN-binding domain.

Wu et al. (2011, in preparation) address specific aim 3. Since the SARS epidemic, various mutations occurred on the RBD of SARS-CoV strains isolated from humans and palm civets. It was poorly understood why these mutations were selected or how SARS-CoV may further evolve. In this study, we investigated the role of each of these mutations in viral interactions with its host receptor ACE2, and provided structural basis for each of these mutations. These mutations were clustered around two "viral adaptation hotspots" on ACE2, enhanced viral interactions with either human or civet ACE2, and were therefore viral adaptations to either receptor. Overall, our study has elucidated detailed molecular and structural mechanism of host receptor adaptation by SARS-CoV, which paves the way for a mechanism-based prediction of potential future evolution of SARS-CoV.

In addition, during our structural studies of coronavirus/receptor complexes, we have developed a novel method for structure determinations of protein complexes (Li and Li, 2011, *Structure*). In this study, we propose a strategy to facilitate structure determinations when only part of a new structure is known: after the molecular replacement (MR) search, structure determination of the unknown part of the new structure can be facilitated by cross-crystal averaging of the known part of the new structure with the search model. This strategy dramatically improves electron density in the unknown part of the new structure. It has enabled us to determine the structures of NL63-CoV and SARS-CoV RBDs each complexed with their receptor ACE2 at moderate resolutions. In a test case, it also enabled automated model building when >50% of an antigen-antibody complex was absent. These results suggest that this averaging strategy can be routinely used after MR to enhance the interpretability of electron density associated with missing model.

C. Significance

The research findings obtained so far underscore the complexity and significance of receptor recognition by coronaviruses. First, our studies are starting to sketch an evolutionary picture of coronaviruses, showing how either drastic changes in modular RBDs or subtle changes within each RBD of their spike proteins can lead to changes in their receptor specificities. It was through these evolutionary changes in their spike proteins that coronaviruses were able to explore novel cellular receptors and expand their host ranges. Second, our studies reveal molecular and structural mechanisms of host receptor adaptation by SARS-CoV, paving the way for further evaluating the chances of SARS-CoV coming back to infect the human populations and for predicting corresponding evolutionary pathways of SARS-CoV. Therefore, our research has important implications for both basic virology and viral epidemics.

D. Plans

For the next funding year, we will keep pursuing the three specific aims. For aim 1, we will examine the interactions between the NL63-CoV spike protein and ACE2 from different animal species. More specifically, we will investigate how residue changes in the hotspot structure on animal ACE2 affects the usage of these animal ACE2 as cellular receptors by NL63-CoV. Furthermore, through the detailed NL63-CoV-spike/animal-ACE2 interactions, we will evaluate the chances of different animals serving as hosts for NL63-CoV and hence these animals as vectors for NL63-CoV-related human common colds.

For aim 2, we will investigate the structural mechanism whereby the TGEV sugar-binding domain binds sugars and APN-binding domain binds APN. We will prepare the complexes of each of these domains complexed with their respective receptor, and set up crystallization screens of these complexes. We will also investigate the mechanism whereby FIPV spike protein kept its APN-binding function but lost its sugar-binding function through evolution.

For aim 3, we will investigate how SARS-CoV may evolve in the future. We will construct two SARS-CoV spike proteins containing the combinations of the residues that were better adapted to human ACE2 or civet ACE2, respectively, and examine their binding affinities for human ACE2 and civet ACE2 using surface plasmon resonance assays, pseudotyped viral infection assays, and X-ray crystallography. We will also investigate several other mutations that have recently been identified in SARS-CoV RBD, examine how these mutations affect viral interactions with human and civet ACE2, and incorporate these mutations in our predictions of future SARS-CoV evolution. In addition, in collaboration with Dr. Zhengli Shi's group in Chinese Academy of Sciences, we will examine the interactions between SARS-CoV and ACE2 molecules from bat species.

E. Publications (from the past project period)

1. Li, W., **Li, F.** (2011) Cross-crystal averaging with search models to improve molecular replacement phases. *Structure*, *19*, 155-161 (PMCID: PMC3037595)

2. Wu, K., Chen, L., Peng, G., Zhou, W., Pennell, C., Mansky, L., Geraghty, R., **Li, F.** (2011) A virus-binding hotspot on human angiotensin-converting enzyme 2 is critical for the binding of two different coronaviruses. *Journal of Virology*, *In Press*

F. Project Generated Resources

This research did not generate cell lines, DNA probes, animal models, protocols, or software. De-identified raw data from the experiments and computer programs (CCP4 macromolecular crystallography scripts) used for protein structure determinations are available to anyone upon written request to the PI. Peer-reviewed publications accepted or published during the past project period are being or have been entered in NIHMS and PubMed Central.

A Virus-binding Hotspot on Human Angiotensin-converting Enzyme 2 is Critical for the Binding of Two Different Coronaviruses

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Running title: NL63 and SARS viruses target a hotspot on human ACE2

Summary

How viruses evolve to select their receptor proteins for host cell entry is puzzling. We recently determined the crystal structures of NL63 coronavirus (NL63-CoV) and SARS coronavirus (SARS-CoV) receptor-binding domains (RBDs) each complexed with their common receptor, human angiotensin-converting enzyme 2 (hACE2), and proposed the existence of a virus-binding hotspot on hACE2. Here we investigated the function of this hypothetical hotspot using structure-guided biochemical and functional assays. The hotspot consists of a salt bridge surrounded by hydrophobic tunnel walls. Mutations that disturb the hotspot structure have significant effects on virus/receptor interactions, revealing critical energy contributions from the hotspot structure. The tunnel structure at the NL63-CoV/hACE2 interface is more compact than that at the SARS-CoV/hACE2 interface, and hence RBD/hACE2 binding affinities are decreased either by NL63-CoV mutations decreasing the tunnel space or by SARS-CoV mutations increasing the tunnel space. Furthermore, NL63-CoV RBD inhibits hACE2-dependent transduction by SARS-CoV spike protein, a successful application of the hotspot theory that has the potential to become a new antiviral strategy against SARS-CoV infections. These results suggest that the structural features of the hotspot on hACE2 were among the driving forces for the convergent evolution of NL63-CoV and SARS-CoV.

Introduction

Host receptor recognition by viruses is the first and essential step for viral infections. During the long history of evolutionary battles between viruses and hosts, viruses have evolved complex strategies for their receptor selections (2). Despite tremendous efforts to understand these strategies, the current picture of how viruses recognize their host receptors is still murky. Viruses exploit a wide variety of host cell surface molecules as their receptors. In addition to serving as receptors for viruses, these molecules are implicated in various host physiological functions such as cell adhesion, immune response, signaling pathways, proteolysis, or ion transport. On one hand, several viruses can share one host receptor. For example, coxsackievirus-adenovirus receptor, an immunoglobulin (Ig) superfamily member, is the receptor for both coxsackieviruses and adenoviruses (3). On the other hand, one virus can recognize several different host receptors. For example, herpes simplex viruses use one of at least three protein receptors: HVEM which is a tumor necrosis factor receptor family member (23), and nectin-1 and nectin-2 both of which are Ig superfamily members (8, 31). Understanding the pattern of host receptor recognition by viruses has important implications for viral evolution, pathogenesis, host range, tropism, cross-species infections, emerging viral epidemics, and virus-mediated gene targeting.

A key question regarding the evolution of host receptor recognition by viruses is what features of these receptor molecules make them targeted by viruses. The receptors for viruses can be proteins, carbohydrates, or lipids (2). Compared with carbohydrates and lipids, protein receptors in general have more structural features and thus are engaged in more specific and high-affinity binding interactions with viruses; they are the focus of

this study. Among protein receptors, some (such as cell adhesion molecules) are more abundant than others (such as proteases and ion transporters). Although the availability of abundant protein receptors to viruses is probably one of the reasons why they were selected by viruses as receptors (30), it is not clear whether receptor proteins, especially non-abundant receptor proteins, contain any structural features that make them targeted by viruses.

The structural features of receptor proteins can be identified by atomic structures of virus/receptor interfaces. Defined structural and functional receptor-binding domains (RBDs) have been identified in many viral surface glycoproteins. One or more receptor-binding motifs (RBMs) on these viral RBDs mediate the interactions with their receptor proteins. To date several crystal structures are available for viral RBDs complexed with their receptor proteins (1, 4, 5, 13, 18, 32, 33). Among these structures, only two reveal how different viral RBDs can bind to their common receptor protein: the structures of NL63 coronavirus (NL63-CoV) and SARS coronavirus (SARS-CoV) RBDs each complexed with their common receptor, human angiotensin-converting enzyme 2 (hACE2) (18, 32). Both NL63-CoV and SARS-CoV are important human viral pathogens. The former causes prevalent respiratory diseases (6, 29), whereas the latter was responsible for the worldwide epidemic of severe acute respiratory diseases (SARS) in 2002-2003 (12, 24). Coronavirus spike glycoproteins are envelope-anchored clove-shaped trimers (16). Each spike trimer contains three monomeric S1 heads that function in receptor binding, and a trimeric S2 stalk that functions in fusing viral envelope and host membrane. NL63-CoV and SARS-CoV RBDs are located in the S1 heads of their respective spike proteins. There is no structural homology in their RBD core structures or

RBMs (Fig. 1). The core structures of NL63-CoV and SARS-CoV RBDs are two-layer β-sandwich and single-layer β-sheet, respectively; the RBMs of NL63-CoV and SARS-CoV are three discontinuous short loops and one continuous long subdomain, respectively. Nevertheless, the two viral RBDs bind to the same three virus-binding motifs (VBMs) on hACE2 (18, 32).

Our previous structural studies lead to the hypothesis that a virus-binding hotspot exists on hACE2 and plays an important role in the binding of both NL63-CoV and SARS-CoV (32). This hypothetical hotspot consists of a critical Lys353-Asp38 salt bridge on hACE2 that is surrounded by four hydrophobic tunnel walls (Fig. 2A, 2B). Two of the tunnel walls, Tyr41 (top wall) and Asp37 (right wall), are contributed by hACE2, whereas the other two tunnel walls are contributed by the viruses: Tyr498 (bottom wall) and Ser535 (left wall) from NL63-CoV, or Tyr491 (bottom wall) and Thr487 (left wall) from SARS-CoV. Details of how this hypothetical hotspot may contribute to the virus/receptor interactions are unknown. In this study we use structure-guided biochemical and functional approaches to investigate the role of each of the components of the hotspot structure in the virus/receptor interactions. We then apply the hotspot theory to developing a potentially new antiviral strategy against SARS-CoV infections. We also discuss how the structural features of the hotspot drove the convergent evolution of two different viruses.

Materials and methods

Protein expression and purification

Soluble proteins including NL63-CoV RBD (residues 461-616), SARS-CoV RBD (residues 306-527) and hACE2 peptidase domain (residues 19-615) were expressed and purified as previously described (17, 18, 32). In brief, the proteins were expressed in insect cells using the Bac-to-Bac system (Life Technologies Inc.). Expression constructs (Invitrogen) all contained an N-terminal honeybee melittin signal sequence and a C-terminal His tag sequence. Mutations were introduced by PCR site-directed mutagenesis to the expression constructs. Recombinant baculoviruses were generated and amplified in Sf9 insect cells. The protein to be purified was harvested from Sf9 cell supernatants, loaded onto a Ni-NTA column, eluted from the Ni-NTA column with 0.5 M imidazole, and further purified by gel filtration chromatography on Superdex 200 (GE Healthcare). Fractions containing the purified protein were pooled together, loaded into an Amicon ultra-15 centrifugal filter unit (10,000 MW cutoff) (Millipore), and centrifuged at 10,000 rpm until the protein concentration reached 10 mg/ml.

RBD/hACE2 binding assays

Surface plasmon resonance assays were carried out using a Biacore 2000 as previously described (32). In brief, to measure the binding affinities between mutant viral RBDs and wild type hACE2, hACE2 was immobilized on a C5 sensor chip through direct covalent coupling via amine groups. The surface of the sensor chip was activated with N-hydroxysuccinimide (NHS), the receptor was injected and immobilized to the surface of the chip, and the remaining activated surface of the chip was blocked with ethanolamine. Soluble RBDs was introduced at a flow rate of 20 μl/min at different concentrations (62.5 nM, 125 nM, and 250 nM). k_{on}, k_{off}, and K_d were determined for the

RBD/receptor binding interactions using BIA-EVALUATIONS software. To measure the binding affinities between mutant hACE2 and wild type viral RBDs, RBDs were immobilized on the sensor chip and hACE2 was the soluble analyte. As negative controls, soluble RBDs or hACE2 were passed through an empty sensor chip, and buffer alone was passed through sensor chips containing RBDs or hACE2 as immobilized ligands.

Pseudotyped viral transduction assays

Transduction was assayed using murine leukemia viruses (MLVs) expressing β-galactosidase and pseudotyped with NL63-CoV or SARS-CoV spike protein. To prepare pseudotyped viruses, HEK293T cells were cotransfected with a spike-protein-encoding pcDNA3.1 and an MLV β-galactosidase-transducing vector, pBAG (25). At 2 days post-transfection, viral supernatants were harvested and concentrated in a spin concentrator. Approximately 4 ml of supernatant was typically concentrated (10,000 MW cutoff) to between 100-200 μl. HEK293T cells transiently expressing hACE2 in pcDNA3.1 were inoculated in 96-well dishes by adding 5 μl of concentrated viral supernatant to 50 μl cell culture medium per well. Transduction efficiency was quantified 2 days later by measuring β-galactosidase activity. The inoculated cells were lysed in PBS containing 0.5% NP40 and 3 mg/ml o-nitrophenyl B-D-glucopyranoside and monitored by spectrometry (O.D. 410 nm). The intracellular C-termini of the spike protein and hACE2 contained a C9 tag and an HA tag, respectively. The concentrations of the spike protein packaged in pseudotyped viruses and of hACE2 expressed on HEK293T cell surface were detected by Western blotting using anti-C9 and anti-HA antibodies, respectively. As

a negative control, the plasmid expressing the spike protein is replaced by a plasmid that does not express any protein.

Results

To investigate the role of the hotspot structure in the virus/receptor binding interactions, we mutated each of the components of the hotspot structure. We then examined how the mutations affect the binding affinities between RBDs and hACE2 using surface plasmon resonance Biacore assays. We also investigated how the mutations impact the interactions between spike proteins and hACE2 using pseudotyped viral transduction assays.

For Biacore assays, we first measured the binding affinities between wild type hACE2 peptidase domain and prototypic NL63-CoV RBD (strain Amsterdam 1) and between wild type hACE2 peptidase domain and prototypic SARS-CoV RBD (strain Tor2 that was isolated during the 2002-2003 SARS epidemic). hACE2 was immobilized on the Biacore sensor chip through direct covalent coupling via amine groups, and NL63-CoV or SARS-CoV RBD was injected over the chip as soluble analyte. The measured K_d between SARS-CoV RBD and hACE2 was 20.8 nM (Fig. 3A), consistent with the K_d of 16.2 nM as measured in a previous study (19). The measured K_d between NL63-CoV RBD and hACE2 was 34.9 nM (Fig. 3A), the first reported K_d between the two proteins. The same RBD fragment as used in this study (residues 461-616) also bound to hACE2 with high affinity in a previous study using a co-immunoprecipitation analysis (21). Interestingly, despite SARS-CoV and NL63-CoV RBDs had similar K_d with hACE2, NL63-CoV RBD bound to hACE2 with significantly lower k_{on} and k_{off}. It has been shown

that k_{off} and k_{on} are dictated by short-range van der Waals interactions and long-range electrostatic interactions between the proteins, respectively (26). Therefore, the lower k_{on} and k_{off} of the NL63-CoV-RBD/hACE2 complex likely reflected a less electrostatic and more hydrophobic interface between the two proteins.

Using Biacore, we also measured the binding affinities between hACE2 and NL63-CoV RBD and between hACE2 and SARS-CoV RBD in a reverse way: NL63-CoV or SARS-CoV RBD was immobilized on the sensor chip, and hACE2 was injected over the chip as soluble analyte. The measured K_d was 68.0 nM between NL63-CoV RBD and hACE2 or 137 nM between SARS-CoV RBD and hACE2, both of which were higher than when hACE2 was immobilized (Fig. 3A). Such discrepancies in measured K_d were mostly due to the differences in measured k_{on}. No matter whether hACE2 or RBDs were immobilized, k_{off} remained similar. When hACE2 was immobilized , however, k_{on} was significantly higher. Why did k_{on} increase when hACE2, instead of RBDs, was immobilized? This is because hACE2 has a larger molecular weight than either of the RBDs, and thus when immobilized, hACE2 can provide more accessible surface area for complex formation, leading to higher k_{on}. Therefore, the surface accessibility of the immobilized protein, but not the dissociation rate, accounted for the discrepancies in measured K_d.

To evaluate how mutations of the hotspot structure affect the binding affinities between RBDs and hACE2, we introduced single mutations to either RBDs or hACE2 that modified every component of the hotspot structure. These mutations were K353A, D38A, D37A, Y41A and Y41F in hACE2, Y498A, S535A and S535T in NL63-CoV RBD, and Y491A, T487A and T487S in SARS-CoV RBD (Fig. 2A, 2B). We expressed

and purified each of the 11 hACE2 and RBD mutants. To measure the binding affinities between mutant RBDs and wild type hACE2, hACE2 was immobilized on the sensor chip and mutant RBDs were the soluble analytes. To measure the binding affinities between wild type RBDs and mutant hACE2, NL63-CoV or SARS-CoV RBD was immobilized on the sensor chip and mutant hACE2 was the soluble analyte. The results were then compared with the binding affinities between wild type hACE2 and wild type RBDs (Fig. 3B, 3C).

In this study we not only measured direct interactions between viral RBDs and hACE2 using recombinant proteins, but also examined the spike/receptor interactions using functional assays. To this end, we carried out pseudotyped viral transduction assays to investigate whether changes in RBD/hACE2 interactions could lead to corresponding changes in viral entry and membrane fusion in the context of the full-length spike proteins and their receptor protein. We prepared retroviral MLVs expressing β-galactosidase and pseudotyped with NL63-CoV or SARS-CoV spike protein. These MLVs were incubated with hACE2-expressing HEK293T cells. The transduction efficiency of the pseudotyped viruses was measured by determining β-galactosidase activity of inoculated cell lysate. To measure the interactions between wild type hACE2 and mutant spike proteins or between mutant hACE2 and wild type spike proteins, we introduced single mutations to hACE2 or the spike proteins that were the same as the mutations used for Biacore assays (Fig. 4A, 4B). The expression levels of the spike proteins in pseudotyped viruses and of hACE2 molecules on HEK293T cells were detected by Western blotting using antibodies against their intracellular C-terminal C9 and HA tags, respectively. The Western blotting results showed that all of the mutant

spike proteins and mutant hACE2 molecules were well expressed, and the expression levels of these mutant proteins were quantified and calibrated against those of the wild type proteins (Fig. 4C). Finally, the measured transduction efficiency for mutant spike proteins or mutant hACE2 was normalized against the transduction efficiency of viruses pseudotyped with wild type spike proteins in cells expressing wild type hACE2 (Fig. 4).

Both Biacore assays and pseudotyped viral transduction assays yielded results that were highly consistent with each other (Fig.3, 4; Table 1). It is worth noting that recombinant SARS-CoV and NL63-CoV RBDs are both monomers in solution, whereas the full-length spike proteins are trimers on virus surfaces (14). Thus the good correlation between the RBD/hACE2 binding affinities and the spike-mediated transduction efficiency strongly suggests that the measured RBD/hACE2 binding activities reflect the native states of the proteins. Our results showed that all of the targeted mutations produced significantly reduced RBD/hACE2 binding affinities and spike-protein-guided transduction compared with the corresponding wild type proteins (*t*-test p values < .01 for both K_d and transduction), with the exception of D37A in hACE2 ($p > .075$ for transduction) and Y498 in NL63-CoV RBD ($p > .10$ for both K_d and transduction). Here we combine these biochemical and functional data with our previous structural data, and discuss molecular and structural features of the virus-binding hotspot that make hACE2 a common target by two different viruses.

The hotspot structures at the NL63-CoV/hACE2 and SARS-CoV/hACE2 interfaces share many common features. The Lys353-Asp38 salt bridge plays a central role in the hotspot structure at both of the interfaces. Because of the hydrophobic environment, the salt bridge not only provides significant amount of energy to the

virus/receptor binding interactions, but also fills a critical void in the hydrophobic stacking interactions at the virus/receptor interfaces. Correspondingly, alanine substitution for either Lys353 or Asp38 in hACE2 significantly decreased the RBD/hACE2 binding affinities and viral transductions (Fig. 3, 4). The hydrophobic tunnel walls of the hotspot structure also make important contributions to the virus/receptor binding interactions; they not only support the side chain of Lys353 to form the salt bridge, but also provide hydrophobic stacking interactions at the virus/receptor interfaces. Some hydrophobic tunnel walls contribute more energy to the virus/receptor binding interactions than others (Fig. 3, 4). For example, Tyr41 in hACE2 (top wall) is more important than Asp37 in hACE2 (right wall), probably because Tyr41 functions better as a tunnel wall with its aromatic ring. Alanine substitution for Tyr41 significantly decreased RBD/hACE2 binding affinities and viral transductions, suggesting that the stacking interaction between Tyr41 and Lys353 is essential for the hotspot structure. Interestingly, although a phenylalanine at the 41 position can potentially function as a tunnel wall with its aromatic ring, the Y41F mutation also significantly decreased RBD/hACE2 binding affinities and viral transductions. Detailed structural analysis reveals that the hydroxyl group of Tyr41 forms a hydrogen bond with receptor Asp355 at the NL63-CoV/hACE2 interface and two hydrogen bonds with receptor Asp355 and RBD Thr486 at the SARS-CoV/hACE2 interface (Fig. 2A, 2B). Thus the side chain of Tyr41 needs to be firmly anchored in order for it to function properly as a tunnel wall. Residue 41 is a histidine in the ACE2 proteins from several bat species (10). Not only is His41 a poor hydrophobic stacker, but it also cannot be anchored properly to function as a tunnel wall. As a result, these bat ACE2 proteins were

poor receptors for human SARS-CoV strains unless an H41Y mutation was introduced (10). Overall, the salt bridge and many of the tunnel walls of the hotspot structure contribute energy to the virus/receptor binding interactions.

The hotspot structures at the NL63-CoV/hACE2 and SARS-CoV/hACE2 interfaces differ in a subtle but functionally important way. The tunnel structure at the NL63-CoV/hACE2 interface is more compact than that at the SARS-CoV/hACE2 interface (Fig. 2A, 2B). At the NL63-CoV/hACE2 interface, the closest distances between the two pairs of opposing tunnel walls, Ser535-Asp37 (left to right) and Tyr41-Tyr498 (top to bottom), are 8.5 Å and 7.7 Å, respectively. At the SARS-CoV/hACE2 interface, if a serine replaces threonine at the 487 position in SARS-CoV RBD, these distances become 9.0 Å and 8.1 Å, respectively. Because the compactness of the tunnel structure at the NL63-CoV/hACE2 interface, S535T mutation in NL63-CoV RBD decreased the tunnel space and was energetically unstable (Fig. 3B, 4A). In contrast, because of the extra space of the tunnel structure at the SARS-CoV/hACE2 interface, T487S mutation in SARS-CoV RBD increased the tunnel space, but was also energetically unstable (Fig. 3C, 4B). Indeed, residue 487 was a serine in RBDs of some low-pathogenicity SARS-CoV strains, and was largely responsible for the lack of human-to-human transmission of these viral strains (18, 19, 27). Thus although S535T mutation in NL63-CoV RBD and T487S mutation in SARS-CoV RBD exerted opposite effects on the same left tunnel wall of the hotspot structure, they both reduced RBD/hACE2 binding affinities and viral transductions. For similar reasons, compared with Tyr498 in NL63-CoV RBD, Tyr491 in SARS-CoV RBD provides more support to the hotspot structure as the bottom tunnel wall in a more spacious tunnel space, and hence alanine substitution for

Tyr491 decreased RBD/hACE2 binding affinities and viral transductions (Fig. 3, 4). Therefore, the seemingly small differences in the hotspot structure at the two virus/receptor interfaces not only have significant impacts on virus/receptor binding interactions, but also have important epidemic implications.

One of the direct implications of our study is the possibility to use NL63-CoV RBD as an inhibitor to block SARS-CoV infections, because NL63-CoV RBD can compete with SARS-CoV for the common virus-binding hotspot on hACE2. To test this possibility, we inoculated MLVs pseudotyped with SARS-CoV spike protein onto hACE2-expressing HEK293T cells in the presence of various concentrations of purified NL63-CoV RBD or SARS-CoV RBD (Fig. 5). Transduction was shown as a percentage of β-galactosidase activity observed in the absence of any inhibitor. The results showed that NL63-CoV RBD indeed inhibited SARS-CoV-spike-mediated transductions. At 10 μg/ml (0.47 μM) concentration, NL63-CoV RBD inhibited SARS-CoV-spike-mediated transductions by over 80%. This method has the potential to become a new antiviral strategy against SARS-CoV infections, as it represents the first case in which SARS-CoV infection can be inhibited by a protein from a different virus. It also represents a successful application of the common virus-binding hotspot theory derived from the present study.

Discussion

Binding to the same hotspot on hACE2 was likely an outcome of convergent evolution by NL63-CoV and SARS-CoV. Our study provides several lines of evidences to support this notion. First, despite no structural homology in their RBDs, NL63-CoV and SARS-CoV both bind to the hotspot region and form highly similar and energetically

stable tunnel structures (Fig. 1, 2). Second, despite no structural homology in their RBMs, both viruses insert an RBM loop between VBM2 and VBM3 on hACE2 (Fig. 1). Third, despite being presented by non-homologous RBM loops, Ser535 in NL63-CoV RBD and Thr487 in SARS-CoV RBD (Ser487 in RBDs of some low-pathogenicity SARS-CoV strains) occupy identical positions as the left tunnel wall in the hotspot structure (Fig. 2A, 2B), and contribute energy to the virus/receptor binding interactions (Fig. 3, 4). Last, despite pointing in opposite directions, Tyr498 in NL63-CoV RBD and Tyr491 in SARS-CoV RBD also occupy identical positions as the bottom tunnel wall in the hotspot structure (Fig. 2A, 2B). These data suggest that NL63-CoV and SARS-CoV likely evolved independent strategies to achieve the same functional goal, supporting convergent evolutionary relationship between the two viruses.

The likely convergent evolution of NL63-CoV and SARS-CoV was at least partly driven by the structural features of the virus-binding hotspot on hACE2. The general structural features of the hotspot favor virus binding: it is located in a region on hACE2 that is furthest from the membrane, relatively flat, free of glycosylation, and thereby easily accessible to viruses (Fig. 1). The detailed structural features of the hotspot such as its potential to form the energetically stable tunnel structure also favor virus binding (Fig. 2A, 2B). In the unbound hACE2 structure where structural restraints from viruses are absent, Lys353 projects into solution; it does not form a salt bridge with Asp38 or stack with Tyr41 or Asp37 (Fig. 2C) (15, 28). Thus the virus-binding hotspot is not pre-existent or pre-organized on hACE2; instead, it is induced to form by virus binding. Therefore, while the hotspot is mainly an intrinsic property of hACE2, it is also a dynamic structure and receives structural contributions from both hACE2 and the viruses, although the

contributions from hACE2 are more pronounced. The virus-binding hotspot on hACE2 is likely different from the hotspots for host protein/protein interactions. Host protein partners have co-evolutionary relationships (9, 11), and hence hotspots for host protein/protein interactions are usually pre-existent and pre-organized in unbound host protein structures (20, 22). Viruses and receptors, however, do not usually have such co-evolutionary relationships; only viruses evolve to adapt to host receptors, but receptors do not evolve to adapt to viruses. Occasionally, if a virus exerts large enough impacts on a host, the host receptor may even evolve away from virus binding (7). So far the virus-binding hotspot on hACE2 is not known for its interaction with any other host proteins. Overall, the potential of the hotspot region on hACE2 to form energetically stable tunnel structures and some general structural features of this region on the receptor surface were among the possible reasons why the hotspot was exploited by two different viruses.

Acknowledgements

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Table 1. Summary of Biacore and pseudotyped transduction data from Figures 3 and 4.

	NL63-CoV			SARS-CoV	
hACE2	K_a (10^{-3} / nMol)	Transduction (%)	hACE2	K_a (10^{-3} / nMol)	Transduction (%)
WT	14.7 (±1.5)	100 (±2.3)	WT	7.30 (±1.2)	100 (±4.8)
K353A	too low	19.3 (±2.9)	K353A	too low	13.5 (±2.2)
D38A	3.37 (±0.49)	47.2 (±2.3)	D38A	2.44 (±0.37)	59.3 (±4.5)
D37A	8.77 (±0.77)	87.6 (±6.8)	D37A	2.72 (±0.46)	85.1 (±2.4)
Y41A	0.69 (±0.14)	28.5 (±2.0)	Y41A	0.63 (±0.21)	24.0 (±2.8)
Y41F	0.96 (±0.33)	35.7 (±2.1)	Y41F	0.69 (±0.24)	25.9 (±4.3)
	hACE2			hACE2	
NL63	K_a (10^{-3} / nMol)	Transduction (%)	SARS	K_a (10^{-3} / nMol)	Transduction (%)
WT	28.7 (±3.8)	100 (±2.3)	WT	48.1 (±4.0)	100 (±4.8)
Y498A	24.3 (±2.7)	91.9 (±3.4)	Y491A	too low	50.4 (±2.4)
S535A	1.25 (±0.32)	64.9 (±2.4)	T487A	too low	57.7 (±6.1)
S535T	0.43 (±0.11)	39.9 (±1.8)	T487S	too low	50.9 (±3.8)

Figure legends

Figure 1. Overall structures of NL63-CoV and SARS-CoV RBDs each complexed with their common receptor human ACE2. (A) Crystal structure of NL63-CoV RBD complexed with hACE2 (PDB 3KBH). hACE2 is in green, virus-binding motifs (VBMs) are in blue, NL63-CoV RBD core structure is in cyan, and receptor-binding motifs (RBMs) are in red. Lys353 and Asp38 in hACE2, which are critical for the RBD/hACE2 interactions, are shown in ball-and-stick format. (B) Crystal structure of SARS-CoV RBD complexed with hACE2 (PDB 2AJF).

Figure 2. Detailed structure of a common virus-binding hotspot on human ACE2. (A) Hotspot structure at the NL63-CoV/hACE2 interface. (B) Hotspot structure at the SARS-CoV/hACE2 interface. (C) Conformation of Lys353 on the surface of unbound human ACE2 (PDB 1R42).

Figure 3. Surface plasmon resonance Biacore analyses of the binding interactions between viral RBDs and human ACE2. Each experiment was repeated 5 times at three different protein concentrations. The corresponding standard errors are shown. (A) Kinetics of the binding interaction between wild type hACE2 and wild type RBDs. (B) Biacore analyses of NL63-CoV RBD and hACE2. Single mutations were introduced to hACE2 or RBD to modify every component of the hotspot structure. (C) Biacore analyses of SARS-CoV RBD and hACE2.

Figure 4. Pseudotyped viral transduction assays of the interactions between viral spike proteins and human ACE2. Retroviral MLVs expressing β-galactosidase and pseudotyped with the NL63-CoV or SARS-CoV spike protein were used to infect hACE2-expressing HEK293T cells. Transduction efficiency of the pseudotyped viruses was measured by β-galactosidase assays. After mutations were introduced into the spike proteins or hACE2, the corresponding transduction efficiency was normalized against the transduction efficiency of viruses pseudotyped with wild type spike proteins in cells expressing wild type hACE2. Each experiment was repeated 6 times. The corresponding standard errors are shown. (A) Transduction of MLVs pseudotyped with NL63-CoV spike protein in hACE2-expressing cells. (B) Transduction of MLVs pseudotyped with SARS-CoV spike protein in hACE2-expressing cells. (C) Western blotting analysis of coronavirus spike proteins and hACE2. The NL63-CoV and SARS-CoV spike proteins packaged in pseutotyped retroviruses both contained a C-terminal C9 tag, and the hACE2 expressed on HEK293T cell surface contained a C-terminal HA tag. The expression levels of the spike proteins and hACE2 were detected by Western blotting using anti-C9 and anti-HA antibodies, respectively. The protein bands were quantified using software Image J (version 1.6).

Figure 5. Inhibition of SARS-CoV-spike-mediated transduction by NL63-CoV RBD. MLVs pseudotyped with SARS-CoV spike protein were used to infect hACE2-expressing HEK293T cells in the presence of various concentrations of purified NL63-CoV RBD, SARS-CoV RBD, SARS-CoV RBD containing T487S mutation, hACE2 (positive control) and BSA (negative control). Transduction is shown as a percentage of

β-galactosidase activity observed in the absence of any inhibitor. Each experiment was repeated 5 times. The corresponding standard errors are shown. The results show that NL63-CoV can efficiently inhibit SARS-CoV-spike-mediate transduction.

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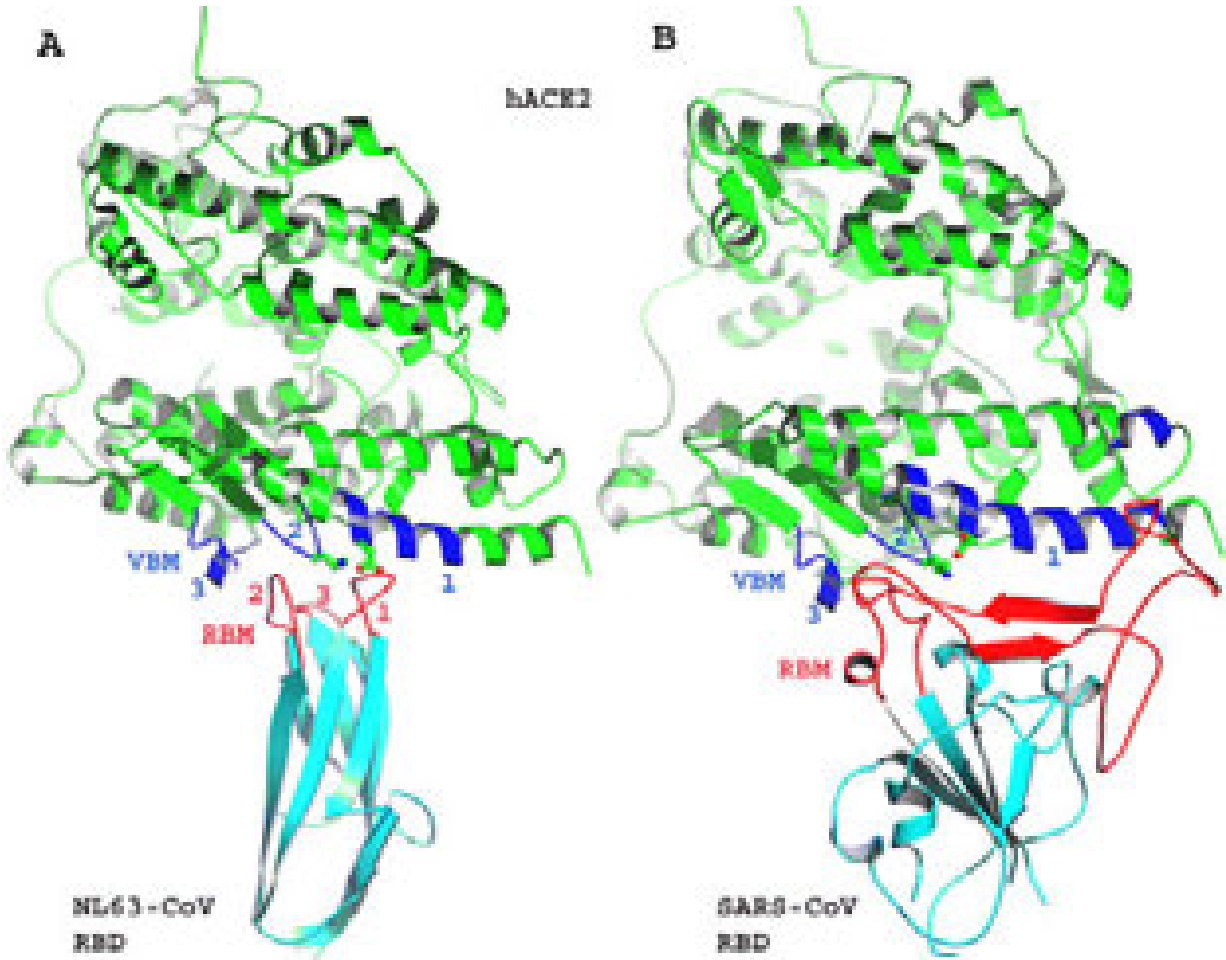
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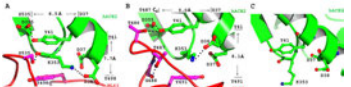
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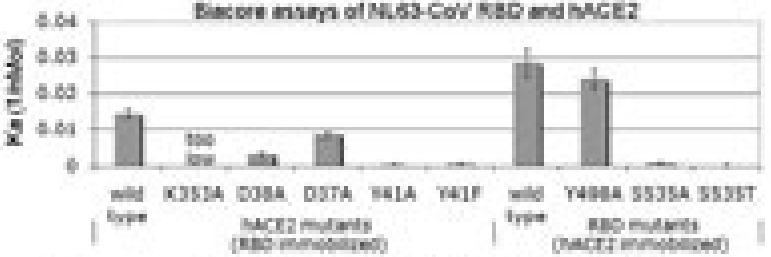

A
hACE2
VBM
RBM
NL63-CoV
RBD
B
VBM
RBM
SARS-CoV
RBD


A
B
C

A

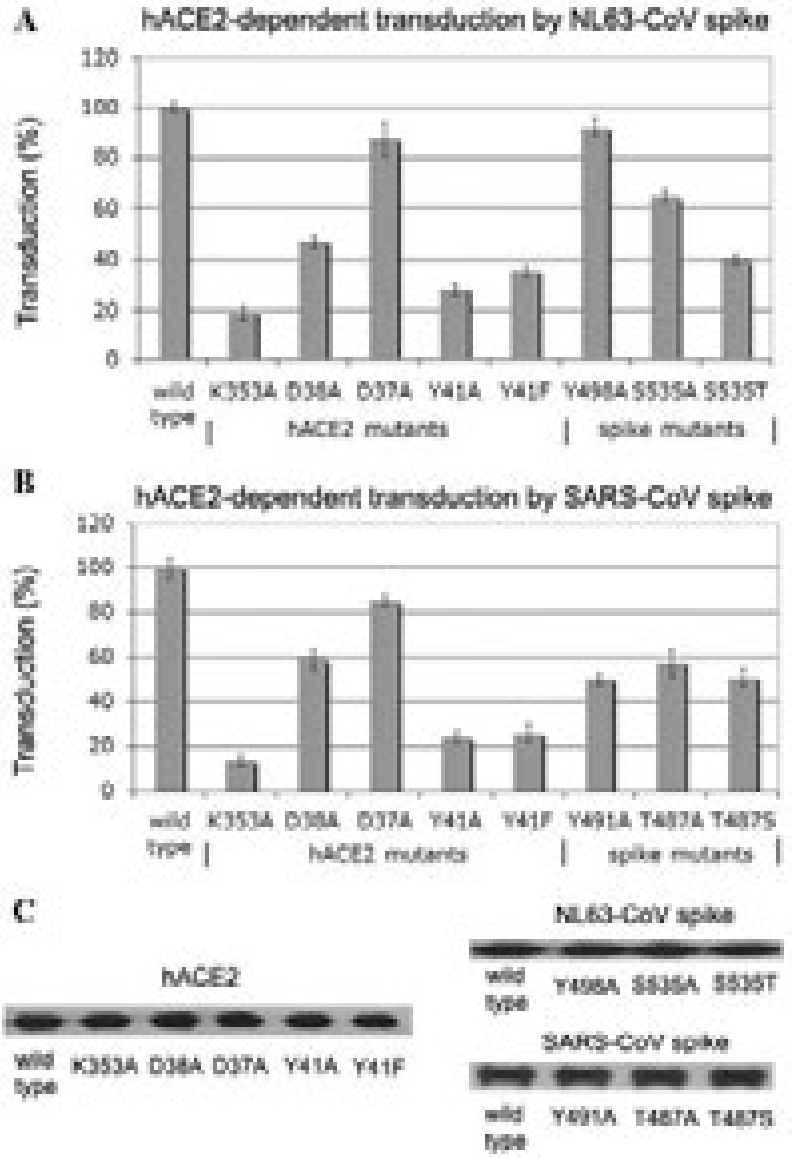
Biacore partners	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_d (M)	K_a (M^{-1})
NL63 RBD (flow through) vs. hACE2 (immobilized)	3.02×10^5	1.05×10^{-3}	3.49×10^{-9}	2.87×10^8
hACE2 (flow through) vs. NL63 RBD (immobilized)	1.42×10^5	0.93×10^{-3}	6.80×10^{-9}	1.47×10^8
SARS RBD (flow through) vs. hACE2 (immobilized)	15.8×10^5	3.26×10^{-3}	2.08×10^{-9}	4.81×10^8
hACE2 (flow through) vs. SARS RBD (immobilized)	2.48×10^5	3.38×10^{-3}	13.7×10^{-9}	0.73×10^8

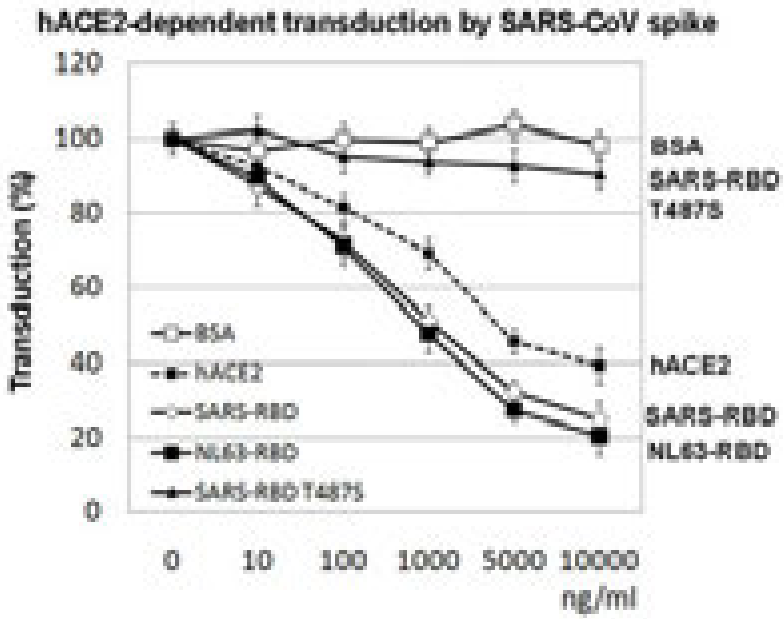
B



C




A
hACE2-dependent transduction by NL63-CoV spike
Transduction (%)
120
100
80
60
40
20
0
wild type
K353A
D38A
D37A
Y41A
Y41F
Y498A
S535A
S535T
hACE2 mutants
spike mutants
B
hACE2-dependent transduction by SARS-CoV spike
Transduction (%)
120
100
80
60
40
20
0
wild type
K353A
D38A
D37A
Y41A
Y41F
Y491A
T487A
T487S
hACE2 mutants
spike mutants
C
hACE2
wild type
K353A
D38A
D37A
Y41A
Y41F
NL63-CoV spike
wild type
Y498A
S535A
S535T
SARS-CoV spike
wild type
Y491A
T487A
T487S


hACE2-dependent transduction by SARS-CoV spike
Transduction (%)
120
100
80
60
40
20
0
BSA
hACE2
SARS-RBD
NL63-RBD
SARS-RBD T487S
0
10
100
1000
5000
10000
ng/ml
BSA
SARS-RBD T487S
hACE2
SARS-RBD
NL63-RBD

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Primary Project: 00013782
PRF #: 651709

AWARD INFORMATION (Online Page Navigation Main Menu > Grants > Awards >Award Profile)

Award Start Date: 05/15/2010
Funds Approved to Date: $717,250 00
Principal Investigator: Li,Fang
Primary Project Dept: PHCL Pharmacology Admin
Sponsor: NIH Nat'l Inst of Allergy & Infect Dis
Sponsor Award #: 5R01AI089728-02
Award Title #: Receptor recognition mechanisms of coronaviruses

Award End Date: 04/30/2015
Award Type: Grant
Purpose: Research

ASSOCIATED PROJECTS (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Funding Tab)

Project #: 00013782
Project PI: Li,Fang
F&A: 51 00 MTDC
Department: 11718 - PHCL Pharmacology Admin
Dates: 05/15/2010 - 04/30/2015
EFFDT: 05/15/2010
Project Title: Receptor recognition mechanism

AWARD HISTORY (Online Page Navigation Main Menu > Grants > Awards > Review Award Modifications)
(Displayed in action order ('Mod Issue Date' in descending order))

Reference Award#: 1R01AI089728-02
Amount: $339,750 00
Period: 2
Begin Date: 05/01/2011 **End Date:** 04/30/2012
Modification Seq: 2 **Modification Type:** Continuation **Mod Issue Date:** 05/05/2011
Comments:

NIH is currently funded through a Continuing Resolution at the FY2010 level Therefore, this non-competing award is made at a level below the committed FY2011 level If final appropriation permits, adjustments may be made up to FY2011 plan level

All other terms and conditions remain the same

Reference Award#: 1R01AI089728-01
Amount: $377,500 00
Period: 1
Begin Date: 05/15/2010 **End Date:** 04/30/2011
Modification Seq: 1 **Modification Type:** Original **Mod Issue Date:** 05/27/2010
Comments:

An unobligated balance may be carried forward to the next budget period without prior agency approval

NOTE In additoin to the PI, Dr Robert Geraghty as been identified as key personnel (co-PI) Any absence, replacement or substantial reduction in effort by key personnel requires prior, written agency approval

Modular award The significant rebudgeting provision does not apply In addition, since costs were averaged over the project period, deficits may be carried forward from one budget period to the next within the same project period

ESTIMATED FUTURE FUNDING / ACTIONS (Online Page Navigation Main Menu > Grants > Awards > Review Award Modifications)
(Displayed by 'Period' and 'Modification Sequence' in ascending order)

Reference Award#: 1R01AI089728-01
Amount: $377,500 00
Period: 3
Begin Date: 05/01/2012 **End Date:** 04/30/2013
Modification Seq: 3 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

Reference Award#: 1R01AI089728-01
Amount: $377,500 00
Period: 4
Begin Date: 05/01/2013 **End Date:** 04/30/2014
Modification Seq: 4 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

11
7-11
70

Reference Award#: 1R01AI089728-01 **Amount:** $377,500 00
Period: 5 **Begin Date:** 05/01/2014 **End Date:** 04/30/2015
Modification Seq: 5 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

COMMENTS
Description: **Entered by:** HOWI0016 **Date & Time:** 5/27/2010 10 00 AM
Date and Time Stamp indicates Proposal-to-Award Process

Description: **Entered by:** HOWI0016 **Date & Time:** 5/27/2010 10 02 AM
Sponsor Contacts

Grants Management
Cheryl Wall
Phone 301-451-2696
Fax 301-493-0597
Email wallcy@niaid nih gov

Program
Frederick J Cassels
Phone 301-402-8601
Fax 301-496-8030
Email casselsf@niaid nih gov

Description: **Entered by:** SPAUL **Date & Time:** 12/23/2010 9 56 AM
LOC
RAI089728

SPECIAL REVIEWS (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Certifications Tab)
Project #: 00013782

Description: rDNA, InfectAgents, BioToxins
Approval Date: 03/10/2011 **Expiration Date:** 03/10/2012 **AssuranceNumber:** 1002H77733
Comments:

BUDGET INFORMATION (Online Page Navigation Main Menu > Grants>Awards>Project Budget)
Please review online budget for valid account string combinations (ex PCBU, Activity, Fund, Dept_Id, Project, Account)

Project #: 00013782 **Cost Share Budget Total:**
Project Start Date: 05/15/2010 **Project End Date:** 04/30/2015
ICR Sharing **Department:** Drug Design, Center for **Percentage:** 11 50
ICR Sharing **Department:** PHCL Pharmacology Admin **Percentage:** 88 50

Period	Start Date	End Date	Direct Cost (Approved)	F & A Cost (Approved)	Direct Cost (Pending)	F & A Cost (Pending)	Total Period Cost
1	05/15/2010	04/30/2011	$252,702 00	$124,798 00	$0 00	$0 00	$377,500 00
2	05/01/2011	04/30/2012	$225,000 00	$114,750 00	$0 00	$0 00	$339,750 00
3	05/01/2012	04/30/2013	$0 00	$0 00	$250,000 00	$127,500 00	$377,500 00
4	05/01/2013	04/30/2014	$0 00	$0 00	$250,000 00	$127,500 00	$377,500 00
5	05/01/2014	04/30/2015	$0 00	$0 00	$250,000 00	$127,500 00	$377,500 00
TOTAL			$477,702 00	$239,548 00	$750,000 00	$382,500 00	$1,849,750 00

Budget Detail (all periods)

Fund Code	DeptID	Program Code	Chartfield 1	Chartfield 2	Fin EmplID	Activity	Analysis Type	Account	Cost Share	Budget Item	Amount	Hold
3002	11718					1	BUD	168100		CAPITAL_EQUIP	$8,000 00	
3002	11718					1	BUD	700100		FACULTY_SALARY	$90,027 00	
3002	11718					1	BUD	700200		PROF_ADM_SALARY	$85,121 00	
3002	11718					1	BUD	700300		GRAD_STU_SALARY	$81,400 00	
3002	11718					1	BUD	700310		GRAD_SAL_NOTUIT	$0 00	
3002	11718					1	BUD	710100		FACULTY_FRINGE	$30,694 00	
3002	11718					1	BUD	710200		PROF_ADM_FRINGE	$22,317 00	
3002	11718					1	BUD	710300		GRAD_STU_FRINGE	$16,117 00	
3002	11718					1	BUD	710310		GRAD_FRG_NOTUIT	$0 00	
3002	11718					1	BUD	720200		LABMED_SUPPLIES	$111,635 00	
3002	11718					1	BUD	720300		GEN_OP_SERVICES	$4,150 00	
3002	11718					1	BUD	720500		TELECOMM	$1,120 00	
3002	11718					1	BUD	720600		TRAVL_MILE_MOVE	$4,700 00	
3002	11718					1	BUD	730100		CONSULTING_SVCS	$19,000 00	
3002	11718					1	BUD	750100		NON_CAP_EQUIP	$571 00	
3002	11718					1	BUD	780100		REPAIRS_MAINT	$2,850 00	
3002	11718					1	BUD	810500		FACADM	$239,548 00	

COST SHARE/MATCH/IN-KIND

CSM - Cost Share, Matching and InKind

CSM05 - Departments must document all cost share that is shown in the proposal

PROGRAM INCOME INFORMATION

PIN - Program Income

PIN03 - Reportable Program Income must be deposited by the department into the Sponsored Program Income Unapplied Cash account

PIN04 - Additional - Program Income can be used in addition to sponsor funds to cover project costs that will further the objectives of the legislation under which the grant was made

TERMS & CONDITIONS (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Terms Tab)

APV - Additional Approval

APV01 - Prior sponsor approval or notification required for reduction of PI's effort by 25% or more

APV04 - Prior sponsor approval required to replace PI

APV07 - Prior sponsor approval required for changes to key personnel that would impact the scope of work

APV10 - Prior sponsor approval required for changes to work scope

APV14 - Prior sponsor approval required for the absence of the PI for 3 months or more

APV15 - Prior sponsor approval required for a second no cost extension or a first no cost extension that is submitted after the agency notification deadline

APV20 - Prior sponsor approval required for the addition of foreign sites or foreign subcontracts

FIS - Fiscal

FIS03 - Reversion of funds at termination / final

FIS07 - Deficits may be carried forward

FIS10 - Grant/contract funds may not be used to pay the salary of an individual at a rate in excess of Executive Level I of the Federal Executive Pay Scale

FIS11 - Modular award - Expenses incurred must be allocated and accounted for in compliance with all applicable cost principles

FIS13 - Deficits may be carried forward within non-competing segments, but may not be carried forward between competing segments

IPR - IP Rights

IPR02 - Sponsor support must be acknowledged in patent applications and patent issuing

IPR04 - Title to U of M, non-exclusive, non-transferable, irrevocable, paid-up license to Federal Government

IPR12 - PI obligation to report inventions (and other IP) to the sponsor (and to PTM per policy)

PRP - Property

PRP02 - U of M receives title to equipment purchased or fabricated under this award

PRP03 - Sponsor may require transfer of equipment and its title upon award completion for those items costing in excess of $5000

PUB - Publications

PUB02 - U of M will place an acknowledgement of sponsor support and a disclaimer, as appropriate, on any publication generated by this award or reporting on the award results and/or activity

PUB07 - Peer-reviewed articles must be submitted to NIH Pubmed Central (PMC) upon acceptance for publication and made publicly available within 12 months Cite PMC ID number in progress reports/proposals

RBD - Rebudgeting

RBD10 - Rebudgeting is allowed as long as there is no change in work scope

REG - Regulatory

REG01 - 45 CFR Part 74 - Code of Federal Regulations as it pertains to institutions of higher education

REG03 - OMB Circular A-21 (www whitehouse gov/omb/circulars/a021/a021 html)

REG04 - OMB Circular A-110 (www whitehouse gov/omb/circulars/a110/a110 html)

REG05 - OMB Circular A-133 (www whitehouse gov/omb/circulars/a133/a133 html)

REG09 - Subject to Streamlined Noncompeting Award Procedures (SNAP)

REG10 - Federal Demonstration Partnership (FDP) guidelines (www thefdp org)

REG19 - NIH Grants Policy Statement (grants nih gov/grants/policy/nihgps_2003)

UMN - U of M Requirements

UMN01 - The U of M Effort Certification system must be used to reflect all U of M staff committed effort on this award, whether or not any salary is charged to the award

UMN02 - Salary cost transfers cannot be made after effort is submitted

UMN03 - All U of M Policy and Guidelines relating to research must be followed and can be read in detail on the web at http //www ospa umn edu/policiesandprocedures/index htm

UMN04 - U of M Cost Transfer guidelines state transfers of non-salary expenses on accounts be clearly documented & made within 30 days after the end of the accounting period the charge was processed

NON-FINANCIAL REPORTING (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Milestones Tab)
Type: Report **Description:** Progress Report **Due Date:**
Comments: PHS 2590 - noncompeting continuation Must be submitted via the eRA Commons Due 45 days prior to the end of the budget period

INVOICE/BILLING INFORMATION

Basis of Payment: COST_REIMBURSE
Method of Payment: Electronic Fund Transfer
Invoice Form: GPC - GM_LOC
Interim Invoice Deadline: 0
Final Invoice Deadline: 0

Contact Name: Jacqueline Jones
Contact Phone:
Contact Fax:
Contact Email:
Contact Address: PSC-FMS (301) 443-3020
PARKLAWN BUILDING ROOM 16A-12
5600 FISHERS LANE
ROCKVILLE MD 20857

FINANCIAL REPORTING

Report Form ID: R_FFR
Reporting Frequency: Termination/Final
Interim Reporting Deadline: 90
Final Reporting Deadline: 90

GENERAL PROVISIONS

This document is intended to provide guidance in managing this award, to the Principal Investigator, Academic Unit, and to those who are noted as receiving this document Its intent is to provide general information regarding University and sponsor policies when given the award In addition, it is intended to reference specific conditions related to this award Specific questions should be addressed to the grant administrator from SPA noted below By accepting this award, the Principal Investigator acknowledges and agrees to abide

by all provisions of the sponsoring agency and the University of Minnesota policies related to sponsored projects management, including the Code of Conduct

U OF M CONTACTS

Administrative questions to SPA staff member:	Sowolo Abdullai	612/626-2724	abdu0092@umn edu
Billing and financial questions to SFR:	Julia Steinkopf	612/624-7033	gordo091@umn edu

Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Issue Date: 04/20/2011

Grant Number: 5R01AI089728-02

Principal Investigator(s):
Fang Li, PHD

Project Title: Receptor recognition mechanisms of coronaviruses

Edward F. Wink
University of Minnesota Twin Cities
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 554552070

Award e-mailed to: awards@umn edu

Budget Period: 05/01/2011 – 04/30/2012
Project Period: 05/15/2010 – 04/30/2015

Dear Business Official

The National Institutes of Health hereby awards a grant in the amount of $339,750 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MINNESOTA TWIN CITIES in support of the above referenced project This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system

Each publication, press release or other document that cites results from NIH grant-supported research must include an acknowledgment of NIH grant support and disclaimer such as "The project described was supported by Award Number R01AI089728 from the National Institute Of Allergy And Infectious Diseases The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Allergy And Infectious Diseases or the National Institutes of Health "

Award recipients are required to comply with the NIH Public Access Policy This includes submission to PubMed Central (PMC), upon acceptance for publication, an electronic version of a final peer-reviewed, manuscript resulting from research supported in whole or in part, with direct costs from National Institutes of Health The author's final peer-reviewed manuscript is defined as the final version accepted for journal publication, and includes all modifications from the publishing peer review process. For additional information, please visit http://publicaccess.nih.gov/

Award recipients must promote objectivity in research by establishing standards to ensure that the design, conduct and reporting of research funded under NIH-funded awards are not biased by a conflicting financial interest of an Investigator Investigator is defined as the Principal Investigator and any other person who is responsible for the design, conduct, or reporting of NIH-funded research or proposed research, including the Investigator's spouse and dependent children. Awardees must have a written administrative process to identify and manage financial conflict of interest and must inform Investigators of the conflict of interest policy and of the Investigators' responsibilities Prior to expenditure of these awarded funds, the Awardee must report to the NIH Awarding Component the existence of a conflicting interest and within 60 days of any new conflicting interests identified after the initial report Awardees must comply with these and all other aspects of 42 CFR Part 50, Subpart F. These requirements also apply to subgrantees, contractors, or collaborators engaged by the Awardee under this award The NIH website http //grants.nih.gov/grants/policy/coi/index.htm provides additional information

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Dawn M Mitchum
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI089728-02

Award Calculation (U.S. Dollars)

Federal Direct Costs	$225,000
Federal F&A Costs	$114,750
Approved Budget	$339,750
Federal Share	$339,750
TOTAL FEDERAL AWARD AMOUNT	$339,750
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$339,750

SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
2	$339,750	$339,750
3	$377,500	$377,500
4	$377,500	$377,500
5	$377,500	$377,500

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:
CFDA Number: 93 855
EIN: 1416007513A1
Document Number: RAI089728A
Fiscal Year: 2011

IC	*CAN*	*2011*	*2012*	*2013*	*2014*
AI	8472364	$339,750	$377,500	$377,500	$377,500

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC M51C S / **OC** 414E / **Processed** MITCHUMD 04/19/2011

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI089728-02

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI089728-02

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following

a The grant program legislation and program regulation cited in this Notice of Award
b Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts
c 45 CFR Part 74 or 45 CFR Part 92 as applicable
d The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period
e This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http //grants nih gov/grants/policy/awardconditions.htm' for certain references cited above)

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase V Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP)

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration Should a consortium/subaward be issued under this award, a DUNS requirement must be included See http //grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information

This award may be subject to the Transparency Act subaward and executive compensation reporting requirements of 2 CFR Part 170 See http //grants nih gov/grants/policy/awardconditions htm for the full NIH award term implementing this requirement and additional award applicability information

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory For more information, see NOT-OD-08-033 and the Public Access website http //publicaccess nih.gov/

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI089728-02

NIH is currently funded through a Continuing Resolution at the FY2010 level (See NIH Guide Notice NOT-OD-11-015) http //grants.nih.gov/grants/guide/notice-files/NOT-OD-11-015 html. Therefore, this non-competing award has been made at a level below that indicated in the previous Notice of Grant Award If the final appropriation permits, adjustments may be made up to the FY2011 funding plan level
****
In addition to the PI, any absence, replacement, or substantial reduction in effort of the following individual(s) below, requires the written prior approval of the National Institutes of Health awarding component

Dr Robert Geraghty,Associate Professor and co-PI

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions The Program Official is responsible for the scientific, programmatic and technical aspects of this project These individuals work together in overall project administration Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist Requests may be made via e-mail

Grants Management Specialist Cheryl Y Wall
Email wallcy@niaid nih gov **Phone** 301-451-2696 **Fax** 301-493-0597

Program Official: Frederick J Cassels
Email casselsf@niaid nih.gov **Phone** 301-402-8601 **Fax** 301-496-8030

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI089728-02

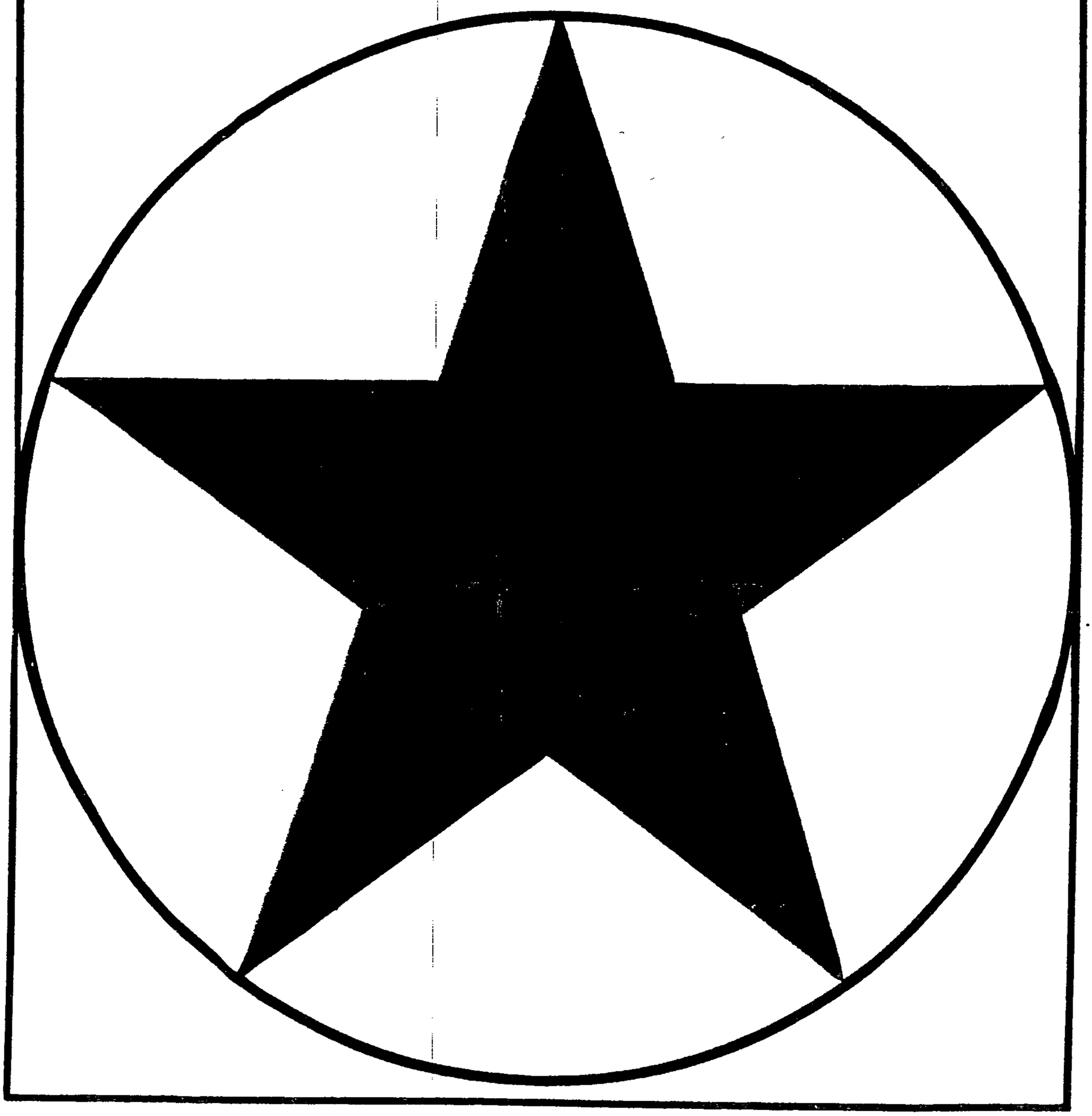
INSTITUTION: UNIVERSITY OF MINNESOTA TWIN CITIES

Budget	*Year 2*	*Year 3*	*Year 4*	*Year 5*
TOTAL FEDERAL DC	$225,000	$250,000	$250,000	$250,000

TOTAL FEDERAL F&A	$114,750	$127,500	$127,500	$127,500
TOTAL COST	$339,750	$377,500	$377,500	$377,500

Facilities and Administrative Costs	***Year 2***	***Year 3***	***Year 4***	***Year 5***
F&A Cost Rate 1	51%	51%	51%	51%
F&A Cost Base 1	$225,000	$250,000	$250,000	$250,000
F&A Costs 1	$114,750	$127,500	$127,500	$127,500

Please Mail All Pages Prior To Page Separator



AWARD SETUP INFORMATION PAGE **GA** CA **DATE** 5/4/11

CON 31200 **PROJECT** 00013782

_____New eTool Completed ✓ No info in eTool _____

_____PreAward – see bottom of page for information

✓ Modification, original Award = CON -22345 (this is the award that will be updated)

_____Stimulus Funding

INVOICING AND FINANCIAL REPORTING INFORMATION CAN BE FOUND ON THE FOLLOWING PAGE(S) IN THE AWARD DOCUMENT _____

TERMS AND CONDITIONS

_____ are attached _____ are in eTool

_____ are saved in MyNetworkPlaces\Award Setup\Award Setup-submissions TAB_____

(\\ovpr.ad.umn.edu\ovpr$\Shared\Groups\Award Setup)

BUDGET INFORMATION FOR FUNDS PROVIDED BY SPONSOR – for new awards only

_____ Fund _____ DeptID

_____ is in eTool

_____See attached PS-friendly budget

COST SHARE INFORMATION – for new awards only (GA updates the budgets for modifications)

_____Fund _____ DeptID _____ Program _____ Chartfield 1 _____ Chartfield 2 _____Emplid

_____ is in eTool

_____See attached PS-friendly budget

_____**NEW PROJECT NEEDED** - For new projects on existing awards, please enter relevant information in the eTool or on separate page and include a completed PS-friendly budget.

ANY ADDITIONAL INFORMATION NEEDED FOR SET UP:

*Please see updates and comments for NOGA in eTool to proposal CON-31200.

PUT AWARD FILE IN THE FOLLOWING ORDER:

Agency award documents
Guidance documents (to be included with award emailed to PI/Dept)
Award Imaging Divider page (yellow star sheet)
Award Setup Information page (this page)
PS-friendly budget (if any)
Terms and Conditions spreadsheet (if any)
Negotiation notes, proposal correspondence, proposal, etc.

SET ALL NEGOTIATE AWARD NEGOTIATION TYPES TO COMPLETED EXCEPT FOR THE ONE FOR CONTRACT ENTRY AND THE ORIGINAL ONE CREATED BY FILES

PREAWARD INFORMATION REQUIRED - Provide information for each project

Note: No cost share information is required for PreAwards.

_____Fund Budget accounts to be set up at $0 are listed below - OR - ____See Attached

_____DeptID Remember to include 810500 if F&A should be charged

_____Project _____ _____ _____ _____ _____

_____Function _____ _____ _____ _____ _____

_____F&A rate & base _____ _____ _____ _____ _____

_____Rate Type *(Research, Other, Training, Off Campus, ClinTrial-nonfed, CREE2, CREES, DOD contract, Equip, Foundation, Hormel, Instruction, SBIR/STTR Phase I, State)*

___/___/_____ End Date of PreAward / Advance Project to be entered in KK_Child and KK_Parent

UNIVERSITY OF MINNESOTA

ReportID: UMFGM017F

PROPOSAL SUMMARY

Run Date: 04/22/2011
Run Time: 09:04:47 AM

Status **4 AwdB Award Sent to GA**

Grant Administrator: Abdullai,Sowolo Armina

PRF #	683893	Proposal #/Version	CON000000031200/A101
EGMS Proposal #		Sponsor Award #	5R01AI089728-02
Proposal Type	Non-Competing Continuation	Concurrent	N
Previous Award		SubContract	N
Purpose			
Title	Receptor recognition mechanisms of coronaviruses		
Principal Investigator	Li,Fang	lifang@umn edu	

Budget Summary

Start Date	05/01/2011	End Date	04/30/2012	
Direct Costs	$250,000 00	Indirect Costs	$127,500 00	Total Costs $377,500 00

Sponsor: NIH Nat'l Inst of Allergy & Infect Dis

Submission Information

Due Date	03/16/2011	Date Type	Receipt Date
Submission Method	Electronically Only	# of Copies Submitted	

Mailing Address

future 5/1/11

Comments/Corrections

REPA	__verified	__email sent for _____		
IRB	__verified	__pending	__email sent	__Not Applicable
IACUC	__verified	__pending	__email sent	__Not Applicable
IBC	__verified	__pending	__email sent	__Not Applicable
F&A rate	__at full or agency limit	__waiver on file	__waiver requested	
Cost share documented?		__yes	__no	__Not Applicable
ICR sharing?		__yes	__no	

3002-11718-00013782

PS Project: 13782

PI NAME: Fang Li

TITLE:

Proposed	$377,500
AWARDED TOTAL	$339,750

0.9000000000

OBJECT	DESCRIPTION	PROPOSED	PRORATED	Difference
700100	Academic Salaries	51,083	45,975	5,108
700300	Acad Sal-Stdts-Grad/Prof/Trng		-	-
700500	Civil Service & Union Salaries		-	-
	Post-Doc	84,803	76,323	8,480
	Acad Professional Salary		-	-
710100	Acad Fringe Benefits	18,391	16,552	1,839
710300	Acad Sal-Stdts Fringe Benefits		-	-
	Acad Professional Fringe		-	-
	Civil Service & Union Fringe		-	-
	Post-Doc	21,542	19,388	
	Undergrad salary		-	-
	Consulant Costs	10,000	9,000	1,000
730200	Professional Services		-	-
810100	Research Subject Payments		-	-
720100	General Operating Supplies		-	-
720300	General Operating Services		-	-
720100	Computer/supplies<2500		-	-
720300	Print/Dupl/Binding/Graphics		-	-
720300	Sponsored Publication Costs	2,500	2,250	250
720200	Lab/Medical Supplies	56,381	50,743	5,638
720400	Lab/Medical Services		-	-
750100	Computer Software		-	-
720300	Courier/Mailing Services		-	-
720100	Postage		-	-
720500	Telephone		-	-
720500	Telephone-Long Distance	800	720	80
810200	Participant Expenses		-	-
730250	Medical/Dental Health Care	-	-	-
720600	Travel-Domestic/Foreign/other	3,000	2,700	300
			-	-
7900	Reserve-SPA	-	-	-
810300	Subcontract		-	-
810300/810402	Subcontract #2		-	-
810300/810403	Subcontract #3		-	-
780100	Repairs & Maintenance	1,500	1,350	150
770100	Rent/Lease		-	-
770150	Rent/Leases-Bldg/Facilities	-	-	-
770200		-	-	-
770250		-	-	-
168100	Equipment		-	-
168100	Computer Equipment		-	-
810600	Institutional Allowance	-	-	-
	TOTAL DIRECT CHARGES	250,000	225,000	25,000
8500	F & A Costs	127,500	114,750	12,750
	TOTAL	377,500	339,750	37,750

GDES INFORMATION:

	GA Code	
	AC Code	
	Grant Grp (CFDA)	
	Grantor Code 0000-	
28591	Proposal No	
	Award No	
12920		
12920		
2751	State Date	
	End Date	
	Report Date	
	Start Month (FY)	
	End Month (FY)	
		N/A
	IDC Rate	51.00%

CON-31200
F:CON-22345
683893
SA
RCR-mut

Subject: NoA: 5 R01 AI089728-02 PI: Li, Fang
From: <era-notify@mail.nih.gov>
Date: Wed, 20 Apr 2011 16:57:24 -0400
To: <awards@umn.edu>

Grant Number: 5 R01 AI089728-02
Principal Investigator: Li, Fang
Project Title: Receptor recognition mechanisms of coronaviruses
Institution: UNIVERSITY OF MINNESOTA
CFDA: 93.855
OC: 414E
PCC: M51C S
Award Issue Date: 04/20/2011
Grants Management Officer: Mitchum, Dawn
Program Official: Cassels, Frederick
Grants Specialist: Wall, Cheryl

*** This is an automated notification - Please do not reply to this message. ***

temp10197.pdf	**Content-Type:**	application/pdf
	Content-Encoding:	base64

(CON-22345)
683893

CON- 31200

Status Information

General Grant Information	
Status:	Pending administrative review. Refer any questions to Program Official or Grants Management Specialist
Institution Name:	UNIVERSITY OF MINNESOTA
School Name:	MEDICAL SCHOOL
School Category:	SCHOOLS OF MEDICINE
Division Name:	NONE
Department Name:	PHARMACOLOGY
PI Name:	Li, Fang
Application ID:	5R01AI089728-02
Proposal Title:	Receptor recognition mechanisms of coronaviruses
Proposal Receipt Date:	03/11/2011
Last Status Update Date:	05/10/2010
Budget Start Date	05/01/2011
Budget End Date	04/30/2012
Progress Report Due Date:	03/16/2011
Current Award Notice Date:	
Application Source:	Esnap
Project Period Begin Date:	05/15/2010
Project Period End Date:	04/30/2015
eApplication Status:	
FOA:	[PA07-070] - RESEARCH PROJECT GRANT (PARENT R01)
NIH Appl. ID:	8072722

Other Relevant Documents
e-Application
Progress Report Face Page
Additions for Review (0 documents)

Status History

Effective Date	Status Message

Institute or Center Assignment

Institute or Center	Assignment Date
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES **(Primary)**	05/10/2010

Application Information		Study Section		Advisory Council(AC) Information
Award Document Number	RAI089728A	Scientific Review Group	VIRA	
FSR Accepted Code	N	Council Meeting Date(YYYY/MM)	2011/00	
Snap Indicator Code	Y			
Review Outcome Available	No			
Summary Statement Available	No			
Early Stage Investigator Eligible				
New Investigator Eligible				

Reference Letter(s)

This list shows Reference Letters associated with this particular Grant Application Principal Investigator can see a list of all Reference Letters within Personal Profile - Reference Letters section on eRA Commons

Contacts

Administration	Name	Phone	Email
Grants Management Specialist(GMS)	Wall, Cheryl Y	301-451-2696	wallcy@niaid nih gov
Program Official(PO)	Cassels, Frederick J	301-402-8601	casselsf@niaid nih gov

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National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland
20892

Department of Health and Human Services

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UNIVERSITY OF MINNESOTA

PROPOSAL SUMMARY

ReportID: UMFGM017F

Run Date: 03/10/2011
Run Time: 03:12:28 PM

Status 2 PropB ~~Elect Received~~ at SPA

INSTITUTION APPROVED
03.11.11 NLB

Grant Administrator Sowlo

PRF #	683893	Proposal #/Version	CON000000031200/V101
EGMS Proposal #		Sponsor Award #	
Proposal Type	Non-Competing Continuation	Concurrent	N
Previous Award		SubContract	N
Purpose	con-22345/00013782		
Title	Receptor recognition mechanisms of coronaviruses		
Principal Investigator	Li,Fang	lifang@umn edu	

Budget Summary

Start Date	05/01/2011	End Date	04/30/2012	
Direct Costs	~~$0 00~~ $250,000	Indirect Costs	~~$0 00~~ $127,500	Total Costs ~~$0 00~~ $377,500
Sponsor	National Institutes of Health (NIH)			

Submission Information

Due Date	03/16/2011	Date Type	Receipt Date
Submission Method		# of Copies Submitted	

Mailing Address

eSNAP

moved/approved
3/11/11

Comments/Corrections

REPA	✓ verified	__email sent for		
IRB	__verified	__pending	__email sent	__Not Applicable
IACUC	__verified	__pending	__email sent	✓ Not Applicable
IBC	✓ verified	__pending	__email sent	__Not Applicable
F&A rate 51% MTDC	✓ at full or agency limit	__waiver on file	__waiver requested	
Cost share documented?		__yes	✓ no	__Not Applicable
ICR sharing?		__yes	✓ no	

*eSNAP 3/11/11 - 2:21pm EDT-ED
*log out

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ESNAP
FEDERAL
NON-FED
G-GOV

NO ATTACHMENT

SUBAWARDS

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E

UNIVERSITY OF MINNESOTA PRF

EGMS ID: PRF ID: 683893

Proposal Routing Form for External Research, Training or Public Service Support — PS Proposal ID

03-10-2011 P03:16

1.	Sponsor Name	**National Institutes of Health**	**Primary Funding Source**
2.	Proposal Title	**Receptor recognition mechanisms of coronaviruses**	
3.	Sponsor Due Date/Time	**03/16/2011 - 5pm CST**	**Receipt**
4.	RFA/RFP/CFDA/FOA#	**PA-07-070** C O N 1 0 0 0 0 0 0 0 3 1 2 0 0	
5.	Submission Medium	**Electronic [other than Grants.gov] Submission via eRA Commons ESNAP.**	
6.	University Contact	**Weber, Lynn F** (612) 626-6902	weber117@umn.edu
7.	Administering Dept	**PHCL PHARMACOLOGY ADMIN**	Department ID 11718

8. Principal Investigator

Li, Fang			Paid	Cost Share	PI	Key
lifang	ID 3694715	(612) 625-6149	50	0	X	X
ASSISTANT PROFESSOR [9403]	PHCL PHARMACOLOGY ADMIN	DeptID 11718				

2009

9. Investigator(s)

Geraghty, Robert J.			Paid	Cost Share	PI	Key
gerag012	ID 1235946	(612) 625-3281	8	0		X
ASSOCIATE PROFESSOR [9402]	DRUG DESIGN, CENTER FOR	DeptID 11345				

2010

10. Proposal is for

	Basic %	Applied %	Development %	Total %
Research	100	0	0	100

11. Proposal is

	Agency Award #	EFS Award #	Primary Project
Continuation	5R01 AI089728-02	30021171800013782	

12. Proposed Duration & Amount Requested

	Start	End	Direct $$	Indirect $$	F&A Rate	Total
Initial/Current Budget	05/01/2011	04/30/2012	250,000	127,500	51% MTDC	377,500
All Years	05/01/2011	04/30/2012	250,000	127,500	51% MTDC	377,500

[X] Departmental Directions to SPA for Award Set Up
Proposal budget has been prepared outside of EGMS: Department will furnish PeopleSoft-friendly budget to SPA prior to receipt of the award; SPA may pro-rate at the time of award if needed to accommodate a reduced award amount.

[X] **F&A Rate Used** 51% MTDC
Full F&A Rate

F&A Comments

13. Project Involves

[X] **Recombinant DNA, Infectious Agents or Biological Toxins** — There will be recombinant DNA, infectious agents or biological toxins used

Registration status of this project with the Institutional Biosafety Committee — Status: Approved; Study Code #: 1002H77733; Approval Date: 03/10/2011

[X] **Chemicals** — There will be chemicals used — The appropriate chemical safety training and hazardous waste training records have been completed

14. Subawards [ ]

15.	Conflict of Interest	☒	**There is not a potential financial and/or business conflict of interest** **REPA #** **Approval Date** **Comments**	
16.	Inventions	☐		
17.	Space	☒	**There is sufficient and suitable space to house this project presently assigned to the principal investigator's or other investigator's department or college.**	**3-284** **Nils Hasselmo Hall**
18.	Resources/Space/Staff	☒	**This project involves University resources, space or staff from more than one department**	
19.	Indirect Cost Recovery	☒	**Academic units have chosen to share ICR revenue and be shared by contributions**	
20.	Matching and Cost Sharing	☐		
21.	Program Income	☐		
22.	International Component	☐		
23.	Fairview Health Services	☐		

24. PROPOSAL ABSTRACT or EXECUTIVE SUMMARY

25. NOTES

03/03/11	**hinsk001**	**Per agreement, 11.5% F&A shared with CDD, deptID 11345**
03/07/11	**weber117**	**Changes made to PRF Geraghty effort was listed incorrectly as 8% and should have been 10%. Budget changed to include 3rd Postdoc instead of a Grad RA.**
03/08/11	**weber117**	**Changes made to PRF. Geraghty effort changed back to 8%.**

APPROVALS and CERTIFICATIONS

Principal Investigator:

(1) I certify the information submitted within the application is true, complete and accurate to the best of the PI's knowledge,
(2) that any false, ficticious, or fraudulent statements or claims may subject the PI to criminal, civil, or administrative penalties, and
(3) that the PI agrees to accept the responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of the application

Investigator(s):

The information provided on this form and in the accompanying proposal is correct to the best of my knowledge Equipment budgeted in this application is not otherwise available for use on this project from existing departmental or collegiate inventories In the event this application is awarded, I (we) agree to abide by all applicable institutional and sponsoring agency policies and procedures including the Intellectual Property Policy of the University of Minnesota and to follow commonly accepted scientific practices in recording and maintaining records of research I (we) certify that I (we) have read the University of Minnesota Code of Conduct policy and agree to abide by the rights and responsibilities as identified therein

Signature Fang Li — **03-08-2011**
Fang Li (lifang) Principal Investigator — **Date**

Department Head(s) and/or Division Head(s)· Academic Dean(s), Chancellor or Provost

We certify that the above statements are correct to the best of our knowledge The commitment of departmental and collegiate resources, if any, has been noted and approved We agree that the scientific objectives of this application are in keeping with departmental and collegiate goals We certify that the individuals from our unit(s) listed on this PRF have filed a current Report of External Professional Activities (REPA)

Signature DeJarlais, Carrie L — **03-08-2011**
DeJarlais, Carrie L (dejar001) Dept Administrator/Representative — **Date**

Signature Loh, Horace H — **03-08-2011**
Loh, Horace H (lohxx001) Dept Head/Delegate — **Date**

Signature Hinske, Liz — **03-08-2011**
Hinske, Liz (hinsk001) Dept Administrator/Representative — **Date**

Signature Vince, Robert — **03-08-2011**
Vince, Robert (vince001) Dept Head/Delegate — **Date**

Signature Paller, Mark S — **03-08-2011**
Paller, Mark S (palle001) Research Associate Dean/Delegate — **Date**

Signature tmeddean — **03-08-2011**
tmeddean (tmeddean) Research Associate Dean/Delegate — **Date**

Signature Segal, Rosalyn A — **03-08-2011**
Segal, Rosalyn A (rsegal) Research Associate Dean/Delegate — **Date**

Subject: PRF# 683893 Fang Li NIH eSNAP Progress Report
From: Lynn Weber <weber117@UMN.EDU>
Date: Thu, 10 Mar 2011 14:54:18 -0600
To: "proposal@umn.edu" <proposal@UMN.EDU>

--

Lynn Weber
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University of Minnesota
Medical School
6-120 Jackson Hall
321 Church Street SE
Minneapolis, MN 55455
Phone 612-626-6902
Fax 612-625-8408
Email weber117@umn edu
http //www pharmacology med umn edu/

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History List

Reviewer Name	Decision	Notification Sent	Decision Rendered
NIH		Fri 03/11/2011 14 21 9 EDT	
Wink, Edward F	Submit	Fri 03/11/2011 11 29 32 EDT	Fri 03/11/2011 14 21 9 EDT
Abdullai, Sowolo	Route	Fri 03/11/2011 11 18 45 EDT	Fri 03/11/2011 11 29 32 EDT
Abdullai, Sowolo	Recall	Fri 03/11/2011 11 18 45 EDT	Fri 03/11/2011 11 18 45 EDT
McKoskey, Kevin		Thu 03/10/2011 15 48 27 EDT	Fri 03/11/2011 11 18 45 EDT
Li, Fang	Route	Fri 01/21/2011 11 49 42 EDT	Thu 03/10/2011 15 48 27 EDT

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Department of Health and Human Services

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OMB No. 0925-0001

Grant Number 5R01AI89728-2	**Total Project Period** From 05/15/2010 To 04/30/2015
EIN: **Review Group:** 1416007513A1 VIRA	**Requested Budget Period:** From 05/01/2011 To 04/30/2012
Title of Project: Receptor recognition mechanisms of coronaviruses	**Due Date:** 03/15/2011 **Submitted Date:** 03/11/2011
Program Director/Principal Investigator: Fang Li UNIVERSITY OF MINNESOTA TWIN CITIES Department of Pharmacology 6-120 Jackson Hall 321 Church Street S E MINNEAPOLIS , MN 554552070 **Phone Number:** 612-625-6149 **Fax Number:** **Email Address:** lifang@umn edu	**Applicant Organization:** UNIVERSITY OF MINNESOTA TWIN CITIES UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS , MN 554552070 **Department:** PHARMACOLOGY **Major Subdivision:** MEDICAL SCHOOL
Administrative Official: Sowolo Abdullai UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS , MN 554552070 **Phone Number:** 6126245599 **Fax Number:** **Email Address:** abdu0092@umn edu	**Signing Official:** Edward F Wink UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS , MN 554552070 **Phone Number:** 612 624-5599 **Fax Number:** **Email Address:** ewink@umn edu
Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** FWA00000312 **Phase III Clinical Trial:** ☒ No ☐ Yes	**Vertebrate Animals:** ☒ No ☐ Yes **Inventions and Patents:** ☒ No ☐ Yes ☐ Previously Reported ☐ Not Previously Reported
Program Income: ☒ No ☐ Yes	

Budget Period	Anticipated Amount	Source
F&A Changes:		

Primary Project/Performance Site Location		
Organizational Name University of Minnesota		
DUNS 5559179960000		
Street 1 3-284 Nils Hasselmo Hall	Street 2 312 Church Street SE	
City Minneapolis	County Hennepin	State MN
Province	Country UNITED STATES	Zip/Postal Code 554550215
Congressional Districts MN-005		

Additional Project/Performance Site Location			
Organizational Name University of of Minnesota			
DUNS 5559179960000			
Street 1 7-227 Phillips Wangensteen Building		Street 2 516 Delaware Street SE	
City Minneapolis	County Hennepin		State MN
Province	Country UNITED STATES		Zip/Postal Code 55455
Project/Performance Site Congressional Districts MN-005			

Program Director/Principal Investigator:	Grant Number
Fang Li	5R01AI89728-2
Applicant Organization:	**Period Covered by this Report:**
UNIVERSITY OF MINNESOTA TWIN CITIES	05/15/2010 - 04/30/2011

Title of Project:

Receptor recognition mechanisms of coronaviruses

SNAP Questions:

Has there been a change in the other support of Senior/Key Personnel since the last reporting period?

☒ No ☐ Yes

Justification:

Will there be, in the next budget period, a significant change in the level of effort for the PD/PI or other Senior/Key Personnel designated on the Notice of Award from what was approved for this project?

☒ No ☐ Yes

Justification:

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☒ No ☐ Yes

Justification:

Changes in Select Agent Research? ☒ No ☐ Yes

Changes in Multiple PD/PI Leadership plan? ☒ No ☐ Yes

Change in human embryonic stem cell (hESC) line(s) used? ☒ No ☐ Yes

Justification:

Human Subject Education Requirement:

Has the Involvement of Human Subjects changed since previous submission? ☒ No ☐ Yes

Has the Involvement of Animal Subjects changed since previous submission? ☒ No ☐ Yes

Publications:

Valid NIHMSID:	Citation ID:	Citation Source:	Citation Text:

OMB No. 0925-0001

Grant Number 5R01AI89728-2	**Total Project Period** From 05/15/2010 To. 04/30/2015
EIN. **Review Group:** 1416007513A1 VIRA	**Requested Budget Period:** From 05/01/2011 To 04/30/2012
Title of Project: Receptor recognition mechanisms of coronaviruses	**Due Date:** 03/15/2011 **Submitted Date:**

Program Director/Principal Investigator: Fang Li UNIVERSITY OF MINNESOTA TWIN CITIES Department of Pharmacology 6-120 Jackson Hall 321 Church Street S E MINNEAPOLIS , MN 554552070 **Phone Number:** 612-625-6149 **Fax Number:** **Email Address:** lifang@umn edu	**Applicant Organization:** UNIVERSITY OF MINNESOTA TWIN CITIES UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS , MN 554552070 **Department:** PHARMACOLOGY **Major Subdivision:** MEDICAL SCHOOL
Administrative Official: Sowolo Abdullai UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS , MN 554552070 **Phone Number:** 6126245599 **Fax Number:** **Email Address:** abdu0092@umn edu	**Signing Official:** BW ~~Kevin McKoskey~~ 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS , MN 554552070 **Phone Number:** 612-624-5599 **Fax Number:** 612-624-4843 **Email Address:** kevin@umn edu
Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** FWA00000312 **Phase III Clinical Trial:** ☒ No ☐ Yes	**Vertebrate Animals:** ☒ No ☐ Yes **Inventions and Patents:** ☒ No ☐ Yes ☐ **Previously Reported** ☐ **Not Previously Reported**
Program Income: ☒ No ☐ Yes	

Budget Period	**Anticipated Amount**	**Source**
F&A Changes:		

Primary Project/Performance Site Location		
Organizational Name University of Minnesota		
DUNS 5559179960000		
Street 1 3-284 Nils Hasselmo Hall	Street 2 312 Church Street SE	
City Minneapolis	County Hennepin	State MN
Province	Country UNITED STATES	Zip/Postal Code 554550215
Congressional Districts MN-005		

Additional Project/Performance Site Location			
Organizational Name University of of Minnesota			
DUNS 5559179960000			
Street 1 7-227 Phillips Wangensteen Building		Street 2 516 Delaware Street SE	
City Minneapolis	County Hennepin		State MN
Province	Country UNITED STATES		Zip/Postal Code 55455
Project/Performance Site Congressional Districts MN-005			

Program Director/Principal Investigator: Fang Li	**Grant Number** 5R01AI89728-2
Applicant Organization: UNIVERSITY OF MINNESOTA TWIN CITIES	**Period Covered by this Report:** 05/15/2010 - 04/30/2011
Title of Project: Receptor recognition mechanisms of coronaviruses	

SNAP Questions:

Has there been a change in the other support of Senior/Key Personnel since the last reporting period?

☒ **No** ☐ **Yes**

Justification:

Will there be, in the next budget period, a significant change in the level of effort for the PD/PI or other Senior/Key Personnel designated on the Notice of Award from what was approved for this project?

☒ **No** ☐ **Yes**

Justification:

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☒ **No** ☐ **Yes**

Justification:

Changes in Select Agent Research? ☒ **No** ☐ **Yes**

Changes in Multiple PD/PI Leadership plan? ☒ **No** ☐ **Yes**

Change in human embryonic stem cell (hESC) line(s) used? ☒ **No** ☐ **Yes**

Justification:

Human Subject Education Requirement:

Has the Involvement of Human Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Has the Involvement of Animal Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Publications:

Valid NIHMSID: **Citation ID:** **Citation Source:** **Citation Text:**

Cover Letter: File is not uploaded
Research Accomplishments: File is not uploaded
Other Support File. File is not uploaded

All Personnel Report

Program Director/Principal Investigator:	Grant Number
Fang Li	5R01AI89728-2

Name:	Commons ID:	Degree(s) Name:	SSN:	Months Devoted to Project		
Fang Li	FANGLI	PHD	XXX-XX [redacted]			
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
PD/PI			[redacted]	6 0		

Name:	Commons ID:	Degree(s) Name:	SSN:	Months Devoted to Project		
Robert James Geraghty	ROBERT GERAGHTY	PhD	XXX-XX [redacted]			
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-Investigator			[redacted]	1 0		

Name:	Commons ID:	Degree(s) Name:	SSN:	Months Devoted to Project		
Guiqing Peng	GUIQINGPENG	PhD	XXX-XX [redacted]			
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			[redacted]	12 0		

Name:	Commons ID:	Degree(s) Name:	SSN:	Months Devoted to Project		
Kailang Wu	KAILANGWU	PhD	XXX-XX [redacted]			
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			[redacted]	12 0		

Name:	Commons ID:	Degree(s) Name:	SSN:	Months Devoted to Project		
Wenbo Zhou	WENBOZHOU	PhD	XXX-XX [redacted]			
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			[redacted]	4 8		

Progress report

A. Specific aims

Coronaviruses use a variety of cellular receptors and co-receptors including proteins and sugars. The diverse usage of receptors has allowed coronaviruses to infect a wide range of mammalian and avian species, and cause respiratory, enteric, systemic and neurological diseases How coronaviruses have evolved to do so has been a major puzzle in virology. To solve this puzzle, this NIH-funded project "receptor recognition mechanisms of coronaviruses" investigates the molecular and structural basis for the complex receptor-recognition mechanisms of coronaviruses.

There are three specific aims in this project Aim 1 focuses on the receptor recognition mechanisms of group-I NL63 coronavirus (NL63-CoV). Aim 2 focuses on the receptor recognition mechanisms of other group-1 coronaviruses such as feline infectious peritonitis (FIPV) and porcine transmissible gastroenteritis virus (TGEV) Aim 3 focuses on the receptor recognition mechanisms of group-II SARS coronavirus (SARS-CoV) The research approaches of this proposal include crystallographic analysis of the virus-receptor interfaces, identification of structural elements important for virus-receptor interactions, and characterization of these elements using crystallographic, biochemical, molecular, and virological tools.

B. Studies and Results

Our research in the past funding year has tapped into all of the three aims. Wu et al. (2011, *Journal of Virology*) addresses specific aim 1. In this study, we investigated the function of a virus-binding hotspot on human angiotensin-conversing enzyme2 (ACE2) that is critical for the binding of both NL63-CoV and SARS-CoV. The hotspot consists of a salt bridge surrounded by hydrophobic tunnel walls Mutations that disturb the hotspot structure have significant effects on virus/receptor interactions, revealing critical energy contributions from the hotspot structure The tunnel structure at the NL63-CoV/ACE2 interface is more compact than that at the SARS-CoV/ACE2 interface, and hence virus/ACE2 binding affinities are decreased either by NL63-CoV mutations that decrease the tunnel space or by SARS-CoV mutations that increase the tunnel space. Furthermore, NL63-CoV receptor-binding domain (RBD) inhibits ACE2-dependent infection by SARS-CoV, a successful application of our hotspot theory that has the potential to become a novel antiviral strategy against SARS-CoV infections These results suggest that the structural features of the hotspot on ACE2 were among the driving forces for the convergent evolution of NL63-CoV and SARS-CoV

Specific aim 2 has been partially addressed by Peng et al. (2011, in preparation) From our previous crystal structure of NL63-CoV RBD complexed with ACE2, we hypothesized that FIPV RBD has the same core structure as NL63-CoV RBD, but changes in three receptor-binding loops switch the receptor specificity from ACE2 in NL63-CoV to aminopeptidase-N (APN) in FIPV. In this study, we mutated the receptor-binding loops in FIPV RBD, and found that FIPV lost its APN-binding specificity, in agreement with our hypothesis. Moreover, because TGEV, but not FIPV, recognizes sugars in addition to APN, we have isolated and characterized the sugar-binding domain of TGEV spike protein that is separate from the APN-binding domain.

Wu et al (2011, in preparation) address specific aim 3. Since the SARS epidemic, various mutations occurred on the RBD of SARS-CoV strains isolated from humans and palm civets. It was poorly understood why these mutations were selected or how SARS-CoV may further evolve. In this study, we investigated the role of each of these mutations in viral interactions with its host receptor ACE2, and provided structural basis for each of these mutations These mutations were clustered around two "viral adaptation hotspots" on ACE2, enhanced viral interactions with either human or civet ACE2, and were therefore viral adaptations to either receptor. Overall, our study has elucidated detailed molecular and structural mechanism of host receptor adaptation by SARS-CoV, which paves the way for a mechanism-based prediction of potential future evolution of SARS-CoV.

In addition, during our structural studies of coronavirus/receptor complexes, we have developed a novel method for structure determinations of protein complexes (Li and Li, 2011, *Structure*) In this study, we propose a strategy to facilitate structure determinations when only part of a new structure is known· after the molecular replacement (MR) search, structure determination of the unknown part of the new structure can be facilitated by cross-crystal averaging of the known part of the new structure with the search model. This strategy dramatically improves electron density in the unknown part of the new structure It has enabled us to determine the structures of NL63-CoV and SARS-CoV RBDs each complexed with their receptor ACE2 at moderate resolutions. In a test case, it also enabled automated model building when >50% of an antigen-antibody complex was absent. These results suggest that this averaging strategy can be routinely used after MR to enhance the interpretability of electron density associated with missing model.

C. Significance

The research findings obtained so far underscore the complexity and significance of receptor recognition by coronaviruses First, our studies are starting to sketch an evolutionary picture of coronaviruses, showing how either drastic changes in modular RBDs or subtle changes within each RBD of their spike proteins can lead to changes in their receptor specificities It was through these evolutionary changes in their spike proteins that coronaviruses were able to explore novel cellular receptors and expand their host ranges. Second, our studies reveal molecular and structural mechanisms of host receptor adaptation by SARS-CoV, paving the way for further evaluating the chances of SARS-CoV coming back to infect the human populations and for predicting corresponding evolutionary pathways of SARS-CoV Therefore, our research has important implications for both basic virology and viral epidemics.

D. Plans

For the next funding year, we will keep pursuing the three specific aims For aim 1, we will examine the interactions between the NL63-CoV spike protein and ACE2 from different animal species. More specifically, we will investigate how residue changes in the hotspot structure on animal ACE2 affects the usage of these animal ACE2 as cellular receptors by NL63-CoV. Furthermore, through the detailed NL63-CoV-spike/animal-ACE2 interactions, we will evaluate the chances of different animals serving as hosts for NL63-CoV and hence these animals as vectors for NL63-CoV-related human common colds

For aim 2, we will investigate the structural mechanism whereby the TGEV sugar-binding domain binds sugars and APN-binding domain binds APN We will prepare the complexes of each of these domains complexed with their respective receptor, and set up crystallization screens of these complexes We will also investigate the mechanism whereby FIPV spike protein kept its APN-binding function but lost its sugar-binding function through evolution.

For aim 3, we will investigate how SARS-CoV may evolve in the future We will construct two SARS-CoV spike proteins containing the combinations of the residues that were better adapted to human ACE2 or civet ACE2, respectively, and examine their binding affinities for human ACE2 and civet ACE2 using surface plasmon resonance assays, pseudotyped viral infection assays, and X-ray crystallography. We will also investigate several other mutations that have recently been identified in SARS-CoV RBD, examine how these mutations affect viral interactions with human and civet ACE2, and incorporate these mutations in our predictions of future SARS-CoV evolution. In addition, in collaboration with Dr. Zhengli Shi's group in Chinese Academy of Sciences, we will examine the interactions between SARS-CoV and ACE2 molecules from bat species

E. Publications (from the past project period)

1 Li, W., **Li, F.** (2011) Cross-crystal averaging with search models to improve molecular replacement phases. *Structure*, *19*, 155-161 (PMCID. PMC3037595)

2. Wu, K , Chen, L , Peng, G , Zhou, W., Pennell, C., Mansky, L , Geraghty, R., **Li, F.** (2011) A virus-binding hotspot on human angiotensin-converting enzyme 2 is critical for the binding of two different coronaviruses. *Journal of Virology*, *In Press*

F. Project Generated Resources

This research did not generate cell lines, DNA probes, animal models, protocols, or software De-identified raw data from the experiments and computer programs (CCP4 macromolecular crystallography scripts) used for protein structure determinations are available to anyone upon written request to the PI. Peer-reviewed publications accepted or published during the past project period are being or have been entered in NIHMS and PubMed Central

A Virus-binding Hotspot on Human Angiotensin-converting Enzyme 2 is Critical for the Binding of Two Different Coronaviruses

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Running title: NL63 and SARS viruses target a hotspot on human ACE2

Summary

How viruses evolve to select their receptor proteins for host cell entry is puzzling. We recently determined the crystal structures of NL63 coronavirus (NL63-CoV) and SARS coronavirus (SARS-CoV) receptor-binding domains (RBDs) each complexed with their common receptor, human angiotensin-converting enzyme 2 (hACE2), and proposed the existence of a virus-binding hotspot on hACE2. Here we investigated the function of this hypothetical hotspot using structure-guided biochemical and functional assays. The hotspot consists of a salt bridge surrounded by hydrophobic tunnel walls. Mutations that disturb the hotspot structure have significant effects on virus/receptor interactions, revealing critical energy contributions from the hotspot structure. The tunnel structure at the NL63-CoV/hACE2 interface is more compact than that at the SARS-CoV/hACE2 interface, and hence RBD/hACE2 binding affinities are decreased either by NL63-CoV mutations decreasing the tunnel space or by SARS-CoV mutations increasing the tunnel space. Furthermore, NL63-CoV RBD inhibits hACE2-dependent transduction by SARS-CoV spike protein, a successful application of the hotspot theory that has the potential to become a new antiviral strategy against SARS-CoV infections. These results suggest that the structural features of the hotspot on hACE2 were among the driving forces for the convergent evolution of NL63-CoV and SARS-CoV.

Introduction

Host receptor recognition by viruses is the first and essential step for viral infections. During the long history of evolutionary battles between viruses and hosts, viruses have evolved complex strategies for their receptor selections (2). Despite tremendous efforts to understand these strategies, the current picture of how viruses recognize their host receptors is still murky. Viruses exploit a wide variety of host cell surface molecules as their receptors. In addition to serving as receptors for viruses, these molecules are implicated in various host physiological functions such as cell adhesion, immune response, signaling pathways, proteolysis, or ion transport. On one hand, several viruses can share one host receptor. For example, coxsackievirus-adenovirus receptor, an immunoglobulin (Ig) superfamily member, is the receptor for both coxsackieviruses and adenoviruses (3). On the other hand, one virus can recognize several different host receptors. For example, herpes simplex viruses use one of at least three protein receptors: HVEM which is a tumor necrosis factor receptor family member (23), and nectin-1 and nectin-2 both of which are Ig superfamily members (8, 31). Understanding the pattern of host receptor recognition by viruses has important implications for viral evolution, pathogenesis, host range, tropism, cross-species infections, emerging viral epidemics, and virus-mediated gene targeting.

A key question regarding the evolution of host receptor recognition by viruses is what features of these receptor molecules make them targeted by viruses. The receptors for viruses can be proteins, carbohydrates, or lipids (2). Compared with carbohydrates and lipids, protein receptors in general have more structural features and thus are engaged in more specific and high-affinity binding interactions with viruses; they are the focus of

this study. Among protein receptors, some (such as cell adhesion molecules) are more abundant than others (such as proteases and ion transporters). Although the availability of abundant protein receptors to viruses is probably one of the reasons why they were selected by viruses as receptors (30), it is not clear whether receptor proteins, especially non-abundant receptor proteins, contain any structural features that make them targeted by viruses.

The structural features of receptor proteins can be identified by atomic structures of virus/receptor interfaces. Defined structural and functional receptor-binding domains (RBDs) have been identified in many viral surface glycoproteins. One or more receptor-binding motifs (RBMs) on these viral RBDs mediate the interactions with their receptor proteins. To date several crystal structures are available for viral RBDs complexed with their receptor proteins (1, 4, 5, 13, 18, 32, 33). Among these structures, only two reveal how different viral RBDs can bind to their common receptor protein: the structures of NL63 coronavirus (NL63-CoV) and SARS coronavirus (SARS-CoV) RBDs each complexed with their common receptor, human angiotensin-converting enzyme 2 (hACE2) (18, 32). Both NL63-CoV and SARS-CoV are important human viral pathogens. The former causes prevalent respiratory diseases (6, 29), whereas the latter was responsible for the worldwide epidemic of severe acute respiratory diseases (SARS) in 2002-2003 (12, 24). Coronavirus spike glycoproteins are envelope-anchored clove-shaped trimers (16). Each spike trimer contains three monomeric S1 heads that function in receptor binding, and a trimeric S2 stalk that functions in fusing viral envelope and host membrane. NL63-CoV and SARS-CoV RBDs are located in the S1 heads of their respective spike proteins. There is no structural homology in their RBD core structures or

RBMs (Fig. 1). The core structures of NL63-CoV and SARS-CoV RBDs are two-layer β-sandwich and single-layer β-sheet, respectively; the RBMs of NL63-CoV and SARS-CoV are three discontinuous short loops and one continuous long subdomain, respectively. Nevertheless, the two viral RBDs bind to the same three virus-binding motifs (VBMs) on hACE2 (18, 32).

Our previous structural studies lead to the hypothesis that a virus-binding hotspot exists on hACE2 and plays an important role in the binding of both NL63-CoV and SARS-CoV (32). This hypothetical hotspot consists of a critical Lys353-Asp38 salt bridge on hACE2 that is surrounded by four hydrophobic tunnel walls (Fig. 2A, 2B). Two of the tunnel walls, Tyr41 (top wall) and Asp37 (right wall), are contributed by hACE2, whereas the other two tunnel walls are contributed by the viruses: Tyr498 (bottom wall) and Ser535 (left wall) from NL63-CoV, or Tyr491 (bottom wall) and Thr487 (left wall) from SARS-CoV. Details of how this hypothetical hotspot may contribute to the virus/receptor interactions are unknown. In this study we use structure-guided biochemical and functional approaches to investigate the role of each of the components of the hotspot structure in the virus/receptor interactions. We then apply the hotspot theory to developing a potentially new antiviral strategy against SARS-CoV infections. We also discuss how the structural features of the hotspot drove the convergent evolution of two different viruses.

Materials and methods

Protein expression and purification

Soluble proteins including NL63-CoV RBD (residues 461-616), SARS-CoV RBD (residues 306-527) and hACE2 peptidase domain (residues 19-615) were expressed and purified as previously described (17, 18, 32). In brief, the proteins were expressed in insect cells using the Bac-to-Bac system (Life Technologies Inc.). Expression constructs (Invitrogen) all contained an N-terminal honeybee melittin signal sequence and a C-terminal His tag sequence. Mutations were introduced by PCR site-directed mutagenesis to the expression constructs. Recombinant baculoviruses were generated and amplified in Sf9 insect cells. The protein to be purified was harvested from Sf9 cell supernatants, loaded onto a Ni-NTA column, eluted from the Ni-NTA column with 0.5 M imidazole, and further purified by gel filtration chromatography on Superdex 200 (GE Healthcare). Fractions containing the purified protein were pooled together, loaded into an Amicon ultra-15 centrifugal filter unit (10,000 MW cutoff) (Millipore), and centrifuged at 10,000 rpm until the protein concentration reached 10 mg/ml.

RBD/hACE2 binding assays

Surface plasmon resonance assays were carried out using a Biacore 2000 as previously described (32). In brief, to measure the binding affinities between mutant viral RBDs and wild type hACE2, hACE2 was immobilized on a C5 sensor chip through direct covalent coupling via amine groups. The surface of the sensor chip was activated with N-hydroxysuccinimide (NHS), the receptor was injected and immobilized to the surface of the chip, and the remaining activated surface of the chip was blocked with ethanolamine. Soluble RBDs was introduced at a flow rate of 20 μl/min at different concentrations (62.5 nM, 125 nM, and 250 nM). k_{on}, k_{off}, and K_d were determined for the

RBD/receptor binding interactions using BIA-EVALUATIONS software. To measure the binding affinities between mutant hACE2 and wild type viral RBDs, RBDs were immobilized on the sensor chip and hACE2 was the soluble analyte. As negative controls, soluble RBDs or hACE2 were passed through an empty sensor chip, and buffer alone was passed through sensor chips containing RBDs or hACE2 as immobilized ligands.

Pseudotyped viral transduction assays

Transduction was assayed using murine leukemia viruses (MLVs) expressing β-galactosidase and pseudotyped with NL63-CoV or SARS-CoV spike protein. To prepare pseudotyped viruses, HEK293T cells were cotransfected with a spike-protein-encoding pcDNA3.1 and an MLV β-galactosidase-transducing vector, pBAG (25). At 2 days post-transfection, viral supernatants were harvested and concentrated in a spin concentrator. Approximately 4 ml of supernatant was typically concentrated (10,000 MW cutoff) to between 100-200 μl. HEK293T cells transiently expressing hACE2 in pcDNA3.1 were inoculated in 96-well dishes by adding 5 μl of concentrated viral supernatant to 50 μl cell culture medium per well. Transduction efficiency was quantified 2 days later by measuring β-galactosidase activity. The inoculated cells were lysed in PBS containing 0.5% NP40 and 3 mg/ml o-nitrophenyl B-D-glucopyranoside and monitored by spectrometry (O.D. 410 nm). The intracellular C-termini of the spike protein and hACE2 contained a C9 tag and an HA tag, respectively. The concentrations of the spike protein packaged in pseudotyped viruses and of hACE2 expressed on HEK293T cell surface were detected by Western blotting using anti-C9 and anti-HA antibodies, respectively. As

a negative control, the plasmid expressing the spike protein is replaced by a plasmid that does not express any protein.

Results

To investigate the role of the hotspot structure in the virus/receptor binding interactions, we mutated each of the components of the hotspot structure. We then examined how the mutations affect the binding affinities between RBDs and hACE2 using surface plasmon resonance Biacore assays. We also investigated how the mutations impact the interactions between spike proteins and hACE2 using pseudotyped viral transduction assays.

For Biacore assays, we first measured the binding affinities between wild type hACE2 peptidase domain and prototypic NL63-CoV RBD (strain Amsterdam 1) and between wild type hACE2 peptidase domain and prototypic SARS-CoV RBD (strain Tor2 that was isolated during the 2002-2003 SARS epidemic). hACE2 was immobilized on the Biacore sensor chip through direct covalent coupling via amine groups, and NL63-CoV or SARS-CoV RBD was injected over the chip as soluble analyte. The measured K_d between SARS-CoV RBD and hACE2 was 20.8 nM (Fig. 3A), consistent with the K_d of 16.2 nM as measured in a previous study (19). The measured K_d between NL63-CoV RBD and hACE2 was 34.9 nM (Fig. 3A), the first reported K_d between the two proteins. The same RBD fragment as used in this study (residues 461-616) also bound to hACE2 with high affinity in a previous study using a co-immunoprecipitation analysis (21). Interestingly, despite SARS-CoV and NL63-CoV RBDs had similar K_d with hACE2, NL63-CoV RBD bound to hACE2 with significantly lower k_{on} and k_{off}. It has been shown

that k_{off} and k_{on} are dictated by short-range van der Waals ınteractions and long-range electrostatıc interactions between the proteins, respectıvely (26). Therefore, the lower k_{on} and k_{off} of the NL63-CoV-RBD/hACE2 complex lıkely reflected a less electrostatic and more hydrophobıc ınterface between the two proteıns.

Usıng Biacore, we also measured the binding affinities between hACE2 and NL63-CoV RBD and between hACE2 and SARS-CoV RBD in a reverse way: NL63-CoV or SARS-CoV RBD was immobilızed on the sensor chip, and hACE2 was injected over the chip as soluble analyte. The measured K_d was 68.0 nM between NL63-CoV RBD and hACE2 or 137 nM between SARS-CoV RBD and hACE2, both of which were higher than when hACE2 was ımmobılized (Fıg. 3A). Such discrepancies in measured K_d were mostly due to the dıfferences in measured k_{on}. No matter whether hACE2 or RBDs were immobilized, k_{off} remaıned sımılar. When hACE2 was immobilized , however, k_{on} was sıgnificantly higher. Why did k_{on} increase when hACE2, instead of RBDs, was immobilized? This is because hACE2 has a larger molecular weight than either of the RBDs, and thus when immobilized, hACE2 can provide more accessible surface area for complex formation, leading to higher k_{on}. Therefore, the surface accessibility of the immobılized protein, but not the dissocıation rate, accounted for the discrepancies in measured K_d.

To evaluate how mutatıons of the hotspot structure affect the binding affinities between RBDs and hACE2, we introduced sıngle mutations to either RBDs or hACE2 that modıfied every component of the hotspot structure. These mutations were K353A, D38A, D37A, Y41A and Y41F ın hACE2, Y498A, S535A and S535T ın NL63-CoV RBD, and Y491A, T487A and T487S in SARS-CoV RBD (Fig. 2A, 2B). We expressed

and purified each of the 11 hACE2 and RBD mutants. To measure the binding affinities between mutant RBDs and wild type hACE2, hACE2 was immobilized on the sensor chip and mutant RBDs were the soluble analytes. To measure the binding affinities between wild type RBDs and mutant hACE2, NL63-CoV or SARS-CoV RBD was immobilized on the sensor chip and mutant hACE2 was the soluble analyte. The results were then compared with the binding affinities between wild type hACE2 and wild type RBDs (Fig. 3B, 3C).

In this study we not only measured direct interactions between viral RBDs and hACE2 using recombinant proteins, but also examined the spike/receptor interactions using functional assays. To this end, we carried out pseudotyped viral transduction assays to investigate whether changes in RBD/hACE2 interactions could lead to corresponding changes in viral entry and membrane fusion in the context of the full-length spike proteins and their receptor protein. We prepared retroviral MLVs expressing β-galactosidase and pseudotyped with NL63-CoV or SARS-CoV spike protein. These MLVs were incubated with hACE2-expressing HEK293T cells. The transduction efficiency of the pseudotyped viruses was measured by determining β-galactosidase activity of inoculated cell lysate. To measure the interactions between wild type hACE2 and mutant spike proteins or between mutant hACE2 and wild type spike proteins, we introduced single mutations to hACE2 or the spike proteins that were the same as the mutations used for Biacore assays (Fig. 4A, 4B). The expression levels of the spike proteins in pseudotyped viruses and of hACE2 molecules on HEK293T cells were detected by Western blotting using antibodies against their intracellular C-terminal C9 and HA tags, respectively. The Western blotting results showed that all of the mutant

spike proteins and mutant hACE2 molecules were well expressed, and the expression levels of these mutant proteins were quantified and calibrated against those of the wild type proteins (Fig. 4C). Finally, the measured transduction efficiency for mutant spike proteins or mutant hACE2 was normalized against the transduction efficiency of viruses pseudotyped with wild type spike proteins in cells expressing wild type hACE2 (Fig. 4).

Both Biacore assays and pseudotyped viral transduction assays yielded results that were highly consistent with each other (Fig.3, 4; Table 1). It is worth noting that recombinant SARS-CoV and NL63-CoV RBDs are both monomers in solution, whereas the full-length spike proteins are trimers on virus surfaces (14). Thus the good correlation between the RBD/hACE2 binding affinities and the spike-mediated transduction efficiency strongly suggests that the measured RBD/hACE2 binding activities reflect the native states of the proteins. Our results showed that all of the targeted mutations produced significantly reduced RBD/hACE2 binding affinities and spike-protein-guided transduction compared with the corresponding wild type proteins (*t*-test p values < .01 for both K_d and transduction), with the exception of D37A in hACE2 (p > .075 for transduction) and Y498 in NL63-CoV RBD (p > .10 for both K_d and transduction). Here we combine these biochemical and functional data with our previous structural data, and discuss molecular and structural features of the virus-binding hotspot that make hACE2 a common target by two different viruses.

The hotspot structures at the NL63-CoV/hACE2 and SARS-CoV/hACE2 interfaces share many common features. The Lys353-Asp38 salt bridge plays a central role in the hotspot structure at both of the interfaces. Because of the hydrophobic environment, the salt bridge not only provides significant amount of energy to the

virus/receptor binding interactions, but also fills a critical void in the hydrophobic stacking interactions at the virus/receptor interfaces. Correspondingly, alanine substitution for either Lys353 or Asp38 in hACE2 significantly decreased the RBD/hACE2 binding affinities and viral transductions (Fig. 3, 4). The hydrophobic tunnel walls of the hotspot structure also make important contributions to the virus/receptor binding interactions; they not only support the side chain of Lys353 to form the salt bridge, but also provide hydrophobic stacking interactions at the virus/receptor interfaces. Some hydrophobic tunnel walls contribute more energy to the virus/receptor binding interactions than others (Fig. 3, 4). For example, Tyr41 in hACE2 (top wall) is more important than Asp37 in hACE2 (right wall), probably because Tyr41 functions better as a tunnel wall with its aromatic ring. Alanine substitution for Tyr41 significantly decreased RBD/hACE2 binding affinities and viral transductions, suggesting that the stacking interaction between Tyr41 and Lys353 is essential for the hotspot structure. Interestingly, although a phenylalanine at the 41 position can potentially function as a tunnel wall with its aromatic ring, the Y41F mutation also significantly decreased RBD/hACE2 binding affinities and viral transductions. Detailed structural analysis reveals that the hydroxyl group of Tyr41 forms a hydrogen bond with receptor Asp355 at the NL63-CoV/hACE2 interface and two hydrogen bonds with receptor Asp355 and RBD Thr486 at the SARS-CoV/hACE2 interface (Fig. 2A, 2B). Thus the side chain of Tyr41 needs to be firmly anchored in order for it to function properly as a tunnel wall. Residue 41 is a histidine in the ACE2 proteins from several bat species (10). Not only is His41 a poor hydrophobic stacker, but it also cannot be anchored properly to function as a tunnel wall. As a result, these bat ACE2 proteins were

poor receptors for human SARS-CoV strains unless an H41Y mutation was introduced (10). Overall, the salt bridge and many of the tunnel walls of the hotspot structure contribute energy to the virus/receptor binding interactions.

The hotspot structures at the NL63-CoV/hACE2 and SARS-CoV/hACE2 interfaces differ in a subtle but functionally important way. The tunnel structure at the NL63-CoV/hACE2 interface is more compact than that at the SARS-CoV/hACE2 interface (Fig. 2A, 2B). At the NL63-CoV/hACE2 interface, the closest distances between the two pairs of opposing tunnel walls, Ser535-Asp37 (left to right) and Tyr41-Tyr498 (top to bottom), are 8.5 Å and 7.7 Å, respectively. At the SARS-CoV/hACE2 interface, if a serine replaces threonine at the 487 position in SARS-CoV RBD, these distances become 9.0 Å and 8.1 Å, respectively. Because the compactness of the tunnel structure at the NL63-CoV/hACE2 interface, S535T mutation in NL63-CoV RBD decreased the tunnel space and was energetically unstable (Fig. 3B, 4A). In contrast, because of the extra space of the tunnel structure at the SARS-CoV/hACE2 interface, T487S mutation in SARS-CoV RBD increased the tunnel space, but was also energetically unstable (Fig. 3C, 4B). Indeed, residue 487 was a serine in RBDs of some low-pathogenicity SARS-CoV strains, and was largely responsible for the lack of human-to-human transmission of these viral strains (18, 19, 27). Thus although S535T mutation in NL63-CoV RBD and T487S mutation in SARS-CoV RBD exerted opposite effects on the same left tunnel wall of the hotspot structure, they both reduced RBD/hACE2 binding affinities and viral transductions. For similar reasons, compared with Tyr498 in NL63-CoV RBD, Tyr491 in SARS-CoV RBD provides more support to the hotspot structure as the bottom tunnel wall in a more spacious tunnel space, and hence alanine substitution for

Tyr491 decreased RBD/hACE2 binding affinities and viral transductions (Fig. 3, 4). Therefore, the seemingly small differences in the hotspot structure at the two virus/receptor interfaces not only have significant impacts on virus/receptor binding interactions, but also have important epidemic implications.

One of the direct implications of our study is the possibility to use NL63-CoV RBD as an inhibitor to block SARS-CoV infections, because NL63-CoV RBD can compete with SARS-CoV for the common virus-binding hotspot on hACE2. To test this possibility, we inoculated MLVs pseudotyped with SARS-CoV spike protein onto hACE2-expressing HEK293T cells in the presence of various concentrations of purified NL63-CoV RBD or SARS-CoV RBD (Fig. 5). Transduction was shown as a percentage of β-galactosidase activity observed in the absence of any inhibitor. The results showed that NL63-CoV RBD indeed inhibited SARS-CoV-spike-mediated transductions. At 10 μg/ml (0.47 μM) concentration, NL63-CoV RBD inhibited SARS-CoV-spike-mediated transductions by over 80%. This method has the potential to become a new antiviral strategy against SARS-CoV infections, as it represents the first case in which SARS-CoV infection can be inhibited by a protein from a different virus. It also represents a successful application of the common virus-binding hotspot theory derived from the present study.

Discussion

Binding to the same hotspot on hACE2 was likely an outcome of convergent evolution by NL63-CoV and SARS-CoV. Our study provides several lines of evidences to support this notion. First, despite no structural homology in their RBDs, NL63-CoV and SARS-CoV both bind to the hotspot region and form highly similar and energetically

stable tunnel structures (Fig. 1, 2). Second, despite no structural homology in their RBMs, both viruses insert an RBM loop between VBM2 and VBM3 on hACE2 (Fig. 1). Third, despite being presented by non-homologous RBM loops, Ser535 in NL63-CoV RBD and Thr487 in SARS-CoV RBD (Ser487 in RBDs of some low-pathogenicity SARS-CoV strains) occupy identical positions as the left tunnel wall in the hotspot structure (Fig. 2A, 2B), and contribute energy to the virus/receptor binding interactions (Fig. 3, 4). Last, despite pointing in opposite directions, Tyr498 in NL63-CoV RBD and Tyr491 in SARS-CoV RBD also occupy identical positions as the bottom tunnel wall in the hotspot structure (Fig. 2A, 2B). These data suggest that NL63-CoV and SARS-CoV likely evolved independent strategies to achieve the same functional goal, supporting convergent evolutionary relationship between the two viruses.

The likely convergent evolution of NL63-CoV and SARS-CoV was at least partly driven by the structural features of the virus-binding hotspot on hACE2. The general structural features of the hotspot favor virus binding: it is located in a region on hACE2 that is furthest from the membrane, relatively flat, free of glycosylation, and thereby easily accessible to viruses (Fig. 1). The detailed structural features of the hotspot such as its potential to form the energetically stable tunnel structure also favor virus binding (Fig. 2A, 2B). In the unbound hACE2 structure where structural restraints from viruses are absent, Lys353 projects into solution; it does not form a salt bridge with Asp38 or stack with Tyr41 or Asp37 (Fig. 2C) (15, 28). Thus the virus-binding hotspot is not pre-existent or pre-organized on hACE2; instead, it is induced to form by virus binding. Therefore, while the hotspot is mainly an intrinsic property of hACE2, it is also a dynamic structure and receives structural contributions from both hACE2 and the viruses, although the

contributions from hACE2 are more pronounced. The virus-binding hotspot on hACE2 is likely different from the hotspots for host protein/protein interactions. Host protein partners have co-evolutionary relationships (9, 11), and hence hotspots for host protein/protein interactions are usually pre-existent and pre-organized in unbound host protein structures (20, 22). Viruses and receptors, however, do not usually have such co-evolutionary relationships; only viruses evolve to adapt to host receptors, but receptors do not evolve to adapt to viruses. Occasionally, if a virus exerts large enough impacts on a host, the host receptor may even evolve away from virus binding (7). So far the virus-binding hotspot on hACE2 is not known for its interaction with any other host proteins. Overall, the potential of the hotspot region on hACE2 to form energetically stable tunnel structures and some general structural features of this region on the receptor surface were among the possible reasons why the hotspot was exploited by two different viruses.

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Table 1. Summary of Biacore and pseudotyped transduction data from Figures 3 and 4.

	NL63-CoV			SARS-CoV	
hACE2	K_a (10^{-3} / nMol)	Transduction (%)	hACE2	K_a (10^{-3} / nMol)	Transduction (%)
WT	14.7 (±1.5)	100 (±2.3)	WT	7.30 (±1.2)	100 (±4.8)
K353A	too low	19.3 (±2.9)	K353A	too low	13.5 (±2.2)
D38A	3.37 (±0.49)	47.2 (±2.3)	D38A	2.44 (±0.37)	59.3 (±4.5)
D37A	8.77 (±0.77)	87.6 (±6.8)	D37A	2.72 (±0.46)	85.1 (±2.4)
Y41A	0.69 (±0.14)	28.5 (±2.0)	Y41A	0.63 (±0.21)	24.0 (±2.8)
Y41F	0.96 (±0.33)	35.7 (±2.1)	Y41F	0.69 (±0.24)	25.9 (±4.3)
	hACE2			hACE2	
NL63	K_a (10^{-3} / nMol)	Transduction (%)	SARS	K_a (10^{-3} / nMol)	Transduction (%)
WT	28.7 (±3.8)	100 (±2.3)	WT	48.1 (±4.0)	100 (±4.8)
Y498A	24.3 (±2.7)	91.9 (±3.4)	Y491A	too low	50.4 (±2.4)
S535A	1.25 (±0.32)	64.9 (±2.4)	T487A	too low	57.7 (±6.1)
S535T	0.43 (±0.11)	39 9 (±1.8)	T487S	too low	50.9 (±3.8)

Figure legends

Figure 1. Overall structures of NL63-CoV and SARS-CoV RBDs each complexed with their common receptor human ACE2. (A) Crystal structure of NL63-CoV RBD complexed with hACE2 (PDB 3KBH). hACE2 is in green, virus-binding motifs (VBMs) are in blue, NL63-CoV RBD core structure is in cyan, and receptor-binding motifs (RBMs) are in red. Lys353 and Asp38 in hACE2, which are critical for the RBD/hACE2 interactions, are shown in ball-and-stick format. (B) Crystal structure of SARS-CoV RBD complexed with hACE2 (PDB 2AJF).

Figure 2. Detailed structure of a common virus-binding hotspot on human ACE2. (A) Hotspot structure at the NL63-CoV/hACE2 interface. (B) Hotspot structure at the SARS-CoV/hACE2 interface. (C) Conformation of Lys353 on the surface of unbound human ACE2 (PDB 1R42).

Figure 3. Surface plasmon resonance Biacore analyses of the binding interactions between viral RBDs and human ACE2. Each experiment was repeated 5 times at three different protein concentrations. The corresponding standard errors are shown. (A) Kinetics of the binding interaction between wild type hACE2 and wild type RBDs. (B) Biacore analyses of NL63-CoV RBD and hACE2. Single mutations were introduced to hACE2 or RBD to modify every component of the hotspot structure. (C) Biacore analyses of SARS-CoV RBD and hACE2.

Figure 4. Pseudotyped viral transduction assays of the interactions between viral spike proteins and human ACE2. Retroviral MLVs expressing β-galactosidase and pseudotyped with the NL63-CoV or SARS-CoV spike protein were used to infect hACE2-expressing HEK293T cells. Transduction efficiency of the pseudotyped viruses was measured by β-galactosidase assays. After mutations were introduced into the spike proteins or hACE2, the corresponding transduction efficiency was normalized against the transduction efficiency of viruses pseudotyped with wild type spike proteins in cells expressing wild type hACE2. Each experiment was repeated 6 times. The corresponding standard errors are shown. (A) Transduction of MLVs pseudotyped with NL63-CoV spike protein in hACE2-expressing cells. (B) Transduction of MLVs pseudotyped with SARS-CoV spike protein in hACE2-expressing cells. (C) Western blotting analysis of coronavirus spike proteins and hACE2. The NL63-CoV and SARS-CoV spike proteins packaged in pseutotyped retroviruses both contained a C-terminal C9 tag, and the hACE2 expressed on HEK293T cell surface contained a C-terminal HA tag. The expression levels of the spike proteins and hACE2 were detected by Western blotting using anti-C9 and anti-HA antibodies, respectively. The protein bands were quantified using software Image J (version 1.6).

Figure 5. Inhibition of SARS-CoV-spike-mediated transduction by NL63-CoV RBD. MLVs pseudotyped with SARS-CoV spike protein were used to infect hACE2-expressing HEK293T cells in the presence of various concentrations of purified NL63-CoV RBD, SARS-CoV RBD, SARS-CoV RBD containing T487S mutation, hACE2 (positive control) and BSA (negative control). Transduction is shown as a percentage of

β-galactosidase activity observed in the absence of any inhibitor. Each experiment was repeated 5 times. The corresponding standard errors are shown. The results show that NL63-CoV can efficiently inhibit SARS-CoV-spike-mediate transduction.

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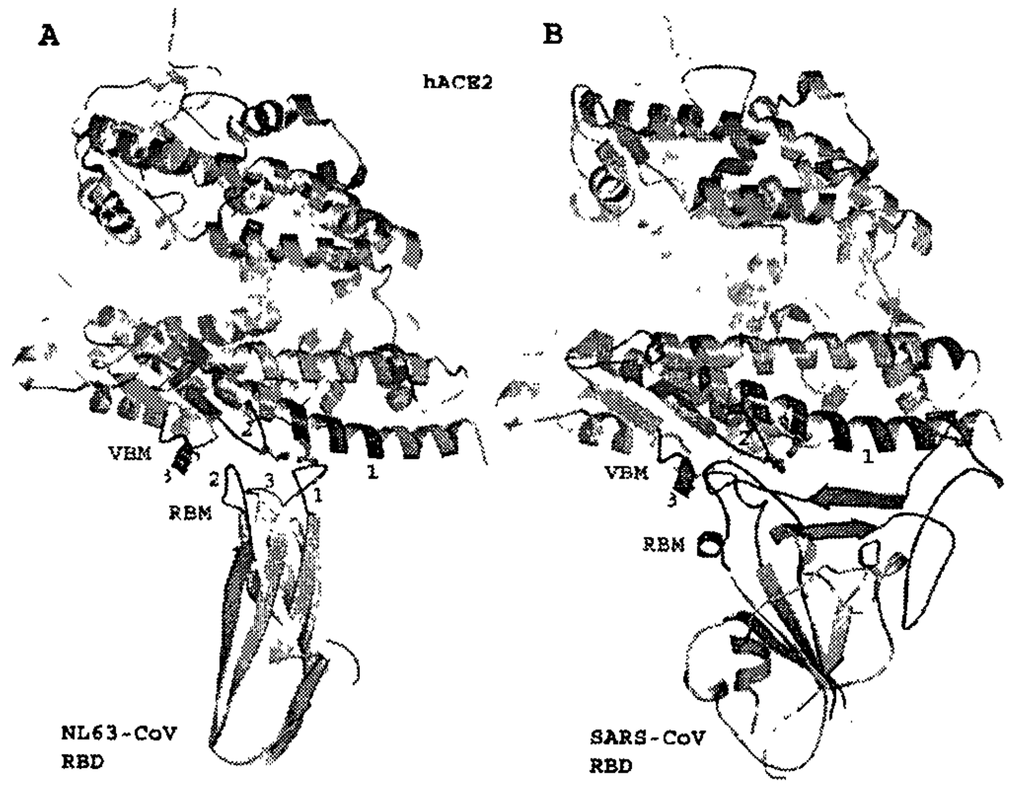
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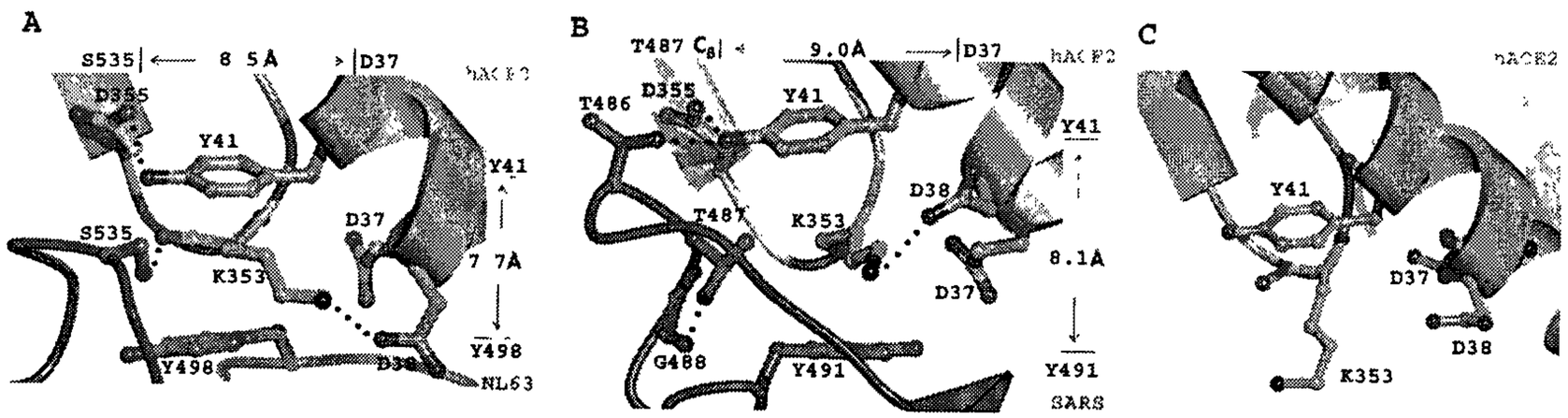
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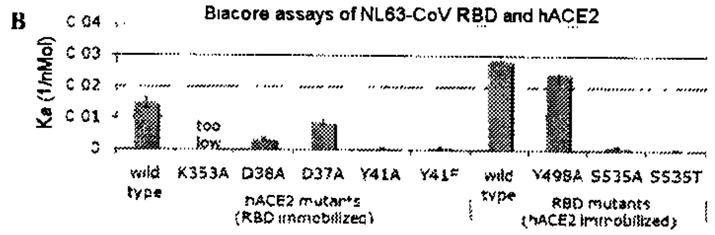

A
hACE2
VBM
RBM
NL63-CoV
RBD
B
VBM
RBM
SARS-CoV
RBD


A
S535
8.5Å
D37
hACE2
D355
Y41
Y41
S535
D37
7.7Å
K353
Y498
Y498
D38
NL63
B
T487 Cβ
9.0Å
D37
hACE2
T486
D355
Y41
Y41
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G488
Y491
Y491
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Y41
D37
D38
K353

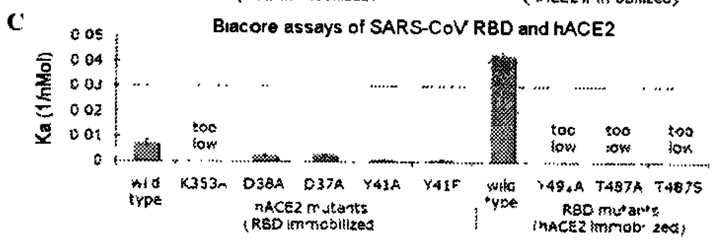
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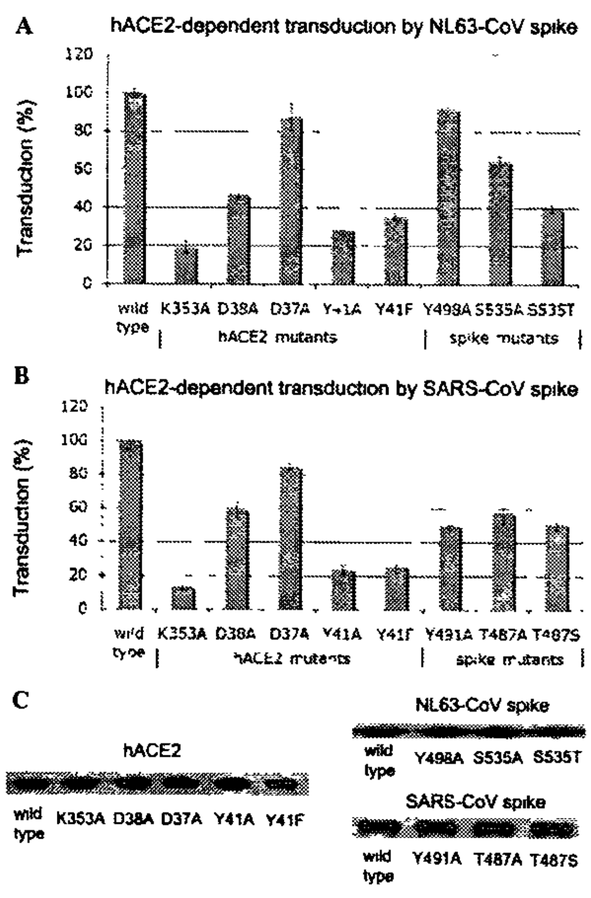
Biacore partners	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (M)	K_A (M^{-1})
NL63 RBD (flow through) vs hACE2 (immobilized)	3.02×10^{4}	7.09×10^{-3}	3.49×10^{-8}	2.87×10^{7}
hACE2 (flow through) vs NL63 RBD (immobilized)	1.42×10^{4}	9.63×10^{-4}	6.80×10^{-8}	1.47×10^{7}
SARS RBD (flow through) vs hACE2 (immobilized)	1.58×10^{5}	3.26×10^{-3}	2.08×10^{-8}	4.81×10^{7}
hACE2 (flow through) vs SARS RBD (immobilized)	2.48×10^{4}	3.35×10^{-3}	13.7×10^{-8}	0.73×10^{7}

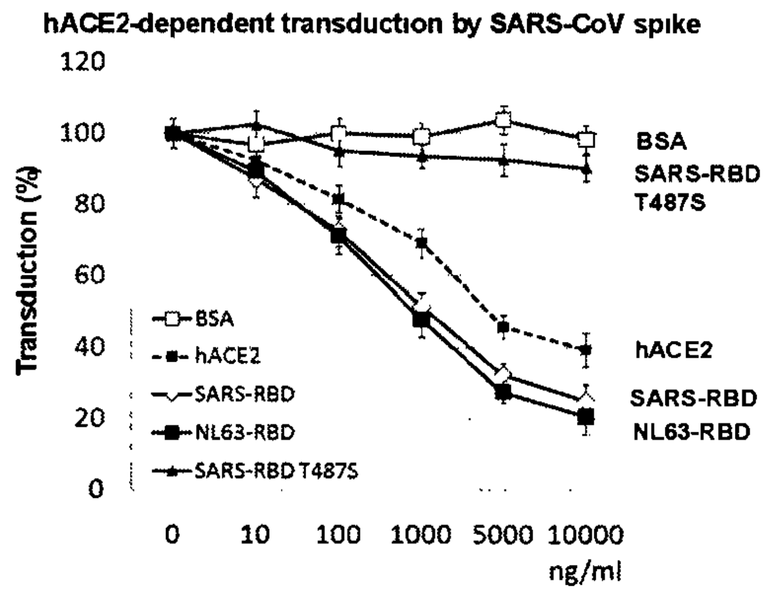
B



C




A
hACE2-dependent transduction by NL63-CoV spike
Transduction (%)
120
100
80
60
40
20
0
wild type
K353A
D38A
D37A
Y41A
Y41F
Y498A
S535A
S535T
hACE2 mutants
spike mutants
B
hACE2-dependent transduction by SARS-CoV spike
Transduction (%)
120
100
80
60
40
20
0
wild type
K353A
D38A
D37A
Y41A
Y41F
Y491A
T487A
T487S
hACE2 mutants
spike mutants
C
hACE2
wild type
K353A
D38A
D37A
Y41A
Y41F
NL63-CoV spike
wild type
Y498A
S535A
S535T
SARS-CoV spike
wild type
Y491A
T487A
T487S


hACE2-dependent transduction by SARS-CoV spike
Transduction (%)
120
100
80
60
40
20
0
BSA
hACE2
SARS-RBD
NL63-RBD
SARS-RBD T487S
0
10
100
1000
5000
10000
ng/ml
BSA
SARS-RBD T487S
hACE2
SARS-RBD
NL63-RBD

Principal Investigator/Program Director (Last First, Middle) Li, Fang

DETAILED BUDGET FOR NEXT BUDGET PERIOD - DIRECT COSTS ONLY	FROM 5/1/11	THROUGH 4/30/12	GRANT NUMBER R01 AI089728-02

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal Mnths	Acad Mnths	Summer Mnths	SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Fang Li	PD/PI	6 00			$42,250	$15,211	$57,461
Robert Geraghty	Co-Invest	1 00			$8,833	$3,180	$12,013
Lang Chen	Postdoctoral	12 00			$34,000	$6,868	$40,868
Guiqing Peng	Postdoctoral	12 00			$36,000	$7,272	$43,272
Zhou, Wenbo	Postdoctoral	4 80			$14,803	$7,402	$22,205
							$0
SUBTOTALS →					$135,886	$39 933	$175,819

CONSULTANT COSTS

Dr Kathryn Holmes	$10,000

EQUIPMENT (*Itemize*)

	$0

SUPPLIES (*Itemize by category*)

Molecular biology supplies	$14,580	
Insect cell culture supplies	$9,500	
Protein purification supplies	$16,000	
Protein crystallization supplies	$13,500	
Plastic & glassware supplies	$2,801	
		$56,381

TRAVEL

PI and/or postdoctoral to attend one meeting per year.	$3,000
INPATIENT CARE COSTS $0	$0
OUTPATIENT CARE COSTS $0	$0

ALTERATIONS AND RENOVATIONS (*Itemize by category*)

OTHER EXPENSES (*Itemize by category*)

Publication costs/reprints	$2,500	
Equipment maintenance	$1,500	
Communication costs	$800	
		$4,800

SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD	$250,000
CONSORTIUM/CONTRACTUAL COSTS — DIRECT COSTS	
CONSORTIUM/CONTRACTUAL COSTS — FACILITIES AND ADMINISTRATION COSTS	
TOTAL DIRECT COSTS FOR NEXT PROJECT PERIOD (*Item 8a Face Page*)	$250,000

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	Years 1-5
Indirect Cost Base	$250,000
IDC @ 51%	$127,500
Total Direct Costs	$250,000
IDC	$127,500
Total Costs	$377,500

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Program Director/Principal Investigator (Last first middle) Li, Fang

GRANT NUMBER
5R01AI89728-2

CHECKLIST

1 PROGRAM INCOME ***(See instructions.)***
All applications must indicate whether program income is anticipated during the period(s) for which grant support is requested If program income is anticipated, use the format below to reflect the amount and source(s)

Budget Period	Anticipated Amount	Source(s)
	0	

2 ASSURANCES/CERTIFICATIONS ***(See instructions.)***
In signing the application Face Page, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the application instructions when applicable Descriptions of individual assurances/certifications are provided in Part III of the PHS 398, and listed in Part I, 4 1 under Item 14 If unable to certify compliance where applicable, provide an explanation and place it after the Progress Report (Form Page 5)

3 FACILITIES AND ADMINSTRATIVE (F&A) COSTS
Indicate the applicant organization's most recent F&A cost rate established with the appropriate DHHS Regional Office, or, in the case of for-profit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office

F&A costs will ***not*** be paid on construction grants, grants to Federal organizations, grants to individuals, and conference grants Follow any additional instructions provided for Research Career Awards, Institutional National Research Service Awards, Small Business Innovation Research/Small Business Technology Transfer Grants, foreign grants, and specialized grant applications

☒ DHHS Agreement dated 3/23/2010 ☐ No Facilities and Administrative Costs Requested

☐ No DHHS Agreement but rate established with _____ Date _____

CALCULATION*

Entire proposed budget period Amount of base $ 250,000 x Rate applied 51 % = F&A costs $ 127,500 ✓

Add to total direct costs from Form Page 2 and enter new total on Face Page, Item 8b

*Check appropriate box(es)

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary)*

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UNIVERSITY OF MINNESOTA
Twin Cities Campus

Department of Pharmacology

Medical School
Academic Health Center

6-120 Jackson Hall
321 Church Street S E
Minneapolis MN 55455 U S A

612 625-9997
FAX. 612 625-8408

FAX COVER PAGE

Date: 3 10 11 Number of Pages (*including cover page*): 7

TO: **PROPOSALS - SPA**

FAX #: 4-4843

FROM: PHARMACOLOGY

Message:

RE: ELECTRONIC GRANT SUBMISSION

PRF #: 683843

PI NAME: Fang Li

Grant Type: NIH RC1 eSNAP Progress Report

Detailed Budget Attached: Yes X No ____

Budget Justification Attached: Yes ____ No X

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Report ID UMGPCSPD

Run Date 3/11/2011
Run Time 9 34 33 AM
Page 1 of 1

ENTERPRISE FINANCIAL SYSTEMS
SPONSORED PROJECT DETAIL
UNIVERSITY OF MINNESOTA
SPONSORED PROJECT 00013782 - Receptor recognition mechanism
ACCOUNTING DATE THROUGH 3/11/2011

Sponsor NIH Nat'l Inst of Allergy & Infect Dis
Award CON000000022345
Department 11718
Project Start Date 5/15/2010 Project End Date 4/30/2015
PI Li,Fang

Project Budget	$	377,500 00
Program Income	$	0 00
TIP Income	$	0 00
F&A RSRCH	MTDC	51 00 %
F&A Distribution		
11345 DrugDesCtr		11 50%
11718 PhclAdmin		88 50%

		SPONSORED						COST SHARE					
			3/1/2011 - 3/11/2011		INCEPTION THROUGH 3/11/2011				3/1/2011 - 3/11/2011		INCEPTION THROUGH 3/11/2011		
DETAIL ACCOUNT		BUDGET AMOUNT	PRE-ENC & ENC	EXPENSES	PRE-ENC & ENC	EXPENSES	AVAILABLE BALANCE	BUDGET AMOUNT	PRE-ENC & ENC	EXPENSES	PRE-ENC & ENC	EXPENSES	AVAILABLE BALANCE
168100	Capital Assets - Fin Bdg Only	8,000 00	0 00	0 00	0 00	27 489 50	(19,489 50)	0 00	0 00	0 00	0 00	0 00	0 00
700100	Salaries-Faculty-Fin Bdg Only	44 052 00	(1 829 33)	1,829 32	8 231 95	36 439 55	(619 50)	0 00	0 00	0 00	0 00	0 00	0 00
700200	Salaries-Prof&Adm-Fin Bdg Only	8 798 00	0 00	0 00	0 00	367 95	8 430 05	0 00	0 00	0 00	0 00	0 00	0 00
700300	Salaries-Grd/Pr w/Tuitn-FinBdg	81 400 00	0 00	0 00	0 00	0 00	81,400 00	0 00	0 00	0 00	0 00	0 00	0 00
700310	Salaries-Grd/Pr NoTuitn-FinBdg	0 00	(2,708 61)	3,054 79	14,092 72	65,050 93	(79,143 65)	0 00	0 00	0 00	0 00	0 00	0 00
710100	Fringe - Faculty-Fin Bdg Only	14,142 00	(604 07)	604 02	2,718 25	10 778 30	645 45	0 00	0 00	0 00	0 00	0 00	0 00
710200	Fringe - P&A-Fin Bdg Only	2,929 00	0 00	0 00	0 00	118 84	2,810 16	0 00	0 00	0 00	0 00	0 00	0 00
710300	Fringe-Grad/Pr-w/Tuitn-FinBdg	16 117 00	0 00	0 00	0 00	0 00	16 117 00	0 00	0 00	0 00	0 00	0 00	0 00
710310	Fringe-Grad/Pr No Tuitn-FinBdg	0 00	(455 59)	525 56	2 435 12	9 781 43	(12,216 55)	0 00	0 00	0 00	0 00	0 00	0 00
720200	Lab & Med Supplies-FinBdg Only	60 893 00	2,265 14	157 02	7 187 10	25 879 91	27,825 99	0 00	0 00	0 00	0 00	0 00	0 00
720300	Gen Oper Services-Fin Bdg Only	1 900 00	0 00	0 00	0 00	41 13	1,858 87	0 00	0 00	0 00	0 00	0 00	0 00
720500	Telecommunications-FinBdg Only	400 00	0 00	0 00	0 00	0 00	400 00	0 00	0 00	0 00	0 00	0 00	0 00
720600	Travel/Mileage/Mov-FinBdg Only	2,000 00	0 00	0 00	0 00	388 59	1,611 41	0 00	0 00	0 00	0 00	0 00	0 00
730100	Consulting Services-FinBdg Onl	10,000 00	0 00	0 00	0 00	0 00	10,000 00	0 00	0 00	0 00	0 00	0 00	0 00
750100	Non-Capital Equip-Fin Bdg Only	571 00	0 00	0 00	0 00	3,201 51	(2,630 51)	0 00	0 00	0 00	0 00	0 00	0 00
780100	Repairs & Maint-FinBdg Only	1,500 00	0 00	0 00	0 00	500 37	999 63	0 00	0 00	0 00	0 00	0 00	0 00
	DIRECT COST TOTALS	252,702 00	(3,332 46)	6,170 71	34,665 14	180,038 01	37,998 85	0 00	0 00	0 00	0 00	0 00	0 00
810500	F&A Costs-Fin Bdg Only	124 798 00	0 00	3,147 06	0 00	77 799 81	46 998 19	0 00	0 00	0 00	0 00	0 00	0 00
	F&A TOTALS	124,798 00	0 00	3,147 06	0 00	77,799 81	46,998 19	0 00	0 00	0 00	0 00	0 00	0 00
	PROJECT TOTALS	377,500 00	(3,332 46)	9,317 77	34,665 14	257,837 82	84,997 04	0 00	0 00	0 00	0 00	0 00	0 00

@23%

UNIVERSITY OF MINNESOTA

Notice of Grant or Contract Award

Sponsored Projects Administration

(Online Page Navigation Main Menu > Grants >Awards > UM_NOGA Report)

Award: CON000000022345
Primary Project: 00013782
PRF #: 651709

AWARD INFORMATION (Online Page Navigation Main Menu > Grants > Awards >Award Profile)

Award Start Date: 05/15/2010
Funds Approved to Date: S751,225 00
Principal Investigator: Li,Fang
Primary Project Dept: PHCL Pharmacology Admin
Sponsor: NIH Nat'l Inst of Allergy & Infect Dis
Sponsor Award #: 5R01AI089728-02 Rev
Award Title #: Receptor recognition mechanisms of coronaviruses

Award End Date: 04/30/2015
Award Type: Grant
Purpose: Research

ASSOCIATED PROJECTS (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Funding Tab)

Project #: 00013782
Project PI: Li,Fang
F&A: 51 00 MTDC
Department: 11718 - PHCL Pharmacology Admin
Dates: 05/15/2010 - 04/30/2015
EFFDT: 05/15/2010
Project Title: Receptor recognition mechanism

AWARD HISTORY (Online Page Navigation Main Menu > Grants > Awards > Review Award Modifications)

(Displayed in action order ('Mod Issue Date' in descending order))

Reference Award#: 5R01AI089728-02 Rev
Amount: S33,975 00
Period: 2
Begin Date: 05/01/2011 **End Date:** 04/30/2012
Modification Seq: 3 **Modification Type:** Additional Funding **Mod Issue Date:** 06/15/2011
Comments:

This revised award increases the total costs authorized consistent with NIAID implementation of the NIH Fiscal policy for FY2011

All other terms and conditions remain the same

Reference Award#: 1R01AI089728-02
Amount: S339,750 00
Period: 2
Begin Date: 05/01/2011 **End Date:** 04/30/2012
Modification Seq: 2 **Modification Type:** Continuation **Mod Issue Date:** 05/05/2011
Comments:

NIH is currently funded through a Continuing Resolution at the FY2010 level Therefore, this non-competing award is made at a level below the committed FY2011 level If final appropriation permits, adjustments may be made up to FY2011 plan level

All other terms and conditions remain the same

Reference Award#: 1R01AI089728-01
Amount: S377,500 00
Period: 1
Begin Date: 05/15/2010 **End Date:** 04/30/2011
Modification Seq: 1 **Modification Type:** Original **Mod Issue Date:** 05/27/2010
Comments:

An unobligated balance may be carried forward to the next budget period without prior agency approval

NOTE In additoin to the PI, Dr Robert Geraghty as been identified as key personnel (co-PI) Any absence, replacement or substantial reduction in effort by key personnel requires prior, written agency approval

Modular award The significant rebudgeting provision does not apply In addition, since costs were averaged over the project period, deficits may be carried forward from one budget period to the next within the same project period

ESTIMATED FUTURE FUNDING / ACTIONS (Online Page Navigation Main Menu > Grants > Awards > Review Award Modifications)

(Displayed by 'Period' and 'Modification Sequence' in ascending order)

Reference Award#: 1R01AI089728-01
Amount: $373,725 00
Period: 5
Begin Date: 05/01/2014 **End Date:** 04/30/2015
Modification Seq: 5 **Modification Type:** Continuation **Mod Issue Date:**

11
7-11
17

Comments:

Reference Award#: 1R01AI089728-01 **Amount:** $373,725 00
Period: 4 **Begin Date:** 05/01/2013 **End Date:** 04/30/2014
Modification Seq: 4 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

Reference Award#: 1R01AI089728-01 **Amount:** $373,725 00
Period: 3 **Begin Date:** 05/01/2012 **End Date:** 04/30/2013
Modification Seq: 3 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

COMMENTS

Description: **Entered by:** HOWI0016 **Date & Time:** 5/27/2010 10 00 AM
Date and Time Stamp indicates Proposal-to-Award Process

Description: **Entered by:** HOWI0016 **Date & Time:** 5/27/2010 10 02 AM
Sponsor Contacts
Grants Management
Cheryl Wall
Phone 301-451-2696
Fax 301-493-0597
Email wallcy@niaid nih gov

Program
Frederick J Cassels
Phone 301-402-8601
Fax 301-496-8030
Email casselsf@niaid nih gov

Description: **Entered by:** SPAUL **Date & Time:** 12/23/2010 9 56 AM
LOC
RAI089728

SPECIAL REVIEWS (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Certifications Tab)

Project #: 00013782
Description: rDNA, InfectAgents, BioToxins
Approval Date: 03/10/2011 **Expiration Date:** 03/10/2012 **AssuranceNumber:** 1002H77733
Comments:

BUDGET INFORMATION (Online Page Navigation Main Menu > Grants>Awards>Project Budget)

Please review online budget for valid account string combinations (ex PCBU, Activity, Fund, Dept_Id, Project, Account)

Project #: 00013782 **Cost Share Budget Total:**
Project Start Date: 05/15/2010 **Project End Date:** 04/30/2015
ICR Sharing **Department:** Drug Design, Center for **Percentage:** 11 50
ICR Sharing **Department:** PHCL Pharmacology Admin **Percentage:** 88 50

Period	Start Date	End Date	Direct Cost (Approved)	F & A Cost (Approved)	Direct Cost (Pending)	F & A Cost (Pending)	Total Period Cost
1	05/15/2010	04/30/2011	$252,702 00	$124,798 00	$0 00	$0 00	$377,500 00
2	05/01/2011	04/30/2012	$247,500 00	$126,225 00	$0 00	$0 00	$373,725 00
3	05/01/2012	04/30/2013	$0 00	$0 00	$247,500 00	$126,225 00	$373,725 00
4	05/01/2013	04/30/2014	$0 00	$0 00	$247,500 00	$126,225 00	$373,725 00
5	05/01/2014	04/30/2015	$0 00	$0 00	$247,500 00	$126,225 00	$373,725 00
TOTAL			$500,202 00	$251,023 00	$742,500 00	$378,675 00	$1,872,400 00

Budget Detail (all periods)

Fund Code	DeptID	Program Code	Chartfield 1	Chartfield 2	Fin EmplID	Activity	Analysis Type	Account	Cost Share	Budget Item	Amount	Hold
3002	11718					1	BUD	168100		CAPITAL_EQUIP	$8,000 00	
3002	11718					1	BUD	700100		FACULTY_SALARY	$94,624 00	
3002	11718					1	BUD	700200		PROF_ADM_SALARY	$92,753 00	
3002	11718					1	BUD	700300		GRAD_STU_SALARY	$81,400 00	
3002	11718					1	BUD	700310		GRAD_SAL_NOTUIT	$0 00	
3002	11718					1	BUD	710100		FACULTY_FRINGE	$32,349 00	
3002	11718					1	BUD	710200		PROF_ADM_FRINGE	$24,256 00	
3002	11718					1	BUD	710300		GRAD_STU_FRINGE	$16,117 00	
3002	11718					1	BUD	710310		GRAD_FRG_NOTUIT	$0 00	
3002	11718					1	BUD	720200		LABMED_SUPPLIES	$116,710 00	
3002	11718					1	BUD	720300		GEN_OP_SERVICES	$4,375 00	
3002	11718					1	BUD	720500		TELECOMM	$1,192 00	
3002	11718					1	BUD	720600		TRAVL_MILE_MOVE	$4,970 00	
3002	11718					1	BUD	730100		CONSULTING_SVCS	$19,900 00	
3002	11718					1	BUD	750100		NON_CAP_EQUIP	$571 00	
3002	11718					1	BUD	780100		REPAIRS_MAINT	$2,985 00	
3002	11718					1	BUD	810500		FACADM	$251,023 00	

COST SHARE/MATCH/IN-KIND
CSM - Cost Share, Matching and InKind

CSM05 - Departments must document all cost share that is shown in the proposal

PROGRAM INCOME INFORMATION
PIN - Program Income

PIN03 - Reportable Program Income must be deposited by the department into the Sponsored Program Income Unapplied Cash account

PIN04 - Additional - Program Income can be used in addition to sponsor funds to cover project costs that will further the objectives of the legislation under which the grant was made

TERMS & CONDITIONS (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Terms Tab)

APV - Additional Approval

APV01 - Prior sponsor approval or notification required for reduction of PI's effort by 25% or more

APV04 - Prior sponsor approval required to replace PI

APV07 - Prior sponsor approval required for changes to key personnel that would impact the scope of work

APV10 - Prior sponsor approval required for changes to work scope

APV14 - Prior sponsor approval required for the absence of the PI for 3 months or more

APV15 - Prior sponsor approval required for a second no cost extension or a first no cost extension that is submitted after the agency notification deadline

APV20 - Prior sponsor approval required for the addition of foreign sites or foreign subcontracts

FIS - Fiscal

FIS03 - Reversion of funds at termination / final

FIS07 - Deficits may be carried forward

FIS10 - Grant/contract funds may not be used to pay the salary of an individual at a rate in excess of Executive Level I of the Federal Executive Pay Scale

FIS11 - Modular award - Expenses incurred must be allocated and accounted for in compliance with all applicable cost principles

FIS13 - Deficits may be carried forward within non-competing segments, but may not be carried forward between competing segments

IPR - IP Rights

IPR02 - Sponsor support must be acknowledged in patent applications and patent issuing

IPR04 - Title to U of M, non-exclusive, non-transferable, irrevocable, paid-up license to Federal Government

IPR12 - PI obligation to report inventions (and other IP) to the sponsor (and to PTM per policy)

PRP - Property

PRP02 - U of M receives title to equipment purchased or fabricated under this award

PRP03 - Sponsor may require transfer of equipment and its title upon award completion for those items costing in excess of $5000

PUB - Publications

PUB02 - U of M will place an acknowledgement of sponsor support and a disclaimer, as appropriate, on any publication generated by this award or reporting on the award results and/or activity

PUB07 - Peer-reviewed articles must be submitted to NIH Pubmed Central (PMC) upon acceptance for publication and made publicly available within 12 months Cite PMC ID number in progress reports/proposals

RBD - Rebudgeting

RBD10 - Rebudgeting is allowed as long as there is no change in work scope

REG - Regulatory

REG01 - 45 CFR Part 74 - Code of Federal Regulations as it pertains to institutions of higher education

REG03 - OMB Circular A-21 (www whitehouse gov/omb/circulars/a021/a021 html)

REG04 - OMB Circular A-110 (www whitehouse gov/omb/circulars/a110/a110 html)

REG05 - OMB Circular A-133 (www whitehouse gov/omb/circulars/a133/a133 html)

REG09 - Subject to Streamlined Noncompeting Award Procedures (SNAP)

REG10 - Federal Demonstration Partnership (FDP) guidelines (www thefdp org)

REG19 - NIH Grants Policy Statement (http //grants nih gov/grants/policy/nihgps_2010/index htm)

UMN - U of M Requirements

UMN01 - The U of M Effort Certification system must be used to reflect all U of M staff committed effort on this award, whether or not any salary is charged to the award

UMN02 - Salary cost transfers cannot be made after effort is submitted

UMN03 - All U of M Policy and Guidelines relating to research must be followed and can be read in detail on the web at http //www ospa umn edu/policiesandprocedures/index htm

UMN04 - U of M Cost Transfer guidelines state transfers of non-salary expenses on accounts be clearly documented & made within 30 days after the end of the accounting period the charge was processed

NON-FINANCIAL REPORTING (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Milestones Tab)
Type: Report **Description:** Progress Report **Due Date:**
Comments: PHS 2590 - noncompeting continuation Must be submitted via the eRA Commons Due 45 days prior to the end of the budget period

INVOICE/BILLING INFORMATION
Basis of Payment: COST_REIMBURSE **Method of Payment:** Electronic Fund Transfer
Invoice Form: GPC - GM_LOC
Interim Invoice Deadline: 0 **Final Invoice Deadline:** 0

Contact Name: Jacqueline Jones
Contact Phone:
Contact Fax:
Contact Email:
Contact Address: PSC-FMS (301) 443-3020
PARKLAWN BUILDING ROOM 16A-12
5600 FISHERS LANE

ROCKVILLE MD 20857

FINANCIAL REPORTING

Report Form ID: R_FFR
Interim Reporting Deadline: 90
Reporting Frequency: Termination/Final
Final Reporting Deadline: 90

GENERAL PROVISIONS

This document is intended to provide guidance in managing this award, to the Principal Investigator, Academic Unit, and to those who are noted as receiving this document Its intent is to provide general information regarding University and sponsor policies when given the award In addition, it is intended to reference specific conditions related to this award Specific questions should be addressed to the grant administrator from SPA noted below By accepting this award, the Principal Investigator acknowledges and agrees to abide by all provisions of the sponsoring agency and the University of Minnesota policies related to sponsored projects management, including the Code of Conduct

U OF M CONTACTS

Administrative questions to SPA staff member:	Sowolo Abdullai	612/626-2724	abdu0092@umn edu
Billing and financial questions to SFR:	Julia Steinkopf	612/624-7033	gordo091@umn edu

Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Issue Date: 06/08/2011

Grant Number: 5R01AI089728-02 REVISED

Principal Investigator(s):
Fang Li, PHD

Project Title: Receptor recognition mechanisms of coronaviruses

Edward F Wink
University of Minnesota Twin Cities
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 554552070

Award e-mailed to: awards@umn edu

Budget Period: 05/01/2011 – 04/30/2012
Project Period: 05/15/2010 – 04/30/2015

Dear Business Official

The National Institutes of Health hereby revises this award to reflect an increase in the amount of $33,975 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MINNESOTA TWIN CITIES in support of the above referenced project This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system

Each publication, press release or other document that cites results from NIH grant-supported research must include an acknowledgment of NIH grant support and disclaimer such as "The project described was supported by Award Number R01AI089728 from the National Institute Of Allergy And Infectious Diseases The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Allergy And Infectious Diseases or the National Institutes of Health "

Award recipients are required to comply with the NIH Public Access Policy This includes submission to PubMed Central (PMC), upon acceptance for publication, an electronic version of a final peer-reviewed, manuscript resulting from research supported in whole or in part, with direct costs from National Institutes of Health The author's final peer-reviewed manuscript is defined as the final version accepted for journal publication, and includes all modifications from the publishing peer review process For additional information, please visit http //publicaccess nih gov/

Award recipients must promote objectivity in research by establishing standards to ensure that the design, conduct and reporting of research funded under NIH-funded awards are not biased by a conflicting financial interest of an Investigator Investigator is defined as the Principal Investigator and any other person who is responsible for the design, conduct, or reporting of NIH-funded research or proposed research, including the Investigator's spouse and dependent children Awardees must have a written administrative process to identify and manage financial conflict of interest and must inform Investigators of the conflict of interest policy and of the Investigators' responsibilities Prior to expenditure of these awarded funds, the Awardee must report to the NIH Awarding Component the existence of a conflicting interest and within 60 days of any new conflicting interests identified after the initial report Awardees must comply with these and all other aspects of 42 CFR Part 50, Subpart F These requirements also apply to subgrantees, contractors, or collaborators engaged by the Awardee under this award The NIH website http //grants nih gov/grants/policy/coi/index htm provides additional information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV

Sincerely yours,

Jane Paull
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI089728-02 REVISED

Award Calculation (U.S. Dollars)

Federal Direct Costs	$247,500
Federal F&A Costs	$126,225
Approved Budget	$373,725
Federal Share	$373,725
TOTAL FEDERAL AWARD AMOUNT	$373,725
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$33,975

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
2	$373,725	$373,725
3	$373,725	$373,725
4	$373,725	$373,725
5	$373,725	$373,725

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93 855
EIN:	1416007513A1
Document Number:	RAI089728A
Fiscal Year:	2011

IC	*CAN*	*2011*	*2012*	*2013*	*2014*
AI	8472364	$373,725	$373,725	$373,725	$373,725

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C S / **OC** 414E / **Processed** PAULLJ 06/08/2011

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI089728-02 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http //grants nih gov/grants/policy/awardconditions htm

SECTION III – TERMS AND CONDITIONS – 5R01AI089728-02 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following

a The grant program legislation and program regulation cited in this Notice of Award.
b Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts
c 45 CFR Part 74 or 45 CFR Part 92 as applicable
d The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period
e This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW

(See NIH Home Page at 'http //grants nih gov/grants/policy/awardconditions.htm' for certain references cited above)

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase V Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP)

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included See http //grants nih gov/grants/policy/awardconditions htm for the full NIH award term implementing this requirement and other additional information

This award may be subject to the Transparency Act subaward and executive compensation reporting requirements of 2 CFR Part 170 See http //grants nih gov/grants/policy/awardconditions htm for the full NIH award term implementing this requirement and additional award applicability information

In accordance with P L 110-161, compliance with the NIH Public Access Policy is now mandatory For more information, see NOT-OD-08-033 and the Public Access website http //publicaccess nih gov/

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI089728-02 REVISED

This revised award increases the total costs authorized consistent with NIAID implementation of the NIH fiscal policy for FY2011 (See NIH Guide Notice NOT-OD-11-068) Future year recommended levels also have been adjusted consistent with the referenced Notice

Supersedes Notice of Award (NoA) issued 04/20/11
****
In addition to the PI, any absence, replacement, or substantial reduction in effort of the following individual(s) below, requires the written prior approval of the National Institutes of Health awarding component

Dr Robert Geraghty,Associate Professor and co-PI

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions The Program Official is responsible for the scientific, programmatic and technical aspects of this project These individuals work together in overall project administration Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist Requests may be made via e-mail

Grants Management Specialist Cheryl Y Wall
Email wallcy@niaid nih gov **Phone** 301-451-2696 **Fax** 301-493-0597

Program Official: Frederick J Cassels
Email casselsf@niaid nih gov **Phone** 301-402-8601 **Fax** 301-496-8030

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI089728-02 REVISED

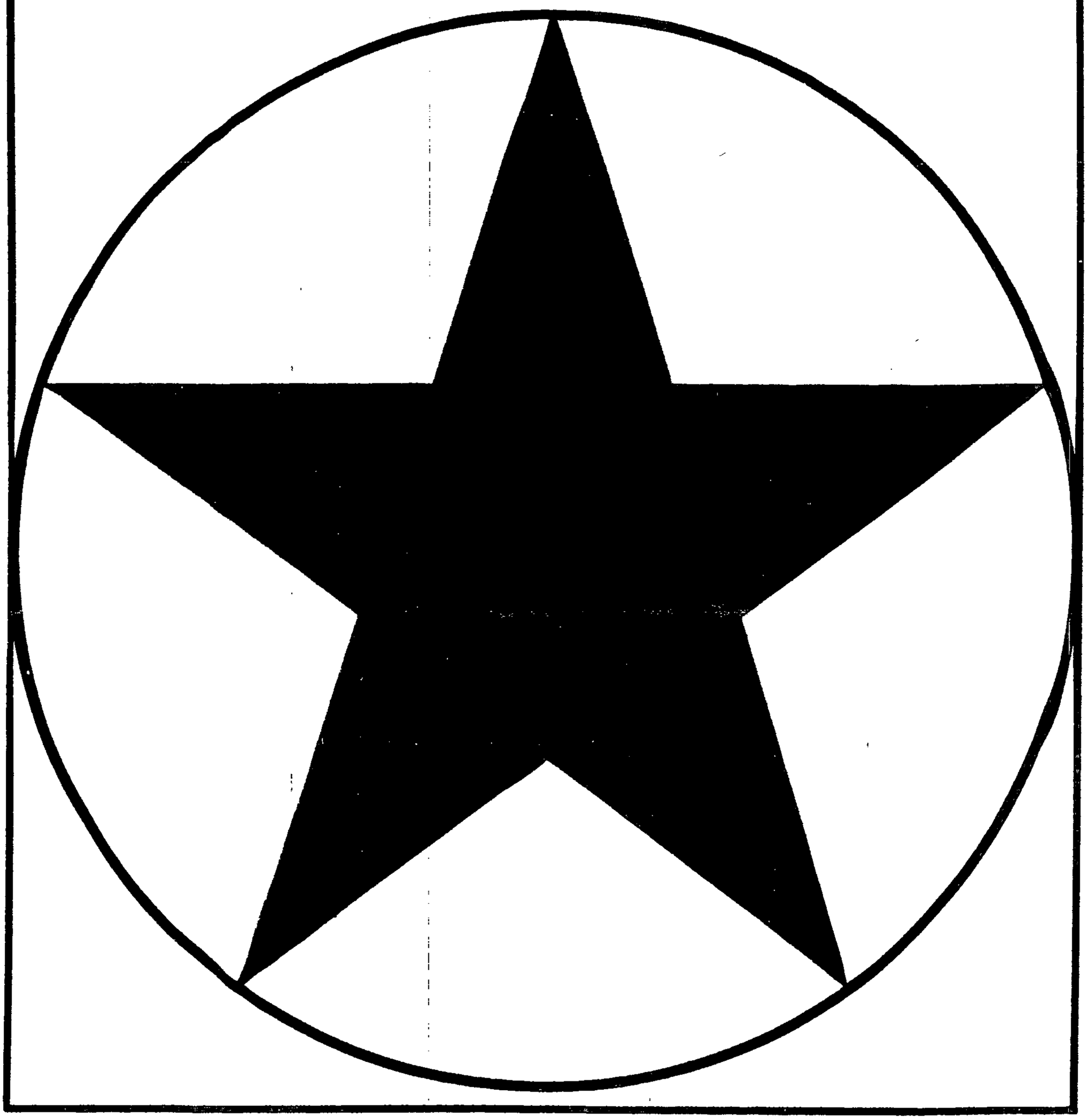
INSTITUTION: UNIVERSITY OF MINNESOTA TWIN CITIES

Budget	***Year 2***	***Year 3***	***Year 4***	***Year 5***
TOTAL FEDERAL DC	$247,500	$247,500	$247,500	$247,500

TOTAL FEDERAL F&A	$126,225	$126,225	$126,225	$126,225
TOTAL COST	$373,725	$373,725	$373,725	$373,725

Facilities and Administrative Costs	***Year 2***	***Year 3***	***Year 4***	***Year 5***
F&A Cost Rate 1	51%	51%	51%	51%
F&A Cost Base 1	$247,500	$247,500	$247,500	$247,500
F&A Costs 1	$126,225	$126,225	$126,225	$126,225

Please Mail All Pages Prior To Page Separator



AWARD SETUP INFORMATION PAGE **GA** SA **DATE** 6/15/11
CON_____ **PROJECT** 00013782
eTool Completed ✓ No info in eTool_____

_____New
_____PreAward – see bottom of page for information
___✓___Modification, original Award = CON – 22345 (this is the award that will be updated)
_____Stimulus Funding

INVOICING AND FINANCIAL REPORTING INFORMATION CAN BE FOUND ON THE FOLLOWING PAGE(S) IN THE AWARD DOCUMENT _____

TERMS AND CONDITIONS
_____ are attached _____ are in eTool
_____ are saved in MyNetworkPlaces\Award Setup\Award Setup-submissions TAB_____
(\\ovpr.ad.umn.edu\ovpr$\Shared\Groups\Award Setup)

BUDGET INFORMATION FOR FUNDS PROVIDED BY SPONSOR – for new awards only
_____ Fund _____ DeptID
_____ is in eTool
_____See attached PS-friendly budget

COST SHARE INFORMATION – for new awards only (GA updates the budgets for modifications)
_____Fund _____ DeptID _____ Program _____ Chartfield 1 _____ Chartfield 2 _____Emplid
_____ is in eTool
_____See attached PS-friendly budget

_____**NEW PROJECT NEEDED** - For new projects on existing awards, please enter relevant information in the eTool or on separate page and include a completed PS-friendly budget.

ANY ADDITIONAL INFORMATION NEEDED FOR SET UP:
*Please see updates and comments for NOGA in eTool

PUT AWARD FILE IN THE FOLLOWING ORDER:
Agency award documents
Guidance documents (to be included with award emailed to PI/Dept)
Award Imaging Divider page (yellow star sheet)
Award Setup Information page (this page)
PS-friendly budget (if any)
Terms and Conditions spreadsheet (if any)
Negotiation notes, proposal correspondence, proposal, etc.

SET ALL NEGOTIATE AWARD NEGOTIATION TYPES TO COMPLETED EXCEPT FOR THE ONE FOR CONTRACT ENTRY AND THE ORIGINAL ONE CREATED BY FILES

PREAWARD INFORMATION REQUIRED - Provide information for each project
Note: No cost share information is required for PreAwards.
_____Fund Budget accounts to be set up at $0 are listed below - OR - ____See Attached
_____DeptID Remember to include 810500 if F&A should be charged
_____Project _____ _____ _____ _____ _____
_____Function _____ _____ _____ _____ _____
_____F&A rate & base _____ _____ _____ _____ _____.
_____Rate Type *(Research, Other, Training, Off Campus, ClinTrial-nonfed, CREE2, CREES, DOD contract, Equip, Foundation, Hormel, Instruction, SBIR/STTR Phase I, State)*

___/___/_____ End Date of PreAward / Advance Project to be entered in KK_Child and KK_Parent

Thursday, June 09, 2011
11.51 AM

Negotiate Award

Proposal ID:	CON000000022345	**Business Unit:**	UMSPR
Version ID:	A101		
Award ID:	CON000000022345	**Sponsor:**	NIH Natl Inst of Allergy & Infect Dis

Type and Status Find | View All First 6 of 6 Last

Sequence: 6 **Event Date:** 06/09/2011

Negotiation Type: 4ModA Mod Request Rece **Negotiate Status:** Working Negotia

Comments: Li. 00013782, Yr 02 REV sent to SA

Details Find | View All First 1 of 1 Last

Name: **Title:**

Telephone: **Fax:**

Email ID:

Screen clipping taken 6/9/2011 11 51 AM

PS Project: 13782

PI NAME: Fang Li

TITLE:

Proposed	$37,750
AWARDED TOTAL	$33,975

0.9000000000

OBJECT	DESCRIPTION	PROPOSED	PRORATED	Difference
700100	Academic Salaries	5,108	4,597	511
700300	Acad Sal-Stdts-Grad/Prof/Trng		-	-
700500	Civil Service & Union Salaries		-	-
700300	Post-Doc	8,480	7,632	848
	Acad Professional Salaries		-	-
710100	Acad Fringe Benefits	1,839	1,655	184
710300	Acad Sal-Stdts Fringe Benefits		-	-
	Acad Professional Fringe		-	-
	Civil Service & Union Fringe		-	-
710300	Post-Doc Fringe	2,154	1,939	215
	Undergrad salary		-	-
	Consultant Cost	1,000	900	100
730200			-	-
810100	Research Subject Payments		-	-
720100	General Operating Supplies		-	-
720300	General Operating Services		-	-
720100	Computer/supplies<2500		-	-
720300	Print/Dupl/Binding/Graphics		-	-
720300	Sponsored Publication Costs	250	225	25
720200	Lab/Medical Supplies	5,639	5,075	564
720400	Lab/Medical Services		-	-
750100	Computer Software		-	-
720300	Courier/Mailing Services		-	-
720100	Postage		-	-
720500	Telephone		-	-
720500	Telephone-Long Distance	80	72	8
810200	Participant Expenses		-	-
730250	Medical/Dental Health Care	-	-	-
720600	Travel-Domestic/Foreign/other	300	270	30
			-	-
7900	Reserve-SPA	-	-	-
810300	Subcontract		-	-
810300/810402	Subcontract #2		-	-
810300/810403	Subcontract #3		-	-
780100	Repairs & Maintenance	150	135	15
770100	Rent/Lease		-	-
770150	Rent/Leases-Bldg/Facilities	-	-	-
770200		-	-	-
770250		-	-	-
168100	Equipment		-	-
168100	Computer Equipment		-	-
810600	Institutional Allowance	-	-	-
	TOTAL DIRECT CHARGES	25,000	22,500	2,500
8500	F & A Costs	12,750	11,475	1,275
	TOTAL	37,750	33,975	3,775

GDES INFORMATION:

	GA Code	
1843	AC Code	
	Grant Grp (CFDA)	
	Grantor Code 0000-	
1249	Proposal No	
	Award No	
	State Date	
	End Date	
	Report Date	
	Start Month (FY)	
	End Month (FY)	
		N/A
	IDC Rate	51.00%

PS Project: 13782

PI NAME: Fang Li

TITLE:

Proposed	$377,500
AWARDED TOTAL	$339,750

0.9000000000

OBJECT	DESCRIPTION	PROPOSED	PRORATED	Difference
700100	Academic Salaries	51,083	45,975	5,108
700300	Acad Sal-Stdts-Grad/Prof/Trng		-	-
700500	Civil Service & Union Salaries		-	-
	Post-Doc	84,803	76,323	8,480
	Acad Professional Salary		-	-
710100	Acad Fringe Benefits	18,391	16,552	1,839
710300	Acad Sal-Stdts Fringe Benefits		-	-
	Acad Professional Fringe		-	-
	Civil Service & Union Fringe		-	-
	Post-Doc	21,542	19,388	
	Undergrad salary		-	-
	Consulant Costs	10,000	9,000	1,000
730200	Professional Services		-	-
810100	Research Subject Payments		-	-
720100	General Operating Supplies		-	-
720300	General Operating Services		-	-
720100	Computer/supplies<2500		-	-
720300	Print/Dupl/Binding/Graphics		-	-
720300	Sponsored Publication Costs	2,500	2,250	250
720200	Lab/Medical Supplies	56,381	50,743	5,638
720400	Lab/Medical Services		-	-
750100	Computer Software		-	-
720300	Courier/Mailing Services		-	-
720100	Postage		-	-
720500	Telephone		-	-
720500	Telephone-Long Distance	800	720	80
810200	Participant Expenses		-	-
730250	Medical/Dental Health Care	-	-	-
720600	Travel-Domestic/Foreign/other	3,000	2,700	300
			-	-
7900	Reserve-SPA	-	-	-
810300	Subcontract		-	-
810300/810402	Subcontract #2		-	-
810300/810403	Subcontract #3		-	-
780100	Repairs & Maintenance	1,500	1,350	150
770100	Rent/Lease		-	-
770150	Rent/Leases-Bldg/Facilities	-	-	-
770200		-	-	-
770250		-	-	-
168100	Equipment		-	-
168100	Computer Equipment		-	-
810600	Institutional Allowance	-	-	-
	TOTAL DIRECT CHARGES	250,000	225,000	25,000
8500	F & A Costs	127,500	114,750	12,750
	TOTAL	377,500	339,750	37,750

GDES INFORMATION:

GA Code		
AC Code		
Grant Grp (CFDA)		
Grantor Code 0000-		
28591	Proposal No	
	Award No	
12920		
12920		
2751	State Date	
	End Date	
	Report Date	
	Start Month (FY)	
	End Month (FY)	
		N/A
	IDC Rate	51.00%

CON- 22345
00013782
SA

Subject: NoA: 5 R01 AI089728-02 PI: Li, Fang
From: <era-notify@mail.nih.gov>
Date: Wed, 8 Jun 2011 19:04:22 -0400
To: <awards@umn.edu>

Grant Number: 5 R01 AI089728-02
Principal Investigator: Li, Fang
Project Title: Receptor recognition mechanisms of coronaviruses
Institution: UNIVERSITY OF MINNESOTA
CFDA: 93.855
OC: 414E
PCC: M51C S
Award Issue Date: 06/08/2011
Grants Management Officer: Paull, Jane
Program Official: Cassels, Frederick
Grants Specialist: Wall, Cheryl

*** This is an automated notification - Please do not reply to this message. ***

temp1890557261313115188.pdf	**Content-Type:** application/pdf **Content-Encoding:** base64

2) Rebudget funds into new Accounts:

00013782.

Sponsored Chartfields:

(4) FUND	(5) DeptID	(5) PCBU	(8) Project	(1) Activity
3002	11718	UMSPR	[redacted]	1

Please remember to include any necessary adjustments to F&A

Move From:

$	200	*from Account*	720200
$		*from Account*	
$		*from Account*	
$		*from Account*	
$		*from Account*	
$		*from Account*	
$		*from Account*	
$		*from Account*	
$		*from Account*	
$		*from Account*	

Move To:

$	200	*to Account*	720401
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	
$		*to Account*	

JUSTIFICATION:

Set up Scientific services for use of Biacore 2000 that is used for detecting the binding affinity of conoravirus spike protein and receptor.
This service is necessary in the research on "Structural studies of the SARS coronavirus spike protein"

By submission of this information, I certify that all parties have agreed to these budget reallocations.

Name (CA)	James Burak #52
Dept ID #	11718
Phone #	612-625-8660
Email	burak001@umn.edu

Sowolo Abdullai <abdu0092@umn.edu>

FW: Rebudget request 00013782

1 message

Jim Burak <burak001@umn.edu> **Tue, Aug 16, 2011 at 9:38 AM**
To: Sowolo Abdullai <abdu0092@umn.edu>
Cc: Carrie DeJarlais <dejar001@umn.edu>

Approved.
--
Jim Burak
MNPI Center &
Department of Pharmacology
University of Minnesota
6-120 Jackson Hall
321 Church Street SE
Minneapolis, MN 55455
612 625-8660

On 8/16/11 8:50 AM, "Carrie DeJarlais" <dejar001@umn.edu> wrote:

>Jim,
>
>If in agreement with this request please forward onto Sowolo
>(abdu0092@UMN.EDU
>) in SPA for account code setup.
>
>Thanks.
>
>Carrie DeJarlais
>Dept. of Pharmacology
>MNPI Center, U of MN
>6-120 Jackson Hall
>Minneapolis, MN 55455
>612-625-4982
>
>
>

Biocor rebudget request.xls
139K

OMB No. 0925-0001

Grant Number R01AI089728-03	**Total Project Period** From: 05/15/2010 To: 04/30/2015
EIN: **Review Group:** 1416007513A1 VIRA	**Requested Budget Period:** From: 05/01/2012 To: 04/30/2013
Title of Project: Receptor recognition mechanisms of coronaviruses	**Due Date:** 03/30/2012 **Submitted Date:** 03/12/2012

Program Director/Principal Investigator: Fang Li UNIVERSITY OF MINNESOTA TWIN CITIES Department of Pharmacology 6-120 Jackson Hall 321 Church Street S.E. MINNEAPOLIS , MN 554552070 **Phone Number:** 612-625-6149 **Fax Number:** **Email Address:** lifang@umn.edu	**Applicant Organization:** UNIVERSITY OF MINNESOTA TWIN CITIES UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS , MN 554552070 **Department:** PHARMACOLOGY **Major Subdivision:** MEDICAL SCHOOL
Administrative Official: Lorrie Awoyinka 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS , MN 554552070 **Phone Number:** 6126245599 **Fax Number:** 6126244843 **Email Address:** lorrie@umn.edu	**Signing Official:** David L Hagen 200 Oak Street SE Suite 450 Minneapolis , MN 55455 **Phone Number:** 612-626-9895 **Fax Number:** 612-624-4843 **Email Address:** hagen027@umn.edu
Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** **Phase III Clinical Trial:** ☒ No ☐ Yes	**Vertebrate Animals:** ☒ No ☐ Yes **Inventions and Patents:** ☒ No ☐ Yes ☐ **Previously Reported** ☒ **Not Previously Reported**
Program Income: ☒ No ☐ Yes	

Budget Period	**Anticipated Amount**	**Source**
F&A Changes:		

Primary Project/Performance Site Location		
Organizational Name: University of Minnesota		
DUNS: 5559179960000		
Street 1: 3-284 Nils Hasselmo Hall	Street 2: 312 Church Street SE	
City: Minneapolis	County: Hennepin	State: MN
Province:	Country: UNITED STATES	Zip/Postal Code: 554550215
Congressional Districts: MN-005		

Additional Project/Performance Site Location		
Organizational Name: University of of Minnesota		
DUNS: 5559179960000		
Street 1: 7-227 Phillips Wangensteen Building	Street 2: 516 Delaware Street SE	
City: Minneapolis	County: Hennepin	State: MN
Province:	Country: UNITED STATES	Zip/Postal Code: 55455
Project/Performance Site Congressional Districts: MN-005		

Program Director/Principal Investigator:	**Grant Number**
Fang Li	R01AI089728-03
Applicant Organization:	**Period Covered by this Report:**
UNIVERSITY OF MINNESOTA TWIN CITIES	05/01/2011 - 04/30/2012

Title of Project:

Receptor recognition mechanisms of coronaviruses

SNAP Questions:

Has there been a change in the other support of Senior/Key Personnel since the last reporting period?

☒ **No** ☐ **Yes**

Justification:

Will there be, in the next budget period, a significant change in the level of effort for the PD/PI or other Senior/Key Personnel designated on the Notice of Award from what was approved for this project?

☒ **No** ☐ **Yes**

Justification:

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☒ **No** ☐ **Yes**

Justification:

Changes in Select Agent Research? ☒ **No** ☐ **Yes**

Changes in Multiple PD/PI Leadership plan? ☒ **No** ☐ **Yes**

Change in human embryonic stem cell (hESC) line(s) used? ☒ **No** ☐ **Yes**

Justification:

Human Subject Education Requirement:

Has the Involvement of Human Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Has the Involvement of Animal Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Publications:

Valid NIHMSID:	Citation ID:	Citation Source:	Citation Text:

Cover Letter: File is not uploaded
Research Accomplishments: File is not uploaded
Other Document File: File is not uploaded
Other Support File: File is not uploaded

All Personnel Report

Program Director/Principal Investigator:			**Grant Number**		
Fang Li			R01AI089728-03		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Fang Li	FANGLI	PHD	XXX-XX [redacted]	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
PD/PI			[redacted]	6.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Lang Chen	LANGCHEN	PhD	XXX-XX [redacted]	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Robert James Geraghty		PhD	XXX-XX [redacted]	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-Investigator				1.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Yi-Lun Lin	YILUNLIN	PhD	XXX-XX [redacted]	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Guiqing Peng	GUIQINGPENG	PhD	XXX-XX [redacted]	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Liqing Xu	XULXUL	PhD	XXX-XX [redacted]	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Wenbo Zhou	WENBOZHOU	PhD	XXX-XX [redacted]	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				4.8		

Progress report

A. Specific aims

Coronaviruses use a variety of cellular receptors and co-receptors including proteins and sugars. The diverse usage of receptors has allowed coronaviruses to infect a wide range of mammalian and avian species, and cause respiratory, enteric, systemic and neurological diseases. Two domains in coronavirus spike protein S1 subunit, the N-terminal domain (NTD) and the C-domain, have evolved functions as receptor-binding domains (RBDs). How coronaviruses have evolved to do so has been a major puzzle in virology. To solve this puzzle, this NIH-funded project "receptor recognition mechanisms of coronaviruses" investigates the molecular and structural basis for the complex receptor-recognition mechanisms of coronaviruses.

There are three specific aims in this project. Aim 1 focuses on the receptor recognition mechanisms of group-I coronaviruses that use angiotensin-converting enzyme 2 as receptors (e.g. NL63 coronavirus). Aim 2 focuses on the receptor recognition mechanisms of group-1 coronaviruses that use aminopeptidase-N as receptors (e.g., porcine transmissible gastroenteritis virus and 229E coronavirus). Aim 3 focuses on the receptor recognition mechanisms of group-2 coronaviruses (e.g. SARS coronavirus, mouse coronavirus, bovine coronavirus, human coronavirus OC43). The research approaches of this proposal include crystallographic analysis of the virus-receptor interfaces, identification of structural elements important for virus-receptor interactions, and characterization of these elements using crystallographic, biochemical, molecular, and virological tools.

B. Studies and Results

Our research in the past funding year has made groundbreaking progress in understanding the evolution and receptor recognition of coronaviruses.

First, Peng et al. (2011, *PNAS*) determined the crystal structure of group-2 mouse coronavirus (MHV) NTD complexed with its receptor murine carcinoembryonic antigen-related cell adhesion molecule 1a (mCEACAM1a). Unexpectedly, MHV NTD contains a core structure that has the same β-sandwich fold as human galectins (S-lectins) and additional structural motifs that bind to the N-terminal Ig-like domain of mCEACAM1a. Despite its galectin fold, MHV NTD does not bind sugars, but instead binds mCEACAM1a through exclusive protein-protein interactions. Critical contacts at the interface have been confirmed by mutagenesis, providing a structural basis for viral and host specificities of coronavirus/CEACAM1 interactions. Sugar-binding assays reveal that galectin-like NTDs of some group-2 coronaviruses such as human coronavirus OC43 (HCoV-OC43) and bovine coronavirus (BCoV) bind sugars. Structural analysis and mutagenesis localize the sugar-binding site in coronavirus NTDs to be above the β-sandwich core. We propose that coronavirus NTDs originated from a host galectin and retained sugar-binding functions in some contemporary coronaviruses, but evolved new structural features in MHV for mCEACAM1a binding.

Second, Wu et al. (2012, *Journal of Biological Chemistry*) elucidated the molecular and structural mechanisms for the host receptor adaptation by group-2 SARS coronavirus (SARS-CoV). We found that all of the naturally selected mutations in SARS-CoV RBD (the S1 C-domain) were viral adaptations to either human or civet receptor angiontensin-converting enzyme 2 (ACE2). Further, we have designed and characterized two optimized RBDs. The human-optimized RBD contains all of the human-ACE2-adapted residues (F442/F472/N479/D480/T487), and possesses exceptionally high affinity for human ACE2 but relative low affinity for civet ACE2. The civet-optimized RBD contains all of the civet-ACE2-adapted residues (P442/Y472/R479/G480/T487), and possesses exceptionally high affinity for civet ACE2 and also substantial affinity for human ACE2. These results not only illustrate the detailed mechanisms of host receptor adaptation by SARS-CoV, but also provide molecular and structural basis for tracking future SARS-CoV evolution in animals.

Third, Li (2012, *Journal of Virology*) identified shared structural topologies (connectivity of secondary structural elements) in S1 domains of different coronavirus genera. The results suggest that coronavirus S1 subunits share a common evolutionary origin but have attained diverse sequences and structures following extensive divergent evolution. The results also increase understanding of the structures and functions of coronavirus S1 domains whose tertiary structures are currently unknown.

Fourth, Wu et al. (2011, *Journal of Virology*) investigated the function of a virus-binding hotspot on human ACE2 that is critical for the binding of both group-1 NL63 coronavirus (NL63-CoV) and group-1 SARS-CoV. The hotspot consists of a salt bridge surrounded by hydrophobic tunnel walls. Mutations that disturb the hotspot structure have significant effects on virus/receptor interactions, revealing critical energy contributions from the hotspot structure. The tunnel structure at the NL63-CoV/ACE2 interface is more compact than that at the SARS-CoV/ACE2 interface, and hence virus/ACE2 binding affinities are decreased either by NL63-CoV

mutations that decrease the tunnel space or by SARS-CoV mutations that increase the tunnel space. Furthermore, NL63-CoV RBD (the S1 C-domain) inhibits ACE2-dependent infection by SARS-CoV, a successful application of our hotspot theory that has the potential to become a novel antiviral strategy against SARS-CoV infections. These results suggest that the structural features of the hotspot on ACE2 were among the driving forces for the convergent evolution of NL63-CoV and SARS-CoV.

C. Significance

Our research has been the major driving force in the coronavirus field to understand coronavirus/receptor interactions. Prior to the SARS epidemic, no structural information was available for any coronavirus S1 subunit, despite decades of extensive research. Since the SARS epidemic, we have successfully determined the crystal structures of group-1 NL63-CoV C-domain, group-2 SARS-CoV C-domain, and group-2 MHV NTD each complexed with their respective host receptor. To date, these structures remain the only structures available for any coronavirus S1 or coronavirus-S1/receptor complexes. By determining these structures, we have solved several important puzzles in virology, such as how animal viruses can cross species barriers to infect humans through one or a few seemingly trivial mutations in their RBDs, how different viruses recognize the same receptor by targeting common virus-binding hotspots on the receptor, how similar viruses recognize different receptors through subtle structural changes in viral RBDs, and how viruses can acquire host proteins and evolve them into viral RBDs with different receptor specificity. Protein biochemistry and virological methods have also been systematically performed to corroborate our structural findings. By elucidating these structures, we have also been able to reconstruct the evolutionary history of coronaviruses. Furthermore, our detailed structural analyses of SARS-CoV/receptor interactions have significantly prepared humans for any potential future SARS epidemics.

D. Plans

For the next funding year, we will focus on specific aims 2 and 3.

For specific aims 2, we will investigate the structural mechanism whereby the group-1 porcine transmissible gastroenteritis virus (TGEV) sugar-binding domain binds sugars and APN-binding domain binds APN. We will prepare the complexes of each of these domains complexed with their respective receptor, and set up crystallization screens of these complexes. We will also investigate the mechanism whereby 229E coroanvirus spike protein kept its APN-binding function but lost its sugar-binding function through evolution. In a pilot study, we have successfully obtained APN crystals that diffract X-ray to 1.85 Å. Breakthroughs will be expected from this study not only for the coronavirus field, but also for other major medical fields such as cancer, pain sensation, mood disorder, blood pressure regulation and high cholesterol level, because of the multifunctional roles of APN in these fields.

For aim 3, we will investigate the structural and biochemical studies of sugar binding mechanisms of group-2 coronavirus NTDs. Here we will determine the crystal structures of group-2 BCoV and HCoV-OC43 NTDs by themselves and in complex with their sugar substrates and examine the functions of important sugar-binding residues in BCoV and HCoV-OC43 NTDs. These studies will elucidate the sugar binding mechanisms of group-2 coronaviruses and reveal how the sugar-binding functions have evolved in coronaviruses. In two pilot studies, we have obtained crystals of BCoV NTD that diffract X-ray to 1.6 Å and have also identified a sugar-binding pocket in coronavirus NTDs that shares both similarities and differences with human galectins.

Also for aim 3, we will study the structural and molecular basis for MHV driven mouse evolution. Pathogen driven host evolution in mammals has long been a topic of debate for lack of clear supporting evidence. Two isoforms of mCEACAM1, 1a and 1b, are present in mice. Despite their high sequence homology, mCEACAM1b is a much less effective MHV receptor than mCEACAM1a, a likely result from MHV-driven mouse evolution. Here we will determine the crystal structure of mCEACAM1b and provide structural basis for mCEACAM1b being an ineffective MHV receptor and engineer mCEACAM1b to allow it to gain functions as MHV receptor. These studies will elucidate the structural and molecular mechanisms for mCEACAM1b being an ineffective MHV receptor and for MHV driven mouse evolution. In a pilot study, we have obtained crystals of mCEACAM1b that diffract X-ray to 3.1 Å.

E. Publications (from the past project period)

1. Wu, K., Chen, L., Peng, G., Zhou, W., Pennell, C., Mansky, L., Geraghty, R., **Li, F.** (June 2011) A virus-binding hotspot on human angiotensin-converting enzyme 2 is critical for binding of two

different coronaviruses. *Journal of Virology*, *85,* 5331-5337 (PMID: 21411533; PMCID: PMC3094985)

2. Peng, G., Sun, D., Rajashankar, K., Qian, Z., Holmes, K., **Li, F.** (June 2011) Crystal structure of mouse coronavirus receptor-binding domain complexed with its receptor murine CEACAM1a. *Proceedings of the National Academy of Sciences USA*, *108,* 10696-10701 (PMID: 21670291; PMCID: PMC3127895)

3. **Li, F.** (March 2012) Evidence for a common evolutionary origin of coronavirus spike protein receptor-binding subunits. *Journal of Virology*, *86,* 2856-2858 (PMID: 22205723)

4. Wu, K., Peng, G., Wilken, M., Geraghty, R., **Li, F.** (March 2012) Mechanisms of host receptor adaptation by SARS coronavirus. *Journal of Biological Chemistry*, *Epub ahead of print,* (PMID: 22291007)

F. Project Generated Resources

This research did not generate cell lines, DNA probes, animal models, protocols, or software. De-identified raw data from the experiments and computer programs (CCP4 macromolecular crystallography scripts) used for protein structure determinations are available to anyone upon written request to the PI. Peer-reviewed publications accepted or published during the past project period are being or have been entered in NIHMS and PubMed Central.

UNIVERSITY OF MINNESOTA

Notice of Grant or Contract Award

Sponsored Projects Administration

(Online Page Navigation Main Menu > Grants >Awards > UM_NOGA Report)

Award: CON000000022345
Primary Project: 00013782
PRF #: 651709

AWARD INFORMATION (Online Page Navigation Main Menu > Grants > Awards >Award Profile)

Award Start Date: 05/15/2010
Award End Date: 04/30/2015
Funds Approved to Date: $1,124,950 00
Principal Investigator: Li,Fang
Award Type: Grant
Primary Project Dept: PHCL Pharmacology Admin
Purpose: Research
Sponsor: NIH Nat'l Inst of Allergy & Infect Dis
Sponsor Award #: 5R01AI089728-03
Award Title #: Receptor recognition mechanisms of coronaviruses

ASSOCIATED PROJECTS (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Funding Tab)

Project #: 00013782 **Department:** 11718 - PHCL Pharmacology Admin
Project PI: Li,Fang **Dates:** 05/15/2010 - 04/30/2015 **Project Title:** Receptor recognition mechanism
F&A: 51 00 MTDC **EFFDT:** 05/15/2010

AWARD HISTORY (Online Page Navigation Main Menu > Grants > Awards > Review Award Modifications)

(Displayed in action order ('Mod Issue Date' in descending order))

Reference Award#: 5R01AI089728-03 **Amount:** $373,725 00
Period: 3 **Begin Date:** 05/01/2012 **End Date:** 04/30/2013
Modification Seq: 3 **Modification Type:** Continuation **Mod Issue Date:** 05/01/2012
Comments:

Notice of Award dated 04/10/2012

None of the funds in this award shall be used to pay the salary of an individual at a rate per year in excess of the amounts reflected in the following NIH Guide Notice http //grants nih gov/grants/guide/notice-files/NOT-OD-12-035 html

All other previous terms and conditions remain unchanged

Reference Award#: 5R01AI089728-02 Rev **Amount:** $33,975 00
Period: 2 **Begin Date:** 05/01/2011 **End Date:** 04/30/2012
Modification Seq: 3 **Modification Type:** Additional Funding **Mod Issue Date:** 06/15/2011
Comments:

This revised award increases the total costs authorized consistent with NIAID implementation of the NIH Fiscal policy for FY2011

All other terms and conditions remain the same

Reference Award#: 1R01AI089728-02 **Amount:** $339,750 00
Period: 2 **Begin Date:** 05/01/2011 **End Date:** 04/30/2012
Modification Seq: 2 **Modification Type:** Continuation **Mod Issue Date:** 05/05/2011
Comments:

NIH is currently funded through a Continuing Resolution at the FY2010 level Therefore, this non-competing award is made at a level below the committed FY2011 level If final appropriation permits, adjustments may be made up to FY2011 plan level

All other terms and conditions remain the same

Reference Award#: 1R01AI089728-01 **Amount:** $377,500 00
Period: 1 **Begin Date:** 05/15/2010 **End Date:** 04/30/2011
Modification Seq: 1 **Modification Type:** Original **Mod Issue Date:** 05/27/2010
Comments:

An unobligated balance may be carried forward to the next budget period without prior agency approval

NOTE In additoin to the PI, Dr Robert Geraghty as been identified as key personnel (co-PI) Any absence, replacement or substantial

11
7-11
39

reduction in effort by key personnel requires prior, written agency approval

Modular award The significant rebudgeting provision does not apply. In addition, since costs were averaged over the project period, deficits may be carried forward from one budget period to the next within the same project period

ESTIMATED FUTURE FUNDING / ACTIONS (Online Page Navigation Main Menu > Grants > Awards > Review Award Modifications)
(Displayed by 'Period' and 'Modification Sequence' in ascending order)

Reference Award#: 1R01AI089728-01 **Amount:** $373,725 00
Period: 4 **Begin Date:** 05/01/2013 **End Date:** 04/30/2014
Modification Seq: 4 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

Reference Award#: 1R01AI089728-01 **Amount:** $373,725 00
Period: 5 **Begin Date:** 05/01/2014 **End Date:** 04/30/2015
Modification Seq: 5 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

COMMENTS

Description: **Entered by:** HOWI0016 **Date & Time:** 5/27/2010 10:00 AM
Date and Time Stamp indicates Proposal-to-Award Process

Description: **Entered by:** HOWI0016 **Date & Time:** 5/27/2010 10 02 AM
Sponsor Contacts

Grants Management
Michael Wells
Phone 301-451-2656
Fax 301-493-0597
Email wellsmj@niaid nih gov

Program
Rachelle Salomon
Phone 301-402-2202
Fax 301-496-8030
Email salomonra@mail nih gov

Description: **Entered by:** SPAUL **Date & Time:** 12/23/2010 9 56 AM
LOC
RAI089728

SPECIAL REVIEWS (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Certifications Tab)
Project #: 00013782

Description: rDNA, InfectAgents, BioToxins
Approval Date: 03/10/2012 **Expiration Date:** 03/09/2013 **AssuranceNumber:** 1002H77733
Comments:

BUDGET INFORMATION (Online Page Navigation Main Menu > Grants>Awards>Project Budget)
Please review online budget for valid account string combinations (ex PCBU, Activity, Fund, Dept_Id, Project, Account)

Project #: 00013782 **Cost Share Budget Total:**
Project Start Date: 05/15/2010 **Project End Date:** 04/30/2015

ICR Sharing **Department:** Drug Design, Center for **Percentage:** 11 50
ICR Sharing **Department:** PHCL Pharmacology Admin **Percentage:** 88.50

Period	Start Date	End Date	Direct Cost (Approved)	F & A Cost (Approved)	Direct Cost (Pending)	F & A Cost (Pending)	Total Period Cost
1	05/15/2010	04/30/2011	$252,702 00	$124,798 00	$0 00	$0 00	$377,500 00
2	05/01/2011	04/30/2012	$247,500 00	$126,225 00	$0 00	$0 00	$373,725 00
3	05/01/2012	04/30/2013	$247,500 00	$126,225.00	$0.00	$0 00	$373,725 00
4	05/01/2013	04/30/2014	$0 00	$0 00	$247,500.00	$126,225 00	$373,725 00
5	05/01/2014	04/30/2015	$0 00	$0 00	$247,500 00	$126,225 00	$373,725 00
TOTAL			$747,702 00	$377,248 00	$495,000 00	$252,450 00	$1,872,400 00

Budget Detail (all periods)

Fund Code	DeptID	Program Code	Chartfield 1	Chartfield 2	Fin EmplID	Activity	Analysis Type	Account	Cost Share	Budget Item	Amount	Hold
3002	11718					1	BUD	168100		CAPITAL_EQUIP	$8,000 00	
3002	11718					1	BUD	700100		FACULTY_SALARY	$136,874 00	
3002	11718					1	BUD	700200		PROF_ADM_SALARY	$186,666 00	
3002	11718					1	BUD	700300		GRAD_STU_SALARY	$81,400 00	
3002	11718					1	BUD	700310		GRAD_SAL_NOTUIT	$0 00	
3002	11718					1	BUD	710100		FACULTY_FRINGE	$47,095 00	
3002	11718					1	BUD	710200		PROF_ADM_FRINGE	$46,666 00	
3002	11718					1	BUD	710300		GRAD_STU_FRINGE	$16,117 00	
3002	11718					1	BUD	710310		GRAD_FRG_NOTUIT	$0 00	
3002	11718					1	BUD	720200		LABMED_SUPPLIES	$172,891 00	
3002	11718					1	BUD	720300		GEN_OP_SERVICES	$6,875 00	
3002	11718					1	BUD	720400		LABMED_SERVICES	$200 00	
3002	11718					1	BUD	720500		TELECOMM	$1,992 00	
3002	11718					1	BUD	720600		TRAVL_MILE_MOVE	$7,970 00	
3002	11718					1	BUD	730100		CONSULTING_SVCS	$29,900 00	
3002	11718					1	BUD	750100		NON_CAP_EQUIP	$571 00	
3002	11718					1	BUD	780100		REPAIRS_MAINT	$4,485 00	
3002	11718					1	BUD	810500		FACADM	$377,248 00	

COST SHARE/MATCH/IN-KIND
CSM - Cost Share, Matching and InKind

CSM05 - Departments must document all cost share that is shown in the proposal

PROGRAM INCOME INFORMATION
PIN - Program Income

PIN03 - Reportable Program Income must be deposited by the department into the Sponsored Program Income Unapplied Cash account

PIN04 - Additional - Program Income can be used in addition to sponsor funds to cover project costs that will further the objectives of the legislation under which the grant was made

TERMS & CONDITIONS (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Terms Tab)

APV - Additional Approval

APV01 - Prior sponsor approval or notification required for reduction of PI's effort by 25% or more

APV04 - Prior sponsor approval required to replace PI

APV07 - Prior sponsor approval required for changes to key personnel that would impact the scope of work

APV10 - Prior sponsor approval required for changes to work scope

APV14 - Prior sponsor approval required for the absence of the PI for 3 months or more

APV15 - Prior sponsor approval required for a second no cost extension or a first no cost extension that is submitted after the agency notification deadline

APV20 - Prior sponsor approval required for the addition of foreign sites or foreign subcontracts

FIS - Fiscal

FIS03 - Reversion of funds at termination / final

FIS07 - Deficits may be carried forward

FIS10 - Grant/contract funds may not be used to pay the salary of an individual at a rate in excess of Executive Level I of the Federal Executive Pay Scale ($199,700)

FIS11 - Modular award - Expenses incurred must be allocated and accounted for in compliance with all applicable cost principles

FIS13 - Deficits may be carried forward within non-competing segments, but may not be carried forward between competing segments

FIS14 - Grant/contract funds may not be used to pay the salary of an individual at a rate in excess of Executive Level II of the Federal Executive Pay Scale ($179,700)

IPR - IP Rights

IPR02 - Sponsor support must be acknowledged in patent applications and patent issuing

IPR04 - Title to U of M, non-exclusive, non-transferable, irrevocable, paid-up license to Federal Government

IPR12 - PI obligation to report inventions (and other IP) to the sponsor (and to PTM per policy)

PRP - Property

PRP02 - U of M receives title to equipment purchased or fabricated under this award

PRP03 - Sponsor may require transfer of equipment and its title upon award completion for those items costing in excess of $5000

PUB - Publications

PUB02 - U of M will place an acknowledgement of sponsor support and a disclaimer, as appropriate, on any publication generated by this award or reporting on the award results and/or activity

PUB07 - Peer-reviewed articles must be submitted to NIH Pubmed Central (PMC) upon acceptance for publication and made publicly available within 12 months Cite PMC ID number in progress reports/proposals

RBD - Rebudgeting

RBD10 - Rebudgeting is allowed as long as there is no change in work scope

REG - Regulatory

REG01 - 45 CFR Part 74 - Code of Federal Regulations as it pertains to institutions of higher education

REG03 - OMB Circular A-21 (www whitehouse gov/omb/circulars/a021/a021 html)

REG04 - OMB Circular A-110 (www whitehouse gov/omb/circulars/a110/a110 html)

REG05 - OMB Circular A-133 (www whitehouse gov/omb/circulars/a133/a133 html)

REG09 - Subject to Streamlined Noncompeting Award Procedures (SNAP)

REG10 - Federal Demonstration Partnership (FDP) guidelines (www thefdp org)

REG19 - NIH Grants Policy Statement (http //grants nih gov/grants/policy/nihgps_2010/index htm)

UMN - U of M Requirements

UMN01 - The U of M Effort Certification system must be used to reflect all U of M staff committed effort on this award, whether or not any salary is charged to the award

UMN02 - Salary cost transfers cannot be made after effort is submitted.

UMN03 - All U of M Policy and Guidelines relating to research must be followed and can be read in detail on the web at http //www ospa umn edu/policiesandprocedures/index htm

UMN04 - U of M Cost Transfer guidelines state transfers of non-salary expenses on accounts be clearly documented & made within 30 days after the end of the accounting period the charge was processed

NON-FINANCIAL REPORTING (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Milestones Tab)

Type: Report **Description:** Progress Report **Due Date:**
Comments: PHS 2590 - noncompeting continuation Must be submitted via the eRA Commons Due 45 days prior to the end of the budget period

INVOICE/BILLING INFORMATION

Basis of Payment: COST_REIMBURSE
Method of Payment: Electronic Fund Transfer
Invoice Form: GPC - GM_LOC
Interim Invoice Deadline: 0
Final Invoice Deadline: 0

Contact Name: Jacqueline Jones
Contact Phone:

Contact Fax:
Contact Email:
Contact Address: PSC-FMS (301) 443-3020
PARKLAWN BUILDING ROOM 16A-12
5600 FISHERS LANE
ROCKVILLE MD 20857

FINANCIAL REPORTING

Report Form ID: R_FFR
Interim Reporting Deadline: 90
Reporting Frequency: Termination/Final
Final Reporting Deadline: 90

GENERAL PROVISIONS

This document is intended to provide guidance in managing this award, to the Principal Investigator, Academic Unit, and to those who are noted as receiving this document Its intent is to provide general information regarding University and sponsor policies when given the award In addition, it is intended to reference specific conditions related to this award Specific questions should be addressed to the grant administrator from SPA noted below By accepting this award, the Principal Investigator acknowledges and agrees to abide by all provisions of the sponsoring agency and the University of Minnesota policies related to sponsored projects management, including the Code of Conduct

U OF M CONTACTS

Administrative questions to SPA staff member:	Sowolo Abdullai	612/626-2724	abdu0092@umn edu
Billing and financial questions to SFR:	Julia Steinkopf	612/624-7033	gordo091@umn edu

Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Issue Date: 04/10/2012

Grant Number: 5R01AI089728-03

Principal Investigator(s):
Fang Li, PHD

Project Title: Receptor recognition mechanisms of coronaviruses

David L Hagen
University of Minnesota Twin Cities
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 554552070

Award e-mailed to: awards@umn edu

Budget Period. 05/01/2012 – 04/30/2013
Project Period: 05/15/2010 – 04/30/2015

Dear Business Official

The National Institutes of Health hereby awards a grant in the amount of $373,725 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MINNESOTA TWIN CITIES in support of the above referenced project This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI089728 The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health " Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with 42 CFR Part 50 Subpart F Subsequent to the compliance date of the 2011 revised FCOI regulation (i e , on or before August 24, 2012), Awardees must be in compliance with all aspects of the 2011 revised regulation, until then, Awardees must comply with the 1995 regulation The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards Consult the NIH website http //grants nih gov/grants/policy/coi/ for a link to the regulation and additional important information

If you have any questions about this award, please contact the individual(s) referenced in Section IV

Sincerely yours,

Artisha Wright
Grants Management Officer

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI089728-03

Award Calculation (U.S. Dollars)

Federal Direct Costs	$247,500
Federal F&A Costs	$126,225
Approved Budget	$373,725
Federal Share	$373,725
TOTAL FEDERAL AWARD AMOUNT	$373,725
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$373,725

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
3	$373,725	$373,725
4	$373,725	$373,725
5	$373,725	$373,725

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number	93 855
EIN:	1416007513A1
Document Number:	RAI089728A
Fiscal Year:	2012

IC	*CAN*	*2012*	*2013*	*2014*
AI	8472364	$373,725	$373,725	$373,725

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC M51C S / **OC** 414E / **Processed** WRIGHTAR 04/04/2012

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI089728-03

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI089728-03

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following

a The grant program legislation and program regulation cited in this Notice of Award
b Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts
c 45 CFR Part 74 or 45 CFR Part 92 as applicable
d The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period
e This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW

(See NIH Home Page at 'http //grants nih gov/grants/policy/awardconditions htm' for certain references cited above)

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase V Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP)

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration Should a consortium/subaward be issued under this award, a DUNS requirement must be included See http //grants nih gov/grants/policy/awardconditions htm for the full NIH award term implementing this requirement and other additional information

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170

In accordance with P L 110-161, compliance with the NIH Public Access Policy is now mandatory For more information, see NOT-OD-08-033 and the Public Access website http //publicaccess nih gov/

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI089728-03

INFORMATION ONLY None of the funds in this award shall be used to pay the salary of an individual at a rate per year in excess of the amounts reflected in the following NIH Guide Notice http //grants nih gov/grants/guide/notice-files/NOT-OD-12-035 html
****

In addition to the PI, any absence, replacement, or substantial reduction in effort of the following individual(s) below, requires the written prior approval of the National Institutes of Health awarding component

Dr Robert Geraghty, Associate Professor and co-PI
****

The research proposed in this grant may involve Select Agents and/or Highly Pathogenic Agents Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving approval from the NIAID,NIH Program Official Awardee is responsible for having its subcomponent/subcontractor comply with the requirements pertaining to the use of Select Agents and/or Highly Pathogenic Agents An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13 b (http //www selectagents gov/Regulations html)

NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that, under some circumstances, may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http //www cdc gov/OD/ohs/biosfty/bmbl5/bmbl5toc htm), and your Institutional Biosafety Committee (IBC) or equivalent body, or appropriate designated institutional biosafety official If there is ambiguity in the BMBL guidelines and/or there is disagreement among the BMBL, an institutional committee or institutional official, the highest recommended containment level must be used

When submitting future Progress Reports indicate at the beginning of the report

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide

o A list of the new and/or additional Agent(s) that will be studied,
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment,
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official It is important to note if the work is being done in a new location

For work with Select Agents performed in the U S provide documentation of Registration status of all domestic organizations/entities where Select Agent(s) will be used For work with Select Agents performed in a non-U S country prior NIAID approval is required

Please be advised that changes in the use of a Select Agent will likely be considered a change in scope and, therefore, require NIH awarding office prior approval

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions The Program Official is responsible for the scientific, programmatic and technical aspects of this project These individuals work together in overall project administration Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist Requests may be made via e-mail

Grants Management Specialist Michael J Wells
Email wellsmj@niaid nih gov **Phone** 301-451-2656 **Fax** 301-493-0597

Program Official: Rachelle Salomon
Email salomonra@mail nih gov **Phone** 301-402-2202 **Fax** 301-496-8030

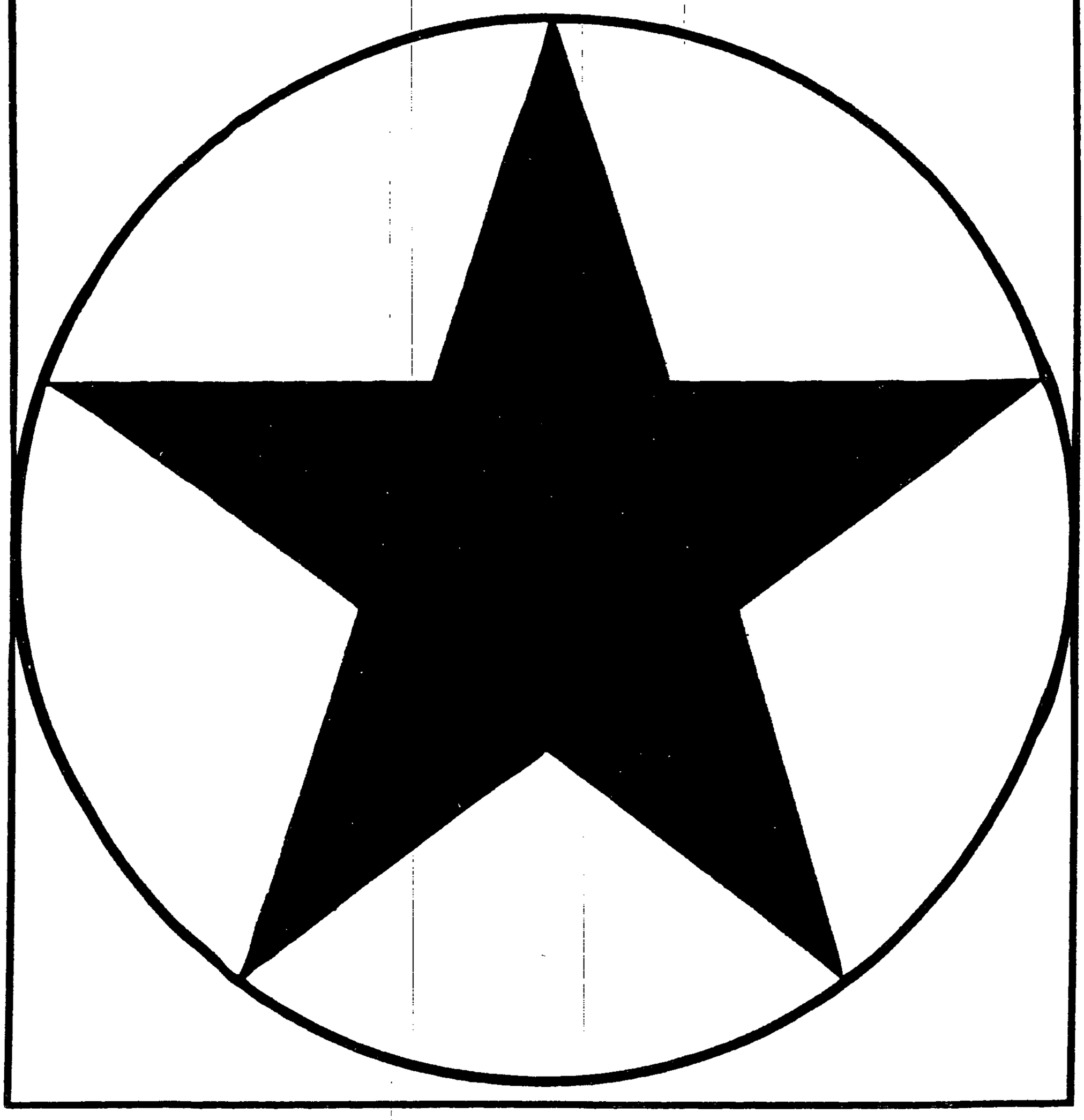
SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI089728-03

INSTITUTION: UNIVERSITY OF MINNESOTA TWIN CITIES

Budget	***Year 3***	***Year 4***	***Year 5***
TOTAL FEDERAL DC	$247,500	$247,500	$247,500
TOTAL FEDERAL F&A	$126,225	$126,225	$126,225
TOTAL COST	$373,725	$373,725	$373,725

Facilities and Administrative Costs	***Year 3***	***Year 4***	***Year 5***
F&A Cost Rate 1	51%	51%	51%
F&A Cost Base 1	$247,500	$247,500	$247,500
F&A Costs 1	$126,225	$126,225	$126,225

Please Mail All Pages Prior To Page Separator



AWARD SETUP INFORMATION PAGE GA Renee for TBN DATE 5/1/13
CON 370201 PROJECT 00013782
eTool Completed ____

_____New
_____ PreAward – see bottom of page for information
__X__ Modification, original Award = CON 23345 (this is the award that will be updated)
_____ Stimulus Funding
_____ FFATA

INVOICING AND FINANCIAL REPORTING INFORMATION CAN BE FOUND ON THE FOLLOWING PAGE(S) IN THE AWARD DOCUMENT Same

TERMS AND CONDITIONS
3002 are attached 11718 are in eTool
_____ are saved in MyNetworkPlaces\Award Setup\Award Setup-submissions TAB_____
(\\ovpr.ad.umn.edu\ovpr$\Shared\Groups\Award Setup)

BUDGET INFORMATION FOR FUNDS PROVIDED BY SPONSOR – for new awards only
3002 Fund 11718 DeptID
_____ is in eTool
_____See attached PS-friendly budget

COST SHARE INFORMATION – for new awards only (GA updates the budgets for modifications)
_____Fund _____ DeptID _____ Program _____ Chartfield 1 _____ Chartfield 2 _____Emplid
_____ is in eTool
_____See attached PS-friendly budget

_____**NEW PROJECT NEEDED** - For new projects on existing awards, please enter relevant information in the eTool or on separate page and include a completed PS-friendly budget.

ANY ADDITIONAL INFORMATION NEEDED FOR SET UP:

Yr 3 funding
Comments in EFS-Modifications

PUT AWARD FILE IN THE FOLLOWING ORDER:
Agency award documents
Guidance documents (to be included with award emailed to PI/Dept)
Award Imaging Divider page (yellow star sheet)
Award Setup Information page (this page)
PS-friendly budget (if any)
Terms and Conditions spreadsheet (if any)
Negotiation notes, proposal correspondence, proposal, etc.

SET ALL NEGOTIATE AWARD NEGOTIATION TYPES TO COMPLETED EXCEPT FOR THE ONE FOR CONTRACT ENTRY AND THE ORIGINAL ONE CREATED BY FILES

PREAWARD INFORMATION REQUIRED - Provide information for each project
Note: No cost share information is required for PreAwards.
_____Fund Budget accounts to be set up at $0 are listed below - OR - ____See Attached
_____DeptID Remember to include 810500 if F&A should be charged
_____Project _____ _____ _____ _____ _____
_____Function _____ _____ _____ _____ _____
_____F&A rate & base _____ _____ _____ _____ _____
_____Rate Type *(Research, Other, Training, Off Campus, ClinTrial-nonfed, CREE2, CREES, DOD contract, Equip, Foundation, Hormel, Instruction, SBIR/STTR Phase I, State)*

___/___/_____ End Date of PreAward / Advance Project to be entered in KK_Child and KK_Parent

PS / SPONSOR PROPOSAL BUDGET WORKSHEET

Please complete the cost share and F&A distribution information at the bottom of the page, if applicable

Award 5R01AI089728-03 Project #, if known **13782** Should this be the Primary Project? x Yes ___ No
Project DeptID 11718 Project PI **Fang Li** trainee-related expenses

		Total of all worksheet tabs	Sponsor $373,725	Cost Share $0	Total Projects $373,725
ITEM			**Sponsor Funding**	**Cost Share or Match***	**TOTAL PROJECT BUDGET**
Personnel					
Salary					
700100	Salary - Faculty		42,250		
700200	Salary - Professional and Admin		93,913		
700300	Salary - Post Doc / Professional Student				
700300	Salary - Grad Research Asst (expense account 700301)				
700400	Salary - Undergrad Student				
700500	Salary - Civil Service Bargaining Unit				
Total Salary			136,163	0	136,163
Fringe					
710100	Fringe - Faculty		14,746		
710200	Fringe - Professional and Admin		22,410		
710300	Fringe - Post Doc / Professional Student				
710300	Fringe - Grad Research Asst (expense account 710301)				
710400	Fringe - Undergrad Student				
710500	Fringe - Civil Service Bargaining Unit				
710600	Fringe - Direct Charge				
Total Fringe			37,156	0	37,156
Total Personnel			173,319	0	173,319
Consulting / Professional Services					
730100	Consulting Services		10,000		
730200	Professional Services				
Total Consulting / Professional Services			10,000	0	10,000
Equipment					
168100	Equipment				0
Supplies					
720100	General Operating Supplies				
720200	Lab & Medical Supplies		56,381		
750100	Non-Capitalized Equipment				
Total Supplies			56,381	0	56,381
Travel					
720600	Travel / Mileage / Moving		3,000		3,000
Other Direct Costs					
720300	General Operating Services		2,500		
720400	Lab & Medical Services				
720500	Telecommunications		800		
770100	Long Term Rents & Leases				
770150	Long Term Rents & Leases (bldg/facility)				
770200	Short Term Rents & Leases				
770250	Short Term Rents & Leases (bldg/facility)				
780100	Repairs & Maintenance		1,500		
810100	Research Subject Payments				
Total Other Direct Costs			4,800	0	4,800
Subawards / Subcontracts					
810300	Subcontracts not subject to F&A				
810400	Subcontracts subject to F&A				
Total Subawards / Subcontracts			0	0	0
Additional Accounts (show budget account and a description, please)					
___	___				0
___	___				0
___	___				0
TOTAL DIRECT COSTS			247,500	0	247,500
810500	Facilities and Administrative Expenses		126,225		126,225
TOTAL COSTS			373,725	0	373,725

UNIVERSITY OF MINNESOTA

ReportID: UMFGM017F

PROPOSAL SUMMARY

Run Date 04/12/2012
Run Time: 03:50:34 PM

Status 4 AwdB Award Sent to GA

May 1st

Grant Administrator	Abdullai,Sowolo Armina		
PRF #	714044	Proposal #/Version	CON000000037020/A101
EGMS Proposal #		Sponsor Award #	5R01AI089728-03
Proposal Type	Non-Competing Continuation	Concurrent	N
Previous Award	CON000000022345	SubContract	N
Purpose			
Title	Receptor recognition mechanisms of coronaviruses		
Principal Investigator	Li,Fang	lifang@umn edu	

Budget Summary

Start Date	05/01/2012	End Date	04/30/2013	
Direct Costs	$250,000 00	Indirect Costs	$127,500 00	Total Costs $377,500 00

Sponsor: NIH Nat'l Inst of Allergy & Infect Dis

Submission Information

Due Date	03/15/2012	Date Type	Receipt Date
Submission Method	Electronically Only	# of Copies Submitted	

Mailing Address

Comments/Corrections

REPA	__verified	__email sent for _____		
IRB	__verified	__pending	__email sent	__Not Applicable
IACUC	__verified	__pending	__email sent	__Not Applicable
IBC	__verified	__pending	__email sent	__Not Applicable
F&A rate	__at full or agency limit	__waiver on file	__waiver requested	
Cost share documented?		__yes	__no	__Not Applicable
ICR sharing?		__yes	__no	

✓ NOGA readd
✓ Need to Finalize Budget last

CON-37020
F: CON-13728
714044
LLA
RCR-met

Subject: NoA: 5 R01 AI089728-03 PI: Li, Fang
From: <era-notify@mail.nih.gov>
Date: 4/9/2012 11:21 PM
To: <awards@umn.edu>

Grant Number: 5 R01 AI089728-03
Principal Investigator: Li, Fang
Project Title: Receptor recognition mechanisms of coronaviruses
Institution: UNIVERSITY OF MINNESOTA
CFDA: 93.855
OC: 414E
PCC: M51C S
Award Issue Date: 04/10/2012
Grants Management Officer: Wright, Artisha
Program Official: Salomon, Rachelle
Grants Specialist: Wells, Michael

*** This is an automated notification - Please do not reply to this message. ***

Attachments

temp2485156342014393263.pdf 111 KB

(CON-13728)
CON-370200 (CON-22345)
714044

Status Information

General Grant Information	
Status:	Pending administrative review Refer any questions to Program Official or Grants Management Specialist
Institution Name:	UNIVERSITY OF MINNESOTA
School Name:	MEDICAL SCHOOL
School Category:	SCHOOLS OF MEDICINE
Division Name:	NONE
Department Name:	PHARMACOLOGY
PI Name:	Li, Fang
Application ID:	5R01AI089728-03
Proposal Title:	Receptor recognition mechanisms of coronaviruses
Proposal Receipt Date:	03/12/2012
Last Status Update Date:	04/20/2011
Budget Start Date.	05/01/2012
Budget End Date	04/30/2013
Progress Report Due Date:	03/16/2012
Current Award Notice Date:	
Application Source:	Esnap
Project Period Begin Date:	05/15/2010
Project Period End Date:	04/30/2015
eApplication Status:	
FOA:	[PA07-070] - RESEARCH PROJECT GRANT (PARENT R01)
NIH Appl. ID:	8259500

Other Relevant Documents

e-Application

Additions for Review (0 documents)

Status History

Effective Date	Status Message

Institute or Center Assignment

Institute or Center	Assignment Date
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES **(Primary)**	04/20/2011
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES **(Primary)**	06/08/2011

Application Information

Award Document Number	RAI089728A
FSR Accepted Code	N
Snap Indicator Code	Y
Review Outcome Available	No
Summary Statement Available	No
Early Stage Investigator Eligible	
New Investigator Eligible	

Study Section

Scientific Review Group	VIRA
Council Meeting Date(YYYY/MM)	2012/00

Advisory Council(AC) Information

Reference Letter(s)

This list shows Reference Letters associated with this particular Grant Application Principal Investigator can see a list of all Reference Letters within Personal Profile - Reference Letters section on eRA Commons

Contacts

Administration	Name	Phone	Email
Grants Management Specialist(GMS)	Wells, Michael J	301-451-2656	wellsmj@niaid nih gov
Program Official(PO)	Salomon, Rachelle	301-402-2202	salomonra@mail nih gov

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National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland
20892

Department of Health and Human Services

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ESNAP due 3/15

UNIVERSITY OF MINNESOTA

ReportID: UMFGM017F

PROPOSAL SUMMARY

Run Date: 03/12/2012
Run Time: 08:27:53 AM

Status ~~2 PropB Elect Received at SPA~~

Grant Administrator. LA for TBN

INSTITUTION APPROVED
03.12.12 NLB

PRF #	714044	Proposal #/Version	CON000000037020/V101
EGMS Proposal #		Sponsor Award #	
Proposal Type	Non-Competing Continuation	Concurrent	N
Previous Award		SubContract	N
Purpose	Project 13728/52/CON - 22345		
Title	Receptor recognition mechanisms of coronaviruses		
Principal Investigator	Li,Fang	lifang@umn edu	

Budget Summary

Start Date	05/01/2012	End Date	04/30/2013	
Direct Costs	~~$0 00~~ 247,500	Indirect Costs	~~$0 00~~ 126,225	Total Costs ~~$0 00~~ 373,725
Sponsor	National Institutes of Health (NIH) /NIAID			

Submission Information

Due Date	03/15/2012	Date Type	Receipt Date
Submission Method		# of Copies Submitted	

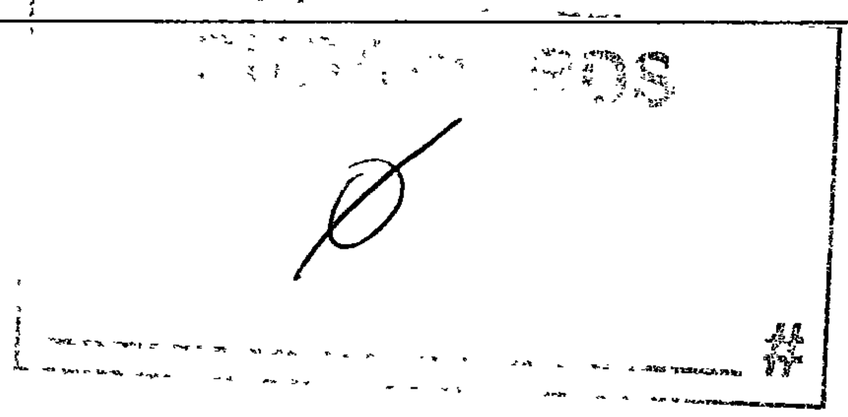
Mailing Address eSNAP 3\12\12 - 11:42 am EDT via ESNAP- Kevin

Comments/Corrections

REPA	X verified	__email sent for _____		
IRB	__verified	__pending	__email sent	X Not Applicable
IACUC	__verified	__pending	__email sent	X Not Applicable
IBC	X verified	__pending	__email sent	__Not Applicable
F&A rate 51% MTDC	X at full or agency limit	__waiver on file	__waiver requested	
Cost share documented?		__yes	__no	X Not Applicable
ICR sharing?		X yes	__no	

eSNAP submission

03.12.12 Logout NLB



ELECTRONIC PROPOSAL
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_NON-FED
_G-GOV

~~NO~~
ATTACHMENT

UNIVERSITY OF MINNESOTA PRF

EGMS ID: PRF ID: 714044

Proposal Routing Form for External Research, Training or Public Service Support — PS Proposal ID

1.	Sponsor Name	**National Institutes of Health** — **Primary Funding Source:**
2.	Proposal Title	**Receptor recognition mechanisms of coronaviruses**
3.	Sponsor Due Date/Time	**03/15/2012 - 5pm CST** — **Receipt** C O N 3 7 0 20 '12 MR 9 09:30
4.	RFA/RFP/CFDA/FOA#	
5.	Submission Medium	**Electronic [other than Grants.gov] Submission via eRA Commons ESNAP.**
6.	University Contact	**Weber, Lynn F** — **(612) 626-6902** — **weber117@umn.edu**
7.	Administering Dept	**PHCL PHARMACOLOGY ADMIN** — **Department ID: 11718**

8. Principal Investigator

			Paid	Cost Share	PI	Key
Li, Fang 2010						
lifang	**ID 3694715**	**(612) 625-6149**	**50**	**0**	**X**	**X**
ASSISTANT PROFESSOR [9403]	**PHCL PHARMACOLOGY ADMIN**	**DeptID 11718**				

9. Investigator(s)

			Paid	Cost Share	PI	Key
Geraghty, Robert J. 2010						
gerag012	**ID 1235946**	**(612) 625-3281**	**8**	**0**		**X**
ASSOCIATE PROFESSOR [9402]	**DRUG DESIGN, CENTER FOR**	**DeptID 11345**				

10. Proposal is for

	Basic %	Applied %	Development %	Total %
Research	**100**	**0**	**0**	**100**

11. Proposal is.

	Agency Award #	EFS Award #	Primary Project
Continuation	**5R01 Ai089728-03**	**30021171800013782**	

13782

12. Proposed Duration & Amount Requested

	Start	End	Direct $$	Indirect $$	F&A Rate	Total
Initial/Current Budget	**05/01/2012**	**04/30/2013**	**250,000**	**127,500**	**51% MTDC**	**377,500**
All Years	**05/01/2012**	**04/30/2013**	**250,000**	**127,500**	**51% MTDC**	**377,500**

[X] Departmental Directions to SPA for Award Set Up
Proposal budget has been prepared outside of EGMS: Department will furnish PeopleSoft-friendly budget to SPA prior to receipt of the award; SPA may pro-rate at the time of award if needed to accommodate a reduced award amount.

[X] **F&A Rate Used** — **51% MTDC**

F&A Comments — **Full F&A Rate**

13. Project Involves

[X] **Recombinant DNA, Infectious Agents or Biological Toxins** — **There will be recombinant DNA, infectious agents or biological toxins used**

Registration status of this project with the Institutional Biosafety Committee

Status	Study Code #	Approval Date
Approved	**1002H77733** ✓	**03/10/2012** ✓

[X] **Chemicals** — **There will be chemicals used** — **The appropriate chemical safety training and hazardous waste training records have been completed**

14. Subawards [ ]

15. Conflict of Interest.	[X]	**There is not a potential financial and/or business conflict of interest** **REPA #.** **Approval Date:** **Comments:**			
16. Inventions	[ ]				
17. Space	[X]	**There is sufficient and suitable space to house this project presently assigned to the principal investigator's or other investigator's department or college.**	**3-284 and 7-227**	**Nils Hasselmo Hall and PWB**	
18. Resources/Space/Staff	[X]	**This project involves University resources, space or staff from more than one department**			
19. Indirect Cost Recovery	[X]	**Academic units have chosen to share ICR revenue and be shared by contributions**	**PHCL PHARMACOLOGY ADMIN**	**11718**	**89**
			DRUG DESIGN, CENTER FOR	**11345**	**11**
		Comments **11.5% of F&A shared with Center for Drug Design, Dept. ID 11345** **88.5 of F&A for Pharmacology Department, Dept. ID 11718**			
20. Matching and Cost Sharing	[ ]				
21. Program Income	[ ]				
22. International Component	[ ]				
23. Fairview Health Services	[ ]				

24. PROPOSAL ABSTRACT or EXECUTIVE SUMMARY

25. NOTES

03/08/12	**dejar001**	**Actual direct cost is $247,500 & $126,225 IDC = Total $373,725.**

APPROVALS and CERTIFICATIONS

Principal Investigator:

(1) I certify the information submitted within the application is true, complete and accurate to the best of the PI's knowledge,
(2) that any false, ficticious, or fraudulent statements or claims may subject the PI to criminal, civil, or administrative penalties, and
(3) that the PI agrees to accept the responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of the application

Investigator(s):

The information provided on this form and in the accompanying proposal is correct to the best of my knowledge Equipment budgeted in this application is not otherwise available for use on this project from existing departmental or collegiate inventories In the event this application is awarded, I (we) agree to abide by all applicable institutional and sponsoring agency policies and procedures including the Intellectual Property Policy of the University of Minnesota and to follow commonly accepted scientific practices in recording and maintaining records of research I (we) certify that I (we) have read the University of Minnesota Code of Conduct policy and agree to abide by the rights and responsibilities as identified therein

Signature **Fang Li** **03-08-2012**
Fang Li (lifang) Principal Investigator **Date**

Department Head(s) and/or Division Head(s). Academic Dean(s), Chancellor or Provost.

We certify that the above statements are correct to the best of our knowledge The commitment of departmental and collegiate resources, if any, has been noted and approved We agree that the scientific objectives of this application are in keeping with departmental and collegiate goals We certify that the individuals from our unit(s) listed on this PRF have filed a current Report of External Professional Activities (REPA)

Signature **DeJarlais, Carrie L** **03-08-2012**
DeJarlais, Carrie L (dejar001) Dept Administrator/Representative **Date**

Signature **Loh, Horace H** **03-08-2012**
Loh, Horace H (lohxx001) Dept Head/Delegate **Date**

Signature **Hinske, Liz** **03-08-2012**
Hinske, Liz (hinsk001) Dept Administrator/Representative **Date**

Signature **Vince, Robert** **03-08-2012**
Vince, Robert (vince001) Dept Head/Delegate **Date**

Signature **Paller, Mark S** **03-08-2012**
Paller, Mark S (palle001) Research Associate Dean/Delegate **Date**

Signature **tmeddean** **03-08-2012**
tmeddean (tmeddean) Research Associate Dean/Delegate **Date**

Signature **Segal, Rosalyn A** **03-08-2012**
Segal, Rosalyn A (rsegal) Research Associate Dean/Delegate **Date**

Signature **LeBien, Tucker W** **03-08-2012**
LeBien, Tucker W (lebie001) Research Associate Dean/Delegate **Date**

Subject: PRF 714044 Fang Li NIH eSNAP Progress Report
From: Lynn Weber <weber117@umn.edu>
Date: 3/12/2012 8:22 AM
To: "proposal@umn.edu" <proposal@umn.edu>
CC: <spateam1@umn.edu>

FYI

Fang Li NIH eSNAP Progress Report has been electronically routed to SPA via eRA Commons

PRF 714044 and budget have been faxed to SPA

Lynn

--

Lynn Weber
Department of Pharmacology
University of Minnesota
Medical School
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321 Church Street SE
Minneapolis, MN 55455
Phone 612-626-6902
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History List

Reviewer Name	Decision	Notification Sent	Decision Rei
McKoskey, Kevin		03/10/2012 06 12 38 PM	03/12/2012 10 31 37 Al
Li, Fang	Route	02/16/2012 12 57 46 PM	03/10/2012 06 12 38 PI
Hagen, David L		03/12/2012 10 44 27 AM	03/12/2012 11 41 21 Al
NIH		03/12/2012 11 42 54 AM	
McKoskey, Kevin	Submit	03/12/2012 11 41 21 AM	03/12/2012 11 42 54 Al
Awoyinka, Lorrie	Route	03/12/2012 10 31 37 AM	03/12/2012 10 44 27 Al

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National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland 20892

Department of Health and Human Services

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OMB No 0925-0001

Grant Number		Total Project Period	
R01AI089728 03		From 05/15/2010	To 04/30/2015
EIN	**Review Group**	**Requested Budget Period**	
1416007513A1	VIRA	From 05/01/2012	To 04/30/2013

Title of Project
Receptor recognition mechanisms of coronaviruses

Due Date 03/30/2012
Submitted Date 03/12/2012

Program Director/Principal Investigator

Fang Li
UNIVERSITY OF MINNESOTA TWIN CITIES
Department of Pharmacology
6-120 Jackson Hall
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MINNEAPOLIS MN 554552070

Phone Number 612 625-6149
Fax Number
Email Address lifang@umn edu

Applicant Organization

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Department PHARMACOLOGY

Major Subdivision MEDICAL SCHOOL

Administrative Official

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Signing Official

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Minneapolis MN 55455

Phone Number 612 626 9895
Fax Number 612-624-4843
Email Address hager027@umn edu

Human Subjects	☒ No ☐ Yes	Vertebrate Animals	☒ No ☐ Yes
Research Exempt	☒ No ☐ Yes	Inventions and Patents	☒ No ☐ Yes
Exemption No	FWA Number	☐	Previously Reported
Phase III Clinical Trial	☒ No ☐ Yes	☒	Not Previously Reported

Program Income ☒ No ☐ Yes

Budget Period	Anticipated Amount	Source

F&A Changes

Primary Project/Performance Site Location

Organizational Name University of Minnesota

DUNS 5559179960000

Street 1 3-284 Nils Hasselmo Hall		Street 2 312 Church Street SE
City Minneapolis	County Hennepin	State MN
Province	Country UNITED STATES	Zip/Postal Code 554555215

Congressional Districts MN-005

Additional Project/Performance Site Location

Organizational Name University of of Minnesota

DUNS 5559179960000

Street 1 7 227 Phillips Wargenstaer Building		Street 2 516 Delaware Street SE
City Minneapolis	County Hennepin	State MN
Province	Country UNITED STATES	Zip/Postal Code 55455

Project/Performance Site Congressional Districts MN-005

Program Director/Principal Investigator	Grant Number
Fang Li	R01AI089728 03
Applicant Organization	**Period Covered by this Report**
UNIVERSITY OF MINNESOTA TWIN CITIES	05/01/2011 04/30/2012

Title of Project
Receptor recognition mechanisms of coronaviruses

SNAP Questions

Has there been a change in the other support of Senior/Key Personnel since the last reporting period?

☒ No ☐ Yes

Justification

Will there be, in the next budget period a significant change in the level of effort for the PD/PI or other Senior/Key Personnel designated on the Notice of Award from what was approved for this project?

☒ No ☐ Yes

Justification

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☒ No ☐ Yes

Justification

Changes in Select Agent Research? ☒ No ☐ Yes

Changes in Multiple PD/PI Leadership plan? ☒ No ☐ Yes

Change in human embryonic stem cell (hESC) line(s) used? ☒ No ☐ Yes

Justification

Human Subject Education Requirement

Has the Involvement of Human Subjects changed since previous submission? ☒ No ☐ Yes

Has the Involvement of Animal Subjects changed since previous submission? ☒ No ☐ Yes

Publications

Valid NIHMSID	Citation ID	Citation Source	Citation Text

Cover Letter

File is not uploaded

Research Accomplishments

File is not uploaded

Other Document File

File is not uploaded

Other Support File

File is not uploaded

All Personnel Report

Program Director/Principal Investigator			Grant Number			
Fang Li			R01AI089728 03			
Name	**Commons ID**	**Degree(s) Name**	**SSN**			
Fang Li	FANGLI	PHD	XXX XX [redacted] Minn Stat 13.43	**Months Devoted to Project**		
Role on Project	**Supplement Support**		**DoB (MM/YY)**	**Cal**	**Acad**	**Sum**
PD/PI			[redacted] Minn Stat 13.43	6.0		

Name	Commons ID	Degree(s) Name	SSN			
Lang Chen	LANGCHEN	PhD	XXX XX [redacted] Minn Stat 13.43	**Months Devoted to Project**		
Role on Project	**Supplement Support**		**DoB (MM/YY)**	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar Fellow or Other Postdoctoral Position				6.0		

Name	Commons ID	Degree(s) Name	SSN			
Robert James Geraghty		PhD	XXX XX [redacted] Minn Stat 13.43	**Months Devoted to Project**		
Role on Project	**Supplement Support**		**DoB (MM/YY)**	**Cal**	**Acad**	**Sum**
Co-Investigator				1.0		

Name	Commons ID	Degree(s) Name	SSN			
Yi-Lun Lin	YILUNLIN	PhD	XXX XX [redacted] Minn Stat 13.43	**Months Devoted to Project**		
Role on Project	**Supplement Support**		**DoB (MM/YY)**	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar Fellow or Other Postdoctoral Position				6.0		

Name	Commons ID	Degree(s) Name	SSN			
Guiqing Peng	GUIQINGPENG	PhD	XXX XX [redacted] Minn Stat 13.43	**Months Devoted to Project**		
Role on Project	**Supplement Support**		**DoB (MM/YY)**	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar Fellow or Other Postdoctoral Position				6.0		

Name	Commons ID	Degree(s) Name	SSN			
Liqing Xu	XULIXJL	PhD	XXX XX [redacted] Minn Stat 13.43	**Months Devoted to Project**		
Role on Project	**Supplement Support**		**DoB (MM/YY)**	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar Fellow or Other Postdoctoral Position				6.0		

Name	Commons ID	Degree(s) Name	SSN			
Wenbo Zhou	WENBOZHOU	PhD	XXX XX [redacted] Minn Stat 13.43	**Months Devoted to Project**		
Role on Project	**Supplement Support**		**DoB (MM/YY)**	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar Fellow or Other Postdoctoral Position				4.8		

Progress report

A Specific aims

Coronaviruses use a variety of cellular receptors and co-receptors including proteins and sugars The diverse usage of receptors has allowed coronaviruses to infect a wide range of mammalian and avian species and cause respiratory enteric systemic and neurological diseases Two domains in coronavirus spike protein S1 subunit the N terminal domain (NTD) and the C domain have evolved functions as receptor-binding domains (RBDs) How coronaviruses have evolved to do so has been a major puzzle in virology To solve this puzzle this NIH funded project receptor recognition mechanisms of coronaviruses investigates the molecular and structural basis for the complex receptor-recognition mechanisms of coronaviruses

There are three specific aims in this project Aim 1 focuses on the receptor recognition mechanisms of group-I coronaviruses that use angiotensin-converting enzyme 2 as receptors (e g NL63 coronavirus) Aim 2 focuses on the receptor recognition mechanisms of group-1 coronaviruses that use aminopeptidase-N as receptors (e g porcine transmissible gastroenteritis virus and 229E coronavirus) Aim 3 focuses on the receptor recognition mechanisms of group-2 coronaviruses (e g SARS coronavirus mouse coronavirus bovine coronavirus human coronavirus OC43) The research approaches of this proposal include crystallographic analysis of the virus-receptor interfaces identification of structural elements important for virus-receptor interactions and characterization of these elements using crystallographic biochemical molecular and virological tools

B Studies and Results

Our research in the past funding year has made groundbreaking progress in understanding the evolution and receptor recognition of coronaviruses

First Peng et al (2011 *PNAS*) determined the crystal structure of group-2 mouse coronavirus (MHV) NTD complexed with its receptor murine carcinoembryonic antigen-related cell adhesion molecule 1a (mCEACAM1a) Unexpectedly MHV NTD contains a core structure that has the same β-sandwich fold as human galectins (S-lectins) and additional structural motifs that bind to the N-terminal Ig-like domain of mCEACAM1a Despite its galectin fold MHV NTD does not bind sugars but instead binds mCEACAM1a through exclusive protein-protein interactions Critical contacts at the interface have been confirmed by mutagenesis providing a structural basis for viral and host specificities of coronavirus/CEACAM1 interactions Sugar-binding assays reveal that galectin-like NTDs of some group-2 coronaviruses such as human coronavirus OC43 (HCoV-OC43) and bovine coronavirus (BCoV) bind sugars Structural analysis and mutagenesis localize the sugar-binding site in coronavirus NTDs to be above the β-sandwich core We propose that coronavirus NTDs originated from a host galectin and retained sugar-binding functions in some contemporary coronaviruses but evolved new structural features in MHV for mCEACAM1a binding

Second Wu et al (2012 *Journal of Biological Chemistry*) elucidated the molecular and structural mechanisms for the host receptor adaptation by group 2 SARS coronavirus (SARS CoV) We found that all of the naturally selected mutations in SARS-CoV RBD (the S1 C-domain) were viral adaptations to either human or civet receptor angiotensin-converting enzyme 2 (ACE2) Further we have designed and characterized two optimized RBDs The human optimized RBD contains all of the human ACE2 adapted residues (F442/F472/N479/D480/T487) and possesses exceptionally high affinity for human ACE2 but relative low affinity for civet ACE2 The civet-optimized RBD contains all of the civet-ACE2-adapted residues (P442/Y472/R479/G480/T487) and possesses exceptionally high affinity for civet ACE2 and also substantial affinity for human ACE2 These results not only illustrate the detailed mechanisms of host receptor adaptation by SARS-CoV but also provide molecular and structural basis for tracking future SARS CoV evolution in animals

Third Li (2012 *Journal of Virology*) identified shared structural topologies (connectivity of secondary structural elements) in S1 domains of different coronavirus genera The results suggest that coronavirus S1 subunits share a common evolutionary origin but have attained diverse sequences and structures following extensive divergent evolution The results also increase understanding of the structures and functions of coronavirus S1 domains whose tertiary structures are currently unknown

Fourth Wu et al (2011 *Journal of Virology*) investigated the function of a virus-binding hotspot on human ACE2 that is critical for the binding of both group-1 NL63 coronavirus (NL63 CoV) and group 1 SARS CoV The hotspot consists of a salt bridge surrounded by hydrophobic tunnel walls Mutations that disturb the hotspot structure have significant effects on virus/receptor interactions revealing critical energy contributions from the hotspot structure The tunnel structure at the NL63-CoV/ACE2 interface is more compact than that at the SARS-CoV/ACE2 interface and hence virus/ACE2 binding affinities are decreased either by NL63-CoV mutations that decrease the tunnel space or by SARS CoV mutations that increase the tunnel space Furthermore NL63-CoV RBD (the S1 C-domain) inhibits ACE2-dependent infection by SARS-CoV a successful application of our hotspot theory that has the potential to become a novel antiviral strategy against SARS-CoV infections These results suggest that the structural features of the hotspot on ACE2 were among the driving forces for the convergent evolution of NL63-CoV and SARS-CoV

C Significance

Our research has been the major driving force in the coronavirus field to understand coronavirus/receptor interactions Prior to the SARS epidemic no structural information was available for any coronavirus S1 subunit despite decades of extensive research Since the SARS epidemic we have successfully determined the crystal structures of group-1 NL63-CoV C-domain group-2 SARS CoV C-domain and group-2 MHV NTD each complexed with their respective host receptor To date these structures remain the only structures available for any coronavirus S1 or coronavirus-S1/receptor complexes By determining these structures we have solved several important puzzles in virology such as how animal viruses can cross species barriers to infect humans through one or a few seemingly trivial mutations in their RBDs how different viruses recognize the same receptor by targeting common virus-binding hotspots on the receptor how similar viruses recognize different receptors through subtle structural changes in viral RBDs and how viruses can acquire host proteins and evolve them into viral RBDs with different receptor specificity Protein biochemistry and virological methods have also been systematically performed to corroborate our structural findings By elucidating these structures we have also been able to reconstruct the evolutionary history of coronaviruses Furthermore our detailed structural analyses of SARS-CoV/receptor interactions have significantly prepared humans for any potential future SARS epidemics

D Plans

For the next funding year we will focus on specific aims 2 and 3

For specific aims 2, we will investigate the structural mechanism whereby the group-1 porcine transmissible gastroenteritis virus (TGEV) sugar binding domain binds sugars and APN-binding domain binds APN We will prepare the complexes of each of these domains complexed with their respective receptor and set up crystallization screens of these complexes We will also investigate the mechanism whereby 229E coroanvirus spike protein kept its APN-binding function but lost its sugar-binding function through evolution In a pilot study we have successfully obtained APN crystals that diffract X-ray to 1 85 Å Breakthroughs will be expected from this study not only for the coronavirus field but also for other major medical fields such as cancer pain sensation mood disorder blood pressure regulation and high cholesterol level because of the multi-functional roles of APN in these fields

For aim 3 we will investigate the structural and biochemical studies of sugar binding mechanisms of group-2 coronavirus NTDs Here we will determine the crystal structures of group-2 BCoV and HCoV-OC43 NTDs by themselves and in complex with their sugar substrates and examine the functions of important sugar-binding residues in BCoV and HCoV-OC43 NTDs These studies will elucidate the sugar binding mechanisms of group 2 coronaviruses and reveal how the sugar binding functions have evolved in coronaviruses In two pilot studies we have obtained crystals of BCoV NTD that diffract X-ray to 1 6 Å and have also identified a sugar-binding pocket in coronavirus NTDs that shares both similarities and differences with human galectins

Also for aim 3 we will study the structural and molecular basis for MHV driven mouse evolution Pathogen driven host evolution in mammals has long been a topic of debate for lack of clear supporting evidence Two isoforms of mCEACAM1 1a and 1b are present in mice Despite their high sequence homology mCEACAM1b is a much less effective MHV receptor than mCEACAM1a a likely result from MHV-driven mouse evolution Here we will determine the crystal structure of mCEACAM1b and provide structural basis for mCEACAM1b being an ineffective MHV receptor and engineer mCEACAM1b to allow it to gain functions as MHV receptor These studies will elucidate the structural and molecular mechanisms for mCEACAM1b being an ineffective MHV receptor and for MHV driven mouse evolution In a pilot study we have obtained crystals of mCEACAM1b that diffract X-ray to 3 1 Å

E Publications (from the past project period)

1 Wu K, Chen L Peng G Zhou W Pennell C Mansky, L Geraghty R **Li, F** (June 2011) A virus-binding hotspot on human angiotensin-converting enzyme 2 is critical for binding of two

different coronaviruses *Journal of Virology* *85* 5331-5337 (PMID 21411533 PMCID PMC3094985)

2 Peng G Sun D Rajashankar K Qian Z Holmes K **Li, F** (June 2011) Crystal structure of mouse coronavirus receptor-binding domain complexed with its receptor murine CEACAM1a *Proceedings of the National Academy of Sciences USA* *108* 10696-10701 (PMID 21670291 PMCID PMC3127895)

3 **Li, F** (March 2012) Evidence for a common evolutionary origin of coronavirus spike protein receptor-binding subunits *Journal of Virology* *86* 2856-2858 (PMID 22205723)

4 Wu K Peng G Wilken M Geraghty R **Li, F** (March 2012) Mechanisms of host receptor adaptation by SARS coronavirus *Journal of Biological Chemistry* *Epub ahead of print* (PMID 22291007)

F Project Generated Resources

This research did not generate cell lines DNA probes animal models protocols or software De identified raw data from the experiments and computer programs (CCP4 macromolecular crystallography scripts) used for protein structure determinations are available to anyone upon written request to the PI Peer reviewed publications accepted or published during the past project period are being or have been entered in NIHMS and PubMed Central

Grant Number R01AI089728-03	**Total Project Period** From: 05/15/2010 To: 04/30/2015
EIN: **Review Group:** 1416007513A1 VIRA	**Requested Budget Period:** From 05/01/2012 To: 04/30/2013
Title of Project: Receptor recognition mechanisms of coronaviruses	**Due Date:** 03/15/2012 **Submitted Date:**

Program Director/Principal Investigator: Fang Li UNIVERSITY OF MINNESOTA TWIN CITIES Department of Pharmacology 6-120 Jackson Hall 321 Church Street S E MINNEAPOLIS , MN 554552070 **Phone Number:** 612-625-6149 **Fax Number:** **Email Address:** lifang@umn edu	**Applicant Organization:** UNIVERSITY OF MINNESOTA TWIN CITIES UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS , MN 554552070 **Department:** PHARMACOLOGY **Major Subdivision:** MEDICAL SCHOOL
Administrative Official: Sowolo Abdullai LA UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS , MN 554552070 **Phone Number:** 6126245599 **Fax Number:** **Email Address:** abdu0092@umn edu	**Signing Official:** Kevin McKoskey DH 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS , MN 554552070 **Phone Number:** 612-624-5599 **Fax Number:** 612-624-4843 **Email Address:** kevin@umn edu
Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** **Phase III Clinical Trial:** ☒ No ☐ Yes	**Vertebrate Animals:** ☒ No ☐ Yes **Inventions and Patents:** ☒ No ☐ Yes ☐ **Previously Reported** ☒ **Not Previously Reported**
Program Income: ☒ No ☐ Yes	

Budget Period	Anticipated Amount	Source
F&A Changes:		

Primary Project/Performance Site Location		
Organizational Name University of Minnesota		
DUNS 5559179960000		
Street 1 3-284 Nils Hasselmo Hall	Street 2 312 Church Street SE	
City Minneapolis	County Hennepin	State MN
Province	Country UNITED STATES	Zip/Postal Code 554550215
Congressional Districts MN-005		

Additional Project/Performance Site Location			
Organizational Name University of of Minnesota			
DUNS 5559179960000			
Street 1 7-227 Phillips Wangensteen Building		Street 2 516 Delaware Street SE	
City Minneapolis	County Hennepin		State MN
Province	Country UNITED STATES		Zip/Postal Code 55455
Project/Performance Site Congressional Districts MN-005			

Program Director/Principal Investigator:	**Grant Number**
Fang Li	R01AI089728-03
Applicant Organization:	**Period Covered by this Report:**
UNIVERSITY OF MINNESOTA TWIN CITIES	05/01/2011 - 04/30/2012

Title of Project:

Receptor recognition mechanisms of coronaviruses

SNAP Questions:

Has there been a change in the other support of Senior/Key Personnel since the last reporting period?

☒ **No** ☐ **Yes**

Justification:

Will there be, in the next budget period, a significant change in the level of effort for the PD/PI or other Senior/Key Personnel designated on the Notice of Award from what was approved for this project?

☒ **No** ☐ **Yes**

Justification:

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☒ **No** ☐ **Yes** ok currently 26 %

Justification:

Changes in Select Agent Research? ☒ **No** ☐ **Yes**

Changes in Multiple PD/PI Leadership plan? ☒ **No** ☐ **Yes**

Change in human embryonic stem cell (hESC) line(s) used? ☒ **No** ☐ **Yes**

Justification:

Human Subject Education Requirement:

Has the Involvement of Human Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Has the Involvement of Animal Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Publications:

Valid NIHMSID:	Citation ID:	Citation Source:	Citation Text:

Cover Letter: File is not uploaded
Research Accomplishments: File is not uploaded
Other Document File: File is not uploaded
Other Support File: File is not uploaded

All Personnel Report					
Program Director/Principal Investigator: Fang Li			**Grant Number** R01AI089728-03		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Fang Li	FANGLI	PHD	XXX-XX-[Minn Stat 13.43]	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
PD/PI			[Minn Stat 13.43]	6 0		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Lang Chen	LANGCHEN ✓	PhD	XXX-XX-[Minn Stat 13.43]	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6 0		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Robert James Geraghty		PhD	XXX-XX-[Minn Stat 13.43]	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-Investigator				1 0		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Yi-Lun Lin	YILUNLIN ✓	PhD	XXX-XX-[Minn Stat 13.43]	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6 0		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Guiqing Peng	GUIQINGPENG ✓	PhD	XXX-XX-[Minn Stat 13.43]	**Months Devoted**	
				to Project	
Role on Project.	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6 0		

Name:	Commons ID:	Degree(s) Name:	SSN:			
Liqing Xu	XULXUL ✓	PhD	XXX-XX [redacted] Minn Stat 13.43	Months Devoted		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6 0		

Name:	Commons ID:	Degree(s) Name:	SSN:			
Wenbo Zhou	Wenbo Zhou added ✓	PhD	XXX-XX- [redacted] Minn Stat 13.43	Months Devoted		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				4 8		

Progress report

A. Specific aims

Coronaviruses use a variety of cellular receptors and co-receptors including proteins and sugars. The diverse usage of receptors has allowed coronaviruses to infect a wide range of mammalian and avian species, and cause respiratory, enteric, systemic and neurological diseases Two domains in coronavirus spike protein S1 subunit, the N-terminal domain (NTD) and the C-domain, have evolved functions as receptor-binding domains (RBDs) How coronaviruses have evolved to do so has been a major puzzle in virology. To solve this puzzle, this NIH-funded project "receptor recognition mechanisms of coronaviruses" investigates the molecular and structural basis for the complex receptor-recognition mechanisms of coronaviruses

There are three specific aims in this project Aim 1 focuses on the receptor recognition mechanisms of group-I coronaviruses that use angiotensin-converting enzyme 2 as receptors (e g. NL63 coronavirus) Aim 2 focuses on the receptor recognition mechanisms of group-1 coronaviruses that use aminopeptidase-N as receptors (e.g , porcine transmissible gastroenteritis virus and 229E coronavirus) Aim 3 focuses on the receptor recognition mechanisms of group-2 coronaviruses (e g. SARS coronavirus, mouse coronavirus, bovine coronavirus, human coronavirus OC43). The research approaches of this proposal include crystallographic analysis of the virus-receptor interfaces, identification of structural elements important for virus-receptor interactions, and characterization of these elements using crystallographic, biochemical, molecular, and virological tools.

B. Studies and Results

Our research in the past funding year has made groundbreaking progress in understanding the evolution and receptor recognition of coronaviruses

First, Peng et al. (2011, *PNAS*) determined the crystal structure of group-2 mouse coronavirus (MHV) NTD complexed with its receptor murine carcinoembryonic antigen-related cell adhesion molecule 1a (mCEACAM1a) Unexpectedly, MHV NTD contains a core structure that has the same β-sandwich fold as human galectins (S-lectins) and additional structural motifs that bind to the N-terminal Ig-like domain of mCEACAM1a. Despite its galectin fold, MHV NTD does not bind sugars, but instead binds mCEACAM1a through exclusive protein-protein interactions. Critical contacts at the interface have been confirmed by mutagenesis, providing a structural basis for viral and host specificities of coronavirus/CEACAM1 interactions. Sugar-binding assays reveal that galectin-like NTDs of some group-2 coronaviruses such as human coronavirus OC43 (HCoV-OC43) and bovine coronavirus (BCoV) bind sugars Structural analysis and mutagenesis localize the sugar-binding site in coronavirus NTDs to be above the β-sandwich core We propose that coronavirus NTDs originated from a host galectin and retained sugar-binding functions in some contemporary coronaviruses, but evolved new structural features in MHV for mCEACAM1a binding.

Second, Wu et al. (2012, *Journal of Biological Chemistry*) elucidated the molecular and structural mechanisms for the host receptor adaptation by group-2 SARS coronavirus (SARS-CoV) We found that all of the naturally selected mutations in SARS-CoV RBD (the S1 C-domain) were viral adaptations to either human or civet receptor angiontensin-converting enzyme 2 (ACE2). Further, we have designed and characterized two optimized RBDs The human-optimized RBD contains all of the human-ACE2-adapted residues (F442/F472/N479/D480/T487), and possesses exceptionally high affinity for human ACE2 but relative low affinity for civet ACE2 The civet-optimized RBD contains all of the civet-ACE2-adapted residues (P442/Y472/R479/G480/T487), and possesses exceptionally high affinity for civet ACE2 and also substantial affinity for human ACE2. These results not only illustrate the detailed mechanisms of host receptor adaptation by SARS-CoV, but also provide molecular and structural basis for tracking future SARS-CoV evolution in animals.

Third, Li (2012, *Journal of Virology*) identified shared structural topologies (connectivity of secondary structural elements) in S1 domains of different coronavirus genera. The results suggest that coronavirus S1 subunits share a common evolutionary origin but have attained diverse sequences and structures following extensive divergent evolution The results also increase understanding of the structures and functions of coronavirus S1 domains whose tertiary structures are currently unknown

Fourth, Wu et al. (2011, *Journal of Virology*) investigated the function of a virus-binding hotspot on human ACE2 that is critical for the binding of both group-1 NL63 coronavirus (NL63-CoV) and group-1 SARS-CoV. The hotspot consists of a salt bridge surrounded by hydrophobic tunnel walls. Mutations that disturb the hotspot structure have significant effects on virus/receptor interactions, revealing critical energy contributions from the hotspot structure The tunnel structure at the NL63-CoV/ACE2 interface is more compact than that at the SARS-CoV/ACE2 interface, and hence virus/ACE2 binding affinities are decreased either by NL63-CoV

mutations that decrease the tunnel space or by SARS-CoV mutations that increase the tunnel space. Furthermore, NL63-CoV RBD (the S1 C-domain) inhibits ACE2-dependent infection by SARS-CoV, a successful application of our hotspot theory that has the potential to become a novel antiviral strategy against SARS-CoV infections. These results suggest that the structural features of the hotspot on ACE2 were among the driving forces for the convergent evolution of NL63-CoV and SARS-CoV

C. Significance

Our research has been the major driving force in the coronavirus field to understand coronavirus/receptor interactions Prior to the SARS epidemic, no structural information was available for any coronavirus S1 subunit, despite decades of extensive research Since the SARS epidemic, we have successfully determined the crystal structures of group-1 NL63-CoV C-domain, group-2 SARS-CoV C-domain, and group-2 MHV NTD each complexed with their respective host receptor. To date, these structures remain the only structures available for any coronavirus S1 or coronavirus-S1/receptor complexes. By determining these structures, we have solved several important puzzles in virology, such as how animal viruses can cross species barriers to infect humans through one or a few seemingly trivial mutations in their RBDs, how different viruses recognize the same receptor by targeting common virus-binding hotspots on the receptor, how similar viruses recognize different receptors through subtle structural changes in viral RBDs, and how viruses can acquire host proteins and evolve them into viral RBDs with different receptor specificity. Protein biochemistry and virological methods have also been systematically performed to corroborate our structural findings By elucidating these structures, we have also been able to reconstruct the evolutionary history of coronaviruses. Furthermore, our detailed structural analyses of SARS-CoV/receptor interactions have significantly prepared humans for any potential future SARS epidemics.

D. Plans

For the next funding year, we will focus on specific aims 2 and 3.

For specific aims 2, we will investigate the structural mechanism whereby the group-1 porcine transmissible gastroenteritis virus (TGEV) sugar-binding domain binds sugars and APN-binding domain binds APN. We will prepare the complexes of each of these domains complexed with their respective receptor, and set up crystallization screens of these complexes. We will also investigate the mechanism whereby 229E coroanvirus spike protein kept its APN-binding function but lost its sugar-binding function through evolution. In a pilot study, we have successfully obtained APN crystals that diffract X-ray to 1.85 Å. Breakthroughs will be expected from this study not only for the coronavirus field, but also for other major medical fields such as cancer, pain sensation, mood disorder, blood pressure regulation and high cholesterol level, because of the multifunctional roles of APN in these fields.

For aim 3, we will investigate the structural and biochemical studies of sugar binding mechanisms of group-2 coronavirus NTDs. Here we will determine the crystal structures of group-2 BCoV and HCoV-OC43 NTDs by themselves and in complex with their sugar substrates and examine the functions of important sugar-binding residues in BCoV and HCoV-OC43 NTDs. These studies will elucidate the sugar binding mechanisms of group-2 coronaviruses and reveal how the sugar-binding functions have evolved in coronaviruses. In two pilot studies, we have obtained crystals of BCoV NTD that diffract X-ray to 1.6 Å and have also identified a sugar-binding pocket in coronavirus NTDs that shares both similarities and differences with human galectins

Also for aim 3, we will study the structural and molecular basis for MHV driven mouse evolution Pathogen driven host evolution in mammals has long been a topic of debate for lack of clear supporting evidence. Two isoforms of mCEACAM1, 1a and 1b, are present in mice. Despite their high sequence homology, mCEACAM1b is a much less effective MHV receptor than mCEACAM1a, a likely result from MHV-driven mouse evolution Here we will determine the crystal structure of mCEACAM1b and provide structural basis for mCEACAM1b being an ineffective MHV receptor and engineer mCEACAM1b to allow it to gain functions as MHV receptor. These studies will elucidate the structural and molecular mechanisms for mCEACAM1b being an ineffective MHV receptor and for MHV driven mouse evolution In a pilot study, we have obtained crystals of mCEACAM1b that diffract X-ray to 3.1 Å.

E. Publications (from the past project period)

1. Wu, K., Chen, L., Peng, G., Zhou, W , Pennell, C , Mansky, L., Geraghty, R., **Li, F.** (June 2011) A virus-binding hotspot on human angiotensin-converting enzyme 2 is critical for binding of two

different coronaviruses. *Journal of Virology*, *85,* 5331-5337 (PMID: 21411533; PMCID: PMC3094985)

2. Peng, G., Sun, D , Rajashankar, K., Qian, Z., Holmes, K , **Li, F.** (June 2011) Crystal structure of mouse coronavirus receptor-binding domain complexed with its receptor murine CEACAM1a. *Proceedings of the National Academy of Sciences USA*, *108,* 10696-10701 (PMID: 21670291, PMCID. PMC3127895)

3 **Li, F.** (March 2012) Evidence for a common evolutionary origin of coronavirus spike protein receptor-binding subunits. *Journal of Virology*, *86,* 2856-2858 (PMID: 22205723)

4. Wu, K., Peng, G., Wilken, M., Geraghty, R., **Li, F.** (March 2012) Mechanisms of host receptor adaptation by SARS coronavirus *Journal of Biological Chemistry*, *Epub ahead of print,* (PMID: 22291007)

F. Project Generated Resources

This research did not generate cell lines, DNA probes, animal models, protocols, or software. De-identified raw data from the experiments and computer programs (CCP4 macromolecular crystallography scripts) used for protein structure determinations are available to anyone upon written request to the PI Peer-reviewed publications accepted or published during the past project period are being or have been entered in NIHMS and PubMed Central.

Principal Investigator/Program Director (Last, Firs Li, Fang

DETAILED BUDGET FOR NEXT E PERIOD - DIRECT COSTS ONL	FROM #####	THROUGH 4/30/2013	GRANT NUMBER R01 AI089728-03

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal Mnths	Acad Mnths	Summer Mnths	SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Fang Li	PD/PI	6 00			$42,250	$14,746	$56,996
Robert Geraghty	Co-Invest	1 00			$8,833	$3,083	$11,916
Lang Chen	Postdoctoral	6 00			$17,000	$3,791	$20,791
Guiqing Peng	Postdoctoral	6 00			$18,000	$4,014	$22,014
Wenbo Zhou	Postdoctoral	4 80			$14,830	$3,411	$18,241
Liqing Xu	Postdoctoral	6 00			$16,500	$3,798	$20,298
Yi-Lun Lin	Postdoctoral	6 00			$18,750	$4,313	$23,063
SUBTOTALS →					$136,163	$37,156	$173,319

CONSULTANT COSTS
Dr Kathryn Holmes — $10,000

EQUIPMENT (*Itemize*) — $0

SUPPLIES (*Itemize by category*)

Molecular biology supplies	$14,580
Insect cell culture supplies	$9,500
Protein purification supplies	$16,000
Protein crystallization supplies	$13,500
Plastic & glassware supplies	$2,801

$56,381

TRAVEL
PI and/or postdoctoral to attend one meeting per year — $3,000

INPATIENT CARE COSTS $0 — $0

OUTPATIENT CARE COST $0 — $0

ALTERATIONS AND RENOVATIONS (*Itemize by category*)

OTHER EXPENSES (*Itemize by category*)

Publication costs/reprints	$2,500
Equipment maintenance	$1,500
Communication costs	$800

$4,800

SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD	$247,500
CONSORTIUM/CONTRACTUAL COSTS — DIRECT COSTS	
CONSORTIUM/CONTRACTUAL COSTS — FACILITIES AND ADMINISTRATION COSTS	
TOTAL DIRECT COSTS FOR NEXT PROJECT PERIOD (*Item 8a, Face Page*)	$247,500

	Years 1-5
Indirect Cost Base	$247,500
IDC @ 51%	$126,225
Total Direct Costs	$247,500
IDC	$126,225

Total Costs	$373,725

Sponsored Award Overview

CON000000022345 - Receptor recognition mechanisms of coronaviruses
Accounting Date Through 03/12/2012

AWARD SPONSOR NIH Nat'l Inst of Allergy & Infect Dis
AWARD START DATE 05/15/2010
AWARD END DATE 04/30/2015
AWARD TIME ELAPSED 37%
AWARD SPENT 93%
F&A RSRCH MTDC 51%

Report Run Date Monday,3/12/2012

Data as of Monday,03/12/2012 03 35 AM

Cost Share (Actuals):	$0 00
Program Income (Actuals)	$0 00
TIP Income (Actuals)·	$0 00
Invoiced Amount ITD	$634,337 75
Open AR Amount ITD	$0 00

						03/01/2012 - 03/12/2012		INCEPTION THROUGH ??03/12/2012		
PROJECT	PP	PI	DETAIL ACCOUNT	FINALIZED BUDGET AMOUNT	PRE-ENC & ENC	EXPENSES	PRE-ENC & ENC	EXPENSES	AVAILABLE BALANCE
00013782	Y	Li,Fang	168100 - Capital Assets - Fin Bdg Only	8,000 00	0 00	0 00	0 00	37,570 84	-29,570 84
00013782	Y	Li,Fang	700100 - Salaries-Faculty-Fin Bdg Only	94,624 00	-1,808 99	1,809 00	8,321 34	90,666 59	-4,363 93
00013782	Y	Li,Fang	700200 - Salaries-Prof&Adm-Fin Bdg Only	92,753 00	-1,558 82	1,558 82	7,170 57	8,378 77	77,203 66
00013782	Y	Li,Fang	700300 - Salaries-Grd/Pr w/Tuitn-FinBdg	81,400 00	0 00	0 00	0 00	0 00	81,400 00
00013782	Y	Li,Fang	700310 - Salaries-Grd/Pr NoTuitn-FinBdg	0 00	-4,819 67	4,819 67	22,170 48	141,406 57	-163 577 05
00013782	Y	Li,Fang	710100 - Fringe - Faculty-Fin Bdg Only	32,349 00	-642 52	642 52	2,955 62	27,143 01	2,250 37
00013782	Y	Li,Fang	710200 - Fringe - P&A-Fin Bdg Only	24,256 00	384 48	280 06	1,952 81	1,865 89	20,437 30
00013782	Y	Li,Fang	710300 - Fringe-Grad/Pr-w/Tuitn-FinBdg	16,117 00	0 00	0 00	0 00	0 00	16,117 00
00013782	Y	Li,Fang	710310 - Fringe-Grad/Pr No Tuitn-FinBdg	0 00	-914 56	914 59	4,207 04	24,240 46	-28 447 50
00013782	Y	Li,Fang	720200 - Lab & Med Supplies-FinBdg Only	116,510 00	932 50	846 65	3,475 16	90,122 51	22,912 33
00013782	Y	Li,Fang	720300 - Gen Oper Services-Fin Bdg Only	4,375 00	0 00	0 00	0 00	4,588 73	-213 73
00013782	Y	Li,Fang	720400 - Lab & Medical Svcs-FinBdg Only	200 00	0 00	81 00	0 00	2,754 97	-2 554 97
00013782	Y	Li,Fang	720500 - Telecommunications-FinBdg Only	1,192 00	0 00	0 00	0 00	0 00	1,192 00
00013782	Y	Li,Fang	720600 - Travel/Mileage/Mov-FinBdg Only	4,970 00	0 00	0 00	0 00	8,808 16	-3,838 16
00013782	Y	Li,Fang	730100 - Consulting Services-FinBdg Onl	19,900 00	0 00	0 00	0 00	1,279 26	18,620 74
00013782	Y	Li,Fang	750100 - Non-Capital Equip-Fin Bdg Only	571 00	0 00	0 00	0 00	3,201 51	-2,630 51
00013782	Y	Li,Fang	780100 - Repairs & Maint-FinBdg Only	2,985 00	0 00	0 00	0 00	1,705 37	1,279 63
			Direct Cost Total	**$500,202 00**	**-$8,427 58**	**$10,952 31**	**$50,253 02**	**$443,732 64**	**$6,216 34**
00013782	Y	Li,Fang	810500 - F&A Costs-Fin Bdg Only	251,023 00	0 00	5,544 38	0 00	207,101 80	43,921 20
			F&A Total	**$251,023.00**	**$0 00**	**$5,544 38**	**$0 00**	**$207,101 80**	**$43,921.20**
CSR $0 00 RFA. $125,287 56			**Project Total**	**$751,225.00**	**-$8,427 58**	**$16,496 69**	**$50,253 02**	**$650,834 44**	**$50,137.54**
CSR $0.00 RFA. $125,287 56			**Award Total:**	**$751,225 00**	**-$8,427 58**	**$16,496 69**	**$50,253 02**	**$650,834 44**	**$50,137 54**

recommended = $373,725

13.4%

100,390.56

= 26.9%

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Grant Number R01AI089728-04	**Total Project Period** From: 05/15/2010 To: 04/30/2015
EIN: **Review Group:** 1416007513A1 VIRA	**Requested Budget Period:** From: 05/01/2013 To: 04/30/2014
Title of Project: Receptor recognition mechanisms of coronaviruses	**Due Date:** 03/15/2013 **Submitted Date:** 03/14/2013

Program Director/Principal Investigator: Fang Li UNIVERSITY OF MINNESOTA TWIN CITIES Department of Pharmacology 6-120 Jackson Hall 321 Church Street S.E. MINNEAPOLIS , MN 554552070 **Phone Number:** 612-625-6149 **Fax Number:** **Email Address:** lifang@umn.edu	**Applicant Organization:** UNIVERSITY OF MINNESOTA UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS , MN 554552070 **Department:** PHARMACOLOGY **Major Subdivision:** MEDICAL SCHOOL
Administrative Official: Paulie Garcia 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE Minneapolis , MN 554552070 **Phone Number:** 612-624-5599 **Fax Number:** **Email Address:** awards@umn.edu	**Signing Official:** David L Hagen 200 Oak Street SE Suite 450 Minneapolis , MN 55455 **Phone Number:** 612-626-9895 **Fax Number:** 612-624-4843 **Email Address:** hagen027@umn.edu
Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** **Phase III Clinical Trial:** ☒ No ☐ Yes	**Vertebrate Animals:** ☒ No ☐ Yes **Inventions and Patents:** ☒ No ☐ Yes ☐ **Previously Reported** ☒ **Not Previously Reported**
Program Income: ☒ No ☐ Yes	

Budget Period	**Anticipated Amount**	**Source**
F&A Changes:		

Primary Project/Performance Site Location		
Organizational Name: University of Minnesota		
DUNS: 5559179960000		
Street 1: 3-284 Nils Hasselmo Hall	Street 2: 312 Church Street SE	
City: Minneapolis	County: Hennepin	State: MN
Province:	Country: UNITED STATES	Zip/Postal Code: 554550215
Congressional Districts: MN-005		

Additional Project/Performance Site Location		
Organizational Name: University of of Minnesota		
DUNS: 5559179960000		
Street 1: 7-227 Phillips Wangensteen Building	Street 2: 516 Delaware Street SE	
City: Minneapolis	County: Hennepin	State: MN
Province:	Country: UNITED STATES	Zip/Postal Code: 55455
Project/Performance Site Congressional Districts: MN-005		

Program Director/Principal Investigator:	**Grant Number**
Fang Li	R01AI089728-04
Applicant Organization:	**Period Covered by this Report:**
UNIVERSITY OF MINNESOTA	05/01/2012 - 04/30/2013

Title of Project:

Receptor recognition mechanisms of coronaviruses

SNAP Questions:

Has there been a change in the other support of Senior/Key Personnel since the last reporting period?

☒ **No** ☐ **Yes**

Justification:

Will there be, in the next budget period, a significant change in the level of effort for the PD/PI or other Senior/Key Personnel designated on the Notice of Award from what was approved for this project?

☒ **No** ☐ **Yes**

Justification:

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☒ **No** ☐ **Yes**

Justification:

Changes in Select Agent Research? ☒ **No** ☐ **Yes**

Changes in Multiple PD/PI Leadership plan? ☒ **No** ☐ **Yes**

Change in human embryonic stem cell (hESC) line(s) used? ☒ **No** ☐ **Yes**

Justification:

Human Subject Education Requirement:

Has the Involvement of Human Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Has the Involvement of Animal Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Publications:

Valid NIHMSID:	**Citation ID:**	**Citation Source:**	**Citation Text:**

Cover Letter: File is not uploaded
Research Accomplishments: File is not uploaded
Other Document File: File is not uploaded
Other Support File: File is not uploaded

All Personnel Report

Program Director/Principal Investigator:			**Grant Number**		
Fang Li			R01AI089728-04		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Fang Li	FANGLI	PHD	XXX-XX [redacted]	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
PD/PI			[redacted]	6.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Lang Chen	LANGCHEN	PhD	XXX-XX [redacted]	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Yaoqing Chen	YAOQING	PhD	XXX-X [redacted]	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			[redacted]	6.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Robert James Geraghty	ROBERT.GERAGHTY	PhD	XXX-XX [redacted]	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-Investigator				1.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Yi-Lun Lin	YILUNLIN	PhD	XXX-XX [redacted]	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Guiqing Peng	GUIQINGPENG	PhD	XXX-X [redacted]	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Liqing Xu	XULXUL	PhD	XXX-XX [redacted]	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
[redacted]		BS	XXX-XX [redacted]	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Graduate Research Assistant				6.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Wenbo Zhou	WENBOZHOU	PhD	XXX-XX [redacted]	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				4.8		

Progress Report

A. Specific Aims

Coronaviruses use a variety of cellular receptors and co-receptors including proteins and sugars. The diverse usage of receptors has allowed coronaviruses to infect a wide range of mammalian and avian species, and cause respiratory, enteric, systemic and neurological diseases. Two domains in coronavirus spike protein S1 subunit, the N-terminal domain (NTD) and the C-domain, have evolved functions as receptor-binding domains (RBDs). How coronaviruses have evolved to do so has been a major puzzle in virology. To solve this puzzle, this NIH-funded project "receptor recognition mechanisms of coronaviruses" investigates the molecular and structural basis for the complex receptor-recognition mechanisms of coronaviruses.

There are three specific aims in this project. Aim 1 focuses on the receptor recognition mechanisms of group-I coronaviruses that use angiotensin-converting enzyme 2 as receptors (e.g. NL63 coronavirus). Aim 2 focuses on the receptor recognition mechanisms of group-1 coronaviruses that use sugars and aminopeptidase N as receptors (e.g., porcine transmissible gastroenteritis virus and 229E coronavirus). Aim 3 focuses on the receptor recognition mechanisms of group-2 coronaviruses (e.g. SARS coronavirus, mouse coronavirus, and bovine coronavirus). The research approaches of this proposal include crystallographic analysis of the virus-receptor interfaces, identification of structural elements important for virus-receptor interactions, and characterization of these elements using crystallographic, biochemical, molecular, and virological tools.

B. Studies and Results

We made the following discoveries in 2012.

First, Chen et al. (2012, *PNAS*) determined crystal structures of porcine aminopeptidase N (APN) at 1.85 Å resolution and its complexes with a peptide substrate and a variety of inhibitors. Mammalian APN plays multifunctional roles in many physiological processes, including peptide metabolism, cell motility and adhesion, and coronavirus entry. Our results show that APN is a cell surface-anchored and seahorse-shaped zinc-aminopeptidase that forms head-to-head dimers. Captured in a catalytically active state, these structures of APN illustrate a detailed catalytic mechanism for its aminopeptidase activity. The active site and peptide-binding channel of APN reside in cavities with wide openings, allowing easy access to peptides. The cavities can potentially open up further to bind the exposed N terminus of proteins. The active site anchors the N-terminal neutral residue of peptides/proteins, and the peptide-binding channel binds the remainder of the peptides/proteins in a sequence-independent fashion. APN also provides an exposed outer surface for coronavirus binding, without its physiological functions being affected. These structural features enable APN to function ubiquitously in peptide metabolism, interact with other proteins to mediate cell motility and adhesion, and serve as a coronavirus receptor. This study elucidates multifunctional roles of APN and can guide therapeutic efforts to treat APN-related diseases.

Second, Peng et al. (2012, *Journal of Biological Chemistry*) determined the crystal structure of bovine coronavirus (BCoV) spike protein lectin domain. The spike protein N-terminal domains (NTDs) of BCoV and mouse hepatitis coronavirus (MHV) recognize sugar and protein receptors, respectively, despite their significant sequence homology. We recently determined the crystal structure of MHV NTD complexed with its protein receptor murine carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) (Peng et al., *PNAS 108*, 10696-10701, 2011), which surprisingly revealed a human galectin (galactose-binding lectin) fold in MHV NTD. Here, we have determined at 1.55 Å resolution the crystal structure of BCoV NTD, which also has the human galectin fold. Using mutagenesis, we have located the sugar-binding site in BCoV NTD, which overlaps with the galactose-binding site in human galectins. Using a glycan array screen, we have identified 5-N-acetyl-9-O-acetylneuraminic acid as the preferred sugar substrate for BCoV NTD. Subtle structural differences between BCoV and MHV NTDs, primarily involving different conformations of receptor-binding loops, explain why BCoV NTD does not bind CEACAM1 and why MHV NTD does not bind sugar. These results suggest a successful viral evolution strategy in which coronaviruses stole a galectin from hosts, incorporated it into their spike protein, and evolved it into viral receptor-binding domains with altered sugar specificity in contemporary BCoV or novel protein specificity in contemporary MHV.

Third, Wu et al. (2012, *Journal of Biological Chemistry*) elucidated the molecular and structural mechanisms for the host receptor adaptation by group-2 SARS coronavirus (SARS-CoV). We found that all of the naturally selected mutations in SARS-CoV RBD (the S1 C-domain) were viral adaptations to either human or civet receptor angiontensin-converting enzyme 2 (ACE2). Further, we have designed and characterized two optimized RBDs. The human-optimized RBD contains all of the human-ACE2-adapted residues (F442/F472/N479/D480/T487), and possesses exceptionally high affinity for human ACE2 but relative low

affinity for civet ACE2. The civet-optimized RBD contains all of the civet-ACE2-adapted residues (P442/Y472/R479/G480/T487), and possesses exceptionally high affinity for civet ACE2 and also substantial affinity for human ACE2. These results not only illustrate the detailed mechanisms of host receptor adaptation by SARS-CoV, but also provide molecular and structural basis for tracking future SARS-CoV evolution in animals.

Fourth, Li (2012, *Journal of Virology*) identified shared structural topologies (connectivity of secondary structural elements) in S1 domains of different coronavirus genera. The results suggest that coronavirus S1 subunits share a common evolutionary origin but have attained diverse sequences and structures following extensive divergent evolution. The results also increase understanding of the structures and functions of coronavirus S1 domains whose tertiary structures are currently unknown.

Fifth, Chen et al. (2013, *Journal of Virology*) analyzed the structural relationships between BCoV spike protein lectin domain and other known viral lectins. Influenza and other viruses use host-cell-surface sugars as receptors. Here we show that the sugar-binding domains in influenza hemagglutinin, BCoV spike protein lectin domain, and other viral lectins share the same structural fold as human galectins (host lectins). Unlike the easily accessible sugar-binding sites in human galectins, the sugar-binding sites in viral lectins are hidden in cavities. We propose that these viral lectins originated from host lectins but have evolved to use hidden sugar-binding sites to evade host immune attacks.

C. Significance

Our research has been the major driving force in the coronavirus field to understand coronavirus/receptor interactions. Prior to the SARS epidemic, no structural information was available for any coronavirus S1 subunit, despite decades of extensive research. By now, we have successfully determined the crystal structures of group-1 NL63-CoV C-domain complexed with its receptor ACE2, group-2 SARS-CoV C-domain complexed with its receptor ACE2, group-2 MHV NTD complexed with its receptor CEACAM1, group-2 BCoV NTD, and group-1 coronavirus receptor APN. To date, these structures account for all but one of the structures available for any coronavirus S1 or coronavirus-S1/receptor complexes. By determining these structures, we have solved several important puzzles in virology, such as how animal viruses can cross species barriers to infect humans through one or a few seemingly trivial mutations in their RBDs, how different viruses recognize the same receptor by targeting common virus-binding hotspots on the receptor, how similar viruses recognize different receptors through subtle structural changes in viral RBDs, and how viruses can acquire host proteins and evolve them into viral RBDs with different receptor specificity. Protein biochemistry and virological methods have also been systematically performed to corroborate our structural findings. By elucidating these structures, we have also been able to reconstruct the evolutionary history of coronaviruses. Furthermore, our detailed structural analyses of SARS-CoV/receptor interactions have significantly prepared humans for any potential future SARS epidemics.

D. Plans

For the next funding year, we will focus on specific aims 2 and 3.

For specific aims 2, we will investigate the structural mechanism whereby the group-1 coronavirus NTDs recognize their sugar and APN receptors. First, we will aim to determine the crystal structures of porcine transmissible gastroenteritis virus (TGEV) NTD by itself and in complex with sugar receptors. Previously we hypothesized that like group-2 coronavirus NTDs, group-1 coronavirus NTDs also have a human galectin fold despite of no sequence homology between group-1 and group-2 coronavirus NTDs (Peng et al., *PNAS 108*, 10696-10701, 2011; Li, *Journal of Virology 86*, 2856-2858, 2012). The new study will elucidate the sugar binding mechanisms of group-1 coronaviruses and reveal how the sugar-binding functions have evolved in coronaviruses. Second, we will aim to determine the crystal structure of the human 229E coronavirus (HCoV-229E) C-domain complexed with human APN. Although the crystal structure of TGEV C-domain complexed with porcine APN was recently determined by another group (Reguera et al., *PloS Pathogens 8*, e1002859, 2012), our preliminary study has revealed significant differences in the APN-binding sites and mechanisms between HCoV-229E C-domain and TGEV C-domain. We will investigate the structural basis for the different APN-binding mechanisms of these group-1 coronaviruses.

For specific aim 3, we will determine the crystal structure of group-2 BCoV NTD complexed with the sugar substrate. Based on the successful structure determination of BCoV NTD by itself, we will synthesize the sugar substrates of BCoV NTD, and use either crystal soaking or co-crystallization to determine the structure

of the complex. This study will reveal detailed structural basis for the sugar-binding mechanism of group-2 coronaviruses.

Also for specific aim 3, we will study the structural and molecular basis for MHV driven mouse evolution. Pathogen driven host evolution in mammals has long been a topic of debate for lack of clear supporting evidence. Two isoforms of mCEACAM1, 1a and 1b, are present in mice. Despite their high sequence homology, mCEACAM1b is a much less effective MHV receptor than mCEACAM1a, a likely result from MHV-driven mouse evolution. Here we will investigate the structural basis for why mCEACAM1b is an ineffective MHV receptor. Furthermore, we will engineer mCEACAM1b to improve its effectiveness as an MHV receptor. These studies will elucidate the structural and molecular mechanisms for MHV driven mouse evolution. In a pilot study, we have determined the crystal structure of mCEACAM1b. Currently we are correlating structural and functional data and will perform structure-based protein engineering to improve the effectiveness of mCEACAM1b as an MHV receptor.

Furthermore, a novel human EMC coronavirus (HCoV-EMC) has recently emerged from Mideast (Zaki et al., *N. Engl. J. Med. 367*, 1814-1820, 2012). HCoV-EMC has been characterized as a highly lethal virus and appears to have passed from animals (most likely bats) to humans easily. HCoV-EMC belongs to group-2 and is related to SARS-CoV. The receptor for HCoV-EMC is still unknown, but is being pursued intensively. As part of specific am 3 where the receptor-recognition mechanisms of group-2 coronaviruses are investigated, we will identify the receptor-binding domain (RBD) of HCoV-EMC and determine its crystal structure. In a pilot study, we have successfully expressed, purified, and crystallized the HCoV-EMC S1 C-domain. Next, we will determine the crystal structure of HCoV-EMC S1 C-domain, and also characterize its capability of blocking the infections of human cells by retroviruses that are pseudotyped with HCoV-EMC spike protein. Once the receptor for HCoV-EMC is known, we will also determine the co-crystal structures of HCoV-EMC RBD complexed with human or bat receptor. Just like our research on SARS-CoV, these studies on HCoV-EMC will provide structural insights into the receptor-recognition mechanisms and cross-species infections of HCoV-EMC. The crystal structure of HCoV-EMC RBD by itself will guide development of subunit vaccines using the RBD as the template.

E. Publications (in 2012)

1. Chen, L., Lin, Y.L., Peng, G., **Li, F.** (2012) Structural basis for multifunctional roles of mammalian aminopeptidase N. *Proceedings of the National Academy of Sciences USA*, 109, 17966-17971 (PMID: 23071329; PMCID: PMC3497818 [Available on 2013/4/30])

2. Peng, G., Xu, L., Lin, Y.L., Chen, L., Pasquarella, J., Holmes, K.V., **Li, F.** (2012) Crystal structure of bovine coronavirus spike protein lectin domain. *Journal of Biological Chemistry*, 287, 41931-41938 (PMID: 23091051; PMCID: PMC3516740 [Available on 2013/12/7])

3. Wu, K., Peng, G., Wilken, M., Geraghty, R., **Li, F.** (March 2012) Mechanisms of host receptor adaptation by SARS coronavirus. *Journal of Biological Chemistry*, *287,* 8904-8911 (PMID: 22291007; PMCID: PMC3308800 [Available on 2013/3/16])

4. **Li, F.** (March 2012) Evidence for a common evolutionary origin of coronavirus spike protein receptor-binding subunits. *Journal of Virology*, *86,* 2856-2858 (PMID: 22205723; PMCID: PMC3302248)

5. Chen, L., **Li, F.** (2013) Structural analysis of evolutionary origins of influenza hemagglutinin and other viral lectins. *Journal of Virology*, [Epub ahead of print] In press (PMID: 23365425)

F. Project Generated Resources

This research did not generate cell lines, DNA probes, animal models, protocols, or software. De-identified raw data from the experiments and computer programs (CCP4 macromolecular crystallography scripts) used for protein structure determinations are available to anyone upon written request to the PI. Peer-reviewed publications accepted or published during the past project period are being or have been entered in NIHMS and PubMed Central.

AWARD INFORMATION (Online Page Navigation Main Menu > Grants > Awards >Award Profile)

Award Start Date:	05/15/2010	**Award End Date:**	04/30/2015
Funds Approved to Date:	$1,461,303 00		
Principal Investigator:	Li,Fang	**Award Type:**	Grant
Primary Project Dept:	PHCL Pharmacology Admin	**Purpose:**	Research
Sponsor:	NIH Nat'l Inst of Allergy & Infect Dis		
Sponsor Award #:	5R01AI089728-04		
Award Title #:	Receptor recognition mechanisms of coronaviruses		

ASSOCIATED PROJECTS (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Funding Tab)

Project #:	00013782	**Department:**	11718 - PHCL Pharmacology Admin
Project PI:	Li,Fang	**Dates:**	05/15/2010 - 04/30/2015
F&A:	51 00 MTDC	**EFFDT:**	05/15/2010

Project Title: Receptor recognition mechanism

AWARD HISTORY (Online Page Navigation Main Menu > Grants > Awards > Review Award Modifications)
(Displayed in action order ('Mod Issue Date' in descending order))

Reference Award#:	5R01AI089728-04	**Amount:**	$336,353 00		
Period:	4	**Begin Date:**	05/01/2013	**End Date:**	04/30/2014
Modification Seq:	4	**Modification Type:**	Continuation	**Mod Issue Date:**	05/02/2013

Comments:

The NOA dated 4/11/2013 awards funding in the amount of $336,353 NIH is currently funded through a Continuing Resolution at the FY2012 level This award has been made at a level below that indicated in the previous NOA If the final appropriation permits, adjustments may be made up to the FY2013 funding plan

In addition to the PI, any absence, replacement or substantial reduction in effort of the following individuals requires prior NIH approval Dr Robert Geraghty

Awardee must notify and receive prior approval for the use of Select Agents Ses NOA for details on Select Agents, Highly Pathogenic Agents and additional Agents or Toxins

All other terms and conditions remain the same

Reference Award#:	5R01AI089728-03	**Amount:**	$373,725 00		
Period:	3	**Begin Date:**	05/01/2012	**End Date:**	04/30/2013
Modification Seq:	3	**Modification Type:**	Continuation	**Mod Issue Date:**	05/01/2012

Comments:

Notice of Award dated 04/10/2012

None of the funds in this award shall be used to pay the salary of an individual at a rate per year in excess of the amounts reflected in the following NIH Guide Notice http //grants nih gov/grants/guide/notice-files/NOT-OD-12-035 html

All other previous terms and conditions remain unchanged

Reference Award#:	5R01AI089728-02 Rev	**Amount:**	$33,975 00		
Period:	2	**Begin Date:**	05/01/2011	**End Date:**	04/30/2012
Modification Seq:	3	**Modification Type:**	Additional Funding	**Mod Issue Date:**	06/15/2011

Comments:

This revised award increases the total costs authorized consistent with NIAID implementation of the NIH Fiscal policy for FY2011

All other terms and conditions remain the same

Reference Award#:	1R01AI089728-02	**Amount:**	$339,750 00

11
7-11
49

Period: 2 **Begin Date:** 05/01/2011 **End Date:** 04/30/2012
Modification Seq: 2 **Modification Type:** Continuation **Mod Issue Date:** 05/05/2011
Comments:

NIH is currently funded through a Continuing Resolution at the FY2010 level Therefore, this non-competing award is made at a level below the committed FY2011 level If final appropriation permits, adjustments may be made up to FY2011 plan level

All other terms and conditions remain the same

Reference Award#: 1R01AI089728-01 **Amount:** $377,500 00
Period: 1 **Begin Date:** 05/15/2010 **End Date:** 04/30/2011
Modification Seq: 1 **Modification Type:** Original **Mod Issue Date:** 05/27/2010
Comments:

An unobligated balance may be carried forward to the next budget period without prior agency approval

NOTE In additoin to the PI, Dr Robert Geraghty as been identified as key personnel (co-PI) Any absence, replacement or substantial reduction in effort by key personnel requires prior, written agency approval

Modular award The significant rebudgeting provision does not apply In addition, since costs were averaged over the project period, deficits may be carried forward from one budget period to the next within the same project period

ESTIMATED FUTURE FUNDING / ACTIONS (Online Page Navigation Main Menu > Grants > Awards > Review Award Modifications)

(Displayed by 'Period' and 'Modification Sequence' in ascending order)

Reference Award#: 1R01AI089728-01 **Amount:** $373,725 00
Period: 5 **Begin Date:** 05/01/2014 **End Date:** 04/30/2015
Modification Seq: 5 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

COMMENTS

Description: **Entered by:** HOWI0016 **Date & Time:** 5/27/2010 10 00 AM

Date and Time Stamp indicates Proposal-to-Award Process

Description: **Entered by:** HOWI0016 **Date & Time:** 5/27/2010 10 02 AM

Sponsor Contacts

Grants Management
Michael Wells
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Program
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Description: **Entered by:** SPAUL **Date & Time:** 12/23/2010 9 56 AM

LOC

RAI089728

Description: **Entered by:** GARC0027 **Date & Time:** 4/18/2013 12 50 PM

Grants Management Specialist Updated

Jennifer Schermerhorn

Email schermerhornj@niaid nih gov
P 301-451-2649

SPECIAL REVIEWS (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Certifications Tab)
Project #: 00013782
Description: rDNA, InfectAgents, BioToxins
Approval Date: 03/10/2012 **Expiration Date:** 03/09/2013 **AssuranceNumber:** 1002H77733
Comments:

BUDGET INFORMATION (Online Page Navigation Main Menu > Grants>Awards>Project Budget)
Please review online budget for valid account string combinations (ex PCBU, Activity, Fund, Dept_Id, Project, Account)

Project #: 00013782
Project Start Date: 05/15/2010
ICR Sharing Department: Drug Design, Center for
ICR Sharing Department: PHCL Pharmacology Admin

Cost Share Budget Total:
Project End Date: 04/30/2015
Percentage: 11 50
Percentage: 88 50

Period	Start Date	End Date	Direct Cost (Approved)	F & A Cost (Approved)	Direct Cost (Pending)	F & A Cost (Pending)	Total Period Cost
1	05/15/2010	04/30/2011	$252,702 00	$124,798 00	$0 00	$0 00	$377,500 00
2	05/01/2011	04/30/2012	$247,500 00	$126,225 00	$0 00	$0 00	$373,725 00
3	05/01/2012	04/30/2013	$247,500 00	$126,225 00	$0 00	$0 00	$373,725 00
4	05/01/2013	04/30/2014	$228,695 00	$107,658 00	$0 00	$0 00	$336,353 00
5	05/01/2014	04/30/2015	$0 00	$0 00	$247,500 00	$126,225 00	$373,725 00
TOTAL			$976,397 00	$484,906 00	$247,500 00	$126,225 00	$1,835,028 00

Budget Detail (all periods)

Fund Code	DeptID	Program Code	Chartfield 1	Chartfield 2	Fin EmplID	Activity	Analysis Type	Account	Cost Share	Budget Item	Amount	Hold
3002	11718					1	BUD	168100		CAPITAL_EQUIP	$8.000 00	
3002	11718					1	BUD	700100		FACULTY_SALARY	$186,874 00	
3002	11718					1	BUD	700200		PROF_ADM_SALARY	$201,666 00	
3002	11718					1	BUD	700300		GRAD_STU_SALARY	$101.400 00	
3002	11718					1	BUD	700310		GRAD_SAL_NOTUIT	$41,000 00	
3002	11718					1	BUD	710100		FACULTY_FRINGE	$64,545 00	
3002	11718					1	BUD	710200		PROF_ADM_FRINGE	$51,901 00	
3002	11718					1	BUD	710300		GRAD_STU_FRINGE	$33,717 00	
3002	11718					1	BUD	710310		GRAD_FRG_NOTUIT	$9,839 00	
3002	11718					1	BUD	720200		LABMED_SUPPLIES	$202,962 00	
3002	11718					1	BUD	720300		GEN_OP_SERVICES	$9,375 00	
3002	11718					1	BUD	720400		LABMED_SERVICES	$4,200 00	
3002	11718					1	BUD	720500		TELECOMM	$1,992 00	
3002	11718					1	BUD	720600		TRAVL_MILE_MOVE	$12,970 00	
3002	11718					1	BUD	730100		CONSULTING_SVCS	$29,900 00	
3002	11718					1	BUD	750100		NON_CAP_EQUIP	$9,571 00	
3002	11718					1	BUD	780100		REPAIRS_MAINT	$6,485 00	
3002	11718					1	BUD	810500		FACADM	$484.906 00	

COST SHARE/MATCH/IN-KIND

CSM - Cost Share, Matching and InKind

CSM05 - Departments must document all cost share/unpaid effort that is shown in the proposal

PROGRAM INCOME INFORMATION
PIN - Program Income

PIN03 - Reportable Program Income must be deposited by the department into the Sponsored Program Income Unapplied Cash account

PIN04 - Additional - Program Income can be used in addition to sponsor funds to cover project costs that will further the objectives of the legislation under which the grant was made

TERMS & CONDITIONS (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Terms Tab)

APV - Additional Approval

APV01 - Prior sponsor approval or notification required for reduction of PI's effort by 25% or more

APV04 - Prior sponsor approval required to replace PI

APV07 - Prior sponsor approval required for changes to key personnel that would impact the scope of work

APV10 - Prior sponsor approval required for changes to work scope

APV14 - Prior sponsor approval required for the absence of the PI for 3 months or more

APV15 - Prior sponsor approval required for a second no cost extension or a first no cost extension that is submitted after the agency notification deadline

APV20 - Prior sponsor approval required for the addition of foreign sites or foreign subcontracts

FIS - Fiscal

FIS03 - Reversion of funds at termination / final

FIS07 - Deficits may be carried forward

FIS10 - Grant/contract funds may not be used to pay the salary of an individual at a rate in excess of Executive Level I of the Federal Executive Pay Scale ($199,700)

FIS11 - Modular award - Expenses incurred must be allocated and accounted for in compliance with all applicable cost principles

FIS13 - Deficits may be carried forward within non-competing segments, but may not be carried forward between competing segments

FIS14 - Grant/contract funds may not be used to pay the salary of an individual at a rate in excess of Executive Level II of the Federal Executive Pay Scale ($179,700)

IPR - IP Rights

IPR02 - Sponsor support must be acknowledged in patent applications and patent issuing

IPR04 - Title to U of M; non-exclusive, non-transferable, irrevocable, paid-up license to Federal Government

IPR12 - PI obligation to report inventions (and other IP) to the sponsor (and to OTC per policy)

PRP - Property

PRP02 - U of M receives title to equipment purchased or fabricated under this award

PRP03 - Sponsor may require transfer of equipment and its title upon award completion for those items costing in excess of $5000

PUB - Publications

PUB02 - U of M will place an acknowledgement of sponsor support and a disclaimer, as appropriate, on any publication generated by this award or reporting on the award results and/or activity

PUB07 - Peer-reviewed articles must be submitted to NIH Pubmed Central (PMC) upon acceptance for publication and made publicly available within 12 months Cite PMC ID number in progress reports/proposals

RBD - Rebudgeting

RBD10 - Rebudgeting is allowed as long as there is no change in work scope

REG - Regulatory

REG01 - 45 CFR Part 74 - Code of Federal Regulations as it pertains to institutions of higher education

REG03 - OMB Circular A-21 (www whitehouse gov/omb/circulars/a021/a021 html)

REG04 - OMB Circular A-110 (www whitehouse gov/omb/circulars/a110/a110 html)

REG05 - OMB Circular A-133 (www whitehouse gov/omb/circulars/a133/a133 html)

REG09 - Subject to Streamlined Noncompeting Award Procedures (SNAP)

REG10 - Federal Demonstration Partnership (FDP) guidelines (www thefdp org)

REG19 - NIH Grants Policy Statement (http //grants nih gov/grants/policy/nihgps_2010/index htm)

UMN - U of M Requirements

UMN01 - The U of M Effort Certification system must be used to reflect all U of M staff committed effort on this award, whether or not any salary is charged to the award

UMN02 - Salary cost transfers cannot be made after effort is submitted

UMN03 - All U of M Policy and Guidelines relating to research must be followed and can be read in detail on the web at http //www ospa umn edu/policiesandprocedures/index htm

UMN04 - U of M Cost Transfer guidelines state transfers of non-salary expenses on accounts be clearly documented & made within 30

days after the end of the accounting period the charge was processed

NON-FINANCIAL REPORTING (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Milestones Tab)

Type: Report **Description:** Progress Report **Due Date:**

Comments: PHS 2590 - noncompeting continuation Must be submitted via the eRA Commons Due 45 days prior to the end of the budget period

INVOICE/BILLING INFORMATION

Basis of Payment:	COST_REIMBURSE	**Method of Payment:**	Electronic Fund Transfer
Invoice Form:	GPC - GM_LOC		
Interim Invoice Deadline:	0	**Final Invoice Deadline:**	0

Contact Name: Jacqueline Jones
Contact Phone:
Contact Fax:
Contact Email:
Contact Address: PSC-FMS (301) 443-3020
PARKLAWN BUILDING ROOM 16A-12
5600 FISHERS LANE
ROCKVILLE MD 20857

FINANCIAL REPORTING

Report Form ID:	R_FFR	**Reporting Frequency:**	Termination/Final
Interim Reporting Deadline:	90	**Final Reporting Deadline:**	90

GENERAL PROVISIONS

This document is intended to provide guidance in managing this award, to the Principal Investigator, Academic Unit, and to those who are noted as receiving this document Its intent is to provide general information regarding University and sponsor policies when given the award In addition, it is intended to reference specific conditions related to this award Specific questions should be addressed to the grant administrator from SPA noted below By accepting this award, the Principal Investigator acknowledges and agrees to abide by all provisions of the sponsoring agency and the University of Minnesota policies related to sponsored projects management, including the Code of Conduct

U OF M CONTACTS

Administrative questions to SPA staff member:	Paulie Garcia	612/626-2724	garc0027@umn edu
Billing and financial questions to SFR:	Julia Steinkopf	612/624-7033	gordo091@umn edu

Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Issue Date. 04/11/2013

Grant Number: 5R01AI089728-04

Principal Investigator(s).
Fang Li, PHD

Project Title· Receptor recognition mechanisms of coronaviruses

David L Hagen
University of Minnesota Twin Cities
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 554552070

Award e-mailed to. awards@umn edu

Budget Period: 05/01/2013 – 04/30/2014
Project Period: 05/15/2010 – 04/30/2015

Dear Business Official

The National Institutes of Health hereby awards a grant in the amount of $336,353 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MINNESOTA in support of the above referenced project This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI089728 The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health " Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with 42 CFR Part 50 Subpart F Subsequent to the compliance date of the 2011 revised FCOI regulation (i e , on or before August 24, 2012), Awardees must be in compliance with all aspects of the 2011 revised regulation, until then, Awardees must comply with the 1995 regulation The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards Consult the NIH website http grants nih gov/grants policy cc for a link to the regulation and additional important information

If you have any questions about this award, please contact the individual(s) referenced in Section IV

Sincerely yours,

Shellie M Wilburn
Grants Management Officer

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI089728-04

Award Calculation (U S. Dollars)

Federal Direct Costs	$222,750
Federal F&A Costs	$113,603
Approved Budget	$336,353
Federal Share	$336,353
TOTAL FEDERAL AWARD AMOUNT	$336,353
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$336,353

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
4	$336,353	$336,353
5	$336,353	$336,353

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93 855
EIN:	1416007513A1
Document Number:	RAI089728A
Fiscal Year:	2013

IC	*CAN*	*2013*	*2014*
AI	8472364	$336,353	$336,353

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:

PCC M51C S / **OC** 414E / **Released** WILBURNS 04/10/2013
Award Processed: 04/11/2013 12 05 24 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI089728-04

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http //grants nih gov/grants/policy/awardconditions htm

SECTION III – TERMS AND CONDITIONS – 5R01AI089728-04

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following

a The grant program legislation and program regulation cited in this Notice of Award
b Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts
c 45 CFR Part 74 or 45 CFR Part 92 as applicable
d The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period
e This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW

(See NIH Home Page at 'http //grants nih gov/grants/policy/awardconditions htm' for certain references cited above)

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase V Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP)

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration Should a consortium/subaward be issued under this award, a DUNS requirement must be included See http //grants nih gov/grants/policy/awardconditions htm for the full NIH award term implementing this requirement and other additional information

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170

In accordance with P L 110-161, compliance with the NIH Public Access Policy is now mandatory For more information, see NOT-OD-08-033 and the Public Access website http //publicaccess nih gov

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI089728-04

NIH is currently funded through a Continuing Resolution (CR) at the FY2012 level (See NIH Guide Notice NOT-OD-13-002) http //grants nih gov/grants/guide/notice-files/NOT-OD-13-002 html Therefore, this non-competing award has been made at a level below that indicated in the previous Notice of Grant Award If the final appropriation permits, adjustments may be made up to the FY2013 funding plan level Grantee institutions are advised to monitor their expenditures carefully during the CR period
****

In addition to the PI, any absence, replacement, or substantial reduction in effort of the following individual(s) below, requires the written prior approval of the National Institutes of Health awarding component
Dr Robert Geraghty, Associate Professor and co-PI
****

Select Agents
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13 b (http //www selectagents gov/Regulations html)

Highly Pathogenic Agent
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http //www cdc gov/OD/ohs/biosfty/bmbl5/bmbl5toc htm) Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used
When submitting future Progress Reports indicate at the beginning of the report

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide

o A list of the new and/or additional Agent(s) that will be studied,
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment,
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official It is important to note if the work is being done in a new location
****

If you have a question on the award calculation or the terms and conditions of the award, your grants management contact should be Philip Smith at (301) 402-3980 or via e-mail at philip smith2@nih gov

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions The Program Official is responsible for the scientific, programmatic and technical aspects of this project These individuals work together in overall project administration Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist Requests may be made via e-mail

Grants Management Specialist Jennifer L Schermerhorn
Email schermerhornj@niaid nih gov **Phone** 301-451-2649 **Fax** 301-493-0597

Program Official: Rachelle Salomon
Email salomonra@mail nih gov **Phone** 301-402-2202 **Fax** 301-496-8030

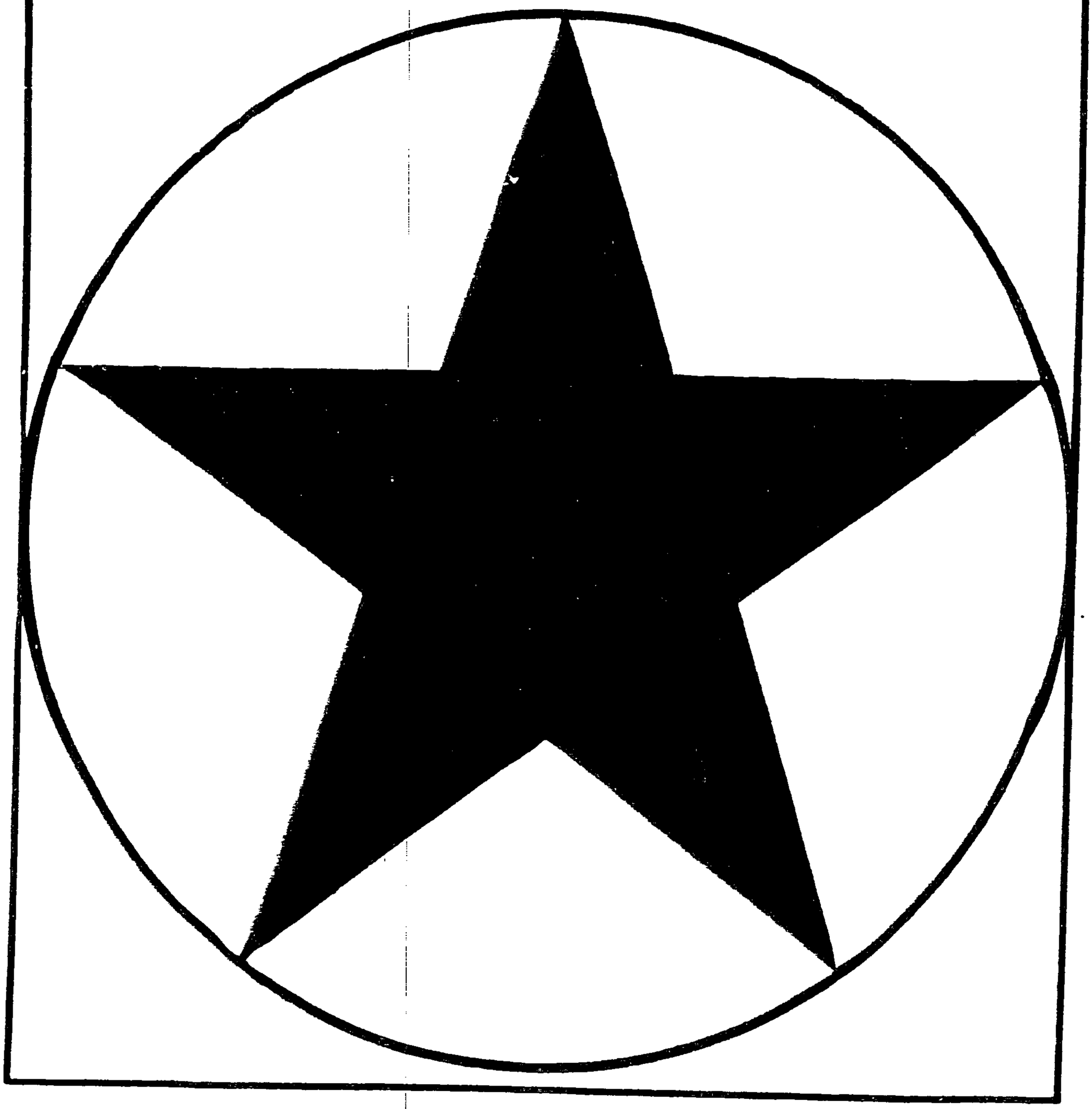
SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI089728-04

INSTITUTION: UNIVERSITY OF MINNESOTA

Budget	***Year 4***	***Year 5***
TOTAL FEDERAL DC	$222,750	$222,750
TOTAL FEDERAL F&A	$113,603	$113,603
TOTAL COST	$336,353	$336,353

Facilities and Administrative Costs	***Year 4***	***Year 5***
F&A Cost Rate 1	51%	51%
F&A Cost Base 1	$222,750	$222,750
F&A Costs 1	$113,603	$113,603

Please Mail All Pages Prior To Page Separator



AWARD SETUP INFORMATION PAGE GA Paulii DATE 4/22/13
CON 22345 7 PROJECT 00013782
eTool Completed N/O

_____New
_____PreAward – see bottom of page for information
__X__ Modification, original Award = CON 42711 (this is the award that will be updated)
_____Stimulus Funding
_____FFATA

INVOICING AND FINANCIAL REPORTING INFORMATION CAN BE FOUND ON THE FOLLOWING PAGE(S) IN THE AWARD DOCUMENT _____ N/A

TERMS AND CONDITIONS
_____ are attached _____ are in eTool
_____ are saved in MyNetworkPlaces\Award Setup\Award Setup-submissions TAB_____ N/A
(\\ovpr.ad.umn.edu\ovpr$\Shared\Groups\Award Setup)

BUDGET INFORMATION FOR FUNDS PROVIDED BY SPONSOR – for new awards only
3002 Fund 11715 DeptID
_____ is in eTool
_____See attached PS-friendly budget

COST SHARE INFORMATION – for new awards only (GA updates the budgets for modifications)
_____Fund _____ DeptID _____ Program _____ Chartfield 1 _____ Chartfield 2 _____Emplid N/A
_____ is in eTool
_____See attached PS-friendly budget

_____**NEW PROJECT NEEDED** - For new projects on existing awards, please enter relevant information in the eTool or on separate page and include a completed PS-friendly budget.

ANY ADDITIONAL INFORMATION NEEDED FOR SET UP:
continuation – 4/30/14
+336,353

PUT AWARD FILE IN THE FOLLOWING ORDER:
Agency award documents
Guidance documents (to be included with award emailed to PI/Dept)
Award Imaging Divider page (yellow star sheet)
Award Setup Information page (this page)
PS-friendly budget (if any)
Terms and Conditions spreadsheet (if any)
Negotiation notes, proposal correspondence, proposal, etc.

SET ALL NEGOTIATE AWARD NEGOTIATION TYPES TO COMPLETED EXCEPT FOR THE ONE FOR CONTRACT ENTRY AND THE ORIGINAL ONE CREATED BY FILES

PREAWARD INFORMATION REQUIRED - Provide information for each project
Note: No cost share information is required for PreAwards.
_____Fund Budget accounts to be set up at $0 are listed below - OR - ____See Attached
_____DeptID Remember to include 810500 if F&A should be charged
_____Project _____ _____ _____ _____ _____
_____Function _____ _____ _____ _____ _____
_____F&A rate & base _____ _____ _____ _____ _____
_____Rate Type *(Research, Other, Training, Off Campus, ClinTrial-nonfed, CREE2, CREES, DOD contract, Equip, Foundation, Hormel, Instruction, SBIR/STTR Phase I, State)*

/ / End Date of PreAward / Advance Project to be entered in KK Child and KK Parent

PS / SPONSOR PROPOSAL BUDGET WORKSHEET

Please complete the cost share and F&A distribution information at the bottom of the page, if applicable

Award CON0000000022345 Project #, if known **00013782** Should this be the Primary Project? ___X_Yes ___ No

Project DeptID 11718 Project PI **Fang Li**

		Total of all worksheet tabs	Sponsor	Cost Share	Total Projects
LINK TO FY 2012 COA					
ITEM			**Sponsor Funding**	**Cost Share or Match***	**TOTAL PROJECT BUDGET**
Personnel					
Salary					
700100	Salary - Faculty		50,000		
700200	Salary - Professional and Admin		15,000		
700310	Salary - Post Doc/Prof Student (expense account 700302)		36,000		
700300	Salary - Grad Research Asst (expense account 700301)		20,000		
700310	Salary - Grad Research Asst (No Tuition)		5,000		
700400	Salary - Undergrad Student				
700500	Salary - Civil Service Bargaining Unit				
Total Salary			126,000	0	126,000
Fringe					
710100	Fringe - Faculty		17,450		
710200	Fringe - Professional and Admin		5,235		
710310	Fringe - Post Doc/Prof Student (expense account 710302)		8,089		
710300	Fringe - Grad Research Asst (expense account 710301)		17,600		
710310	Fringe - Grad Research Asst (No Tuition)		1,750		
710400	Fringe - Undergrad Student				
710500	Fringe - Civil Service Bargaining Unit				
710600	Fringe - Direct Charge				
Total Fringe			50,124	0	50,124
Total Personnel			176,124	0	176,124
Consulting / Professional Services					
730100	Consulting Services				
730200	Professional Services				
Total Consulting / Professional Services			0	0	0
Equipment					
168100	Equipment				0
Supplies					
720100	General Operating Supplies				
720200	Lab & Medical Supplies		30,071		
750100	Non-Capitalized Equipment		9,000		
Total Supplies			39,071	0	39,071
Travel					
720600	Travel / Mileage / Moving		5,000		5,000
Other Direct Costs					
720300	General Operating Services		2,500		
720400	Lab & Medical Services		4,000		
720500	Telecommunications				
770100	Long Term Rents & Leases				
770150	Long Term Rents & Leases (bldg/facility)				
770200	Short Term Rents & Leases				
770250	Short Term Rents & Leases (bldg/facility)				
780100	Repairs & Maintenance		2,000		
810100	Research Subject Payments				
810200	Participant Payments				
Total Other Direct Costs			8,500	0	8,500
Subawards / Subcontracts					
810300	Subcontracts not subject to F&A				
810400	Subcontracts subject to F&A				
Total Subawards / Subcontracts			0	0	0
Additional Accounts (show budget account and a description, please)					
					0
					0
					0
TOTAL DIRECT COSTS			228,695	0	228,695
810500	Facilities and Administrative Expenses		107,658		107,658
TOTAL COSTS			336,353	0	336,353

Account descriptions are provided in comments for each category While SPA will try to keep the account codes and descriptions updated, you should refer to the current FY COA for the most up to date information.

***Cost Share/Match account string**

Does your Program FUNC match your Project FUNC? Check the COA for FUNC and Program descriptions

For any amounts shown as Cost Share or Match, the Fund, DeptID, Program and optional fields CF1, CF2, and FinEmplID providing the funding must be identified If these codes will be the same for all lines with Cost Share or Match, please enter them here

Fund _____ DeptID _____ Program _____

CF1 _____ CF2 _____ FinEmplID _____

If the Fund, DeptID, Program, CF1, CF2, and FinEmplID will differ among the cost share or match amounts, please enter the information next to the specific cost share or match amount so that budget lines can be correctly established

F&A Distribution

If the F&A revenue is going to be shared between departments, please identify the department(s) and percentages below If all the F&A revenue should go to the department identified at the top of the page, this section may be left blank

F&A Distribution - Approved by (name)

DeptID _____ Dept Name _____ _____ % F&A rec'd _____

DeptID _____ Dept Name _____ _____ % F&A rec'd _____

DeptID _____ Dept Name _____ _____ % F&A rec'd _____

DeptID _____ Dept Name _____ _____ % F&A rec'd _____

Total F&A % (must = 100%) 0%

By submission of this information, I certify that the proper authorizing officials are named above and that I have retained evidence within the department/unit confirming their agreement with the distributions above

Name _____

DeptID _____

Phone # _____

Email _____

See current rates (fringe benefits, F&A, etc) http //www ospa umn edu/forms/index html

For a crosswalk between CUFS object codes and PS Accounts, see

http //www budget umn edu/FY09docs/CUFS_PS_conversion_grid xls

Subject: NoA: 5 R01 AI089728-04 PI: Li, Fang
From: <era-notify@mail.nih gov>
Date: 4/10/2013 11:10 PM
To: <awards@umn.edu>

Grant Number. 5 R01 AI089728-04
Principal Investigator Li, Fang
Project Title· Receptor recognition mechanisms of coronaviruses
Institution· UNIVERSITY OF MINNESOTA
CFDA· 93 855
OC. 414E
PCC M51C S
Award Issue Date· 04/11/2013
Grants Management Officer· Wilburn, Shellie
Program Official Salomon, Rachelle
Grants Specialist. Schermerhorn, Jennifer

*** This is an automated notification - Please do not reply to this message. ***

Attachments

temp80109075499168757 47 pdf 110 KB

CON-427N
F: CON-22345
755486 / 00036630
L
PGM Amount

UNIVERSITY OF MINNESOTA

ReportID: UMFGM017F

PROPOSAL SUMMARY

Run Date: 04/11/2013
Run Time: 10:43:08 AM

Status **4 AwdB Award Sent to GA**

Grant Administrator	Garcia Paulie			
PRF #	755486	Proposal #/Version	CON000000042711/A101	
EGMS Proposal #		Sponsor Award #	5R01AI089728-04	
Proposal Type	Non-Competing Continuation	Concurrent	N	
Previous Award		SubContract	N	
Purpose				
Title	Receptor recognition mechanisms of coronaviruses			
Principal Investigator	Li,Fang	lifang@umn edu		
Budget Summary				
Start Date	05/01/2013	End Date	04/30/2014	
Direct Costs	$252,317 00	Indirect Costs	$121.408 00	Total Costs $373,725.00
Sponsor	National Institutes of Health (NIH)			
Submission Information				
Due Date	03/15/2013	Date Type	Receipt Date	
Submission Method	Electronically Only	# of Copies Submitted	_____	
Mailing Address				

Comments/Corrections

REPA	__verified	__email sent for _____		
IRB	__verified	__pending	__email sent	__Not Applicable
IACUC	__verified	__pending	__email sent	__Not Applicable
IBC	__verified	__pending	__email sent	__Not Applicable
F&A rate	__at full or agency limit	__waiver on file	__waiver requested	
Cost share documented?		__yes	__no	__Not Applicable
ICR sharing?		__yes	__no	

(CON-22345)
CON-42711
755486

Status Information

General Grant Information	
Status:	Pending administrative review Refer any questions to Program Official or Grants Management Specialist
Institution Name:	UNIVERSITY OF MINNESOTA
School Name:	MEDICAL SCHOOL
School Category:	SCHOOLS OF MEDICINE
Division Name:	NONE
Department Name:	PHARMACOLOGY
PI Name:	Li, Fang
Application ID:	5R01AI089728-04
Proposal Title:	Receptor recognition mechanisms of coronaviruses
Proposal Receipt Date.	03/14/2013
Last Status Update Date:	04/10/2012
Budget Start Date	05/01/2013
Budget End Date	04/30/2014
Progress Report Due Date:	03/15/2013
Current Award Notice Date.	
Application Source:	Esnap
Project Period Begin Date:	05/15/2010
Project Period End Date:	04/30/2015
eApplication Status:	
FOA.	[PA07-070] - RESEARCH PROJECT GRANT (PARENT R01)
NIH Appl ID:	8458050

Other Relevant Documents

e-Application

Additions for Review (0 documents)

Correspondence

Referral

Date	Description	Action

Status History

Effective Date	Status Message

Institute or Center Assignment

Institute or Center	Assignment Date
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES **(Primary)**	04/10/2012
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES **(Primary)**	04/10/2012

Application Information

Award Document Number	RAI089728A
FSR Accepted Code	N
Snap Indicator Code	Y
Review Outcome Available	No
Summary Statement Available	No
Early Stage Investigator Eligible	
New Investigator Eligible	
Eligible for FFATA Reporting	No

Study Section

Scientific Review Group	VIRA
Council Meeting Date(YYYY/MM)	2013/00

Advisory Council(AC) Information

Reference Letter(s)

This list shows Reference Letters associated with this particular Grant Application Principal Investigator can see a list of all Reference Letters within Personal Profile - Reference Letters section on eRA Commons

Contacts

Administration	Name	Phone	Email

Grants Management Specialist(GMS)	Schermerhorn, Jennifer L	301-451-2649	schermerhornj@niaid nih gov
Program Official(PO)	Salomon, Rachelle	301-402-2202	salomonra@mail nih gov

Close

[Contact Us | Privacy Notice | Disclaimer | Accessibility | Popup Block Check]

National Institutes of Health (NIH) 9000 Rockville Pike Bethesda, Maryland 20892

Department of Health and Human Services

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Screen Rendered 03/18/2013 10 25 18 EDT
Screen Id STA4002@3245

Version 1 09 01

GRANTS.GOV™ FIND APPLY SUCCEED™

Approved

UNIVERSITY OF MINNESOTA

PROPOSAL SUMMARY

ReportID: UMFGM017F

Page No: 1
Run Date: 03/13/2013
Run Time: 10:55:37 AM

Status ~~--2 PropB Elect Received at SPA~~

INSTITUTION APPROVED
3/14/13

Grant Administrator Paulie

PRF #	755486	Proposal #/Version	CON000000042711/V101
EGMS Proposal #		Sponsor Award #	
Proposal Type	Non-Competing Continuation	Concurrent	N
Previous Award		SubContract	N
Purpose	CON-22345		
Title	Receptor recognition mechanisms of coronaviruses		
Principal Investigator	Li,Fang	lifang@umn edu	

Budget Summary

Start Date	05/01/2013	End Date	04/30/2014
Direct Costs	~~$0.00~~ 252,317	Indirect Costs	~~$0.00~~ 121,408 Total Costs ~~$0.00~~ 373,725
Sponsor	National Institutes of Health (NIH)		

Submission Information

Due Date	03/15/2013	Date Type	Receipt Date
Submission Method		# of Copies Submitted	

Mailing Address

ESNAP

3/14/13 Submitted -AC

Comments/Corrections

REPA	X verified	__email sent for _____		
IRB	__verified	__pending	__email sent	X Not Applicable
IACUC	__verified	__pending	__email sent	X Not Applicable
IBC	__verified	__pending	__email sent	__Not Applicable
F&A rate	MTDC 1 at full or agency limit	__waiver on file	__waiver requested	
Cost share documented?		__yes	__no	X Not Applicable
ICR sharing?		__yes	X no	

SUBAWARDS

	FDP/DB	Forms 1&2
PHS Subrecipient	☐	☐
PHS Subrecipient	☐	☐
PHS Subrecipient	☐	☐
PHS Subrecipient	☐	☐

Logged Out
3/14/13
KE

✓ ELECTRONIC PROPOSAL
✓ ESNAP
- FEDERAL
- NON-FED
- G-GOV

NO ATTACHMENT

✓p

E

UNIVERSITY OF MINNESOTA PRF

EGMS ID PRF ID 755486

Proposal Routing Form for External Research, Training or Public Service Support — PS Proposal ID

1	Sponsor Name	**National Institutes of Health**	**Primary Funding Source**
2	Proposal Title	**Receptor recognition mechanisms of coronaviruses**	
3.	Sponsor Due Date/Time	**03/15/2013 - 5pm CST**	**Receipt**
4	RFA/RFP/CFDA/FOA#		
5.	Submission Medium	**Electronic [other than Grants gov] Submission via eRA Commons ESNAP**	

CON-42711

6.	University Contact	**Weber, Lynn F**	**(612) 626-6902**	**weber117@umn.edu**
7.	Administering Dept	**PHCL PHARMACOLOGY ADMIN**	**Department ID 11718**	

8	Principal Investigator	**Li, Fang**			**Paid**	**Cost Share**	**PI**	**Key**
		lifang	**ID 3694715**	**(612) 625-6149**	**50**	**0**	**X**	**X**
		ASSISTANT PROFESSOR [9403]	**PHCL PHARMACOLOGY ADMIN**	**DeptID 11718**	Checked			
9.	Investigator(s)	**Geraghty, Robert J.**			**Paid**	**Cost Share**	**PI**	**Key**
		gerag012	**ID 1235946**	**(612) 625-3281**	**8**	**0**		**X**
		ASSOCIATE PROFESSOR [9402]	**DRUG DESIGN, CENTER FOR**	**DeptID 11345**	Checked			

10.	Proposal is for		**Basic %**	**Applied %**	**Development %**	**Total %**
		Research	**100**	**0**	**0**	**100**

11	Proposal is		**Agency Award #**	**EFS Award #**	**Primary Project**
		Continuation	**5R01 AI089728-04**	**30021171800013782**	

12.	Proposed Duration & Amount Requested		**Start**	**End**	**Direct $$**	**Indirect $$**	**F&A Rate**	**Total**
		Initial/Current Budget	**05/01/2013**	**04/30/2014**	**252,317**	**121,408**	**51% MTDC**	**373,725**
		All Years	**05/01/2013**	**04/30/2014**	**252.317**	**121,408**	**51% MTDC**	**373,725**

X Departmental Directions to SPA for Award Set Up
Proposal budget has been prepared outside of EGMS: Department will furnish PeopleSoft-friendly budget to SPA prior to receipt of the award; SPA may pro-rate at the time of award if needed to accommodate a reduced award amount.

X **F&A Rate Used** **51% MTDC**
Full F&A Rate

F&A Comments

13.	Project Involves				
	X	**Recombinant DNA, Infectious Agents or Biological Toxins**	**There will be recombinant DNA, infectious agents or biological toxins used**		
		Application status of this project with the Institutional Biosafety Committee	**Status**	**Study Code/Protocol ID**	**Approval Date**
			Approved	**121230150H**	**03/04/2013**
	X	**Chemicals**	**There will be chemicals used**	**The appropriate chemical safety training and hazardous waste training records have been completed**	

14 Subawards

UNIVERSITY OF MINNESOTA PRF EGMS ID PRF ID 755486

Proposal Routing Form for External Research Training or Public Service Support PS Proposal ID

15. Conflict of Interest	X	**There is not a potential financial and/or business conflict of interest** **REPA #** **Approval Date** **Comments**			
16. Inventions	--				
17 Space	X	**There is sufficient and suitable space to house this project presently assigned to the principal investigator's or other investigator's department or college**	**3-284 and 7-227**	**Nils Hasselmo Hall and PWB**	
18. Resources/Space/Staff	X	**This project involves University resources, space or staff from more than one department**			
19 Indirect Cost Recovery	X	**Academic units have chosen to share ICR revenue and be shared by contributions**	**PHCL PHARMACOLOGY ADMIN**	**11718**	**89**
			DRUG DESIGN, CENTER FOR	**11345**	**11**
		Comments **11 5% of F&A shared with Center for Drug Design, Dept ID 11345** **88 5 of F&A for Pharmacology Department, Dept. ID 11718**			
20 Matching and Cost Sharing					
21. Program Income					
22 International Component					
23. Fairview Health Services					

24 PROPOSAL ABSTRACT or EXECUTIVE SUMMARY

25. NOTES

UNIVERSITY OF MINNESOTA PRF EGMS ID: PRF ID 755486

Proposal Routing Form for External Research Training or Public Service Support PS Proposal ID

APPROVALS and CERTIFICATIONS

Principal Investigator

(1) I certify the information submitted within the application is true complete and accurate to the best of the PI's knowledge
(2) that any false ficticious, or fraudulent statements or claims may subject the PI to criminal civil or administrative penalties, and
(3) that the PI agrees to accept the responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of the application

Investigator(s).

The information provided on this form and in the accompanying proposal is correct to the best of my knowledge Equipment budgeted in this application is not otherwise available for use on this project from existing departmental or collegiate inventories In the event this application is awarded I (we) agree to abide by all applicable institutional and sponsoring agency policies and procedures including the Intellectual Property Policy of the University of Minnesota and to follow commonly accepted scientific practices in recording and maintaining records of research I (we) certify that I (we) have read the University of Minnesota Code of Conduct policy and agree to abide by the rights and responsibilities as identified therein

Signature Fang Li 03-11-2013
Fang Li (lifang) Principal Investigator **Date**

Department Head(s) and/or Division Head(s) Academic Dean(s). Chancellor or Provost

We certify that the above statements are correct to the best of our knowledge The commitment of departmental and collegiate resources if any has been noted and approved We agree that the scientific objectives of this application are in keeping with departmental and collegiate goals We certify that the individuals from our unit(s) listed on this PRF have filed a current Report of External Professional Activities (REPA)

Signature DeJarlais, Carrie L 03-11-2013
DeJarlais, Carrie L (dejar001) Dept Administrator/Representative **Date**

Signature Marchant, Jonathan S 03-11-2013
Marchant, Jonathan S (march029) Dept Head/Delegate **Date**

Signature Hinske, Liz 03-12-2013
Hinske, Liz (hinsk001) Dept Administrator/Representative **Date**

Signature Vince, Robert 03-11-2013
Vince, Robert (vince001) Dept Head/Delegate **Date**

Signature tmeddean 03-11-2013
tmeddean (tmeddean) Research Associate Dean/Delegate **Date**

Signature Segal, Rosalyn A 03-11-2013
Segal, Rosalyn A (rsegal) Research Associate Dean/Delegate **Date**

Signature LeBien, Tucker W 03-13-2013
LeBien, Tucker W (lebie001) Research Associate Dean/Delegate **Date**

Final

ESNAP Report **DRAFT**

Grant Number R01AI089728-04		**Total Project Period** From 05/15/2010 To 04/30/2015	
EIN: 1416007513A1	**Review Group:** VIRA	**Requested Budget Period:** From 05/01/2013 To 04/30/2014	
Title of Project: Receptor recognition mechanisms of coronaviruses			**Due Date:** 03/15/2013 **Submitted Date:**
Program Director/Principal Investigator: Fang Li UNIVERSITY OF MINNESOTA TWIN CITIES Department of Pharmacology 6-120 Jackson Hall 321 Church Street S E MINNEAPOLIS , MN 554552070 **Phone Number:** 612-625-6149 **Fax Number:** **Email Address:** lifang@umn edu		**Applicant Organization:** UNIVERSITY OF MINNESOTA UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS , MN 554552070 **Department:** PHARMACOLOGY **Major Subdivision:** MEDICAL SCHOOL	
Administrative Official: Paulie Garcia 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE Minneapolis , MN 554552070 **Phone Number:** 612-624-5599 **Fax Number:** **Email Address:** awards@umn edu		**Signing Official:** David L Hagen 200 Oak Street SE Suite 450 Minneapolis , MN 55455 **Phone Number:** 612-626-9895 **Fax Number:** 612-624-4843 **Email Address:** hagen027@umn edu	
Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** **Phase III Clinical Trial:** ☒ No ☐ Yes		**Vertebrate Animals:** ☒ No ☐ Yes **Inventions and Patents:** ☒ No ☐ Yes ☐ Previously Reported ☒ Not Previously Reported	
Program Income: ☒ No ☐ Yes			
Budget Period	**Anticipated Amount**	**Source**	
F&A Changes:			
Primary Project/Performance Site Location			
Organizational Name University of Minnesota			
DUNS 5559179960000			
Street 1 3-284 Nils Hasselmo Hall		Street 2 312 Church Street SE	
City Minneapolis	County Hennepin		State MN
Province	Country UNITED STATES		Zip/Postal Code 554550215
Congressional Districts MN-005			

Additional Project/Performance Site Location			
Organizational Name University of of Minnesota			
DUNS 5559179960000			
Street 1 7-227 Phillips Wangensteen Building		Street 2 516 Delaware Street SE	
City Minneapolis	County Hennepin		State MN
Province	Country UNITED STATES		Zip/Postal Code 55455
Project/Performance Site Congressional Districts MN-005			

Program Director/Principal Investigator:	Grant Number
Fang Li	R01AI089728-04
Applicant Organization:	**Period Covered by this Report:**
UNIVERSITY OF MINNESOTA	05/01/2012 - 04/30/2013
Title of Project:	
Receptor recognition mechanisms of coronaviruses	

SNAP Questions:

Has there been a change in the other support of Senior/Key Personnel since the last reporting period?

☒ **No** ☐ **Yes**

Justification:

Will there be, in the next budget period, a significant change in the level of effort for the PD/PI or other Senior/Key Personnel designated on the Notice of Award from what was approved for this project?

☒ **No** ☐ **Yes**

Justification:

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☒ **No** ☐ **Yes**

Justification:

Changes in Select Agent Research? ☒ **No** ☐ **Yes**

Changes in Multiple PD/PI Leadership plan? ☒ **No** ☐ **Yes**

Change in human embryonic stem cell (hESC) line(s) used? ☒ **No** ☐ **Yes**

Justification:

Human Subject Education Requirement:

Has the Involvement of Human Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Has the Involvement of Animal Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Publications:

Valid NIHMSID:	Citation ID:	Citation Source:	Citation Text:

Cover Letter: File is not uploaded
Research Accomplishments: File is not uploaded
Other Document File: File is not uploaded
Other Support File: File is not uploaded

All Personnel Report						
Program Director/Principal Investigator: Fang Li			**Grant Number** R01AI089728-04			
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Fang Li	FANGLI	PHD	XXX-XX[Minn Stat 13.43]	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
PD/PI			[Minn Stat 13.43]	6 0		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Lang Chen	LANGCHEN	PhD	XXX-XX[Minn Stat 13.43]	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6 0		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Yaoqing Chen	YAOQING	PhD	XXX-XX[Minn Stat 13.43]	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			[Minn Stat 13.43]	6 0		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Robert James Geraghty	ROBERT GERAGHTY	PhD	XXX-XX[Minn Stat 13.43]	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-Investigator				1 0		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Yi-Lun Lin	YILUNLIN	PhD	XXX-XX[Minn Stat 13.43]	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6 0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Guiqing Peng	GUIQINGPENG	PhD	XXX-XX[Minn Stat 13.43]	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6 0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Liqing Xu	XULXUL	PhD	XXX-XX-[Minn Stat 13.43]	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6 0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
[Minn Stat 13.32]		BS	XXX-XX-[Minn Stat 13.32]	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Graduate Research Assistant				6 0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Wenbo Zhou	WENBOZHOU	PhD	XXX-XX[Minn Stat 13.43]	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				4 8		

Progress Report

A. Specific Aims

Coronaviruses use a variety of cellular receptors and co-receptors including proteins and sugars. The diverse usage of receptors has allowed coronaviruses to infect a wide range of mammalian and avian species, and cause respiratory, enteric, systemic and neurological diseases Two domains in coronavirus spike protein S1 subunit, the N-terminal domain (NTD) and the C-domain, have evolved functions as receptor-binding domains (RBDs) How coronaviruses have evolved to do so has been a major puzzle in virology To solve this puzzle, this NIH-funded project "receptor recognition mechanisms of coronaviruses" investigates the molecular and structural basis for the complex receptor-recognition mechanisms of coronaviruses

There are three specific aims in this project Aim 1 focuses on the receptor recognition mechanisms of group-I coronaviruses that use angiotensin-converting enzyme 2 as receptors (e g NL63 coronavirus) Aim 2 focuses on the receptor recognition mechanisms of group-1 coronaviruses that use sugars and aminopeptidase N as receptors (e g., porcine transmissible gastroenteritis virus and 229E coronavirus) Aim 3 focuses on the receptor recognition mechanisms of group-2 coronaviruses (e g SARS coronavirus, mouse coronavirus, and bovine coronavirus). The research approaches of this proposal include crystallographic analysis of the virus-receptor interfaces, identification of structural elements important for virus-receptor interactions, and characterization of these elements using crystallographic, biochemical, molecular, and virological tools

B. Studies and Results

We made the following discoveries in 2012.

First, Chen et al (2012, *PNAS*) determined crystal structures of porcine aminopeptidase N (APN) at 1 85 Å resolution and its complexes with a peptide substrate and a variety of inhibitors. Mammalian APN plays multifunctional roles in many physiological processes, including peptide metabolism, cell motility and adhesion, and coronavirus entry. Our results show that APN is a cell surface-anchored and seahorse-shaped zinc-aminopeptidase that forms head-to-head dimers Captured in a catalytically active state, these structures of APN illustrate a detailed catalytic mechanism for its aminopeptidase activity The active site and peptide-binding channel of APN reside in cavities with wide openings, allowing easy access to peptides. The cavities can potentially open up further to bind the exposed N terminus of proteins. The active site anchors the N-terminal neutral residue of peptides/proteins, and the peptide-binding channel binds the remainder of the peptides/proteins in a sequence-independent fashion APN also provides an exposed outer surface for coronavirus binding, without its physiological functions being affected These structural features enable APN to function ubiquitously in peptide metabolism, interact with other proteins to mediate cell motility and adhesion, and serve as a coronavirus receptor. This study elucidates multifunctional roles of APN and can guide therapeutic efforts to treat APN-related diseases

Second, Peng et al (2012, *Journal of Biological Chemistry*) determined the crystal structure of bovine coronavirus (BCoV) spike protein lectin domain The spike protein N-terminal domains (NTDs) of BCoV and mouse hepatitis coronavirus (MHV) recognize sugar and protein receptors, respectively, despite their significant sequence homology We recently determined the crystal structure of MHV NTD complexed with its protein receptor murine carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) (Peng et al , *PNAS 108*, 10696-10701, 2011), which surprisingly revealed a human galectin (galactose-binding lectin) fold in MHV NTD Here, we have determined at 1 55 Å resolution the crystal structure of BCoV NTD, which also has the human galectin fold Using mutagenesis, we have located the sugar-binding site in BCoV NTD, which overlaps with the galactose-binding site in human galectins Using a glycan array screen, we have identified 5-N-acetyl-9-O-acetylneuraminic acid as the preferred sugar substrate for BCoV NTD. Subtle structural differences between BCoV and MHV NTDs, primarily involving different conformations of receptor-binding loops, explain why BCoV NTD does not bind CEACAM1 and why MHV NTD does not bind sugar. These results suggest a successful viral evolution strategy in which coronaviruses stole a galectin from hosts, incorporated it into their spike protein, and evolved it into viral receptor-binding domains with altered sugar specificity in contemporary BCoV or novel protein specificity in contemporary MHV

Third, Wu et al. (2012, *Journal of Biological Chemistry*) elucidated the molecular and structural mechanisms for the host receptor adaptation by group-2 SARS coronavirus (SARS-CoV). We found that all of the naturally selected mutations in SARS-CoV RBD (the S1 C-domain) were viral adaptations to either human or civet receptor angiontensin-converting enzyme 2 (ACE2) Further, we have designed and characterized two optimized RBDs The human-optimized RBD contains all of the human-ACE2-adapted residues (F442/F472/N479/D480/T487), and possesses exceptionally high affinity for human ACE2 but relative low

affinity for civet ACE2. The civet-optimized RBD contains all of the civet-ACE2-adapted residues (P442/Y472/R479/G480/T487), and possesses exceptionally high affinity for civet ACE2 and also substantial affinity for human ACE2 These results not only illustrate the detailed mechanisms of host receptor adaptation by SARS-CoV, but also provide molecular and structural basis for tracking future SARS-CoV evolution in animals.

Fourth, Li (2012, *Journal of Virology*) identified shared structural topologies (connectivity of secondary structural elements) in S1 domains of different coronavirus genera The results suggest that coronavirus S1 subunits share a common evolutionary origin but have attained diverse sequences and structures following extensive divergent evolution The results also increase understanding of the structures and functions of coronavirus S1 domains whose tertiary structures are currently unknown

Fifth, Chen et al (2013, *Journal of Virology*) analyzed the structural relationships between BCoV spike protein lectin domain and other known viral lectins Influenza and other viruses use host-cell-surface sugars as receptors. Here we show that the sugar-binding domains in influenza hemagglutinin, BCoV spike protein lectin domain, and other viral lectins share the same structural fold as human galectins (host lectins) Unlike the easily accessible sugar-binding sites in human galectins, the sugar-binding sites in viral lectins are hidden in cavities We propose that these viral lectins originated from host lectins but have evolved to use hidden sugar-binding sites to evade host immune attacks.

C. Significance

Our research has been the major driving force in the coronavirus field to understand coronavirus/receptor interactions Prior to the SARS epidemic, no structural information was available for any coronavirus S1 subunit, despite decades of extensive research By now, we have successfully determined the crystal structures of group-1 NL63-CoV C-domain complexed with its receptor ACE2, group-2 SARS-CoV C-domain complexed with its receptor ACE2, group-2 MHV NTD complexed with its receptor CEACAM1, group-2 BCoV NTD, and group-1 coronavirus receptor APN. To date, these structures account for all but one of the structures available for any coronavirus S1 or coronavirus-S1/receptor complexes By determining these structures, we have solved several important puzzles in virology, such as how animal viruses can cross species barriers to infect humans through one or a few seemingly trivial mutations in their RBDs, how different viruses recognize the same receptor by targeting common virus-binding hotspots on the receptor, how similar viruses recognize different receptors through subtle structural changes in viral RBDs, and how viruses can acquire host proteins and evolve them into viral RBDs with different receptor specificity. Protein biochemistry and virological methods have also been systematically performed to corroborate our structural findings. By elucidating these structures, we have also been able to reconstruct the evolutionary history of coronaviruses Furthermore, our detailed structural analyses of SARS-CoV/receptor interactions have significantly prepared humans for any potential future SARS epidemics

D. Plans

For the next funding year, we will focus on specific aims 2 and 3.

For specific aims 2, we will investigate the structural mechanism whereby the group-1 coronavirus NTDs recognize their sugar and APN receptors First, we will aim to determine the crystal structures of porcine transmissible gastroenteritis virus (TGEV) NTD by itself and in complex with sugar receptors Previously we hypothesized that like group-2 coronavirus NTDs, group-1 coronavirus NTDs also have a human galectin fold despite of no sequence homology between group-1 and group-2 coronavirus NTDs (Peng et al., *PNAS 108*, 10696-10701, 2011, Li, *Journal of Virology 86*, 2856-2858, 2012) The new study will elucidate the sugar binding mechanisms of group-1 coronaviruses and reveal how the sugar-binding functions have evolved in coronaviruses. Second, we will aim to determine the crystal structure of the human 229E coronavirus (HCoV-229E) C-domain complexed with human APN Although the crystal structure of TGEV C-domain complexed with porcine APN was recently determined by another group (Reguera et al , *PloS Pathogens 8*, e1002859, 2012), our preliminary study has revealed significant differences in the APN-binding sites and mechanisms between HCoV-229E C-domain and TGEV C-domain We will investigate the structural basis for the different APN-binding mechanisms of these group-1 coronaviruses

For specific aim 3, we will determine the crystal structure of group-2 BCoV NTD complexed with the sugar substrate Based on the successful structure determination of BCoV NTD by itself, we will synthesize the sugar substrates of BCoV NTD, and use either crystal soaking or co-crystallization to determine the structure

of the complex This study will reveal detailed structural basis for the sugar-binding mechanism of group-2 coronaviruses

Also for specific aim 3, we will study the structural and molecular basis for MHV driven mouse evolution. Pathogen driven host evolution in mammals has long been a topic of debate for lack of clear supporting evidence Two isoforms of mCEACAM1, 1a and 1b, are present in mice Despite their high sequence homology, mCEACAM1b is a much less effective MHV receptor than mCEACAM1a, a likely result from MHV-driven mouse evolution Here we will investigate the structural basis for why mCEACAM1b is an ineffective MHV receptor Furthermore, we will engineer mCEACAM1b to improve its effectiveness as an MHV receptor These studies will elucidate the structural and molecular mechanisms for MHV driven mouse evolution. In a pilot study, we have determined the crystal structure of mCEACAM1b Currently we are correlating structural and functional data and will perform structure-based protein engineering to improve the effectiveness of mCEACAM1b as an MHV receptor.

Furthermore, a novel human EMC coronavirus (HCoV-EMC) has recently emerged from Mideast (Zaki et al , *N. Engl. J. Med 367*, 1814-1820, 2012). HCoV-EMC has been characterized as a highly lethal virus and appears to have passed from animals (most likely bats) to humans easily HCoV-EMC belongs to group-2 and is related to SARS-CoV The receptor for HCoV-EMC is still unknown, but is being pursued intensively As part of specific am 3 where the receptor-recognition mechanisms of group-2 coronaviruses are investigated, we will identify the receptor-binding domain (RBD) of HCoV-EMC and determine its crystal structure. In a pilot study, we have successfully expressed, purified, and crystallized the HCoV-EMC S1 C-domain. Next, we will determine the crystal structure of HCoV-EMC S1 C-domain, and also characterize its capability of blocking the infections of human cells by retroviruses that are pseudotyped with HCoV-EMC spike protein Once the receptor for HCoV-EMC is known, we will also determine the co-crystal structures of HCoV-EMC RBD complexed with human or bat receptor Just like our research on SARS-CoV, these studies on HCoV-EMC will provide structural insights into the receptor-recognition mechanisms and cross-species infections of HCoV-EMC. The crystal structure of HCoV-EMC RBD by itself will guide development of subunit vaccines using the RBD as the template

E. Publications (in 2012)

1 Chen, L , Lin, Y.L., Peng, G , **Li, F.** (2012) Structural basis for multifunctional roles of mammalian aminopeptidase N *Proceedings of the National Academy of Sciences USA*, 109, 17966-17971 (PMID 23071329, PMCID PMC3497818 [Available on 2013/4/30])

2 Peng, G , Xu, L., Lin, Y L , Chen, L , Pasquarella, J , Holmes, K V , **Li, F.** (2012) Crystal structure of bovine coronavirus spike protein lectin domain. *Journal of Biological Chemistry*, 287, 41931-41938 (PMID· 23091051; PMCID. PMC3516740 [Available on 2013/12/7])

3 Wu, K., Peng, G , Wilken, M , Geraghty, R , **Li, F.** (March 2012) Mechanisms of host receptor adaptation by SARS coronavirus. *Journal of Biological Chemistry*, *287,* 8904-8911 (PMID· 22291007, PMCID PMC3308800 [Available on 2013/3/16])

4 **Li, F.** (March 2012) Evidence for a common evolutionary origin of coronavirus spike protein receptor-binding subunits *Journal of Virology*, *86,* 2856-2858 (PMID: 22205723; PMCID: PMC3302248)

5. Chen, L., **Li, F.** (2013) Structural analysis of evolutionary origins of influenza hemagglutinin and other viral lectins *Journal of Virology*, [Epub ahead of print] In press (PMID 23365425)

F. Project Generated Resources

This research did not generate cell lines, DNA probes, animal models, protocols, or software. De-identified raw data from the experiments and computer programs (CCP4 macromolecular crystallography scripts) used for protein structure determinations are available to anyone upon written request to the PI Peer-reviewed publications accepted or published during the past project period are being or have been entered in NIHMS and PubMed Central

orginal

ESNAP Report **DRAFT**

Grant Number R01AI089728-04	**Total Project Period** From 05/15/2010 To 04/30/2015
EIN: **Review Group:** 1416007513A1 VIRA	**Requested Budget Period:** From 05/01/2013 To 04/30/2014
Title of Project: Receptor recognition mechanisms of coronaviruses	**Due Date:** 03/15/2013 **Submitted Date:**

Program Director/Principal Investigator: Fang Li UNIVERSITY OF MINNESOTA TWIN CITIES Department of Pharmacology 6-120 Jackson Hall 321 Church Street S E MINNEAPOLIS , MN 554552070 **Phone Number:** 612-625-6149 **Fax Number:** **Email Address:** lifang@umn edu	**Applicant Organization:** UNIVERSITY OF MINNESOTA UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS , MN 554552070 **Department:** PHARMACOLOGY **Major Subdivision:** MEDICAL SCHOOL
Administrative Official: Paulie Garcia 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE Minneapolis , MN 554552070 **Phone Number:** 612-624-5599 **Fax Number:** **Email Address:** awards@umn edu	**Signing Official:** Kevin McKoskey 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS , MN 554552070 **Phone Number:** 612-624-5599 **Fax Number:** 612-624-4843 **Email Address:** kevin@umn edu
Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** **Phase III Clinical Trial:** ☒ No ☐ Yes	**Vertebrate Animals:** ☒ No ☐ Yes **Inventions and Patents:** ☒ No ☐ Yes ☐ Previously Reported ☒ Not Previously Reported
Program Income: ☒ No ☐ Yes	

Budget Period	Anticipated Amount	Source
F&A Changes:		

Primary Project/Performance Site Location		
Organizational Name UNIVERSITY OF MINNESOTA		
DUNS 5559179960000		
Street 1 UNIVERSITY OF MINNESOTA TWIN CITIES	Street 2 450 MCNAMARA ALUMNI CENTER	
City MINNEAPOLIS	County Hennepin	State MN
Province	Country UNITED STATES	Zip/Postal Code 554552070
Congressional Districts MN-005		

Additional Project/Performance Site Location		
Organizational Name University of Minnesota		
DUNS 5559179960000		
Street 1 3-284 Nils Hasselmo Hall	Street 2 312 Church Street SE	
City Minneapolis	County Hennepin	State MN
Province	Country UNITED STATES	Zip/Postal Code 554550215
Project/Performance Site Congressional Districts MN-005		

Additional Project/Performance Site Location		
Organizational Name University of of Minnesota		
DUNS 5559179960000		
Street 1 7-227 Phillips Wangensteen Building	Street 2 516 Delaware Street SE	
City Minneapolis	County Hennepin	State MN
Province	Country UNITED STATES	Zip/Postal Code 55455
Project/Performance Site Congressional Districts MN-005		

Program Director/Principal Investigator:	**Grant Number**
Fang Li	R01AI089728-04
Applicant Organization:	**Period Covered by this Report:**
UNIVERSITY OF MINNESOTA	05/01/2012 - 04/30/2013

Title of Project:

Receptor recognition mechanisms of coronaviruses

SNAP Questions:

Has there been a change in the other support of Senior/Key Personnel since the last reporting period?

☒ **No** ☐ **Yes**

Justification:

Will there be, in the next budget period, a significant change in the level of effort for the PD/PI or other Senior/Key Personnel designated on the Notice of Award from what was approved for this project?

☒ **No** ☐ **Yes**

Justification:

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☒ **No** ☐ **Yes**

Justification:

Changes in Select Agent Research? ☒ **No** ☐ **Yes**

Changes in Multiple PD/PI Leadership plan? ☒ **No** ☐ **Yes**

Change in human embryonic stem cell (hESC) line(s) used? ☒ **No** ☐ **Yes**

Justification:

Human Subject Education Requirement:

Has the Involvement of Human Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Has the Involvement of Animal Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Publications:

Valid NIHMSID:	**Citation ID:**	**Citation Source:**	**Citation Text:**

Cover Letter: File is not uploaded
Research Accomplishments: File is not uploaded
Other Document File: File is not uploaded
Other Support File: File is not uploaded

All Personnel Report

Program Director/Principal Investigator:			Grant Number		
Fang Li			R01AI089728-04		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Fang Li	FANGLI	PHD	XXX-XX Minn Stat 13.43	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
PD/PI			Minn Stat 13.43	6 0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Lang Chen	LANGCHEN	PhD	XXX-XX Minn Stat 13.43	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6 0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Yaoqing Chen	YAOQING	PhD	XXX-XX Minn Stat 13.43	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			Minn Stat 13.43	6 0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Robert James Geraghty	ROBERT GERAGHTY	PhD	XXX-XX Minn Stat 13.43	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-Investigator				1 0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**		
Yi-Lun Lin	YILUNLIN	PhD	XXX-XX Minn Stat 13.43	**Months Devoted**	
				to Project	
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6.0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Guiqing Peng	GUIQINGPENG	PhD	XXX-XX Minn Stat 13.43	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6 0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Liqing Xu	XULXUL	PhD	XXX-XX Minn Stat 13.43	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				6 0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Minn Stat 13.32		BS	XXX-XX Minn Stat 13.32	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Graduate Research Assistant				6 0		

Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Wenbo Zhou	WENBOZHOU	PhD	XXX-XX Minn Stat 13.43	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				4 8		

Progress Report

A. Specific Aims

Coronaviruses use a variety of cellular receptors and co-receptors including proteins and sugars. The diverse usage of receptors has allowed coronaviruses to infect a wide range of mammalian and avian species, and cause respiratory, enteric, systemic and neurological diseases Two domains in coronavirus spike protein S1 subunit, the N-terminal domain (NTD) and the C-domain, have evolved functions as receptor-binding domains (RBDs) How coronaviruses have evolved to do so has been a major puzzle in virology To solve this puzzle, this NIH-funded project "receptor recognition mechanisms of coronaviruses" investigates the molecular and structural basis for the complex receptor-recognition mechanisms of coronaviruses

There are three specific aims in this project. Aim 1 focuses on the receptor recognition mechanisms of group-I coronaviruses that use angiotensin-converting enzyme 2 as receptors (e g NL63 coronavirus) Aim 2 focuses on the receptor recognition mechanisms of group-1 coronaviruses that use sugars and aminopeptidase N as receptors (e g , porcine transmissible gastroenteritis virus and 229E coronavirus) Aim 3 focuses on the receptor recognition mechanisms of group-2 coronaviruses (e g SARS coronavirus, mouse coronavirus, and bovine coronavirus) The research approaches of this proposal include crystallographic analysis of the virus-receptor interfaces, identification of structural elements important for virus-receptor interactions, and characterization of these elements using crystallographic, biochemical, molecular, and virological tools.

B. Studies and Results

We made the following discoveries in 2012.

First, Chen et al (2012, *PNAS*) determined crystal structures of porcine aminopeptidase N (APN) at 1 85 Å resolution and its complexes with a peptide substrate and a variety of inhibitors Mammalian APN plays multifunctional roles in many physiological processes, including peptide metabolism, cell motility and adhesion, and coronavirus entry. Our results show that APN is a cell surface-anchored and seahorse-shaped zinc-aminopeptidase that forms head-to-head dimers. Captured in a catalytically active state, these structures of APN illustrate a detailed catalytic mechanism for its aminopeptidase activity The active site and peptide-binding channel of APN reside in cavities with wide openings, allowing easy access to peptides The cavities can potentially open up further to bind the exposed N terminus of proteins The active site anchors the N-terminal neutral residue of peptides/proteins, and the peptide-binding channel binds the remainder of the peptides/proteins in a sequence-independent fashion APN also provides an exposed outer surface for coronavirus binding, without its physiological functions being affected These structural features enable APN to function ubiquitously in peptide metabolism, interact with other proteins to mediate cell motility and adhesion, and serve as a coronavirus receptor. This study elucidates multifunctional roles of APN and can guide therapeutic efforts to treat APN-related diseases.

Second, Peng et al. (2012, *Journal of Biological Chemistry*) determined the crystal structure of bovine coronavirus (BCoV) spike protein lectin domain The spike protein N-terminal domains (NTDs) of BCoV and mouse hepatitis coronavirus (MHV) recognize sugar and protein receptors, respectively, despite their significant sequence homology. We recently determined the crystal structure of MHV NTD complexed with its protein receptor murine carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) (Peng et al , *PNAS 108*, 10696-10701, 2011), which surprisingly revealed a human galectin (galactose-binding lectin) fold in MHV NTD. Here, we have determined at 1 55 Å resolution the crystal structure of BCoV NTD, which also has the human galectin fold Using mutagenesis, we have located the sugar-binding site in BCoV NTD, which overlaps with the galactose-binding site in human galectins Using a glycan array screen, we have identified 5-N-acetyl-9-O-acetylneuraminic acid as the preferred sugar substrate for BCoV NTD. Subtle structural differences between BCoV and MHV NTDs, primarily involving different conformations of receptor-binding loops, explain why BCoV NTD does not bind CEACAM1 and why MHV NTD does not bind sugar. These results suggest a successful viral evolution strategy in which coronaviruses stole a galectin from hosts, incorporated it into their spike protein, and evolved it into viral receptor-binding domains with altered sugar specificity in contemporary BCoV or novel protein specificity in contemporary MHV

Third, Wu et al. (2012, *Journal of Biological Chemistry*) elucidated the molecular and structural mechanisms for the host receptor adaptation by group-2 SARS coronavirus (SARS-CoV) We found that all of the naturally selected mutations in SARS-CoV RBD (the S1 C-domain) were viral adaptations to either human or civet receptor angiontensin-converting enzyme 2 (ACE2) Further, we have designed and characterized two optimized RBDs The human-optimized RBD contains all of the human-ACE2-adapted residues (F442/F472/N479/D480/T487), and possesses exceptionally high affinity for human ACE2 but relative low

affinity for civet ACE2. The civet-optimized RBD contains all of the civet-ACE2-adapted residues (P442/Y472/R479/G480/T487), and possesses exceptionally high affinity for civet ACE2 and also substantial affinity for human ACE2 These results not only illustrate the detailed mechanisms of host receptor adaptation by SARS-CoV, but also provide molecular and structural basis for tracking future SARS-CoV evolution in animals.

Fourth, Li (2012, *Journal of Virology*) identified shared structural topologies (connectivity of secondary structural elements) in S1 domains of different coronavirus genera. The results suggest that coronavirus S1 subunits share a common evolutionary origin but have attained diverse sequences and structures following extensive divergent evolution The results also increase understanding of the structures and functions of coronavirus S1 domains whose tertiary structures are currently unknown.

Fifth, Chen et al (2013, *Journal of Virology*) analyzed the structural relationships between BCoV spike protein lectin domain and other known viral lectins Influenza and other viruses use host-cell-surface sugars as receptors. Here we show that the sugar-binding domains in influenza hemagglutinin, BCoV spike protein lectin domain, and other viral lectins share the same structural fold as human galectins (host lectins) Unlike the easily accessible sugar-binding sites in human galectins, the sugar-binding sites in viral lectins are hidden in cavities. We propose that these viral lectins originated from host lectins but have evolved to use hidden sugar-binding sites to evade host immune attacks.

C. Significance

Our research has been the major driving force in the coronavirus field to understand coronavirus/receptor interactions Prior to the SARS epidemic, no structural information was available for any coronavirus S1 subunit, despite decades of extensive research By now, we have successfully determined the crystal structures of group-1 NL63-CoV C-domain complexed with its receptor ACE2, group-2 SARS-CoV C-domain complexed with its receptor ACE2, group-2 MHV NTD complexed with its receptor CEACAM1, group-2 BCoV NTD, and group-1 coronavirus receptor APN To date, these structures account for all but one of the structures available for any coronavirus S1 or coronavirus-S1/receptor complexes By determining these structures, we have solved several important puzzles in virology, such as how animal viruses can cross species barriers to infect humans through one or a few seemingly trivial mutations in their RBDs, how different viruses recognize the same receptor by targeting common virus-binding hotspots on the receptor, how similar viruses recognize different receptors through subtle structural changes in viral RBDs, and how viruses can acquire host proteins and evolve them into viral RBDs with different receptor specificity Protein biochemistry and virological methods have also been systematically performed to corroborate our structural findings By elucidating these structures, we have also been able to reconstruct the evolutionary history of coronaviruses Furthermore, our detailed structural analyses of SARS-CoV/receptor interactions have significantly prepared humans for any potential future SARS epidemics

D. Plans

For the next funding year, we will focus on specific aims 2 and 3

For specific aims 2, we will investigate the structural mechanism whereby the group-1 coronavirus NTDs recognize their sugar and APN receptors First, we will aim to determine the crystal structures of porcine transmissible gastroenteritis virus (TGEV) NTD by itself and in complex with sugar receptors. Previously we hypothesized that like group-2 coronavirus NTDs, group-1 coronavirus NTDs also have a human galectin fold despite of no sequence homology between group-1 and group-2 coronavirus NTDs (Peng et al., *PNAS 108*, 10696-10701, 2011, Li, *Journal of Virology 86*, 2856-2858, 2012) The new study will elucidate the sugar binding mechanisms of group-1 coronaviruses and reveal how the sugar-binding functions have evolved in coronaviruses. Second, we will aim to determine the crystal structure of the human 229E coronavirus (HCoV-229E) C-domain complexed with human APN Although the crystal structure of TGEV C-domain complexed with porcine APN was recently determined by another group (Reguera et al , *PloS Pathogens 8*, e1002859, 2012), our preliminary study has revealed significant differences in the APN-binding sites and mechanisms between HCoV-229E C-domain and TGEV C-domain We will investigate the structural basis for the different APN-binding mechanisms of these group-1 coronaviruses.

For specific aim 3, we will determine the crystal structure of group-2 BCoV NTD complexed with the sugar substrate. Based on the successful structure determination of BCoV NTD by itself, we will synthesize the sugar substrates of BCoV NTD, and use either crystal soaking or co-crystallization to determine the structure

of the complex This study will reveal detailed structural basis for the sugar-binding mechanism of group-2 coronaviruses

Also for specific aim 3, we will study the structural and molecular basis for MHV driven mouse evolution. Pathogen driven host evolution in mammals has long been a topic of debate for lack of clear supporting evidence Two isoforms of mCEACAM1, 1a and 1b, are present in mice Despite their high sequence homology, mCEACAM1b is a much less effective MHV receptor than mCEACAM1a, a likely result from MHV-driven mouse evolution Here we will investigate the structural basis for why mCEACAM1b is an ineffective MHV receptor. Furthermore, we will engineer mCEACAM1b to improve its effectiveness as an MHV receptor. These studies will elucidate the structural and molecular mechanisms for MHV driven mouse evolution In a pilot study, we have determined the crystal structure of mCEACAM1b Currently we are correlating structural and functional data and will perform structure-based protein engineering to improve the effectiveness of mCEACAM1b as an MHV receptor.

Furthermore, a novel human EMC coronavirus (HCoV-EMC) has recently emerged from Mideast (Zaki et al., *N Engl. J. Med. 367*, 1814-1820, 2012) HCoV-EMC has been characterized as a highly lethal virus and appears to have passed from animals (most likely bats) to humans easily. HCoV-EMC belongs to group-2 and is related to SARS-CoV The receptor for HCoV-EMC is still unknown, but is being pursued intensively As part of specific am 3 where the receptor-recognition mechanisms of group-2 coronaviruses are investigated, we will identify the receptor-binding domain (RBD) of HCoV-EMC and determine its crystal structure. In a pilot study, we have successfully expressed, purified, and crystallized the HCoV-EMC S1 C-domain Next, we will determine the crystal structure of HCoV-EMC S1 C-domain, and also characterize its capability of blocking the infections of human cells by retroviruses that are pseudotyped with HCoV-EMC spike protein Once the receptor for HCoV-EMC is known, we will also determine the co-crystal structures of HCoV-EMC RBD complexed with human or bat receptor Just like our research on SARS-CoV, these studies on HCoV-EMC will provide structural insights into the receptor-recognition mechanisms and cross-species infections of HCoV-EMC The crystal structure of HCoV-EMC RBD by itself will guide development of subunit vaccines using the RBD as the template

E. Publications (in 2012)

1 Chen, L , Lin, Y L , Peng, G , **Li, F.** (2012) Structural basis for multifunctional roles of mammalian aminopeptidase N *Proceedings of the National Academy of Sciences USA*, 109, 17966-17971 (PMID: 23071329; PMCID PMC3497818 [Available on 2013/4/30])

2. Peng, G., Xu, L., Lin, Y L , Chen, L., Pasquarella, J , Holmes, K V., **Li, F.** (2012) Crystal structure of bovine coronavirus spike protein lectin domain. *Journal of Biological Chemistry*, 287, 41931-41938 (PMID. 23091051, PMCID PMC3516740 [Available on 2013/12/7])

3. Wu, K., Peng, G., Wilken, M., Geraghty, R , **Li, F.** (March 2012) Mechanisms of host receptor adaptation by SARS coronavirus. *Journal of Biological Chemistry, 287,* 8904-8911 (PMID 22291007, PMCID PMC3308800 [Available on 2013/3/16])

4 **Li, F.** (March 2012) Evidence for a common evolutionary origin of coronavirus spike protein receptor-binding subunits. *Journal of Virology, 86,* 2856-2858 (PMID 22205723; PMCID. PMC3302248)

5 Chen, L , **Li, F.** (2013) Structural analysis of evolutionary origins of influenza hemagglutinin and other viral lectins. *Journal of Virology*, [Epub ahead of print] In press (PMID: 23365425)

F. Project Generated Resources

This research did not generate cell lines, DNA probes, animal models, protocols, or software De-identified raw data from the experiments and computer programs (CCP4 macromolecular crystallography scripts) used for protein structure determinations are available to anyone upon written request to the PI Peer-reviewed publications accepted or published during the past project period are being or have been entered in NIHMS and PubMed Central

UNIVERSITY OF MINNESOTA

Twin Cities Campus

Department of Pharmacology

Medical School
Academic Health Center

6-120 Jackson Hall
321 Church Street S E
Minneapolis MN 55455 U S A

612 625-9997
FAX 612 625-8408

FAX COVER PAGE

Date. 3/13/13 Number of Pages (*including cover page*): 6

TO: **PROPOSALS - SPA**

FAX #: 4-4843

FROM: PHARMACOLOGY

Message:

RE: ELECTRONIC GRANT SUBMISSION

PRF #: 755486

PI NAME: Fang Li

Grant Type: NIH K01 Progress Report eSNAP

Detailed Budget Attached: Yes X No ____

Budget Justification Attached: Yes ____ No X

Subject: PRF# 755486 Fang Li NIH eSNAP Progress Report
From: Lynn Weber <weber117@umn.edu>
Date: 3/13/2013 10:29 AM
To: "proposal@umn.edu" <proposal@umn.edu>
CC: Paulie Garcia <garc0027@umn.edu>

--
Lynn Weber
Department of Pharmacology
University of Minnesota
Medical School
6-120 Jackson Hall
321 Church Street SE
Minneapolis, MN 55455
Phone 612-626-6902
Fax 612-625-8408
Email weber117@umn edu
http //www pharmacology med umn edu/

UNIVERSITY OF MINNESOTA
Driven to Discover℠

D528 Mayo Memorial Building
420 Delaware Street S E
MMC 820
Minneapolis, MN 55455
Office 612-626-5654
Fax 612-626-6061
Email irb@umn edu or ibc@umn edu
Website http //research umn edu/subjects/

Date March 04, 2013

To Li, Fang

From Institutional Biosafety Committee (IBC)

Subject *Receptor recognition mechanisms of coronaviruses*

IBC Code Number 1212-30150H

Notification of IBC Action

IBC Action Final Approval

NIH Guidelines Designation III-E-1

Approval for this project includes the following:

Genes/Sequence(s)
Genes, DNA or RNA As described in approved protocol

Gene Transfer Method(s) transfection of recombinant bacmid into insect cells

Infectious Agents/Microorganism(s) N/A

Biologically-derived toxin(s) N/A

IBC Approved Laboratory Biosafety Level: 1
IBC Approved Animal Housing Biosafety Level: Not Applicable

The protocol number and the title of your study should be used in all communication with the IBC. The approval date of this study is March 04, 2013 Approval will expire one year from this date A continuing review request will be sent one month prior to that time IBC protocols expire every three years

As Principal Investigator of this project, you are required by federal regulations to report any research-related accidents or incidents to the IBC, and to inform the IBC of any proposed changes to your research Changes should not be initiated until written IBC approval is received. Upon receipt of this letter, you may initiate your research.

The IBC wishes you success with this research If you have questions, please call the IBC office at 612-626-5654

Sincerely,
Tracy Rouse
Research Compliance Supervisor

Paulie Garcia <garc0027@umn edu>

PRF 755486, PI Li

4 messages

Paulie Garcia <garc0027@umn edu> Wed, Mar 13, 2013 at 2 52 PM
To Lynn Weber <weber117@umn edu>
Cc Carrie DeJarlais <dejar001@umn edu>

Lynn,

Could you please verify the primary place of performance for the PRF referenced above It is currently listed as SPA's address and I need to change this The other two addresses are in the Hasselmo Hall and Wangensteen Hall I have to designate one as the Primary Place of Performance

Thanks,
Paulie
--
****
Paulie Garcia
Grant and Contract Administrator
University of Minnesota
Office of the Vice President for Research
Sponsored Projects Administration
McNamara Alumni Center, Suite 450
200 Oak Street S E
Minneapolis, MN 55455

Phone (612) 626-2724
Fax (612) 624-4843
Email garc0027@umn edu

Paulie Garcia <garc0027@umn.edu> Wed, Mar 13, 2013 at 4 06 PM
To Lynn Weber <weber117@umn.edu>
Cc Carrie DeJarlais <dejar001@umn.edu>

Lynn,

In addition, could you please provide me with the IBC approval letter for 3/4/13? The Study Code is 121230150H on the PRF It is not in the RSPP system and we are not able to look it up in the eProtocol system as of yet

Thank you,
Paulie
[Quoted text hidden]

Lynn Weber <weber117@umn edu> Wed, Mar 13, 2013 at 4 07 PM
To Paulie Garcia <garc0027@umn edu>

Hi Paulie,

The SPA address is a default of NIH eSNAP system
I used to remove it but it always reappears the next time so I just left it
The NHH location is Fang Li's lab so would be the Prime then

Lynn

--

Lynn Weber
Department of Pharmacology
University of Minnesota
Medical School
6-120 Jackson Hall
321 Church Street SE
Minneapolis MN 55455
Phone 612-626-6902
Fax 612-625-8408
Email weber117@umn edu
http //www pharmacology med umn edu

From. Paulie Garcia <garc0027@umn edu>
Date. Wednesday, March 13, 2013 2 52 PM
To. Lynn Weber <weber117@umn edu>
Cc Carrie DeJarlais <dejar001@umn edu>
Subject: PRF 755486, PI Li
[Quoted text hidden]

Paulie Garcia <garc0027@umn.edu> Wed, Mar 13, 2013 at 4 24 PM
To Lynn Weber <weber117@umn.edu>

Lynn,

If you don't have a the approval letter, I will send you the standard IBC letter Please let me know if you have it

Thanks,
Paulie

[Quoted text hidden]

Grant Number R01AI089728-04	**Total Project Period** From 05/15/2010 To 04/30/2015
EIN: **Review Group:** 1416007513A1 VIRA	**Requested Budget Period:** From 05/01/2013 To 04/30/2014
Title of Project: Receptor recognition mechanisms of coronaviruses	**Due Date:** 03/15/2013 **Submitted Date:** 03/14/2013

Program Director/Principal Investigator: Fang Li UNIVERSITY OF MINNESOTA TWIN CITIES Department of Pharmacology 6-120 Jackson Hall 321 Church Street S E MINNEAPOLIS , MN 554552070 **Phone Number:** 612-625-6149 **Fax Number:** **Email Address:** lifang@umn edu	**Applicant Organization:** UNIVERSITY OF MINNESOTA UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS , MN 554552070 **Department:** PHARMACOLOGY **Major Subdivision:** MEDICAL SCHOOL
Administrative Official: Paulie Garcia 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE Minneapolis , MN 554552070 **Phone Number:** 612-624-5599 **Fax Number:** **Email Address:** awards@umn edu	**Signing Official:** David L Hagen 200 Oak Street SE Suite 450 Minneapolis , MN 55455 **Phone Number:** 612-626-9895 **Fax Number:** 612-624-4843 **Email Address:** hagen027@umn edu

Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** **Phase III Clinical Trial:** ☒ No ☐ Yes	**Vertebrate Animals:** ☒ No ☐ Yes **Inventions and Patents:** ☒ No ☐ Yes ☐ Previously Reported ☒ Not Previously Reported
Program Income: ☒ No ☐ Yes	

Budget Period	Anticipated Amount	Source
F&A Changes:		

Primary Project/Performance Site Location

Organizational Name University of Minnesota		
DUNS 5559179960000		
Street 1 3-284 Nils Hasselmo Hall	Street 2 312 Church Street SE	
City Minneapolis	County Hennepin	State MN
Province	Country UNITED STATES	Zip/Postal Code 554550215
Congressional Districts MN-005		

Principal Investigator/Program Director (Last First Middle) Li, Fang

DETAILED BUDGET FOR NEXT BUDGET PERIOD - DIRECT COSTS ONLY	FROM 5/1/13	THROUGH 4/30/14	GRANT NUMBER 5 R01 AI89728-4

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal Mnths	Acad Mnths	Summer Mnths	SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Fang Li	PD/PI	6 00			$45,695	$15,454	$61 149
Robert Geraghty	Co-Invest	1 00			$9,087	$3,073	$12,160
Yaoqing Chen	Postdoctoral	7 00			$20,417	$4,553	$24,970
Yi-Lun Lin	Postdoctoral	6 00			$16,500	$3,680	$20,180
Wenbo Zhou	Research Asso	4 80			$15,275	$5,166	$20,441
Minn Stat 13.32	Research Asst	6 00			$25,000	$14,263	$39,263
						$0	$0
SUBTOTALS →					$131,974	$46,189	$178,163

F&A

CONSULTANT COSTS

Dr Kathryn Holmes	$10,000

EQUIPMENT (*Itemize*)

	$0

SUPPLIES (*Itemize by category*)

Molecular biology supplies	$14,553
Insect cell culture supplies	$9,500
Protein purification supplies	$16,000
Protein crystallization supplies	$13,500
Plastic & glassware supplies	$2,801
	$56,354

TRAVEL

PI and/or postdoctoral to attend one meeting per year	$3,000

INPATIENT CARE COSTS	$0	$0
OUTPATIENT CARE COSTS	$0	$0

ALTERATIONS AND RENOVATIONS (*Itemize by category*)

OTHER EXPENSES (*Itemize by category*)

Publication costs/reprints	$2,500
Equipment maintenance	$1,500
Communication costs	$800
	$4,800

SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD	$252,317
CONSORTIUM/CONTRACTUAL COSTS — DIRECT COSTS	
CONSORTIUM/CONTRACTUAL COSTS — FACILITIES AND ADMINISTRATION COSTS	
TOTAL DIRECT COSTS FOR NEXT PROJECT PERIOD (*Item 8a, Face Page*)	$252.317

	Years 1-5
Indirect Cost Base	$238.054
IDC @ 51%	$121,408
Total Direct Costs	$252,317
IDC	$121,408
Total Costs	$373,725

F&A OK

Program Director/Principal Investigator (Last, first, middle) Li, Fang

GRANT NUMBER
5R01 AI89728-4

CHECKLIST

1 PROGRAM INCOME ***(See instructions.)***
All applications must indicate whether program income is anticipated during the period(s) for which grant support is requested If program income is anticipated, use the format below to reflect the amount and source(s)

Budget Period	Anticipated Amount	Source(s)
	0	

2 ASSURANCES/CERTIFICATIONS ***(See instructions)***
In signing the application Face Page the authorized organizational representative agrees to comply with the policies assurances and/or certifications listed in the application instructions when applicable Descriptions of individual assurances/certifications are provided in Part III of the PHS 398, and listed in Part I, 4 1 under Item 14 If unable to certify compliance, where applicable, provide an explanation and place it after the Progress Report (Form Page 5)

3 FACILITIES AND ADMINSTRATIVE (F&A) COSTS
Indicate the applicant organization's most recent F&A cost rate established with the appropriate DHHS Regional Office or, in the case of for-profit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office

F&A costs will ***not*** be paid on construction grants, grants to Federal organizations, grants to individuals, and conference grants Follow any additional instructions provided for Research Career Awards, Institutional National Research Service Awards, Small Business Innovation Research/Small Business Technology Transfer Grants, foreign grants, and specialized grant applications

☐ DHHS Agreement dated 04/20/12 ☐ No Facilities and Administrative Costs Requested

☐ No DHHS Agreement, but rate established with _____ Date _____

CALCULATION*

Entire proposed budget period Amount of base $ 238,054 x Rate applied 51 % = F&A costs $ 121,408

Add to total direct costs from Form Page 2 and enter new total on Face Page, Item 8b

*Check appropriate box(es)

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet if necessary)*
Excludes Grad RA fringe

Help

Prompt Path: Acctg Date→Award

Sponsored Award Overview

Award: CON000000022345 - Receptor recognition mechanisms of coronaviruses
Accounting Date Through: 03/13/2013
Encumbered Facilities and Admin: Included
Award Sponsor: NIH Nat'l Inst of Allergy & Infect Dis
Award Start Date: 05/15/2010
Award End Date: 04/30/2015
Award Time Elapsed: 57%
Award %Left: -5%
F&A: RSRCH MTDC 51%

Report Run Date: Wednesday,3/13/2013
Data as of Wednesday,03/13/2013 03 39 AM

Cost Share (Actuals):	$0 00
Program Income (Actuals):	$0 00
TIP Income (Actuals):	$0 00
Invoiced Amount ITD:	$1,124,950 00
Open AR Amount ITD:	$2,117 14

						03/01/2013 - 03/13/2013		INCEPTION THROUGH 03/13/2013		
Project	PP	PI	Detail Account	A Finalized Budget Amount	B Pre-Enc & Enc	C Expenses	D Pre-Enc & Enc	E Expenses	F = A - D - E Available Balance
00013782	Y	Li,Fang	168100 - Capital Assets - Fin Bdg Only	8,000 00	0 00	0 00	0 00	44,954 63	-36,954 63
00013782	Y	Li,Fang	700100 - Salaries-Faculty-Fin Bdg Only	136,874 00	-2,055 64	2,055 64	9,661 51	144,058 72	-16,846 23
00013782	Y	Li,Fang	700200 - Salaries-Prof&Adm-Fin Bdg Only	186,666 00	-591 33	591 33	2,779 25	35,451 27	148,435 48
00013782	Y	Li,Fang	700300 - Salaries-Grd/Pr w/Tuitn-FinBdg	81,400 00	-942 40	942 40	4,429 28	12,251 20	64,719 52
00013782	Y	Li,Fang	700310 - Salaries-Grd/Pr NoTuitn-FinBdg	0 00	-1,346 16	1,346 16	6,326 95	239,026 49	-245,353 44
00013782	Y	Li,Fang	710100 - Fringe - Faculty-Fin Bdg Only	47,095 00	-717 42	717 39	3,371 87	45,534 84	-1,811 71
00013782	Y	Li,Fang	710200 - Fringe - P&A-Fin Bdg Only	46,666 00	-206 37	206 37	969 96	9,830 99	35,865 05
00013782	Y	Li,Fang	710300 - Fringe-Grad/Pr-w/Tuitn-FinBdg	16,117 00	-830 74	830 75	3,904 51	10,799 75	1,412 74
00013782	Y	Li,Fang	710310 - Fringe-Grad/Pr No	0 00	-302 48	203 67	1,421 67	44,215 90	-45,637 57

			Tuitn-FinBdg						
00013782	Y	Li,Fang	720200 - Lab & Med Supplies-FinBdg Only	172,891 00	-295 05	0 00	506 75	148,692 53	23,691 72
00013782	Y	Li,Fang	720300 - Gen Oper Services-Fin Bdg Only	6,875 00	0 00	0 00	0 00	9,858 32	-2,983 32
00013782	Y	Li,Fang	720400 - Lab & Medical Svcs-FinBdg Only	200 00	0 00	51 76	0 00	7,789 73	-7,589 73
00013782	Y	Li,Fang	720500 - Telecommunications-FinBdg Only	1,992 00	0 00	0 00	0 00	0 00	1,992 00
00013782	Y	Li,Fang	720600 - Travel/Mileage/Mov-FinBdg Only	7,970 00	0 00	0 00	0 00	8,808 16	-838 16
00013782	Y	Li,Fang	730100 - Consulting Services-FinBdg Onl	29,900 00	0 00	0 00	0 00	1,279 26	28,620 74
00013782	Y	Li,Fang	750100 - Non-Capital Equip-Fin Bdg Only	571 00	0 00	0 00	0 00	5,153 67	-4,582 67
00013782	Y	Li,Fang	780100 - Repairs & Maint-FinBdg Only	4,485 00	0 00	0 00	0 00	1,705 37	2,779 63
			Direct Cost Total:	**$747,702.00**	**-$7,287.59**	**$6,945.47**	**$33,371.75**	**$769,410.83**	**-$55,080.58**
00013782	Y	Li,Fang	810500 - F&A Costs-Fin Bdg Only	377,248 00	0 00	3,118 51	13,282 41	363,965 59	0 00
			F&A Total:	**$377,248.00**	**$0.00**	**$3,118.51**	**$13,282.41**	**$363,965.59**	**$0.00**
CSR: $0.00 RFA: $273,169.57			**Project Total:**	**$1,124,950.00**	**-$7,287.59**	**$10,063.98**	**$46,654.16**	**$1,133,376.42**	**-$55,080.58**
CSR: $0.00 RFA: $273,169.57			**Award Total:**	**$1,124,950.00**	**-$7,287.59**	**$10,063.98**	**$46,654.16**	**$1,133,376.42**	**-$55,080.58**

Back to top

Encumbered Facilities and Admin is included on this report.

$8,426.42
OK

UNIVERSITY OF MINNESOTA

Notice of Grant or Contract Award

Sponsored Projects Administration

(Online Page Navigation Main Menu > Grants >Awards > UM_NOGA Report)

Award: CON000000022345
Primary Project: 00013782
PRF #: 651709

AWARD INFORMATION (Online Page Navigation Main Menu > Grants > Awards >Award Profile)

Award Start Date:	05/15/2010	**Award End Date:**	04/30/2015
Funds Approved to Date:	$1,476,252 00		
Principal Investigator:	Li,Fang	**Award Type:**	Grant
Primary Project Dept:	PHCL Pharmacology Admin	**Purpose:**	Research
Sponsor:	NIH Nat'l Inst of Allergy & Infect Dis		
Sponsor Award #:	5R01AI089728-04 REVISED		
Award Title #:	Receptor recognition mechanisms of coronaviruses		

ASSOCIATED PROJECTS (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Funding Tab)

Project #: 00013782 **Department:** 11718 - PHCL Pharmacology Admin
Project PI: Li,Fang **Dates:** 05/15/2010 - 04/30/2015 **Project Title:** Receptor recognition mechanism
F&A: 51 00 MTDC **EFFDT:** 05/15/2010

AWARD HISTORY (Online Page Navigation Main Menu > Grants > Awards > Review Award Modifications)

(Displayed in action order ('Mod Issue Date' in descending order))

Reference Award#: 5R01AI089728-04 REVISED **Amount:** $14,949 00
Period: 4 **Begin Date:** 05/01/2013 **End Date:** 04/30/2014
Modification Seq: 5 **Modification Type:** Additional Funding **Mod Issue Date:** 06/19/2013
Comments:

The NOA dated 6/5/13 awards funding in the amount of $14,949 5/1/13 - 4/30/14

This award was revised to restore funding near or at NIAID Funding Plan Levels, and consistent with NIH FY 2013 Fiscal Policy per NOT-OD-13-064

If you have any questions on the award calculation or terms and conditions of the award, your grants management contact is Stephanie Burt at 301-451-2672 or via email at stephanie burt@nih gov

In addition to the PI, any absence, replacement or substantial reduction in effort of the individual below requires written prior approval of the NIH awarding component, Dr Robert Geraghty, Associate Professor and Co-PI

All other terms and conditions remain the same

Reference Award#: 5R01AI089728-04 **Amount:** $336,353 00
Period: 4 **Begin Date:** 05/01/2013 **End Date:** 04/30/2014
Modification Seq: 4 **Modification Type:** Continuation **Mod Issue Date:** 05/02/2013
Comments:

The NOA dated 4/11/2013 awards funding in the amount of $336,353 NIH is currently funded through a Continuing Resolution at the FY2012 level This award has been made at a level below that indicated in the previous NOA If the final appropriation permits, adjustments may be made up to the FY2013 funding plan

In addition to the PI, any absence, replacement or substantial reduction in effort of the following individuals requires prior NIH approval Dr Robert Geraghty

Awardee must notify and receive prior approval for the use of Select Agents Ses NOA for details on Select Agents, Highly Pathogenic Agents and additional Agents or Toxins

All other terms and conditions remain the same

Reference Award#: 5R01AI089728-03 **Amount:** $373,725 00
Period: 3 **Begin Date:** 05/01/2012 **End Date:** 04/30/2013
Modification Seq: 3 **Modification Type:** Continuation **Mod Issue Date:** 05/01/2012

12
8-12
20

Comments:
Notice of Award dated 04/10/2012

None of the funds in this award shall be used to pay the salary of an individual at a rate per year in excess of the amounts reflected in the following NIH Guide Notice http //grants nih gov/grants/guide/notice-files/NOT-OD-12-035 html

All other previous terms and conditions remain unchanged

Reference Award#: 5R01AI089728-02 Rev **Amount:** $33,975 00
Period: 2 **Begin Date:** 05/01/2011 **End Date:** 04/30/2012
Modification Seq: 3 **Modification Type:** Additional Funding **Mod Issue Date:** 06/15/2011
Comments:
This revised award increases the total costs authorized consistent with NIAID implementation of the NIH Fiscal policy for FY2011

All other terms and conditions remain the same

Reference Award#: 1R01AI089728-02 **Amount:** $339,750 00
Period: 2 **Begin Date:** 05/01/2011 **End Date:** 04/30/2012
Modification Seq: 2 **Modification Type:** Continuation **Mod Issue Date:** 05/05/2011
Comments:
NIH is currently funded through a Continuing Resolution at the FY2010 level Therefore, this non-competing award is made at a level below the committed FY2011 level If final appropriation permits, adjustments may be made up to FY2011 plan level

All other terms and conditions remain the same

Reference Award#: 1R01AI089728-01 **Amount:** $377,500 00
Period: 1 **Begin Date:** 05/15/2010 **End Date:** 04/30/2011
Modification Seq: 1 **Modification Type:** Original **Mod Issue Date:** 05/27/2010
Comments:
An unobligated balance may be carried forward to the next budget period without prior agency approval

NOTE In additoin to the PI, Dr Robert Geraghty as been identified as key personnel (co-PI) Any absence, replacement or substantial reduction in effort by key personnel requires prior, written agency approval

Modular award The significant rebudgeting provision does not apply In addition, since costs were averaged over the project period, deficits may be carried forward from one budget period to the next within the same project period

ESTIMATED FUTURE FUNDING / ACTIONS (Online Page Navigation Main Menu > Grants > Awards > Review Award Modifications)

(Displayed by 'Period' and 'Modification Sequence' in ascending order)

Reference Award#: 1R01AI089728-01 **Amount:** $373,725 00
Period: 5 **Begin Date:** 05/01/2014 **End Date:** 04/30/2015
Modification Seq: 5 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

COMMENTS

Description: **Entered by:** HOWI0016 **Date & Time:** 5/27/2010 10 00 AM
Date and Time Stamp indicates Proposal-to-Award Process

Description: **Entered by:** HOWI0016 **Date & Time:** 5/27/2010 10 02 AM
Sponsor Contacts

Grants Management
Michael Wells
Phone 301-451-2656
Fax 301-493-0597
Email wellsmj@niaid nih gov

Program
Rachelle Salomon
Phone 301-402-2202
Fax 301-496-8030
Email salomonra@mail nih gov

Description: **Entered by:** SPAUL **Date & Time:** 12/23/2010 9 56 AM
LOC
RAI089728

Description: **Entered by:** GARC0027 **Date & Time:** 4/18/2013 12 50 PM
Grants Management Specialist Updated
Jennifer Schermerhorn
Email schermerhornj@niaid nih gov
P 301-451-2649

SPECIAL REVIEWS (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Certifications Tab)
Project #: 00013782

Description: rDNA, InfectAgents, BioToxins
Approval Date: 03/10/2012 **Expiration Date:** 03/09/2013 **AssuranceNumber:** 1002H77733
Comments:

BUDGET INFORMATION (Online Page Navigation Main Menu > Grants>Awards>Project Budget)
Please review online budget for valid account string combinations (ex PCBU, Activity, Fund, Dept_Id, Project, Account)

Project #: 00013782
Project Start Date: 05/15/2010
ICR Sharing **Department:** Drug Design, Center for
ICR Sharing **Department:** PHCL Pharmacology Admin

Cost Share Budget Total:
Project End Date: 04/30/2015
Percentage: 11 50
Percentage: 88 50

Period	Start Date	End Date	Direct Cost (Approved)	F & A Cost (Approved)	Direct Cost (Pending)	F & A Cost (Pending)	Total Period Cost
1	05/15/2010	04/30/2011	$252,702 00	$124,798 00	$0 00	$0 00	$377,500 00
2	05/01/2011	04/30/2012	$247,500 00	$126,225 00	$0 00	$0 00	$373,725 00
3	05/01/2012	04/30/2013	$247,500 00	$126,225 00	$0 00	$0 00	$373,725 00
4	05/01/2013	04/30/2014	$238,595 00	$112,707 00	$0 00	$0 00	$351,302 00
5	05/01/2014	04/30/2015	$0 00	$0 00	$247,500 00	$126,225 00	$373,725 00
TOTAL			$986,297 00	$489,955 00	$247,500 00	$126,225 00	$1,849,977 00

Budget Detail (all periods)

Fund Code	DeptID	Program Code	Chartfield 1	Chartfield 2	Fin EmplID	Activity	Analysis Type	Account	Cost Share	Budget Item	Amount	Hold
3002	11718					1	BUD	168100		CAPITAL_EQUIP	$8 000 00	
3002	11718					1	BUD	700100		FACULTY_SALARY	$186,874 00	
3002	11718					1	BUD	700200		PROF_ADM_SALARY	$201,666 00	
3002	11718					1	BUD	700300		GRAD_STU_SALARY	$101,400 00	
3002	11718					1	BUD	700310		GRAD_SAL_NOTUIT	$49,084 00	

Fund Code	DeptID	Program Code	Chartfield 1	Chartfield 2	Fin EmplID	Activity	Analysis Type	Account	Cost Share	Budget Item	Amount	Hold
3002	11718					1	BUD	710100		FACULTY_FRINGE	$64,545 00	
3002	11718					1	BUD	710200		PROF_ADM_FRINGE	$51,901 00	
3002	11718					1	BUD	710300		GRAD_STU_FRINGE	$33,717 00	
3002	11718					1	BUD	710310		GRAD_FRG_NOTUIT	$11,655 00	
3002	11718					1	BUD	720200		LABMED_SUPPLIES	$202,962 00	
3002	11718					1	BUD	720300		GEN_OP_SERVICES	$9,375 00	
3002	11718					1	BUD	720400		LABMED_SERVICES	$4,200 00	
3002	11718					1	BUD	720500		TELECOMM	$1,992 00	
3002	11718					1	BUD	720600		TRAVL_MILE_MOVE	$12,970 00	
3002	11718					1	BUD	730100		CONSULTING_SVCS	$29,900 00	
3002	11718					1	BUD	750100		NON_CAP_EQUIP	$9,571 00	
3002	11718					1	BUD	780100		REPAIRS_MAINT	$6,485 00	
3002	11718					1	BUD	810500		FACADM	$489,955 00	

COST SHARE/MATCH/IN-KIND
CSM - Cost Share, Matching and InKind

CSM05 - Departments must document all cost share/unpaid effort that is shown in the proposal

PROGRAM INCOME INFORMATION
PIN - Program Income

PIN03 - Reportable Program Income must be deposited by the department into the Sponsored Program Income Unapplied Cash account

PIN04 - Additional - Program Income can be used in addition to sponsor funds to cover project costs that will further the objectives of the legislation under which the grant was made

TERMS & CONDITIONS (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Terms Tab)

APV - Additional Approval

APV01 - Prior sponsor approval or notification required for reduction of PI's effort by 25% or more

APV04 - Prior sponsor approval required to replace PI

APV07 - Prior sponsor approval required for changes to key personnel that would impact the scope of work

APV10 - Prior sponsor approval required for changes to work scope

APV14 - Prior sponsor approval required for the absence of the PI for 3 months or more

APV15 - Prior sponsor approval required for a second no cost extension or a first no cost extension that is submitted after the agency notification deadline

APV20 - Prior sponsor approval required for the addition of foreign sites or foreign subcontracts

FIS - Fiscal

FIS03 - Reversion of funds at termination / final

FIS07 - Deficits may be carried forward

FIS10 - Grant/contract funds may not be used to pay the salary of an individual at a rate in excess of Executive Level I of the Federal Executive Pay Scale (S199,700)

FIS11 - Modular award - Expenses incurred must be allocated and accounted for in compliance with all applicable cost principles

FIS13 - Deficits may be carried forward within non-competing segments, but may not be carried forward between competing segments

FIS14 - Grant/contract funds may not be used to pay the salary of an individual at a rate in excess of Executive Level II of the Federal Executive Pay Scale ($179,700)

IPR - IP Rights

IPR02 - Sponsor support must be acknowledged in patent applications and patent issuing

IPR04 - Title to U of M, non-exclusive, non-transferable, irrevocable, paid-up license to Federal Government

IPR12 - PI obligation to report inventions (and other IP) to the sponsor (and to OTC per policy)

PRP - Property

PRP02 - U of M receives title to equipment purchased or fabricated under this award

PRP03 - Sponsor may require transfer of equipment and its title upon award completion for those items costing in excess of $5000

PUB - Publications

PUB02 - U of M will place an acknowledgement of sponsor support and a disclaimer, as appropriate, on any publication generated by this award or reporting on the award results and/or activity

PUB07 - Peer-reviewed articles must be submitted to NIH Pubmed Central (PMC) upon acceptance for publication and made publicly available within 12 months Cite PMC ID number in progress reports/proposals

RBD - Rebudgeting

RBD10 - Rebudgeting is allowed as long as there is no change in work scope

REG - Regulatory

REG01 - 45 CFR Part 74 - Code of Federal Regulations as it pertains to institutions of higher education

REG03 - OMB Circular A-21 (www whitehouse gov/omb/circulars/a021/a021 html)

REG04 - OMB Circular A-110 (www whitehouse gov/omb/circulars/a110/a110 html)

REG05 - OMB Circular A-133 (www whitehouse gov/omb/circulars/a133/a133 html)

REG09 - Subject to Streamlined Noncompeting Award Procedures (SNAP)

REG10 - Federal Demonstration Partnership (FDP) guidelines (www thefdp org)

REG19 - NIH Grants Policy Statement (http //grants nih gov/grants/policy/nihgps_2010/index htm)

UMN - U of M Requirements

UMN01 - The U of M Effort Certification system must be used to reflect all U of M staff committed effort on this award, whether or not any salary is charged to the award

UMN02 - Salary cost transfers cannot be made after effort is submitted

UMN03 - All U of M Policy and Guidelines relating to research must be followed and can be read in detail on the web at http //www ospa umn edu/policiesandprocedures/index htm

UMN04 - U of M Cost Transfer guidelines state transfers of non-salary expenses on accounts be clearly documented & made within 30 days after the end of the accounting period the charge was processed

NON-FINANCIAL REPORTING (Online Page Navigation Main Menu > Grants >Awards > Award Profile – Milestones Tab)
Type: Report **Description:** Progress Report **Due Date:**
Comments: PHS 2590 - noncompeting continuation Must be submitted via the eRA Commons Due 45 days prior to the end of the budget period

INVOICE/BILLING INFORMATION

Basis of Payment: COST_REIMBURSE
Method of Payment: Electronic Fund Transfer
Invoice Form: GPC - GM_LOC
Interim Invoice Deadline: 0
Final Invoice Deadline: 0

Contact Name: Jacqueline Jones
Contact Phone:
Contact Fax:
Contact Email:
Contact Address: PSC-FMS (301) 443-3020
PARKLAWN BUILDING ROOM 16A-12
5600 FISHERS LANE
ROCKVILLE MD 20857

FINANCIAL REPORTING

Report Form ID: R_FFR
Reporting Frequency: Termination/Final
Interim Reporting Deadline: 90
Final Reporting Deadline: 90

GENERAL PROVISIONS

This document is intended to provide guidance in managing this award, to the Principal Investigator, Academic Unit, and to those who are noted as receiving this document Its intent is to provide general information regarding University and sponsor policies when given the award In addition, it is intended to reference specific conditions related to this award Specific questions should be addressed to the grant administrator from SPA noted below By accepting this award, the Principal Investigator acknowledges and agrees to abide by all provisions of the sponsoring agency and the University of Minnesota policies related to sponsored projects management, including the Code of Conduct

UNIVERSITY OF MINNESOTA

Notice of Grant or Contract Award

Sponsored Projects Administration

(Online Page Navigation Main Menu > Grants >Awards > UM_NOGA Report)

Award: CON000000022345
Primary Project: 00013782
PRF #: 651709

U OF M CONTACTS

Administrative questions to SPA staff member:	Paulie Garcia	612/626-2724	garc0027@umn edu
Billing and financial questions to SFR:	Julia Steinkopf	612/624-7033	gordo091@umn edu

Notice of Award

RESEARCH **Issue Date:** 06/05/2013

Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI089728-04 REVISED

Principal Investigator(s):
Fang Li, PHD

Project Title: Receptor recognition mechanisms of coronaviruses

David L Hagen
University of Minnesota Twin Cities
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 554552070

Award e-mailed to: awards@umn edu

Budget Period: 05/01/2013 – 04/30/2014
Project Period: 05/15/2010 – 04/30/2015

Dear Business Official·

The National Institutes of Health hereby revises this award to reflect an increase in the amount of $14,949 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MINNESOTA in support of the above referenced project This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI089728 The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health " Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with 42 CFR Part 50 Subpart F Subsequent to the compliance date of the 2011 revised FCOI regulation (i.e., on or before August 24, 2012), Awardees must be in compliance with all aspects of the 2011 revised regulation, until then, Awardees must comply with the 1995 regulation The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards Consult the NIH website http //grants nih gov/grants/policy/coi/ for a link to the regulation and additional important information

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Michael W Fato
Grants Management Officer

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI089728-04 REVISED

Award Calculation (U.S. Dollars)

Federal Direct Costs	$232,650
Federal F&A Costs	$118,652
Approved Budget	$351,302
Federal Share	$351,302
TOTAL FEDERAL AWARD AMOUNT	$351,302
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$14,949

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
4	$351,302	$351,302
5	$373,725	$373,725

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93 855
EIN:	1416007513A1
Document Number:	RAI089728A
Fiscal Year:	2013

IC	*CAN*	*2013*	*2014*
AI	8472364	$351,302	$373,725

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC M51C S / **OC** 414E / **Released** FATOM 06/04/2013
Award Processed: 06/05/2013 12 39 38 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI089728-04 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http //grants nih gov/grants/policy/awardconditions htm

SECTION III – TERMS AND CONDITIONS – 5R01AI089728-04 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following

a The grant program legislation and program regulation cited in this Notice of Award
b Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts
c 45 CFR Part 74 or 45 CFR Part 92 as applicable
d The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period
e This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW

(See NIH Home Page at 'http //grants nih gov/grants/policy/awardconditions htm' for certain references cited above)

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase V Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP)

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration Should a consortium/subaward be issued under this award, a DUNS requirement must be included See http //grants nih gov/grants/policy/awardconditions htm for the full NIH award term implementing this requirement and other additional information

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170

In accordance with P L 110-161, compliance with the NIH Public Access Policy is now mandatory For more information, see NOT-OD-08-033 and the Public Access website http.//publicaccess nih gov/

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI089728-04 REVISED

REVISED AWARD. This award was revised to restore funding near or at NIAID Funding Plan Levels, and consistent with NIH FY2013 Fiscal Policy per NOT-OD-13-064, http //grants nih gov/grants/guide/notice-files/NOT-OD-13-064 html.

Supersedes Notice of Award (NoA) issued 04/11/13.

If you have a question on the award calculation or the terms and conditions of the award, your grants management contact should be Stephanie Burt at (301)451-2672 or via e-mail at stephanie burt@nih gov

In addition to the PI, any absence, replacement, or substantial reduction in effort of the following individual(s) below, requires the written prior approval of the National Institutes of Health awarding component
Dr Robert Geraghty, Associate Professor and co-PI

Select Agents.
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13 b (http //www.selectagents gov/Regulations.html)

Highly Pathogenic Agent.
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL)
(http //www cdc gov/OD/ohs/biosfty/bmbl5/bmbl5toc htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used
When submitting future Progress Reports indicate at the beginning of the report

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide

o A list of the new and/or additional Agent(s) that will be studied;
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment,
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions The Program Official is responsible for the scientific, programmatic and technical aspects of this project These individuals work together in overall project administration Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail

Grants Management Specialist Jennifer L Schermerhorn
Email. schermerhornj@niaid nih gov **Phone** 301-451-2649 **Fax** 301-493-0597

Program Official: Rachelle Salomon
Email salomonra@mail nih gov **Phone** 301-402-2202 **Fax**· 301-496-8030

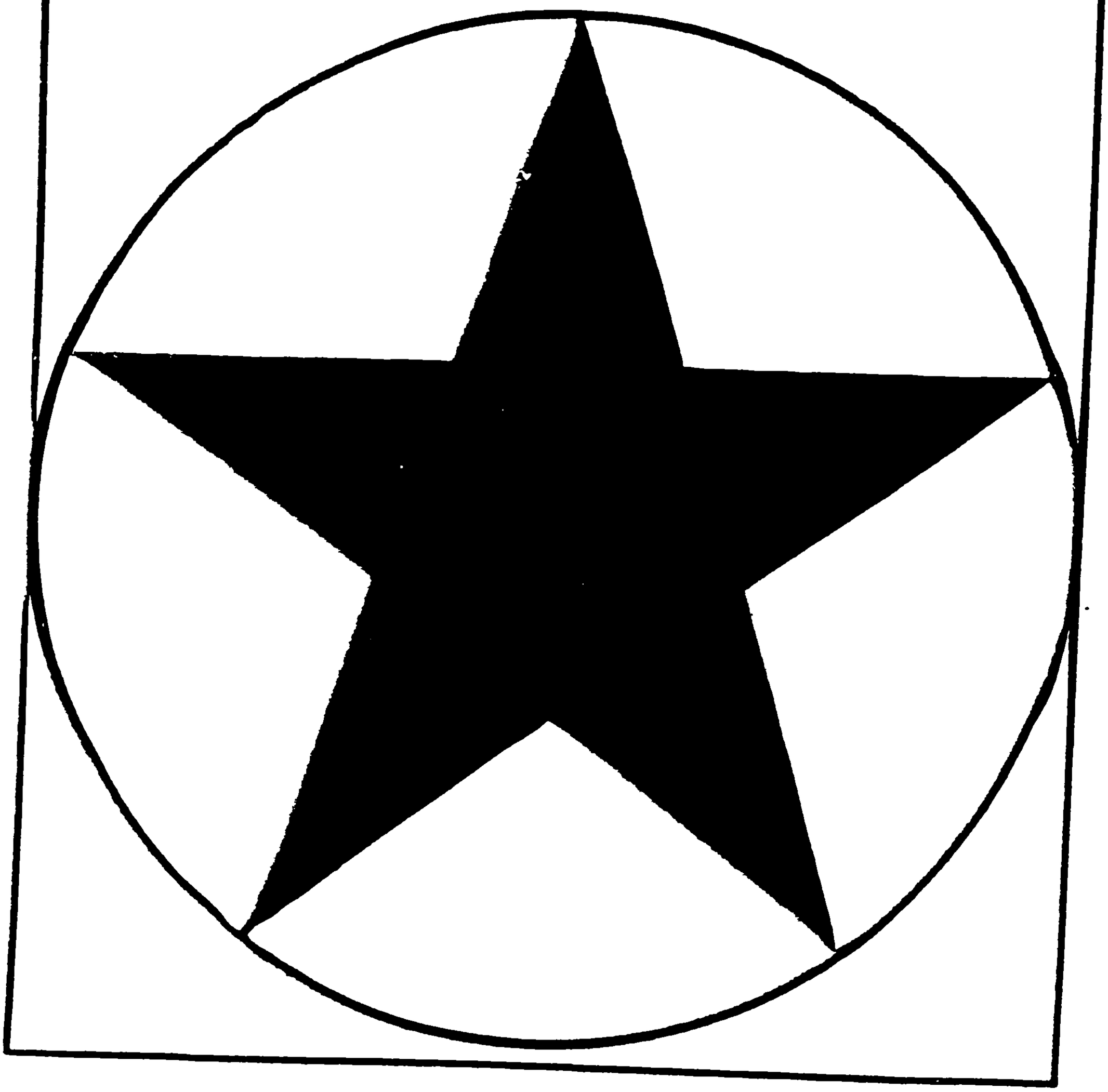
SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI089728-04 REVISED

INSTITUTION: UNIVERSITY OF MINNESOTA

Budget	***Year 4***	***Year 5***
TOTAL FEDERAL DC	$232,650	$247,500
TOTAL FEDERAL F&A	$118,652	$126,225
TOTAL COST	$351,302	$373,725

Facilities and Administrative Costs	***Year 4***	***Year 5***
F&A Cost Rate 1	51%	51%
F&A Cost Base 1	$232,650	$247,500
F&A Costs 1	$118,652	$126,225

Please Mail All Pages Prior To Page Separator



AWARD SETUP INFORMATION PAGE

GA Paulii DATE 6/18/13

_____ New CON#_____ Primary Project _____ In PreAward/Adv Account___

X Modification to CON# 22345 Primary Project 0010137782 Related Proposal: CON#_____

*If a mod has a related proposal, use the Negotiate Award notes on the proposal, not on the existing award

NO FFATA

NO eTool completed

INVOICING AND FINANCIAL REPORTING INFORMATION CAN BE FOUND ON THE FOLLOWING PAGE(S) IN THE AWARD DOCUMENT _____ NA

TEMPLATE TERMS AND CONDITIONS (pick one)

___ AHA_SCI_DEV	___ DOD	___ DOE	___ EPA_Fellowship	
___ MMF	___ MNDOT	___ STATE_OF_MN	___ NASA	
___ NIH_Modular	___ NIH_Expanded	___ NIH_Not_Expanded	___ NIH_IND_TR_FSER	___NIH_INST_TR_T32
___ NSF	___ USDA_FDP	___ USDA_NON-FDP	___ USDE	___VHO AND FNDN

Terms to be added _____

Terms to be Removed _____ N/A

IF ANY CHANGES MADE TO A TEMPLATE ARE NOT ABLE TO BE SHOWN ABOVE, SAVE THE TERMS IN THE AWARD SET UP FOLDER.

NON-TEMPLATE TERMS AND CONDITIONS or TEMPLATE TERMS with CHANGES

_____ are saved in MyNetworkPlaces\Award Setup\Award Setup-submissions TAB_____ N/A

(\\ovpr.ad.umn.edu\ovpr$\Shared\Groups\Award Setup)

_____ are in eTool

BUDGET INFORMATION FOR FUNDS PROVIDED BY SPONSOR – for new awards only (GA enters budgets on modifications)

N/A

_____ Fund _____ DeptID

_____ is in eTool

_____ See attached PS-friendly budget

COST SHARE INFORMATION – for new awards only (GA updates the budgets for modifications)

_____ Fund _____ DeptID _____ Program _____ Chartfield 1 _____ Chartfield 2 _____Emplid N/A

_____ is in eTool

_____ See attached PS-friendly budget

_____**NEW PROJECT NEEDED** – enter information on the Information Required to Establish a New Project form and include a completed PS-friendly budget. N/A

ANY ADDITIONAL INFORMATION NEEDED FOR SET UP:

additional funding $14,949

PUT AWARD FILE IN THE FOLLOWING ORDER:

Agency award documents

Guidance documents (to be included with award emailed to PI/Dept)

Award Imaging Divider page (yellow star sheet)

Award Setup Information page (this page)

PS-friendly budget (if any)

Negotiation notes, proposal correspondence, proposal, etc.

SET ALL NEGOTIATE AWARD NEGOTIATION TYPES TO COMPLETED EXCEPT FOR THE ONE FOR CONTRACT ENTRY AND THE ORIGINAL ONE CREATED BY FILES

PS / SPONSOR PROPOSAL BUDGET WORKSHEET

Please complete the cost share and F&A distribution information at the bottom of the page, if applicable

Award CON0000000022345 Project #, if known **00013782** Should this be the Primary Project? ___X_Yes ___ No

Project DeptID 11718 Project PI **Fang Li**

		Total of all worksheet tabs	Sponsor	Cost Share	Total Projects
LINK TO FY 2012 COA					
ITEM			**Sponsor Funding**	**Cost Share or Match***	**TOTAL PROJECT BUDGET**
Personnel					
Salary					
700100	Salary - Faculty		0		
700200	Salary - Professional and Admin		0		
700310	Salary - Post Doc/Prof Student (expense account 700302)		8,084		
700300	Salary - Grad Research Asst (expense account 700301)		0		
700310	Salary - Grad Research Asst (No Tuition)		0		
700400	Salary - Undergrad Student				
700500	Salary - Civil Service Bargaining Unit				
Total Salary			8,084	0	8,084
Fringe					
710100	Fringe - Faculty		0		
710200	Fringe - Professional and Admin		0		
710310	Fringe - Post Doc/Prof Student (expense account 710302)		1,816		
710300	Fringe - Grad Research Asst (expense account 710301)		0		
710310	Fringe - Grad Research Asst (No Tuition)		0		
710400	Fringe - Undergrad Student				
710500	Fringe - Civil Service Bargaining Unit				
710600	Fringe - Direct Charge				
Total Fringe			1,816	0	1,816
Total Personnel			9,900	0	9,900
Consulting / Professional Services					
730100	Consulting Services				
730200	Professional Services				
Total Consulting / Professional Services			0	0	0
Equipment					
168100	Equipment				0
Supplies					
720100	General Operating Supplies				
720200	Lab & Medical Supplies		0		
750100	Non-Capitalized Equipment		0		
Total Supplies			0	0	0
Travel					
720600	Travel / Mileage / Moving		0		0
Other Direct Costs					
720300	General Operating Services		0		
720400	Lab & Medical Services		0		
720500	Telecommunications				
770100	Long Term Rents & Leases				
770150	Long Term Rents & Leases (bldg/facility)				
770200	Short Term Rents & Leases				
770250	Short Term Rents & Leases (bldg/facility)				
780100	Repairs & Maintenance		0		
810100	Research Subject Payments				
810200	Participant Payments				
Total Other Direct Costs			0	0	0
Subawards / Subcontracts					
810300	Subcontracts not subject to F&A				
810400	Subcontracts subject to F&A				
Total Subawards / Subcontracts			0	0	0
Additional Accounts (show budget account and a description, please)					
					0
					0
					0
TOTAL DIRECT COSTS			9,900	0	9,900
810500	Facilities and Administrative Expenses		5,049		5,049
TOTAL COSTS			14,949	0	14,950
			14,949	0	

Account descriptions are provided in comments for each category. While SPA will try to keep the account codes and descriptions updated, you should refer to the current FY COA for the most up to date information.

***Cost Share/Match account string**

Does your Program FUNC match your Project FUNC? Check the COA for FUNC and Program descriptions

For any amounts shown as Cost Share or Match, the Fund, DeptID, Program and optional fields CF1, CF2, and FinEmplID providing the funding must be identified If these codes will be the same for all lines with Cost Share or Match, please enter them here

Fund _____ DeptID _____ Program _____
CF1 _____ CF2 _____ FinEmplID _____

If the Fund, DeptID, Program, CF1, CF2, and FinEmplID will differ among the cost share or match amounts, please enter the information next to the specific cost share or match amount so that budget lines can be correctly established

F&A Distribution

If the F&A revenue is going to be shared between departments, please identify the department(s) and percentages below If all the F&A revenue should go to the department identified at the top of the page, this section may be left blank

F&A Distribution - Approved by (name)

DeptID _____ Dept Name _____ _____ % F&A rec'd _____
DeptID _____ Dept Name _____ _____ % F&A rec'd _____
DeptID _____ Dept Name _____ _____ % F&A rec'd _____
DeptID _____ Dept Name _____ _____ % F&A rec'd _____
Total F&A % (must = 100%) 0%

By submission of this information, I certify that the proper authorizing officials are named above and that I have retained evidence within the department/unit confirming their agreement with the distributions above

Name _____
DeptID _____
Phone # _____
Email _____

See current rates (fringe benefits, F&A, etc) http //www ospa umn edu/forms/index html

For a crosswalk between CUFS object codes and PS Accounts, see
http //www budget umn edu/FY09docs/CUFS_PS_conversion_grid xls

PS / SPONSOR PROPOSAL BUDGET WORKSHEET

Please complete the cost share and F&A distribution information at the bottom of the page, if applicable

Award CON0000000022345 Project #, if known **00013782** Should this be the Primary Project? ___X_Yes ___ No

Project DeptID 11718 Project PI **Fang Li**

LINK TO FY 2012 COA

ITEM		Total of all worksheet tabs Sponsor Funding	Sponsor Cost Share or Match*	Cost Share TOTAL PROJECT BUDGET	Total Projects
Personnel					
Salary					
700100	Salary - Faculty	50,000			
700200	Salary - Professional and Admin	15,000			
700310	Salary - Post Doc/Prof Student (expense account 700302)	36,000			
700300	Salary - Grad Research Asst (expense account 700301)	20,000			
700310	Salary - Grad Research Asst (No Tuition)	5,000			
700400	Salary - Undergrad Student				
700500	Salary - Civil Service Bargaining Unit				
Total Salary		126,000	0	126,000	
Fringe					
710100	Fringe - Faculty	17,450			
710200	Fringe - Professional and Admin	5,235			
710310	Fringe - Post Doc/Prof Student (expense account 710302)	8,089			
710300	Fringe - Grad Research Asst (expense account 710301)	17,600			
710310	Fringe - Grad Research Asst (No Tuition)	1,750			
710400	Fringe - Undergrad Student				
710500	Fringe - Civil Service Bargaining Unit				
710600	Fringe - Direct Charge				
Total Fringe		50,124	0	50,124	
Total Personnel		176,124	0	176,124	
Consulting / Professional Services					
730100	Consulting Services				
730200	Professional Services				
Total Consulting / Professional Services		0	0	0	
Equipment					
168100	Equipment			0	
Supplies					
720100	General Operating Supplies				
720200	Lab & Medical Supplies	30,071			
750100	Non-Capitalized Equipment	9,000			
Total Supplies		39,071	0	39,071	
Travel					
720600	Travel / Mileage / Moving	5,000		5,000	
Other Direct Costs					
720300	General Operating Services	2,500			
720400	Lab & Medical Services	4,000			
720500	Telecommunications				
770100	Long Term Rents & Leases				
770150	Long Term Rents & Leases (bldg/facility)				
770200	Short Term Rents & Leases				
770250	Short Term Rents & Leases (bldg/facility)				
780100	Repairs & Maintenance	2,000			
810100	Research Subject Payments				
810200	Participant Payments				
Total Other Direct Costs		8,500	0	8,500	
Subawards / Subcontracts					
810300	Subcontracts not subject to F&A				
810400	Subcontracts subject to F&A				
Total Subawards / Subcontracts		0	0	0	
Additional Accounts (show budget account and a description, please)					
				0	
				0	
				0	
TOTAL DIRECT COSTS		228,695	0	228,695	
810500	Facilities and Administrative Expenses	107,658		107,658	
TOTAL COSTS		336,353	0	336,353	

Account descriptions are provided in comments for each category. While SPA will try to keep the account codes and descriptions updated, you should refer to the current FY COA for the most up to date information.

***Cost Share/Match account string**

Does your Program FUNC match your Project FUNC? Check the COA for FUNC and Program descriptions

For any amounts shown as Cost Share or Match, the Fund, DeptID, Program and optional fields CF1, CF2, and FinEmplID providing the funding must be identified If these codes will be the same for all lines with Cost Share or Match, please enter them here

Fund _____ DeptID _____ Program _____
CF1 _____ CF2 _____ FinEmplID _____

If the Fund, DeptID, Program, CF1, CF2, and FinEmplID will differ among the cost share or match amounts, please enter the information next to the specific cost share or match amount so that budget lines can be correctly established

F&A Distribution

If the F&A revenue is going to be shared between departments, please identify the department(s) and percentages below If all the F&A revenue should go to the department identified at the top of the page, this section may be left blank

F&A Distribution - Approved by (name)

DeptID _____ Dept Name _____ _____ % F&A rec'd _____
DeptID _____ Dept Name _____ _____ % F&A rec'd _____
DeptID _____ Dept Name _____ _____ % F&A rec'd _____
DeptID _____ Dept Name _____ _____ % F&A rec'd _____
Total F&A % (must = 100%) 0%

By submission of this information, I certify that the proper authorizing officials are named above and that I have retained evidence within the department/unit confirming their agreement with the distributions above

Name _____
DeptID _____
Phone # _____
Email _____

See current rates (fringe benefits, F&A, etc) http //www ospa umn edu/forms/index html

For a crosswalk between CUFS object codes and PS Accounts, see
http //www budget umn edu/FY09docs/CUFS_PS_conversion_grid xls

Wednesday, June 05, 2013
10 15 AM

Negotiate Award

Proposal ID:	CON000000022345	**Business Unit:**	UMSPR	
Version ID:	A101			
Award ID:	CON000000022345	**Sponsor:**	NIH Nat'l Inst of Allergy & Infect Dis	UM GM Dates and Notes

Type and Status — Find | View All — First 11 of 11 Last

Sequence: 11 **Event Date:** 06/05/2013

Negotiation Type: 4ModA Mod Request Rec **Negotiate Status:** Working Negotia

Comments: Li. 00013782, Yr 04 Rev
sent to PG

Details — Find | View All — First 1 of 1 Last

Name: **Title:**

Telephone: **Fax:**

Email ID:

Screen clipping taken 6/5/2013 10 15 AM

Subject: NoA: 5 R01 AI089728-04 PI: Li, Fang
From: <era-notify@mail.nih.gov>
Date: 6/4/2013 11:39 PM
To: <awards@umn.edu>

Grant Number: 5 R01 AI089728-04
Principal Investigator: Li, Fang
Project Title. Receptor recognition mechanisms of coronaviruses
Institution: UNIVERSITY OF MINNESOTA
CFDA 93.855
OC 414E
PCC. M51C S
Award Issue Date: 06/05/2013
Grants Management Officer: Fato, Michael
Program Official: Salomon, Rachelle
Grants Specialist: Schermerhorn, Jennifer

*** This is an automated notification - Please do not reply to this message ***

Attachments

temp8787348535986318747.pdf	111 KB

Cn-12345
0813782
Li
PG

A. COVER PAGE

Project Title: Receptor recognition mechanisms of coronaviruses	
Grant Number: 5R01AI089728-05	**Project/Grant Period:** 05/15/2010 - 04/30/2015
Reporting Period: 05/01/2013 - 04/30/2014	**Requested Budget Period:** 05/01/2014 - 04/30/2015
Report Term Frequency: Annual	**Date Submitted:** 03/07/2014
Program Director/Principal Investigator Information: FANG LI , PHD **Phone number:** 612-625-6149 **Email:** lifang@umn.edu	**Recipient Organization:** UNIVERSITY OF MINNESOTA UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS, MN 554552070 **DUNS:** 555917996 **EIN:** 1416007513A1 **RECIPIENT ID:** PRF 786631
Change of Contact PD/PI: No	
Administrative Official: PAULIE GARCIA 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE Minneapolis, MN 554552070 **Phone number:** 612-624-5599 **Email:** awards@umn.edu	**Signing Official:** DAVID L HAGEN 200 Oak Street SE Suite 450 Minneapolis, MN 55455 **Phone number:** 612-626-9895 **Email:** hagen027@umn.edu
Human Subjects: No	**Vertebrate Animals:** No
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Coronaviruses use a variety of cellular receptors and co-receptors including proteins and sugars. The diverse usage of receptors has allowed coronaviruses to infect a wide range of mammalian and avian species, and cause respiratory, enteric, systemic and neurological diseases. Two domains in coronavirus spike protein S1 subunit, the N-terminal domain (NTD) and the C-domain, have evolved functions as receptor-binding domains (RBDs). How coronaviruses have evolved to do so has been a major puzzle in virology. To solve this puzzle, this NIH-funded project receptor recognition mechanisms of coronaviruses investigates the molecular and structural basis for the complex receptor-recognition mechanisms of coronaviruses.

There are three specific aims in this project. Aim 1 focuses on the receptor recognition mechanisms of group-I coronaviruses that use S1 C-domains as their RBDs (e.g., NL63 coronavirus (NL63-CoV) and porcine epidemic diarrhea virus (PEDV). Aim 2 focuses on the receptor recognition mechanisms of group-2 coronaviruses that use S1 NTD as their RBDs (e.g., murine hepatitis virus (MHV) and bovine coronavirus (BCoV)). Aim 3 focuses on the receptor recognition mechanisms of group-2 coronaviruses that use S1 C-domain as their RBDs (e.g., MERS coronavirus (MERS-CoV) and SARS coronavirus (SARS-CoV)). The research approaches of this proposal include crystallographic analysis of the virus-receptor interfaces, identification of structural elements important for virus-receptor interactions, and characterization of these elements using crystallographic, biochemical, molecular, and virological tools.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Fang_Li_RESULTS_SIGNIF_030314.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITITES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

For the next funding year, we will continue to focus on all three specific aims.
For specific Aim 1, we will investigate the structural mechanism whereby the group-1 PEDV RBD (i.e., S1 C-domain) recognizes its receptor porcine APN. In recent years, PEDV has become increasingly problematic in USA and many other countries, and causes huge losses in pork industry. The mortality rates of PEDV vary from 50 to 100%, and different PEDV strains have been isolated containing changes in the RBD. We will determine the crystal structures of the RBD from different PEDV strains in complex with porcine APN. In addition, we will examine the binding interactions between the RBD from different PEDV strains and porcine APN using a variety of biochemical assays such as surface plasmon resonance, AlphaScreen, and pseudovirus entry assays. These studies will reveal the receptor recognition mechanisms and host adaptive evolution of PEDV, and provide structural bases for design of novel and highly effective PEDV subunit vaccines.
For specific Aim 2, we will determine the crystal structure of group-2 BCoV NTD complexed with its sugar substrate. Based on the successful structure determination of BCoV NTD by itself, we will synthesize the sugar substrates of BCoV NTD, and use either crystal soaking or co-crystallization to determine the structure of the complex. This study will reveal detailed structural basis for the sugar-binding mechanism of group-2 coronaviruses.
For specific Aim 3, we will study the host range and cross-species infections of MERS-CoV. More specifically, we will examine how MERS-CoV RBD (i.e., S1 C-domain) recognizes DPP4 orthologues from different animal species and how we can convert those DPP4 orthologues with low receptor activities into efficient receptors for MERS-CoV. The approaches include structure determinations of MERS-CoV RBD in complex with DPP4 orthologues, structure-based rational design, biochemical assays, and pseudovirus entry assays. These studies will provide structural bases for animal model development for MERS-CoV infections.
Also for specific Aim 3, we will investigate the receptor recognition mechanisms and potential cross-species infections of MERS-CoV-like and SARS-CoV-like bat coronaviruses. A constant and long-term threat to human health is cross-species transmission of MERS-CoV-like and SARS-CoV-like coronaviruses from bats to humans. This process is poorly understood. We will investigate the receptor usage and

cell entry mechanisms of bat coronavirus HKU4 and HKU5, which are genetically related to MERS-CoV, and bat coronaviruses WIV1, which is genetically related to SARS-CoV. These studies will be critical for evaluating emerging disease potentials of bat coronaviruses and for preventing and controlling their spreads in humans.

B. Studies and Results

We made the following discoveries in 2013.

First, Chen et al. (October 2013, Journal of Virology) have identified the receptor-binding domain (RBD) for the newly emerged MERS coronavirus (MERS-CoV) and determined its crystal structure. This study also presents a structural comparison of MERS-CoV RBD with other coronavirus RBDs, successfully positioning MERS-CoV on the landscape of coronavirus evolution and providing insights into receptor binding by MERS-CoV. Furthermore, we found that MERS-CoV RBD functions as an effective entry inhibitor of MERS-CoV. The identified MERS-CoV RBD may also serve as a potential candidate for MERS-CoV subunit vaccines. Overall, this study enhances our understanding of the evolution of coronavirus RBDs, provides insights into receptor recognition by MERS-CoV, and may help control the transmission of MERS-CoV in humans.

Second, Li (October 2013, Antiviral Research) reviews the latest knowledge about how RBDs from different SARS-CoV strains interact with ACE2 from several animal species. Detailed research on these RBD/ACE2 interactions has established important principles on host receptor adaptations, cross-species infections, and future evolution of SARS-CoV. These principles may apply to other emerging animal viruses, including the recently emerged Middle East respiratory syndrome coronavirus (MERS-CoV).

Third, Chen et al. (April 2013, Journal of Virology) analyzed the structural relationships between BCoV spike protein lectin domain and other known viral lectins. Influenza and other viruses use host-cell-surface sugars as receptors. Here we show that the sugar-binding domains in influenza hemagglutinin, BCoV spike protein lectin domain, and other viral lectins share the same structural fold as human galectins (host lectins). Unlike the easily accessible sugar-binding sites in human galectins, the sugar-binding sites in viral lectins are hidden in cavities. We propose that these viral lectins originated from host lectins but have evolved to use hidden sugar-binding sites to evade host immune attacks.

Fourth, Chen at al. (2014, Journal of Virology, under revision) demonstrated that dipeptidyl peptidase 4 (DPP4) from humans, not mice, serves as a receptor for MERS-CoV. Further, we identified residue differences between human and mouse DPP4 that largely account for their differential receptor activities. These residue differences form a species barrier between humans and mice for MERS-CoV infection. An improved understanding of MERS-CoV host range may facilitate development of transgenic mouse model for MERS-CoV infection.

Fifth, Du et al. (2014, Journal of Virology under revision) have developed a neutralizing monoclonal antibody (mAb), designated Mersmab1, which potently blocks MERS-CoV entry into host cells. Biochemical assays reveal that Mersmab1 binds to the receptor-binding domain (RBD) of the MERS-CoV spike protein to, in turn, competitively block the binding of the RBD to its cellular dipeptidyl peptidase 4 (DPP4) receptor. Furthermore, alanine scanning of the RBD has identified several residues at the DPP4-binding surface that serve as neutralizing epitopes for Mersmab1. These results suggest that Mersmab1 can be humanized as a promising immunotherapeutic for treating and preventing MERS-CoV infection. Additionally, it is also useful to study the conformation and antigenicity of MERS-CoV spike protein S1 subunit for guiding the design of anti-MERS-CoV vaccines.

C. Significance

Our research has been the major driving force in the coronavirus field to understand coronavirus/receptor interactions. Prior to the SARS epidemic, no structural information was available for any coronavirus S1 subunit, despite decades of extensive research. By now, we have successfully determined the crystal structures of group-1 NL63-CoV C-domain complexed with its receptor ACE2, group-2 SARS-CoV C-domain complexed with its receptor ACE2, group-2 MHV NTD complexed with its receptor CEACAM1, group-2 BCoV NTD, group-2 MERS-CoV C-domain, and group-1 coronavirus receptor APN. To date, these structures account for the majority of the structures available for any coronavirus S1 or coronavirus-S1/receptor complexes. By determining these structures, we have solved several important puzzles in virology, such as how animal viruses can cross species barriers to infect humans through one or a few seemingly trivial mutations in their RBDs, how different viruses recognize the same receptor by targeting common virus-binding hotspots on the receptor, how similar viruses recognize different receptors through subtle structural changes in viral RBDs, and how viruses can acquire proteins from their hosts and evolve these host proteins into viral RBDs with different receptor specificity. Protein biochemistry and virological methods have also been systematically performed to corroborate our structural findings. By elucidating these structures, we have also been able to reconstruct the evolutionary history of coronaviruses. Furthermore, our detailed structural analyses of SARS-CoV/receptor interactions have significantly prepared humans for any potential future SARS epidemics.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Complete	Chen L, Li F. Structural analysis of the evolutionary origins of influenza virus hemagglutinin and other viral lectins. J Virol. 2013 Apr;87(7):4118-20. PubMed PMID: 23365425; PubMed Central PMCID: PMC3624229.
Complete	Chen Y, Rajashankar KR, Yang Y, Agnihothram SS, Liu C, Lin YL, Baric RS, Li F. Crystal structure of the receptor-binding domain from newly emerged Middle East respiratory syndrome coronavirus. J Virol. 2013 Oct;87(19):10777-83. PubMed PMID: 23903833; PubMed Central PMCID: PMC3807420.
Complete	Li F. Receptor recognition and cross-species infections of SARS coronavirus. Antiviral Res. 2013 Oct;100(1):246-54. PubMed PMID: 23994189; PubMed Central PMCID: PMC3840050.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

File uploaded: Fang_Li_NIH_report_030314 RESOURCES GEN.pdf

F. Project Generated Resources

This research did not generate cell lines, DNA probes, animal models, protocols, or software. De-identified raw data from the experiments and computer programs (CCP4 macromolecular crystallography scripts) used for protein structure determinations are available to anyone upon written request to the PI. Peer-reviewed publications accepted or published during the past project period are being or have been entered in NIHMS and PubMed Central.

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
FANGLI	Y	Li, Fang,	Minn Stat 13.4	Minn Stat 13.43	PHD	PD/PI	6	0	0			NA
ROBERT.GERAGHTY	Y	Geraghty, Robert,	Minn Stat 13.	Minn Stat 13.43	BS,PHD	Co-Investigator	1	0	0			NA
WENBOZHOU	N	Zhou, Wenbo,	Minn Stat 13.4	Minn Stat 13.43	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	3	0	0			NA
Minn Stat 13.32	N	Minn Stat 13.32	Minn Stat 13.	Minn Stat 13.32	MS	Graduate Student (research assistant)	4	0	0			NA
YAOQING	N	Chen, Yaoqing,	Minn Stat 13.4	Minn Stat 13.43	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	7	0	0			NA
Minn Stat 13.32	N	Minn Stat 13.32	Minn Stat 13.	Minn Stat 13.32	BS	Graduate Student (research assistant)	6	0	0			NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

NOTHING TO REPORT

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

NOTHING TO REPORT

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

NOTHING TO REPORT

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

NOTHING TO REPORT

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

No

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: University of Minnesota	555917996	MN-005	3-284 Nils Hasselmo Hall 312 Church Street SE Minneapolis MN 554550215
University of of Minnesota	555917996	MN-005	7-227 Phillips Wangensteen Building 516 Delaware Street SE Minneapolis MN 55455

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

UNIVERSITY OF MINNESOTA

Notice of Grant or Contract Award

Sponsored Projects Administration

(Online Page Navigation: Main Menu > Grants >Awards > UM_NOGA Report)

Award: CON000000022345
Primary Project: 00013782
PRF #: 651709

AWARD INFORMATION (Online Page Navigation: Main Menu > Grants > Awards >Award Profile)

Award Start Date:	05/15/2010	**Award End Date:**	04/30/2015
Funds Approved to Date:	$1,849,977.00		
Principal Investigator:	Li,Fang	**Award Type:**	Grant
Primary Project Dept:	PHCL Pharmacology Admin	**Purpose:**	Research
Sponsor:	NIH Nat'l Inst of Allergy & Infect Dis		
Sponsor Award #:	5R01AI089728-05		
Award Title #:	Receptor recognition mechanisms of coronaviruses		

ASSOCIATED PROJECTS (Online Page Navigation: Main Menu > Grants >Awards > Award Profile – Funding Tab)

Project #: 00013782 **Department:** 11718 - PHCL Pharmacology Admin
Project PI: Li,Fang **Dates:** 05/15/2010 - 04/30/2015 **Project Title:** Receptor recognition mechanism
F&A: 51.00 MTDC **EFFDT:** 05/15/2010

AWARD HISTORY (Online Page Navigation: Main Menu > Grants > Awards > Review Award Modifications)

(Displayed in action order ('Mod Issue Date' in descending order))

Reference Award#: 5R01AI089728-05 **Amount:** $373,725.00
Period: 5 **Begin Date:** 05/01/2014 **End Date:** 04/30/2015
Modification Seq: 5 **Modification Type:** Continuation **Mod Issue Date:** 05/01/2014
Comments:

The NOA dated 4/2/14 awards $373,725 for the budget period 5/1/14 - 4/30/15.

In addition to the PI, any absence, replacement or substantial reduction in effort of the following individual requires the written prior approval of the NIH awarding component: Robert Geraghty

Please see the NOA for important information regarding Highly Pathogenic Agents, Select Agents and IBC. Specific information may be needed on progress reports regarding these.

All other terms and conditions remain the same.

Reference Award#: 5R01AI089728-04 REVISED **Amount:** $14,949.00
Period: 4 **Begin Date:** 05/01/2013 **End Date:** 04/30/2014
Modification Seq: 5 **Modification Type:** Additional Funding **Mod Issue Date:** 06/19/2013
Comments:

The NOA dated 6/5/13 awards funding in the amount of $14,949 5/1/13 - 4/30/14.

This award was revised to restore funding near or at NIAID Funding Plan Levels, and consistent with NIH FY 2013 Fiscal Policy per NOT-OD-13-064.

If you have any questions on the award calculation or terms and conditions of the award, your grants management contact is Stephanie Burt at 301-451-2672 or via email at stephanie.burt@nih.gov.

In addition to the PI, any absence, replacement or substantial reduction in effort of the individual below requires written prior approval of the NIH awarding component, Dr. Robert Geraghty, Associate Professor and Co-PI.

All other terms and conditions remain the same.

Reference Award#: 5R01AI089728-04 **Amount:** $336,353.00
Period: 4 **Begin Date:** 05/01/2013 **End Date:** 04/30/2014
Modification Seq: 4 **Modification Type:** Continuation **Mod Issue Date:** 05/02/2013
Comments:

The NOA dated 4/11/2013 awards funding in the amount of $336,353. NIH is currently funded through a Continuing Resolution at the

13
8-13
71

FY2012 level. This award has been made at a level below that indicated in the previous NOA. If the final appropriation permits, adjustments may be made up to the FY2013 funding plan.

In addition to the PI, any absence, replacement or substantial reduction in effort of the following individuals requires prior NIH approval: Dr. Robert Geraghty

Awardee must notify and receive prior approval for the use of Select Agents. Ses NOA for details on Select Agents, Highly Pathogenic Agents and additional Agents or Toxins.

All other terms and conditions remain the same.

Reference Award#: 5R01AI089728-03 **Amount:** $373,725.00
Period: 3 **Begin Date:** 05/01/2012 **End Date:** 04/30/2013
Modification Seq: 3 **Modification Type:** Continuation **Mod Issue Date:** 05/01/2012
Comments:
Notice of Award dated 04/10/2012

None of the funds in this award shall be used to pay the salary of an individual at a rate per year in excess of the amounts reflected in the following NIH Guide Notice: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-12-035.html

All other previous terms and conditions remain unchanged.

Reference Award#: 5R01AI089728-02 Rev **Amount:** $33,975.00
Period: 2 **Begin Date:** 05/01/2011 **End Date:** 04/30/2012
Modification Seq: 3 **Modification Type:** Additional Funding **Mod Issue Date:** 06/15/2011
Comments:
This revised award increases the total costs authorized consistent with NIAID implementation of the NIH Fiscal policy for FY2011.

All other terms and conditions remain the same.

Reference Award#: 1R01AI089728-02 **Amount:** $339,750.00
Period: 2 **Begin Date:** 05/01/2011 **End Date:** 04/30/2012
Modification Seq: 2 **Modification Type:** Continuation **Mod Issue Date:** 05/05/2011
Comments:
NIH is currently funded through a Continuing Resolution at the FY2010 level. Therefore, this non-competing award is made at a level below the committed FY2011 level. If final appropriation permits, adjustments may be made up to FY2011 plan level.

All other terms and conditions remain the same.

Reference Award#: 1R01AI089728-01 **Amount:** $377,500.00
Period: 1 **Begin Date:** 05/15/2010 **End Date:** 04/30/2011
Modification Seq: 1 **Modification Type:** Original **Mod Issue Date:** 05/27/2010
Comments:
An unobligated balance may be carried forward to the next budget period without prior agency approval.

NOTE: In additoin to the PI, Dr. Robert Geraghty as been identified as key personnel (co-PI). Any absence, replacement or substantial reduction in effort by key personnel requires prior, written agency approval.

Modular award. The significant rebudgeting provision does not apply. In addition, since costs were averaged over the project period, deficits may be carried forward from one budget period to the next within the same project period.

ESTIMATED FUTURE FUNDING / ACTIONS (Online Page Navigation: Main Menu > Grants > Awards > Review Award Modifications)
(Displayed by 'Period' and 'Modification Sequence' in ascending order)

COMMENTS

Description: **Entered by:** HOWI0016 **Date & Time:** 5/27/2010 10:00 AM
Date and Time Stamp indicates Proposal-to-Award Process

Description: **Entered by:** HOWI0016 **Date & Time:** 5/27/2010 10:02 AM
Sponsor Contacts:
Grants Management:
Michael Wells
Phone: 301-451-2656
Fax: 301-493-0597
Email: wellsmj@niaid.nih.gov
Program:
Rachelle Salomon
Phone: 301-402-2202
Fax: 301-496-8030
Email: salomonra@mail.nih.gov

Description: **Entered by:** SPAUL **Date & Time:** 12/23/2010 9:56 AM
LOC:
RAI089728

Description: **Entered by:** GARC0027 **Date & Time:** 4/18/2013 12:50 PM
Grants Management Specialist Updated
Jennifer Schermerhorn
Email: schermerhornj@niaid.nih.gov
P: 301-451-2649

SPECIAL REVIEWS (Online Page Navigation: Main Menu > Grants >Awards > Award Profile – Certifications Tab)

Project #: 00013782

Description: rDNA, InfectAgents, BioToxins
Approval Date: 03/10/2012 **Expiration Date:** 03/09/2013 **AssuranceNumber:** 1002H77733
Comments:

BUDGET INFORMATION (Online Page Navigation: Main Menu > Grants>Awards>Project Budget)

Please review online budget for valid account string combinations (ex. PCBU, Activity, Fund, Dept_Id, Project, Account)

Project #: 00013782
Project Start Date: 05/15/2010
ICR Sharing **Department:** Drug Design, Center for
ICR Sharing **Department:** PHCL Pharmacology Admin

Cost Share Budget Total:
Project End Date: 04/30/2015
Percentage: 11.50
Percentage: 88.50

Period	Start Date	End Date	Direct Cost (Approved)	F & A Cost (Approved)	Direct Cost (Pending)	F & A Cost (Pending)	Total Period Cost
1	05/15/2010	04/30/2011	$252,702.00	$124,798.00	$0.00	$0.00	$377,500.00
2	05/01/2011	04/30/2012	$247,500.00	$126,225.00	$0.00	$0.00	$373,725.00
3	05/01/2012	04/30/2013	$247,500.00	$126,225.00	$0.00	$0.00	$373,725.00
4	05/01/2013	04/30/2014	$240,283.00	$111,019.00	$0.00	$0.00	$351,302.00
5	05/01/2014	04/30/2015	$252,322.00	$121,403.00	$0.00	$0.00	$373,725.00
TOTAL			$1,240,307.00	$609,670.00	$0.00	$0.00	$1,849,977.00

Budget Detail (all periods)

(Online Page Navigation: Main Menu > Grants >Awards > UM_NOGA Report)

Fund Code	DeptID	Program Code	Chartfield 1	Chartfield 2	Fin EmplID	Activity	Analysis Type	Account	Cost Share	Budget Item	Amount	Hold
3002	11718					1	BUD	168100		CAPITAL_EQUIP	$8,000.00	
3002	11718					1	BUD	700100		FACULTY_SALARY	$248,289.00	
3002	11718					1	BUD	700200		PROF_ADM_SALARY	$201,666.00	
3002	11718					1	BUD	700300		GRAD_STU_SALARY	$126,400.00	
3002	11718					1	BUD	700310		GRAD_SAL_NOTUIT	$100,084.00	
3002	11718					1	BUD	710100		FACULTY_FRINGE	$85,316.00	
3002	11718					1	BUD	710200		PROF_ADM_FRINGE	$51,901.00	
3002	11718					1	BUD	710300		GRAD_STU_FRINGE	$47,993.00	
3002	11718					1	BUD	710310		GRAD_FRG_NOTUIT	$23,028.00	
3002	11718					1	BUD	720200		LABMED_SUPPLIES	$250,337.00	
3002	11718					1	BUD	720300		GEN_OP_SERVICES	$11,875.00	
3002	11718					1	BUD	720400		LABMED_SERVICES	$6,500.00	
3002	11718					1	BUD	720500		TELECOMM	$1,992.00	
3002	11718					1	BUD	720600		TRAVL_MILE_MOVE	$15,970.00	
3002	11718					1	BUD	730100		CONSULTING_SVCS	$39,900.00	
3002	11718					1	BUD	750100		NON_CAP_EQUIP	$9,571.00	
3002	11718					1	BUD	780100		REPAIRS_MAINT	$6,485.00	
3002	11718					1	BUD	810500		FACADM	$609,670.00	
3002	11718					1	BUD	850100		CAPITAL_EQ_5K	$5,000.00	

COST SHARE/MATCH/IN-KIND

CSM - Cost Share, Matching and InKind

CSM05 - Departments must document all cost share/unpaid effort that is shown in the proposal.

PROGRAM INCOME INFORMATION

PIN - Program Income

PIN03 - Reportable Program Income must be deposited by the department into the Sponsored Program Income Unapplied Cash account.

PIN04 - Additional - Program Income can be used in addition to sponsor funds to cover project costs that will further the objectives of the legislation under which the grant was made.

TERMS & CONDITIONS (Online Page Navigation: Main Menu > Grants >Awards > Award Profile – Terms Tab)

APV - Additional Approval

APV01 - Prior sponsor approval or notification required for reduction of PI's effort by 25% or more.

APV04 - Prior sponsor approval required to replace PI.

APV07 - Prior sponsor approval required for changes to key personnel that would impact the scope of work.

APV10 - Prior sponsor approval required for changes to work scope.

APV14 - Prior sponsor approval required for the absence of the PI for 3 months or more.

APV15 - Prior sponsor approval required for a second no cost extension or a first no cost extension that is submitted after the agency notification deadline.

APV20 - Prior sponsor approval required for the addition of foreign sites or foreign subcontracts.

EQP - Equipment

EQP13 - Equipment purchases are subject to the previous capitalization threshold of $2500 for F&A calculations until 7/1/15 or until renewal funding is received, whichever occurs first.

FIS - Fiscal

FIS03 - Reversion of funds at termination / final

FIS07 - Deficits may be carried forward.

FIS10 - Grant/contract funds may not be used to pay the salary of an individual at a rate in excess of Executive Level I of the Federal Executive Pay Scale

FIS11 - Modular award - Expenses incurred must be allocated and accounted for in compliance with all applicable cost principles

FIS13 - Deficits may be carried forward within non-competing segments, but may not be carried forward between competing segments.

FIS14 - Grant/contract funds may not be used to pay the salary of an individual at a rate in excess of Executive Level II of the Federal Executive Pay Scale

IPR - IP Rights

IPR02 - Sponsor support must be acknowledged in patent applications and patent issuing.

IPR04 - Title to U of M; non-exclusive, non-transferable, irrevocable, paid-up license to Federal Government.

IPR12 - PI obligation to report inventions (and other IP) to the sponsor (and to OTC per policy).

PRP - Property

PRP02 - U of M receives title to equipment purchased or fabricated under this award.

PRP03 - Sponsor may require transfer of equipment and its title upon award completion for those items costing in excess of $5000.

PUB - Publications

PUB02 - U of M will place an acknowledgement of sponsor support and a disclaimer, as appropriate, on any publication generated by this award or reporting on the award results and/or activity.

PUB07 - Peer-reviewed articles must be submitted to NIH Pubmed Central (PMC) upon acceptance for publication and made publicly available within 12 months. Cite PMC ID number in progress reports/proposals.

RBD - Rebudgeting

RBD10 - Rebudgeting is allowed as long as there is no change in work scope

REG - Regulatory

REG01 - 45 CFR Part 74 - Code of Federal Regulations as it pertains to institutions of higher education.

REG03 - OMB Circular A-21 (www.whitehouse.gov/omb/circulars/a021/a021.html)

REG04 - OMB Circular A-110 (www.whitehouse.gov/omb/circulars/a110/a110.html)

REG05 - OMB Circular A-133 (www.whitehouse.gov/omb/circulars/a133/a133.html)

REG09 - Subject to Streamlined Noncompeting Award Procedures (SNAP)

REG10 - Federal Demonstration Partnership (FDP) guidelines (www.thefdp.org)

REG19 - NIH Grants Policy Statement (http://grants.nih.gov/grants/policy/nihgps_2010/index.htm)

UMN - U of M Requirements

UMN01 - The U of M Effort Certification system must be used to reflect all U of M staff committed effort on this award, whether or not any salary is charged to the award.

UMN02 - Salary cost transfers cannot be made after effort is submitted.

UMN03 - All U of M Policy and Guidelines relating to research must be followed and can be read in detail on the web at http://www.ospa.umn.edu/policiesandprocedures/index.htm

UMN04 - U of M Cost Transfer guidelines state transfers of non-salary expenses on accounts be clearly documented & made within 30 days after the end of the accounting period the charge was processed.

NON-FINANCIAL REPORTING (Online Page Navigation: Main Menu > Grants >Awards > Award Profile – Milestones Tab)

Type: Report **Description:** Progress Report **Due Date:**

Comments: PHS 2590 - noncompeting continuation: Must be submitted via the eRA Commons. Due 45 days prior to the end of the budget period.

INVOICE/BILLING INFORMATION

Basis of Payment: COST_REIMBURSE
Method of Payment: Electronic Fund Transfer
Invoice Form: GPC - GM_LOC
Interim Invoice Deadline: 0
Final Invoice Deadline: 0

Contact Name: Jacqueline Jones
Contact Phone: 410/594-1175
Contact Fax:
Contact Email: jacqueline.jones@cms.hhs.gov
Contact Address: PSC-FMS (301) 443-3020
PARKLAWN BUILDING ROOM 16A-12
5600 FISHERS LANE
ROCKVILLE MD 20857

FINANCIAL REPORTING

Report Form ID: R_FFR
Interim Reporting Deadline: 90

Reporting Frequency: Termination/Final
Final Reporting Deadline: 90

GENERAL PROVISIONS

This document is intended to provide guidance in managing this award, to the Principal Investigator, Academic Unit, and to those who are noted as receiving this document. Its intent is to provide general information regarding University and sponsor policies when given the award. In addition, it is intended to reference specific conditions related to this award. Specific questions should be addressed to the grant administrator from SPA noted below. By accepting this award, the Principal Investigator acknowledges and agrees to abide by all provisions of the sponsoring agency and the University of Minnesota policies related to sponsored projects management, including the Code of Conduct.

U OF M CONTACTS

Administrative questions to SPA staff member:	Paulie Garcia	612/626-2724	garc0027@umn.edu
Billing and financial questions to SFR:	Diane Grimley	612/624-3848	dgrimley@umn.edu

Notice of Award

RESEARCH **Issue Date:** 04/02/2014

Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI089728-05
FAIN: R01AI089728

Principal Investigator(s):
Fang Li, PHD

Project Title: Receptor recognition mechanisms of coronaviruses

David L. Hagen
University of Minnesota Twin Cities
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 554552070

Award e-mailed to: awards@umn.edu

Budget Period: 05/01/2014 – 04/30/2015
Project Period: 05/15/2010 – 04/30/2015

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $373,725 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MINNESOTA in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI089728. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with 42 CFR Part 50 Subpart F. Subsequent to the compliance date of the 2011 revised FCOI regulation (i.e., on or before August 24, 2012), Awardees must be in compliance with all aspects of the 2011 revised regulation; until then, Awardees must comply with the 1995 regulation. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Devon R. Bumbray-Quarles
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI089728-05

Award Calculation (U.S. Dollars)

Federal Direct Costs	$247,500
Federal F&A Costs	$126,225
Approved Budget	$373,725
Federal Share	$373,725
TOTAL FEDERAL AWARD AMOUNT	$373,725
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$373,725

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
5	$373,725	$373,725

Fiscal Information:

CFDA Number:	93.855
EIN:	1416007513A1
Document Number:	RAI089728A
PMS Account Type:	G (Pooled)
Fiscal Year:	2014

IC	CAN	2014
AI	8472364	$373,725

NIH Administrative Data:
PCC: M51C S / **OC**: 414E / **Released**: BUMBRAYD 04/01/2014
Award Processed: 12/26/2013 10:57:56 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI089728-05

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI089728-05

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase V Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI089728. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award represents the final year of the competitive segment for this grant. See the NIH Grants Policy Statement Section 8.6 Closeout for complete closeout requirements at: http://grants.nih.gov/grants/policy/#gps .

A final Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 90 days of the expiration date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/#gps, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) cash transaction data.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 90 days of the expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm.

Unless an application for competitive renewal is submitted, a final progress report must also be submitted within 90 days of the expiration date. Instructions for preparing a Final Progress Report are at: http://grants.nih.gov/grants/funding/finalprogressreport.pdf. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the final progress report. Institute/Centers may accept the progress report contained in competitive renewal (type 2) in lieu of a separate final progress report. Contact the awarding IC for IC-specific policy regarding acceptance of a progress report contained in a competitive renewal application in lieu of a separate final progress report.

NIH **strongly encourages** electronic submission of the final progress report and the final invention statement through the Closeout feature in the Commons, but will accept an email or hard copy submission as indicated below.

Email: The final progress report and final invention statement may be e-mailed as PDF attachments to the NIH Central Closeout Center at: DeasCentralized@od.nih.gov.

Hard copy: Paper submissions of the final progress report and the final invention statement may be faxed to the NIH Division of Central Grants Processing at 301-480-2304, or mailed to:

NIH Division of Central Grants Processing, OER
6705 Rockledge Drive
Suite 5016, Room 5109
MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express mail delivery only)

NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final Progress Report is not required. However, a final FFR is required and should be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI089728-05

In addition to the PI, any absence, replacement, or substantial reduction in effort of the following individual(s) below, requires the written prior approval of the National Institutes of Health awarding component.

Robert Geraghty

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

o A list of the new and/or additional Agent(s) that will be studied;
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;

o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

If you have a question on the award calculation or the terms and conditions of the award, your grants management contact should be Ashley Dash at (301) 402-5936 or via e-mail at dashar@mail.nih.gov.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Devon R. Bumbray-quarles
Email: dbumbray@niaid.nih.gov **Phone**: 301-402-6213 **Fax**: 301-493-0597

Program Official: Erik J. Stemmy
Email: erik.stemmy@nih.gov **Phone**: (301)-402-3947

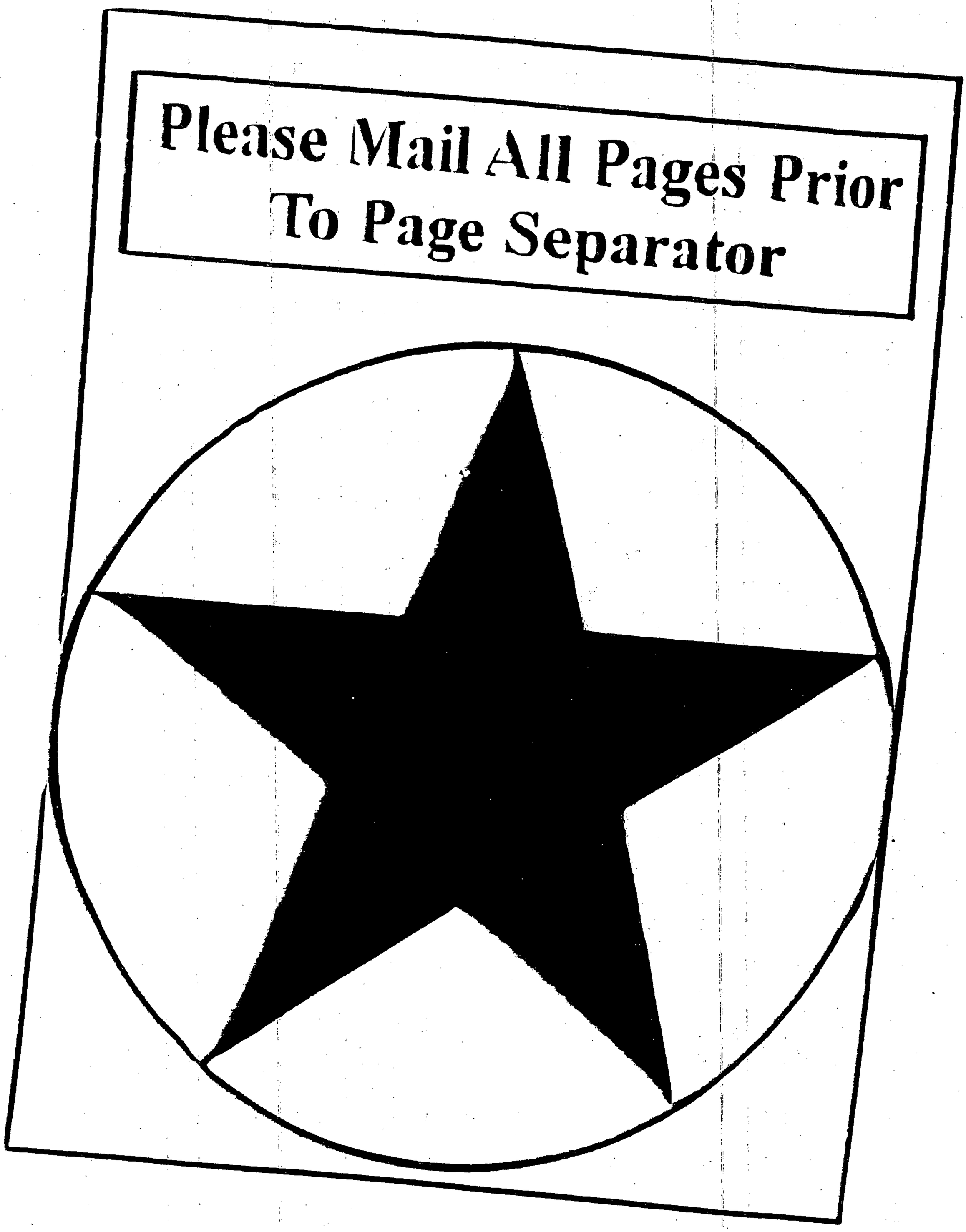
SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI089728-05

INSTITUTION: UNIVERSITY OF MINNESOTA

Budget	Year 5
TOTAL FEDERAL DC	$247,500
TOTAL FEDERAL F&A	$126,225
TOTAL COST	$373,725

Facilities and Administrative Costs	Year 5
F&A Cost Rate 1	51%
F&A Cost Base 1	$247,500
F&A Costs 1	$126,225

Please Mail All Pages Prior To Page Separator



AWARD SETUP INFORMATION PAGE GA Paula DATE 4/10/14

_____ New CON#_____ Primary Project _____ In PreAward/Adv Account____

Parent CON# _____ For DHHS Renewals and DHHS continuations transitioning to P subaccount LOC (includes NIH)

__X__ Modification to CON# 22345 Primary Project 00013782 Related Proposal: CON# 48300

*If a mod has a related proposal, use the Negotiate Award notes on the proposal, not on the existing award

__X__ FFATA
_____ NOT FFATA
For all awards received directly from the federal government, pick appropriate value. Does not apply when we are a subrecipient under a prime federal award.

__X__ eTool completed Already in EFS

INVOICING AND FINANCIAL REPORTING INFORMATION CAN BE FOUND ON THE FOLLOWING PAGE(S) IN THE AWARD DOCUMENT _____

TEMPLATE TERMS AND CONDITIONS (pick one)

___ AHA_SCI_DEV	___ DOD	___ DOE	___ EPA_Fellowship				
___ MMF	___ MNDOT	___ STATE_OF_MN	___ NASA				
___ NIH_Modular	___ NIH_Expanded	___ NIH_Not_Expanded	___ NIH_IND_TR_FSER	___NIH_INST_TR_T32			
___ NSF	___ USDA_FDP	___ USDA_NON-FDP	___ USDE	___VHO AND FNDN			
Terms to be added	_____	_____	_____	_____	_____	_____	_____
Terms to be Removed	_____	_____	_____	_____	_____	_____	_____

N/A

IF ANY CHANGES MADE TO A TEMPLATE ARE NOT SHOWN ABOVE, SAVE THE TERMS IN THE AWARD SET UP FOLDER.

NON-TEMPLATE TERMS AND CONDITIONS or TEMPLATE TERMS with CHANGES

_____ are saved in MyNetworkPlaces\Award Setup\Award Setup-submissions TAB_____ N/A
(\\ovpr.ad.umn.edu\ovpr$\Shared\Groups\Award Setup)

_____ are in eTool

BUDGET INFORMATION FOR FUNDS PROVIDED BY SPONSOR – new awards only (GA enters budgets on mods)

3002 Fund 17HS DeptID N/A

_____ is in eTool

_____ See attached PS-friendly budget

COST SHARE INFORMATION – for new awards only (GA updates the budgets for modifications)

_____ Fund _____ DeptID _____ Program _____ Chartfield 1 _____ Chartfield 2 _____Emplid

_____ is in eTool N/A

_____ See attached PS-friendly budget

_____**NEW PROJECT NEEDED** – enter information on the Information Required to Establish a New Project form and include a completed PS-friendly budget. N/A

ANY ADDITIONAL INFORMATION NEEDED FOR SET UP:

Continuation - 4/30/15
+ $373,725

PUT AWARD FILE IN THE FOLLOWING ORDER:

Agency award documents
Guidance documents (to be included with award emailed to PI/Dept)
Award Imaging Divider page (yellow star sheet)
Award Setup Information page (this page)
PS-friendly budget (if any)
Negotiation notes, proposal correspondence, proposal, etc.

SET ALL NEGOTIATE AWARD NEGOTIATION TYPES TO COMPLETED EXCEPT FOR THE ONE FOR CONTRACT ENTRY AND THE ORIGINAL ONE CREATED BY FILES

INSTRUCTIONS / INFORMATION

NEW – If this award should be set up as a new award/contract in EFS, enter the CON# and Primary Project. If the award is currently in Preaward or Advance Project status, please put an "X" where indicated. Sometimes the dates change from the PreAward to actual award and the system doesn't automatically update the project dates even if you change them on the Proposal pages, so this will alert Award Setup staff to double-check all the dates.

PARENT CON# (DHHS awards only) - Identify the previous CON# of any DHHS renewal awards and DHHS continuation awards that transition to the P subaccount LOC. The previous CON# and renewal/continuation CON#s need to be 'attached' so that cumulative reports can be run in UM Reports.

MODIFICATION – Provide the CON# to be updated and the Primary Project. If this modification has a proposal, also indicate the Proposal CON#.

FFATA – If the award is subject to FFATA, put an "X" in the space provided. FFATA only applies to awards we receive directly from the federal government. FFATA does not apply when we receive a subaward agreement under a federal award.

INVOICING AND FINANCIAL REPORTING INFORMATION – Identify where in the award documents the invoicing and financial reporting information can be found.

TEMPLATE TERMS AND CONDITIONS – Put an "X" in front of the template to be used. Identify specific terms that should be added to or deleted from the template (example: IPR12). If no text needs to be added to any terms, you should NOT save or print out the terms. If text does need to be added to any term on one of the templates see info below.

NON-TEMPLATE TERMS AND CONDITIONS or TEMPLATE TERMS with CHANGES – For awards with terms that are not part of a template or for templates that need added text, check the appropriate terms and add text as needed and save to MyNetworkPlaces\Award Setup\Award Setup-submissions.

BUDGET INFORMATION for SPONSOR FUNDS – Fund and DeptID information is required for each project

COST SHARE INFORMATION - Include Fund, DeptID, Program. Chartfield 1, Chartfield 2, and FinEmplID are optional fields

NEW PROJECT NEEDED – If one of more projects need to be added to the award, enter the information on the Information Required to Establish a New Project form and include a completed PS-friendly budget for each project to be added.

ADDITIONAL INFORMATION NEEDED FOR SET UP – Include information about adjustments needed or items that should be entered on the award - for example, revising F&A by $1 to match the awarded amount, or only 1 project on a multiple-project award needs to be extended, or whether to finalize or not finalize future budget years.

UNIVERSITY OF MINNESOTA

ReportID: UMFGM017F

PROPOSAL SUMMARY

Run Date: 04/02/2014
Run Time: 10:11:10 AM

Status 4 AwdB Award Sent to GA

Grant Administrator:	Garcia,Paulie		
PRF #	786631	Proposal #/Version	CON000000048300/A101
EGMS Proposal #		Sponsor Award #	5R01AI089728-05
Proposal Type	Non-Competing Continuation	Concurrent	N
Previous Award		SubContract	N
Purpose			
Title	Receptor recognition mechanisms of coronaviruses		
Principal Investigator	Li,Fang	lifang@umn.edu	

Budget Summary

Start Date	05/01/2014	End Date	04/30/2016	
Direct Costs	$252,322.00	Indirect Costs	$121,403.00	Total Costs $373,725.00

Sponsor: NATIONAL INSTITUTES OF HEALTH (NIH)

Submission Information

Due Date	03/15/2014	Date Type	Receipt Date
Submission Method	Electronically Only	# of Copies Submitted	_____

Mailing Address

Comments/Corrections

REPA	__verified	__email sent for _____		
IRB	__verified	__pending	__email sent	__Not Applicable
IACUC	__verified	__pending	__email sent	__Not Applicable
IBC	__verified	__pending	__email sent	__Not Applicable
F&A rate	__at full or agency limit	__waiver on file	__waiver requested	
Cost share documented?		__yes	__no	__Not Applicable
ICR sharing?		__yes	__no	

Subject: NoA: 5 R01 AI089728-05 PI: Li, Fang
From: <era-notify@mail.nih.gov>
Date: 4/1/2014 11:10 PM
To: <awards@umn.edu>

Grant Number: 5 R01 AI089728-05
Principal Investigator: Li, Fang
Project Title: Receptor recognition mechanisms of coronaviruses
Institution: UNIVERSITY OF MINNESOTA
CFDA: 93.855
OC: 414E
PCC: M51C S
Award Issue Date: 04/02/2014
Grants Management Officer: Bumbray-Quarles, Devon
Program Official: Stemmy, Erik
Grants Specialist: Bumbray-Quarles, Devon

*** This is an automated notification - Please do not reply to this message. ***

Attachments:

temp78107451797247635556.pdf 98.2 KB

Con-48800
F-ON-22345
786371/88428446
Li
PI
Renewal

(CON-22345)
CON-48300
786631

Status Information

General Grant Information	
Status:	Pending administrative review. Refer any questions to Program Official or Grants Management Specialist.
Institution Name:	UNIVERSITY OF MINNESOTA
School Name:	MEDICAL SCHOOL
School Category:	SCHOOLS OF MEDICINE
Division Name:	NONE
Department Name:	PHARMACOLOGY
PI Name:	Li, Fang
Application ID:	5R01AI089728-05
Proposal Title:	Receptor recognition mechanisms of coronaviruses
Proposal Receipt Date:	03/07/2014
Last Status Update Date:	04/11/2013
Budget Start Date:	05/01/2014
Budget End Date:	04/30/2015
Progress Report Due Date:	03/15/2014
Current Award Notice Date:	
Application Source:	RPPR
Project Period Begin Date:	05/15/2010
Project Period End Date:	04/30/2015
eApplication Status:	
FOA:	[PA07-070] - RESEARCH PROJECT GRANT (PARENT R01)
NIH Appl. ID:	8651407

Other Relevant Documents

e-Application

Additions for Review (0 documents)

Correspondence

Referral Date	Description	Action

Status History

Effective Date	Status Message

Institute or Center Assignment

Institute or Center	Assignment Date
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES **(Primary)**	04/11/2013
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES **(Primary)**	06/05/2013

Application Information

Award Document Number:	RAI089728A
FSR Accepted Code:	N
Snap Indicator Code:	Y
Review Outcome Available:	No
Summary Statement Available:	No
Early Stage Investigator Eligible:	
New Investigator Eligible:	
Eligible for FFATA Reporting:	No

Study Section

Scientific Review Group: VIRA
Council Meeting 2014/00
Date(YYYY/MM):

Advisory Council(AC) Information

Reference Letter(s)

This list shows Reference Letters associated with this particular Grant Application. Principal Investigator can see a list of all Reference Letters within Personal Profile - Reference Letters section on eRA Commons

Contacts

Administration	Name	Phone	Email

Grants Management Specialist(GMS)	Bumbray-Quarles, Devon R.	301-402-6213	dbumbray@niaid.nih.gov
Program Official(PO)	Stemmy, Erik J.	(301)-402-3947	erik.stemmy@nih.gov

Close

[Contact Us | Privacy Notice | Disclaimer | Accessibility | Popup Block Check]

National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland 20892

Department of Health and Human Services

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Screen Id: STA4002@3245

Version 1.13.01

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Approved

UNIVERSITY OF MINNESOTA

PROPOSAL SUMMARY

ReportID: UMFGM017F

Page No: 1
Run Date: 03/07/2014
Run Time: 09:44:58 AM

Status 2 ~~PropB Elect Received at SPA~~

INSTITUTION APPROVED
03.10.14

Grant Administrator: Paulii

PRF #	786631	Proposal #/Version	CON000000048300/V101
EGMS Proposal #		Sponsor Award #	
Proposal Type	Non-Competing Continuation	Concurrent	N
Previous Award		SubContract	N
Purpose	CON-22345		
Title	Receptor recognition mechanisms of coronaviruses		
Principal Investigator	Li,Fang	lifang@umn.edu	

Budget Summary

Start Date	05/01/2014	End Date	04/30/2016		
Direct Costs	~~$0.00~~ 252,322	Indirect Costs	~~$0.00~~ 121,403	Total Costs	~~$0.00~~ 373,725
Sponsor	NATIONAL INSTITUTES OF HEALTH (NIH)				

Submission Information

Due Date	03/15/2014	Date Type	Receipt Date
Submission Method		# of Copies Submitted	

Mailing Address

RPPR DH 3/7/14 @ 5:08pm

Comments/Corrections

REPA	X verified	__email sent for		
IRB	__verified	__pending	__email sent	X Not Applicable
IACUC	X verified	__pending	__email sent	X Not Applicable
IBC	cant check in new system __verified	__pending	__email sent	__Not Applicable
F&A rate	mtc 51% __at full or agency limit	__waiver on file	__waiver requested	
Cost share documented?		__yes	__no	X Not Applicable
ICR sharing?		X yes	__no	

SUBAWARDS 0 #

For more than 4 subawards please record on seperate page

	IDP/DB	Forms 1 & 2	Entered in EF*	New Vendor*
Subrecipient	☐	☐	☐	☐
Subrecipient	☐	☐	☐	☐
Subrecipient	☐	☐	☐	☐
Subrecipient	☐	☐	☐	☐

* Temporary New Vendor Code: 0000002365

03.10.14 logout NLB

E

ELECTRONIC PROPOSAL
_ESNAP
X FEDERAL
_NON-FED.
_ G-GOV.

NO ATTACHMENT

E

UNIVERSITY OF MINNESOTA PRF

EGMS ID: PRF ID: 786631

Proposal Routing Form for External Research, Training or Public Service Support

PS Proposal ID:

1. Sponsor Name: **National Institutes of Health** **Primary Funding Source:**

2. Proposal Title: **Receptor recognition mechanisms of coronaviruses**

3. Sponsor Due Date/Time: **03/15/2014 - 5pm CST** **Receipt** CON-48300

4. RFA/RFP/CFDA/FOA#:

03-07-14 09:46 RCVD

5. Submission Medium: **Electronic [other than Grants.gov]**
Submission via eRA Commons RPPR.

6. University Contact: **Weber, Lynn F** | **(612) 626-6902** | **weber117@umn.edu**

7. Administering Dept: **PHCL PHARMACOLOGY ADMIN** **Department ID: 11718**

8. Principal Investigator:

			Paid	Cost Share	PI	Key
Li, Fang						
lifang	**ID 3694715**	**(612) 625-6149**	**50**	**0**	**X**	**X**
ASSISTANT PROFESSOR [9403]	**PHCL PHARMACOLOGY ADMIN**	**DeptID 11718**				

✔FCOI

9. Investigator(s):

			Paid	Cost Share	PI	Key
Geraghty, Robert J.						
gerag012	**ID 1235946**	**(612) 625-3281**	**8**	**0**		**X**
ASSOCIATE PROFESSOR [9402]	**DRUG DESIGN, CENTER FOR**	**DeptID 11345**				

✔FCOI

10. Proposal is for:

	Basic %	Apllied %	Development %	Total %
Research	**100**	**0**	**0**	**100**

11. Proposal is:

	Agency Award #	EFS Award #	Primary Project
Continuation	**5R01 AI089728-04**	**30021171800013782**	

12. Proposed Duration & Amount Requested:

	Start	End	Direct $$	Indirect $$	F&A Rate	Total
Initial/Current Budget	**05/01/2014**	**04/30/2015**	**252,322**	**121,403**	**51% MTDC**	**373,725**
All Years	**05/01/2014**	**04/30/2016**	**252,322**	**121,403**	**51% MTDC**	**373,725**

[X] Departmental Directions to SPA for Award Set Up
Proposal budget has been prepared outside of EGMS: Department will furnish PeopleSoft-friendly budget to SPA prior to receipt of the award; SPA may pro-rate at the time of award if needed to accommodate a reduced award amount.

[X] **F&A Rate Used** **51% MTDC**
Full F&A Rate

F&A Comments

13. Project Involves:

[X] **Recombinant DNA, Infectious Agents or Biological Toxins** — **There will be recombinant DNA, infectious agents or biological toxins used**

Application status of this project with the Institutional Biosafety Committee

Status	Study Code/Protocol ID	Approval Date
Approved	**121230150H**	**03/04/2013**

[X] **Chemicals** — **There will be chemicals used** — **The appropriate chemical safety training and hazardous waste training records have been completed**

14. Subawards: [ ]

15. Conflict of Interest: ☒

There is not a potential financial and/or business conflict of interest
REPA #: **Approval Date:**
Comments:

16. Inventions: ☐

17. Space: ☒

There is sufficient and suitable space to house this project presently assigned to the principal investigator's or other investigator's department or college.	**3-284 and 7-227**	**Nils Hasselmo Hall and PWB**

18. Resources/Space/Staff: ☒

This project involves University resources, space or staff from more than one department

19. Indirect Cost Recovery: ☒

Academic units have chosen to share ICR revenue and be shared by contributions

PHCL PHARMACOLOGY ADMIN	**11718**	**89**
DRUG DESIGN, CENTER FOR	**11345**	**11**

Comments:
11.5% of F&A shared with Center for Drug Design, Dept. ID 11345
88.5 of F&A for Pharmacology Department, Dept. ID 11718

20. Matching and Cost Sharing: ☐

21. Program Income: ☐

22. International Component: ☐

23. Fairview Health Services: ☐

24. PROPOSAL ABSTRACT or EXECUTIVE SUMMARY

25. NOTES

APPROVALS and CERTIFICATIONS

Principal Investigator:

(1) I certify the information submitted within the application is true, complete and accurate to the best of the PI's knowledge;
(2) that any false, ficticious, or fraudulent statements or claims may subject the PI to criminal, civil, or administrative penalties; and
(3) that the PI agrees to accept the responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of the application.

Investigator(s):

The information provided on this form and in the accompanying proposal is correct to the best of my knowledge. Equipment budgeted in this application is not otherwise available for use on this project from existing departmental or collegiate inventories. In the event this application is awarded, I (we) agree to abide by all applicable institutional and sponsoring agency policies and procedures including the Intellectual Property Policy of the University of Minnesota and to follow commonly accepted scientific practices in recording and maintaining records of research. I (we) certify that I (we) have read the University of Minnesota Code of Conduct policy and agree to abide by the rights and responsibilities as identified therein.

Signature Fang Li 03-06-2014

Fang Li (lifang) : Principal Investigator **Date**

Department Head(s) and/or Division Head(s): Academic Dean(s), Chancellor or Provost:

We certify that the above statements are correct to the best of our knowledge. The commitment of departmental and collegiate resources, if any, has been noted and approved. We agree that the scientific objectives of this application are in keeping with departmental and collegiate goals. We certify that the individuals from our unit(s) listed on this PRF have filed a current Report of External Professional Activities (REPA).

Signature DeJarlais, Carrie L 03-06-2014

DeJarlais, Carrie L (dejar001) : Dept Administrator/Representative **Date**

Signature Siegfried, Jill M 03-06-2014

Siegfried, Jill M (jsiegfri) : Dept Head/Delegate **Date**

Signature Hinske, Liz 03-06-2014

Hinske, Liz (hinsk001) : Dept Administrator/Representative **Date**

Signature Vince, Robert 03-06-2014

Vince, Robert (vince001) : Dept Head/Delegate **Date**

Signature tmeddean 03-06-2014

tmeddean (tmeddean) : Research Associate Dean/Delegate **Date**

Signature Segal, Rosalyn A 03-06-2014

Segal, Rosalyn A (rsegal) : Research Associate Dean/Delegate **Date**

Signature LeBien, Tucker W 03-06-2014

LeBien, Tucker W (lebie001) : Research Associate Dean/Delegate **Date**

Subject: eRA Commons: RPPR for Grant R01AI089728-05 Submitted to NIH
From: <era-notify@mail.nih.gov>
Date: 3/7/2014 5:08 PM
To: <lifang@umn.edu>, <awards@umn.edu>, <hagen027@umn.edu>
CC: <weber117@umn.edu>

*** This is an automated notification - Please do not reply to this message. ***

Hagen, David L has submitted RPPR for Grant R01AI089728-05 associated with Program Director/Principal Investigator Fang Li and may now be viewed in the eRA Commons by going to Status, executing a query for the grant and selecting link associated with the Grant Number. If you have any questions about this email, please contact Hagen, David L at hagen027@umn.edu , who initiated this action.

For any further questions about this email, please contact the eRA Help Desk at our preferred method of contact http://era.nih.gov/help/ or call 1-866-504-9552 (tty: 301-451-5939) or commons@od.nih.gov .

Please access the commons at https://public.era.nih.gov/commons/

For more information please visit http://era.nih.gov/

Welcome: David Hagen
ID: HAGEN027
Institution: UNIVERSITY OF MINNESOTA
Roles: SO

RPPR Menu

Warning Messages

D.1. In the near future, Commons IDs will be required for individuals with the Undergraduate and Graduate Student roles. Completion of a Commons Personal Profile for these individuals is strongly encouraged now. (ID: 200257)

D.1. In the near future all individuals with a Graduate Student or Post Doc role will be required to add the institution associated with their degree to their Commons Profile. (ID: 200258)

D.1. Individuals with Undergraduate, Graduate Student, and Postdoctoral roles on a project will be required to complete the following fields in the Commons Personal Profile : Birthdate, Gender, Race/Ethnicity, U.S. Citizenship Status, and Country of Citizenship, or indicate that they do not wish to respond. Individuals with a Graduate Student role must enter at least one degree and those with a Postdoctoral role must enter a doctoral degree. The profile must also include the name of institution issuing the degree. Completion of these data fields is strongly encouraged now. (ID: 200256)

Application Information

Grant Number:	5R01AI089728-05
Institution:	UNIVERSITY OF MINNESOTA
PD/PI Name:	Li, Fang
Project Title:	Receptor recognition mechanisms of coronaviruses
Due Date:	03/15/2014
Current Reviewer:	Hagen, David L
Status:	Reviewer Work in Progress

Edit | Check for Errors | View | View Routing History | Route | Recall | Submit | Cancel

National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland 20892

Department of Health and Human Services

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Welcome: David Hagen
ID: HAGEN027
Institution: UNIVERSITY OF MINNESOTA
Roles: SO

Routing History

Reviewer Name	Action	Notification Sent	Date of Action	Next Reviewer Name	Comments
Li, Fang	Initiate	02-07-2014 09:03:18	02-07-2014 09:03:18	Li, Fang	
Li, Fang	Route	03-07-2014 10:14:38	03-07-2014 10:14:38	Albertson, Curt	
Garcia, Paulie	Recall	03-07-2014 02:26:30	03-07-2014 02:26:30	Garcia, Paulie	
Garcia, Paulie	Route	03-07-2014 04:55:08	03-07-2014 04:55:08	Hagen, David	
Hagen, David	Submit	03-07-2014 06:08:25	03-07-2014 06:08:25	NIH	

Back

National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland 20892

Department of Health
and Human Services

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Screen Rendered: 03/07/2014 06:08:33 EST
Screen Id: RPPR0010@3254
Version: 3.11.02

GRANTS.GOV
FIND. APPLY. SUCCEED

A. COVER PAGE

Project Title: Receptor recognition mechanisms of coronaviruses	
Grant Number: 5R01AI089728-05	**Project/Grant Period:** 05/15/2010 - 04/30/2015
Reporting Period: 05/01/2013 - 04/30/2014	**Requested Budget Period:** 05/01/2014 - 04/30/2015
Report Term Frequency: Annual	**Date Submitted:** 03/07/2014
Program Director/Principal Investigator Information: FANG LI , PHD **Phone number:** 612-625-6149 **Email:** lifang@umn.edu	**Recipient Organization:** UNIVERSITY OF MINNESOTA UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS, MN 554552070 **DUNS:** 555917996 **EIN:** 1416007513A1 **RECIPIENT ID:** PRF 786631
Change of Contact PD/PI: No	
Administrative Official: PAULIE GARCIA 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE Minneapolis, MN 554552070 **Phone number:** 612-624-5599 **Email:** awards@umn.edu	**Signing Official:** DAVID L HAGEN 200 Oak Street SE Suite 450 Minneapolis, MN 55455 **Phone number:** 612-626-9895 **Email:** hagen027@umn.edu
Human Subjects: No	**Vertebrate Animals:** No
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Coronaviruses use a variety of cellular receptors and co-receptors including proteins and sugars. The diverse usage of receptors has allowed coronaviruses to infect a wide range of mammalian and avian species, and cause respiratory, enteric, systemic and neurological diseases. Two domains in coronavirus spike protein S1 subunit, the N-terminal domain (NTD) and the C-domain, have evolved functions as receptor-binding domains (RBDs). How coronaviruses have evolved to do so has been a major puzzle in virology. To solve this puzzle, this NIH-funded project receptor recognition mechanisms of coronaviruses investigates the molecular and structural basis for the complex receptor-recognition mechanisms of coronaviruses.

There are three specific aims in this project. Aim 1 focuses on the receptor recognition mechanisms of group-I coronaviruses that use S1 C-domains as their RBDs (e.g., NL63 coronavirus (NL63-CoV) and porcine epidemic diarrhea virus (PEDV). Aim 2 focuses on the receptor recognition mechanisms of group-2 coronaviruses that use S1 NTD as their RBDs (e.g., murine hepatitis virus (MHV) and bovine coronavirus (BCoV)). Aim 3 focuses on the receptor recognition mechanisms of group-2 coronaviruses that use S1 C-domain as their RBDs (e.g., MERS coronavirus (MERS-CoV) and SARS coronavirus (SARS-CoV)). The research approaches of this proposal include crystallographic analysis of the virus-receptor interfaces, identification of structural elements important for virus-receptor interactions, and characterization of these elements using crystallographic, biochemical, molecular, and virological tools.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITITES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

For the next funding year, we will continue to focus on all three specific aims.
For specific Aim 1, we will investigate the structural mechanism whereby the group-1 PEDV RBD (i.e., S1 C-domain) recognizes its receptor porcine APN. In recent years, PEDV has become increasingly problematic in USA and many other countries, and causes huge losses in pork industry. The mortality rates of PEDV vary from 50 to 100%, and different PEDV strains have been isolated containing changes in the RBD. We will determine the crystal structures of the RBD from different PEDV strains in complex with porcine APN. In addition, we will examine the binding interactions between the RBD from different PEDV strains and porcine APN using a variety of biochemical assays such as surface plasmon resonance, AlphaScreen, and pseudovirus entry assays. These studies will reveal the receptor recognition mechanisms and host adaptive evolution of PEDV, and provide structural bases for design of novel and highly effective PEDV subunit vaccines.
For specific Aim 2, we will determine the crystal structure of group-2 BCoV NTD complexed with its sugar substrate. Based on the successful structure determination of BCoV NTD by itself, we will synthesize the sugar substrates of BCoV NTD, and use either crystal soaking or co-crystallization to determine the structure of the complex. This study will reveal detailed structural basis for the sugar-binding mechanism of group-2 coronaviruses.
For specific Aim 3, we will study the host range and cross-species infections of MERS-CoV. More specifically, we will examine how MERS-CoV RBD (i.e., S1 C-domain) recognizes DPP4 orthologues from different animal species and how we can convert those DPP4 orthologues with low receptor activities into efficient receptors for MERS-CoV. The approaches include structure determinations of MERS-CoV RBD in complex with DPP4 orthologues, structure-based rational design, biochemical assays, and pseudovirus entry assays. These studies will provide structural bases for animal model development for MERS-CoV infections.
Also for specific Aim 3, we will investigate the receptor recognition mechanisms and potential cross-species infections of MERS-CoV-like and SARS-CoV-like bat coronaviruses. A constant and long-term threat to human health is cross-species transmission of MERS-CoV-like and SARS-CoV-like coronaviruses from bats to humans. This process is poorly understood. We will investigate the receptor usage and

cell entry mechanisms of bat coronavirus HKU4 and HKU5, which are genetically related to MERS-CoV, and bat coronaviruses WIV1, which is genetically related to SARS-CoV. These studies will be critical for evaluating emerging disease potentials of bat coronaviruses and for preventing and controlling their spreads in humans.

B. Studies and Results

We made the following discoveries in 2013.

First, Chen et al. (October 2013, Journal of Virology) have identified the receptor-binding domain (RBD) for the newly emerged MERS coronavirus (MERS-CoV) and determined its crystal structure. This study also presents a structural comparison of MERS-CoV RBD with other coronavirus RBDs, successfully positioning MERS-CoV on the landscape of coronavirus evolution and providing insights into receptor binding by MERS-CoV. Furthermore, we found that MERS-CoV RBD functions as an effective entry inhibitor of MERS-CoV. The identified MERS-CoV RBD may also serve as a potential candidate for MERS-CoV subunit vaccines. Overall, this study enhances our understanding of the evolution of coronavirus RBDs, provides insights into receptor recognition by MERS-CoV, and may help control the transmission of MERS-CoV in humans.

Second, Li (October 2013, Antiviral Research) reviews the latest knowledge about how RBDs from different SARS-CoV strains interact with ACE2 from several animal species. Detailed research on these RBD/ACE2 interactions has established important principles on host receptor adaptations, cross-species infections, and future evolution of SARS-CoV. These principles may apply to other emerging animal viruses, including the recently emerged Middle East respiratory syndrome coronavirus (MERS-CoV).

Third, Chen et al. (April 2013, Journal of Virology) analyzed the structural relationships between BCoV spike protein lectin domain and other known viral lectins. Influenza and other viruses use host-cell-surface sugars as receptors. Here we show that the sugar-binding domains in influenza hemagglutinin, BCoV spike protein lectin domain, and other viral lectins share the same structural fold as human galectins (host lectins). Unlike the easily accessible sugar-binding sites in human galectins, the sugar-binding sites in viral lectins are hidden in cavities. We propose that these viral lectins originated from host lectins but have evolved to use hidden sugar-binding sites to evade host immune attacks.

Fourth, Chen at al. (2014, Journal of Virology, under revision) demonstrated that dipeptidyl peptidase 4 (DPP4) from humans, not mice, serves as a receptor for MERS-CoV. Further, we identified residue differences between human and mouse DPP4 that largely account for their differential receptor activities. These residue differences form a species barrier between humans and mice for MERS-CoV infection. An improved understanding of MERS-CoV host range may facilitate development of transgenic mouse model for MERS-CoV infection.

Fifth, Du et al. (2014, Journal of Virology under revision) have developed a neutralizing monoclonal antibody (mAb), designated Mersmab1, which potently blocks MERS-CoV entry into host cells. Biochemical assays reveal that Mersmab1 binds to the receptor-binding domain (RBD) of the MERS-CoV spike protein to, in turn, competitively block the binding of the RBD to its cellular dipeptidyl peptidase 4 (DPP4) receptor. Furthermore, alanine scanning of the RBD has identified several residues at the DPP4-binding surface that serve as neutralizing epitopes for Mersmab1. These results suggest that Mersmab1 can be humanized as a promising immunotherapeutic for treating and preventing MERS-CoV infection. Additionally, it is also useful to study the conformation and antigenicity of MERS-CoV spike protein S1 subunit for guiding the design of anti-MERS-CoV vaccines.

C. Significance

Our research has been the major driving force in the coronavirus field to understand coronavirus/receptor interactions. Prior to the SARS epidemic, no structural information was available for any coronavirus S1 subunit, despite decades of extensive research. By now, we have successfully determined the crystal structures of group-1 NL63-CoV C-domain complexed with its receptor ACE2, group-2 SARS-CoV C-domain complexed with its receptor ACE2, group-2 MHV NTD complexed with its receptor CEACAM1, group-2 BCoV NTD, group-2 MERS-CoV C-domain, and group-1 coronavirus receptor APN. To date, these structures account for the majority of the structures available for any coronavirus S1 or coronavirus-S1/receptor complexes. By determining these structures, we have solved several important puzzles in virology, such as how animal viruses can cross species barriers to infect humans through one or a few seemingly trivial mutations in their RBDs, how different viruses recognize the same receptor by targeting common virus-binding hotspots on the receptor, how similar viruses recognize different receptors through subtle structural changes in viral RBDs, and how viruses can acquire proteins from their hosts and evolve these host proteins into viral RBDs with different receptor specificity. Protein biochemistry and virological methods have also been systematically performed to corroborate our structural findings. By elucidating these structures, we have also been able to reconstruct the evolutionary history of coronaviruses. Furthermore, our detailed structural analyses of SARS-CoV/receptor interactions have significantly prepared humans for any potential future SARS epidemics.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Complete	Chen L, Li F. Structural analysis of the evolutionary origins of influenza virus hemagglutinin and other viral lectins. J Virol. 2013 Apr;87(7):4118-20. PubMed PMID: 23365425; PubMed Central PMCID: PMC3624229.
Complete	Chen Y, Rajashankar KR, Yang Y, Agnihothram SS, Liu C, Lin YL, Baric RS, Li F. Crystal structure of the receptor-binding domain from newly emerged Middle East respiratory syndrome coronavirus. J Virol. 2013 Oct;87(19):10777-83. PubMed PMID: 23903833; PubMed Central PMCID: PMC3807420.
Complete	Li F. Receptor recognition and cross-species infections of SARS coronavirus. Antiviral Res. 2013 Oct;100(1):246-54. PubMed PMID: 23994189; PubMed Central PMCID: PMC3840050.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

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F. Project Generated Resources

This research did not generate cell lines, DNA probes, animal models, protocols, or software. De-identified raw data from the experiments and computer programs (CCP4 macromolecular crystallography scripts) used for protein structure determinations are available to anyone upon written request to the PI. Peer-reviewed publications accepted or published during the past project period are being or have been entered in NIHMS and PubMed Central.

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
FANGLI	Y	Li, Fang,	Minn Stat 13.43	Minn Stat 13.43	PHD	PD/PI	6	0	0			NA
ROBERT.GERAGHTY	Y	Geraghty, Robert,	Minn Stat 13.43	Minn Stat 13.43	BS,PHD	Co-Investigator	1	0	0			NA
WENBOZHOU	N	Zhou, Wenbo,	Minn Stat 13.43	Minn Stat 13.43	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	3	0	0			NA
Minn Stat 13.32	N	Minn Stat 13.32	Minn Stat 13.32	Minn Stat 13.32	MS	Graduate Student (research assistant)	4	0	0			NA
YAOQING	N	Chen, Yaoqing,	Minn Stat 13.43	Minn Stat 13.43	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	7	0	0			NA
Minn Stat 13.32	N	Minn Stat 13.32	Minn Stat 13.32	Minn Stat 13.32	BS	Graduate Student (research assistant)	6	0	0			NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

NOTHING TO REPORT

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

NOTHING TO REPORT

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

NOTHING TO REPORT

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

NOTHING TO REPORT

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

No

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: University of Minnesota	555917996	MN-005	3-284 Nils Hasselmo Hall 312 Church Street SE Minneapolis MN 554550215
University of of Minnesota	555917996	MN-005	7-227 Phillips Wangensteen Building 516 Delaware Street SE Minneapolis MN 55455

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

 final

A. COVER PAGE

Project Title: Receptor recognition mechanisms of coronaviruses	
Grant Number: 5R01AI089728-05	**Project/Grant Period:** 05/15/2010 - 04/30/2015
Reporting Period: 05/01/2013 - 04/30/2014	**Requested Budget Period:** 05/01/2014 - 04/30/2015
Report Term Frequency: Annual	**Date Submitted:**
Program Director/Principal Investigator Information: FANG LI , PHD **Phone number:** 612-625-6149 **Email:** lifang@umn.edu	**Recipient Organization:** UNIVERSITY OF MINNESOTA UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS, MN 554552070 **DUNS:** 555917996 **EIN:** 1416007513A1 **RECIPIENT ID:** PRF 786631
Change of Contact PD/PI: No	
Administrative Official: PAULIE GARCIA 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE Minneapolis, MN 554552070 **Phone number:** 612-624-5599 **Email:** awards@umn.edu	**Signing Official:** DAVID L HAGEN 200 Oak Street SE Suite 450 Minneapolis, MN 55455 **Phone number:** 612-626-9895 **Email:** hagen027@umn.edu
Human Subjects: No	**Vertebrate Animals:** No
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Coronaviruses use a variety of cellular receptors and co-receptors including proteins and sugars. The diverse usage of receptors has allowed coronaviruses to infect a wide range of mammalian and avian species, and cause respiratory, enteric, systemic and neurological diseases. Two domains in coronavirus spike protein S1 subunit, the N-terminal domain (NTD) and the C-domain, have evolved functions as receptor-binding domains (RBDs). How coronaviruses have evolved to do so has been a major puzzle in virology. To solve this puzzle, this NIH-funded project receptor recognition mechanisms of coronaviruses investigates the molecular and structural basis for the complex receptor-recognition mechanisms of coronaviruses.

There are three specific aims in this project. Aim 1 focuses on the receptor recognition mechanisms of group-I coronaviruses that use S1 C-domains as their RBDs (e.g., NL63 coronavirus (NL63-CoV) and porcine epidemic diarrhea virus (PEDV). Aim 2 focuses on the receptor recognition mechanisms of group-2 coronaviruses that use S1 NTD as their RBDs (e.g., murine hepatitis virus (MHV) and bovine coronavirus (BCoV)). Aim 3 focuses on the receptor recognition mechanisms of group-2 coronaviruses that use S1 C-domain as their RBDs (e.g., MERS coronavirus (MERS-CoV) and SARS coronavirus (SARS-CoV)). The research approaches of this proposal include crystallographic analysis of the virus-receptor interfaces, identification of structural elements important for virus-receptor interactions, and characterization of these elements using crystallographic, biochemical, molecular, and virological tools.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITITES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

For the next funding year, we will continue to focus on all three specific aims.
For specific Aim 1, we will investigate the structural mechanism whereby the group-1 PEDV RBD (i.e., S1 C-domain) recognizes its receptor porcine APN. In recent years, PEDV has become increasingly problematic in USA and many other countries, and causes huge losses in pork industry. The mortality rates of PEDV vary from 50 to 100%, and different PEDV strains have been isolated containing changes in the RBD. We will determine the crystal structures of the RBD from different PEDV strains in complex with porcine APN. In addition, we will examine the binding interactions between the RBD from different PEDV strains and porcine APN using a variety of biochemical assays such as surface plasmon resonance, AlphaScreen, and pseudovirus entry assays. These studies will reveal the receptor recognition mechanisms and host adaptive evolution of PEDV, and provide structural bases for design of novel and highly effective PEDV subunit vaccines.
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For specific Aim 3, we will study the host range and cross-species infections of MERS-CoV. More specifically, we will examine how MERS-CoV RBD (i.e., S1 C-domain) recognizes DPP4 orthologues from different animal species and how we can convert those DPP4 orthologues with low receptor activities into efficient receptors for MERS-CoV. The approaches include structure determinations of MERS-CoV RBD in complex with DPP4 orthologues, structure-based rational design, biochemical assays, and pseudovirus entry assays. These studies will provide structural bases for animal model development for MERS-CoV infections.
Also for specific Aim 3, we will investigate the receptor recognition mechanisms and potential cross-species infections of MERS-CoV-like and SARS-CoV-like bat coronaviruses. A constant and long-term threat to human health is cross-species transmission of MERS-CoV-like and SARS-CoV-like coronaviruses from bats to humans. This process is poorly understood. We will investigate the receptor usage and

cell entry mechanisms of bat coronavirus HKU4 and HKU5, which are genetically related to MERS-CoV, and bat coronaviruses WIV1, which is genetically related to SARS-CoV. These studies will be critical for evaluating emerging disease potentials of bat coronaviruses and for preventing and controlling their spreads in humans.

B. Studies and Results

We made the following discoveries in 2013.

First, Chen et al. (October 2013, Journal of Virology) have identified the receptor-binding domain (RBD) for the newly emerged MERS coronavirus (MERS-CoV) and determined its crystal structure. This study also presents a structural comparison of MERS-CoV RBD with other coronavirus RBDs, successfully positioning MERS-CoV on the landscape of coronavirus evolution and providing insights into receptor binding by MERS-CoV. Furthermore, we found that MERS-CoV RBD functions as an effective entry inhibitor of MERS-CoV. The identified MERS-CoV RBD may also serve as a potential candidate for MERS-CoV subunit vaccines. Overall, this study enhances our understanding of the evolution of coronavirus RBDs, provides insights into receptor recognition by MERS-CoV, and may help control the transmission of MERS-CoV in humans.

Second, Li (October 2013, Antiviral Research) reviews the latest knowledge about how RBDs from different SARS-CoV strains interact with ACE2 from several animal species. Detailed research on these RBD/ACE2 interactions has established important principles on host receptor adaptations, cross-species infections, and future evolution of SARS-CoV. These principles may apply to other emerging animal viruses, including the recently emerged Middle East respiratory syndrome coronavirus (MERS-CoV).

Third, Chen et al. (April 2013, Journal of Virology) analyzed the structural relationships between BCoV spike protein lectin domain and other known viral lectins. Influenza and other viruses use host-cell-surface sugars as receptors. Here we show that the sugar-binding domains in influenza hemagglutinin, BCoV spike protein lectin domain, and other viral lectins share the same structural fold as human galectins (host lectins). Unlike the easily accessible sugar-binding sites in human galectins, the sugar-binding sites in viral lectins are hidden in cavities. We propose that these viral lectins originated from host lectins but have evolved to use hidden sugar-binding sites to evade host immune attacks.

Fourth, Chen at al. (2014, Journal of Virology, under revision) demonstrated that dipeptidyl peptidase 4 (DPP4) from humans, not mice, serves as a receptor for MERS-CoV. Further, we identified residue differences between human and mouse DPP4 that largely account for their differential receptor activities. These residue differences form a species barrier between humans and mice for MERS-CoV infection. An improved understanding of MERS-CoV host range may facilitate development of transgenic mouse model for MERS-CoV infection.

Fifth, Du et al. (2014, Journal of Virology under revision) have developed a neutralizing monoclonal antibody (mAb), designated Mersmab1, which potently blocks MERS-CoV entry into host cells. Biochemical assays reveal that Mersmab1 binds to the receptor-binding domain (RBD) of the MERS-CoV spike protein to, in turn, competitively block the binding of the RBD to its cellular dipeptidyl peptidase 4 (DPP4) receptor. Furthermore, alanine scanning of the RBD has identified several residues at the DPP4-binding surface that serve as neutralizing epitopes for Mersmab1. These results suggest that Mersmab1 can be humanized as a promising immunotherapeutic for treating and preventing MERS-CoV infection. Additionally, it is also useful to study the conformation and antigenicity of MERS-CoV spike protein S1 subunit for guiding the design of anti-MERS-CoV vaccines.

C. Significance

Our research has been the major driving force in the coronavirus field to understand coronavirus/receptor interactions. Prior to the SARS epidemic, no structural information was available for any coronavirus S1 subunit, despite decades of extensive research. By now, we have successfully determined the crystal structures of group-1 NL63-CoV C-domain complexed with its receptor ACE2, group-2 SARS-CoV C-domain complexed with its receptor ACE2, group-2 MHV NTD complexed with its receptor CEACAM1, group-2 BCoV NTD, group-2 MERS-CoV C-domain, and group-1 coronavirus receptor APN. To date, these structures account for the majority of the structures available for any coronavirus S1 or coronavirus-S1/receptor complexes. By determining these structures, we have solved several important puzzles in virology, such as how animal viruses can cross species barriers to infect humans through one or a few seemingly trivial mutations in their RBDs, how different viruses recognize the same receptor by targeting common virus-binding hotspots on the receptor, how similar viruses recognize different receptors through subtle structural changes in viral RBDs, and how viruses can acquire proteins from their hosts and evolve these host proteins into viral RBDs with different receptor specificity. Protein biochemistry and virological methods have also been systematically performed to corroborate our structural findings. By elucidating these structures, we have also been able to reconstruct the evolutionary history of coronaviruses. Furthermore, our detailed structural analyses of SARS-CoV/receptor interactions have significantly prepared humans for any potential future SARS epidemics.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Complete	Chen L, Li F. Structural analysis of the evolutionary origins of influenza virus hemagglutinin and other viral lectins. J Virol. 2013 Apr;87(7):4118-20. PubMed PMID: 23365425; PubMed Central PMCID: PMC3624229.
Complete	Chen Y, Rajashankar KR, Yang Y, Agnihothram SS, Liu C, Lin YL, Baric RS, Li F. Crystal structure of the receptor-binding domain from newly emerged Middle East respiratory syndrome coronavirus. J Virol. 2013 Oct;87(19):10777-83. PubMed PMID: 23903833; PubMed Central PMCID: PMC3807420.
Complete	Li F. Receptor recognition and cross-species infections of SARS coronavirus. Antiviral Res. 2013 Oct;100(1):246-54. PubMed PMID: 23994189; PubMed Central PMCID: PMC3840050.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

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F. Project Generated Resources

This research did not generate cell lines, DNA probes, animal models, protocols, or software. De-identified raw data from the experiments and computer programs (CCP4 macromolecular crystallography scripts) used for protein structure determinations are available to anyone upon written request to the PI. Peer-reviewed publications accepted or published during the past project period are being or have been entered in NIHMS and PubMed Central.

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
FANGLI	Y	Li, Fang,	Minn Stat 13.43	Minn Stat 13.43	PHD	PD/PI	6	0	0			NA
ROBERT.GERAGHTY	Y	Geraghty, Robert,	Minn Stat 13.43	Minn Stat 13.43	BS,PHD	Co-Investigator	1	0	0			NA
WENBOZHOU	N	Zhou, Wenbo,	Minn Stat 13.43	Minn Stat 13.43	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	3	0	0			NA
Minn Stat 13.32	N	Minn Stat 13.32	Minn Stat 13.32	Minn Stat 13.32	MS	Graduate Student (research assistant)	4	0	0			NA
YAOQING	N	Chen, Yaoqing,	Minn Stat 13.43	Minn Stat 13.43	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	7	0	0			NA
Minn Stat 13.32	N	Minn Stat 13.32	Minn Stat 13.32	Minn Stat 13.32	BS	Graduate Student (research assistant)	6	0	0			NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

NOTHING TO REPORT

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

NOTHING TO REPORT

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

NOTHING TO REPORT

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

NOTHING TO REPORT

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES? Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE? NOTHING TO REPORT
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER? Not Applicable
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)? NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

No

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: University of Minnesota	555917996	MN-005	3-284 Nils Hasselmo Hall 312 Church Street SE Minneapolis MN 554550215
University of of Minnesota	555917996	MN-005	7-227 Phillips Wangensteen Building 516 Delaware Street SE Minneapolis MN 55455

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

A. COVER PAGE

Project Title: Receptor recognition mechanisms of coronaviruses	
Grant Number: 5R01AI089728-05	**Project/Grant Period:** 05/15/2010 - 04/30/2015
Reporting Period: 05/01/2013 - 04/30/2014	**Requested Budget Period:** 05/01/2014 - 04/30/2015
Report Term Frequency: Annual	**Date Submitted:**
Program Director/Principal Investigator Information: FANG LI , PHD **Phone number:** 612-625-6149 **Email:** lifang@umn.edu	**Recipient Organization:** UNIVERSITY OF MINNESOTA UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS, MN 554552070 **DUNS:** 555917996 **EIN:** 1416007513A1 **RECIPIENT ID:** PRF 786631
Change of Contact PD/PI: No	
Administrative Official: PAULIE GARCIA 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE Minneapolis, MN 554552070 **Phone number:** 612-624-5599 **Email:** awards@umn.edu	**Signing Official:** KEVIN MCKOSKEY 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS, MN 554552070 **Phone number:** 612-624-5599 **Email:** kevin@umn.edu
Human Subjects: No	**Vertebrate Animals:** No
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Coronaviruses use a variety of cellular receptors and co-receptors including proteins and sugars. The diverse usage of receptors has allowed coronaviruses to infect a wide range of mammalian and avian species, and cause respiratory, enteric, systemic and neurological diseases. Two domains in coronavirus spike protein S1 subunit, the N-terminal domain (NTD) and the C-domain, have evolved functions as receptor-binding domains (RBDs). How coronaviruses have evolved to do so has been a major puzzle in virology. To solve this puzzle, this NIH-funded project receptor recognition mechanisms of coronaviruses investigates the molecular and structural basis for the complex receptor-recognition mechanisms of coronaviruses.

There are three specific aims in this project. Aim 1 focuses on the receptor recognition mechanisms of group-I coronaviruses that use S1 C-domains as their RBDs (e.g., NL63 coronavirus (NL63-CoV) and porcine epidemic diarrhea virus (PEDV). Aim 2 focuses on the receptor recognition mechanisms of group-2 coronaviruses that use S1 NTD as their RBDs (e.g., murine hepatitis virus (MHV) and bovine coronavirus (BCoV)). Aim 3 focuses on the receptor recognition mechanisms of group-2 coronaviruses that use S1 C-domain as their RBDs (e.g., MERS coronavirus (MERS-CoV) and SARS coronavirus (SARS-CoV)). The research approaches of this proposal include crystallographic analysis of the virus-receptor interfaces, identification of structural elements important for virus-receptor interactions, and characterization of these elements using crystallographic, biochemical, molecular, and virological tools.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Fang_Li_RESULTS_SIGNIF_030314.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITITES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

For the next funding year, we will continue to focus on all three specific aims.
For specific Aim 1, we will investigate the structural mechanism whereby the group-1 PEDV RBD (i.e., S1 C-domain) recognizes its receptor porcine APN. In recent years, PEDV has become increasingly problematic in USA and many other countries, and causes huge losses in pork industry. The mortality rates of PEDV vary from 50 to 100%, and different PEDV strains have been isolated containing changes in the RBD. We will determine the crystal structures of the RBD from different PEDV strains in complex with porcine APN. In addition, we will examine the binding interactions between the RBD from different PEDV strains and porcine APN using a variety of biochemical assays such as surface plasmon resonance, AlphaScreen, and pseudovirus entry assays. These studies will reveal the receptor recognition mechanisms and host adaptive evolution of PEDV, and provide structural bases for design of novel and highly effective PEDV subunit vaccines.
For specific Aim 2, we will determine the crystal structure of group-2 BCoV NTD complexed with its sugar substrate. Based on the successful structure determination of BCoV NTD by itself, we will synthesize the sugar substrates of BCoV NTD, and use either crystal soaking or co-crystallization to determine the structure of the complex. This study will reveal detailed structural basis for the sugar-binding mechanism of group-2 coronaviruses.
For specific Aim 3, we will study the host range and cross-species infections of MERS-CoV. More specifically, we will examine how MERS-CoV RBD (i.e., S1 C-domain) recognizes DPP4 orthologues from different animal species and how we can convert those DPP4 orthologues with low receptor activities into efficient receptors for MERS-CoV. The approaches include structure determinations of MERS-CoV RBD in complex with DPP4 orthologues, structure-based rational design, biochemical assays, and pseudovirus entry assays. These studies will provide structural bases for animal model development for MERS-CoV infections.
Also for specific Aim 3, we will investigate the receptor recognition mechanisms and potential cross-species infections of MERS-CoV-like and SARS-CoV-like bat coronaviruses. A constant and long-term threat to human health is cross-species transmission of MERS-CoV-like and SARS-CoV-like coronaviruses from bats to humans. This process is poorly understood. We will investigate the receptor usage and

cell entry mechanisms of bat coronavirus HKU4 and HKU5, which are genetically related to MERS-CoV, and bat coronaviruses WIV1, which is genetically related to SARS-CoV. These studies will be critical for evaluating emerging disease potentials of bat coronaviruses and for preventing and controlling their spreads in humans.

B. Studies and Results

We made the following discoveries in 2013.

First, Chen et al. (October 2013, Journal of Virology) have identified the receptor-binding domain (RBD) for the newly emerged MERS coronavirus (MERS-CoV) and determined its crystal structure. This study also presents a structural comparison of MERS-CoV RBD with other coronavirus RBDs, successfully positioning MERS-CoV on the landscape of coronavirus evolution and providing insights into receptor binding by MERS-CoV. Furthermore, we found that MERS-CoV RBD functions as an effective entry inhibitor of MERS-CoV. The identified MERS-CoV RBD may also serve as a potential candidate for MERS-CoV subunit vaccines. Overall, this study enhances our understanding of the evolution of coronavirus RBDs, provides insights into receptor recognition by MERS-CoV, and may help control the transmission of MERS-CoV in humans.

Second, Li (October 2013, Antiviral Research) reviews the latest knowledge about how RBDs from different SARS-CoV strains interact with ACE2 from several animal species. Detailed research on these RBD/ACE2 interactions has established important principles on host receptor adaptations, cross-species infections, and future evolution of SARS-CoV. These principles may apply to other emerging animal viruses, including the recently emerged Middle East respiratory syndrome coronavirus (MERS-CoV).

Third, Chen et al. (April 2013, Journal of Virology) analyzed the structural relationships between BCoV spike protein lectin domain and other known viral lectins. Influenza and other viruses use host-cell-surface sugars as receptors. Here we show that the sugar-binding domains in influenza hemagglutinin, BCoV spike protein lectin domain, and other viral lectins share the same structural fold as human galectins (host lectins). Unlike the easily accessible sugar-binding sites in human galectins, the sugar-binding sites in viral lectins are hidden in cavities. We propose that these viral lectins originated from host lectins but have evolved to use hidden sugar-binding sites to evade host immune attacks.

Fourth, Chen at al. (2014, Journal of Virology, under revision) demonstrated that dipeptidyl peptidase 4 (DPP4) from humans, not mice, serves as a receptor for MERS-CoV. Further, we identified residue differences between human and mouse DPP4 that largely account for their differential receptor activities. These residue differences form a species barrier between humans and mice for MERS-CoV infection. An improved understanding of MERS-CoV host range may facilitate development of transgenic mouse model for MERS-CoV infection.

Fifth, Du et al. (2014, Journal of Virology under revision) have developed a neutralizing monoclonal antibody (mAb), designated Mersmab1, which potently blocks MERS-CoV entry into host cells. Biochemical assays reveal that Mersmab1 binds to the receptor-binding domain (RBD) of the MERS-CoV spike protein to, in turn, competitively block the binding of the RBD to its cellular dipeptidyl peptidase 4 (DPP4) receptor. Furthermore, alanine scanning of the RBD has identified several residues at the DPP4-binding surface that serve as neutralizing epitopes for Mersmab1. These results suggest that Mersmab1 can be humanized as a promising immunotherapeutic for treating and preventing MERS-CoV infection. Additionally, it is also useful to study the conformation and antigenicity of MERS-CoV spike protein S1 subunit for guiding the design of anti-MERS-CoV vaccines.

C. Significance

Our research has been the major driving force in the coronavirus field to understand coronavirus/receptor interactions. Prior to the SARS epidemic, no structural information was available for any coronavirus S1 subunit, despite decades of extensive research. By now, we have successfully determined the crystal structures of group-1 NL63-CoV C-domain complexed with its receptor ACE2, group-2 SARS-CoV C-domain complexed with its receptor ACE2, group-2 MHV NTD complexed with its receptor CEACAM1, group-2 BCoV NTD, group-2 MERS-CoV C-domain, and group-1 coronavirus receptor APN. To date, these structures account for the majority of the structures available for any coronavirus S1 or coronavirus-S1/receptor complexes. By determining these structures, we have solved several important puzzles in virology, such as how animal viruses can cross species barriers to infect humans through one or a few seemingly trivial mutations in their RBDs, how different viruses recognize the same receptor by targeting common virus-binding hotspots on the receptor, how similar viruses recognize different receptors through subtle structural changes in viral RBDs, and how viruses can acquire proteins from their hosts and evolve these host proteins into viral RBDs with different receptor specificity. Protein biochemistry and virological methods have also been systematically performed to corroborate our structural findings. By elucidating these structures, we have also been able to reconstruct the evolutionary history of coronaviruses. Furthermore, our detailed structural analyses of SARS-CoV/receptor interactions have significantly prepared humans for any potential future SARS epidemics.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Complete	Chen L, Li F. Structural analysis of the evolutionary origins of influenza virus hemagglutinin and other viral lectins. J Virol. 2013 Apr;87(7):4118-20. PubMed PMID: 23365425; PubMed Central PMCID: PMC3624229.
Complete	Chen Y, Rajashankar KR, Yang Y, Agnihothram SS, Liu C, Lin YL, Baric RS, Li F. Crystal structure of the receptor-binding domain from newly emerged Middle East respiratory syndrome coronavirus. J Virol. 2013 Oct;87(19):10777-83. PubMed PMID: 23903833; PubMed Central PMCID: PMC3807420.
Complete	Li F. Receptor recognition and cross-species infections of SARS coronavirus. Antiviral Res. 2013 Oct;100(1):246-54. PubMed PMID: 23994189; PubMed Central PMCID: PMC3840050.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

File uploaded: Fang_Li_NIH_report_030314 RESOURCES GEN.pdf

F. Project Generated Resources

This research did not generate cell lines, DNA probes, animal models, protocols, or software. De-identified raw data from the experiments and computer programs (CCP4 macromolecular crystallography scripts) used for protein structure determinations are available to anyone upon written request to the PI. Peer-reviewed publications accepted or published during the past project period are being or have been entered in NIHMS and PubMed Central.

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
FANGLI	Y	Li, Fang,	Minn Stat 13.43	Minn Stat 13.43	PHD	PD/PI	6	0	0			NA
ROBERT.GERAGHTY	Y	Geraghty, Robert,	Minn Stat 13.43	Minn Stat 13.43	BS,PHD	Co-Investigator	1	0	0			NA
WENBOZHOU	N	Zhou, Wenbo,	Minn Stat 13.43	Minn Stat 13.43	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	3	0	0			NA
Minn Stat 13.32	N	Minn Stat 13.32	Minn Stat 13.32	Minn Stat 13.32	MS	Graduate Student (research assistant)	4	0	0			NA
YAOQING	N	Chen, Yaoqing,	Minn Stat 13.43	Minn Stat 13.43	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	7	0	0			NA
Minn Stat 13.32	N	Minn Stat 13.32	Minn Stat 13.32	Minn Stat 13.32	BS	Graduate Student (research assistant)	6	0	0			NA

OK

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)

Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

NOTHING TO REPORT

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

NOTHING TO REPORT

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

NOTHING TO REPORT

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

NOTHING TO REPORT

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES? Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE? NOTHING TO REPORT
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER? Not Applicable
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)? NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

No

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: University of Minnesota	555917996	MN-005	3-284 Nils Hasselmo Hall 312 Church Street SE Minneapolis MN 554550215
University of of Minnesota	555917996	MN-005	7-227 Phillips Wangensteen Building 516 Delaware Street SE Minneapolis MN 55455
UNIVERSITY OF MINNESOTA	555917996	MN-005	UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER MINNEAPOLIS MN 554552070

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

Subject: PRF# 786631 Fang Li NIH RPPR
From: Lynn Weber <weber117@umn.edu>
Date: 3/7/2014 9:29 AM
To: "proposal@umn.edu" <proposal@umn.edu>

--

Lynn Weber
Department of Pharmacology
University of Minnesota
Medical School
6-120 Jackson Hall
321 Church Street SE
Minneapolis, MN 55455
Phone: 612-626-6902
Fax: 612-625-8408
Email: weber117@umn.edu
http://www.pharmacology.med.umn.edu/

Paulie Garcia< garc0027@umn.edu>

PRF 786631, PI: Dr. Li

7 messages

Paulie Garcia< garc0027@umn.edu> Fri, Mar 7, 2014 at 10:59 AM
To: Lynn Weber <weber117@umn.edu>

Lynn,

Dr. Li has not routed the RPPR to me in eRA Commons. Could you do it, or could you ask him to so I can review it?

Thanks,
Paulie
--
****
Paulie Garcia
Grant and Contract Administrator
University of Minnesota
Office of the Vice President for Research
Sponsored Projects Administration
McNamara Alumni Center, Suite 450
200 Oak Street S.E.
Minneapolis, MN 55455

Phone: (612) 626-2724
Fax: (612) 624-4843
Email: garc0027@umn.edu

Paulie Garcia< garc0027@umn.edu> Fri, Mar 7, 2014 at 1:39 PM
To: Lynn Weber <weber117@umn.edu>
Cc: Carrie DeJarlais <dejar001@umn.edu>

Lynn,

I reviewed the PRF referenced above and have the following comment:

1. The unobligated balance is -$38,033. Do you plan on spending up to the 25% limit?

Thanks,
Paulie

--
****
Paulie Garcia
Grant and Contract Administrator
University of Minnesota
Office of the Vice President for Research
Sponsored Projects Administration
McNamara Alumni Center, Suite 450
200 Oak Street S.E.
Minneapolis, MN 55455

Phone: (612) 626-2724
Fax: (612) 624-4843
Email: garc0027@umn.edu

Lynn Weber< weber117@umn.edu> Fri, Mar 7, 2014 at 1:50 PM
To: Paulie Garcia <garc0027@umn.edu>

Hi Paulie,

This was the response Yang Chong the accountant gave me:

BTW, the balance of this grant will be in deficit which is allowable on this grant.

Lynn

--

Lynn Weber
Department of Pharmacology
University of Minnesota
Medical School
6-120 Jackson Hall
321 Church Street SE
Minneapolis, MN 55455
Phone: 612-626-6902
Fax: 612-625-8408
Email: weber117@umn.edu
http://www.pharmacology.med.umn.edu/

From: Paulie Garcia <garc0027@umn.edu>
Date: Friday, March 7, 2014 1:39 PM
To: Lynn Weber <weber117@umn.edu>
Cc: Carrie DeJarlais <dejar001@umn.edu>
Subject: PRF 786631, PI: Dr. Li
[Quoted text hidden]

Paulie Garcia< garc0027@umn.edu> Fri, Mar 7, 2014 at 1:55 PM
To: Lynn Weber <weber117@umn.edu>

Lynn,

Yes, deficits are allowable. The only thing is that SPA has to verify this info. when the deficits get a little high.

Thanks,
Paulie
[Quoted text hidden]

Lynn Weber< weber117@umn.edu> Fri, Mar 7, 2014 at 2:06 PM
To: Paulie Garcia <garc0027@umn.edu>

Hi Paulie,

So is ok now?
I wasn't trying to be snippy with the quote I included below.
I thought your question was about whether or not we were aware there was a deficit., hence the response.
If you need more information, let me know and I will have an accountant address it.

Lynn

--

Lynn Weber
Department of Pharmacology
University of Minnesota
Medical School
6-120 Jackson Hall
321 Church Street SE
Minneapolis, MN 55455
Phone: 612-626-6902
Fax: 612-625-8408
Email: weber117@umn.edu
http://www.pharmacology.med.umn.edu/

From: Paulie Garcia <garc0027@umn.edu>
Date: Friday, March 7, 2014 1:55 PM
To: Lynn Weber <weber117@umn.edu>
Subject: Re: PRF 786631, PI: Dr. Li
[Quoted text hidden]

Carrie DeJarlais< dejar001@umn.edu> Fri, Mar 7, 2014 at 2:12 PM
To: Paulie Garcia <garc0027@umn.edu>
Cc: Lynn Weber <weber117@umn.edu>

Paulie,

I'm confused, the account is in deficit why would we plan on spending up to 25%? This is a modular award and deficits are allowed to be carried over into the next budget period unless something has changed that I'm not aware of?

Carrie
[Quoted text hidden]
--

Carrie DeJarlais
Dept. of Pharmacology Administrator
MNPI Center Sr. Finance Manage
University of Minnesota
6-120 Jackson Hall
612-625-4982

Paulie Garcia < garc0027@umn.edu> Fri, Mar 7, 2014 at 2:27 PM
To: Lynn Weber <weber117@umn.edu>

Lynn,

Yes, it is okay. I just talked to Carrie. When deficits get higher, or when the unobligated balance is higher, SPA just needs to verify that spending is under control. Carrie did that.

thanks,
Paulie
[Quoted text hidden]

Principal Investigator/Program Director (Last, First, Middle): Li, Fang

DETAILED BUDGET FOR NEXT BUDGET PERIOD - DIRECT COSTS ONLY	FROM 5/1/14	THROUGH 4/30/15	GRANT NUMBER 5 R01 AI89728

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Fang Li	PD/PI	6.00			$50,000	$16,910	$66,910
Robert Geraghty	Co-Invest	1.00			$11,415	$3,861	$15,276
Yaoqing Chen	Postdoctoral	12.00			$35,000	$7,805	$42,805
TBN	Postdoctoral	6.00			$16,000	$3,568	$19,568
Minn Stat 13.32	Research Asst.	3.00			$12,500	$7,138	$19,638
Minn Stat 13.32	Research Asst.	3.00			$12,500	$7,138	$19,638
						$0	$0
SUBTOTALS →					$137,415	$46,420	$183,835

CONSULTANT COSTS

Dr. Kathryn Holmes	$10,000

EQUIPMENT (*Itemize*)

	$0

SUPPLIES (*Itemize by category*)

Molecular biology supplies	$14,178	
Insect cell culture supplies	$9,500	
Protein purification supplies	$10,708	
Protein crystallization supplies	$13,500	
Plastic & glassware supplies	$2,801	
		$50,687

TRAVEL

PI and/or postdoctoral to attend one meeting per year.	$3,000
INPATIENT CARE COSTS $0	$0
OUTPATIENT CARE COSTS $0	$0

ALTERATIONS AND RENOVATIONS (*Itemize by category*)

OTHER EXPENSES (*Itemize by category*)

Publication costs/reprints	$2,500	
Equipment maintenance	$1,500	
Communication costs	$800	
		$4,800

SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD	$252,322
CONSORTIUM/CONTRACTUAL COSTS — DIRECT COSTS	
CONSORTIUM/CONTRACTUAL COSTS — FACILITIES AND ADMINISTRATION COSTS	
TOTAL DIRECT COSTS FOR NEXT PROJECT PERIOD (*Item 8a, Face Page*)	$252,322

	Years 1-5
Indirect Cost Base	$238,046
IDC @ 51%	$121,403
Total Direct Costs	$252,322
IDC	$121,403
Total Costs	$373,725

Program Director/Principal Investigator (Last, first, middle): Li, Fang

GRANT NUMBER
5R01 AI89728-4

CHECKLIST

1. PROGRAM INCOME ***(See instructions.)***
All applications must indicate whether program income is anticipated during the period(s) for which grant support is requested. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)
	0	

2. ASSURANCES/CERTIFICATIONS ***(See instructions.)***
In signing the application Face Page, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the application instructions when applicable. Descriptions of individual assurances/certifications are provided in Part III of the PHS 398, and listed in Part I, 4.1 under Item 14. If unable to certify compliance, where applicable, provide an explanation and place it after the Progress Report (Form Page 5).

3. FACILITIES AND ADMINSTRATIVE (F&A) COSTS
Indicate the applicant organization's most recent F&A cost rate established with the appropriate DHHS Regional Office, or, in the case of for-profit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office.

F&A costs will ***not*** be paid on construction grants, grants to Federal organizations, grants to individuals, and conference grants. Follow any additional instructions provided for Research Career Awards, Institutional National Research Service Awards, Small Business Innovation Research/Small Business Technology Transfer Grants, foreign grants, and specialized grant applications.

☐ DHHS Agreement dated: 01/07/13 ☐ No Facilities and Administrative Costs Requested.

☐ No DHHS Agreement, but rate established with _____ Date _____

CALCULATION*

Entire proposed budget period: Amount of base $ 238,046 x Rate applied 51 % = F&A costs $ 121,403

Add to total direct costs from Form Page 2 and enter new total on Face Page, Item 8b.

*Check appropriate box(es):

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.)*:
Excludes Grad RA fringe

- grad fringe
$14,276
FA OK

Help

Prompt Path: Acctg Date→Award

Sponsored Award Overview

Report Run Date: Friday,3/7/2014

Data as of Friday,03/07/2014 03:52 AM

Award: CON000000022345 - Receptor recognition mechanisms of coronaviruses
Accounting Date Through: 03/07/2014
Encumbered Facilities and Admin: Included
Award Sponsor: NIH Nat'l Inst of Allergy & Infect Dis
Award Start Date: 05/15/2010
Award End Date: 04/30/2015
Award Time Elapsed: 77%
Award %Left: -5%
F&A: RSRCH MTDC 51%

Cost Share (Actuals):	$0.00
Program Income (Actuals):	$0.00
TIP Income (Actuals):	$0.00
Invoiced Amount ITD:	$1,476,252.00
Open AR Amount ITD:	$0.00

						03/01/2014 - 03/07/2014		INCEPTION THROUGH 03/07/2014		
Project	PP	PI	Detail Account	A Finalized Budget Amount		B Pre-Enc & Enc	C Expenses	D Pre-Enc & Enc	E Expenses	F = A - D - E Available Balance
00013782	Y	Li,Fang	168100 - Capital Assets - Fin Bdg Only	8,000.00		0.00	0.00	0.00	44,954.63	-36,954.63
00013782	Y	Li,Fang	700100 - Salaries-Faculty-Fin Bdg Only	186,874.00		-2,030.73	2,030.73	9,747.50	196,944.53	-19,818.03
00013782	Y	Li,Fang	700200 - Salaries-Prof&Adm-Fin Bdg Only	201,666.00		-549.55	549.55	2,637.85	49,891.72	149,136.43
00013782	Y	Li,Fang	700300 - Salaries-Grd/Pr w/Tuitn-FinBdg	101,400.00		-1,923.20	1,923.20	9,231.36	43,378.40	48,790.24
00013782	Y	Li,Fang	700310 - Salaries-Grd/Pr No Tuit-FinBdg	49,084.00		0.00	0.00	0.00	272,075.29	-222,991.29
00013782	Y	Li,Fang	710100 - Fringe - Faculty-Fin Bdg Only	64,545.00		-682.32	682.36	3,275.16	63,517.19	-2,247.35
00013782	Y	Li,Fang	710200 - Fringe - P&A-Fin Bdg Only	51,901.00		-184.65	184.63	886.32	14,741.82	36,272.86
00013782	Y	Li,Fang	710300 - Fringe-Grad/Pr-w/Tuitn-FinBdg	33,717.00		-521.94	521.94	2,505.32	34,407.72	-3,196.04
00013782	Y	Li,Fang	710310 - Fringe-Grad/Pr No Tuitn-FinBdg	11,655.00		0.00	0.00	0.00	48,978.62	-37,323.62
00013782	Y	Li,Fang	720200 - Lab & Med Supplies-FinBdg Only	199,650.00		0.00	0.00	1,665.65	204,982.72	-6,998.37
00013782	Y	Li,Fang	720300 - Gen Oper Services-Fin Bdg Only	9,375.00		0.00	0.00	0.00	12,150.75	-2,775.75
00013782	Y	Li,Fang	720400 - Lab & Medical Svcs-FinBdg Only	4,200.00		0.00	0.00	0.00	14,407.12	-10,207.12
00013782	Y	Li,Fang	720500 -	1,992.00		0.00	0.00	0.00	0.00	1,992.00

			Telecommunications-FinBdg Only						
00013782	Y	Li,Fang	720600 - Travel/Mileage/Mov-FinBdg Only	12,970.00	0.00	0.00	0.00	8,808.16	4,161.84
00013782	Y	Li,Fang	730100 - Consulting Services-FinBdg Onl	29,900.00	0.00	0.00	0.00	1,279.26	28,620.74
00013782	Y	Li,Fang	750100 - Non-Capital Equip-Fin Bdg Only	9,571.00	0.00	0.00	0.00	5,153.67	4,417.33
00013782	Y	Li,Fang	780100 - Repairs & Maint-FinBdg Only	6,485.00	0.00	0.00	0.00	1,705.37	4,779.63
00013782	Y	Li,Fang	850100 - Capital Equip - Fin Bdg Only	5,000.00	0.00	0.00	0.00	18,518.95	-13,518.95
			Direct Cost Total:	**$987,985.00**	**-$5,892.39**	**$5,892.41**	**$29,949.16**	**$1,035,895.92**	**-$77,860.08**
00013782	Y	Li,Fang	810500 - F&A Costs-Fin Bdg Only	488,267.00	0.00	2,738.93	9,878.71	478,388.29	0.00
			F&A Total:	**$488,267.00**	**$0.00**	**$2,738.93**	**$9,878.71**	**$478,388.29**	**$0.00**
CSR: $0.00 RFA: $404,136.42			**Project Total:**	**$1,476,252.00**	**-$5,892.39**	**$8,631.34**	**$39,827.87**	**$1,514,284.21**	**-$77,860.08**
CSR: $0.00 RFA: $404,136.42			**Award Total:**	**$1,476,252.00**	**-$5,892.39**	**$8,631.34**	**$39,827.87**	**$1,514,284.21**	**-$77,860.08**

Back to top

Encumbered Facilities and Admin is included on this report.

-$38,073

FINAL PROGRESS REPORT

Grant Number: R01 AI089728

Project Title: Receptor recognition mechanisms of coronaviruses

Grantee Organization: University of Minnesota

Funding Period: 05/15/10 – 04/30/15

Principal Investigator: FANG LI

A. Scientific goals

Coronaviruses cause epidemic outbreaks of diseases in humans and animals, symbolized by the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and porcine epidemic diarrhea coronavirus (PEDV). Receptor recognition and cell entry are two essential steps in coronavirus infection cycle, critical determinants of coronavirus host range and tissue tropism, and primary targets for antiviral intervention. An envelope-anchored coronavirus spike protein recognizes host receptors through its S1 subunit and fuses viral and host membranes through its S2 subunit. Coronaviruses recognize a variety of host receptors through its spike S1 domains (S1-NTD and S1-CTD), one or both of which function as the viral receptor-binding domain (RBD). Coronaviruses are also regulated for cell entry by host proteases that cleave at the S1/S2 boundary. Understanding the receptor recognition and cell entry by coronaviruses has important implications for virology, medicine, and epidemics.

During the five-year funding cycle of this R01 grant, we started with structural studies of the receptor recognition mechanisms of coronaviruses, and later expanded our research to the functional studies of the cell entry mechanisms of coronaviruses. Overall, this grant has covered four specific aims: (i) animal origins and cross-species transmissions of SARS-CoV and MERS-CoV; (ii) receptor recognition mechanisms of coronaviruses; (iii) cell entry mechanisms of coronaviruses; (iv) design and development of vaccines and therapeutic antibodies against coronavirus infections.

We have successfully achieved all of the above goals. (i) We have continued our pioneering work on the receptor recognition and cell entry of SARS-CoV and and MERS-CoV, which reveal the animal origins and cross-species transmissions of these viruses. (ii) We have determined more novel structures of coronavirus RBDs, including those of mouse hepatitis coronavirus, bovine coronavirus and MERS-CoV, in addition to our previously determined structures of the RBDs from SARS-CoV and NL63 respiratory coronavirus. (iii) We have also identified the host receptors for bat coronavirus HKU4 and porcine epidemic diarrhea coronavirus, and elucidated the cell entry mechanisms for a number of coronaviruses. (iv) We have collaborated with other groups on the design and development of vaccines and therapeutic antibodies against MERS-CoV infections.

B. Achievements

We made the following discoveries during the five-year funding cycle of this R01 grant:

B1. Animal origins and cross-species transmissions of SARS and MERS coronaviruses

SARS-CoV and MERS-CoV have both originated from animals and transmitted to humans, causing serious threat to global health. One of the goals of my research is to elucidate the molecular mechanisms by which emerging animal viruses transmit to humans. To this end, I study the structures and functions of the coronavirus spike protein, which guides coronavirus entry into host cells.

During the five-year funding cycle, my lab determined the crystal structures of the spike S1-CTD from different SARS-CoV strains complexed with their receptor angiotensin-converting enzyme 2 (ACE2) from different animal species (**Wu, K., et al., *Journal of Biological Chemistry*, 2012**). Through comparative studies of these S1-CTDs from different SARS-CoV strains and ACE2 from different animal species, my research has established that receptor recognition serves a critical barrier for the cross-species transmission of SARS-CoV and that a few RBD mutations allowed SARS-CoV virus to transmit from animals to humans (**Li, F., *Antiviral Research*, 2013)**.

Moreover, my lab examined the cell entry mechanisms of MERS-CoV. My research showed for the first time that cellular proteases from different hosts serve as a barrier for the cross-species transmission of MERS-CoV (**Yang, Y., et al., *PNAS*, 2014)**. Moreover, we discovered that two mutations in the protease cleavage sites of MERS-CoV spike allowed MERS-CoV to transmit from bats to humans, either directly or through intermediate hosts (**Yang, Y., et al., *Journal of Virology*, 2015)**.

Overall, my research has contributed critically to the knowledge about the animal origins and cross-species transmissions of viruses, and has important implications on epidemic monitoring, prevention, and control.

B2. Receptor recognition mechanisms of coronaviruses

Coronaviruses recognize a variety of receptors, and display a complex receptor recognition pattern. Another major goal of my research is to elucidate the receptor recognition mechanisms of coronaviruses, and provide insight into how coronaviruses have evolved such a complex receptor recognition pattern. During the five-year funding cycle, my lab has determined a number of crystal structures of coronavirus RBDs, either alone or in complex with their respective receptor. We have also performed functional studies to further our understanding of the receptor usage by coronaviruses.

First, my lab determined the crystal structure of the S1-NTD of β-genus mouse hepatitis CoV (MHV) complexed with its protein receptor CEACAM1 **(Peng, G., et al., *PNAS*, 2011)**. Surprisingly, MHV S1-NTD has the same structural fold as human galectins (galactose-binding host lectin), although it binds to CEACAM1 through exclusive protein-protein interactions. We then found that the S1-NTDs of β-genus bovine CoV (BCoV) and human OC43 CoV both bind sugar and function as viral lectins, and identified the type of sugar that BCoV S1-NTD recognizes using glycan screen array. We further determined the crystal structure of BCoV S1-NTD, which also has a galectin fold **(Peng, G, et al., *Journal of Biological Chemistry*, 2012)**. We performed structure-based mutagenesis and identified the sugar-binding site in BCoV S1-NTD, which overlaps with the galactose-binding site in galectins. Based on these studies, we hypothesized that ancient coronaviruses acquired their spike S1-NTD from the host, and have evolved it into viral RBD with novel specificity for protein receptors (as in MHV) or for novel sugar specificities (as in BCoV). We expanded our observation by showing that other viral lectins such as influenza hemagglutinin also have the same structural fold as human galectins, and hence all of these viral lectins may have originated from the host **(Chen, L. and Li, F., *Journal of Virology*, 2013)**.

Second, my lab continued to investigate why the S1-CTDs of α-genus NL63 respiratory coronavirus (NL63-CoV) and β-genus SARS-CoV have very different structures, but recognize the same receptor protein ACE2. We found that both viruses bind to a virus-binding hotspot on ACE2 by forming an energetically favorable tunnel structure **(Wu, K., et al., *Journal of Virology*, 2011)**. In addition, we detected a related structural topology (i.e., connectivity of secondary structural elements) between NI63-CoV and SARS-CoV S1-CTDs, suggesting a common evolutionary origin of the spikes from different genera **(Li, F., *Journal of Virology*, 2012)**.

Third, my lab determined the crystal structure of MERS-CoV S1-CTD. The structure revealed that MERS-CoV and SARS-CoV S1-CTDs have very similar core structures. However, the two S1-CTDs differ markedly in their receptor-binding motif regions, explaining why MERS-CoV and SARS-CoV recognize two different protein receptors – dipeptidyl peptidase 4 (DPP4) for MERS-CoV and ACE2 for SARS-CoV **(Chen, Y., et al., *Journal of Virology*, 2013)**.

Fourth, my lab determined the crystal structure of aminopeptidase N (APN), which is the receptor for several α-genus coronaviruses. The structure not only reveals the binding sites for these α-genus coronaviruses, but also has important implications for many other physiological functions of APN such as tumor motility and pain sensation **(Chen, L., et al., *PNAS*, 2012)**. Moreover, we identified DPP4 as the receptor for bat coronavirus HKU4 **(Yang, Y., et al, *PNAS*, 2014)**, and found that both human and pig APN molecules serve as the primary receptor and sugar as the co-receptor for α-genus porcine epidemic diarrhea coronavirus (PEDV) **(Liu, C., et al., *Journal of Virology*, 2015)**.

Overall, my research on the coronavirus-receptor interactions described above has established the following virology principles. First, drastic structural changes in viral RBDs can still lead to recognition of a virus-binding hot spot on the same receptor protein. Supporting this principle is the finding that SARS-CoV and NL63-CoV recognize a common virus-binding hot spot on ACE2 using structurally divergent S1-CTDs. Second, subtle structural changes in viral RBDs can lead to a complete receptor switch. For example, MHV and BCoV S1-NTDs recognize a protein receptor and a sugar receptor, respectively, through subtle conformational changes in receptor-binding loops. Third, it is a successful viral strategy to steal a host protein and evolve it into viral RBDs with novel protein receptor specificities or altered sugar receptor specificities. For example, MHV and BCoV S1-NTDs have the same structural fold as human galectins, but they recognize a novel protein receptor and a different sugar receptor, respectively. Fourth, a few residue changes at the receptor binding interface can lead to efficient cross-species infection and human-to-human transmission of a virus. For example, SARS-CoV needed only one or two mutations in its RBD to transmit from animal to humans. These virology principles may be extended from the coronavirus family to other virus families **(Li, F., *Journal of Virology*, 2015)**.

B3. Cell entry mechanisms of coronaviruses

For coronaviruses to enter host cells, their spike must be proteolytically activated by one or more host proteins and then undergo a dramatic conformational change. During the five-year funding period of the R01 grant, my lab identified the specific proteases that activate the cell entry of MERS-CoV and bat coronavirus HKU4 **(Yang, Y., et al., *PNAS*, 2014)**. Further, we successfully located the corresponding protease motifs in the spikes of these viruses. We also identified two mutations in the protease motifs of MERS-CoV spike that were critical for MERS-CoV to transmit from bats to humans, either directly or through intermediate hosts **(Yang, Y., et al., *Journal of Virology*, 2015)**. Importantly, as mentioned earlier, we showed for the first time that different activities of host protease serve a critical barrier for the cross-species transmission of viruses. In addition, we have elucidated the cell entry mechanisms of PEDV **(Liu, C., et al., *Journal of Virology*, 2015)**. Overall, my research has revealed how host proteases regulate viral entry, pathogenesis, host range, and tissue tropism.

B4. Design and development of vaccines and therapeutic antibodies against coronavirus infections

Coronaviruses, particularly SARS-CoV and MERS-CoV, pose serious threat to global health. Coronavirus spike RBDs are the prime vaccine candidates for preventing coronavirus infections, and monoclonal antibodies targeting coronavirus spike RBDs have the great potential to serve as therapeutics to treat coronavirus infections. During the five-year funding period of the R01 grant, we have collaborated with other groups to rationally design and develop RBD-based vaccines **(Ma, C., et al., *Vaccine*, 2014)** as well as RBD-targeting antibodies to combat MERS-CoV infections **(Du, L., et al., *Journal of Virology*, 2014)**.

C. Significance

Overall, my research has elucidated how coronaviruses transmitted from animals to humans to cause viral epidemics, and how coronaviruses explore different host receptors and other host factors to expand their host ranges. We use multidisciplinary research tools such as structural biology, protein biochemistry, molecular

virology, and vaccine design. Our long-term goals are to identify novel principles in virology that govern viral evolution, receptor recognition, cell entry, cross-species transmission and epidemics, and to develop novel therapy strategies to treat viral infections in humans.

D. Publications resulting from this R01 grant

Publication Summary

Using funding from this R01 grant, I have published 15 papers as the corresponding author, including 3 in *PNAS*, 1 in *Structure*, 8 in the *Journal of Virology*, 2 in the *Journal of Biological Chemistry,* and 1 in *Antiviral Research*. I have also published 1 other paper as a middle author in *Vaccine*.

List of Publications (in chronological order) (* indicates corresponding authorship)

1. Li, W., **Li, F. *** (2011) Cross-crystal averaging with search models to improve molecular replacement phases. *Structure*, *19*, 155-161 (PMID: 21300285; PMCID: PMC3037595)

2. Wu, K., Chen, L., Peng, G., Zhou, W., Pennell, C., Mansky, L., Geraghty, R., **Li, F. *** (2011) A virus-binding hotspot on human angiotensin-converting enzyme 2 is critical for binding of two different coronaviruses. *Journal of Virology*, *85,* 5331-5337 (PMID: 21411533; PMCID: PMC3094985)

3. Peng, G., Sun, D., Rajashankar, K., Qian, Z., Holmes, K., **Li, F. *** (2011) Crystal structure of mouse coronavirus receptor-binding domain complexed with its receptor murine CEACAM1a. *Proceedings of the National Academy of Sciences USA*, *108,* 10696-10701 (PMID: 21670291; PMCID: PMC3127895)

4. **Li, F. *** (2012) Evidence for a common evolutionary origin of coronavirus spike protein receptor-binding subunits. *Journal of Virology*, *86,* 2856-2858 (PMID: 22205723; PMCID: PMC3302248)

5. Wu, K., Peng, G., Wilken, M., Geraghty, R., **Li, F.** * (2012) Mechanisms of host receptor adaptation by severe acute respiratory syndrome coronavirus. *Journal of Biological Chemistry*, *287,* 8904-8911 (PMID: 22291007; PMCID: PMC3308800)

6. Chen, L., Lin, Y.L., Peng, G., **Li, F.** * (2012) Structural basis for multifunctional roles of mammalian aminopeptidase N. *Proceedings of the National Academy of Sciences USA*, *109*, 17966-17971 (PMID: 23071329; PMCID: PMC3497818)

7. Peng, G., Xu, L., Lin, Y.L., Chen, L., Pasquarella, J., Holmes, K.V., **Li, F. *** (2012) Crystal structure of bovine coronavirus spike protein lectin domain. *Journal of Biological Chemistry*, *287*, 41931-41938 (PMID: 23091051; PMCID: PMC3516740)

8. Chen, L., **Li, F. *** (2013) Structural analysis of the evolutionary origins of influenza virus hemagglutinin and other viral lectins. *Journal of Virology*, *87*, 4118-4120 (PMID: 23365425; PMCID: PMC3624229)

9. Chen, Y., Rajashankar, K.R., Yang, Y., Agnihothram, S.S., Liu, C., Lin, Y.L., Baric, R.S., **Li, F. *** (2013) Crystal structure of the receptor-binding domain from newly emerged middle East respiratory syndrome coronavirus. *Journal of Virology*, *87*, 10777-10783 (PMID: 23903833; PMCID: PMC3807420)

10. **Li, F. *** (2013) Receptor recognition and cross-species infections of SARS coronavirus. *Antiviral Research*, *100*, 246-254 (PMID: 23994189; PMCID: PMC3840050)

11. Du, L., Zhao, G., Yang, Y., Qiu, H., Wang, L., Kou, Z., Tao, X., Yu, H., Sun, S., Tseng, K.C., Jiang. S., **Li, F. ***, Zhou, Y. ***** (***** co-correspondence) (2014) A conformation-dependent neutralizing monoclonal antibody specifically targeting receptor-binding domain in Middle East respiratory syndrome coronavirus spike protein. *Journal of Virology*, *88*, 7045-7053 (PMID: 24719424; PMCID: PMC4054355)

12. Yang, Y., Du, L., Liu, C., Wang, L., Ma, C., Tang, J., Baric, R.S., Jiang, S., **Li, F. *** (2014) Receptor usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human transmission of MERS coronavirus. *Proceedings of the National Academy of Sciences USA*, *111*, 12516-12521 (PMID: 25114257; PMCID: PMC4151778)

13. Ma, C., Wang, L., Tao, X., Zhang, N., Yang, Y., Tseng, K.C., **Li, F.**, Zhou, Y., Jiang, S., Du, L. (2014) Searching for an ideal vaccine candidate among different MERS coronavirus receptor-binding fragments – the importance of immunofocusing in subunit vaccine design. *Vaccine*, *32*, 6170-6176 (PMID: 25240756; PMCID: PMC4194190 [Available on 2015-10-21])

14. **Li, F. *** (2015) Receptor recognition mechanisms of coronaviruses: a decade of structural studies. *Journal of Virology*, *89,* 1954-1964 (PMID: 25428871; PMCID: PMC4338876 [Available on 2015-08-15])

15. Liu, C., Tang, J., Ma, Y., Liang, X., Yang, Y., Peng, G., Qi, Q., Jiang, S., Li, J., Du, L., **Li, F. *** (2015) Receptor usage and cell entry to porcine epidemic diarrhea coronavirus. *Journal of Virology*, *89, 6121-6125* (PMID: 25787280; PMCID: PMC4442452 [Available on 2015-12-01])

16. Yang, Y., Liu, C., Du, L., Jiang, S., Shi, Z., Baric, R.S., **Li, F. *** (2015) Two mutations were critical for bat-to-human transmission of MERS coronavirus. *Journal of Virology*, *In press.* 2015 Jun 10. pii: JVI.01279-15. [Epub ahead of print] (PMID: 26063432)

E. Project Generated Resources

This research did not generate cell lines, DNA probes, animal models, protocols, or software. De-identified raw data from the experiments and computer programs (CCP4 macromolecular crystallography scripts) used for protein structure determinations are available to anyone upon written request to the PI. Peer-reviewed publications accepted or published during the past project period are being or have been entered in NIHMS and PubMed Central.

PI: **Li, Fang**	Title: Receptor recognition and cell entry of coronaviruses	
Received: 11/03/2015	FOA: PA13-302	Council: 05/2016
Competition ID: FORMS-C	FOA Title: RESEARCH PROJECT GRANT (PARENT R01)	
2 R01 AI089728-06	Dual:	Accession Number: 3877580
IPF: 1450402	Organization: UNIVERSITY OF MINNESOTA	
Former Number:	Department: Pharmacology	
IRG/SRG: VIRA	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 6: 300,000 Year 7: 300,000 Year 8: 300,000 Year 9: 300,000 Year 10: 300,000	Animals: N Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Fang Li Ph.D	Regents of the University of Minnesota	PD/PI
Ralph Baric Ph.D	University of North Carolina - Chapel Hill	Co-Investigator

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier
AI089728

b. Agency Routing Number

2. DATE SUBMITTED
2015-11-03

Application Identifier
835469 LI NIH

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: 5559179960000

Legal Name*: Regents of the University of Minnesota
Department: Sponsored Projects Admin
Division:
Street1*: 450 McNamara Alumni Center
Street2: 200 Oak Street SE
City*: Minneapolis
County: Hennepin
State*: MN: Minnesota
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 55455-2070

Person to be contacted on matters involving this application
Prefix: Ms. First Name*: Erin Middle Name: Last Name*: Jondahl Suffix:
Position/Title: Grant Administrator
Street1*: 450 McNamara Alumni Center
Street2: 200 Oak Street SE
City*: Minneapolis
County: Hennepin
State*: MN: Minnesota
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 55455-2070
Phone Number*: 612-624-5599 Fax Number: 612-624-4843 Email: awards@umn.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 41-6007513

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

❍ New ❍ Resubmission
● Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Receptor recognition and cell entry of coronaviruses

12. PROPOSED PROJECT
Start Date* 07/01/2016
Ending Date* 06/30/2021

13. CONGRESSIONAL DISTRICTS OF APPLICANT
MN-005

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Fang Middle Name: Last Name*: Li Suffix: Ph.D

Position/Title: Associate Professor
Organization Name*: Regents of the University of Minnesota
Department: Pharmacology
Division: Medical School
Street1*: 6-120 Jackson Hall
Street2: 321 Church Street SE
City*: Minneapolis
County: Hennepin
State*: MN: Minnesota
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 55455-0217
Phone Number*: 612-625-6149 Fax Number: 612-625-8408 Email*: lifang@umn.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$2,308,400.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$2,308,400.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Kevin Middle Name: Last Name*: McKoskey Suffix:

Position/Title*: Director
Organization Name*: University of Minnesota
Department: Sponsored Projects Admin
Division:
Street1*: 450 McNamara Alumni Center
Street2: 200 Oak Street SE
City*: Minneapolis
County: Hennepin
State*: MN: Minnesota
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 55455-2070
Phone Number*: 612-624-5599 Fax Number: 612-624-4843 Email*: awards@umn.edu

Signature of Authorized Representative*
Kevin McKoskey

Date Signed*
11/03/2015

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:1235-Fang_Li_CoV_R01_renew_2015_cover.pdf

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OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Regents of the University of Minnesota
Duns Number:	5559179960000
Street1*:	3-284 Nils Hasselmo Hall
Street2:	312 Church Street SE
City*:	Minneapolis
County:	Hennepin
State*:	MN: Minnesota
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	55455-0215
Project/Performance Site Congressional District*:	MN-005

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of North Carolina - Chapel Hill
DUNS Number:	6081952770000
Street1*:	3109 Hooker Research Center
Street2:	135 Dauer Drive
City*:	Chapel Hill
County:	Orange
State*:	NC: North Carolina
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	27599-7435
Project/Performance Site Congressional District*:	NC-004

File Name

Additional Location(s)

Funding Opportunity Number: PA-13-302. Received Date: 2015-11-03T17:08:03.000-05:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	1236-R01-CoV-renew-2015-project-summary-final.pdf
8. Project Narrative*	1237-R01-CoV-renew-2015-narrative-final.pdf
9. Bibliography & References Cited	1238-R01-CoV-renew-2015-cited-references-final.pdf
10.Facilities & Other Resources	1239-Li Baric R01_resources_2015.pdf
11.Equipment	1240-R01_equipment_Li_2015.pdf

Funding Opportunity Number: PA-13-302. Received Date: 2015-11-03T17:08:03.000-05:00

Project Summary

Receptor recognition and cell entry by viruses are two initial and essential steps in viral infection cycles. They are important determinants of viral host ranges, tissue tropisms and pathogenesis, and are primary targets for human intervention. Coronaviruses (CoVs) pose serious health threats to humans and other animals. SARS-CoV and MERS-CoV have infected thousands of people with significant fatality, whereas porcine epidemic diarrhea CoV is currently causing ~100% fatality in piglets. A virus-surface spike protein guides CoV entry into host cells by binding to its host receptor via its S1 subunit and fusing viral and host membranes via its S2 subunit. S1 from different CoVs recognizes a variety of host receptors through one or both of its domains (S1-NTD and S1-CTD), and the S1/S2 boundary is cleaved by host proteases for activation of membrane fusion by S2. Our previous research has determined a number of crystal structures of CoV S1 domains by themselves or in complex with their respective receptor, and also shown how proteolysis regulates the cell entry of some CoVs. Our research has contributed critically to the current knowledge about the molecular mechanisms for CoV receptor recognition, cell entry, and cross-species transmission. In this competitive renewal of R01, we will continue to investigate how CoVs exploit host receptors and host proteases for cell entry. This proposal has three specific aims. Aim 1 examines receptor binding by CoV S1-NTDs. Specifically, we will investigate whether S1-NTDs from different CoV genera have the same structural fold and evolutionary origin as host galectins (galactose-binding lectins). We will also examine how CoV S1-NTDs recognize sugar receptors. These studies will reveal the evolutionary origins of CoV S1-NTDs, enhance understanding of sugar recognition by CoVs, and may facilitate future design of sugar analogues and subunit vaccines to inhibit CoV infections. Aim 2 focuses on receptor binding by CoV S1-CTDs. Specifically, we will analyze the interactions between the S1-CTDs of bat SARS-like CoVs (SLCoVs) and the protein receptor homologues from humans and other animals, and elucidate how bat SLCoVs transmitted to humans and other animals to cause the SARS epidemic through evolutionary changes in their S1-CTDs. These studies will provide critical information for understanding emergence potential of bat SLCoVs and for facilitating epidemic monitoring and control. Aim 3 investigates cell entry by CoVs. Specifically, we will investigate what host proteases activate CoV entry and how the proteases motifs in CoV spikes have evolved to modulate CoV entry. These studies will reveal how host proteases regulate CoV entry to meet their specific need for host range, tissue tropism and pathogenesis, and may facilitate future design of protease inhibitors to block CoV entry. Overall, this proposal investigates the molecular and structural mechanisms for receptor recognition, cell entry, cross-species transmission, and tissue tropism of CoVs, which will lead to novel principles in virology. This research is also important for evaluating the emerging disease potentials of CoVs and for preventing, controlling and treating CoV infections in humans and other animals.

Narrative

This research investigates the molecular and structural mechanisms for the receptor recognition and cell entry of coronaviruses. It explores novel principles governing viral evolution, receptor recognition, cell entry, host ranges, cross-species infections, and tissue tropisms. These studies are critical for evaluating the emerging disease potentials of coronaviruses and for preventing, controlling and treating the spread of coronaviruses in humans or other animals.

Resources

(1) Laboratory:

Biochemical and structural portions of the proposal will be conducted in Dr. Fang Li's laboratory at the University of Minnesota. Dr. Li's laboratory has six benches, each allowing two experimenters to work simultaneously.

(2) Computers:

We have free access to computers in the Basic Sciences Computing Laboratory at the University of Minnesota.

(3) Other:

Crystallization screens will be performed using Rigaku's CrystalMation (crystal growth automation equipment).

In house X-ray data will be collected at the Kahlert Structural Biology Laboratory at the University of Minnesota.

Synchrotron X-ray data will be collected at national synchrotron facilities including the Advanced Photon Source near Chicago IL and Advanced Light Source at Berkeley CA.

Negative-stain electron microscopic images will be collected with a new FEI electron microscope operating at 300 kV at the University of Minnesota.

Surface plasmon resonance experiments to measure protein-protein binding interactions will be performed using a Biacore 3000 at the University of Minnesota.

We also have access to the University of Minnesota Masonic Cancer Center Flow Cytometry facility, which contains BD LSRII and 3 FacsCalibur flow cytometers as well as high-speed cell sorting capabilities up to 22,000 events/sec with the FACSDiVa and FACSAria cell sorters.

FACILITIES AND OTHER RESOURCES

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL-BARIC

Research Environment. The Department of Epidemiology is internationally recognized as a leader in epidemiologic research and training. The Department offers research training in most specialized areas including cancer, cardiovascular diseases, environmental and occupational health, health services/clinical epidemiology, reproductive health and infectious diseases. For the fiscal year 2010/2011, the Department was awarded in excess of $28 million in sponsored funding (research, training and public service) and ranks in the top five largest units at the University of North Carolina at Chapel Hill in the area of sponsored research awards. The department's current faculty consists of 51 regular full-time faculty and 151 adjunct faculty members. The department has 218 graduate students enrolled, including 20 in the MPH program, 5 in the MSPH program, 20 in the MSCR program and 173 in the Ph.D. program. The Department of Epidemiology is headquartered in the four-story McGavran-Greenberg Building, adjacent to Rosenau Hall directly across the street from the Carolina Vaccine Center in the School of Medicine. The epidemiology administrative and office space occupies 10,928 sq. ft. and provides additional classroom space. Most of the department's research staff occupies a research annex consisting of approximately 7,000 square feet of contiguous rental space in a commercial office building that is a 10-minute walk from McGavran/Greenberg Hall.

BSL2 Facility. Dr. Baric has three laboratories of ~2400 sq. ft. equipped as BL2 space for the molecular biology, virology, immunology and recombinant DNA techniques proposed in the application in Hooker Research Center. Equipment includes gel electrophoresis equipment, power supplies, thermal cyclers, a programmable heat block, heat blocks, water baths, CO_2 incubators (2), several -70°C freezers, one -140°C freezer, refrigerators, DNA documentation system, DNA sequencing and computer assisted sequence analysis programs, several microfuges, two Nikon microscopes with photographic and fluorescent capabilities, several class 2 environmental hoods, refrigerated water baths, several IBM and Apple Pentium II/III computers with accompanying software, three thermocyclers, a fume hood, Nuclisens reader, hybridization oven, real time thermocyclers, three fluorescent inverted scopes with computer software (Olympus IX51), and a spectrophotometer. A Roche Light Cycler 480II is available for real time measurements. The laboratory has an ELISA plate reader, an illuminometer, 200 cages for animal maintenance and breeding in Seal-Safe housing, Bio Rad low pressure chromatography system, ELISA plate washer, spectrophotometers, and other equipment that is routinely used in characterizing antibody-protein interactions.

BSL 3 Facility. The Baric laboratory contains an additional 1200+ square feet of newly renovated or new BSL3 facilities with enhanced features including 1) shower in/shower out facility, 2) dual anteroom access, 3) Hepa filtered exhaust, 4) redundant exhaust fans, 4) Card key access, alarm system to Public Health/Campus Police, Lab controlled combination lock, and 5) Techniplast SealsafeTM Hepa filtered animal housing for rodents (mice (~300 cages). PAPR and tyvek suits are worn at all times in the BSL3 facility. The BL3 facilities are in an adjacent, attached building (3221D McGavern-Greenberg Hall) or in Room 3104 Hooker Research Center, the latter space is directly adjacent to Dr. Baric's BSL2 laboratory resources. Each facility is equipped with sterile hoods (BSCIIA), four CO_2 incubators, gel electrophoresis equipment, thermal cyclers and power supplies, and related equipment necessary for virus cultivation and molecular genetic research. The facilities each house a -70C freezer, an inverted Nikon fluorescent microscope with an assortment of filters, magnifications and digital camera, an ELISA plate reader and illuminometer. Both facility contain rodent-sized Seal-Safe systems (~192 cages) for maintaining animals in a Hepa-filtered Air in/out environment, exhausted into the BSL3 Hepa-filtered exhaust system. An 8 chamber Buxco plethysmography system which allows for repetitive, noninvasive measures of the number of breaths, tidal volume, airway responsiveness, enhanced pause, respiratory gases, etc. from live control and infected mice in a contained system is available in the main BSL3 laboratory in McGavran Greenberg Hall (http://www.buxco.com/FinePointe.aspx?Page=FinePointe).

Departmental and University Services. The department provides cold-room, autoclave, centralized dishwashing and a darkroom with an automated developer. The campus has central facilities for DNA oligonucleotide synthesis, histopathology, DNA sequencing, EM, light and confocal microscopy, automated PCR genotyping and Taqman facilities, and Fluorescent activated cell sorter facilities (FAC). As a member of the Department of Microbiology and Immunology and UNC Cancer center, our laboratory has access to these facilities and receives discounts. The University provides a variety of core services including: sequencing and

deep sequencing cores, genomics cores, oligonucleotide synthesis cores, hybridoma cores, transgenic cores, structural biology cores, etc. typical of any world class research institution. Campus wide core facilities are available for oligonucleotide synthesis, Sanger and 454 sequencing, RNAseq, pathology and histology services, and Flow Cytometry. Approximately, 40,000 cages are available for CC RIX production in the Genetic Medicine Building on UNC Campus.

Major Equipment

The following major equipments are available at the PI's laboratory or other facilities at the University of Minnesota:

Protein purification facilities: AKTA prime protein purification system, Pall Minimate concentrator, protein purification chromatography columns. Protein binding measurement facilities: Biacore 3000, AlphaScreen, BLITZ, Isothermal Titration Calorimeter. Cell culture hoods: two 4 ft. Thermo Sci Class II A2 biosafety hoods. Dishwasher: Miele G7804 lab glass and plastic washer. Freezers: Thermo Sci Revco UltraPlus -80°C, Thermo Sci Isotemp –20°C. Refrigerator: Thermo Sci chromatography sliding double-door refrigerator. Incubators: Thermo Sci Excellar E25 incubator/shaker, Thermo Sci MaxQ 4000 incubator/shaker, three Thermo Sci Forma Steri-Cycle CO_2 incubators with gas cylinder monitor and two CO_2 regulators, and two Bellco Bench Top Incubators with Bellco Rocker Platform, Bellco Orbital Shaker, and Bellco Cell-Production Roller Apparatus. Microscopy: Leica DMIL inverted-stand microscope with 3.2, 10, 20, and 40X phase objectives and fluorescence capabilities. Microcentrifuges: Sorvall Biofuge Pico and Sorvall Biofuge Fresco. Table-top centrifuge: Sorvall Legend RT plus refrigerated centrifuge with swinging bucket rotor and adaptors for 50, 15, and 5ml tubes, and 96-well plates. Floor model centrifuge: Beckman Avanti JE with JA-17 and JLA 10.5 rotors. Sonicator: Fisher Scientific 60 Sonic Dismembrator with Sonabox Accoustic Enclosure. Thermal cycler: BioRad DNA engine. Plate reader: Molecular Devices SpectraMaxM5 Plate Reader with SOFTmax PRO Software. Power supplies: BioRad PowerPac Universal, BioRad Power Pac 200, BioRad Power Pac 1000, and EC Apparatus Corporation EC-150 units. Electrophoresis: Two BioRad Transblot Cells, two BioRad Mini PROTEAN 3 Cells, two Continental Lab Products 12 cm x 14 cm horizontal electrophoresis devices, 1 Continental Lab Products mini-gel horizontal electrophoresis device, two BioRad Protean II xi vertical electrophoresis rigs. Miscellaneous: Mettler-Toledo PB-S/FACT series balance, Denver Instrument PI series analytical balance, Fisher Scientific Accumet Basic AB15 pH Meter, and Panasonic Microwave. Bellco pipet plugger. Biomate 3 spectrophotometer. Thermo Sci drying oven. FEI electron microscope operating at 300 kV.

Kahlert Structural Biology Lab: one RigakuMSC Micromax 007 X-ray generator with a Cr anode, VariMax Confocal Max-Flux Cr optics, R-axis IV++ image plate, helium cone, and X-stream low temperature device for *ab initio* structure solution by sulfur single wavelength anomalous diffraction (S-SAD); one RigakuMSC Micromax HF X-ray generator with a Cu anode, and two data collection systems; (1) VariMax Confocal Max-Flux optics, R-axis IV++ image plate, 2θ stage, and RigakuMSC XStream low temperature device for high resolution in-house X-ray data collection; (2) Confocal Blue optics, inverse phi, and Oxford Instruments low temperature Cryojet for crystal screening; one RigakuMSC Micromax 007 HFM X-ray generator with a Cu anode, VariMax-HF Confocal Max-Flux optics, an AFC11 goniometer, a RigakuMSC Saturn 944+ CCD detector, and an X-Stream 2000 low temperature system. Multiple crystal incubators, running at a range of temperatures, are available for crystallization trials, and multiple stereomicroscopes enable crystals to be visualized and photographed. Two data processing computers. Rigaku's CrystalMation (crystal growth automation equipment).

Basic Sciences Computing Laboratory: three shared memory systems (SGI Altix 3700, SGI Onyx4, Sun V880), sixteen workstations (linux, SGI, and PC), and all major software for structural biology.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Fang Middle Name Last Name*: Li Suffix: Ph.D

Position/Title*: Associate Professor
Organization Name*: Regents of the University of Minnesota
Department: Pharmacology
Division: Medical School
Street1*: 6-120 Jackson Hall
Street2: 321 Church Street SE
City*: Minneapolis
County: Hennepin
State*: MN: Minnesota
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 55455-0217

Phone Number*: 612-625-6149 Fax Number: 612-625-8408 E-Mail*: lifang@umn.edu

Credential, e.g., agency login: fangli

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 2002

	File Name
Attach Biographical Sketch*:	1245-FangLi_Biosketch_October2015.pdf
Attach Current & Pending Support:	

Tracking Number: GRANT12031055
Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

PROFILE - Senior/Key Person				
Prefix:	First Name*: Ralph	Middle Name	Last Name*: Baric	Suffix: Ph.D
Position/Title*:	Professor			
Organization Name*:	University of North Carolina - Chapel Hill			
Department:				
Division:	Epidemiology/Microbio/Immunolo			
Street1*:	3304 Michael Hooker Res. Bldg.			
Street2:	CB# 7435			
City*:	Chapel Hill			
County:	Orange			
State*:	NC: North Carolina			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	27599-7435			
Phone Number*: 919-966-3895	Fax Number: 919-966-2089	E-Mail*: rbaric@email.unc.edu		
Credential, e.g., agency login: ralph_baric				
Project Role*: Co-Investigator		Other Project Role Category:		
Degree Type: PhD		Degree Year: 1982		
		File Name		
Attach Biographical Sketch*:		1246-Baric CoV biosketch Oct 2015 Final-1.pdf		
Attach Current & Pending Support:				

Tracking Number: GRANT12031055

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

BIOGRAPHICAL SKETCH

NAME: **Fang Li**

POSITION TITLE: Associate Professor with Tenure

eRA COMMONS USER NAME (credential, e.g., agency login): fangli

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Beijing University, Beijing, China	B.S.	07/1996	Biochemistry and Molecular Biology
Yale University, New Haven, CT	Ph.D.	05/2002	Molecular Biophysics and Biochemistry
Yale University, New Haven, CT	Postdoctoral	06/2002 - 06/2003	Structural Biology
Harvard Medical School, Children's Hospital, Boston, MA	Postdoctoral	07/2003 - 12/2006	Structural Virology

A. Personal Statement.

As a structural and molecular biologist, I study host receptor recognition and host cell entry of viruses. I received rigorous training in X-ray crystallography from Nobel Laureate Thomas A. Steitz at Yale and NAS member Steven C. Harrison at Harvard. I have an established program of research in the structural and functional studies of host receptor recognition and host cell entry by coronaviruses. I have done pioneering work on the receptor recognition and cell entry of SARS and MERS coronaviruses, which have revealed the animal origins and cross-species transmissions of SARS and MERS coronaviruses.

My other work includes novel structure determinations of the receptor-binding proteins of human NL63 respiratory coronavirus, mouse hepatitis coronavirus, and bovine coronavirus, in addition to the receptor-binding proteins of SARS and MERS coronaviruses. My group has also identified the host receptors for bat coronavirus HKU4 and porcine epidemic diarrhea coronavirus, and elucidated the cell entry mechanisms for a number of coronaviruses.

Overall, my research has elucidated how coronaviruses transmitted from animals to humans to cause viral epidemics, and how coronaviruses exploit different host receptors and other host factors to expand their host ranges and regulate their tissue tropisms. I use multidisciplinary research tools such as structural biology, protein biochemistry, molecular virology, and vaccine design. My long-term goals are to identify novel principles in virology that govern viral evolution, receptor recognition, cell entry, cross-species transmission and epidemics, and to develop novel therapy strategies to treat viral infections in humans.

1. **Li, F.,** Li, W., Farzan, M., Harrison S.C. (2005) Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science*, 309, 1864-1868 (PMID: 16166518)
2. Wu, K., Li, W., Peng, G., **Li, F.** (2009) Crystal structure of NL63 respiratory coronavirus receptor-binding domain complexed with its human receptor. *Proceedings of the National Academy of Sciences USA*, *106*, 19970-19974 (PMID: 19901337; PMCID: PMC2785276)
3. Peng, G., Sun, D., Rajashankar, K., Qian, Z., Holmes, K., **Li, F.** (2011) Crystal structure of mouse coronavirus receptor-binding domain complexed with its murine receptor. *Proceedings of the National Academy of Sciences USA*, 108, 10696-10701 (PMID: 21670291; PMCID: PMC3127895)
4. Yang, Y., Du, L., Liu, C., Wang, L., Ma, C., Tang, J., Baric, R.S., Jiang, S., **Li, F.** (2014) Receptor usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human transmission of MERS coronavirus. *Proceedings of the National Academy of Sciences USA*, 111, 12516-12521 (PMID: 25114257: PMCID: PMC4151778)

B. Positions and Honors.

1996 – 2002	Ph.D. Student, Department of Molecular Biophysics and Biochemistry, Yale University, (Advisor: Nobel laureate Dr. Thomas A. Steitz), New Haven, CT
2002 – 2003	Postdoctoral Fellow, Department of Molecular Biophysics and Biochemistry, Yale University, (Sponsor: Dr. Thomas A. Steitz), New Haven, CT
2003 – 2006	Postdoctoral Fellow, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston Children's Hospital (Sponsor: Dr. Stephen C. Harrison) Boston, MA
2007 – 2014	Assistant Professor (tenure track), Department of Pharmacology, University of Minnesota, Minneapolis, MN
2014 – present	Associate Professor (with tenure), Department of Pharmacology, University of Minnesota, Minneapolis, MN

Professional Memberships

American Society for Virology – lifetime member

American Society for Microbiology – full member

American Crystallographic Association – full member

American Society for Biochemistry and Molecular Biology – full member

C. Contribution to Science.

1. Animal origins and cross-species transmissions of SARS and MERS coronaviruses: SARS coronavirus (SARS-CoV) and MERS coronaviruses (MERS-CoV) both originated from animals and transmitted to humans, causing serious threat to global health. One of the main goals of my research is to elucidate the molecular mechanisms by which emerging animal viruses transmit to humans. To this end, I study the structures and functions of coronavirus spike proteins, which guide coronavirus entry into host cells. I determined the crystal structures of the spike receptor-binding domains (RBDs) from different SARS-CoV strains complexed with their receptor from different host species. My research has revealed that receptor recognition serves as a critical barrier for cross-species transmissions of viruses and that a few RBD mutations allowed SARS-CoV to transmit from animals to humans. Moreover, I examined the cell entry mechanisms of MERS-CoV. My research showed for the first time that cellular proteases from different host species serve as a barrier for cross-species transmissions of viruses and that two mutations in the protease cleavage sites in MERS-CoV spike protein allowed MERS-CoV to transmit from animals to humans. Overall, my research has contributed critically to the knowledge about animal origins and cross-species transmissions of viruses, and has important implications on epidemic monitoring, prevention, and control.
    a. **Li, F.,** Li, W., Farzan, M., Harrison S.C. (2005) Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science, 309,* 1864-1868 (PMID: 16166518)
    b. **Li, F.** (2008) Structural analysis of major species barriers between humans and palm civets for severe acute respiratory syndrome coronavirus infections. *Journal of Virology, 82,* 6984-6991 (PMID: 18448527; PMCID: PMC2446986)
    c. Wu, K., Peng, G., Wilken, M., Geraghty, R., **Li, F.** (2012) Mechanisms of host receptor adaptation by severe acute respiratory syndrome coronavirus. *Journal of Biological Chemistry*, *287,* 8904-8911 (PMID: 22291007; PMCID: PMC3308800)
    d. Yang, Y., Du, L., Liu, C., Wang, L., Ma, C., Tang, J., Baric, R.S., Jiang, S., **Li, F.** (2014) Receptor usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human transmission of MERS coronavirus. *Proceedings of the National Academy of Sciences USA*, *111*, 12516-12521 (PMID: 25114257; PMCID: PMC4151778)

2. Receptor recognition mechanisms of coronaviruses: Coronaviruses recognize a variety of receptors, and display a complex receptor recognition pattern. Another major goal of my research is to elucidate the receptor recognition mechanisms of coronaviruses and to provide insight into how coronaviruses have evolved such a complex receptor recognition pattern. To this end, I have determined a number of crystal structures of coronavirus spike RBDs either alone or in complex with their respective receptor. In

addition to the aforementioned SARS-CoV RBD complexed with its receptor, my lab has also determined the structures of the RBDs of human NL63 respiratory coronavirus and mouse hepatitis coronavirus in complex with their respective receptor, and the RBDs of MERS-CoV and bovine coronavirus by themselves. These structures account for the majority of the structures in the field. My lab also identified the receptor for bat coronavirus HKU4 and porcine epidemic diarrhea coronavirus. My research has shown that coronaviruses have undergone both divergent and convergent evolutions, resulting in (i) very different coronavirus RBDs recognizing a virus-binding hotspot on the same receptor, (ii) very similar coronavirus RBDs recognizing completely different receptors though subtle structural changes in their RBDs, and (iii) coronaviruses stealing a host protein and evolving it into viral RBD with novel receptor binding specificity. Overall, my research has established novel principles on the evolution and receptor recognition of viruses.

a. Wu, K., Li, W., Peng, G., **Li, F.** (2009) Crystal structure of NL63 respiratory coronavirus receptor-binding domain complexed with its human receptor. *Proceedings of the National Academy of Sciences USA*, *106*, 19970-19974 (PMID: 19901337; PMCID: PMC2785276)
b. Peng, G., Sun, D., Rajashankar, K., Qian, Z., Holmes, K., **Li, F.** (2011) Crystal structure of mouse coronavirus receptor-binding domain complexed with its murine receptor. *Proceedings of the National Academy of Sciences USA*, 108, 10696-10701 (PMID: 21670291; PMCID: PMC3127895)
c. Chen, Y., Rajashankar, K.R., Yang, Y., Agnihothram, S.S., Liu, C., Lin, Y.L., Baric, R.S., **Li, F.** (2013) Crystal structure of the receptor-binding domain from newly emerged Middle East respiratory syndrome coronavirus. *Journal of Virology*, *87*, 10777-10783 (PMID: 23903833; PMCID: PMC3807420)
d. **Li, F.** (2015) Receptor recognition mechanisms of coronaviruses: a decade of structural studies. *Journal of Virology*, *89,* 1954-1964 (PMID: 25428871; PMCID: PMC4338876)

3. Cell entry mechanisms of coronaviruses: For coronaviruses to enter host cells, their spike protein must be proteolytically activated by one or more host proteases and then undergo dramatic conformational changes. Using electron microscopy, I established two distinct conformations of the SARS-CoV spike, corresponding to its conformations before and after cell entry, respectively. My lab has also identified the specific proteases that activate the cell entry of MERS-CoV and porcine epidemic diarrhea coronavirus, and the corresponding protease motifs in the spikes of these viruses. Importantly, we showed for the first time that different activities of host protease serve as a critical barrier for the cross-species transmissions of viruses. Overall, my research has revealed how host proteases regulate viral entry, host range, tissue tropism, and pathogenesis.

a. **Li, F.,** Berardi, M., Li, W., Farzan, M., Dormitzer, P.R., Harrison, S.C. (2006) Conformational states of the severe acute respiratory syndrome coronavirus spike protein ectodomain. *Journal of Virology, 80,* 6794-6800 (PMID: 16809285; PMCID: PMC1489032)
b. Huang, I.C., Bosch, B.J., **Li, F.,** Li, W., Lee, K.H., Ghiran, H., Vasilieva, N., Dermody, T.S., Harrison, S.C., Dormitzer, P.R., Farzan, M., Rottier, P.M., Choe, H. (2006) SARS coronavirus, but not human coronavirus NL63, utilizes cathepsin L to infect ACE2-expressing cells. *Journal of Biological Chemistry, 281,* 3198-3203 (PMID: 16339146)
c. Liu, C., Tang, J., Ma, Y., Liang, X., Yang, Y., Peng, G., Qi, Q., Jiang, S., Li, J., Du, L., **Li, F.** (2015) Receptor usage and cell entry to porcine epidemic diarrhea coronavirus. *Journal of Virology*, *89, 6121-6125* (PMID: 25787280; PMCID: PMC4442452 [Available on 2015-12-01])
d. Yang, Y., Liu, C., Du, L., Jiang, S., Shi, Z., Baric, R.S., **Li, F.** (2015) Two mutations were critical for bat-to-human transmission of MERS coronavirus. *Journal of Virology*, *89*, 9119-9123 (PMID: 26063432; PMCID: PMC4524054 [Available on 2016-03-01])

4. Multifunctional roles of proteases in cancer growth, cancer treatment, and blood pressure regulation: Human cell-surface aminopeptidases cleave residues from the N-terminus of proteins, and play multifunctional roles in cancer growth and blood pressure regulation. My lab has determined the crystal structures of two such enzymes, aminopeptidase N (APN) and aminopeptidase A (APA). My research on APN elucidated the detailed catalytic mechanism of APN, demonstrated the unique structural features of APN that allow many of its ligands to access its enzymatic site, and revealed a unified mechanism for the APN-based tumor cell motility and tumor-homing therapy. Moreover, my research on APA provided a structural basis for the calcium-modulated substrate specificity of APA in blood pressure regulation and can guide the design and development of antihypertensive APA inhibitors.

Overall, my research explores the structural and molecular mechanisms of cancer growth and blood pressure regulation, and seeks treatment of these diseases based on these mechanisms.

a. Chen, L., Lin, Y.L., Peng, G., **Li, F.** (2012) Structural basis for multifunctional roles of mammalian aminopeptidase N. *Proceedings of the National Academy of Sciences USA*, *109*, 17966-17971 (PMID: 23071329; PMCID: PMC3497818)
b. Yang, Y., Liu, C., Lin, Y.L., **Li F.** (2013) Structural insights into central hypertension regulation by human aminopeptidase A. *Journal of Biological Chemistry*, *288*, 25638-25645 (PMID: 23888046; PMCID: PMC3757224)
c. Liu, C., Yang, Y., Chen, L., Lin, Y.L., **Li, F.** (2014) A unified mechanism for aminopeptidase N-based tumor cell motility and tumor-homing therapy. *Journal of Biological Chemistry*, *289,* 34520-34529 (PMID: 25359769; PMCID: PMC4263860)

5. Template-independent synthesis of tRNA CCA-adding enzymes. My PhD thesis was about the structures and functions of tRNA CCA-adding enzymes, which polymerize CCA onto the 3' terminus of immature tRNAs without using a nucleic acid template. I determined the first crystal structures of tRNA CCA-adding enzymes. From these structures, I identified a novel protein template in the enzymes that specifically recognizes and accommodates the incoming ATP or CTP. My PhD research provided insight into the template-independent nucleic acid synthesis.
   a. **Li, F.,** Xiong, Y., Wang, J., Cho, H.D., Tomita, K., Weiner, A.M., Steitz, T.A. (2002) Crystal structures of the Bacillus stearothermophilus CCA-adding enzyme and its complexes with ATP or CTP. *Cell, 111,* 815-824 (PMID: 12526808)
   b. Xiong, Y., **Li, F.,** Wang, J., Weiner, A.M., Steitz, T.A. (2003) Crystal structures of an archaeal class I CCA-adding enzyme and its nucleotide complexes. *Molecular Cell, 12,* 1165-1172 (PMID: 14636575)
   c. **Li, F.,** Wang, J., Steitz, T.A. (2000) Sulfolobus shibatae CCA-adding enzyme forms a tetramer upon binding two tRNA molecules: A scrunching-shuttling model of CCA specificity. *Journal of Molecular Biology, 304,* 483-492 (PMID: 11090289)

Complete List of Published Work:
http://www.msi.umn.edu/~lifang/flpublications.html

D. Research Support.

Active

National Institutes of Health 04/1/15 – 03/31/20
R01AI110700
Role: Co-Principal Investigator (contact Co-PI: Ralph Baric, University of North Carolina)
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
Goal: This research investigates genetic pathways regulating MERS coronavirus cross species transmission and receptor homolog usage, establishes robust animal models of human disease, and discovers critical reagents for therapeutic and vaccine testing.

Completed

National Institutes of Health 05/15/10 – 04/30/15
R01AI089728
Role: Principal Investigator
Receptor recognition mechanisms of coronaviruses
Goal: This research investigates how coronaviruses recognize their receptors and how they interact with receptors from different hosts. It explores novel principles governing viral evolution, virus-receptor interactions, viral host ranges and cross-species infections, and may lead to new approaches in the prevention and treatment of coronavirus infections in humans and other animals.

AHC Faculty Research Development Grant, University of Minnesota 01/01/09 – 12/31/11
Receptor recognition and cross-species infections by human coronaviruses
Role: Principal Investigator

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: **RALPH STEVEN BARIC**

eRA COMMONS USER NAME (credential, e.g., agency login): RALPH_BARIC

POSITION TITLE: **PROFESSOR**

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
N.C. State University, Raleigh, NC	BS	1977	Zoology
N.C. State University, Raleigh, NC	PhD	1982	Microbiology
University of Southern CA, School of Med,(Los Angeles, CA)	Post-Doc	1986	Microbiology

A. Personal Statement: The Baric laboratory uses genetic, biochemical, molecular and immunologic approaches to study the molecular mechanisms regulating viral evolution, virus immunity, virus-host interactions and vaccine mediated protective immunity using coronaviruses, noroviruses and flaviviruses (Dengue) as models. This program focuses on identifying the molecular and structural correlates for Coronavirus (CoV) entry and cross species transmission, using a variety of human, animal, epidemic and pre-epidemic strains from natural sources. CoV have a long history of host shifting and our group has made major contributions toward understanding Mouse Hepatitis Virus (MHV), SARS-CoV and MERS-CoV cross species transmission, receptor usage and entry. Key reagents provided by the Baric laboratory are a panel of CoV molecular cDNA clones from which recombinant viruses can be isolated from MHV, SARS-CoV, SARS-like bat coronaviruses (SL-CoV), porcine epidemic diarrhea virus (PEDV) and human coronavirus NL63 (HCoV-NL63). These molecular clones will be used to introduce targeted mutations, identified by experimental evolution in vitro or by structural, biochemical and mutagenesis of recombinant proteins in biochemical assays, and evaluated for impact on virus entry, receptor usage and proteolytic processing of the S glycoprotein during entry. **Major Contributions**: We have made significant contributions to our understanding of all aspects of CoV biology, including: i) CoV genetics and reverse genetics for SARS-CoV, MHV, MERS-CoV, HCoV NL63, PEDV, TGEV, bat SL-CoV, BtCoV HKU-5 and others, ii) demonstration of proof-reading activities in the CoV genome, iii) identification and characterization of bat SL-CoV with prepandemic potential, iii) coronavirus transcription mechanisms, iv) mechanisms of interferon antagonism and interferon stimulated gene expression control, v) virus host susceptibility allele mapping, vi) epitope mapping of human monoclonal antibodies, vii) identification of broad spectrum human monoclonal antibodies against SARS-CoV and MERS-CoV, viii) mouse models of human disease (MERS-CoV and SARS-CoV), ix) aging and emerging coronavirus vaccine efficacy, and x) live and attenuated vaccine design in young and aged animal models of human disease. As we have ongoing and well-developed collaborations with Dr. Li, we are well positioned to contribute to the proposal.

A1. Four Papers that Specifically Highlight Qualifications for Project.

1. Menachery, VD, Yount, BL, Debbink, K, Agnihothram, S., Gralinski, LE, Plante, JA, Graham, RL, Scobey, T., Ge, S-Y, Donaldson, E.F., Randell, S.H., Lanzavecchia, A., Marasco, W.A., Shi, Z-L, **Baric, R.S.** 2015. Novel platform identifies threat posed by a SARS-like cluster of circulating bat coronaviruses. In Press, **Nature Medicine.**
2. Yount, B, Curtis, K., Fritz L, Hensley, L., Jahrling P., Prentice E., Denison M., Geisbert T and **Baric, RS**. 2003. Reverse Genetics with a full length infectious cDNA for the SARS Coronavirus. **Proc Natl Acad Sci USA** 100(22):12995-13000. PMCID: PMC240733. 242 Citations
3. Yang Y, Du L, Liu C, Wang L, Ma C, Tang J, **Baric RS**, Jiang S, **Li F**. Receptor usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human transmission of MERS coronavirus. **Proc Natl Acad Sci U S A**. 2014 Aug 26;111(34):12516-21. PMC4151778. >8800 downloads.

4. Donaldson EF, Yount B, Sims AC, Burkett S, Pickles RJ, **Baric** RS. Systematic assembly of a full-length infectious clone of human coronavirus NL63. **J Virol**. 2008 Dec;82(23):11948-57. PMC2583659. Cited 40 times.

B. Positions and Honors.

Employment Experience:

1986-1992 Assistant Professor, Department of Parasitology and Laboratory Practice and Department of Epidemiology, University of North Carolina (UNC), Chapel Hill, NC
1992-2001 Associate Professor, Departments of Epidemiology and Microbiology & Immunology, UNC Chapel Hill
2001- Professor, Departments of Epidemiology and Microbiology and Immunology, UNC Chapel Hill

Selected Awards:

2015 Natl. Acad. Of Sciences **"**China-U.S. Workshop on the Challenges of Emerging Infections, Laboratory Safety, and Global Health Security" September 28-30 in Beijing, China
2015 MERS-CoV Stakeholders Workshop, Invited panelist., NIH
2014 National Academy of Sciences: Working Group on Risks and Benefits of Gain of Function Research
2005-2014 Review Board, J. Virology
2008-2014 Senior Editor, Plos Pathogens
2008- Member-Biological Sciences Expert Group (BSEG)
2008 National Academy Sciences: Working Group: Gene Sequence Methods for Classification of Select Agents
2007-2008 Associate Editor, Plos Pathogens
2005-2009 Permanent Member, NIH VirB Study Section
2003 Finalist/Runner-up, World Technology Award
1989-1994 Established Investigator: American Heart Association
1984-1986.1 Harvey Weaver Scholar, National Multiple Sclerosis Society

C. Most Significant Contributions to Virology: The Baric laboratory has made significant contributions to coronavirus, norovirus and flavivirus immunology, molecular biology, virus-host interactions, susceptibility allele mapping, virus genetics, pathogenesis, vaccine and therapeutic design, using traditional and new technologies like structure guided immunogen design, synthetic genome design, systems biology and systems genetic approaches. Some representative major contributions outside and within the CoV field include:

C1. **Virus-Host Interactions and Susceptibility.** We have identified human susceptibility alleles and the molecular mechanisms that regulate norovirus susceptibility and persistence in human populations, developed platform strategies to map host susceptibility alleles in the mice, and identified the major targets of long-term protective immunity in DENV2.

1. Lindesmith L, Moe C, Marionneau S, Ruvoen N, Jiang X, Lindblad L, Stewart P, LePendu J, **Baric R**. Human susceptibility and resistance to Norwalk virus infection. **Nat Med.** 2003;9(5):548-53. PMID:12692541. 630 Citations
2. Gralinski LE, Ferris MT, Aylor DL, Whitmore AC, Green R, Frieman MB, Deming D, Menachery VD, Miller DR, Buus RJ, Bell TA, Churchill GA, Threadgill DW, Katze MG, McMillan L, Valdar W, Heise MT, Pardo-Manuel de Villena F, **Baric RS**. Genome Wide Identification of SARS-CoV Susceptibility Loci Using the Collaborative Cross. **PLoS Genet**. 2015 Oct 9;11(10):e1005504. PMID:26452100. (over 1,000 views)
3. Gallichotte EN, Widman DG, Yount BL, Wahala WM, Durbin A, Whitehead S, Sariol CA, Crowe JE Jr, de Silva AM, Baric RS. A New Quaternary Structure Epitope on Dengue Virus Serotype 2 Is the Target of Durable Type-Specific Neutralizing Antibodies. **MBio**. 2015 Oct 13;6(5). PMID:26463165.
4. Lindesmith LC, Donaldson EF, Lobue AD, Cannon JL, Zheng DP, Vinje J, **Baric** RS. Mechanisms of GII.4 **norovirus** persistence in human populations. **PLoS Med**. 2008 Feb;5(2):e31. PMC2235898. 343 citations

C2. Virus Reverse Genetics-Evolution/Disease Emergence. The Baric laboratory has pioneered strategies for performing reverse genetic analyses in coronaviruses and dengue viruses. Several coronavirus infectious cDNA clones are available in the lab, including recently emerged strains like SARS-CoV, MERS-CoV, PEDV, conventional human and model coronaviruses like MHV and HCoV NL63, and several bat coronaviruses. Using a similar strategy to solve flavivirus genome cDNA instability, we have built full length infectious cDNA clones for DENV1-4. The availability of these genetic platforms allows for detailed studies into the role of viral genes in pathogenesis, innate immune antiviral immunity, vaccine performance and design, virus-receptor interactions, entry and virus evolution.

1. Graham RL, Becker MM, Eckerle LD, Bolles M, Denison MR, **Baric RS**. 2012. A live, impaired-fidelity coronavirus vaccine protects in an aged, immunocompromised mouse model of lethal disease. **Nat Med**. Dec 6;18(12):1820-6. doi: 10.1038/nm.2972. PMCID: PMC3518599. Cited 56 times
2. Scobey T, Yount BL, Sims AC, Donaldson EF, Agnihothram SS, Menachery VD, Graham RL, Swanstrom J, Bove PF, Kim JD, Grego S, Randell SH, **Baric R.S**. Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus. **PNAS USA**. 2013 Oct 1;110(40):16157-62. PMC3791741.Cited 41 times
3. **Yount** B, Denison MR, Weiss SR, **Baric** RS. Systematic assembly of a full-length infectious cDNA of mouse hepatitis virus strain A59. J Virol. 2002 Nov;76(21):11065-78. Cited 228 times.
4. Becker MM, Graham RL, Donaldson EF, Rockx B, Sims AC, Sheahan T, Pickles RJ, Corti D, Johnston RE, **Baric RS**, Denison MR. Synthetic recombinant bat SARS-like coronavirus is infectious in cultured cells and in mice. **Proc Natl Acad Sci U S A**. 2008 Dec 16;105(50):19944-9. PMC2588415. Cited 95 time
5. Beall, A., Yount, B, Lin, C-M, Hou, Y, Wang, Q, Saif, L, and **Baric, R**. Characterization of a pathogenic full length cDNA clone and transmission model of porcine epidemic diarrhea virus strain PC22A. **mBIO** (positively reviewed and resubmitted.

C3. Coronavirus Host Range-Experimental Evolution. Our group has studied coronavirus host range expansion using experimental evolution and SARS-CoV, MERS-CoV, civet SL-CoV, bat SL-CoV, and bat CoV HKU5 as models. In many instances, this first required the synthetic reconstruction of civet and bat CoV from in silico sequence databases, recovery of recombinant bat viruses for the first time, and then characterization of the virus host range phenotypes both in vitro and in vivo. Applications of experimental evolution have typically focused on understanding the molecular mechanisms associated with virus-receptor interactions in viral persistence, host range adaptation in vitro and virus adaptation to increased virulence in mice.

1. Agnihothram S, Yount BL Jr, Donaldson EF, Huynh J, Menachery VD, Gralinski LE, Graham RL, Becker MM, Tomar S, Scobey TD, Osswald HL, Whitmore A, Gopal R, Ghosh AK, Mesecar A, Zambon M, Heise M, Denison MR, **Baric RS**. A mouse model for Betacoronavirus subgroup 2c using a bat coronavirus strain **HKU5** variant. **MBio**. 2014 Mar 25;5(2):e00047-14. PMC3977350. Cited 71 times.
2. Yang Y, Liu C, Du L, Jiang S, Shi Z, **Baric** RS, **Li** F. Two Mutations Were Critical for Bat-to-Human Transmission of Middle East Respiratory Syndrome Coronavirus. J Virol. 2015 Sep;89(17):9119-23. doi: 10.1128/JVI.01279-15. PMID: 26063432.
3. Huynh J, **Li** S, Yount B, Smith A, Sturges L, Olsen JC, Nagel J, Johnson JB, Agnihothram S, Gates JE, Frieman MB, **Baric** RS, Donaldson EF. Evidence supporting a zoonotic origin of human coronavirus strain NL63. **J Virol**. 2012 Dec;86(23):12816-25. PMC3497669. Cited 37
4. Sheahan T, Rockx B, Donaldson E, Corti D, **Baric R**. Pathways of cross-species transmission of synthetically reconstructed zoonotic severe acute respiratory syndrome coronavirus. **J Virol**. 2008 Sep;82(17):8721-32. PMC2519660. Cited 35 times.
5. Sheahan T, Rockx B, Donaldson E, Sims A, Pickles R, Corti D, **Baric R**. Mechanisms of zoonotic severe acute respiratory syndrome coronavirus host range expansion in human airway epithelium. **J Virol**. 2008 Mar;82(5):2274-85. PMC2258931. Cited 45 times.

C4. Virus Vaccine Design and Antiviral Immunotherapy. Impact requires the translation of basic science findings into novel strategies for the detection, control and prevention of human diseases. Coronaviruses, noroviruses and DENV are major causes of human morbidity and mortality worldwide. We have used structure-guided immunogen design and epitope exchange between norovirus and denv to broaden immunogenicity and build multivalent immunogens for increased vaccine breadth and diagnostic potential.

1. Deming, D.J., Sheahan, T., Heise, M, Yount, B., Davis, N., Sims, A., Suthar, M, Harkema J. Whitmore, A., Pickles R, West, A., Donaldson, E., Curtis, K., Johnston, RE, and **RS. Baric**. 2006. Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants. **PLoS Med** 3(12)e525 PMCID: PMC1716185. Cited 78 times.
2. Tang XC, Agnihothram SS, Jiao Y, Stanhope J, Graham RL, Peterson EC, Avnir Y, Tallarico AS, Sheehan J, Zhu Q, **Baric RS**, Marasco WA. 2014. Identification of human neutralizing antibodies against MERS-CoV and their role in virus adaptive evolution. **PNAS USA**. 2014 May 13;111(19):E2018-26. PMC4024880
3. Rockx B, Sheahan T, Donaldson E, Harkema J, Sims A, Heise M, Pickles R, Cameron M, Kelvin D, **Baric R**. Synthetic reconstruction of zoonotic and early human severe acute respiratory syndrome coronaviruses that produce fatal disease in aged mice. **J Virol.** 2007 Jul;81(14):7410-23.PMC1933338. Cited 47 times.
4. Lindesmith LC, Ferris MT, Mullan CW, Ferreira J, Debbink K, Swanstrom J, Richardson C, Goodwin RR, Baehner F, et al. 2015. Broad blockade antibody responses in human volunteers after immunization with a

multivalent norovirus VLP candidate vaccine: immunological analyses from a phase I clinical trial. **PLoS Med.** 2015 Mar 24;12(3):e1001807 PMC4371888. >4,000 views since published

C5. CoV Pathogenesis Studies. Our group has studied the role of virus-host interactions in coronavirus pathogenesis using genetically defined mice, systems biology and virus reverse genetics.

1. **Frieman** MB, Chen J, Morrison TE, Whitmore A, Funkhouser W, Ward JM, Lamirande EW, Roberts A, Heise M, Subbarao K, **Baric** RS. SARS-CoV pathogenesis is regulated by a STAT1 dependent but a type I, II and III interferon receptor independent mechanism. PLoS Pathog. 2010 Apr 8;6(4):e1000849. PMC2851658. Cited 34 times.
2. Sheahan T, Morrison TE, Funkhouser W, Uematsu S, Akira S, **Baric RS**, Heise MT. MyD88 is required for protection from lethal infection with a mouse-adapted SARS-CoV. **PLoS Pathog**. 2008 Dec;4(12): e1000240. PMC2587915. Cited 55 times
3. Menachery VD, Eisfeld AJ, Schäfer A, Josset L, Sims AC, Proll S, Fan S, Li C, Neumann G, Tilton SC, Chang J, Gralinski LE, Long C, Green R, Williams CM, Weiss J, Matzke MM, Webb-Robertson BJ, Schepmoes AA, Shukla AK, Metz TO, Smith RD, Waters KM, Katze MG, Kawaoka Y, **Baric RS**. 2014. Pathogenic influenza and coronaviruses utilize similar and contrasting approaches to control interferon-stimulated gene responses. **MBio.**2014 May20;5(3):e01174-14. PMC4030454. >3400 downloads.
4. Gralinski LE, Bankhead A 3rd, Jeng S, Menachery VD, Proll S, Belisle SE, Matzke M, Webb-Robertson BJ, Luna ML, Shukla AK, Ferris MT, Bolles M, Chang J, Aicher L, Waters KM, Smith RD, Metz TO, Law GL, Katze MG, McWeeney S, **Baric RS**. Mechanisms of severe acute respiratory syndrome coronavirus-induced acute lung injury. **MBio**. 2013 Aug 6;4(4). pii: e00271-13. >2800 downloads.

Complete List of Published Work in MyBibliography: >245 total publications, 102 since 2010, >5,000 citations.
http://www.ncbi.nlm.nih.gov/sites/myncbi/ralph.baric.1/bibliography/40583903/public/?sort=date&direction=ascending.

D. Research Support.

1. **U19 AI100625 (Baric, Heise MPI) 08/05/2012-7/31/2017**
   NIH/NIAID : Systems Immunogenetics of Biodefense Pathogens in the Collaborative Cross
   The Collaborative Cross, a mouse resource designed to study complex genetic interactions in diverse populations, to identify novel polymorphic genes regulating immune responses to SARS, influenza and West Nile viruses, gain new insights into genetic interactions that shape immune phenotypes in mice and humans, and generate panels of genetically defined mice to probe how sets of polymorphic genes affect immune responses against a variety of pathogens or other immune stimuli.
2. **R01 AI085524 (PI: Marasco) 06/09/10-05/31/14**
   Dana Farber/NIH Broad Spectrum Neutralizing Human Abs to SARS-CoV and Zoonotic CoV.
   Specific Aims: We will use SARS-CoV as a model to: 1) establish new paradigms for developing universal therapeutic platforms that protect against new emerging and deliberately designed human pathogens; 2) define pathways of virus escape as a function therapeutic composition and evaluate pathogenic consequences and 3) evaluate therapeutic potential in robust animal models, especially in vulnerable populations that develop acute respiratory distress syndrome (ARDS). Role: Consortium PI
3. **U19 AI107810 (PI: Baric) 07/01/13-06/30/18**
   NIH/NIAID Characterization of novel genes encoded by RNA and DNA viruses
   Using highly pathogenic human respiratory and systemic viruses which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and severe disease outcomes.
   U19 AI 107810-Supplement (PI: Baric) 09/01/14-05/31/15
   NIH/NIAID Characterization of novel genes encoded by RNA and DNA viruses
   One year administrative supplement to identify viral gene products encoded by pathogenic human viruses that manipulate the host protein synthesis machinery and related signaling pathways.
4. **R01 AI 107731 (PI: De Silva) 07/01/13-06/30/18**
   NIH/NIAID Molecular Basis of Dengue Virus Neutralization by Human Antibodies
   These studies proposed here are directly relevant to developing simple assays to predict the performance, safety and efficacy of the leading dengue vaccine candidates Role: Co-Investigator
5. **R01 AI108197 (MPI: Denison/Baric) 08/01/13-07/31/18**
   Vanderbilt Univ./NIH/NIAID Determinants of Coronavirus Fidelity in Replication and Pathogenesis

Experiments in this aim will test the hypothesis nsp1 functions in maintaining high replication fidelity and viral RNA synthesis are coupled and that targeted engineered mutations across nsp14 alter: a) RNA fidelity outcomes; b) sensitivity nucleoside mutagens, terminators and polymerase inhibitors; c) the kinetics and magnitude of positive, negative, genomic and subgenomic RNA synthesis; and d) RNA recombination.

6. **U19-AI106772-02 (PI: Kawaoka) 08/01/13-05/31/18**
   Univ of Wisconsin/NIH/NIAID Modeling Host Responses to Understand Severe Human Virus
   The proposed studies will provide a more detailed look at the intracellular environment by taking "snapshots" of the lipids, metabolytes, and proteins present during viral infection time courses. These assays will allow us to determine the innate immune response occurring immediately following virus infection and to determine how the virus and cell interact over a 72 hour window. Role: Investigator
7. **HHSN272201000019I-HHSN27200003 (PI: Palese) 09/30/13-07/14/15**
   MSSM/NIH MERS-CoV Mouse Model for Vaccine & Therapeutic Testing (Task Order A57)
   Specific Aims: Use generation of transgenic mice and modifications to the MERS-CoV genome to identify a mouse model for MERS-CoV that recapitulates human disease phenotypes for evaluating vaccine platforms and therapeutics. Role: Consortium PI
8. **U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19**
   UAB/NIH/NIAID Antiviral Drug Discovery and Development Center
   The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.Role: Co-Investigator
9. **U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19**
   Columbia/NIH/NIAID Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
   The goal is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection. Role: Project Leader, Consortium PI
10. **Not Assigned (PI: Baric) 01/27/2015-09/16/2015**
    PNNL/DHS Generation of Predictive Models of Viral Pathogenesis
    Using advances in transcriptomics, proteomics, and metabolomics, we will identify changes in the virus-host interaction expression networks associated with DENV infection of Aedes aegypti cells or human immune cells in vitro, the latter model after natural receptor-mediated or after ADE mediated entry.
11. **Not assigned (PI: deSilva) 02/01/2015-01/31/18**
    Johns Hopkins U/Gates Foundation: The dengue human infection model: Defining correlates of protection and advancing vaccine development
    The goal of these studies conducted by the Baric laboratory are to use recombinant dengue viruses encoding multiple homotypic neutralizing sites from multiple strains, as well as a collection of null mutants, to characterize the homotypic immune response elicited in humans following natural infection and after challenge in GSK DENV tetravalent vaccinated individuals. Role: Co-Investigator
12. **R01 AI110700 (PI: Baric) 04/20/15-03/31/20**
    NIH/NIAID Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
    The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis. Co-Director with Dr. Fang Li.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 5559179960000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Regents of the University of Minnesota

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Fang		Li	Ph.D	PD/PI	128,135.00	3.00			32,033.00	10,795.00	42,828.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **42,828.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12.00			40,000.00	8,959.00	48,959.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Postdoc Researcher	9.00			30,000.00	6,719.00	36,719.00
1	Jr. Scientist	6.00			16,000.00	4,383.00	20,383.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**106,061.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**148,889.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 5559179960000

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Regents of the University of Minnesota

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 5559179960000

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Regents of the University of Minnesota

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	60,611.00
2. Publication Costs	2,200.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	121,600.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Equipment repair and maintenance services	2,500.00
9 . Lab services	2,500.00
10 . Communications	300.00
Total Other Direct Costs	**189,711.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**341,600.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	52.00	245,000.00	127,400.00
		Total Indirect Costs	**127,400.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS Arif Karim (214) 767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**469,000.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: 1234-R01-budget-justification-Li-2015.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 5559179960000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Regents of the University of Minnesota

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Fang		Li	Ph.D	PD/PI	128,135.00	3.00			32,033.00	10,795.00	42,828.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **42,828.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12.00			40,000.00	8,959.00	48,959.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Postdoc Researcher	9.00			30,000.00	6,719.00	36,719.00
1	Jr. Scientist	6.00			16,000.00	4,383.00	20,383.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**106,061.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**148,889.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 5559179960000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: Regents of the University of Minnesota

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12031055
Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 5559179960000

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Regents of the University of Minnesota

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	60,611.00
2. Publication Costs	2,200.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	121,600.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Equipment repair and maintenance services	2,500.00
9 . Lab services	2,500.00
10 . Communications	300.00
Total Other Direct Costs	**189,711.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**341,600.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1 . Modified Total Direct Costs	53.00	220,000.00	116,600.00
		Total Indirect Costs	**116,600.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS Arif Karim (214) 767-3600

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**458,200.00**

J. Fee	**Funds Requested ($)***

K. Budget Justification*	File Name: 1234-R01-budget-justification-Li-2015.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12031055
Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 5559179960000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Regents of the University of Minnesota

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Fang		Li	Ph.D	PD/PI	128,135.00	3.00			32,033.00	10,795.00	42,828.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:			File Name:								**Total Senior/Key Person**	**42,828.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12.00			40,000.00	8,959.00	48,959.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Postdoc Researcher	9.00			30,000.00	6,719.00	36,719.00
1	Jr. Scientist	6.00			16,000.00	4,383.00	20,383.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**106,061.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**148,889.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 5559179960000

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Regents of the University of Minnesota

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 5559179960000

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Regents of the University of Minnesota

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	60,611.00
2. Publication Costs	2,200.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	121,600.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Equipment repair and maintenance services	2,500.00
9 . Lab services	2,500.00
10 . Communications	300.00
Total Other Direct Costs	**189,711.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**341,600.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	54.00	220,000.00	118,800.00
		Total Indirect Costs	**118,800.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS Arif Karim (214) 767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**460,400.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: 1234-R01-budget-justification-Li-2015.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 5559179960000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Regents of the University of Minnesota

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Fang		Li	Ph.D	PD/PI	128,135.00	3.00			32,033.00	10,795.00	42,828.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:			File Name:								**Total Senior/Key Person**	**42,828.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12.00			40,000.00	8,959.00	48,959.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Postdoc Researcher	9.00			30,000.00	6,719.00	36,719.00
1	Jr. Scientist	6.00			16,000.00	4,383.00	20,383.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**106,061.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**148,889.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 5559179960000

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Regents of the University of Minnesota

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 5559179960000

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Regents of the University of Minnesota

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

F. Other Direct Costs		Funds Requested ($)*
1. Materials and Supplies		60,611.00
2. Publication Costs		2,200.00
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		121,600.00
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8 . Equipment repair and maintenance services		2,500.00
9 . Lab services		2,500.00
10 . Communications		300.00
	Total Other Direct Costs	**189,711.00**

G. Direct Costs		Funds Requested ($)*
	Total Direct Costs (A thru F)	**341,600.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	54.00	220,000.00	118,800.00
		Total Indirect Costs	**118,800.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS Arif Karim (214) 767-3600

I. Total Direct and Indirect Costs		Funds Requested ($)*
	Total Direct and Indirect Institutional Costs (G + H)	**460,400.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: 1234-R01-budget-justification-Li-2015.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 5559179960000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Regents of the University of Minnesota

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Fang		Li	Ph.D	PD/PI	128,135.00	3.00			32,033.00	10,795.00	42,828.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **42,828.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12.00			40,000.00	8,959.00	48,959.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Postdoc Researcher	9.00			30,000.00	6,719.00	36,719.00
1	Jr. Scientist	6.00			16,000.00	4,383.00	20,383.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**106,061.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**148,889.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 5559179960000

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Regents of the University of Minnesota

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12031055

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 5559179960000

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Regents of the University of Minnesota

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	60,611.00
2. Publication Costs	2,200.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	121,600.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Equipment repair and maintenance services	2,500.00
9 . Lab services	2,500.00
10 . Communications	300.00
Total Other Direct Costs	**189,711.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**341,600.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	54.00	220,000.00	118,800.00
		Total Indirect Costs	**118,800.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS Arif Karim (214) 767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**460,400.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: 1234-R01-budget-justification-Li-2015.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget justification

Personnel Justification

Fang Li, PhD, Principal Investigator (3.0 calendar months), 25% effort, will be responsible for laboratory management and supervision of the projects, including experimental design, execution, data analysis, and write-up.

One Postdoctoral Scientist (To be named) (12.00 calendar months), 100% effort, will be responsible for conducting experiments on (i) crystallographic studies of coronavirus spike RBDs and their receptors; (ii) electron microscopic studies of coronavirus spike proteins.

One Postdoctoral Scientist (To be named) (9.00 calendar months), 75% effort, will be responsible for conducting experiments on (i) biochemical studies of the interactions between coronavirus spike RBDs and their receptors; (ii) coronavirus-spike-mediated pseudovirus entry into cells.

One Junior Scientist (To be named) (6.00 calendar months), 50% effort, will provide support in the expression and purification of coronavirus spike RBDs and their receptors.

Research Supplies Justification

Research supplies will include reagents for molecular biology, insect and mammalian cell culture, protein expression and purification, protein-protein binding assays, pseudovirus entry assays, negative-stain electron microscopy, and protein crystallography. More specifically, these reagents will include cloning enzymes, restriction enzymes, plasmid purification kits, DNA retraction kits, DNA transfection reagents, insect cells, insect cell culture media, mammalian cells, mammalian cell culture media, protein purification columns, Biacore chips, electron microscopy grids and tools, crystallization kits and plates, and crystal freezing tools.

Travel

For the PI, the postdoctoral scientists and junior scientist, on average there will be two trips to National synchrotron laboratories for X-ray data collection, and one trip to a national conference per year.

Publication

On average three publications will be expected per year.

Communications

Fax, long distance phone call, and shipping of experimental materials to collaborators

Equipment maintenance

Purchase of service contracts for equipments such as AKTA purification system, centrifuges, plate readers, and insect cell shaker/incubators.

Lab service fees

Use of services from several on-campus labs, such as the electron microscopy lab, robot crystallization lab, mass spectrometry lab, and protein sequencing lab.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		214,140.00
Section B, Other Personnel		530,305.00
Total Number Other Personnel	15	
Total Salary, Wages and Fringe Benefits (A+B)		744,445.00
Section C, Equipment		
Section D, Travel		15,000.00
1. Domestic	15,000.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		948,555.00
1. Materials and Supplies	303,055.00	
2. Publication Costs	11,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	608,000.00	
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	12,500.00	
9. Other 2	12,500.00	
10. Other 3	1,500.00	
Section G, Direct Costs (A thru F)		1,708,000.00
Section H, Indirect Costs		600,400.00
Section I, Total Direct and Indirect Costs (G + H)		2,308,400.00
Section J, Fee		

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Ralph	S	Baric	PhD	PD/PI	183,300.00	0.36			5,499.00	1,415.00	6,914.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **6,914.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.80			17,822.00	3,351.00	21,173.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician, Trevor Scobey	4.80			14,859.00	5,568.00	20,427.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**41,600.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**48,514.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: The University of North Carolina at Chapel Hill

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: The University of North Carolina at Chapel Hill

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	23,486.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Maintenance Contracts and repair	3,000.00
Total Other Direct Costs	**28,486.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**80,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	52.00	80,000.00	41,600.00
		Total Indirect Costs	**41,600.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**121,600.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: 1241-Baric-Budget Justification-rsb.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Ralph	S	Baric	PhD	PD/PI	183,300.00	0.36			5,499.00	1,415.00	6,914.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **6,914.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.80			17,822.00	3,351.00	21,173.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician, Trevor Scobey	4.80			14,859.00	5,568.00	20,427.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**41,600.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**48,514.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 6081952770000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: The University of North Carolina at Chapel Hill

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 6081952770000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: The University of North Carolina at Chapel Hill

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	23,486.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Maintenance Contracts and repair	3,000.00
Total Other Direct Costs	**28,486.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**80,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	52.00	80,000.00	41,600.00
		Total Indirect Costs	**41,600.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**121,600.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: 1241-Baric-Budget Justification-rsb.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Ralph	S	Baric	PhD	PD/PI	183,300.00	0.36			5,499.00	1,415.00	6,914.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **6,914.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.80			17,822.00	3,351.00	21,173.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician, Trevor Scobey	4.80			14,859.00	5,568.00	20,427.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**41,600.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**48,514.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: The University of North Carolina at Chapel Hill

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: The University of North Carolina at Chapel Hill

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	23,486.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Maintenance Contracts and repair	3,000.00
Total Other Direct Costs	**28,486.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**80,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	52.00	80,000.00	41,600.00
		Total Indirect Costs	**41,600.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**121,600.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: 1241-Baric-Budget Justification-rsb.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Ralph	S	Baric	PhD	PD/PI	183,300.00	0.36			5,499.00	1,415.00	6,914.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **6,914.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.80			17,822.00	3,351.00	21,173.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician, Trevor Scobey	4.80			14,859.00	5,568.00	20,427.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**41,600.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**48,514.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 6081952770000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: The University of North Carolina at Chapel Hill

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: The University of North Carolina at Chapel Hill

Start Date*: 07-01-2019 **End Date*:** 06-30-2020 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	23,486.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Maintenance Contracts and repair	3,000.00
Total Other Direct Costs	**28,486.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**80,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	52.00	80,000.00	41,600.00
		Total Indirect Costs	**41,600.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**121,600.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: 1241-Baric-Budget Justification-rsb.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Ralph	S	Baric	PhD	PD/PI	183,300.00	0.36			5,499.00	1,415.00	6,914.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **6,914.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	4.80			17,822.00	3,351.00	21,173.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician, Trevor Scobey	4.80			14,859.00	5,568.00	20,427.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**41,600.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**48,514.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: The University of North Carolina at Chapel Hill

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Funding Opportunity Number: PA-13-302 . Received Date: 2015-11-03T17:08:03.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: The University of North Carolina at Chapel Hill

Start Date*: 07-01-2020 **End Date*:** 06-30-2021 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	23,486.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Maintenance Contracts and repair	3,000.00
Total Other Direct Costs	**28,486.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**80,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	52.00	80,000.00	41,600.00
		Total Indirect Costs	**41,600.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**121,600.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: 1241-Baric-Budget Justification-rsb.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION – BARIC UNC

PERSONNEL ($48,514):

Ralph S. Baric, PhD, Consortium PI (0.36 calendar months, 3% effort). Dr. Baric is an expert in human emerging coronavirus (SARS-CoV/MERS-CoV) replication, cross species transmission, genetics and pathogenesis and his group is well positioned to conduct the studies proposed in the application. His group has developed molecular clones to a large number of human and animal coronaviruses, including the strains germane to this proposal. Dr. Baric will work closely with Drs. Li to conduct the basic research on virus-receptor interactions, protease usage and entry. Within his own group, he will interact closely with Dr. Kocher and Mr. Scobey to ensure steady progress during the course of the proposal, evaluate results and propose alternative experiments. Dr. Baric will communicate his group's findings, share data, discuss and solve problems by interacting with the rest of the scientific team at monthly meetings and on at an ad hoc basis for specialized topics that are in need immediate solutions. Drs. Baric and Li have worked together over the past year, published papers on MERS-CoV RBD structure and function and in studying coronavirus cross species transmission. Dr. Baric and the members of his group will attend yearly meetings to discuss the progress of the project.

Dr. Baric's salary reflects the current NIH salary cap of $183,300. The remainder of his salary will be supported by other institutional funds.

Jacob Kocher, PhD, Postdoctoral Research Associate (4.8 calendar months, 40% effort). Dr. **Kocher** will assist in the generation of recombinant viruses including panels of mutants with defects in receptor binding and/or protease processing. He has considerable expertise in working in a BSL3 facility, has considerable experience working with human and animal coronaviruses, appropriate cell culture systems and entry based assays. He will work closely with Dr Baric, help direct and follow the progress of Mr. Scobey to coordinate recombinant virus production, phenotypic characterization, sample collection and data interpretation. He will assist in the experimental workflow. He will attend lab meetings, present data and present findings/write papers, and participate in group meetings.

Mr. Drew Scobey, Research Technician (4.8 calendar months, 40% effort). Mr. Scobey is certified to work with high containment pathogens under BSL3 and has considerable experience in working with coronavirus under BSL2 conditions as well. He is expert in recombinant virus recover, virus enumeration, cell fractionation, FACs based analyses, safety testing of samples, and in the preparation of RNA and protein samples. He developed the MERS-CoV molecular clone in our laboratory. Thus, he is well versed in molecular biology and virus reverse genetics and has isolated and characterized a large number of coronavirus mutants. He will also assist and help Dr. Kocher in the genetic and phenotypic characterization of mutants. Finally, he will ensure that the BSL3 and BSL2 laboratory is well stocked and compliant in terms of regulations and day to day maintenance.

Fringe Benefits Faculty/Staff: 22.741% Social Security and Retirement; $5,471/FTE Health Insurance. Post-doctoral Research Associates: 8.99% Social Security and benefits; $4,373/FTE Health Insurance.

EQUIPMENT: None

SUPPLIES ($23,486):

Synthetic DNAs and Sequencing Costs ($4,000/year). The Baric lab routinely uses synthetic genomics for the creation of virus mutants potentially used in validation studies, as it is cheaper and faster than using standard recombinant DNA approaches (considering personnel time, resequencing costs, time, materials, etc). Synthetic DNAs will be used to build new virologic reagents for measuring the impact of target mutations on receptor interactions, protease processing and entry. Synthetic DNAs are primarily purchased from small biotec companies like Blue Heron or Bio Basic Inc. at costs of about 0.30$/base, sufficient for our needs over the course of this project. We will also require funds for sequence validation of recombinant viruses.

Serum and Media ($5,500/year). A large amount of cell culture work is associated with the project, requiring media, serum and culturing flasks. This includes virus tittering and neutralization assays. Consequently, funds are requested for cell culture supplies to maintain Vero and related permissive cells for each coronavirus under study, and for measuring virus growth kinetics, evaluate entry kinetics and virus viability, titer virus stocks, and to isolate recombinant viruses for in vitro characterization or other phenotypes. These monies support cell culture work in BSL2 and BSL3 facilities.

Personnel Protective Gear ($3,000/year). PAPRs, tyvek suits and other protective gear. Personnel wear powered air purifying HEPA filtered breathing apparatuses, wear tyvek suits, tyvek aprons, hoods, booties and are double gloved when entering the BSL3 facility. These materials are expensive as the HEPA, organic chemical filters and even batteries must be replaced every ~6-12 months, and the tyvek suits are disposable. Moreover, the PAPRs (powered air breathing apparatus) are expensive and must be replaced every ~2 years from normal wear and tear, and daily contact with EPA disinfectants. Personnel also use high quantities of EPA approved disinfectants like ethanol alcohol, Clorox and other EPA approved disinfectants in maintaining a safe working environment in the BSL3. Personnel spray down tyvek suits, etc. with alcohol or related disinfectants in the process of deconing and leaving the BSL3 facility. All materials that leave the BSL3 must be disinfected, packaged in disinfected, sealed containers which are disinfected prior to removal from the BSL3 facility.

Consumables ($4,500/year). A large amount of plastic ware will be consumed during the course of this project including eppendorf tubes, flasks, plates, pipettes, pipette tips, etc. This includes plastic ware for virus growth kinetics, plaque assays, TCID50 quantization's, etc. used to evaluate and compare virus growth efficiency and to evaluate neutralization titers following vaccination. Small quantities of chemicals will be purchased, including Brefeldin-A and formalin for fixing cells.

Enzymes, Kits and Reagents ($2,800/year). Oligodeoxynucleotide synthesis and kits for quantitative RT-PCR for measuring concentrations of viral and discrete cellular mRNAs will be used during the course of the project. A variety of reagents needed for tissue fixation and immunohistochemistry. A variety of chemicals will be purchased during the course of the project that will be used for making buffers, reagents, and other solutions. These costs include reagents (e.g., plasticware/EIA kits, etc.) for EIA analyses. A variety of antibodies and immunological reagents will be used during the course of the proposal to characterize S topology in the context of recombinant viruses.

Miscellaneous ($2,186/yr). Funds are requested to purchase chemicals, reagents, paper products, gloves, micropipetors, autoclave supplies, plastic tips, waterbaths, and other small equipment items that typically have short half lives in laboratory settings.

Computers ($1,500/year). Funds are requested to provide NoV specific computer hardware and software updates required over the course of the project. Statistical data analysis and modeling of data require up to date equipment and software.

TRAVEL ($3,000/year):
Scientific Meetings Travel funds are requested for the PI and fellow to attend the scientific meeting each year, such as ASV, during the course of the proposal and visit University of Minnesota. This allows program faculty to communicate results, develop collaborations and share research interests.

OTHER EXPENSES ($5,000)

Maintenance and Service Contracts ($3,000/year). Autoclave service contract and BSL3 maintenance costs for decontaminating/maintaining the facility are requested for each year of the program. These dollars are critical for maintaining a safe environment for BSL3 research and for maintenance contracts for the BSL-2 equipment, like the autoclave in the BSL3.

Publication Costs ($2,000/year). Funds are requested for publication of manuscripts associated with this research.

F&A In an agreement with DHHS dated 5/16/2012 the indirect cost rate for The University of North Carolina is 52% of MTDC, excluding equipment and tuition.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		34,570.00
Section B, Other Personnel		208,000.00
Total Number Other Personnel	10	
Total Salary, Wages and Fringe Benefits (A+B)		242,570.00
Section C, Equipment		
Section D, Travel		15,000.00
1. Domestic	15,000.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		142,430.00
1. Materials and Supplies	117,430.00	
2. Publication Costs	10,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	15,000.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		400,000.00
Section H, Indirect Costs		208,000.00
Section I, Total Direct and Indirect Costs (G + H)		608,000.00
Section J, Fee		

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	300,000	300,000	300,000	300,000	300,000	**1,500,000**

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OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
First Name*: Fang
Middle Name:
Last Name*: Li
Suffix: Ph.D

2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

● Yes ○ No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

Funding Opportunity Number: PA-13-302. Received Date: 2015-11-03T17:08:03.000-05:00

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ❍ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ● Yes ❍ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ● Yes ❍ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

Tracking Number: GRANT12031055

Funding Opportunity Number: PA-13-302. Received Date: 2015-11-03T17:08:03.000-05:00

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	1242-R01-CoV-renew-2015-specific-aims-final.pdf
3. Research Strategy*	1243-R01-CoV-renew-2015-research-strategy-final.pdf
4. Progress Report Publication List	1244-R01-CoV-renewal-2015-progress-report.pdf
Human Subjects Sections	
5. Protection of Human Subjects	
6. Inclusion of Women and Minorities	
7. Inclusion of Children	
Other Research Plan Sections	
8. Vertebrate Animals	
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	1247-UNC 161597SignedFacePage.pdf
12. Letters of Support	1248-HOLMES li letter 2015.pdf
13. Resource Sharing Plan(s)	1249-R01-CoV-renew-2015-resources-sharing.pdf
Appendix (if applicable)	
14. Appendix	

Specific Aims

Coronaviruses (CoVs) cause epidemic outbreaks of diseases in humans and other animals, symbolized by the recent emergence of SARS-CoV, MERS-CoV, and porcine epidemic diarrhea CoV (PEDV). Receptor recognition and cell entry are two essential steps in the CoV infection cycle and function as critical determinants of CoV host range and tissue tropism. They are also primary targets for antiviral intervention. An envelope-anchored CoV spike protein recognizes host receptors through its S1 subunit and fuses viral and host membranes through its S2 subunit. CoVs recognize a variety of host receptors through one or both of its spike S1 domains (S1-NTD and S1-CTD), and are also regulated for cell entry by host proteases that cleave at the S1/S2 boundary. In the previous funding cycle, we have determined the crystal structures of a number of CoV S1 domains alone or in complex with their receptor. We have also identified host receptors as well as the cell entry mechanisms for a number of different CoVs. Our studies have revealed how CoVs exploit host receptors and proteases to regulate their host range, tropism and pathogenesis, and how SARS-CoV and MERS-CoV transmit from animals to humans to cause epidemics. In this competitive grant renewal, we will continue to investigate fundamental questions regarding receptor recognition and cell entry of CoVs from different genera. The long-term goals of these studies are to develop global models of CoV-receptor interactions and protease processing in evolution, entry, tropism, pathogenesis and cross-species transmission of CoVs.

Aim 1: Receptor binding by CoV S1-NTDs. We previously determined the structures of β-genus mouse CoV (MHV) S1-NTD complexed with its protein receptor CEACAM1 and of β-genus bovine CoV (BCoV) S1-NTD by itself. We also showed that S1-NTDs of BCoV and α-genus PEDV both recognize sugar receptors. Our studies revealed that β-CoV S1-NTDs have the same structural fold as human galectins (galactose-binding lectins), but bind either protein or non-galactose sugar receptors. This aim will investigate how S1-NTDs from different genera have evolved to bind sugar receptors. Our overall hypothesis is that S1-NTDs from different CoV genera originated from host galectins, but have evolved molecular mechanisms to recognize novel receptors. To test this hypothesis, we will determine the molecular and structural basis for sugar binding by BCoV and PEDV S1-NTDs. These studies will reveal evolution of CoV S1-NTDs, help evaluate sugar binding by other CoVs, and may facilitate future design of sugar analogue inhibitors and subunit vaccines against CoVs.

Aim 2: Receptor biding by CoV S1-CTDs. We determined the structures of several CoV S1-CTDs in complex with their protein receptors, including β-genus SARS-CoV S1-CTD complexed with its protein receptor ACE2. Our research suggested that two mutations played critical roles in the transmission of SARS-CoV from palm civets to humans. However, recent studies isolated several bat SARS-like CoVs (SLCoVs) with potentials to infect humans directly. This aim will investigate the cross-species transmission pathway of bat SLCoVs by examining the interactions between their S1-CTDs and ACE2 from different species. Our overall hypothesis is that to transmit from bats to another species, bat SLCoVs need to adapt to ACE2 from that species through selective mutations in their S1-CTD. To test the hypothesis, we will (i) identify receptor and viral determinants for transmission of bat SLCoVs to other species (civet, human and mouse); (ii) determine the crystal structures of bat SLCoV S1-CTDs and ACE2 from other species. These studies will reveal the molecular mechanisms for cross-species transmission of bat SLCoVs, inform future bat SLCoV emergence pathways to other mammals, and facilitate epidemic monitoring and the development of broadly cross-protective intervention strategies.

Aim 3: Cell entry by CoVs. Previously we characterized the pre-fusion and post-fusion conformations of SARS-CoV spike, demonstrated that host proteases serve as a cross-species barrier for MERS-CoV, and showed that a glycosylated protease motif attenuates human cell entry of bat CoV HKU4. Our overall hypothesis is that CoV spikes have evolved a variety of mechanisms for protease activation that can meet their specific need for host range, tissue tropism, and pathogenesis. We will investigate the following questions regarding CoV entry. (i) How does neurotropic MHV enter neurons where receptor expression is low? (ii) How does HCoV-NL63 modulate its cell entry and pathogenesis? (iii) How does PEDV enter intestinal cells where extracellular proteases are abundant? These studies will reveal how cell entry regulates the host range, tropism and pathogenesis of CoVs, and may facilitate future design of protease inhibitors against CoVs.

Approaches: We take multi-disciplinary approaches including crystallographic studies of CoV S1 domains complexed with their receptor, biochemical assays on S1 domains/receptor binding, biochemical analysis of protease cleavages of CoV spikes, electron microscopic analysis of conformational states of CoV spikes, pseudovirus entry assays, reverse genetics, and live virus infection assays.

Impact: This research uses CoVs as model systems to establish novel principles in virology that govern viral evolution, receptor recognition, cell entry, cross-species infections and tropism, which may be extended to other virus families. These studies are also critical for evaluating the emerging disease potentials of CoVs and for preventing, controlling and treating the spread of CoVs in humans and other animals.

A. Significance.

A.1. Overview of coronaviruses: Coronaviruses (CoVs) pose serious health threats to humans and other animals. In 2002-2003, SARS-CoV infected 8000 people with a fatality rate of ~10% (16-19). Since 2012, MERS-CoV has infected over 1500 people with a fatality rate of ~35% (20, 21). Since 2013, porcine epidemic diarrhea CoV (PEDV) has swept throughout the United States, causing ~100% fatality rate in piglets and wiping out more than 10% of America's population in less than a year (22-24). Many other CoVs, such as human NL63 respiratory CoV (HCoV-NL63) and bovine CoV (BCoV), cause widespread diseases in humans and animals, leading to significant economical losses (25-28). Importantly, CoVs have the largest genome among all RNA viruses, and are capable of adapting through mutations and recombinations to new environments with relative ease (25-28). Consequently, CoVs are programmed to efficiently alter host range and tissue tropism. It is believed that most or all of human CoVs originated from animals (25-28). Understanding the host range, cross-species infection, and tissue tropism of CoVs have important implications on virology, medicine, global health and global economic stability.

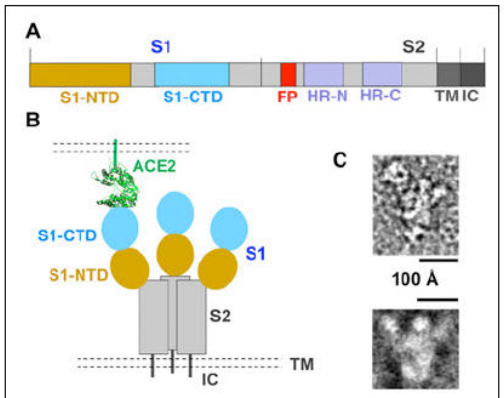


Figure 1. Overall structure of SARS-CoV spike revealed by Dr. Li's electron microscopic study. (A) Schematic structure of CoV spike. S1-NTD: N-terminal domain of S1. S1-CTD: C-terminal domain of S1. FP: fusion peptide. HR-N and HR-C: heptad repeat regions N and C. FP, HR-N and HR-C are required for membrane fusion. TM: transmembrane anchor. IC: intracellular tail. (B) Schematic overall structure of trimeric SARS-CoV spike complexed with its receptor ACE2. (C) Electron microscopic images of SARS-CoV spike by itself (bottom right) and in complex with ACE2 (top right) (10).

A.2. CoV-Receptor Interactions: Receptor recognition is an important determinant of CoV host range, tropism, pathogenesis and a target of neutralizing antibody-mediated protective immunity (25-29). An envelope-anchored spike protein mediates CoV entry into host cells by first binding to a host receptor via its S1 subunit and then fusing the host and viral membranes via its S2 subunit (25, 26, 28) (Fig. 1). S1 contains two domains, S1-NTD and S1-CTD, both of which can function as receptor-binding domains (RBDs) (28, 30). S1-NTDs and S1-CTDs from three major CoV genera, α, β and γ, recognize at least four protein receptors and four sugar receptors, demonstrating a complex receptor recognition pattern (28, 30). For example, highly similar CoV S1-CTDs within the same genus can recognize different receptors, whereas very different CoV S1-CTDs from different genera can recognize the same receptor. CoV S1-NTDs can also recognize either protein or sugar receptors. A fundamental yet poorly understood puzzle in virology is how viruses evolve to recognize their receptors (31). The complex receptor recognition pattern of CoVs presents a unique opportunity for understanding viral evolution using CoVs as model systems. Moreover, studying receptor recognition by CoVs is critical for understanding the host range, tissue tropism, pathogenesis and cross-species transmission of CoVs, for evaluating the emerging disease potentials of CoVs, and for preventing, controlling and treating the spread of CoVs in humans and other animals.

Our previous research has determined the crystal structures of a number of CoV RBDs from different genera by themselves and in complex with their receptor (Fig.2). These structures account for the majority of the structures determined in the field. We have also identified and characterized the receptors for β-genus bat CoV HKU4 and α-genus PEDV (4, 32). Importantly, our studies have built a systematic framework for understanding CoV evolution and receptor recognition, and established a number of important virology principles (28, 30). First, drastic structural changes in viral RBDs can still lead to recognition of a virus-binding hotspot on the same protein receptor. Supporting this principle is our finding that β-genus SARS-CoV and α-genus

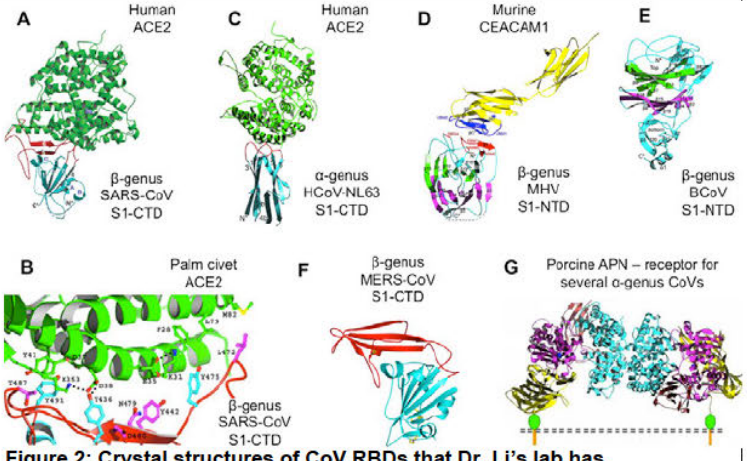


Figure 2: Crystal structures of CoV RBDs that Dr. Li's lab has determined. (A) SARS-CoV S1-CTD complexed with human ACE2 (2). (B) SARS-CoV S1-CTD complexed with palm civet ACE2 (1, 3); (C) Human coronavirus NL63 (HCoV-NL63) S1-CTD complexed with human ACE2 (3, 13); (D) Mouse CoV (MHV) S1-NTD complexed with murine CEACAM1 (11); (E) Bovine CoV (BCoV) S1-NTD (12); (F) MERS-CoV S1-CTD (14); (G) Aminopeptidase N (APN), receptor for several α-genus CoVs (15).

HCoV-NL63 recognize a common virus-binding hotspot on their common protein receptor, angiotensin-converting enzyme 2 (ACE2), using structurally divergent S1-CTDs. Second, small structural changes in viral RBDs can lead to a complete receptor switch. For example, β-genus SARS-CoV and MERS-CoV use structurally similar S1-CTDs to bind different protein receptors: ACE2 for SARS-CoV and dipeptidyl peptidase 4 (DPP4) for MERS-CoV. Third, it is a successful viral strategy to steal a host protein and evolve it into viral RBDs with novel protein receptor specificities or altered sugar receptor specificities. For example, S1-NTDs from β-genus mouse CoV (MHV) and BCoV have the same structural fold as human galectins (galactose-binding lectins), but recognize a protein receptor CEACAM1 and a non-galactose sugar receptor, respectively. Fourth, a few residue changes at the receptor binding interface can lead to efficient cross-species infection and human-to-human transmission of a virus. For example, SARS-CoV needed only a few mutations in its RBD to transmit from palm civets to humans by enhancing its recognition of human ACE2. These virology principles may be extended from CoVs to other virus families.

This proposal will investigate unresolved questions regarding receptor recognition of CoVs. These questions can be generalized as following. (i) What are the detailed sugar binding mechanisms for CoV S1-NTDs from different genera, and what are the structural folds of CoV S1-NTDs outside β-genus? (ii) Did SARS-CoV transmit from bats to humans directly or through intermediate host palm civets, and how did bat SARS-CoV S1-CTD adapt to ACE2 from other species during the cross-species transmission process? Overall, Aims 1 and 2 of this proposal investigate how CoVs have evolved to recognize their protein or sugar receptor and how receptor recognition regulates CoV host range, cross-species transmission, and pathogenesis.

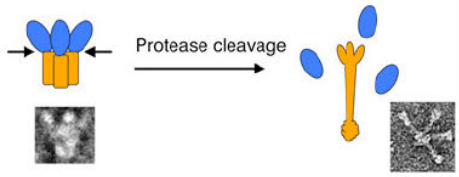
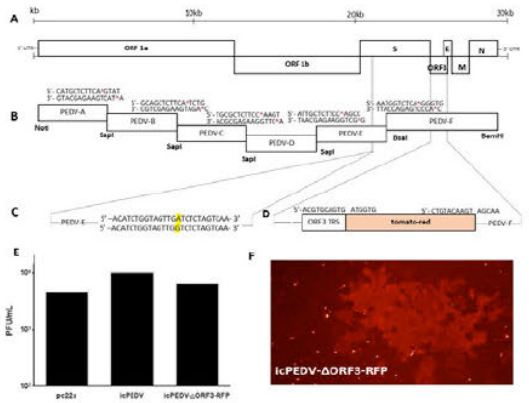


Figure 3. Conformational changes of SARS-CoV spike revealed by Dr. Li's electron microscopic study (10). Shown are schematic drawings of SARS-CoV spike in two different states and their corresponding electron microscopic images. The molecule in the pre-fusion state (left) is a clove-shaped trimer, with three individual S1 heads (blue) and a trimeric S2 stalk (orange). The molecule in the post-fusion state (right) corresponds to structurally rearranged trimeric S2 and dissociated monomeric S1. Conformational changes require proteolysis at the S1/S2 boundary and ensuing dissociation of S1.

A.3. CoV Entry into Host Cells: CoV entry into host cells is limited not only by receptor recognition, but also by membrane fusion. For S2 to fuse membranes, the CoV spike needs to be cleaved at the S1/S2 boundary (i.e., the region between S1-CTD and S2 fusion peptide; see Fig.1A) by one or more host proteases (33-37). The availability of these host proteases to CoVs and the specificities of these host proteases on CoV spikes can contribute to the host range and tissue tropism of CoVs. CoV-spike-processing host proteases may come from four different stages of the virus infection cycle: (i) proprotein convertases during virus packaging in virus-producing cells (e.g., furin); (ii) extracellular proteases after virus release from virus-producing cells (e.g. elastase); (iii) cell-surface proteases after virus attachment to virus-targeting cells (e.g. type II transmembrane serine proteases (TMPRSS2)); (iv) endosomal proteases after virus endocytosis in virus-targeting cells (e.g. cathepsins L and cathepsin B) (33, 34). In previous studies, we characterized the pre-fusion and post-fusion conformations of SARS-CoV spike using electron microscopy, and identified proteolysis as a trigger for the conformational change of SARS-CoV spike (Fig.3) (10). We also identified the host proteases that activate cell entry of SARS-CoV, MERS-CoV, HKU4, and PEDV (4, 5, 32, 38). Moreover, we discovered that the functional differences between human and bat proteases contribute to the bat-to-human transmission of MERS-CoV, and showed that a glycosylated protease motif attenuates the infection of bat CoV HKU4 in human cells (4, 5). While making these discoveries, we have established a series of biochemical and molecular assays to investigate the relationships between spike cleavage and CoV entry. These studies have paved the way for us to study the following important questions regarding CoV entry. (i) Are glycosylated protease motifs in CoV spike a common mechanism for modulating entry and pathogenesis? (ii) How does proteolytic activation regulate the tissue tropism of CoVs? (iii) What proteases activate the entry of newly emerged CoVs? Overall, Aim 3 of this proposal will further investigate how CoVs have evolved to adapt to host proteases and how host proteases regulate CoV host range, tropism, and pathogenesis.

Figure 4. Organization of PEDV Strain PC22A Molecular Clone. (A) Genome Organization, (B) Organization of cDNA Clone, (C&D) Replacement of ORF3 with RFP, (E) Recombinant virus titers, (F) RFP expression.



A.4. CoV reverse genetics to complement biochemical and structural approaches. The Baric lab has established reverse genetics for many human and animal CoVs including human SARS-CoV, MERS-CoV, several bat SARS-like-CoVs, several bat MERS-related CoVs, HCoV-NL63, MHV, and PEDV (Fig.4) (39-43). The genetic approaches developed by the Baric lab will complement the biochemical and structural approaches used by the Li lab to study the receptor recognition and cell entry of CoVs. In parallel, the Baric laboratory has used experimental evolution to select for virus variants in vitro and in vivo with mutations that alter receptor interactions or spike-mediated protease processing, providing parallel tracts for identifying novel mutational pathways that promote entry and/or alter tissue tropism and pathogenesis (44-50). The Li laboratory's capacity for high throughput biochemical characterization of spike mutants, coupled with experimental evolution and reverse genetics in the context of live viruses, provides unparalleled power for dissecting the mutational spectra and structure-function relationships that regulate the evolution of structures in receptor interactions, spike proteolytic processing, entry and cross species transmission.

B. Innovation: One salient characteristic of this proposal is the use of structural approaches to investigate CoV evolution, receptor recognition, cross-species infections, tissue tropism, and epidemics. First, we use the crystallographic method to solve the puzzling receptor recognition patterns of CoVs. This strategy has advantages over other methods in virus/receptor binding studies because the structures of spike-receptor complexes present complete views of the virus/receptor binding interfaces. Our strategy has proven to result in novel discoveries (see Section A2). Second, we use structural analysis of CoV spike proteins to reconstruct evolutionary history of CoVs. This strategy provides unique insight into viral evolutions because protein tertiary structures are associated with functions and hence are often better conserved through evolution than other available information such as protein primary sequences. Third, we use detailed structural analysis of CoV/receptor interactions to examine how seemingly small changes of the viruses at the CoV/receptor interfaces can have dramatic epidemic outcomes in our society. This strategy provides clear molecular insight into the evolutionary pathways driving viral epidemics by providing a direct structural view of virus changes at atomic levels. Indeed, our research stresses that it is critical to understand viral epidemics, evolution, and receptor recognition under detailed structural framework.

Although we are not the first or only group to use structural methods to explore puzzles about CoVs, we have been highly successful in the application of the methods. Prior to the SARS epidemic, no structural information was available for any CoV S1 subunit, despite decades of extensive research. Since the SARS epidemic, we have successfully determined numerous structures of CoV S1 domains by themselves or in complex with their receptor (Fig.2). The key to our structure determinations is to identify the most suitable protein targets for crystallographic studies, using a combination of biochemical and bioinformatics methods. More specifically, we carefully define the domain boundaries of CoV spike proteins based on information from limited proteolysis of the spike proteins, secondary and tertiary structure predictions of the spike proteins, and published mutation and truncation data on the spike proteins. Overall, our previous studies have demonstrated the promise of using crystallography to reveal innovative virology and epidemic findings. In this proposal, we will use the same innovative approach to investigate the structures and functions of CoV spikes, and their impacts on CoV evolution, cross-species transmission, and tissue tropism.

Our research on CoV cell entry is also innovative because we use a combination of molecular, biochemical, and structural approaches. Our molecular approaches include pseudovirus entry assays that we have established for all major CoV spikes. Our biochemical approaches include use of purified total lysosomal proteases or individual recombinant lysosomal proteases to characterize proteolytic cleavage of CoV spikes. Our structural approaches include use of electron microscopy to examine the conformational changes of purified recombinant CoV spikes associated with CoV entry. The combination of all these approaches has advantages over molecular approaches alone because the biochemical and structural approaches can provide direct evidence for CoV entry mechanisms that molecular approaches alone cannot provide.

Furthermore, our approach is innovative in blending both *in vitro* and *in vivo* approaches to study CoV entry and pathogenesis. Whereas Dr. Li has extensive expertise in receptor recognition and cell entry of CoVs, Dr. Baric is the global expert in reverse genetics and pathogenesis of CoVs. Dr. Baric and Dr. Li have jointly published several important studies on CoVs (4, 5, 14), and are collaborating on several additional projects. Importantly, the approaches from Dr. Baric and Dr. Li's labs are complementary, which enrich the depth and breadth of experimental investigations and can lead to important findings in the roles of receptor and entry factors in CoV host range, tissue tropism, and pathogenesis. Dr. Li will lead studies involving structural biology and biochemistry. Dr. Baric will complement Dr. Li's research with reverse genetics and *in vivo* experiments. Studies from both groups integrate across and support each other's research objectives. This model should maximize productivity and strengthen program outcomes.

C. Approach

C.1 Specific Aim 1: Receptor binding by CoV S1-NTDs.

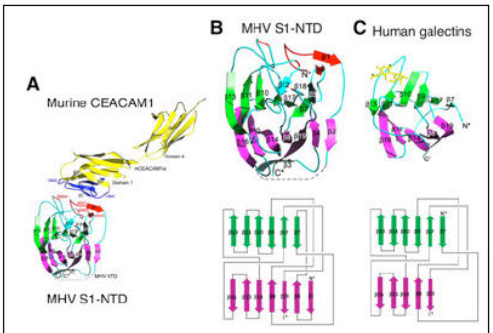


Figure 5. β-genus MHV S1-NTD has a galectin fold. (A) Crystal structure of MHV S1-NTD complexed with its receptor CEACAM1 (11). (B) Structure of MHV S1-NTD (top) and its schematic topology (bottom). (C) Structure of human galectins (top) and their schematic topology (bottom).

Preliminary data supporting Aim 1: We previously determined the crystal structure of β-genus mouse CoV (MHV) S1-NTD complexed with its protein receptor CEACAM1 (Fig.5) (11). Surprisingly, MHV S1-NTD has the same structural fold as human galectins (galactose-binding lectin), although it binds to CEACAM1 through exclusive protein-protein interactions. We then found that the S1-NTDs of β-genus bovine CoV (BCoV) and human CoV OC43 both bind sugar and function as viral lectins, and characterized their sugar receptor (i.e., 5-N-acetyl-9-O-acetylneuraminic acid (Neu5,9Ac2)) using glycan screen arrays. We further determined the crystal structure of BCoV S1-NTD, which also has a galectin fold (Fig.5) (12). Although we didn't have sugar in the structure, structure-based mutagenesis allowed us to identify the sugar-binding site in BCoV S1-NTD, which overlaps with the galactose-binding site in galectins (Fig.6A, 6B). Based on these studies, we hypothesized that ancient β-CoVs acquired their spike S1-NTD from the host, and have evolved it into viral RBDs with novel specificity for protein receptors (as in MHV) or for novel sugar receptors (as in BCoV).

We also characterized receptors for α-genus PEDV (32). We expressed PEDV S1-NTD-CTD as one fragment, and showed that it binds protein receptor APN and sugar receptor N-acetylneuraminic acid (Neu5Ac). We further showed that viral entry is reduced by blocking sugar receptor binding, demonstrating the importance of sugar receptor binding in viral entry (32).

Overview of Aim 1: Despite little sequence similarity between them, S1-NTDs from α-CoVs and β-CoVs both bind sugar receptors, allowing us to further hypothesize that α-CoV S1-NTDs also originated from host galectins and have a galectin fold. This aim will test this hypothesis, shedding light on the evolution of CoV S1-NTDs. In addition, this aim will investigate detailed sugar binding mechanisms for both α-CoV and β-CoV S1-NTDs. Because sugar binding by CoV S1-NTDs has been associated with broadened host range and tissue tropism of CoVs (51-54), results from this aim will allow prediction of sugar binding by S1-NTDs from CoVs that are poorly characterized or will emerge in the future, providing insight into their host range and tissue tropism. Furthermore, understanding the detailed interactions between CoV S1-NTDs and their sugar receptor may help future design of small molecule inhibitors that block sugar binding and thus cell entry of CoVs. The structures of CoV S1-NTDs may also facilitate future design of subunit vaccines against CoV infections.

Overall hypotheses of Aim 1: S1-NTDs from different CoV genera originated from host galectins, but have evolved molecular mechanisms to recognize novel sugar receptors.

C.1.1 Subaim 1. Sugar receptor binding by β-genus BCoV

Hypothesis: The spike receptor-binding domain (RBD) and receptor-destroying enzyme (RDE) of BCoV co-evolved to recognize the same type of sugar, which is critical for balancing viral entry and viral release.

Rationale: Some β-CoVs, including BCoV, encode a hemagglutinin-esterase that destroys virus-bound sugar on cell surface during viral release and thereby functions as the viral RDE (55). BCoV S1-NTD and RDE recognize the same type of sugar using structurally different sugar-binding pockets (12, 56). Thus, the two proteins likely have co-evolved, such that viral attachment and release can be coordinated to achieve optimal viral replication efficiency. The crystal structure of BCoV RDE complexed with a sugar substrate analogue has been determined (56). In this subaim, we will determine the crystal structure of BCoV S1-NTD complexed with its sugar receptor analogue. Comparison of the two structures will reveal how BCoV S1-NTD and RDE have co-evolved to recognize the same type of sugar using structurally different sugar-binding pockets.

Experimental designs and expected results: (i) To obtain the co-crystals of BCoV S1-NTD and its sugar receptor analogue, we will use a sugar compound that is locked in its natural α-configuration without switching to other configurations in solution. Switching to other configurations likely makes the sugar compound lose its binding affinity and specificity for viral lectins (57). 5-N-acetyl-4,9-di-*O*-acetylneuraminic acid α-methylglycoside (αNeu4,5,9Ac$_3$2Me) is a compound locked in the α-configuration because it contains an α-2-O-methyl group (Fig.6C). At this position, the natural sugar receptor would be linked to the penultimate residue of the sugar chain. But in the sugar receptor analogue, this position is methylated and is thereby locked in the α-configuration. The sugar receptor analogue has been successfully used in crystallographic studies of BCoV RDE (56). We have already obtained this compound from Dr. Raoul J. de Groot at Utrecht University, The

Netherlands, in a collaborative effort to determine the structure of BCoV S1-NTD complexed with this compound.

(ii) To determine the structure of BCoV S1-NTD complexed with its sugar receptor analogue, two alternative approaches will be taken. First, the sugar compound will be soaked into existing BCoV S1-NTD crystals. Second, co-crystallization of BCoV S-NTD and the sugar compound will be performed. After obtaining the co-crystals, X-ray diffraction data will be collected at a synchrotron radiation source. The structure of the sugar-bound BCoV S1-NTD will be determined by molecular replacement using the structure of BCoV S1-NTD as the search template.

(iii) The structures of sugar-bound BCoV S1-NTD and sugar-bound BCoV RDE will be compared to reveal the different sugar-binding mechanisms used by the two proteins, which will provide insight into the co-evolutionary relationship between BCoV spike and RDE.

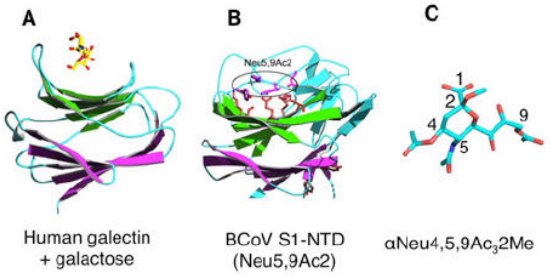


Figure 6. β-genus BCoV S1-NTD and human galectins share the same structural fold and use the same pocket to bind different types of sugar. (A) Structure of human galectin complexed with galactose. (B) Crystal structure of BCoV S1-NTD by itself (12). Residues in brown are sugar-binding residues as identified using mutagenesis. Neu5,9Ac2 is the sugar receptor for BCoV as identified using glycan screen arrays. Circle indicates the sugar-binding pocket in BCoV S1-NTD. (C) A sugar receptor analogue for both BCoV S1-NTD and RDE.

(iv) Although our previous biochemical studies identified several critical sugar-binding residues in BCoV S1-NTD (12), we expect that the structure of sugar-bound BCoV S1-NTD will reveal precise interacting coordinate pairs. We will examine the roles of these residues in sugar binding using biochemical assays such as dot blot hybridization, surface plasmon resonance, and pseudovirus entry assay. We have had extensive experience with these assays (3, 11, 12, 32, 58).

Potential pitfalls and alternative approaches: The crystals of BCoV S1-NTD that we used for structure determination will be used again for obtaining co-crystals of BCoV S1-NTD and the sugar compound, because these crystals diffract to 1.55 Å resolution. In the meanwhile, we obtained a number of other crystal forms that diffracted to lower resolutions (2.5 – 3.0 Å). These other crystal forms will also be used to maximize the chance of obtaining the co-crystals of BCoV S1-NTD and the sugar compound.

In addition to BCoV, several other β-CoVs including human CoVs OC43 and HKU1 also bind sugar receptor using their S1-NTDs and at the same time encode an active RDE (11, 59). As alternative approaches, we will determine the crystal structures of their S1-NTDs alone and in complex with their sugar compound.

C.1.2 Subaim 2 – Sugar receptor binding by α-genus PEDV

Hypothesis: Despite their low sequence homology, α-CoV S1-NTDs share the same galectin fold and sugar-binding pocket as β-CoV S1-NTDs, but structural changes in the sugar-binding pockets lead to their different sugar binding specificity.

Rationale: We previously made the following observations (28, 30). (i) α-CoV and β-CoV S1-CTDs have low or no sequence homology, but share a related structural topology (i.e., connectivity of secondary structural elements), suggesting a common evolutionary origin of the S1 subunits from different CoV genera. (ii) S1-NTDs from different CoV genera function as viral lectins, suggesting a conserved galectin fold among these S1-NTDs. (iii) S1-NTDs from different genera recognize sugar with different modifications in the N-terminal sialic acid ring, suggesting structural changes in their sugar-binding pockets. We will use a combination of biochemical, bioinformatics, and structural approaches to investigate sugar recognition by α-genus PEDV S1-NTD. In addition, we will use a combination of sugar binding assays, pseudovirus entry assay, and live PEDV reverse genetics assay to identify or confirm critical sugar-binding residues in PEDV S1-NTD.

Experimental designs and expected results: (i) Based on our previous experience working with CoV S1 domains, the key to crystallizing S1 domains is to identify the most stable fragment for crystallization studies. We will do limited proteolysis of the PEDV S1-NTD-CTD fragment to identify the most stable S1-NTD fragment for crystallographic studies. After obtaining the crystals of PEDV S1-NTD, X-ray diffraction data will be collected at a synchrotron

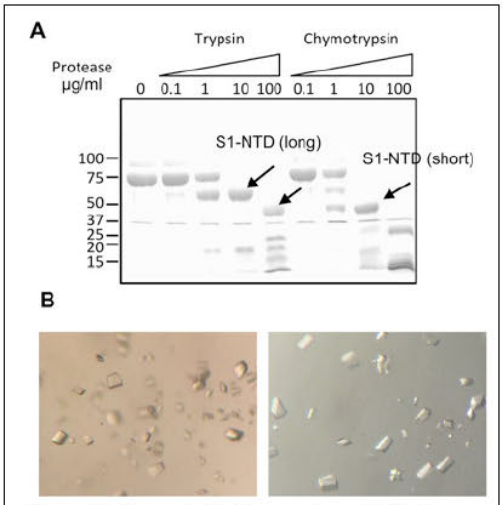


Figure 7. Characterization and crystallization of PEDV S1-NTD. (A) Limited proteolysis of PEDV S1-NTD-CTD produced two stable versions of S1-NTD, one from residues 14-490 and the other from residues 128-490. (B) The longer fragment has been crystallized under several conditions. The short one is being expressed.

radiation source. To obtain structural phases, we will use three alternative approaches. First, we will perform molecular replacement search using the structural model of BCoV S1-NTD as the search template. Second, we will soak heavy atoms into PEDV S1-NTD crystals, and determine the structure using MIRAD method. Third, we will prepare seleno-methionine-displaced PEDV S1-NTD crystals, and determine the structure using MAD method. We have had extensive experience with each of the above approaches (2, 3, 11-15, 60).

(ii) To determine the co-crystal structure of PEDV S1-NTD and its sugar receptor, we will either soak sugar receptor analogues into existing S1-NTD crystals or co-crystallize them. Several compounds are commercially available that are analogues to Neu5Ac, the receptor for PEDV S1-NTD. One such compound is *2-O-methyl-α-D-N-acetyl neuraminic acid*, which contains an α-2-O-methyl group and is thus locked in the α-configuration. This compound has been used in the structural studies of Rotavirus VP4 (61). The co-crystal structure will be determined by molecular replacement using the structure of PEDV S1-NTD as the search template.

(iii) Based on the structure of PEDV S1-NTD complexed with sugar, we will identify those residues that contact sugar and examine their roles in sugar binding and viral entry. To this end, we will mutate the selected residues, express and purify the recombinant S1-NTD, and perform sugar-binding assays. We will also perform PEDV-spike-mediated pseudovirus entry assay to analyze the roles of sugar-binding residues. To assist in these studies, the Baric laboratory has reconstructed recombinant PEDV that (a) encodes RFP for easy visualization and quantification, (b) has adapted to tissue culture through several spike enhancing mutations (titers 10^6-10^7 PFU/ml), and (c) shown well defined growth curves and plaque assays for virus quantification. As PEDV encodes distinct APN receptor binding and sugar binding residues, it seems likely that viable recombinant viruses can be isolated that preferentially use sugar or protein receptors for entry. To assist in the analyses of these mutants, we also have a number of porcine and human cell lines that are susceptible to PEDV pseudovirus entry and for enumeration of live PEDV infections (32).

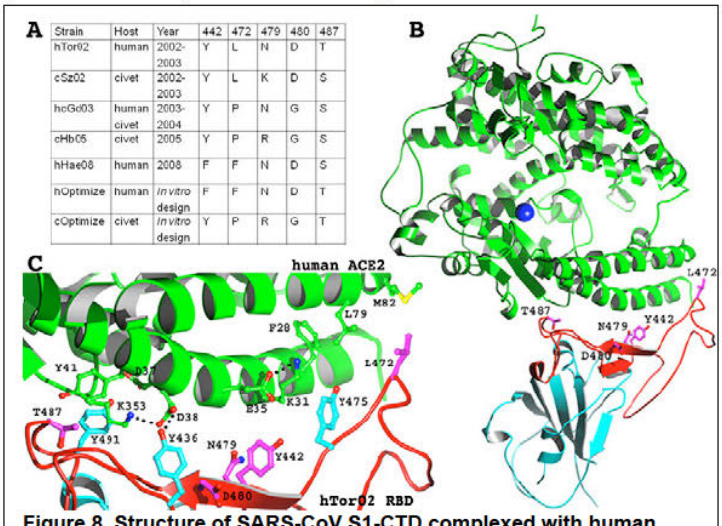
Potential pitfalls and alternative approaches: As an alternative approach to determining the co-crystal structure of PEDV S1-NTD and sugar, mutagenesis studies can be performed to identify sugar-binding residues in S1-NTD. Based on previous studies of protein/sugar interactions, three aromatic residues, tryptophan, tyrosine and phenylalanine, are frequently overrepresented in interacting with the hydrophobic face of sugar rings through CH-π effects (62). These aromatic residues will be top candidates for mutagenesis studies. Several other residues often involved in sugar binding (e.g. aspartate and arginine) will also considered.

The S1-NTD of another α-CoV, transmissible gastroenteritis CoV (TGEV), binds to the same sugar receptor as PEDV S1-NTD (63-66). In addition, α-genus feline CoV (FCoV) is also associated with sugar binding. As alternative approaches, we will investigate the sugar binding mechanism for these other α-CoVs.

Pilot study: We performed limited proteolysis of PEDV S1-NTD-CTD, and identified a stable S1-NTD fragment that ranges from residues 19 to 490 (Fig.7A). We further crystallized this fragment under several crystallization conditions (Fig.7B).

C.2 Specific Aim 2: Receptor binding by bat SARS-like CoV S1-CTDs.

Preliminary data supporting Aim 2: We previously determined a number of crystal structures of CoV S1-CTDs alone or complexed with their respective protein receptor, including β-genus SARS-CoV S1-CTD complexed with ACE2, α-genus NL63-CoV S1-CTD complexed with ACE2, and β-genus MERS-CoV S1-CTD (Fig.2). These structures have revealed the complex evolutionary pathways of CoV S1-CTDs (28). In particular, our research on SARS-CoV has implications on the animal origin and cross-species transmission of SARS-CoV (1-3, 27). Specifically, we examined the interactions between S1-CTDs from different SARS-CoV strains and ACE2 from human and palm civet (Fig.8). These comparative studies suggested that SARS-CoV transmitted from palm civets to humans through stepwise mutations in its S1-CTD. SARS-CoV S1-CTD contains two subdomains, a core and a receptor-binding motif (RBM) that binds ACE2. Two



Strain	Host	Year	442	472	479	480	487
hTor02	human	2002-2003	Y	L	N	D	T
cSz02	civet	2002-2003	Y	L	K	D	S
hcGd03	human civet	2003-2004	Y	P	N	G	S
cHb05	civet	2005	Y	P	R	G	S
hHae08	human	2008	F	F	N	D	S
hOptmize	human	*In vitro* design	F	F	N	D	T
cOptmize	civet	*In vitro* design	Y	P	R	G	T

Figure 8. Structure of SARS-CoV S1-CTD complexed with human ACE2 (1-3). (A) List of mutations in the RBMs of various SARS-CoV strains including five existing strains and two predicted strains. (B) Overall structure of human SARS-CoV S1-CTD complexed with human ACE2. ACE2 is in green, and S1-CTD is in cyan (core) and red (RBM). RBM residues that underwent mutations are displayed. (C) Detailed structure of the S1-CTD/ACE2 interface. ACE2 residues are in green, SARS-CoV residues that underwent mutations are in magenta, and SARS-CoV residues that played significant roles in the mutations are in cyan.

of these RBM mutations, K479N and S487T, significantly increased the binding affinity of SARS-CoV S1-CTD for human ACE2 (Fig.9) (1, 2, 67). These two residues likely played critical roles in the civet-to-human and human-to-human transmission of SARS-CoV, respectively (67, 68). Furthermore, we identified the existence of two virus-binding hotspots on human ACE2, one surrounding Lys353 and the other Lys31, which are critical for the binding of SARS-CoV S1-CTD (3). We showed that all naturally occurring mutations in SARS-CoV S1-CTD surround these two virus-binding hotspots and impact the binding of SARS-CoV S1-CTD to human ACE2. Based on these structural analyses, we engineered two SARS-CoV S1-CTDs with super binding affinity for human ACE2 and civet ACE2, respectively, which might represent the future forms of highly evolved SARS-CoV in human and civet, respectively.

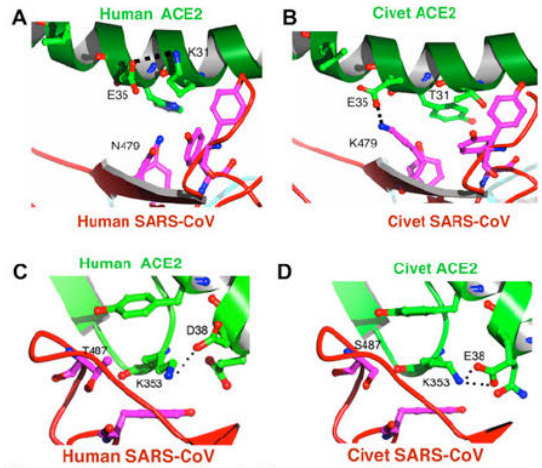


Figure 9. Structural basis for some of the major species barriers between human and civet for SARS-CoV infection (1, 2). Show are interfaces between human or civet SARS-CoV and human or civet ACE2. Asn479, but not Lys479, on SARS-CoV S1-CTD is compatible with human ACE2. Both Asn479 and Lys479 are compatible with civet ACE2. Thr487, but not Ser487, on SARS-CoV S1-CTD is compatible with human ACE2. Both Thr487 and Ser487 are compatible with civet ACE2.

Overview of Aim 2: Recently several SARS-like-CoV (SLCoV) strains have been isolated from bats and shown to use human ACE2 and infect human cells, and thus they have the potentials to transmit directly to humans (69). To investigate the cross-species transmission of bat SLCoVs, we will examine the interactions between bat SLCoV S1-CTDs and ACE2 from bat, civet, human and mouse, and also compare evolutionary changes between bat SLCoV S1-CTDs and S1-CTDs from human and civet SARS-CoV strains. Mouse ACE2 is included here because understanding the cross-species barrier between bat and mouse will help establish an animal model for bat SLCoV infections. Through these studies, we aim to characterize the cross-species barrier between bat and other species for bat SLCoV infections and identify the evolutionary changes allowing bat SLCoVs to transmit to other species. This aim will provide key data for understanding emergence potential of bat SLCoVs and for facilitating epidemic monitoring and the development of broadly cross-protective intervention control strategies. We will use a combination of molecular, biochemical and crystallographic methods. Our previous research has demonstrated the importance of these approaches in understanding animal origins and cross-species transmissions of viruses. Our expertise puts us in a unique position to carry out these studies.

A

```
ACE2   21          31          41
Human  IEEQAKTFLD  KFNHEAEDLF  YQSSLASWNY
Civet  TEELAKTFLE  TFNYEAQELS  YQSSVASWNY
Bat    TEDEAKMFLD  KFNTKAEDLS  HQSSLASWDY
Mouse  TEENAKTFLN  NFNQEAEDLS  YQSSLASWNY
        *: ** **: .** :*::*   :***:***:*

ACE2   71          81          321         351
Human  AFLKEQSTLA  QMYPLQEIQN  PNMTQGFWEN  LGKGDFRILM
Civet  AYYEEQSKLA  QTYPLAEIQD  PNMTQGFWEN  LGKGDFRIKM
Bat    AFYEEQSKLA  KNYSLEQIQN  PNMTEGFWNN  LGKGDFRIKM
Mouse  AFYEEQSKTA  QSFSLQEIQT  PHMTQGFWAN  LGHGDFRIKM
       *: :***. * : : * :**   *.**:*** *  **:***** *
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B

SARS-CoV	436	442	472	479	480	487
Human (Tor02)	Y	Y	L	N	D	T
Civet (Sz02)	Y	Y	L	K	D	S
Human/Civet (Gd03)	Y	Y	P	N	G	S
Mouse (PMID: 22072787)	H	Y	L	N	D	T
Bat (rs3366)	Y	S	F	N	D	N
Bat (shc014)	Y	W	P	R	P	A
Bat (wiv1)	Y	S	F	N	D	N

Figure 10. Receptor and viral determinants of SLCoV/ACE2 interactions. (A) Sequence comparisons of ACE2 from different animal species. In red are residues that are critical to the major species barriers between hosts for SARS-CoV infections. In blue are residues that directly contact SARS-CoV. Asterisks indicate positions which have a single, fully conserved residue. Colons indicate positions which have strongly conserved residues. Periods indicate positions which have weakly conserved residues. (B) Sequence comparisons of S1-CTDs from different SARS-CoV strains and bat SLCoVs. The strain names are in parenthesis. Shown are six residue positions that are critical for ACE2 binding and are also prone to evolution.

C.2.1 Subaim 1 – Receptor and viral determinants of the cross-species transmission of bat SLCoVs

Hypothesis: Residue differences between bat ACE2 and ACE2 from other species form a critical barrier for the cross-species transmission of bat SLCoVs; to overcome this barrier, bat SLCoVs needed to undergo evolutionary changes in their S1-CTD for adaptation to ACE2 from other species.

Rationale: Recent studies showed that several bat SLCoVs can infect human cells and use human ACE2 (69). Here we will investigate the interactions between bat SLCoV S1-CTDs and ACE2 from bat, civet, human and mouse. This subaim will provide quantitative measures of relative efficiency of receptor homologue usage by bat SLCoVs, which will enhance understanding of the animal origin and cross-species transmission pathways of SARS-CoV.

We previously showed that residue differences among human, civet, mouse and rat ACE2 formed a critical cross-species barrier for SARS-CoV (1-3, 27). For example, two residue differences between human and civet ACE2 (at residues 31 and 38) allowed civet SARS-CoV to only infect civets, but not humans. In addition, two residue differences (at residues 353 and 82) between rat and human ACE2 prevented SARS-CoV from infecting rats. In this subaim, we will identify the residue differences between bat ACE2 and ACE2 from other species that form barriers for bat SLCoVs to transmit to other species.

Our previous research also demonstrated that in order for SARS-CoV to transmit from civets to humans, two mutations in its S1-CTD, K479N and S487T, play critical roles by enhancing viral binding to human ACE2 (1-3, 27). In addition, many mouse-adapted SARS-CoV strains contain mutation Y436H in their S1-CTD (27, 70, 71). Therefore, SARS-CoV has the capability to adapt to ACE2 from other species through adaptive mutations in its S1-CTD to enhance its binding affinity for ACE2 from other species. Here we will identify the evolutionary changes in bat SLCoV S1-CTDs that either played critical roles in the past transmission of bat SLCoVs to other species (e.g., human or civet) or will be needed for bat SLCoV to transmit to other species (e.g., mouse).

Experimental designs and expected results: (i) We will construct, express and purify bat SLCoV S1-CTDs and ACE2 from bat, civet, human and mouse, using protocols as previously described (1-3). We will then measure the binding affinities between purified recombinant bat SLCoV S1-CTDs and ACE2 from different species. Several alternative approaches will be used including ELISA, surface plasmon resonance, and AlphaScreen as previously described (3, 4, 72). We will also perform bat SLCoV spike-mediated pseudovirus entry into cells expressing one of the ACE2 molecules. We expect that bat SLCoV S1-CTDs show relatively different binding affinities for ACE2 from these species.

(ii) Based on the sequence comparisons of bat, civet, human and mouse ACE2 (Fig.10A), we can identify a number of residue differences among them that potentially form cross-species barriers for bat SLCoVs. These residues surround the two virus-binding hotspots that we previously identified on human ACE2. We will introduce mutations into bat ACE2 corresponding to the residue differences between bat ACE2 and ACE2 from other species, and measure the binding between the mutant bat ACE2 and bat SLCoV S1-CTDs. We expect that some of the mutations will reduce the binding between bat SLCoV S1-CTDs and bat ACE2, because these residue differences between bat ACE2 and ACE2 from non-bat species form part of the cross-species barriers for bat SLCoVs. In addition, we will introduce the reverse mutations into ACE2 from non-bat species, and measure the binding between bat SLCoV S1-CTDs and mutant ACE2 from other species. We expect that these mutations will enhance the binding between bat SLCoV S1-CTDs and ACE2 from other species.

(iii) Based on our knowledge about SARS-CoV/ACE2 interactions, we will compare the sequences of the S1-CTDs from bat SLCoVs and SARS-CoV isolated from other species, and identify the residue differences among them that potentially impact the binding of bat SLCoV S1-CTDs to ACE2 from other species. These S1-CTD residues should also surround the two virus-binding hotspots that we previously identified on human ACE2 (Fig.10B). We will introduce mutations into bat SLCoV S1-CTDs corresponding to the identified residue differences between bat SLCoV S1-CTDs and human, civet or mouse SARS-CoV S1-CTDs. We will express and purify the mutant bat SLCoV S1-CTDs, and examine the binding between the mutant bat SLCoV S1-CTDs and human, civet or mouse ACE2. It is expected that the mutations will enhance the binding affinity between bat SLCoV S1-CTDs and human, civet or mouse ACE2, because these mutations likely correspond the evolutionary changes of bat SLCoVs that are needed for adaptation to non-bat ACE2.

(iv) The Baric laboratory used synthetic genome design to reconstruct full length WIV-1 and SHC014 molecular clones from which recombinant viruses have been isolated and characterized (Menachery et al., Nat Medicine, In press). We will also synthetically construct a full-length molecular clone of SL-CoV rs3366. As informed by mutagenesis studies in the Li laboratory, we will introduce a series of mutations into the bat SLCoV S1-CTD and evaluate virus usage of human, civet, bat and mouse ACE2 receptors (expressed in nonpermissive DBT cells). We will also query virus replication kinetics in primary human airway epithelial cells.

(v) In parallel, the Baric laboratory will passage bat SLCoVs in HAE cultures and in DBT cells expressing human, civet, mouse and bat ACE2, using experimental evolution to select for novel receptor enhancing mutations in the bat SLCoV S1-CTDs. Using similar strategies, we had previously selected for civet SARS-CoV strains that would evolve efficient usage of the human, but not civet, ACE2 (73, 74). In vitro adapted virus S1-CTD mutation sets will be provided to Dr. Li, allowing detailed biochemical characterization of experimentally evolved S1-CTD enhancing mutations that mitigate efficient human, civet, bat and mouse ACE2 usage.

Potential pitfalls and alternative approaches: Subaim 2 will determine the crystal structures of bat SLCoV S1-CTDs complexed with bat, human, civet or mouse ACE2, which will help identify more ACE2 residues that are part of the cross-species barriers for bat SLCoVs as well as more S1-CTD residues that underwent or need to undergo evolutionary changes during cross-species transmission of bat SLCoVs.

C.2.2 Subaim 2 – Crystallographic studies of bat SLCoV S1-CTDs and ACE2 from different species

Hypothesis: The crystal structures of bat SLCoV S1-CTDs in complex with ACE2 from different species can provide insight into cross-species transmission by bat SLCoVs.

Rationale: Our previous research has demonstrated that close examination of the SARS-CoV S1-CTD/receptor structural interface can provide critical information about the animal origin, cross-species transmission, and host receptor adaptation by SARS-CoV (1-3). Here we will determine the crystal structures of bat SLCoV S1-CTDs complexed with ACE2 from bat, civet, human or mouse.

Experimental designs and expected results: (i) We will prepare the complexes of bat SLCoV S1-CTD and ACE2 from bat, civet, human or mouse, using protocols as previously described (1-3).

(ii) After obtaining the co-crystals of bat SLCoV S1-CTDs and ACE2 from different species, X-ray diffraction data will be collected. These structures will be determined by molecular replacement using the structure of human SARS-CoV S1-CTD complexed with human ACE2 as the search template.

(iii) After determining the structures of bat SLCoV S1-CTDs complexed with bat, civet or human ACE2, we will examine their structural interfaces, and identify critical interactions that contribute to the cross-species transmission and host receptor adaptation by bat SLCoVs. Particularly, we will focus on those residues near the two virus-binding hotspots that we previously identified on human ACE2.

Potential pitfalls and alternative approaches: If the binding between bat SLCoV S1-CTDs and mouse ACE2 is too weak for them to form a stable complex, we will use either mutant mouse ACE2 with enhanced affinity for bat SLCoV S1-CTDs and/or mutant bat SLCoV S1-CTDs with enhanced affinity for mouse ACE2 (as discussed in Subaim 1) for crystallization studies.

As an alternative to crystallizing the complexes of bat SLCoV S1-CTDs and ACE2 from other species, we will engineer a chimeric S1-CTD containing ACE2-contacting residues from bat SLCoV S1-CTDs and the other residues from human SARS-CoV S1-CTD. In other words, this chimeric S1-CTD has a backbone from human SARS-CoV S1-CTD for crystallization and an ACE2-binding region from bat SLCoVs for functional analysis. Thus, this chimeric S1-CTD can display the same crystallization behavior as human SARS-CoV S1-CTD, but the same receptor-binding behavior as bat SLCoV S1-CTDs. Similarly, we can engineer a chimeric ACE2 containing a backbone from human ACE2 and the virus-binding region from non-human ACE2. The complexes of the chimeric S1-CTD and chimeric ACE2 will likely crystallize in the same crystal form as the complex of human SARS-CoV S1-CTD and human ACE2, but reveal the interactions between bat SLCoV S1-CTDs and non-human ACE2. We successfully characterized, established, and applied this approach in our crystallographic studies of civet SARS-CoV S1-CTD and civet ACE2 (1, 3).

C.3 Specific Aim 3: Cell entry by CoVs

Preliminary data supporting Aim 3: We obtained a low-resolution view of SARS-CoV spike in pre-fusion and post-fusion states using negative-stain electron microscopy (Fig.3) (10). The pre-fusion molecule is a clove-shaped trimer, with three S1 heads and a trimeric S2 stalk. The post-fusion molecule corresponds to structurally rearranged trimeric S2 that associates together through exposed hydrophobic fusion peptides to form rosettes. We further showed that the transition of SARS-CoV spike from pre-fusion to post-fusion states requires proteolysis at the S1/S2 boundary. The states and transitions suggest conformational changes of the spike that mediate viral entry into cells (10). These results provided, for the first time, a direct view of the proteolysis-triggered conformational changes of a CoV spike.

In other previous studies, we elucidated the cell entry of MERS-CoV and its related bat CoV HKU4 (Fig.11) (4, 5). We showed that although MERS-CoV and HKU4 S1-CTDs recognize the same receptor DPP4, MERS-CoV pseudovirus enters both human and bat cells, while HKU4 pseudovirus only enters bat cells. We further demonstrated that human cellular proteases

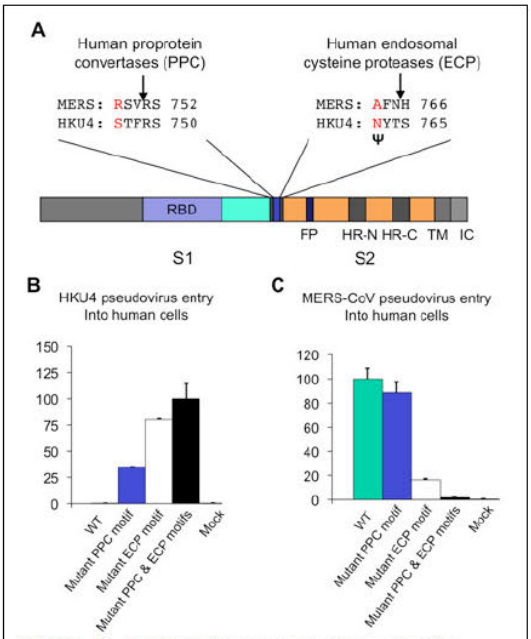


Figure 11. Host proteases as a cross-species barrier for MERS-CoV and bat CoV HKU4 (4, 5). (A) Domain structure of MERS-CoV and HKU4 spikes. MERS-CoV spike contains a PPC and an ECP motif. Arrows indicate the protease cleavage sites. The corresponding PPC motif in HKU4 spike contains mutations, and the corresponding ECP motif in HKU4 spike contains an N-linked glycosylation site (indicated by ψ). (B) Wild type HKU4 pseudovirus does not enter human cells, but mutating the two protease motifs in its spike to those recognized by human proteases allows HKU4 pseudovirus to enter human cells. Wild type MERS-CoV pseudovirus enters human cells, but mutating the two protease motifs in its spike to those in HKU4 spike prevents MERS-CoV pseudovirus from entering human cells.

including proprotein convertases (PPCs) and endosomal proteases (ECPs) activate MERS-CoV pseudovirus entry, but not HKU4 pseudovirus entry. On the other hand, bat cellular proteases activate both MERS-CoV and HKU4 pseudoviruses for cell entry. We identified two residue differences between MERS-CoV and HKu4 spikes that render MERS-CoV spike, but not HKU4 spike, susceptible to activation by human cellular proteases. One of these residue differences is located in a motif for human ECPs. This motif is glycosylated in HKU4 spike and thus cannot be recognized by human ECPs, whereas the corresponding motif in MERS-CoV spike is not glycosylated and can be activated by human ECPs. In our unpublished study, we showed that purified total ECPs from human cells do not cleave HKU4 spike containing the glycosylated ECP motif, but cleave mutant HKU4 spike with the glycosylation removed. On the other hand, purified human ECPs cleave MERS-CoV spike, but do not cleave mutant MERS-CoV spike containing a glycosylated ECP motif (Fig.12). We obtained similar results using purified recombinant cathepin L. Overall, our research reveals, for the first time, that host proteases form a critical cross-species barrier for viruses.

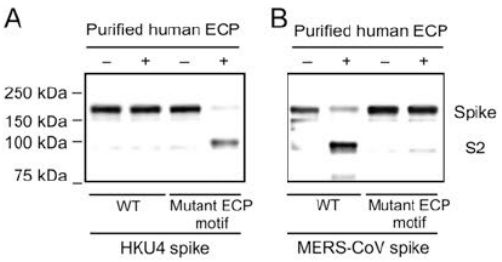


Figure 12. Cleavage of MERS-CoV and HKU4 spikes by purified human total ECPs (unpublished data from Dr. Li's lab). (A) Western blot analysis showed that purified human ECPs do not cleave HKU4 spike containing a glycosylated ECP motif, but cleave mutant HKU4 spike with the glycosylation removed. (B) Purified human ECPs cleave MERS-CoV spike, but do not cleave mutant MERS-CoV spike containing a glycosylated ECP motif.

Overview of Aim 2: Following receptor recognition, membrane fusion takes place to complete viral entry into host cells. Protease activation of CoV spikes is essential for membrane fusion, and is tightly regulated during the entry process. Although protease activations of SARS-CoV and MERS-CoV spikes have been extensively studied, it is less understood how other CoVs are regulated by proteolysis for entry. Importantly, recent progress in protease substrate profiling has yielded new information about the cleavage motifs for host proteases, which previously was unavailable for studying CoV entry (6-9). We are particularly interested in the proteolytic activation of MHV, HCoV-NL63 and PEDV, each of which has a unique need for cell entry: neurotrophic MHV strains enter neurons where receptor expression is low, HCoV-NL63 displays modulated entry and pathogenesis, and PEDV infects pig intestines where extracellular proteases are abundant. This aim will use these CoVs as models to investigate how host proteases regulate CoV host range, tropism, and pathogenesis, and may also guide future design of protease inhibitors against health- or economy-threatening CoVs (75).

Overall hypotheses of Aim 2: CoV spikes have evolved a variety of mechanisms for protease activation that can meet their specific need for host range, tissue tropism, and pathogenesis.

C.3.1 Subaim 1 – Cell entry of MHV

Hypothesis: To adapt to the low CEACAM1 receptor level in the brain, the spike of neurotropic MHV strain has evolved to be more prone to protease activation.

Rationale: The expression level of MHV receptor CEACAM1 is very low in the brain (76), raising the question of how neurotropic MHV strains enter neurons. The spike protein is a major determinant of MHV neurovirulence (77). Because S1-NTDs of hepatotropic MHV-A59 and neurotropic MHV-JHM both bind CEACAM1 with high affinity (78), a likely determinant of MHV-JHM neurotropism is the membrane fusing capability of its spike. Indeed, it was shown that compared to the spike of MHV-A59 strain, the spike of MHV-JHM strain undergoes conformational changes more easily and hence fuses membranes less dependent on CEACAM1 binding (79-81). However, the molecular mechanism behind this observation is not clear. We have examined the sequences of MHV-A59 and MHV-JHM spikes, and identified a PPC site in both of them (Fig.13). Compared to the PPC motif in MHV-A59 that contains two arginine residues, the PPC motif in MHV-JHM is enhanced by containing three arginines (9). This subaim will investigate how the enhanced PPC motif affects the neurotropism of MHV-JHM.

Experimental designs and expected results: (i) The Baric laboratory has isolated stable swine testicular cell lines expressing high and low levels of CEACAM1 (47, 82). To confirm that MHV-JHM spike fuses membranes more easily, we will perform MHV-spike-mediated pseudovirus entry into these cells. We expect that MHV-JHM spike mediates viral entry into low-CEACAM1-expressing cells more effectively than the MHV-A59 spike does.

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MHV-A59:    714 RADR 717 (PPC motif)
MHV-JHM:    766 RARR 769 (Enhanced PPC motif)
HCoV-NL63:  863 RSSR 866 (PPC motif)
            666 NVST 669 (glycosylated ECP/cathepsin-L motif)
SARS-CoV:   676 AYTM 679 (ECP/cathepsin-L motif)
PEDV:       901 LFNK 904 (ECP/cathepsin-L motif)
            937 GVMV 940 (ECP/cathepsin-B motif)
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Figure 13. Identified cellular protease motifs in CoV spikes based on published literature on protease specificities (6-9).

(ii) To examine whether the enhanced PPC motif contributes to the enhanced entry efficiency of MHV-JHM, we will mutate the enhanced PPC

motif in MHV-JHM spike to the regular PPC motif and perform pseudovirus entry assay. We will also introduce the opposite mutation into MHV-A59 spike. We expect that MHV-JHM pseudovirus with a regular PPC motif shows decreased entry, whereas MHV-A59 pseudovirus with an enhanced PPC motif has increased entry.

(iii) To detect the efficiency of PPC in cleaving MHV-A59 and MHV-JHM spikes, we will express the two spikes on cell surfaces, and perform Western blot to examine the cleavage state of the two spikes. We expect that MHV-JHM spike has been cleaved more extensively than MHV-A59 spike.

(iv) To provide a direct view of the conformational changes of MHV-JHM and MHV-A59 spikes, we will express and purify their ectodomains in the trimeric form, and characterize their conformations using negative-strain electron microscopy. We expect that at 4°C both spikes are in the prefusion state, but after incubation at 37°C without CEACAM1, more MHV-JHM spike molecules switch to the post-fusion state than MHV-A59 spike. We have had successful experience with SARS-CoV spike (10). Please also refer to Dr. Kathryn Holmes' letter of support for the availability of the purified recombinant trimeric MHV-A59 spike.

(v) The Baric laboratory has generated molecular clones of MHV-A59 and MHV-JHM (83-85). Using these reagents, we will introduce the corresponding mutations into live MHV-A59 and MHV-JHM viruses (expressing GFP) and examine their entry kinetics into low-CEACAM1-expressing cells. We expect that the results will be similar to those from pseudovirus entry assay. If necessary, we can also swap the spike genes between strains in the presence and absence of these mutations.

Potential pitfalls and alternative approaches: As an alternative approach to electron microscopy, an liposome assay will be performed to detect the conformational changes of MHV-JHM and MHV-A59 spikes (86).

There may be other sequence and structural differences between MHV-A59 and MHV-JHM spikes that contribute to their different membrane fusing capabilities. This subaim focuses on protease activation.

Fig.13 focuses on cellular proteases with well-defined specificity motifs (e.g. PPCs and ECPs). Extracellular proteases (e.g. trypsin) are not included because they in general have lower specificities than cellular proteases and also are less reliable sources in activating CoV entry than cellular protease (e.g. ECPs are ubiquitous to host cells). Cell-surface proteases (e.g. TMPRSS2) are not included either because their substrate specificities are not well defined. Nevertheless, as alternatively approaches, we will select a few representative extracellular proteases and cell-surface proteases, use them to cleave the recombinant MHV spikes, and identify their cleavage sites in MHV spikes. We successful identified the trypsin cleavage site in SARS-CoV spike (10).

C.3.2 Subaim 2 – Cell entry of HCoV-NL63

Hypothesis: HCoV-NL63 entry is activated by PPC and is modulated by a glycosylated ECP spike motif.

Rationale: We previously showed that HCoV-NL63 and SARS-CoV S1-CTDs recognize human ACE2 with similar affinity (13, 58). Yet, HCoV-NL63 is significantly less pathogenic than SARS-CoV (87, 88). Using inhibitor assays, we also showed that cell entry of SARS-CoV, but not HCoV-NL63, is activated by human ECPs (38). Thus, what protease(s) activate HCoV-NL63 entry is unknown. Examination of the spike sequences reveals that HCoV-NL63 spike contains a PPC motif and a glycosylated ECP motif, whereas SARS-CoV spike contains a regular ECP motif (Fig.13). As discussed earlier, glycosylated ECP motifs attenuate human cell entry of HKU4. Hence HCoV-NL63 entry likely differs from SARS-CoV entry in that the former is activated by PPC and is modulated by a glycosylated ECP motif. This subaim will investigate what proteases activate HCoV-NL63 entry and how the glycosylated ECP motif modulates HCoV-NL63 entry.

Experimental designs and expected results: (i) To examine whether PPC cleaves HCoV-NL63 spike, we will prepare HCoV-NL63 pseudovirus particles and detect whether HCoV-NL63 spike is cleaved during molecular maturation. We expect HCoV-NL63 to be partially cleaved.

(ii) We will perform HCoV-NL63 pseudovirus entry in the presence of inhibitors targeting PPCs, cell-surface proteases, or ECPs. We will also perform HCoV-NL63 pseudovirus entry in the presence of trypsin. We expect that entry is blocked by PPC inhibitors. Cell-surface proteases and trypsin may or may not impact entry.

(iii) We will mutate the PPC motif in HCoV-NL63 spike, and perform pseudovirus entry again. We expect the mutation to decrease or even abolish entry. We will also remove the glycosylation site in the ECP motif of HCoV-NL63 spike, and expect that mutant HCoV-NL63 pseudovirus enters human cells more efficiently.

(iv) We will express HCoV-NL63 spike, either wild type or containing the unglycosylated ECP motif, on cell surface, and cleave it using purified total ECPs or individual recombinant ECPs. We expect that HCoV-NL63 spike containing the unglycosylated ECP motif, but not wild type spike, can be cleaved by ECPs.

(v) We will take two alternative approaches to confirm that the wild type ECP motif is glycosylated whereas the mutant ECP motif is not. First, we will express wild type or mutant HCoV-NL63 spikes on cell surface, and perform Western blot analysis carefully to detect the potential subtle change in their molecular weights. We have used this method successfully to confirm the glycosylation states of both MERS-CoV and HKU4 spikes

(5). Second, we will express and purify recombinant wild type and mutant HCoV-NL63 spike ectodomains, and carry out mass spectrometry to determine their molecular weights.

(vi) The Baric laboratory has assembled a full length infectious cDNA for HCoV-NL63 (42). We will introduce the corresponding mutations into live NL63-CoV virus encoding GFP, and perform live virus infections of Vero and human airway epithelial cells. In the presence of specific inhibitors, targeted mutations or protease knockdown/overexpression, we expect to get similar results to those obtained from pseudovirus entry.

Potential pitfalls and alternative approaches: We acknowledge there may be other host and viral factors that modulate the virulence of HCoV-NL63. This subaim focuses on its entry.

C.3.3 Subaim 3 – Cell entry of PEDV

Hypothesis: Host cellular proteases activate PEDV entry.

Rationale: It has been a long-standing puzzle regarding what proteases activate PEDV entry into host cells. Previous studies identified extracellular protease trypsin as required for PEDV infection in cell culture. These studies led to the conclusion that extracellular proteases, which are abundant in pig intestines, are essential for PEDV entry (89-91). However, these previous studies all used live PEDV viruses, and thereby were unable to differentiate between PEDV entry and other steps in the PEDV infection cycle such as PEDV replication or release. Indeed, an electron microscopic study showed that PEDV release is a limiting step in the PEDV infection cycle and that trypsin is required for PEDV release (92). We recently established PEDV pseudovirus entry assay, which only concerns PEDV entry, but not PEDV replication or release (32). We have identified two ECP motifs in PEDV spike, one recognized by cathepsin L and the other by cathepsin B (Fig.13). In this subaim, we will investigate the role of host cellular proteases in PEDV entry.

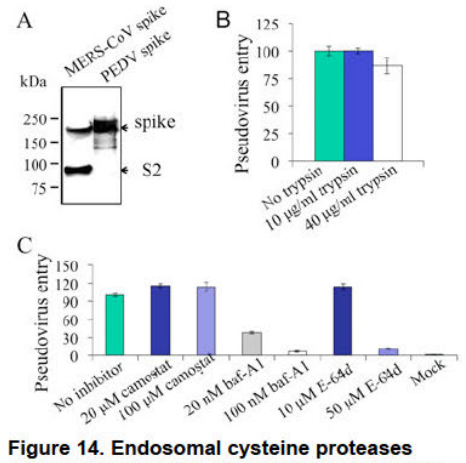


Figure 14. Endosomal cysteine proteases activate PEDV entry. (A) Western blot analysis of PEDV spike in pseudovirus particles. MERS-CoV pseudovirus was used as a control. During the viral maturation process, PEDV spike remained intact, while MERS-CoV spike underwent significant cleavage. (B) PEDV pseudovirus was used to enter porcine cells in the absence or presence of exogenous trypsin. (C) Before PEDV pseudovirus infection, porcine cells were pre-treated with different cellular protease inhibitors: camostat (cell-surface protease inhibitor), Baf-A1 (Bafilomycin A1) (endosomal acidification inhibitor), or E-64d (ECP inhibitor).

Experimental designs and expected results: (i) To identify the host cellular proteases activating PEDV entry, we will perform PEDV pseudovirus entry in the presence of inhibitors specifically targeting PPCs, TMPRSS2, or ECPs. We expect that pseudovirus entry is blocked by ECP inhibitors and endosomal acidification inhibitors.

(ii) To examine whether PPCs cleave PEDV spike, we will prepare PEDV pseudovirus and detect whether the spike is cleaved during molecular maturation. We expect it to remain uncleaved.

(iii) After expressing PEDV spike on cell surface, we will treat PEDV spike with purified total ECPs or individual recombinant ECPs, and perform Western blot to detect whether PEDV spike is cleaved by ECPs. We expect PEDV spike to be directly cleaved by ECPs.

(iv) We will mutate the ECP motifs to turn them off, and repeat the above assays. We expect that the mutations will abolish pseudovirus entry and resist ECP cleavage.

(v) Using the PEDV molecular clone, we will isolate recombinant live PEDV virus encoding these protease altering mutation sets, and compare the infection efficiency of wild type and mutant PEDV viruses encoding RFP. We expect that the mutated ECP motif in PEDV spike will decrease PEDV entry into host cells.

Potential pitfalls and alternative approaches: Previously we showed that human and bat ECPs demonstrate different activities in activating HKU4 spikes. Here we need to make sure that human and porcine ECPs have the same activities in activating PEDV spike. Therefore, we will use both human and porcine cell lines.

Pilot study: We have shown that PEDV pseudovirus entry depends on ECPs, not trypsin (Fig.14).

C.4 Summary and Timeline. The overarching goal is to identify the roles and mechanisms of entry determinants for CoVs, and to use CoVs as model systems for establishing novel principles in virology that govern viral evolution, receptor recognition, cell entry, cross-species infections, tissue tropism, and pathogenesis. Our previous research on CoVs has led to a number of important discoveries, some of which have contributed to the understanding and control of newly emerged CoVs such as SARS-CoV, MERS-CoV, and PEDV. The new findings coming from this proposal will further advance the field and contribute to virology and human health. The PI and co-investigator's labs blend complementary and multi-disciplinary approaches including structural, molecular, biochemical, and genetic methods, which should maximize productivity and strengthen program outcomes. We note that because all three aims are independent from each other, they will be initiated simultaneously.

PROGRESS REPORT PUBLICATION LIST

Grant Number: R01 AI089728

Project Title: Receptor recognition mechanisms of coronaviruses

Grantee Organization: University of Minnesota

Funding Period: 05/15/10 – 04/30/15

Principal Investigator: FANG LI

A. Publications resulting from this R01 grant

Publication Summary

Using funding from this R01 grant, I have published 15 papers as the corresponding author, including 3 in *PNAS*, 1 in *Structure*, 8 in the *Journal of Virology*, 2 in the *Journal of Biological Chemistry,* and 1 in *Antiviral Research*. I have also published 1 other paper as a middle author in *Vaccine*.

List of Publications (in chronological order) (* indicates corresponding authorship)

1. Li, W., **Li, F. *** (2011) Cross-crystal averaging with search models to improve molecular replacement phases. *Structure*, *19*, 155-161 (PMID: 21300285; PMCID: PMC3037595)

2. Wu, K., Chen, L., Peng, G., Zhou, W., Pennell, C., Mansky, L., Geraghty, R., **Li, F. *** (2011) A virus-binding hotspot on human angiotensin-converting enzyme 2 is critical for binding of two different coronaviruses. *Journal of Virology*, *85,* 5331-5337 (PMID: 21411533; PMCID: PMC3094985)

3. Peng, G., Sun, D., Rajashankar, K., Qian, Z., Holmes, K., **Li, F. *** (2011) Crystal structure of mouse coronavirus receptor-binding domain complexed with its receptor murine CEACAM1a. *Proceedings of the National Academy of Sciences USA*, *108,* 10696-10701 (PMID: 21670291; PMCID: PMC3127895)

4. **Li, F. *** (2012) Evidence for a common evolutionary origin of coronavirus spike protein receptor-binding subunits. *Journal of Virology*, *86,* 2856-2858 (PMID: 22205723; PMCID: PMC3302248)

5. Wu, K., Peng, G., Wilken, M., Geraghty, R., **Li, F.** * (2012) Mechanisms of host receptor adaptation by severe acute respiratory syndrome coronavirus. *Journal of Biological Chemistry*, *287,* 8904-8911 (PMID: 22291007; PMCID: PMC3308800)

6. Chen, L., Lin, Y.L., Peng, G., **Li, F.** * (2012) Structural basis for multifunctional roles of mammalian aminopeptidase N. *Proceedings of the National Academy of Sciences USA*, *109*, 17966-17971 (PMID: 23071329; PMCID: PMC3497818)

7. Peng, G., Xu, L., Lin, Y.L., Chen, L., Pasquarella, J., Holmes, K.V., **Li, F. *** (2012) Crystal structure of bovine coronavirus spike protein lectin domain. *Journal of Biological Chemistry*, *287*, 41931-41938 (PMID: 23091051; PMCID: PMC3516740)

8. Chen, L., **Li, F. *** (2013) Structural analysis of the evolutionary origins of influenza virus hemagglutinin and other viral lectins. *Journal of Virology*, *87*, 4118-4120 (PMID: 23365425; PMCID: PMC3624229)

9. Chen, Y., Rajashankar, K.R., Yang, Y., Agnihothram, S.S., Liu, C., Lin, Y.L., Baric, R.S., **Li, F. *** (2013) Crystal structure of the receptor-binding domain from newly emerged middle East respiratory

syndrome coronavirus. *Journal of Virology*, *87*, 10777-10783 (PMID: 23903833; PMCID: PMC3807420)

10. **Li, F. *** (2013) Receptor recognition and cross-species infections of SARS coronavirus. *Antiviral Research*, *100*, 246-254 (PMID: 23994189; PMCID: PMC3840050)

11. Du, L., Zhao, G., Yang, Y., Qiu, H., Wang, L., Kou, Z., Tao, X., Yu, H., Sun, S., Tseng, K.C., Jiang. S., **Li, F. ***, Zhou, Y. * (* co-correspondence) (2014) A conformation-dependent neutralizing monoclonal antibody specifically targeting receptor-binding domain in Middle East respiratory syndrome coronavirus spike protein. *Journal of Virology*, *88*, 7045-7053 (PMID: 24719424; PMCID: PMC4054355)

12. Yang, Y., Du, L., Liu, C., Wang, L., Ma, C., Tang, J., Baric, R.S., Jiang, S., **Li, F. *** (2014) Receptor usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human transmission of MERS coronavirus. *Proceedings of the National Academy of Sciences USA*, *111*, 12516-12521 (PMID: 25114257; PMCID: PMC4151778)

13. Ma, C., Wang, L., Tao, X., Zhang, N., Yang, Y., Tseng, K.C., **Li, F.**, Zhou, Y., Jiang, S., Du, L. (2014) Searching for an ideal vaccine candidate among different MERS coronavirus receptor-binding fragments – the importance of immunofocusing in subunit vaccine design. *Vaccine*, *32*, 6170-6176 (PMID: 25240756; PMCID: PMC4194190 [Available on 2015-10-21])

14. **Li, F. *** (2015) Receptor recognition mechanisms of coronaviruses: a decade of structural studies. *Journal of Virology*, *89,* 1954-1964 (PMID: 25428871; PMCID: PMC4338876 [Available on 2015-08-15])

15. Liu, C., Tang, J., Ma, Y., Liang, X., Yang, Y., Peng, G., Qi, Q., Jiang, S., Li, J., Du, L., **Li, F. *** (2015) Receptor usage and cell entry to porcine epidemic diarrhea coronavirus. *Journal of Virology*, *89, 6121-6125* (PMID: 25787280; PMCID: PMC4442452 [Available on 2015-12-01])

16. Yang, Y., Liu, C., Du, L., Jiang, S., Shi, Z., Baric, R.S., **Li, F. *** (2015) Two mutations were critical for bat-to-human transmission of MERS coronavirus. *Journal of Virology*, *In press.* 2015 Jun 10. pii: JVI.01279-15. [Epub ahead of print] (PMID: 26063432)

B. Breaking down the publications resulting from this R01 grant

B1. Animal origins and cross-species transmissions of SARS and MERS coronaviruses

SARS-CoV and MERS-CoV have both originated from animals and transmitted to humans, causing serious threat to global health. One of the goals of my research is to elucidate the molecular mechanisms by which emerging animal viruses transmit to humans. To this end, I study the structures and functions of the coronavirus spike protein, which guides coronavirus entry into host cells.

During the five-year funding cycle, my lab determined the crystal structures of the spike S1-CTD from different SARS-CoV strains complexed with their receptor angiotensin-converting enzyme 2 (ACE2) from different animal species (**Wu, K., et al., *Journal of Biological Chemistry*, 2012**). Through comparative studies of these S1-CTDs from different SARS-CoV strains and ACE2 from different animal species, my research has established that receptor recognition serves a critical barrier for the cross-species transmission of SARS-CoV and that a few RBD mutations allowed SARS-CoV virus to transmit from animals to humans (**Li, F., *Antiviral Research*, 2013)**.

Moreover, my lab examined the cell entry mechanisms of MERS-CoV. My research showed for the first time that cellular proteases from different hosts serve as a barrier for the cross-species transmission of MERS-CoV (**Yang, Y., et al., *PNAS*, 2014)**. Moreover, we discovered that two mutations in the protease cleavage

sites of MERS-CoV spike allowed MERS-CoV to transmit from bats to humans, either directly or through intermediate hosts (**Yang, Y., et al., *Journal of Virology*, 2015)**.

Overall, my research has contributed critically to the knowledge about the animal origins and cross-species transmissions of viruses, and has important implications on epidemic monitoring, prevention, and control.

B2. Receptor recognition mechanisms of coronaviruses

Coronaviruses recognize a variety of receptors, and display a complex receptor recognition pattern. Another major goal of my research is to elucidate the receptor recognition mechanisms of coronaviruses, and provide insight into how coronaviruses have evolved such a complex receptor recognition pattern. During the five-year funding cycle, my lab has determined a number of crystal structures of coronavirus RBDs, either alone or in complex with their respective receptor. We have also performed functional studies to further our understanding of the receptor usage by coronaviruses.

First, my lab determined the crystal structure of the S1-NTD of β-genus mouse hepatitis CoV (MHV) complexed with its protein receptor CEACAM1 **(Peng, G., et al., *PNAS*, 2011)**. Surprisingly, MHV S1-NTD has the same structural fold as human galectins (galactose-binding host lectin), although it binds to CEACAM1 through exclusive protein-protein interactions. We then found that the S1-NTDs of β-genus bovine CoV (BCoV) and human OC43 CoV both bind sugar and function as viral lectins, and identified the type of sugar that BCoV S1-NTD recognizes using glycan screen array. We further determined the crystal structure of BCoV S1-NTD, which also has a galectin fold **(Peng, G, et al., *Journal of Biological Chemistry*, 2012)**. We performed structure-based mutagenesis and identified the sugar-binding site in BCoV S1-NTD, which overlaps with the galactose-binding site in galectins. Based on these studies, we hypothesized that ancient coronaviruses acquired their spike S1-NTD from the host, and have evolved it into viral RBD with novel specificity for protein receptors (as in MHV) or for novel sugar specificities (as in BCoV). We expanded our observation by showing that other viral lectins such as influenza hemagglutinin also have the same structural fold as human galectins, and hence all of these viral lectins may have originated from the host **(Chen, L. and Li, F., *Journal of Virology*, 2013)**.

Second, my lab continued to investigate why the S1-CTDs of α-genus NL63 respiratory coronavirus (NL63-CoV) and β-genus SARS-CoV have very different structures, but recognize the same receptor protein ACE2. We found that both viruses bind to a virus-binding hotspot on ACE2 by forming an energetically favorable tunnel structure **(Wu, K., et al., *Journal of Virology*, 2011)**. In addition, we detected a related structural topology (i.e., connectivity of secondary structural elements) between Nl63-CoV and SARS-CoV S1-CTDs, suggesting a common evolutionary origin of the spikes from different genera **(Li, F., *Journal of Virology*, 2012)**.

Third, my lab determined the crystal structure of MERS-CoV S1-CTD. The structure revealed that MERS-CoV and SARS-CoV S1-CTDs have very similar core structures. However, the two S1-CTDs differ markedly in their receptor-binding motif regions, explaining why MERS-CoV and SARS-CoV recognize two different protein receptors – dipeptidyl peptidase 4 (DPP4) for MERS-CoV and ACE2 for SARS-CoV **(Chen, Y., et al., *Journal of Virology*, 2013)**.

Fourth, my lab determined the crystal structure of aminopeptidase N (APN), which is the receptor for several α-genus coronaviruses. The structure not only reveals the binding sites for these α-genus coronaviruses, but also has important implications for many other physiological functions of APN such as tumor motility and pain sensation **(Chen, L., et al., *PNAS*, 2012)**. Moreover, we identified DPP4 as the receptor for bat coronavirus HKU4 **(Yang, Y., et al, *PNAS*, 2014)**, and found that both human and pig APN molecules serve as the primary receptor and sugar as the co-receptor for α-genus porcine epidemic diarrhea coronavirus (PEDV) **(Liu, C., et al., *Journal of Virology*, 2015)**.

Overall, my research on the coronavirus-receptor interactions described above has established the following virology principles. First, drastic structural changes in viral RBDs can still lead to recognition of a

virus-binding hot spot on the same receptor protein. Supporting this principle is the finding that SARS-CoV and NL63-CoV recognize a common virus-binding hot spot on ACE2 using structurally divergent S1-CTDs. Second, subtle structural changes in viral RBDs can lead to a complete receptor switch. For example, MHV and BCoV S1-NTDs recognize a protein receptor and a sugar receptor, respectively, through subtle conformational changes in receptor-binding loops. Third, it is a successful viral strategy to steal a host protein and evolve it into viral RBDs with novel protein receptor specificities or altered sugar receptor specificities. For example, MHV and BCoV S1-NTDs have the same structural fold as human galectins, but they recognize a novel protein receptor and a different sugar receptor, respectively. Fourth, a few residue changes at the receptor binding interface can lead to efficient cross-species infection and human-to-human transmission of a virus. For example, SARS-CoV needed only one or two mutations in its RBD to transmit from animal to humans. These virology principles may be extended from the coronavirus family to other virus families **(Li, F., *Journal of Virology*, 2015)**.

B3. Cell entry mechanisms of coronaviruses

For coronaviruses to enter host cells, their spike must be proteolytically activated by one or more host proteins and then undergo a dramatic conformational change. During the five-year funding period of the R01 grant, my lab identified the specific proteases that activate the cell entry of MERS-CoV and bat coronavirus HKU4 **(Yang, Y., et al., *PNAS*, 2014)**. Further, we successfully located the corresponding protease motifs in the spikes of these viruses. We also identified two mutations in the protease motifs of MERS-CoV spike that were critical for MERS-CoV to transmit from bats to humans, either directly or through intermediate hosts **(Yang, Y., et al., *Journal of Virology*, 2015)**. Importantly, as mentioned earlier, we showed for the first time that different activities of host protease serve a critical barrier for the cross-species transmission of viruses. In addition, we have elucidated the cell entry mechanisms of PEDV **(Liu, C., et al., *Journal of Virology*, 2015)**. Overall, my research has revealed how host proteases regulate viral entry, pathogenesis, host range, and tissue tropism.

B4. Design and development of vaccines and therapeutic antibodies against coronavirus infections

Coronaviruses, particularly SARS-CoV and MERS-CoV, pose serious threat to global health. Coronavirus spike RBDs are the prime vaccine candidates for preventing coronavirus infections, and monoclonal antibodies targeting coronavirus spike RBDs have the great potential to serve as therapeutics to treat coronavirus infections. During the five-year funding period of the R01 grant, we have collaborated with other groups to rationally design and develop RBD-based vaccines **(Ma, C., et al., *Vaccine*, 2014)** as well as RBD-targeting antibodies to combat MERS-CoV infections **(Du, L., et al., *Journal of Virology*, 2014)**.

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Form Approved Through 8/31/2015

OMB No. 0925-0001

Department of Health and Human Services Public Health Services

SUBAWARD Grant Application

Do not exceed character length restrictions indicated.

LEAVE BLANK—FOR PHS USE ONLY.		
Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT *(Do not exceed 81 characters, including spaces and punctuation.)*
Receptor recognition and cell entry of coronaviruses

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☒ NO ☐ YES
(If "Yes," state number and title)
Number: Title:

3. SUBAWARD PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. eRA Commons User Name
Baric, Ralph	PhD	ralph_baric

3c. POSITION TITLE
Professor

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Epidemiology/Microbiology/immunology

3f. MAJOR SUBDIVISION

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: 919-966-3895 FAX: 919-966-2089

3d. **SUBAWARD** MAILING ADDRESS *(Street, city, state, zip code)*
Department of Epidemiology
University of North Carolina – Chapel Hill
CB# 7435, McGavran-Greenberg Hall
Chapel Hill, NC 27599-7435

E-MAIL ADDRESS:
rbaric@email.unc.edu

4. **SUBAWARD** HUMAN SUBJECTS RESEARCH	4a. Research Exempt	If "Yes," Exemption No.
☒ No ☐ Yes	☐ No ☐ Yes	

4b. Federal-Wide Assurance No.	4c. Clinical Trial	4d. NIH-defined Phase III Clinical Trial
	☐ No ☐ Yes	☐ No ☐ Yes

5. **SUBAWARD** VERTEBRATE ANIMALS ☒ No ☐ Yes — 5a. Animal Welfare Assurance No.

6. **SUBAWARD** DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. **SUBAWARD** COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. **SUBAWARD** COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
07/01/2016	06/30/2021	80000	121600	400000	608000

9. **SUBAWARD** APPLICANT ORGANIZATION
Name: The University of North Carolina at Chapel Hill
Address: Office of Sponsored Research (OSR)
Administrative Office Bldg., Suite 2200
104 Ariport Drive, CB# 1350
Chapel Hill, NC 27599-1350

10. **SUBAWARD** TYPE OF ORGANIZATION
Public: → ☐ Federal ☒ State ☐ Local
Private: → ☐ Private Nonprofit
For-profit: → ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. **SUBAWARD** ENTITY IDENTIFICATION NUMBER
1566001393A1
DUNS NO. 608195277 Cong. District NC-004

12. **SUBAWARD** ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name: Robin Cyr
Title: Director, Office of Sponsored Research
Address: Office of Sponsored Research (OSR)
Administrative Office Bldg., Suite 2200
104 Airport Drive, CB# 1350
Chapel Hill, NC 27599-1350
Tel: 919-966-3411 FAX: 919-962-3352
E-Mail: resadminosr@unc.edu

13. **SUBAWARD** OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: Barbara Entwisle, PhD
Title: Vice Chancellor for Research
Address: Office of Sponsored Research (OSR)
Administrative Office Bldg., Suite 2200
104 Airport Drive, CB# 1350
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E-Mail: resadminosr@unc.edu

14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF SUBAWARD OFFICIAL NAMED IN 13. *(In ink. "Per" signature not acceptable.)* DATE

Carol J. Burkhart 10/22/15

Barbara Entwisle
Vice Chancellor for Research

University of Colorado
Anschutz Medical Campus

School of Medicine

Department of Immunology and Microbiology
Kathryn V. Holmes, PhD
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Dr. Fang Li
Department of Pharmacology
University of Minnesota
6-120 Jackson Hall
321 Church St. SE
Minneapolis, MN 55455-0217
email: lifang@umn.edu

Dear Fang,

I will be happy to help you in any way with your continuing highly productive research on coronavirus receptor interactions. For the competing renewal application for your grant #R01 AI089728 entitled 'Receptor Recognition Mechanisms of Coronaviruses' you will need soluble trimeric MHV spike glycoprotein and monoclonal antibodies that recognize various conformations of the spike.

Since I have retired and closed my laboratory, I have passed my coronavirus reagents to my colleague Dr. Zhaohui Qian, Associate Professor at the Ministry of Health Key Laboratory, Institute of Pathogen Biology, Chinese Academy of Medical Sciences, Beijing 100176, China. Dr. Qian has an active research program on coronavirus entry and pathogenesis. He has agreed to send you the clone encoding the trimeric spike protein of MHV strain A59 for your research. The ectodomain of the spike (aa1-1248) was inserted into pcDNA3 between the BamH1/Not1 sites behind a Kozak sequence "CACC" that precedes the ATG, and followed by a GCN4 trimerization domain, a GGGGS linker and 6his tag. The spike protein is expressed well in 293T cells and is secreted into the supernatant medium in the form of trimers. This protein is susceptible to trypsin cleavage between the S1 and S2 domains as seen on the spikes on MHV virions. Dr. Qian can also send a similar clone encoding the H716D mutation of the MHV-A59 spike protein that is resistant to trypsin cleavage and might therefore be more stable for some of your experiments.

In our 1990 paper on conformation-dependent epitopes on the MHV-A59 spike glycoprotein we identified monoclonal antibodies to different regions of the viral spike protein, some of which recognized conformation-dependent epitopes. I have called my MHV colleagues and located sources for most of these antibodies for your research.

Both Dr. Qian and I will be delighted to share with you our expertise on coronavirus spike proteins, receptor interactions and virus entry as you continue your exciting research.

With warm regards,

Kathryn V Holmes

Kathryn V. Holmes, PhD

Resources sharing

We will adhere to NIH's Grant Policy on Sharing of Unique Research Resources including the Sharing of Biomedical Research Resources Principles and Guidelines for Recipients of NIH Grants and Contracts issued in December, 1999: http://ott.od.nih.gov/policy/rt_guide_final.html.

All 'model organisms' generated by this project will be distributed freely or deposited into a repository/stock center, making them available to the broader research community, either before or immediately after publication. If we assume responsibility for distributing the newly generated model organisms, we will fill requests in a timely fashion. In addition, we will provide relevant protocols and published genetic and phenotypic data upon request.

Material transfers will be made with terms that are no more restrictive than in the Simple Letter Agreement (SLA) or the Uniform Biological Materials Transfer Agreement (UBMTA) and without reach through requirements. Should any intellectual property arise which requires a patent, we will ensure that the technology (materials and data) remains widely available to the research community in accordance with the NIH Principles and Guidelines document.

Publications derived from this proposal will be made freely available to the public through NIH's Publication Central.

UNIVERSITY OF MINNESOTA

Notice of Grant or Contract Award

Sponsored Projects Administration

(Online Page Navigation Main Menu > Grants >Awards > UM_NOGA Report)

Award: CON000000058289
Primary Project: 00054028
PRF #: 835469

AWARD INFORMATION (Online Page Navigation Main Menu > Grants > Awards >Award Profile)

Award Start Date:	06/07/2016	**Award End Date:**	05/31/2021
Funds Approved to Date:	S464,400 00		
Principal Investigator:	Li,Fang	**Award Type:**	Grant
Primary Project Dept:	PHCL Pharmacology Admin	**Purpose:**	Research
Sponsor:	NIH Nat'l Inst of Allergy & Infect Dis		
Sponsor Award #:	2R01AI089728-06		
Award Title #:	Receptor recognition and cell entry of coronaviruses		

ASSOCIATED PROJECTS (Online Page Navigation Main Menu > Grants >Awards > Award Profile - Funding Tab)

Project #:	00054028	**Department:** 11718 - PHCL Pharmacology Admin	
Project PI:	Li,Fang	**Dates:** 06/07/2016 - 05/31/2021	**Project Title:** Receptor recognition and cell
F&A:	54 00 MTDC	**EFFDT:** 07/01/2018	
F&A:	53 00 MTDC	**EFFDT:** 07/01/2017	
F&A:	52 00 MTDC	**EFFDT:** 06/07/2016	

AWARD HISTORY (Online Page Navigation Main Menu > Grants > Awards > Review Award Modifications)
(Displayed in action order ('Mod Issue Date', 'Period', 'Modification Sequence' in descending order))

Reference Award#: 2R01AI089728-06 **Amount:** S464,400 00
Period: 1 **Begin Date:** 06/07/2016 **End Date:** 05/31/2017
Modification Seq: 1 **Modification Type:** Original **Mod Issue Date:** 06/24/2016
Comments:
$464,440 for 06/07/2016 - 05/31/2017

Subject to 45 CFR Part 75
An unobligated balance may be carried forward to the next budget period without prior, agency approval

No funds are provided and no funds can be used to support gain-of-function research covered under the October 17, 2014 White House Announcement (NIH Guide Notice NOT-OD-15-011)

Direct costs awarded are limited to a 20% increase over the direct costs awarded in the last non-competing continuation (see NIH Guide, Vol 26, No 35, 10/17/97)

This award includes funds awarded for subrecipient activity with University of North Carolina, Chapel Hill in the amount of S120,384 (S79,200 direct costs + S41,184 facilities and administrative costs)

See NOA for information on Select and Highly Pathogenic Agents

**Per the letter dated May 18, 2016 to Mr McKoskey at the University of Minnesota, NIAID funding cannot be used to generate SARS-like viruses with enhanced affinity for human receptors (via either reverse genetics or serial passaging) NIAID funding will instead be used to pursue alternative loss-of-function strategies or mutation of the ACE2 receptor as described in the letter from the University of Minnesota dated April 15, 2016 If any of the alternative experiments proposed, or any experiments proposed under the other Aims, result in a virus with a phenotype of enhanced pathogenicity and/or transmissibility via the respiratory route, or enhanced growth by more than 1 log compared to wild type strains, you must immediately stop the work and notify the NIAID Program Officer and Grants Management Specialist

ESTIMATED FUTURE FUNDING / ACTIONS (Online Page Navigation Main Menu > Grants > Awards > Review Award Modifications)
(Displayed by 'Period' and 'Modification Sequence' in ascending order)

Reference Award#: 2R01AI089728-06 **Amount:** S453,437 00
Period: 2 **Begin Date:** 06/01/2017 **End Date:** 05/31/2018
Modification Seq: 2 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

15
7-15
141

Reference Award#: 2R01AI089728-06 **Amount:** $455.615 00
Period: 3 **Begin Date:** 06/01/2018 **End Date:** 05/31/2019
Modification Seq: 3 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

Reference Award#: 2R01AI089728-06 **Amount:** $455,796 00
Period: 4 **Begin Date:** 06/01/2019 **End Date:** 05/31/2020
Modification Seq: 4 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

Reference Award#: 2R01AI089728-06 **Amount:** $455,796 00
Period: 5 **Begin Date:** 06/01/2020 **End Date:** 05/31/2021
Modification Seq: 5 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

COMMENTS

Description: **Entered by:** SACHI003 **Date & Time:** 6/24/2016 6 49 PM
Date and Time Stamp indicates Proposal-to-Award Process

Description: **Entered by:** SACHI003 **Date & Time:** 6/24/2016 8 53 PM
NIH Contacts
Program Official Erik J Stemmy, Email erik stemmy@nih gov Phone 240-627-3380
Grants Management Specialist Devon R Bumbray-quarles, Email dbumbray@niaid nih gov Phone 240-669-2950 Fax 301-493-0597

SPECIAL REVIEWS (Online Page Navigation Main Menu > Grants >Awards > Award Profile - Certifications Tab)

Project #: 00054028

Description: rDNA, InfectAgents, BioToxins
Approval Date: 01/01/1901 **Expiration Date:** 01/01/1901 **AssuranceNumber:**
Comments: No IBC costs can be incurred on this grant until all approvals are in place with the IBC Committee

BUDGET INFORMATION (Online Page Navigation Main Menu > Grants>Awards>Project Budget)

Please review online budget for valid account string combinations (ex PCBU, Activity, Fund. Dept_Id. Project. Account)

Project #: 00054028 **Cost Share Budget Total:**
Project Start Date: 06/07/2016 **Project End Date:** 05/31/2021
ICR Sharing **Department:** PHCL Pharmacology Admin **Percentage:** 100 00

Period	Start Date	End Date	Direct Cost (Approved)	F & A Cost (Approved)	Direct Cost (Pending)	F & A Cost (Pending)	Total Period Cost
1	06/07/2016	05/31/2017	$339,868 00	$124,532 00	$0 00	$0 00	$464.400 00
2	06/01/2017	05/31/2018	$0 00	$0 00	$453.437 00	$0 00	$453,437 00
3	06/01/2018	05/31/2019	$0 00	$0 00	$455,615 00	$0 00	$455,615 00
4	06/01/2019	05/31/2020	$0 00	$0 00	$455,796 00	$0 00	$455,796 00
5	06/01/2020	05/31/2021	$0 00	$0 00	$455,796 00	$0 00	$455,796 00
TOTAL			$339,868 00	$124,532 00	$1.820,644 00	$0 00	$2,285,044 00

Budget Detail (all periods)

Fund Code	DeptID	Program Code	Chartfield 1	Chartfield 2	Fin EmplID	Activity	Analysis Type	Account	Cost Share	Budget Item	Amount	Hold
3002	11718					1	BUD	700100		FACULTY_SALARY	$32,033 00	
3002	11718					1	BUD	700210		POSTDOC_SALARY	$50,000 00	
3002	11718					1	BUD	700500		CS_BU_SALARY	$35,000 00	
3002	11718					1	BUD	710100		FACULTY_FRINGE	$10,795 00	
3002	11718					1	BUD	710210		POSTDOC_FRINGE	$11,200 00	
3002	11718					1	BUD	710500		CS_BU_FRINGE	$9,590 00	
3002	11718					1	BUD	720200		LABMED_SUPPLIES	$50,566 00	
3002	11718					1	BUD	720300		GEN_OP_SERVICES	$2 000 00	
3002	11718					1	BUD	720400		LABMED_SERVICES	$2,500 00	
3002	11718					1	BUD	720500		TELECOMM	$300 00	
3002	11718					1	BUD	720600		TRAVL_MILE_MOVE	$3,000 00	
3002	11718					1	BUD	750100		NON_CAP_EQUIP	$5,000 00	
3002	11718					1	BUD	780100		REPAIRS_MAINT	$2,500 00	
3002	11718					1	BUD	810300		SUB_NO_FACADM	$95,384 00	
3002	11718					1	BUD	810400		SUB_WITH_FACADM	$25,000 00	
3002	11718					1	BUD	810500		FACADM	$124,532 00	
3002	11718					1	BUD	850100		CAPITAL_EQ_5K	$5,000 00	

COST SHARE/MATCH/IN-KIND

Project #: 00054028

CSM - Cost Share, Matching and InKind

CSM05 - Departments must document all cost share/unpaid effort that is shown in the proposal

PROGRAM INCOME INFORMATION

Project #: 00054028

PIN - Program Income

PIN03 - Reportable Program Income must be deposited by the department into the Sponsored Program Income Unapplied Cash account

PIN04 - Additional - Program Income can be used in addition to sponsor funds to cover project costs that will further the objectives of the legislation under which the grant was made

TERMS & CONDITIONS (Online Page Navigation Main Menu > Grants >Awards > Award Profile - Terms Tab)

Project #: 00054028

APV - Additional Approval

APV10 - Prior sponsor approval required for a change in the scope or objective of the project or program (even if there is no associated budget revision requiring prior written approval)

APV17 - Prior sponsor approval required for patient care costs or rebudgeting in or out of this category

APV27 - Prior sponsor approval required for PD/PI and other Senior/Key Personnel named in the NOA's reduction in effort of 25% or more

APV28 - Prior sponsor approval required to replace PD/PI or other Senior/Key Personnel named in the NOA

APV29 - Prior sponsor approval required for the absence of the PD/PI and/or other Senior/Key Personnel named in the NOA for 3 months or more

APV30 - Prior sponsor approval required for a no cost extension that is submitted after the agency notification deadline

APV31 - Prior sponsor approval required for the addition of a foreign component

APV44 - Particpant costs are only allowable when identified in the funding opportunity announcement (FOA)

APV62 - Prior sponsor approval required for fixed amount subawards that exceed the Simplified Acquisition Threshold of $150,000

APV74 - Prior sponsor approval required to charge admin and clerical costs that are not identified in the approved budget Costs may be allowable if integral to project

EQP - Equipment

EQP70 - Allocable computing devices deemed essential and with unit cost less than $5,000 may be charged

FIS - Fiscal

FIS03 - Reversion of funds at termination / final

FIS08 - Deficits may not be carried forward

FIS14 - Grant/contract funds may not be used to pay the salary of an individual at a rate in excess of Executive Level II of the Federal Executive Pay Scale

IPR - IP Rights

IPR02 - Sponsor support must be acknowledged in patent applications and patent issuing

IPR04 - Title to U of M, non-exclusive, non-transferable, irrevocable, paid-up license to Federal Government

IPR12 - PI obligation to report inventions (and other IP) to the sponsor (and to OTC per policy)

PRP - Property

PRP02 - U of M receives title to equipment purchased or fabricated under this award

PRP03 - Sponsor may require transfer of equipment and its title upon award completion for those items costing in excess of $5000

PUB - Publications

PUB02 - U of M will place an acknowledgement of sponsor support and a disclaimer, as appropriate, on any publication generated by this award or reporting on the award results and/or activity

PUB07 - Peer-reviewed articles must be submitted to NIH Pubmed Central (PMC) upon acceptance for publication and made publicly available within 12 months Cite PMC ID number in progress reports/proposals

PUB70 - Costs for award publications or sharing of results may be charged and posted up to 60 days after the award end date Page charges can only be charged if the journal's policy ensures that charges are calculated the same regardless of fund source

RBD - Rebudgeting

RBD10 - Rebudgeting is allowed as long as there is no change in work scope

REG - Regulatory

REG09 - Subject to Streamlined Noncompeting Award Procedures (SNAP)

REG10 - Federal Demonstration Partnership (FDP) guidelines (www thefdp org)

REG16 - If applicable, no funds may be expended for human subject related activities until all appropriate human subject related approvals are in place

REG17 - If applicable, no funds may be expended for animal related activities until all appropriate animal related approvals are in place

REG18 - If applicable, any personnel involved in the design or conduct of human subject activity must satisfy the requirement for education on the protection of human subjects prior to participating in the study

REG19 - NIH Grants Policy Statement - 10/1/2013 (http //grants nih gov/grants/policy/policy htm)

REG21 - If applicable, no funds may be expended for activities involving recombinant DNA. Infectious Agents or Biological Toxins until Institutional Biosafety Committee (IBC) approval has been obtained

REG26 - Federal Funding Accountability and Transparency Act (FFATA)

REG41 - All University personnel meeting the FCOI definition of key personnel must maintain a current REPA and must have completed FCOI training

REG42 - Final grant reports (Final FFR. Final Progress Report & Final Invention Statement) must be submitted within 120 days of the grant end date

REG43 - NIH Interim Grant General Conditions located at (http //grants nih gov/grants/policy/policy htm)

REG44 - 45 CFR Part 75 - Uniform Administrative Requirements. Cost Principles, and Audit Requirements for Federal Awards ("Uniform Guidance")

REG70 - UNIFORM GUIDANCE - 2 CFR 200 (http //www ecfr gov/cgi-bin/text-idx?SID=704835d27377ef5213a51c149de40cab&node=2 1 1 2 2 1&rgn=div5)

TRV - Travel Restrictions

TRV16 - Regulations implementing the Fly America Act provide that federal funds may be used to pay for air travel only on U S flag carriers, except in specified cases 41 C F R section 301-10 131, et seq For more about Fly America Act requirements and exceptions. see http //travel umn edu/flyamerica php

UMN - U of M Requirements

UMN01 - The U of M Effort Certification system must be used to reflect all U of M staff committed effort on this award, whether or not any salary is charged to the award

UMN02 - Salary cost transfers cannot be made after effort is submitted

UMN03 - All U of M Policy and Guidelines relating to research must be followed and can be read in detail on the web at http //www ospa umn edu/policiesandprocedures/index htm

UMN04 - U of M Cost Transfer guidelines state transfers of non-salary expenses on accounts be clearly documented & made within 30 days after the end of the accounting period the charge was processed

NON-FINANCIAL REPORTING (Online Page Navigation Main Menu > Grants >Awards > Award Profile - Milestones Tab)

Type: Final Report **Description:** Final Technical Report **Due Date:**
Comments: Due 90 days after termination/final or included in renewal proposal

Type: Final Report **Description:** Final Invention Statement **Due Date:**
Comments: Due 90 days after termination/final or included in renewal proposal

Type: Report **Description:** PHS 2590-noncompeting continua **Due Date:**
Comments: Progress Report - Noncompeting continuation must be submitted via RPPR

INVOICE/BILLING INFORMATION

Contract Type: COST_REIMBURSE
Invoicing Method: Cost Reimbursable
Invoice Form: GPC - GM_LOC
Interim Invoice Deadline: 30
Final Invoice Deadline: 90

Contact Name:
Contact Phone:
Contact Fax:
Contact Email:
Contact Address: PSC-FMS (301) 443-3020
PARKLAWN BUILDING ROOM 16A-12
5600 FISHERS LANE
ROCKVILLE MD 20857

FINANCIAL REPORTING

Report Form ID: R_FFR
Reporting Frequency: Termination/Final
Interim Reporting Deadline: 90
Final Reporting Deadline: 90

GENERAL PROVISIONS

This document is intended to provide guidance in managing this award, to the Principal Investigator, Academic Unit, and to those who are noted as receiving this document Its intent is to provide general information regarding University and sponsor policies when given the award In addition, it is intended to reference specific conditions related to this award Specific questions should be addressed to the grant administrator from SPA noted below By accepting this award, the Principal Investigator acknowledges and agrees to abide by all provisions of the sponsoring agency and the University of Minnesota policies related to sponsored projects management, including the Code of Conduct

U OF M CONTACTS

Administrative questions to SPA staff member: Erin Knudsen 612/626-2724 jond0006@umn edu
Billing and financial questions to SFR: Tonya Knutson 612/624-7850 knuts055@umn edu

Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 06/07/2016

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 2R01AI089728-06
FAIN: R01AI089728

Principal Investigator(s):
Fang Li, PHD

Project Title: Receptor recognition and cell entry of coronaviruses

Ms Jondahl, Erin
Grant Administrator
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 554552070

Award e-mailed to: awards@umn edu

Period Of Performance:
Budget Period: 06/07/2016 – 05/31/2017
Project Period: 06/07/2016 – 05/31/2021

Dear Business Official

The National Institutes of Health hereby awards a grant in the amount of $464,440 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MINNESOTA in support of the above referenced project This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI089728 The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health " Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards Consult the NIH website http //grants nih gov/grants/policy/coi/ for a link to the regulation and additional important information

If you have any questions about this award, please contact the individual(s) referenced in Section IV

Sincerely yours,

Devon R Bumbray-Quarles
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 2R01AI089728-06

Award Calculation (U.S. Dollars)

Salaries and Wages	$116,853
Fringe Benefits	$30,547
Materials & Supplies	$60,005
Travel	$2,970
Other	$5,247
Subawards/Consortium/Contractual Costs	$120.384
Publication Costs	$2,178
Federal Direct Costs	$338,184
Federal F&A Costs	$126,256
Approved Budget	$464,440
Total Amount of Federal Funds Obligated (Federal Share)	$464,440
TOTAL FEDERAL AWARD AMOUNT	$464,440
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$464,440

SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
6	$464,440	$464,440
7	$453,437	$453,437
8	$455,615	$455,615
9	$455,796	$455,796
10	$455,796	$455,796

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information.

CFDA Name. Allergy, Immunology and Transplantation Research
CFDA Number: 93 855
EIN. 1416007513A1
Document Number: RAI089728B
PMS Account Type: P (Subaccount)
Fiscal Year: 2016

IC	CAN	2016	2017	2018	2019	2020
AI	8472364	$464,440	$453,437	$455,615	$455,796	$455,796

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC M51C B / **OC** 414B / **Released** BUMBRAYD 05/31/2016
Award Processed: 06/07/2016 12 02 20 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 2R01AI089728-06

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http //grants nih gov/grants/policy/awardconditions htm

SECTION III – TERMS AND CONDITIONS – 2R01AI089728-06

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following

a The grant program legislation and program regulation cited in this Notice of Award
b Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts

c 45 CFR Part 75
d National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period
e Federal Award Performance Goals As required by the periodic report in the RPPR or in the final progress report when applicable
f This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW

(See NIH Home Page at http //grants nih gov/grants/policy/awardconditions htm for certain references cited above)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75 2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA) The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs NIH recognizes that some awards may have another classification for purposes of indirect costs The auditor is not required to report the disconnect (i e , the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s)

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP)

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM) Should a consortium/subaward be issued under this award, a DUNS requirement must be included See http //grants nih gov/grants/policy/awardconditions htm for the full NIH award term implementing this requirement and other additional information

This award has been assigned the Federal Award Identification Number (FAIN) R01AI089728 Recipients must document the assigned FAIN on each consortium/subaward issued under this award

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170

In accordance with P L 110-161, compliance with the NIH Public Access Policy is now mandatory For more information, see NOT-OD-08-033 and the Public Access website http //publicaccess nih gov/

In accordance with the regulatory requirements provided at 45 CFR 75 113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period The recipient must also make semiannual disclosures regarding such proceedings Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)) Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75 This term does not apply to NIH fellowships

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 2R01AI089728-06

No funds are provided and no funds can be used to support gain-of-function research covered under the October 17, 2014 White House Announcement (NIH Guide Notice NOT-OD-15-011)

Per the letter dated May 18, 2016 to Mr McKoskey at the University of Minnesota, NIAID funding cannot be used to generate SARS-like viruses with enhanced affinity for human receptors (via either reverse genetics or serial passaging) NIAID funding will instead be used to pursue alternative loss-of-function strategies or mutation of the ACE2 receptor as described in the letter from the University of Minnesota dated April 15, 2016 If any of the alternative experiments proposed, or any experiments proposed under the other Aims, result in a virus with a phenotype of enhanced pathogenicity and/or transmissibility via the respiratory route, or enhanced growth by more than 1 log compared to wild type strains, you must immediately stop the work and notify the NIAID Program Officer and Grants Management Specialist
*****
Direct costs awarded are limited to a 20% increase over the direct costs awarded in the last non-competing continuation (see NIH Guide, Vol 26, No 35, 10/17/97)
*****
This award includes funds awarded for subrecipient activity with ***University of North Carolina, Chapel Hill*** in the amount of **$120,384** (**$79,200** direct costs + **$41,184** facilities and administrative costs)

Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS) The referenced section of the NIH Grants Policy Statement is available at http //grants nih gov/grants/policy/nihgps/HTML5/section_15/15_consortium_agreements htm
*****
Select Agents
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13 b (http //www selectagents gov/Regulations html)

Highly Pathogenic Agent
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL)
(http //www cdc gov/OD/ohs/biosfty/bmbl5/bmbl5toc htm) Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used
When submitting future Progress Reports indicate at the beginning of the report

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide

o A list of the new and/or additional Agent(s) that will be studied,
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment,
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official It is important to note if the work is being done in a new location
*****
The budget period anniversary start date for future year(s) will be **June** 1

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions The Program Official is responsible for the scientific, programmatic and technical aspects of this project These individuals work together in overall project administration Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist Requests may be made via e-mail

Grants Management Specialist Devon R Bumbray-quarles
Email dbumbray@niaid nih gov **Phone** 240-669-2950 **Fax** 301-493-0597

Program Official: Erik J Stemmy
Email erik stemmy@nih gov **Phone** 240-627-3380

SPREADSHEET SUMMARY
GRANT NUMBER: 2R01AI089728-06

INSTITUTION: UNIVERSITY OF MINNESOTA

Budget	Year 6	Year 7	Year 8	Year 9	Year 10
Salaries and Wages	$116,853	$116,853	$116,853	$116,853	$116,853
Fringe Benefits	$30,547	$30,547	$30,547	$30,547	$30,547
Materials & Supplies	$60,005	$60,005	$60,005	$60,005	$60,005
Travel	$2,970	$2,970	$2,970	$2,970	$2,970
Other	$5,247	$5,247	$5,247	$5,247	$5,247
Subawards/Consortium/Contractual Costs	$120,384	$120,384	$120,384	$120,384	$120,384
Publication Costs	$2,178	$2,178	$2,178	$2,178	$2,178
TOTAL FEDERAL DC	$338,184	$338,184	$338,184	$338,184	$338,184
TOTAL FEDERAL F&A	$126,256	$115,253	$117,431	$117,612	$117,612
TOTAL COST	$464,440	$453,437	$455,615	$455,796	$455,796

Facilities and Administrative Costs	Year 6	Year 7	Year 8	Year 9	Year 10
F&A Cost Rate 1	52%	52%	53%	54%	54%
F&A Cost Base 1	$242,800	$18,150	$18,150	$217,800	$217,800
F&A Costs 1	$126,256	$9,438	$9,620	$117,612	$117,612
F&A Cost Rate 2		53%	54%		
F&A Cost Base 2		$199,650	$199,650		
F&A Costs 2		$105,815	$107,811		

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Institute of Allergy
and Infectious Diseases
Bethesda, Maryland 20892

May 18, 2016

Mr Kevin McKoskey
Director, Sponsored Projects
University of Minnesota
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 55455-2070

RE. 2 R01AI089728-06

Dear Mr McKoskey:

Thank you for your correspondence of April 15, 2016, regarding the October 17, 2014 White House announcement of a U S. Government-wide pause on certain gain-of-function (GoF) experiments and its potential impact on your research (http //www whitehouse.gov/blog/2014/10/17/doing-diligence-assess-risks-and-benefits-life-sciences-gain-function-research). The research funding pause pertains to GoF research projects that may be reasonably anticipated to confer attributes to influenza, MERS, or SARS viruses such that the resulting virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route

NIAID reviewed the original grant application, and the additional information provided by you, and made the following assessments regarding Subaim 2 1 of the above-referenced grant:

- NIAID is in agreement that the work you proposed under Experiments 1, 2, and 3 is not subject to the GoF research funding pause This determination is based on the fact that the work will be carried out using either recombinant proteins or replication-deficient pseudoviruses, and will not involve the generation of a replicating SARS-CoV variant with enhanced pathogenicity and/or transmissibility via the respiratory route.
- NIAID's determination is that the work proposed under Experiment 4 to generate SARS-Like viruses with enhanced affinity for human receptors (via both reverse genetics and serial passaging) is subject to the GoF research funding pause, and therefore may not be conducted under this grant. Given the lack of empiric evidence that increased receptor binding alone is insufficient to increase pathogenicity, NIAID has determined that the proposed work is reasonably anticipated to result in a SARS-Like virus with enhanced pathogenicity. NIAID acknowledges that in lieu of generating SARS-Like viruses with enhanced affinity for human receptors you will pursue alternative strategies using *loss-of-function* mutations, or mutations targeting the ACE2 receptor. NIAID also acknowledges your statement that if any mutant

viruses you generate demonstrate either enhanced virus growth >1 log compared to the wildtype parental backbone strain or more efficient growth in primary human airway epithelial cells, you will immediately stop all experiments with the mutant and notify NIAID and the IBCs at the University of Minnesota and the University of North Carolina at Chapel Hill of these results

Please remember that the institution must comply in full with all terms and conditions placed on this grant. If your research evolves to include experiments that may be subject to the GoF research funding pause or you observe enhanced pathogenicity and/or transmissibility of SARS or SARS-Like viruses in mammals via the respiratory route at any time during the course of conducting these experiments, you must immediately stop these research activities and provide the NIAID Program Officer and Grants Management Specialist with the relevant data and information related to these unanticipated outcomes

As indicated above, NIAID determinations are based on information from multiple sources, but primarily on our communication with you about the details of your proposed experiments and your research results Should NIAID's determination change based on information obtained through the U S Government GoF deliberative process, described here http://www.phe.gov/s3/dualuse/Documents/gain-of-function.pdf, you will be notified, however, until such time, or until the GoF research funding pause is lifted, NIAID's determination, indicated above, is final

Please let us know if you have any questions, or if you require additional information

Sincerely,

Devon Bumbray-Quarles
Grants Management Specialist
NIAID/NIH/DHHS

Erik J. Stemmy, Ph D.
Program Officer
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS

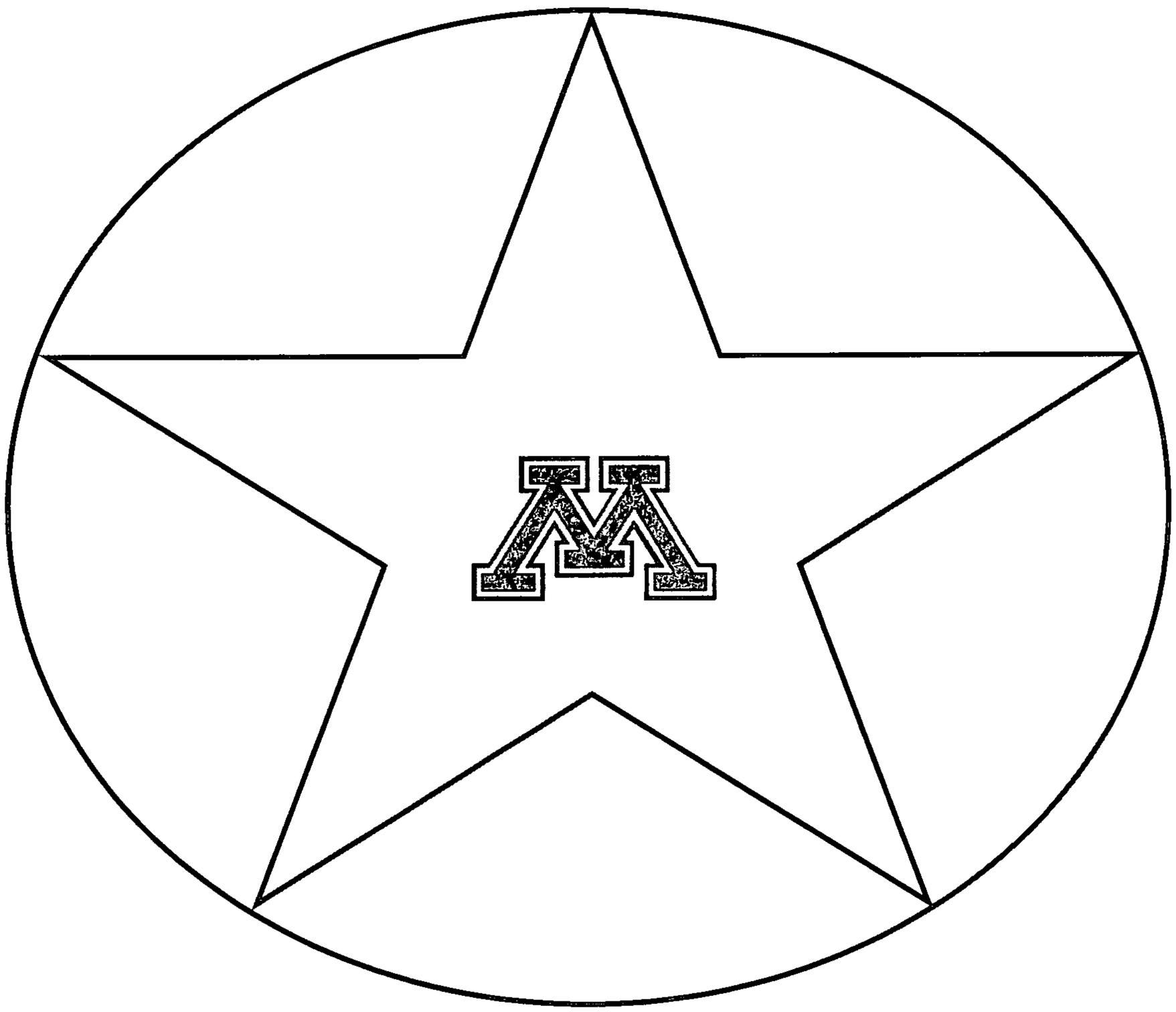
CC. Dr. Fang Li
Ms Erin Knudsen
Ms. Mary Kirker
Dr Irene Glowinski
Dr Andrew Ford
Dr Ralph Baric

GA: ERINK

Awarded CON #: 58289

(If Applicable) Related CON #: 22345

Please Mail All Pages Prior To Page Separator



New Award

VERSION 1 0

GA	Erin
Date	6/24/2016
CON#	58289
Primary Project	00054028
In PreAward/Adv Account	No
Parent CON#	
Award Amount	$ 464,440 00
Award Dates	06/07/2016 - 05/31/2017

For DHHS Renewals and DHHS continuations transitioning to P subaccount LOC (includes NIH)

FAIN (Federal Award ID Number)	R01AI089728
Reference Award Number	2R01AI089728-06
Award Type	Grant

Required for all federal assistance awards and federal subawards

BUDGET INFORMATION FOR FUNDS PROVIDED BY SPONSOR

Fund	3002 - Federal Government - Sponsored
Primary Project DeptID	11718
Child Project DeptID	

PHCL Pharmacology Admin

Sponsor Restricted Funds	
Release Sponsor Restricted Funds	$ -

(set up in 810800)
(moved out of 810800)

Cost Share Information

Fund	
DeptID	
Program	
Chartfield 1	
Chartfield 2	
EmplID	
- OR - See attached PS-friendly budget	

FFATA	
Not FFATA	x

For all awards received directly from the federal government, pick appropriate value Does not apply when we are a subrecipient under a prime federal award

Additional Information hyperlink (GA enters Additional Information on proposal record)

PRF Number Comment	835469
Flow Through Ref Award	
Other Comment	

Sponsor Contact Info

Admin Contact	
Grants Management Specialist Devon R Bumbray-quarles Email dbumbray@niaid nih gov Phone 240-669-2950 Fax 301-493-0597	
Technical Contact	
Program Official Erik J Stemmy Email erik stemmy@nih gov Phone 240-627-3380	
Invoicing Contact	
Other Contact	

Attributes

		Comments
	CLIN_TRIALS	
x	EXP_AUTH	
x	FDP	
	MN-IP	
	MODULAR	
	NON_EXPAUTH	
	OTHER	
	RPPR	

x	SNAP	

INVOICING & FINANCIAL REPORTING INFO – See Page(s)/Article(s)	NIH	
Billing method (how sponsor wants to be billed)	Rate Based	Rate based = bill by expenses Amount based = pay by deliverable, set hourly amount, scheduled dates etc
Contract Type (do funds revert?)	Cost Reimbursable - funds revert	

Excel terms and conditions or terms templates with changes

which tab?	

Template Terms and Conditions		**NOTE TO AWARD SET UP use template terms insert**
Terms apply to all projects		
Non UG Terms		
UG Terms	UG-NIH_Expanded	
Terms to be Removed		

Project Specific Terms to be added (Note Explanation field is limited to 150 characters Any term exceeding that limit will be added to Award Modification section)

Term Type	**Comment**
PIN04	

Project Specific Terms

Project Number	

Milestones

Milestone Type	RPT	Report
Milestone Code	PHS 2590	PHS 2590-noncompeting continua
Comments	Progress Report	

Milestones

Milestone Type	FRPT	Final Report
Milestone Code	FINAL_TECH_RPT	Final Technical Report
Comments	Due 90 days after termination/final or included in renewal proposal	

Milestones

Milestone Type	FRPT	Final Report
Milestone Code	FINAL_HHS568	Final Invention Statement
Comments	Due 90 days after termination/final or included in renewal proposal	

Milestones

Milestone Type	
Milestone Code	

Comments	
Function Code	1100 - Research Sponsored
Award Comments	$464,440 for 06/07/2016 - 05/31/2017 Subject to 45 CFR Part 75 An unobligated balance may be carried forward to the next budget period without prior, agency approval No funds are provided and no funds can be used to support gain-of-function research covered under the October 17, 2014 White House Announcement (NIH Guide Notice NOT-OD-15-011) Direct costs awarded are limited to a 20% increase over the direct costs awarded in the last non-competing continuation (see NIH Guide, Vol 26, No 35, 10/17/97)
Comments to Award set up team	

PUT AWARD FILE IN THE FOLLOWING ORDER
Agency award documents
Guidance documents (to be included with award emailed to PI/Dept)
Award Imaging Divider page (yellow star sheet)
PS-friendly budget (if any)
Negotiation notes, proposal correspondence, proposal, etc

Set all negotiate award negotiation types to completed except for the one for contract entry and the original one created by files

PS / SPONSOR PROPOSAL BUDGET WORKSHEET

6/7/16-5/31/17

Please complete the cost share and F&A distribution information at the bottom of the page, if applicable

Award R01AI089728-06 Project #, if known 0 Should this be the Primary Project? X Yes ___ No

Project DeptID 11718 Project PI **Li, Fang** **PRF#835469**

LINK TO CHART OF ACCOUNT' **Includes new FY15 personnel budget accounts**

ITEM		Sponsor Funding	Cost Share or Match*	TOTAL PROJECT BUDGET
Personnel				
Salary				
700100	Salary - Faculty	32,033		
700200	Salary - Professional/Admin/Police	0		
700210	Salary - Post Doc/Research Spc	50,000		
700300	Salary - Grad Research Asst (with tuition)	0		
700310	Salary - Grad Research Asst (no tuition)			
700320	Salary - Salary - Residents/Fellows			
700400	Salary - Undergrad Student	0		
700500	Salary - Civil Service	35,000		
700510	Salary - Labor Represented			
700520	Salary - Trades			
700530	Salary - Temp/Casual			
Total Salary		117,033	0	117.033
Fringe				
710100	Fringe - Faculty	10,795		
710200	Fringe - Professional/Admin/Police	0		
710210	Fringe - Post Doc/Research Spc	11,200		
710300	Fringe - Grad Research Asst (with tuition)	0		
710310	Fringe - Grad Research Asst (no tuition)			
710320	Fringe - Residents/Fellows			
710400	Fringe - Undergrad Student			
710500	Fringe - Civil Service	9,590		
710510	Fringe - Labor Represented			
710520	Fringe - Trades			
710530	Fringe - Temp/Casual			
710600	Fringe - Direct Charge			
Total Fringe		31,585	0	31,585
Total Personnel		148,618	0	148,618
Consulting / Professional Services				
730100	Consulting Services			
730200	Professional Services			
Total Consulting / Professional Services		0	0	0
Equipment				
850100	Equipment	5,000		5,000
Supplies				
720100	General Operating Supplies			
720200	Lab & Medical Supplies	50,566		
750100	Non-Capitalized Equipment	5,000		
Total Supplies		55,566	0	55,566
Travel				
720600	Travel / Mileage / Moving	3,000		3,000
Other Direct Costs				
720300	General Operating Services	2,000		
720400	Lab & Medical Services	2,500		
720500	Telecommunications	300		
770100	Long Term Rents & Leases			
770150	Long Term Rents & Leases (bldg/facility)			
770200	Short Term Rents & Leases			
770250	Short Term Rents & Leases (bldg/facility)			
780100	Repairs & Maintenance	2,500		
810100	Research Subject Payments			
810200	Participant Payments			
Total Other Direct Costs		7,300	0	7,300
Subawards / Subcontracts				
810300	Subcontracts not subject to F&A	95,384		
810400	Subcontracts subject to F&A	25,000		
Total Subawards / Subcontracts		120,384	0	120,384
Additional Accounts (show budget account and a description, please)				
				0
				0
				0
TOTAL DIRECT COSTS		339,868	0	339,868
810500	Facilities and Administrative Expenses	124,532		124,532
TOTAL COSTS		464,400	0	464,400

***Cost Share/Match account string**

Does your Program FUNC match your Project FUNC? Check the COA for FUNC and Program descriptions

For any amounts shown as Cost Share or Match, the Fund, DeptID, Program and optional fields CF1, CF2, and FinEmplID providing the funding must be identified If these codes will be the same for all lines with Cost Share or Match, please enter them here

Fund _____ DeptID _____ Program _____
CF1 _____ CF2 _____ FinEmplID _____

If the Fund, DeptID, Program, CF1, CF2, and FinEmplID will differ among the cost share or match amounts, please enter the information next to the specific cost share or match amount so that budget lines can be correctly established

F&A Distribution

If the F&A revenue is going to be shared between departments, please identify the department(s) and percentages below If all the F&A revenue should go to the department identified at the top of the page, this section may be left blank

F&A Distribution - Approved by (name)

DeptID _____ Dept Name _____ _____ % F&A rec'd _____
DeptID _____ Dept Name _____ _____ % F&A rec'd _____
DeptID _____ Dept Name _____ _____ % F&A rec'd _____
DeptID _____ Dept Name _____ _____ % F&A rec'd _____
Total F&A % (must = 100%)

By submission of this information, I certify that the proper authorizing officials are named above and that I have retained evidence within the department/unit confirming their agreement with the distributions above

Name _____
DeptID _____
Phone # _____
Email _____

See current rates (fringe benefits, F&A, etc) http //www ospa umn edu/forms/index html

For a crosswalk between CUFS object codes and PS Accounts, see
http //www budget umn edu/FY09docs/CUFS_PS_conversion_grid xls

UNIVERSITY OF MINNESOTA

ReportID UMFGM017F

PROPOSAL SUMMARY

Run Date. 06/07/2016
Run Time 09.19·05 AM

Status 4 AwdB Award Sent to GA

Grant Administrator Knudsen,Erin Elizabeth

PRF #	835469	Proposal #/Version	CON000000058289/A101
EGMS Proposal #	442741	Sponsor Award #	2R01AI089728-06
Proposal Type	Renewal	Concurrent	N
Previous Award		SubContract	N
Purpose			

Title Receptor recognition and cell entry of coronaviruses

Principal Investigator Li,Fang lifang@umn edu

Budget Summary

Start Date	07/01/2016	End Date	06/30/2021	
Direct Costs	$1,708,000 00	Indirect Costs	$600,400 00	Total Costs $2,308,400 00

Sponsor NATIONAL INSTITUTES OF HEALTH (NIH)

Submission Information

Due Date	11/05/2015	Date Type	Receipt Date
Submission Method	Electronically Only	# of Copies Submitted	_____

Mailing Address

Comments/Corrections

REPA	__verified	__email sent for _____		
IRB	__verified	__pending	__email sent	__Not Applicable
IACUC	__verified	__pending	__email sent	__Not Applicable
IBC	__verified	__pending	__email sent	__Not Applicable
F&A rate	__at full or agency limit	__waiver on file	__waiver requested	
Cost share documented?		__yes	__no	__Not Applicable
ICR sharing?		__yes	__no	

√ RCR 6/7/2016
√ IRB
√ IBC

RCR
MET
NOT MET

Erin Knudsen <jond0006@umn.edu>

Fwd: Grant Number: 2 R01 AI 089728 - 06, Li (PI)

Kevin McKoskey <kevin@umn edu> Fri, Apr 15, 2016 at 1 25 PM
To DBumbray@niaid nih gov, erin stempinski@nih gov, IGlowinski@niaid nih gov, MKIRKER@niaid nih gov
Cc Fang Li <lifang@umn edu>, Lynn F Weber-1 <weber117@umn edu>, Carrie L DeJarlais-1 <dejar001@umn edu>, Erin Jondahl <jond0006@umn edu>

Dear Devon Bumbray-Quarles,

Institutional endorsement on behalf of the Regents of the University of Minnesota is provided for Fang Li's attached response to your request for additional information on the above referenced grant

Please contact Fang Li with programmatic matters and Erin Knudsen, of my staff, at 612-626-2724 or jond0006@umn edu if you have administrative questions

Thank you for your support of the University of Minnesota

Regards,

Kevin McKoskey
Director

-
[Quoted text hidden]

3 attachments

Li 2R01AI 089728-06 GOF Response 2016-v3-1.pdf
976K

sars improve binding mouse ace2 schedule g-1.pdf
59K

schedule g sars improve ace2 binding-1.pdf
56K

Erin Knudsen <jond0006@umn.edu>

Fwd: Grant Number: 2 R01 AI 089728 - 06, Li (PI)

Erin Knudsen <jond0006@umn edu> Fri, Apr 15, 2016 at 12 16 PM
To Kevin Mc Koskey <kevin@umn edu>

Kevin,

I recommend Fang Li's response to NIH on the GoF request FYI there is a subaward with UNC on this award If you concur, please add institutional endorsement and forward this request to the following NIH contacts

Grants Officer Bumbray-Quarles, Devon (NIH/NIAID) [E] <DBumbray@niaid nih gov>
cc Stempinski, Erin (NIH/NHLBI) [C] <erin stempinski@nih gov>
Ford, Andrew (NIH/NIAID) [E] <andrew ford@nih gov>
Glowinski, Irene (NIH/NIAID) [E] <IGlowinski@niaid nih gov>
Kirker, Mary (NIH/NIAID) [E] <MKIRKER@niaid nih gov>

and copy
PI Fang Li <lifang@umn edu>
Dept Lynn Weber at weber117@umn edu
Carrie DeJarlais at dejar001@umn edu
GA Erin at jond0006@umn edu

Thanks,
Erin

Dear Devon Bumbray-Quarles,

Institutional endorsement on behalf of the Regents of the University of Minnesota is provided for Fang Li's attached response to your request for additional information on the above referenced grant

Please contact Fang Li with programmatic matters and Erin Knudsen, of my staff, at 612-626-2724 or jond0006@umn edu if you have administrative questions Thank you for your support of the University of Minnesota

Regards,

Kevin McKoskey
Director

[Quoted text hidden]

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3 attachments

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schedule g sars improve ace2 binding-1.pdf
56K

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
National Institute of Allergy
and Infectious Diseases
Bethesda, Maryland 20892

March 31, 2016

Mr Kevin McKoskey
Director, Sponsored Projects
University of Minnesota
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 55455-2070

RE. 2 R01AI089728-06

Dear Mr McKoskey

The above referenced grant is being considered for funding

NIAID has determined that this grant may include Gain of Function (GoF) research that is subject to the recently-announced U S Government (USG) funding pause (http //www phe gov/s3/dualuse/Documents/gain-of-function pdf), issued on October 17, 2014 The following specific aim may involve research covered under the pause

Aim 2.1: Receptor and viral determinants of the cross-species transmission of bat SLCoVs

As per the funding pause announcement, new USG funding will not be released for GoF research projects that may be reasonably anticipated to confer attributes to influenza, MERS, or SARS viruses such that the virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route. The research funding pause would not apply to characterization or testing of naturally occurring influenza, MERS, or SARS viruses, unless the tests are reasonably anticipated to increase transmissibility and/or pathogenicity

NIAID requests that you provide the following information within 15 days of the date of this letter:

- **Determination as to whether the above research does or does not include GoF work subject to the funding pause.** Please provide a detailed explanation for this determination, including, but not limited to, the specific strains of SLCoVs to be used in the proposed work, the mutations planned to be introduced, and details of the planned passaging work Your determination should also include whether it is reasonably anticipated that any of this work would result in SLCoVs that have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route relative to SARS- or MERS-CoV.

- **In addition, the application makes reference to strains of SARS-CoV that the PI has engineered with "super binding affinity" for human and civet ACE2 receptors.** NIAID requests additional information on these strains of SARS-CoV, including: the dates the strains were engineered; whether these "highly evolved" strains exhibit enhanced pathogenicity and/or transmissibility in mammals via the respiratory route relative to wild type SARS-CoV; and how these strains will be used in the proposed work
- **If it is determined that the above research <u>DOES</u> include GoF work subject to the funding pause, provide detailed information on what research will remain viable with the removal of the GoF work and appropriate budget adjustments. Options include:**
  a) For the specific aims that propose GoF work, provide a detailed description of changes that can be made to remove the GoF work but maintain the specific aim(s); or
  b) Remove the specific aims and experiments that are subject to the pause from the Research Plan and request to have the award budget renegotiated

If you have any questions about this matter please do not hesitate to contact the NIAID Program Officer.

Sincerely,

Devon Bumbray-Quarles
Grants Management Specialist
NIAID/NIH/DHHS

Erik J. Stemmy, Ph D
Program Officer
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS

CC Dr Fang Li
Ms. Erin Jondahl
Ms Mary Kirker
Dr Irene Glowinski
Dr Andrew Ford

Response to GOF Pause Letter – 2R01AI 089728–06, Li (PI)/Baric (co-I)

On March 31, 2016, NIAID informed us that our grant application 2R01AI089728–06 may include GOF research that is subject to the recently-announced U S Government funding pause issued on October 17, 2014 Program has identified subaim 2 1 that may involve research covered under the pause Therefore, we have been requested to address (1) whether the mutations introduced to bat SLCoVs as well as passaging of bat SLCoVs in cell culture may result in SLCoVs that have enhanced pathogenicity and/or transmissibility in mammals, (2) information on the strains of SARS-CoV that we have engineered with "super binding affinity" for human and civet ACE2 receptors, (3) if subaim 2 1 does include GOF work, what changes (either in research design or in budget) can be made to maintain subaim 2 1

Summary of subaim 2.1:

Rationale for subaim 2 1 Recently three bat SLCoVs have been identified that can infect human cells and use human ACE2 as their receptor (PMID 24172901) The overall goal of this subaim is to identify the receptor and viral determinants of the cross-species transmission of these three bat SLCoVs

Experiment #1 We will construct, express, and purify the RBDs from these three bat SLCoVs and ACE2 from different species (including human and bat) We will then measure the binding affinity between each of the RBDs and each of the ACE2 molecules The research tools we will use include ELISA, surface plasmon resonance, AlphaScreen, and pseudovirus entry

Experiment #2 Based on the sequence comparisons of ACE2 from different species (Fig 10A), we can identify a number of residue differences among them that potentially regulate cross-species determinants for bat SLCoVs We will introduce mutations into bat ACE2 corresponding to the residue differences between bat ACE2 and ACE2 from other species, and measure the binding between the mutant bat ACE2 and bat SLCoV RBDs In addition, we will introduce the reverse mutations into ACE2 from non-bat species, and measure the binding between bat SLCoV RBDs and mutant ACE2 from other species

A

```
ACE2   21          31          41
Human  IEEQAKTFLD  KFNHEAEDLF  YQSSLASWNY
Civet  TEELAKTFLE  TFNYEAQELS  YQSSVASWNY
Bat    TEDEAKMFLD  KFNTKAEDLS  HQSSLASWDY
Mouse  TEENAKTFLN  NFNQEAEDLS  YQSSLASWNY
         *  ** **   ** :*: *   *** ***·*

ACE2   71          81          321         351
Human  AFLKEQSTLA  QMYPLQEIQN  PNMTQGFWEN  LGKGDFRILM
Civet  AYYEEQSKLA  QTYPLAEIQD  PNMTQGFWEN  LGKGDFRIKM
Bat    AFYEEQSKLA  KNYSLEQIQN  PNMTEGFWNN  LGKGDFRIKM
Mouse  AFYEEQSKTA  QSFSLQEIQT  PHMTQGFWAN  LGHGDFRIKM
       *·  ***  *     * :**  * ** *** * ** ***** *
```

B

SARS-CoV	436	442	472	479	480	487
Human (Tor02)	Y	Y	L	N	D	T
Civet (Sz02)	Y	Y	L	K	D	S
Human/Civet (Gd03)	Y	Y	P	N	G	S
Mouse (PMID 22072787)	H	Y	L	N	D	T
Bat (rs3366)	Y	S	F	N	D	N
Bat (shc014)	Y	W	P	R	P	A
Bat (wiv1)	Y	S	F	N	D	N

Figure 10 Receptor and viral determinants of SLCoV/ACE2 interactions (A) Sequence comparisons of ACE2 from different animal species In red are residues that are critical to the major species barriers between hosts for SARS-CoV infections In blue are residues that directly contact SARS-CoV Asterisks indicate positions which have a single, fully conserved residue Colons indicate positions which have strongly conserved residues Periods indicate positions which have weakly conserved residues (B) Sequence comparisons of S1-CTDs from different SARS-CoV strains and bat SLCoVs The strain names are in parenthesis Shown are six residue positions that are critical for ACE2 binding and are also prone to evolution

Experiment #3 Based on our knowledge about the RBD residues that play key roles in receptor binding (Fig 10B), we will introduce mutations into bat SLCoV RBDs corresponding to the residue differences between bat SLCoV RBDs and human, civet or mouse SARS-CoV RBDs We will then measure the binding between mutant bat SLCoV RBDs and ACE2 from different species

Experiment #4 Based on results from Experiments #1-3, we will introduce mutations into recombinant bat

SLCoVs using reverse genetics, and evaluate virus usage of ACE2 from different species in cell culture In parallel, we will passage bat SLCoVs in cell culture, using experimental evolution to select for novel receptor enhancing mutations in the bat SLCoVs

Response:

Experiments #1, #2, and #3: These experiments will all be carried out using either recombinant proteins or pseudoviruses The pseudoviruses consist of replication-deficient retroviruses that are pseudotyped with SLCoV spike proteins, and hence can only enter cells without replicating themselves No infectious SARS-CoV or SLCoV will be involved, and thus these experiments are not subject to the GOF pause

Experiment #4: This is the only experiment in the entire proposal that involves infectious SLCoVs

Rationale for Experiment #4: A growing body of evidence supports the hypothesis that a potentially large population of SARS-like bat coronaviruses (SLCoV) exist in nature that efficiently use the human, bat and civet ACE2 receptor, can cross the species barrier and replicate efficiently in human cells (PMC4801244, PMC4797993, PMC4810638, PMID 24172901) However, it remains uncertain whether these SLCoV will require additional adaptive mutations to efficiently utilize the human ACE2 receptor, especially in the context of primary airway epithelial cells Mutations which alter ACE2 interactions across species will likely forecast the potential for more serious human disease patterns, as seen during the SARS-CoV epidemic Therefore, identifying key mutations and mutation patterns that might forecast SLCoV emergence would i) assist policy makers in resource allocation decisions, ii) forecast disease patterns in human populations, and iii) inform human monoclonal antibody therapeutic efficacy While several groups have used phylogenetic approaches and some biochemistry to predict emerging coronavirus epidemic and disease severity patterns in human populations (PMC4810480, PMC4702133), these studies are highly speculative because they lack robust empirical support, potentially misinforming epidemic risk potential and outbreak management prioritizations We hypothesize that detailed studies on RBD mutation and evolution may provide critical information for informing the greater public health risk potential during an expanded outbreak Consequently, the experiments in this section are therefore of very high impact These experiments are designed to reveal fundamental mechanisms and patterns of SLCoV RBD mutation that regulate emergence (e g , civet, mouse, human), identify new strains with high epidemic risk potential in humans and animals based on sequence patterns, and inform early choice selection for immunotherapeutic control in an outbreak setting This information will potentially mitigate disease spread to health care workers, predict and reduce the severity of the expanded epidemic in human populations

List of mutations that will be introduced to bat SLCoVs using reverse genetics:

(i) For bat SLCoV rs3366 and bat SLCoV wiv1 Y442S, L472F, T487N (from strain Tor02), Y442S, L472F, K479N, S487N (from strain Sz02), Y442S, P472F, G480D, S487N (from strain Gd03), H436Y, Y442S, L472F, T487N (from mouse-adapted SARS-CoV)

(ii) For bat SLCoV shc014 Y442W, L472P, N479R, D480P, T487A (from strain Tor02), Y442W, L472P, K479R, D480P, S487A (from strain Sz02), Y442W, N479R, G480P, S487A (from strain Gd03), H436Y, Y442W, L472P, N479R, D480P, T487A (from mouse-adapted SARS-CoV)

Expected results from Experiment #4: When the residues from human SARS-CoV strain Tor02 are introduced to bat SLCoVs, the mutant bat SLCoVs may have enhanced binding affinity for human ACE2 because human SARS-CoV strain likely has been adapted to use human ACE2 more efficiently than bat

SLCoVs do Similarly, when the residues from civet or mouse SARS-CoV strains are introduced to bat SLCoVs, the mutant bat SLCoVs may have enhanced binding affinity for civet ACE2 or mouse ACE2, respectively In addition, passage of bat SLCoVs in human cell culture may lead to mutant bat SLCoVs with enhanced affinity for human ACE2 However, the enhanced affinity of SLCoVs for ACE2 doesn't necessarily lead to enhanced viral infectivity or transmissibility (see below)

GOF concerns over introducing mutations into SLCoVs using reverse genetics: The current paradigm argues that increased virus-receptor interactions correlate directly with increased virus growth and pathogenesis However, this has never been empirically tested It is possible that increased virus-receptor interactions attenuate viral infectivity by hindering viral release from infected cells It is also possible that RBD-receptor interactions are highly tuned to regulate associations and dissociations during entry, and as such, both lower and higher affinity RBD-ACE2 interactions may actually ablate receptor biding or downstream S protein conformational reprogramming events that are critical for activation of the fusion domain and entry Moreover, higher affinity interactions can also result in dominant negative effects on virus replication, e g , the production of defective "spike less" progeny virion because high affinity S glycoprotein-ACE2 complexes become tethered on membranes and S cannot participate in virion maturation and release A similar phenomena on virus maturation and release occurs when the receptor is over-expressed in cells (PMID 9123839) Finally, it is also important to consider that RBD interactions are dependent on backbone sequence context, which varies in rs3366, WIV1, SHC014 and SARS-MA, thus, mutations that enhance SARS-MA interaction with a particular ACE2 receptor may subtly enhance, attenuate or ablate interactions across different RBD sequence backbones Because WIV1, SHC014 and SARS-MA can already use the human, civet, mouse and bat ACE2, it is anticipated that most cross mammal responses will be subtle and less than 10 fold over the parental backbone, in either direction Thus, it cannot be reasonably expected that these mutations will result in increased pathogenesis or transmission in any mammalian host

Should any of these recombinants show evidence of enhanced virus growth >1 log in cells expressing the human, bat, mouse or civet ACE2 receptor over wildtype parental backbone SARS-CoV strain or grow more efficiently in primary human airway epithelial cells (HAE), we will immediately i) stop all experiments with the mutant, ii) inform program and the UNC IBC of these results and iii) participate in decision making trees to decide appropriate paths forward

GOF concerns over in vitro passage experiments: The gain of function documents are very clear and focus on experiments that are reasonably anticipated to increase transmissibility or pathogenesis in any mammal Cells in culture are not mammals and in fact in vitro passage has long been used as a strategy to attenuate virus pathogenesis, as viruses adapt to the in vitro environment, losing the capacity to replicate efficiently and produce disease in vivo We had proposed to passage viruses in primary human airway epithelial cell cultures (HAE) or in DBT cells expressing ACE2 receptors from different mammals (human, civet, mouse, bat) Recognizing that primary human cells may be pushing the envelope and the intent of the original GOF document, we will only passage recombinant viruses in mouse astraglial cells (DBT) expressing different ACE2 receptors We believe these experiments are safe as entry is governed by programmed interactions with receptors, entry proteases and other cellular components, which clearly will be different in mouse DBT cells and primary HAE cells, the latter being the normal target for virus infection Thus, entry protease and innate immune program differences in continuous astraglial cell types (as compared to HAE) would likely select for different adaptive mutations resulting in less fit experimentally evolved virus phenotypes when measured in primary HAE and in vivo After in vitro passage, we can evaluate the replication efficiently and perform fitness competition assays of wildtype and experimentally evolved viruses in HAE and DBT cells expressing various receptors, providing definitive information on the replication kinetics and fitness in vitro

Should any of these recombinants show evidence of enhanced virus growth >1 log in cells expressing the human, bat, mouse or civet ACE2 receptor over wildtype parental backbone SARS-CoV strain or grow more

efficiently in primary human airway epithelial cells (HAE), we will immediately i) stop all experiments with the mutant, ii) inform program and the UNC IBC of these results and iii) participate in decision making trees to decide appropriate paths forward

GOF concerns over the engineered SARS-CoV RBDs with "super binding affinity" for human or civet ACE2 receptor Previously we have engineered SARS-CoV RBDs with "super binding affinity" for human mouse or civet ACE2 receptor, respectively (PMID 22291007/ PMC3308800) The study was carried out in 2012 The initial study only involved recombinant proteins and pseudoviruses No infectious SARS-CoV was involved The study was mentioned as background information for subaim 2 1 Independently, the Baric laboratory obtained IBC approval for engineering the hPREDICT and mPREDICT recombinant viruses (see appended recombinant DNA documents) in 2012-2013 Shortly thereafter, the Baric group isolated recombinant hPREDICT and mPREDICT recombinant viruses, however these viruses have not demonstrated any substantial change in disease phenotype in mice or expanded growth phenotypes in Vero cells, as compared to the parental controls Recombinant virus growth kinetics have not been evaluated in primary human airway epithelial cells nor in DBT cells, expressing the civet. human or bat ACE2 receptor For safety considerations, the Baric laboratory is willing to evaluate the growth of these recombinants in HAE cultures and inform program of the results In relationship to this grant application, these experiments are not part of the proposal, and we have no plan to revisit these recombinant viruses encoding these mutant RBDs Thus there are no GOF concerns over these mutant RBDs with respect to this proposal

Alternative plans if NIAID still has concerns over these potential gain-of-function mutations that may enhance the binding affinity of SLCoVs for host ACE2, but may not enhance viral infectivity or transmissibility (we note that loss of function is not anywhere near as powerful a determination of causality as gain of function experiments, which provide more certain determinations of sequence and structure guided function)

(i) We could replace the potential gain-of-function mutations with loss-of-function mutations Specifically, because human SARS-CoV strain likely has been adapted to use human ACE2 more efficiently than bat SLCoVs, instead of introducing residues from human SARS-CoV strains into bat SLCoVs, we will introduce residues from bat SLCoVs into human SARS-CoV strains Correspondingly, instead of generating mutant bat SLCoV strains with enhanced affinity for human ACE2, we will generate mutant human SARS-CoV strains with reduced affinity for human ACE2 The new set of mutations are the reverse of the mutations listed previously in this response We will characterize the receptor-binding affinity of mutant viruses using biochemical assays before we introduce these mutations into infectious viruses

A

```
ACE2   21          31          41
Human  IEEQAKTFLD  KFNHEAEDLF  YQSSLASWNY
Civet  TEELAKTFLE  TFNYEAQELS  YQSSVASWNY
Bat    TEDEAKMFLD  KFNTKAEDLS  HQSSLASWDY
Mouse  TEENAKTFLN  NFNQEAEDLS  YQSSLASWNY
         *  ** **  .** .*..*  :*** *** *

ACE2   71          81          321         351
Human  AFLKEQSTLA  QMYPLQEIQN  PNMTQGFWEN  LGKGDFRILM
Civet  AYYEEQSKLA  QTYPLAEIQD  PNMTQGFWEN  LGKGDFRIKM
Bat    AFYEEQSKLA  KNYSLEQIQN  PNMTEGFWNN  LGKGDFRIKM
Mouse  AFYEEQSKTA  QSFSLQEIQT  PHMTQGFWAN  LGHGDFRIKM
       *:  ***. * :   * :**  * ** *** * **:***** *
```

B

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Human (Tor02)	Y	Y	L	N	D	T
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Human/Civet (Gd03)	Y	Y	P	N	G	S
Mouse (PMID 22072787)	H	Y	L	N	D	T
Bat (rs3366)	Y	S	F	N	D	N
Bat (shc014)	Y	W	P	R	P	A
Bat (wiv1)	Y	S	F	N	D	N
Bat (LYRall) (PMID 26719272)	F	S	E	S	T	V

Modified Figure 10 Receptor and viral determinants of SLCoV/ACE2 interactions (A) Sequence comparisons of ACE2 from different animal species In red are residues that are critical to the major species barriers between hosts for SARS-CoV infections In blue are residues that directly contact SARS-CoV Asterisks indicate positions which have a single, fully conserved residue Colons indicate positions which have strongly conserved residues Periods indicate positions which have weakly conserved residues (B) Sequence comparisons of S1-CTDs from different SARS-CoV strains and bat SLCoVs The strain names are in parenthesis Shown are six residue positions that are critical for ACE2 binding

(ii) Similarly, loss-of-function mutations will also be introduced into civet SARS-CoV strains and mouse-adapted SARS-CoV strains, respectively, to generate mutant civet SARS-CoV strains and mutant mouse-adapted SARS-CoV strains with reduced affinity for civet ACE2 and mouse ACE2, respectively The new sets of mutations are the reverse of the mutations listed previously in this response We will characterize the receptor-binding affinity of mutant viruses using biochemical assays before we introduce these mutations into infectious viruses

Although we expect these new sets of mutations to cause loss-of-function of human, civet, and mouse-adapted SARS-CoV strains, we will carefully monitor the receptor usage and cell infectivity of these mutant SARS-CoV strains on cell lines constitutively expressing the civet, human, bat and mouse ACE2 receptor Should any of these recombinants show evidence of enhanced virus growth >1 log in cells expressing the human, bat, mouse or civet ACE2 receptor over wildtype parental backbone strain or grow more efficiently in primary human airway epithelial cells (HAE), we will immediately i) stop all experiments with the mutant, ii) inform program and the UNC IBC of these results and iii) participate in decision making trees to decide appropriate paths forward

(iii) We could remove the experiment involving the passage of bat SLCoVs in cell culture However, we caution against this decision as virus passage very likely reveal novel and unanticipated mutational pathways to improved receptor usage, critical information for public health planning, epidemic monitoring and human antibody immunotherapeutic treatment regimen selection

(iv) We will expand Experiment #4 by incorporating a newly published bat SLCoV strain (LYRall) (PMID 26719272 / PMC4810638), which is listed in the modified Figure 10b Again, we will only study potentially loss-of-function mutations, and carefully monitor the changes of receptor usage and cell infectivity of any mutant SARS-CoV strains The potential loss-of-function mutations that will be introduced from bat SLCoV strain LYRall to other SARS-CoV strains include F436Y, S442Y, E472L, S479N, T480D, V487T (to strain Tor02), F436Y, S442Y, E472L, S479K, T480D, V487S (to strain Sz02), F436Y, S442Y, E472P, S479N, T480G, V487S (to strain Gd03), F436H, S442Y, E472L, S479N, T480D, V487T (to mouse-adapted SARS-CoV strain)

(v) We will also expand Experiment #4 by examining the interactions between wild-type bat SLCoVs and mutant ACE2 from human, civet, mouse, and bat (similar experiments will be done in Experiment #2 using recombinant proteins and pseudoviruses) The goal is to identify receptor determinants of the cross-species transmission of bat SLCoVs using wild-type infectious viruses To this end, mutations will be introduced to ACE2 molecules from non-bat species, the mutant ACE2 molecules will be expressed in cultured cells, and then the infectivity of bat SLCoVs in these cells will be measured *Because all of the mutations will be introduced to host ACE2, not viruses, these experiments are not subject to GOF pause*

In the event that the committee decides that our GOF determinations prove unexceptablethen in the modified Experiment #4 involving infectious viruses, we will focus on loss-of-function mutations in different SARS-CoV strains, expand the scope of the experiment to incorporate a newly published bat SLCoV strain, and also introduce mutations to host ACE2 receptors

Conclusion: We can either (1) maintain the originally proposed Experiment #4 through carefully monitoring any changes in viral infectivity or (2) modify Experiment #4 to remove all potential gain-of-function mutations in infectious viruses We prefer the former In either case, the budget for Experiment 4 in subaim 2 1 can be maintained

LSP ID 20157190
Sched G ID 11361

UNIVERSITY OF NORTH CAROLINA
LABORATORY SAFETY PLAN

Principal Investigator: RALPH S BARIC **Building: Hooker Research Center**

SCHEDULE G RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES

CLASSIFICATION OF EXPERIMENTS ACCORDING TO NIH GUIDELINES:

You must provide this information to obtain IBC approval. Please consult the Classification Summary Page for NIH Guidelines to determine which section defines your experiments. Choose one category: III-D

DESCRIPTION OF RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES RESEARCH (Section II)

1. Project Title: Generating a SARS-CoV Mutant to Improve Binding Efficiency for the Human ACE2 Receptor

2. If experiment involve a deliberate attempt to obtain an expression of foreign gene, identify what proteins will be produced:
No foreign genes will be expressed

To enhance the binding of the SARS-CoV attachment protein, spike, to the cellular receptor human angiotensin converting enzyme 2 (hACE), we would like to introduce two substitutions into the receptor binding domain

3. Will recombinant or synthetic nucleic acid molecules, recombinant RNA, virus particles or other micro-organisms containing recombinant or synthetic nucleic acid molecules or RNA, or cells containing recombinant or synthetic nucleic acid molecules or RNA be introduced into whole plants or animals (including insects)? NO

4. Will recombinant or synthetic nucleic acid molecules, recombinant RNA, virus particles or other micro-organisms containing recombinant or synthetic nucleic acid molecules or RNA, or cells containing recombinant or synthetic nucleic acid molecules or RNA be introduced into humans for clinical trial(s)? NO

5. What is the source/how was the DNA obtained? (Is it synthetic-for example synthesized de novo from sequence information by a core facility or company OR was it derived/amplified from a biological source?):

6. Containment conditions specified in the NIH Guidelines:

☐ **BSL 1** ☐ **BSL 2** ☑ **BSL 3**

7. IACUC # (or web ID):

8. Recipient organisms or cells to be used (e.g. E.coli, mouse, Arabidopsis, mouse primary liver cells, strain type of animal.): E coli to propagate fragments of the infectious clone, VeroE6 cells or murine delayed brain tumor cells expressing either the human or civet cat ACE2 molecules to propagate virus, in addition, we will infect primary airway epithelial cell cultures

9. Vectors to be used and source (e.g. pCMV plasmids, E1A deleted adenovirus): all of the infectious clone fragments are cloned into the E coli vector pSMART, the SARS-CoV infectious clone backbone will be used to introduce the changes into the receptor binding domain, fragments ordered from BioBasics will be shipped in pUC57

10. Nature of the inserted sequences (payload) and original source of the DNA (e.g. E.coli galactosidase driven by CMV-IE promoter, mus creating kinase promotor driving duck myosin gene, synthetic, hormone-responsive promotor drivingrat TGFa): we will purchase the DNA insert containing the desired mutations from BioBasics, which will then be ligated in place of the wild type receptor binding domain

11. Description of the Experiments: The "hPredict" for the human ACE2 mutant virus is a SARS-CoV variant incorporating two substitutions in the receptor binding domain (RBD) of the Spike glycoprotein designed to increase the specificity of binding for humanACE2 by reducing its affinity for the civet ACE2 These two substitutions, Y442F and L472F, are predicted to remove a potential steric clash with K31 of the human ACE2 and introduce a clash with T82 of the civet ACE2 (this residue is M82 in the human ACE2)

This construction will be used to test our ability to make functional predictions about the RBD-receptor interface The hPredict virus is predicted to increase the binding affinity for hACE2 while significantly reducing the affinity for the civet ACE2 We intend to assess replication on hACE2-DBT cells, cACE2-DBT cells, and human airway epithelial cells The hPredict RBD will be generated by synthesis of a 600bp cassette by Biobasic and then incorporated into the standard reverse genetics clone of the Urbani strain of SARS-CoV

12. Do these experiments use recombinant or synthetic nucleic acid molecules or RNA that encode microbial toxins? NO

13. Do these experiments involve more than 10 liters of culture at one time? NO

14. Do these experiments involve release of genetically modified organisms to the environment? NO

Life sciences research is essential to scientific advances that underpin improvements in public health and safety, agricultural crops and other plants, animals, the environment, materiel, and national security Despite its value and benefits, some research may provide knowledge, information, products, or technologies that could be misused for harmful purposes The updated Dual Use Policy, including the list of regulated agents and toxins can be found at
http //oba od nih gov/oba/biosecurity/pdf/united_states_government_policy_for_oversight_of_durc_final_version_032812 pdf

Research is not necessarily prohibited, however a risk analysis must be made to determine its Dual Use potential

LSP ID 20157190
Sched G ID 11361

15. Is the material a select agent/toxin or is it derived from a select agent or toxin? NO

16. Could the experiments proposed in this Schedule G change the select agent/toxin status of the material? NO

17. Do these experiments raise dual concern? NO

Categories of experiments that are regulated are those that
a) Enhance the harmful consequences of the agent or toxin,
b) Disrupt immunity or the effectiveness of an immunization against the agent or toxin without clinical or agricultural justification,
c) Confer to the agent or toxin resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies,
d) Increase the stability, transmissibility, or the ability to disseminate the agent or toxin,
e) Alter the host range or tropism of the agent or toxin,
f) Enhance the susceptibility of a host population to the agent or toxin, or
g) Generate or reconstitute an eradicated or extinct agent or toxin listed in the link above

18. Do these experiments meet the definition of a Major Action?
"The deliberate transfer of a drug resistance trait to a microorganism, when such a transfer could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture, is a Major Action (see Section III-A-1-a of the NIH Guidelines). Because of the potential implications for public health, animals or agriculture such experiments warrant special review."NO

On behalf of the institution, the Principal Investigator is responsible for complying fully with UNC policies and NIH Guidelines in conducting any recombinant or synthetic nucleic acid molecules research.
Agreed and **Signed By:** AMY C SIMS

This registration was approved by IBC Chair, _____ on 12/06/2011

LSP ID 20157190
Sched G ID 11374

UNIVERSITY OF NORTH CAROLINA
LABORATORY SAFETY PLAN

Principal Investigator: RALPH S BARIC **Building: Hooker Research Center**

SCHEDULE G RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES

CLASSIFICATION OF EXPERIMENTS ACCORDING TO NIH GUIDELINES:

You must provide this information to obtain IBC approval. Please consult the Classification Summary Page for NIH Guidelines to determine which section defines your experiments. Choose one category: III-D

DESCRIPTION OF RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES RESEARCH (Section II)

1. Project Title: Generating a SARS-CoV Mutant to Improve Binding Efficiency for the Mouse ACE2 Receptor

2. If experiment involve a deliberate attempt to obtain an expression of foreign gene, identify what proteins will be produced:

No foreign genes will be expressed

To enhance binding of the SARS-CoV attachment protein, spike to the cellular receptor murine angtiotensin converting enzyme 2, we would like to combine the mutations in the receptor binding domain (RBD) obtained from three viral passage/adaptation experiments SARS-CoV was intranasally inoculated into mice, allowed to replicate, the nares and lungs harvested and used to inoculate subsequent sets of mice Three separate point mutations in the spike RBD were obtained and we would like to engineer them into a single mutant construct

3. Will recombinant or synthetic nucleic acid molecules, recombinant RNA, virus particles or other micro-organisms containing recombinant or synthetic nucleic acid molecules or RNA, or cells containing recombinant or synthetic nucleic acid molecules or RNA be introduced into whole plants or animals (including insects)? YES

4. Will recombinant or synthetic nucleic acid molecules, recombinant RNA, virus particles or other micro-organisms containing recombinant or synthetic nucleic acid molecules or RNA, or cells containing recombinant or synthetic nucleic acid molecules or RNA be introduced into humans for clinical trial(s)? NO

5. What is the source/how was the DNA obtained? (Is it synthetic-for example synthesized de novo from sequence information by a core facility or company OR was it derived/amplified from a biological source?):

6. Containment conditions specified in the NIH Guidelines:

☐ **BSL 1** ☐ **BSL 2** ☑ **BSL 3**

7. IACUC # (or web ID):

8. Recipient organisms or cells to be used (e.g. E.coli, mouse, Arabidopsis, mouse primary liver cells, strain type of animal.):
E coli to propagate the pieces of the infectious clone, VeroE6 or murine delayed brain tumor cells expressing mouse ACE2 (mACE2-DBT), cells to propagate the virus, and mice

9. Vectors to be used and source (e.g. pCMV plasmids, E1A deleted adenovirus): all of the fragments in our infectious clone are in pSMART, the SARS-CoV infectious clone backbone will be used to introduce the changes into the RBD, fragments ordered from BioBasics will be shipped in pUC57

10. Nature of the inserted sequences (payload) and original source of the DNA (e.g. E.coli galactosidase driven by CMV-IE promoter, mus creating kinase promotor driving duck myosin gene, synthetic, hormone-responsive promotor drivingrat TGFa): we will purchase a DNA fragment from BioBasics containing the three desired point mutations which will then be ligated in place of the wild type receptor binding domain

11. Description of the Experiments: The mPredict virus is a SARS-CoV variant incorporating three substitutions in the receptor binding domain of the SARS Spike glycoprotein These three substitutions are designed to improve the efficiency of murine-ACE2 binding

Of the three independently derived mouse-adapted SARS-CoVs, each contain substitutions in the receptor binding domain (RBD) of the spike glycoprotein The substitutions are not identical, but there are conserved sites between the three mouse-adapted viruses Combining this sequence information with crystal structures and homology models of the SARS-RBD bound to the ACE2 receptor, we predict that a RBD combining three substitutions (Y442L, N479K, Y436H) will provide enhanced binding to the murine ACE2 receptor beyond that of the individual mouse-adapted viruses We predict that this enhanced binding will increase pathogenesis in the mouse model independent of mouse-adapted substitutions in viral proteins other than Spike

These three substitutions in spike will be generated by synthesis of a 1200bp cassette by Biobasic and then incorporated into the standard reverse genetics clone of the Urbani strain of SARS-CoV In addition, the cassette/substitutions will be combined with 1-5 additional mouse-adapted substitutions from the MA15 virus

Assessment of the mPredict virus will include both in vitro studies (i e growth curves on ACE2-limited DBT cell lines) and pathogenesis studies in mice Initial characterization will compare the mPredict virus to the MA15 and Urbani strains, including measures of mortality, weight loss, lung titer, and histology Future studies may assess the effectiveness of neutralizing antibodies against mPredict, both in vivo and in vitro, as we expect this virus to more effectively bind the murine ACE2 receptor, potentially altering neutralization profiles

12. Do these experiments use recombinant or synthetic nucleic acid molecules or RNA that encode microbial toxins? NO

LSP ID 20157190
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13. Do these experiments involve more than 10 liters of culture at one time? NO

14. Do these experiments involve release of genetically modified organisms to the environment? NO

Life sciences research is essential to scientific advances that underpin improvements in public health and safety, agricultural crops and other plants, animals, the environment, materiel, and national security Despite its value and benefits, some research may provide knowledge, information, products, or technologies that could be misused for harmful purposes The updated Dual Use Policy, including the list of regulated agents and toxins can be found at
http //oba od nih gov/oba/biosecurity/pdf/united_states_government_policy_for_oversight_of_durc_final_version_032812 pdf

Research is not necessarily prohibited, however a risk analysis must be made to determine its Dual Use potential

15. Is the material a select agent/toxin or is it derived from a select agent or toxin? NO

16. Could the experiments proposed in this Schedule G change the select agent/toxin status of the material? NO

17. Do these experiments raise dual concern? NO

Categories of experiments that are regulated are those that
a) Enhance the harmful consequences of the agent or toxin.
b) Disrupt immunity or the effectiveness of an immunization against the agent or toxin without clinical or agricultural justification,
c) Confer to the agent or toxin resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies,
d) Increase the stability, transmissibility. or the ability to disseminate the agent or toxin,
e) Alter the host range or tropism of the agent or toxin,
f) Enhance the susceptibility of a host population to the agent or toxin, or
g) Generate or reconstitute an eradicated or extinct agent or toxin listed in the link above

18. Do these experiments meet the definition of a Major Action?
"The deliberate transfer of a drug resistance trait to a microorganism, when such a transfer could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture, is a Major Action (see Section III-A-1-a of the NIH Guidelines). Because of the potential implications for public health, animals or agriculture such experiments warrant special review."NO

GENE TRANSFER EXPERIMENTS INVOLVING WHOLE ANIMALS OR PLANTS (Section III)

1. Provide a description of the vector. (Provide relevant literature citations, if available, or a preprint.): the SARS-CoV molecular clone published in 2003 Yount et al PNAS 100(22) 12995-13000

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2. Provide a physical map of the vector: 20101706-704220087-20111024-570-45 pdf

3. Who constructed this vector? (Name, institution): Boyd Yount, Jr Damon Deming and Meagan Bolles at the University of North Carolina at Chapel Hill

4. List specific DNA or RNA segments from any human, veterinary, or plant pathogen included in the recombinant or synthetic nucleic acid molecules (include regulatory elements): SARS-CoV

5. How was the vector produced or packaged: Individual clones will be manipulated at BL2 but full length assembly and in vitro transcription will be performed in the BL3 facility In vitro transcripts will be electroporated in Vero cells and viruses isolated by plaque purification or blind serial passage

6. If a viral packaging or expression system is used, have experiments been done to show that replication competent viruses are excluded?: No

7. Which lab (Principal Investigator, Institution, Company, etc.) produced the DNA to be used in these experiments: Ralph Baric, University of North Carolina at Chapel Hill

8. Will attempts be made to insert recombinant or synthetic nucleic acid molecules into germ lines in order to establish a trangenic animal or plant line: No

On behalf of the institution, the Principal Investigator is responsible for complying fully with UNC policies and NIH Guidelines in conducting any recombinant or synthetic nucleic acid molecules research.

Agreed and **Signed By:** AMY C SIMS

This registration was approved by IBC Chair, _____ on 12/06/2011

U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses

October 17, 2014

U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses

Gain-of-function studies, or research that improves the ability of a pathogen to cause disease, help define the fundamental nature of human-pathogen interactions, thereby enabling assessment of the pandemic potential of emerging infectious agents, informing public health and preparedness efforts, and furthering medical countermeasure development Gain-of-function studies may entail biosafety and biosecurity risks; therefore, the risks and benefits of gain-of-function research must be evaluated, both in the context of recent U S biosafety incidents and to keep pace with new technological developments, in order to determine which types of studies should go forward and under what conditions

In light of recent concerns regarding biosafety and biosecurity, effective immediately, the U.S Government (USG) will pause new USG funding for gain-of-function research on influenza, MERS or SARS viruses, as defined below. This research funding pause will be effective until a robust and broad deliberative process is completed that results in the adoption of a new USG gain-of-function research policy[1] Restrictions on new funding will apply as follows

> *New USG funding will not be released for gain-of-function research projects that may be reasonably anticipated to confer attributes to influenza, MERS, or SARS viruses such that the virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route The research funding pause would not apply to characterization or testing of naturally occurring influenza, MERS, and SARS viruses, unless the tests are reasonably anticipated to increase transmissibility and/or pathogenicity*

In parallel, we will encourage the currently-funded USG and non-USG funded research community to join in adopting a voluntary pause on research that meets the stated definition.

The deliberative process that will ensue during the period of the research pause will explicitly evaluate the risks and potential benefits of gain-of-function research with potential pandemic pathogens The presumptive benefits that are generally identified in pursuing this type of research are stated in terms of enhanced ability for earlier awareness of naturally emerging dangerous pandemic pathogens or in the development of medical products in anticipation of such emergence

However the relative merits of gain-of-function experimental approaches must be compared ultimately to potentially safer approaches The deliberative process will offer recommendations for risk mitigation, potential courses of action in light of this assessment, and propose methodologies for the objective and rigorous assessment of risks and potential benefits that might be applied to the approval and conduct of individual experiments or classes of experiments Although the gain-of-function studies that fall within the scope of research subject to the funding pause will be a starting point for deliberations, the suitability of other types of gain-of-function studies will be discussed. It is feasible that the discussion could lead to suggestions of broadening the funding pause to include research with additional pathogens,

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[1] An exception from the research pause may be obtained if the head of the USG funding agency determines that the research is urgently necessary to protect the public health or national security

however, federal Departments and Agencies who fund, support, or perform research should be consulted prior to any additional pathogens being added to the scope of the funding pause

The deliberative process is envisioned to be time-limited, to involve two distinct, but collaborating, entities, and to be structured to enable robust engagement with the life sciences community As a first step, the National Science Advisory Board for Biosecurity (NSABB) will be asked to conduct the deliberative process described above and to draft a set of resulting recommendations for gain-of-function research that will be reviewed by the broader life sciences community. The NSABB will serve as the official federal advisory body for providing advice on oversight of this area of dual use research, in keeping with federal rules and regulations

As a second step, coincident with NSABB recommendations, the National Research Council (NRC) of the National Academies then will be asked to convene a scientific conference focused on the issues associated with gain-of-function research and will include the review and discussion of the NSABB draft recommendations This NRC conference will provide a mechanism both to engage the life sciences community as well as solicit feedback on optimal approaches to ensure effective federal oversight of gain-of-function research The life sciences community will be encouraged to provide input through both the NRC and NSABB deliberative processes

The NSABB, informed by NRC feedback, will deliver recommendations to the Secretary of Health and Human Services, the Director of the National Institutes of Health, and the heads of all federal entities that conduct, support, or have an interest in life sciences research (including the Assistants to the President for Homeland Security and Counterterrorism and for Science and Technology) The final NSABB recommendations and the outcomes of the NRC conference will inform the development and adoption of a new U S. Government policy governing the funding and conduct of gain-of-function research Upon adoption of a federal gain-of-function policy, the U S Government will declare the end of the research funding pause

The life sciences community will be informed of progress at regular intervals The estimated time-line is six months for completion of the two deliberative steps (culminating in delivery of the NSABB recommendations to the HHS Secretary) and three months for the development, approval, and publication of the policy, with the goal of completing the entire process in less than one year from declaration of the research funding pause

Funding Pause for Certain Types of Gain-of-Function Research Projects

Notice Number:

NOT-OD-15-011

Key Dates

Release Date October 17, 2014

Related Announcements

None

Issued by

National Institutes of Health (NIH)

Purpose

The purpose of this Guide Notice is to notify applicants that in accordance with the October 17 White House announcement, the National Institutes of Health is instituting a funding pause on the provision of new funding for certain gain-of-function research projects, Federal-wide, so that a deliberative process can be undertaken to assess the potential benefits and risks associated with these types of studies

Background

The recent series of laboratory biosafety incidents at U S Government research facilities has caused the federal government to re-assess the risk/benefit calculus underpinning funding decisions for a certain subset of gain-of-function research involving agents that pose a significant risk to public health

On October 17, 2014, the U S Government announced that it would be instituting a funding pause on gain-of-function research projects that reasonably may be anticipated to confer attributes to influenza, MERS, or SARS viruses such that the resulting virus has enhanced pathogenicity and/or transmissibility (via the respiratory route) in mammals In addition to the funding pause, the U S Government will undertake a deliberative process to assess the potential benefits and risks associated with these types of studies The results of the deliberative process will assist the U S Government in developing a framework that will guide future funding decisions

The funding pause does not apply to characterization or testing of naturally occurring influenza, MERS, and SARS viruses, unless these tests reasonably are anticipated to increase transmissibility or pathogenicity An exception to the funding pause may also be granted by the head of a federal funding agency if that official determines that the research is urgently necessary to protect public health or national security Lastly, the U S Government encourages the currently-funded research community to join in adopting a voluntary pause as well

Impact

NIH will continue to accept new applications for research projects involving gain-of-function studies, including those that may be reasonably anticipated to confer attributes to influenza, MERS, or SARS viruses such that the resulting virus has enhanced pathogenicity and/or transmissibility (via the respiratory route) in mammals However, as described in the background section of this Guide Notice, NIH will not provide any new or continuation funding for research subject to the pause while the funding pause is in effect In cases where reviewers identify possible gain-of-function research as described above, this will be recorded as an administrative note, but the presence of such research in the application will not affect the impact scores Program officials will then review the proposed research to determine if the research meets the above criteria, and work with the grantee and grants management officials, as appropriate, to determine how best to proceed, e g , if the proposed research may be redirected to experiments that are not covered by the pause, etc Ongoing NIH awards will be addressed with the grantees on a case-by-case basis

Inquiries

Please direct all inquiries to the Program Official that is listed in the Notice of Award under the Contacts Section

Weekly TOC for this Announcement
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UNDER THE POLISCOPE

Bringing Science Policy Into Focus

The Gain-of-Function Deliberative Process

September 25, 2015

In biomedical research, scientists commonly perform experiments that involve enhancing or diminishing the function of a gene, which may change the observable characteristics of a model organism Such gain- and loss-of-function studies are a cornerstone of many fields of experimental biology and are routinely performed to help understand genetic pathways, infer the function of genes and proteins, and more

Key to our understanding of what causes infectious disease, whether it's influenza, HIV/AIDS, or the common cold, is figuring out the characteristics of what makes microorganisms cause disease When applied to the study of infectious diseases, loss- and gain-of-function experiments can help identify determinants of virulence, pathogenesis, or other pathogen (disease-causing agents) characteristics These types of experiments aid in the discovery of potential targets or strategies for the development of vaccines or other medical countermeasures or inform disease surveillance efforts

In recent years, there has been a lot of attention paid to certain types of gain-of-function studies resulting in pathogens with enhanced pathogenicity (ability to cause disease) or transmissibility (ability to be passed from one individual to another), especially in mammals These are what the popular press sometimes calls "superbugs," and concerns have been raised about whether they might be intentionally or accidentally released Gain-of-function studies also present "dual use" concerns—that is, although the experiments are performed to provide insight into disease or to develop treatments, they may also generate research products or information that could be misused to harm public health or national security These biosafety and

biosecurity issues are particularly acute with studies involving viruses, like influenza, that have a history of causing pandemics in humans Indeed, some people have argued that certain gain-of-function studies should not be undertaken at all

The U S government (USG) launched a deliberative process in October 2014 to re-evaluate the potential risks and benefits associated with gain-of-function research involving certain pathogens with the potential to cause a pandemic This process involves both the National Science Advisory Board for Biosecurity (NSABB), which will develop formal recommendations to the USG, and the National Academies, which in December 2014 hosted the first of two meetings to foster broad stakeholder discussions In addition, a risk-benefit assessment is being conducted by a private company, Gryphon Scientific, to inform the deliberations During this process, the USG has paused the release of new funding for gain-of-function studies that could enhance the pathogenicity or transmissibility of influenza, Middle East Respiratory Syndrome (MERS), or Severe Acute Respiratory Syndrome (SARS) viruses

The initial task of the NSABB was to advise on the design and conduct of the risk-benefit assessment In May, the Board approved its *Framework for Guiding Risk and Benefit Assessments of Gain-of-Function Studies* This framework outlines principles that should guide the risk-benefit assessment, describes the types of risks and benefits that should be analyzed, and makes recommendations on the types of pathogens, pathogen characteristics, and types of gain-of-function studies that should be analyzed The National Institutes of Health (NIH) and Gryphon Scientific are using this framework to guide the analysis of the risks and benefits

The NSABB's second task is to develop recommendations to the USG on a conceptual framework for evaluating research proposals involving gain-of-function studies that raise concerns The Board will convene on September 28th at the NIH campus in Bethesda, Maryland to discuss its progress and continue its deliberations This meeting will also include an update on the risk-benefit assessment and a panel discussion of the ethical, legal, and policy issues associated with the gain-of-function issue The meeting is open to the public and will also be webcast live Additional information, including links to the pre-registration website and meeting agenda, can be found on the NSABB meeting webpage

The results of the risk-benefit assessment are anticipated this fall and the Board's draft recommendations are anticipated in early 2016 These draft recommendations will then be discussed at a second National Academies meeting next spring before being finalized The USG will consider the Board's recommendations as it develops policy for the funding and oversight of gain-of-function studies and expects to revisit the funding pause at that time as well

Public input is key to this process NSABB meetings, as well as those hosted by the National Academies, are free and open to the public Public participation is encouraged and all NSABB meetings include time for

members of the public to provide comments Written comments can be submitted to the Board at any time at NSABB@od nih gov

The debate about gain-of-function studies involving pathogens with pandemic potential are related to broader ongoing discussions about laboratory safety and security However, even more broadly, the gain-of-function debate is about public trust in the scientific enterprise The life sciences are rapidly evolving and we have the ability to manipulate biological systems in ways that were not possible, even 10 years ago New discoveries and emerging technologies hold immense promise but they will also continue to test our policy frameworks It is an exciting time, but also a sobering one for those in science policy It is our job to help ensure that science can advance rapidly as well as safely, ethically, and responsibly

Tags Office of Clinical Research and Bioethics Policy, Office of Biotechnology Activities, Office of Science Management and Reporting | Leave a comment
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UNDER THE POLISCOPE

Bringing Science Policy Into Focus



Carrie D. Wolinetz, Ph.D.
Associate Director for Science Policy, NIH

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Subject: Grant Number: 2 R01 AI 089728 - 06, Li (PI)
From: "Bumbray-Quarles, Devon (NIH/NIAID) [E]" <DBumbray@niaid nih.gov>
Date: 5/18/2016 6:10 AM
To: 'Kevin McKoskey' <kevin@umn.edu>
CC: "'lifang@umn.edu'" <lifang@umn.edu>, "awards@umn.edu" <awards@umn.edu>, "Kirker, Mary (NIH/NIAID) [E]" <MKIRKER@niaid.nih.gov>, "Glowinski, Irene (NIH/NIAID) [E]" <IGlowinski@niaid.nih.gov>, "Ford, Andrew (NIH/NIAID) [E]" <andrew.ford@nih.gov>, "Stemmy, Erik (NIH/NIAID) [E]" <erik.stemmy@nih.gov>, "'jond0006@umn.edu'" <jond0006@umn.edu>, "Baric, Ralph" <rbaric@email.unc.edu>, "Bumbray-Quarles, Devon (NIH/NIAID) [E]" <DBumbray@niaid.nih.gov>

Dear Mr McKoskey,

Please find attached NIAID's response for Gain of Function (GoF) on the above subject grant

If you have any questions or concerns, please do not hesitate to ask

Thank you

Sincerely,
Ms. Devon Bumbray-Quarles
Grants Management Specialist
Grants Management Program
DHHS, NIH, NIAID, GMP
5601 Fishers Lane, Room 4E28, MSC 9824
Bethesda, MD 20892-9824
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National Institute of Allergy and Infectious Diseases

"Effective October 1, 2014, NIH closeout policy has changed (see NOT-OD-14-084) In order to avoid unilateral closeout, final reports must be submitted in a timely manner Failure to submit accurate final reports could result in enforcement actions such as revisions to NOA funding levels, or delay in future funding "

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Attachments

NIAID Response to GoF- 2R01AI089728-06.pdf	82.6 KB

Erin Knudsen <jond0006@umn.edu>

Fwd: Grant Number: 2R01AI089728 - 06 PI Name: Li, Fang

Erin Knudsen <jond0006@umn edu> Wed, Apr 27, 2016 at 9 21 AM
To dbumbray@niaid nih gov
Cc Fang Li <lifang@umn edu>, Carrie DeJarlais <dejar001@umn edu>, Lynn Weber <weber117@umn edu>

Ms Devon Bumbray-Quarles,

Attached is Fang Li's response to your request for additional information on the above referenced grant

Please contact Fang Li with programmatic matters and myself, at 612-626-2724 or jond0006@umn edu if you have administrative questions Thank you for your support of the University of Minnesota

Regards,

Erin Knudsen
Grants & Contracts Administrator
Sponsored Project Administration
University of Minnesota
McNamara Alumni Center
200 Oak Street SE, Suite 450
Minneapolis, MN 55455

Phone 612 626-2724
Fax 612 624 4843
Email jond0006@umn edu

[Quoted text hidden]

Fang Li 2R01AI089728_06.pdf
428K

Response to Summary Statement – 2R01AI 089728–06, Li (PI)/Baric (co-I)

On April 20, 2016, NIAID informed us that our grant application 2R01AI089728–06 needs to address two concerns from the summary statement (1) **BIOHAZARD COMMENT:** There are concerns that recombinant coronaviruses altered to enhance proteolytic cleavage or binding of the human angiotensin converting enzyme 2 (ACE 2) receptor may have novel and unexpected virulence phenotypes Therefore, biosafety level-3 (BSL-3) protections, training, and monitoring procedures should be considered unless otherwise indicated, (2) **BUDGETARY OVERLAP:** There is potential for budgetary overlap with the project R01AI110700 titled, "Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis" awarded to PI Ralph Baric (co-investigator on this proposal) Our response is as following

Response to BIOHAZARD COMMENT:

Biosafety level-3 (BSL-3) protections, training, and monitoring procedures will be used for any work related to SARS-CoV and bat SARS-like CoVs (SEE SECTION 11, APPENDED BELOW)

Response to BUDGETARY OVERLAP:

The project R01AI110700 titled "Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis" studies MERS-CoV and bat MERS-like CoVs such as HKU4 and HKU5 The current grant application 2R01AI089728–06 titled "Receptor recognition and cell entry of coronaviruses" proposes to study all of the other coronaviruses except MERS-CoV and bat MERS-like CoVs Therefore, there is no budgetary overlap between these two projects

****

SECTION 11. Select Agent Research/Biohazards/Dual Use. SARS-CoV is a select agent, requires appropriate BSL3 facilities and UNC Chapel Hill has a license to work with this select agent pathogen Bat SARS-like Coronaviruses and civet SARS-like coronaviruses are not designated as select agents We recover and culture all SARS like viruses (Bt-SLCoV and Civet-SLCoV) under BSL3 conditions We are profoundly aware of the necessity for meeting the most rigorous standards for safe use of the viruses described in this proposal Dr Baric has certified and approved BSL3 laboratories dedicated for SARS-CoV research, which has been in use since 2003 Drs Baric has directed his own BSL3 laboratories for the past 12 years, primarily studying the SARS-CoV Detailed protocols for safe use of SARS-CoV, MERS-CoV and related BtSLCoV and viral derivitives, have been presented to and approved by the Institutional Biosafety Committee at UNC Detailed protocols were written, designed, and approved with the goal of ensuring the safety of the investigators and the general public, while allowing invaluable research to proceed If any BtSLCoV become select agents, we will register and abide by federal regulations, noting that our facilities are select agent certified to work with these agents In response to GOF requirements, we have responded to all noted expeirments of concerns in a timely fashion and provided these documents to NIH for review We have also provided alternative approaches, should certain experiments be deemed unexceptable During the course of this program, should experimental findings dictate the development of new recombinant viruses, we will notify the NIH of potential GOF concerns, while applying for approval from both the NIH and the local IBC

We recognize that experiments are proposed that might subtly alter the host range of the Bt SLCoV (WIV1, SHC014, etc) and discussed potential GOF concerns with the NIH and local IBC We have generated a molecular clone to SARS-CoV, WIV1 and SHC014 and isolated recombinant viruses Other emerging coronaviruses in the facilities are MERS-CoV and some other BtSARS-like coronaviruses HKU5-S and HKU3-BSRBD

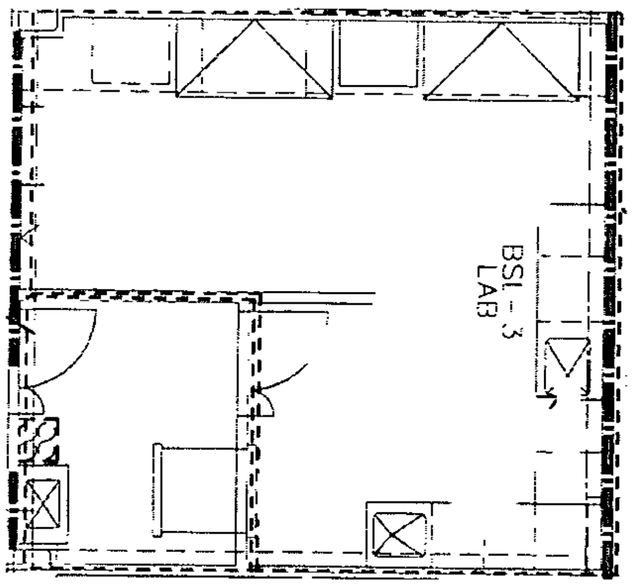
Biosafety:

- All work will be performed with approved standard operating procedures (SOPs) and safety conditions for wildtype and recombinant SARS-CoV, SHC014, WIV1, other BtSLCoV and derivative viruses For recovery

of SARS-CoV and SARS like CoV recombinant viruses, all transcription, electroporation and analysis will be performed at BSL3 as previously approved by UNC's institutional IBC

- Any derived viruses will be stored in -80 in the BSL3, as with any BSL3 recombinant virus isolate

Fig 12. MERS-CoV BSL3 Facility.



- Analysis will be by plaque assay, $TCID_{50}$, single cycle growth assays, protein detection by immunoblot and immunofluorescence or by RNA abundance using real time RT-PCR
- Biological samples will be inactivated prior to removal and shipment to Dr Li, using validated and approved SOPs Protocols have been approved for destroying full length viral genomes for SARS-CoV
- Any wildtype or recombinant BSL3 viruses will be shipped using FAA-approved protocols and permits from the CDC All laboratories have designated "shipping" trained personnel
- Some SARS-CoV variants required passage in cell culture to enhance adaptation to various civet, bat and/or human ACE2 receptors As in vitro passage oftentimes attenuates virus pathogenesis for the original host, we anticipate that these viruses are less pathogenic in humans, although direct evidence is lacking We have requested GOF approval for these experiments
- All experimental approaches, handling, and analysis will be based on the presumption that any recombinant or chimeric viruses will retain the potential for human infection and have the same safety risks as the parental strains SARS-CoV and btSLCoV isolates will be studied under BSL3
- Specifically, the transcription of genome RNA, electroporation of genome RNA into cells, infection of cells, analysis of virus growth, as well as all storage, will performed at BSL3 with full protections as described in approved SOPs
- Human antibodies are now available that neutralize SARS-CoV, and IFN cocktails may reduce virus growth significantly in some culture conditions Nucleoside analogues from Gildead Scientific have robust in vitro and in vivo activity against SARS and these SARS like CoV (Baric unpublished, manuscript in preparation)

Biosafety and Biocontainment. Baric Lab

Agent Information

Agent (Category C) Biosafety Level Diseases, Symptoms Caused by Agent

SARS-CoV-ARDS and fatal respiratory infection in humans BSL3

SHC014, WIV1 and other BtSLCoV- These viruses replicate in various primary human cells No infections have ever been reported in humans so their pathogenic potential in humans is unknown Recombinant viruses harboring mutations that enhance or attenuate ACE2 usage in various species may alter pathogenic outcomes, ranging from attenuation to potentially increased growth in primary human cells Should any mutants replicate 10 fold more efficiently in primary human airway epithelial cultures, we will halt experiments, notify our local IBC and NIH program officers

BSL3 SARS-CoV BSL3 Facility The Baric Labs BSL3 facilities have been designed to conform to the safety requirements recommended in Biosafety in Microbiological and Biomedical Laboratories (BMBL), U S Department of Health and Human Services, Public Health Service, CDC and National Institutes of Health Laboratory safety plans have been submitted and the facility has been approved for use by UNC Department of Environmental Health and Safety (EHS) Electronic card access will be required for entering into the facility All workers will be trained by EHS to safely use PAPR respirators, and appropriate work habits in a BSL3 facility and active medical surveillance plans are in place The Baric Hooker BSL3 facility (**Figure 12**) has been operational since 2004, contains redundant fans, emergency power to fans, biological safety cabinets and freezers, and accommodates two double-sided SealSafe mouse racks housing ~196 cages The Baric laboratory also has a second BSL3 suite in McGavran-Greenberg Research Hall, directly adjacent to the main BSL2 laboratory, which is about 800 sq feet, is supported by redundant fans, contains two biological safety cabinet and 2 Seal Safe Mouse Racks (~100 cages) (facility not shown) This facility is also registered as a Select Agent Facility for SARS-CoV research

BSL3 Materials Materials classified as BSL3 will consist of the wildtype and recombinant SARS-CoV, Civet and BtSLCoV (WIV1, SHC014, etc) and derivative viruses Within the BSL3 facility, experimentation with infectious virus, or with SARS-CoV RNA genomes over 2/3 length will be carried out in a certified Class II BSC All staff wear portal air breathing apparatus, tyvek suits and apron, booties, and hands are double gloved

Medical Monitoring and Exposure Management Plan
Laboratory Personnel Working with SARS-CoV and related BtSLCoV (Designed by discussion with the Baric laboratory, Department of Environmental Health and Safety(EHS), UEOHC (University Employee Occupational Health Clinic), UNC Division of Infectious Disease, UNC Division of Hospital Epidemiology) This monitoring plan is identical for SARS-CoV, MERS-CoV and BtSLCoV

General
All workers with potential exposure to SARS-CoV and BtSLCoV will be issued a thermometer and 2 surgical masks to keep at home IAV antivirals will be provided to research staff as well, noting that antivirals aren't available for the emerging coronaviruses They will be responsible to keep these items in working condition and in an accessible location The laboratory will maintain a supply of these items in the event that they are lost or damaged Serum samples from laboratory personnel who work in the BSL3 lab will be collected and analyzed on timely intervals to determine if subtle exposures resulting in sub-clinical infections have occurred Samples will be taken at the UEOHC and analyzed at an appropriate laboratory (UNC Hospitals, NC State Public Health Laboratories, or CDC laboratories)

Exposure Event
Definition: 1) animal bite, cut, or needle stick with a contaminated sharp, 2) mucous membrane of eyes, nose, or mouth exposure resulting from splash or contact with contaminated hands, 3) aerosol generating activity or event coinciding with failure primary containment such as of biological safety cabinet, PPE, and/or work practices
Procedure: Dr Ralph Baric and UEOHC are to be notified Worker confirms to UEOHC that they have surgical masks, antivirals and thermometer accessible at home or are provided replacements by before leaving work after appropriate decontamination procedures Worker will remain out of work for at least 10 days and is advised to minimize contacts outside of household Worker monitors temperature and symptoms Worker calls UEOHC daily to report on temperature and symptoms UEOHC asks standard question list for symptoms and advises worker regarding self-quarantine or need for medical evaluation
No Symptoms: If no temperature elevation above 100 degrees F or no symptoms consistent with SARS-CoV and BtSLCoV, which appear by day 10 after exposure, UEOHC will arrange to see employee to give final clearance to return to work without further monitoring Worker will be given specific appointment time and is to report directly to the designated location for the evaluation wearing a surgical mask This encounter may take place during normal working hours and in a place deemed to be appropriate for the circumstances by UEOHC This may be UEOHC itself or a negative pressure room in the Pulmonary Clinic of the ACC
Symptoms: If temperature elevates above 100 degrees F or if symptoms are consistent with SARS, BtSLCoV or MERS-CoV infection appear by day 10 after exposure, worker will be advised to self-quarantine (i e to minimize contacts) and to take precautions to minimize droplet generation including covering nose and mouth when coughing or sneezing UEOHC will arrange to have patient seen after hours in an appropriate location for clinical evaluation The site will likely be the ID clinic since the evaluation may need to include a physical examination, chest radiograph, and blood sampling and SARS/MERS-CoV laboratory test Depending on availability and clinical circumstances the evaluating MD may be the Medical Director of UEOHC, the attending from the ID consultation service or the attending for the General Medical Service on call for UEOHC after hours Disposition will depend on the outcome of the evaluation

Potential Exposure Event
Definition: A release of virus outside of primary containment with PPE intact Examples include 1) a spill in the open laboratory, 2) failure of biological safety cabinet during work with virus, 3) splash outside of a biological safety cabinet, 4) leakage of contaminated research material through gloves or other PPE, or 5) failure of secondary containment, e g negative pressure in lab, at the same time as a spill outside of the biological safety cabinet
Procedure: Dr Ralph Baric and UEOHC are to be notified Worker confirms to UEOHC they have surgical masks, and thermometer accessible at home or gets replacements at UEOHC before leaving work after appropriate decontamination procedures in laboratory Worker will monitor temperature and symptoms for 10 days Worker will communicate with UEOHC daily before work to report on temperature and symptoms UEOHC will ask a standard question list for symptoms and will advise worker whether they may report to work directly or should remain home
No Symptoms: If no temperature elevation above 100 degrees F or no symptoms consistent with SARS/MERS-CoV appears by day 10 after exposure, UEOHC will arrange to see employee to give final clearance to return to work without further monitoring Worker will be given specific appointment time and is to

report directly to the place for the evaluation wearing a surgical mask This encounter may take place during normal working hours and in a place deemed to be appropriate for the circumstances by UEOHC This may be UEOHC itself or a negative pressure room in the Pulmonary Clinic of the ACC

Symptoms: If temperature elevation above 100 degrees F or symptoms appears within days 10 after exposure, employee will remain away from work for a minimum of 72 hours after developing fever or symptoms They will be advised to self-quarantine (i e minimize contacts) and to take precautions to minimize droplet generation including covering nose and mouth when coughing or sneezing Worker will communicate with UEOHC daily to report temperature and symptoms

If temperature and symptoms are gone within 72 hours of appearance, UEOHC will arrange to see worker in clinic to give final approval to return to work Employee will be given specific appointment time and is to report directly to the place for the evaluation wearing a surgical mask This encounter may take place during normal working hours and in a place deemed to be appropriate for the circumstances by UEOHC This may be UEOHC itself or a negative pressure room in the Pulmonary Clinic of the ACC

If symptoms and /or fever are not gone after 72 hours, employee will continue to monitor himself/herself and report results to UEOHC by telephone Employee will be advised when/if arrangements for clinical evaluation (same as last step for high risk exposure management above) will be needed

Employee Symptoms with No Recognized Exposure

Definition: Employee develops respiratory symptoms or fever, but there has been no recognized exposure or laboratory accident involving breach of PPE or inadequate work practices, but employee develops respiratory symptoms or fever These procedures do not apply to personnel who have not worked in the SARS/MERS-CoV/BtSLCoV laboratory for 10 days or more prior to onset of symptoms They should, however, report their symptoms to the principal investigator and the UEOHC

Procedure: Employee notifies PI and UEOHC and confirms they have thermometer, and surgical mask available The Baric lab will make arrangements to replace these items if symptoms or fever are noted while employee is not at work If employee is at work, they will go home and take the required items with them from a supply maintained at the laboratory

Symptoms: Employee will remain away from work for a minimum of 72 hours after developing fever or symptoms Employee will be advised to self-quarantine (i e minimize contacts) and take precautions to minimize droplet generation including covering nose and mouth when coughing or sneezing Worker will communicate with UEOHC daily to report temperature and symptoms

If symptoms and fever are not gone after 72 hours, worker will continue to monitor his/her condition and will report results to UEOHC daily Employee will be advised when/if arrangements for clinical evaluation after hours will be needed

Resolution of Symptoms: If temperature and symptoms are gone within 72 hours of appearance, UEOHC will arrange to see worker to give final approval to return to work UEOHC will arrange to see employee to give final clearance to return to work without further monitoring and the supervisor will be notified Worker will be given specific appointment time and is to report directly to the location for the evaluation wearing a surgical mask This encounter may take place during normal working hours and in a place deemed to be appropriate for the circumstances by UEOHC This may be UEOHC itself or a negative pressure room in the Pulmonary Clinic of the ACC

Severe Illness: If employee develops a respiratory illness which may require specific medical treatment or evaluation for admission to hospital (including any degree of pneumonia diagnosed by a physician, PA, or NP) within 14 days of any direct contact with the laboratory or during any period of symptom monitoring required by UOEHC, the Orange County Health Department and the County Health Department in the county of residence of the worker, if different, must be notified Workers with a respiratory illness of severity, which may require specific medical treatment or hospitalization, will be evaluated by staff of the UNC Hospitals Emergency Department (ED) The patient will be instructed to wear a surgical mask when reporting to the ED and to come to a specified entrance, which minimizes contact with other patients and ED personnel Disposition will be determined by the evaluating physician with consultation with the ID consultation attending and UEOHC

Respiratory Disease Protection: For laboratory personnel, inactivated flu vaccines will be offered each year to minimize likelihood of severe respiratory disease unrelated to SARS-CoV, BtSLCoV or MERS-CoV exposure in the BSL3 laboratory Serum samples have been taken and stored at the University Employee Occupational Health Clinic on a twice yearly basis for future reference

SARS-CoV, BtSLCoV Identification The reverse genetic system developed for SARS-CoV, SHC014 and WIV1 results in recombinant viruses that contain unique marker mutations that allows the precise identification

of the source of virus responsible for any particular infection of laboratory personnel Similar markers exist for the other SLCoV studied in this proposal Primer sets are available in the laboratory to determine which particular recombinant virus was responsible for infection and to distinguish between naturally acquired (should SARS/MERS-CoV spreads) and laboratory acquired infections Primer sets are also available for distinguishing among the many different recombinant viruses generated in the laboratory

Fang Li, Ph D

April Coon, Assistant Director
Sponsored Projects Administration

Erin Knudsen <jond0006@umn.edu>

Fwd: Grant Number: 2R01AI089728 - 06 PI Name: Li, Fang

spamon <spamon@umn edu> Wed, Apr 20, 2016 at 11 19 AM
To Erin E Knudsen-1 <jond0006@umn edu>, Lorrie Awoyinka <lorrie@umn edu>

-------- Forwarded Message --------
Subject: Grant Number 2R01AI089728 - 06 PI Name Li, Fang
Date: Wed, 20 Apr 2016 16 18 25 +0000
From: Bumbray-Quarles, Devon (NIH/NIAID) [E] <DBumbray@niaid nih gov>
To:'lifang@umn edu' <lifang@umn edu>, 'awards@umn edu' <awards@umn edu>
CC: Bumbray-Quarles, Devon (NIH/NIAID) [E] <DBumbray@niaid nih gov>

Dear Dr Li,

Please provide a response to the Summary Statement concerns as noted below for the above subject grant

BIOHAZARD COMMENT: There are concerns that recombinant coronaviruses altered to enhance proteolytic cleavage or binding of the human angiotensin converting enzyme 2 (ACE 2) receptor may have novel and unexpected virulence phenotypes Therefore, biosafety level-3 (BSL-3) protections, training, and monitoring procedures should be considered unless otherwise indicated

BUDGETARY OVERLAP: There is potential for budgetary overlap with the project R01AI110700 titled, "Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis" awarded to PI

Ralph Baric (co-investigator on this proposal)

Please ensure all responses submitted to my attention are countersigned by an authorized institutional signing official

Thank you

Sincerely,

Ms. Devon Bumbray-Quarles

Grants Management Specialist

Grants Management Program

DHHS, NIH, NIAID, GMP

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-

National Institute of Allergy and Infectious Diseases

"Effective October 1, 2014, NIH closeout policy has changed (see NOT-OD-14-084) In order to avoid unilateral closeout, final reports must be submitted in a timely manner Failure to submit accurate final reports could result in enforcement actions such as revisions to NOA funding levels, or delay in future funding "

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Subject: NoA. 2 R01 AI089728-06 PI: Li, Fang
From: era-notify@mail.nih.gov
Date: 6/6/2016 11:02 PM
To: awards@umn.edu

Grant Number: 2 R01 AI089728-06
Principal Investigator: Li, Fang
Project Title: Receptor recognition and cell entry of coronaviruses
Institution: UNIVERSITY OF MINNESOTA
CFDA: 93.855
OC: 414B
PCC: M51C B
Award Issue Date: 06/07/2016
Grants Management Officer: Bumbray-Quarles, Devon
Program Official: Stemmy, Erik
Grants Specialist: Bumbray-Quarles, Devon

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Attachments

temp6079383602979248 61.pdf 27 bytes

Filed: CON-[redacted] CON-S8289 00054028
22345

(P)

DC 15-16 BOX #22

LI 1

Erin Knudsen <jond0006@umn.edu>

Just in Time Submitted for Grant Application: R01AI089728-06 to the NIH

1 message

era-notify@mail.nih.gov <era-notify@mail nih gov> Thu, Mar 17, 2016 at 4 15 PM
Reply-To commons@od nih gov
To jond0006@umn edu, lifang@umn edu

A Just in Time Submission was submitted by Signing Official Knudsen, Erin E to the NIH for grant application R01AI089728-06 associated with Principal Investigator Li, Fang using the NIH Commons

The Just In Time information submitted to NIH is available for review in the Grant Detail section of the Commons Status module To access this information, go to Status, retrieve the grant application and click on the Application Id link available in the result set
If you have any questions about this email, please contact Erin E Knudsen at jond0006@umn edu, who initiated this action

For any further questions about this email, call the eRA Service Desk at 1-866-504-9552 or refer to http //grants nih gov/support for additional methods of contact Please access Commons at http //public era nih gov/commons/
For more information please visit http //era nih gov/

11/23/15

Just In Time Report

IRB Confirmation.	No IRB Certification was required
Human Subjects Assurance Number	No Human Subjects Assurance was required
Human Subjects Education	No Human Subjects Education was provided
IACUC Confirmation	No IACUC Certification was required

OTHER SUPPORT

Provide active and pending support information for all individuals designated in an application as senior/key personnel – those devoting measurable effort to a project

Other Support includes all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors, including but not limited to research grants, cooperative agreements, contracts, and/or institutional awards Training awards, prizes, or gifts do not need to be included

Fang Li

ACTIVE

R01AI110700 (Contact Co-PI Ralph Baric) 04/01/15 - 03/31/20 3 00 cal mo
Sponsor National Institutes of Health Subaward Annual Direct Costs $247,699
Title **Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis**
Role Co-Principal Investigator
Goals This research investigates genetic pathways regulating MERS coronavirus cross species transmission and receptor homolog usage, establishes robust animal models of human disease, and discovers critical reagents for therapeutic and vaccine testing
Overlap There is no scientific overlap between the above grant and the pending NIH R01 grant (2 R01 AI089728-06)

OTHER SUPPORT

RALPH S. BARIC

ACTIVE:

U19 AI107810 (PI Baric) 06/21/13 - 05/31/18 2 16 cal mos
NIH/NIAID $1,558,483
Characterization of novel genes encoded by RNA and DNA viruses
Using highly pathogenic human respiratory and systemic viruses, which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and host responses to determine severe disease outcomes

U19 AI100625 (PI Baric/Heise-MPI) 08/05/12 - 07/31/17 2 04 cal mos
NIH/NIAID $3,152,632
Systems Immunogenetics of Biodefense Pathogens in the Collaborative Cross
Specific Aims In this proposal, we are utilizing the Collaborative Cross (CC), a novel panel of reproducible, recombinant inbred (RI) mouse lines to identify genes and gene interactions, which regulate the induction, kinetics, and magnitude of the innate, inflammatory and adaptive arms of the immune response following virus infection Specifically, we will develop novel modeling algorithms to predict and validate the causal relationships between natural genetic variation and host signaling networks, immune cell recruitment, and immune function

00008956 (PI De Silva) 07/29/15 - 06/30/16 0 60 cal mos
UCB/NIH $275,000
Protective immunity following dengue virus natural infections and vaccination
We will perform studies to characterize the B-cell/ antibody (responses in people who receive dengue live attenuated virus vaccines (DLAV)
Role Co-Investigator

R01 AI107731 (PI De Silva) 08/05/13 - 07/31/17 0 24 cal mos
NIH/NIAID $378,675
Molecular Basis of Dengue Virus Neutralization by Human Antibodies
These studies proposed here are directly relevant to developing simple assays to predict the performance of the leading dengue vaccine candidates and also for developing the next generation of safe and effective dengue vaccines
Role Co-Investigator

R01 AI108197 (MPI Denison/Baric) 08/01/13 - 07/31/17 0 60 cal mos
Vanderbilt University/NIH/NIAID $450,129
Determinants of Coronavirus Fidelity in Replication and Pathogenesis
Experiments in this aim will test the hypothesis nsp1 functions in maintaining high replication fidelity and viral RNA synthesis are coupled and that targeted engineered mutations across nsp14 alter a) RNA fidelity outcomes, b) sensitivity nucleoside mutagens, terminators and polymerase inhibitors, c) the kinetics and magnitude of positive, negative, genomic and subgenomic RNA synthesis, and d) RNA recombination frequencies

U19 AI106772-01 (PI Kawaoka) 06/01/13 - 05/31/16 0 12 cal mos
University of Wisconsin/NIH $780,782
MERS-CoV Supplement for OMICs Proposal
The proposed studies will provide a more detailed look at the intracellular environment by taking "snapshots" of the lipids, metabolytes, and proteins present during viral infection time courses These assays will allow us to determine the innate immune response occurring immediately following virus infection and to determine how the virus and cell interact over a 72 hour window
Role Investigator

HHSN272201000019I-HHSN27200003 (PI Baric) 09/30/11 - 04/14/16 0 60 cal mos
MSSM/NIH 481,223
MERS-CoV Mouse Model for Vaccine and Therapeutic Testing (Task Order A57)
Specific Aims Use generation of transgenic mice and modifications to the MERS-CoV genome to identify a mouse model for MERS-CoV that recapitulates human disease phenotypes for evaluating vaccine platforms and therapeutics

U19 AI109680 CETR (PI Whitley) 03/01/14 - 02/28/19 1 02 cal mos
UAB/NIH/NIAID $314,437
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using *in vivo* mouse models of acute and persistent CoV disease
Role Co-Investigator

U19 AI109761 CETR (PI Lipkin) 03/01/14 - 02/28/19 1 02 cal mos
Columbia/NIH/NIAID $597,812
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung
Role Project Leader, Consortium PI

246823 (PI Baric) 01/27/15 - 09/16/16 0 48 cal mos
PNNL/DHS $205,569
The Generation of Predictive Models of Viral Pathogenesis
Using advances in transcriptomics, proteomics, and metabolomics, we will identify changes in the virus-host interaction expression networks associated with DENV infection of Aedes aegypti cells or human immune cells *in vitro*, the latter model after natural receptor-mediated or after ADE mediated entry processes

R56 AI106006 (PI Baric) 09/01/14 - 08/31/16 1 20 cal mos
NIAID/NIH $597,745
Mechanisms of Norovirus Protective Immunity
The goal of this grant is to prevent future norovirus outbreaks We propose to identify molecular markers for long-term protective immunity and characterize the breadth of the protective antibody response after vaccination Our studies will identify key norovirus neutralizing epitopes which mediate type specific and broadly cross reactive short and long-term protective immunity, develop robust platforms for discriminating between short and long-term memory B cell response following human vaccination and inform second generation norovirus vaccine design as certain strains evolve quickly

Not assigned (PI deSilva) 11/05/14 - 09/30/17 0 36 cal mos
Johns Hopkins U/Gates Foundation $726,498
The dengue human infection model: Defining correlates of protection and advancing vaccine development
The goal of these studies conducted by the Baric laboratory are to use recombinant dengue viruses encoding multiple homotypic neutralizing sites from multiple strains, as well as a collection of null mutants, to characterize the homotypic immune response elicited in humans following natural infection and after challenge in GSK DENV tetravalent vaccinated individuals This grant has been funded by Gates
Role Co-Investigator

R01 AI110700 (PI Baric) 04/01/15 - 03/31/20 0 96 cal mos
NIH/NIAID $617,400
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis

PENDING:

Not Assigned	(PI Baric)	01/01/16 - 12/31/18	0 36 cal mos
Taked Vaccines, Inc		$1,243,048	

In Vitro and In Vivo Characterization of Bivalent DENV Live Virus Vaccines

To provide expertise in molecular virology required for creating recombinant dengue viruses for *in vitro* and *in vivo* testing

OVERLAP: None

~~(CON-222595)~~
CON-566289
635469

Status Information

General Grant Information	
Status.	Scientific Review Group review pending
Institution Name.	UNIVERSITY OF MINNESOTA
School Name:	MEDICAL SCHOOL
School Category.	SCHOOLS OF MEDICINE
Division Name:	NONE
Department Name.	PHARMACOLOGY
PI Name:	Li, Fang
Application ID:	2R01AI089728-06
Proposal Title:	Receptor recognition and cell entry of coronaviruses
Proposal Receipt Date:	11/03/2015
Last Status Update Date:	11/11/2015
Current Award Notice Date.	
Application Source:	Grants gov
Project Period Begin Date:	05/15/2010
Project Period End Date:	06/30/2021
eApplication Status:	Submission Complete
FOA:	[PA13-302] - RESEARCH PROJECT GRANT (PARENT R01)
NIH Appl ID:	9174759

Other Relevant Documents

e-Application

eSubmission Cover Letter

Additions for Review (0 documents)

Status History

Effective Date	Status Message
11/03/2015	Application entered into system

Institute or Center Assignment

Institute or Center	Assignment Date
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES **(Primary)**	11/03/2015
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES **(Primary)**	11/06/2015

Application Information

Award Document Number	RAI089728B
FSR Accepted Code	N
Snap Indicator Code	
Review Outcome Available	No
Summary Statement Available	No
Early Stage Investigator Eligible	N
New Investigator Eligible	N
Eligible for FFATA Reporting	No

Study Section

Scientific Review Group	VIRA
Council Meeting Date(YYYY/MM)	2016/05
Meeting Date	02/25/2016
Meeting Time	08 00
Study Roster	

Advisory Council(AC) Information

Reference Letter(s)

This list shows Reference Letters associated with this particular Grant Application Principal Investigator can see a list of all Reference Letters within Personal Profile - Reference Letters section on eRA Commons

Contacts

Administration	Name	Phone	Email
Scientific Review Administrator(SRO)	Izumi, Kenneth M	301-496-6980	izumikm@csr nih gov
Grants Management Specialist(GMS)	Bumbray-Quarles, Devon R	240-669-2950	dbumbray@niaid nih gov
Program Official(PO)	Stemmy, Erik J	240-627-3380	erik stemmy@nih gov

Close

[Contact Us | Privacy Notice | Disclaimer | Accessibility | Popup Block Check]

National Institutes of Health (NIH) 9000 Rockville Pike Bethesda, Maryland 20892

Department of Health and Human Services

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Version 1 20 01

GRANTS.GOV FIND APPLY SUCCEED

CON-22345 5-15-10→
DC 15-16 4-30-15
Box #22
Prop + EFS Noted gap IN time
CON-58289
will be set up as
New at time
of Award

UNIVERSITY OF MINNESOTA

ReportID. UMFGM017F

PROPOSAL SUMMARY

Page No: 1
Run Date: 10/30/2015
Run Time: 08:29:56 AM

Status ~~1-Draft~~

Grant Administrator ERIN

INSTITUTION APPROVED
11.03.15

approved - moved

PRF #	835469	Proposal #/Version	CON000000058289/V101
EGMS Proposal #		Sponsor Award #	
Proposal Type	Renewal	Concurrent	N
Previous Award		SubContract	N
Purpose	UMN-22345		

Title: Receptor recognition and cell entry of coronaviruses

Principal Investigator: Li,Fang — lifang@umn edu

Budget Summary

Start Date	07/01/2016	End Date	06/30/2021
Direct Costs	~~$0.00~~ 1,708,000	Indirect Costs	~~$0.00~~ 600,400
Sponsor	NATIONAL INSTITUTES OF HEALTH (NIH)	Total Costs	~~$0.00~~ 2,308,400

Submission Information

Due Date	11/05/2015	Date Type	Receipt Date
Submission Method	g.gov	# of Copies Submitted	
Mailing Address		11/3/15 - 5:08pm EST - Kevin	

grants.gov: 12031055

Comments/Corrections

REPA	X verified	__email sent for _____		
IRB	__verified	__pending	__email sent	X Not Applicable
IACUC	__verified	__pending	__email sent	X Not Applicable
IBC	__verified	X pending	X email sent	__Not Applicable
F&A rate	52% / 53/54 X at full or agency limit	__waiver on file	__waiver requested	
Cost share documented?		__yes	X no	__Not Applicable
ICR sharing?		__yes	X no	

SUBAWARDS 1 #

~~For more than 4 subawards please record on separate page~~

11.09.15 Request NLB

	EDP/DE	Forms 1 & 2	Entered in EFS	New Vendor*
✓ University of North Carolina Chapel Hill — Subrecipient	☑	☐	☒	☐
_____ Subrecipient	☐	☐	☐	☐
_____ Subrecipient	☐	☐	☐	☐
_____ Subrecipient	☐	☐	☐	☐

ELECTRONIC PROPOSAL

- FEDERAL
- NON-FED.
- X (b)-GOV.

NO ATTACHMENT

* Temporary New Vendor Code 0000002885

x3 E

UNIVERSITY OF MINNESOTA PRF

EGMS ID. 442741 PRF ID: 835469

Proposal Routing Form for External Research, Training or Public Service Support — PS Proposal ID

1	Sponsor Name	National Institutes of Health	Primary Funding Source
2.	Proposal Title	Receptor recognition and cell entry of coronaviruses	
3.	Sponsor Due Date/Time	11/05/2015 - 5pm CST	Receipt
4.	RFA/RFP/CFDA/FOA#	PA-13-302	CON-S8289
5.	Submission Medium	Grants gov	
6	University Contact	Weber, Lynn F — (612) 626-6902 — weber117@umn.edu	
7	Administering Dept	PHCL PHARMACOLOGY ADMIN	Department ID 11718

8 Principal Investigator

Li, Fang			Paid	Cost Share	PI	Key
lifang	ID 3694715	(612) 625-6149	25	0	X	X
Associate Professor [9402]	PHCL PHARMACOLOGY ADMIN	DeptID 11718				

FCOI ✓ OK ✓2015

9 Investigator(s)

10. Proposal is for

	Basic %	Applied %	Development %	Total %
Research	100	0	0	100

11 Proposal is

	Agency Award #	EFS Award #	Primary Project
Renewal	R01 AI089728	3002117183782	

12 Proposed Duration & Amount Requested

	Start	End	Direct $$	Indirect $$	F&A Rate	Total
Initial/Current Budget ✓	07/01/2016	06/30/2017	341,600	127,400	52% MTDC	469,000
All Years ✓	07/01/2016	06/30/2021	1,708,000	600,400	52% MTDC	2,308,400

[X] Departmental Directions to SPA for Award Set Up
Proposal budget has been established in EGMS this budget should be used to set up the award, SPA may pro-rate at time of award if needed to accommodate award amount

[X] F&A Rate Used: 52% MTDC
Full F&A Rate

F&A Comments:

13 Project Involves

[X] Recombinant DNA, Infectious Agents or Biological Toxins — There will be recombinant DNA, infectious agents or biological toxins used

Application status of this project with the Institutional Biosafety Committee — Status: Pending — Study Code/Protocol ID — Approval Date

[X] Chemicals — There will be chemicals used — The appropriate chemical safety training and hazardous waste training records have been completed

[X] 14 Subawards — University of North Carolina - Chapel Hill

[ ] Outside Entities

[X] 15. Conflict of Interest — a There is not a significant financial interest OR business interest in a business entity that could benefit from the results of this project

REPA # — Approval Date

Comments

		X	b There is not a familial connection OR financial or business interest (of any amount) with a proposed subrecipient or collaborator Comments	
16	Inventions	X	Inventions have been conceived or reduced to practice under prior research on project This has been previously reported	
17.	Space	X	There is sufficient and suitable space to house this project presently assigned to the principal investigator's or other investigator's department or college	3-284 NHH
18.	Resources/Space/Staff			
19	Indirect Cost Recovery	X	ICR revenue will not be shared	
20	Matching and Cost Sharing	☐		
21.	Program Income	☐		
22.	International Component	☐		
23.	Fairview Health Services	☐		

24 **PROPOSAL ABSTRACT or EXECUTIVE SUMMARY**

25 **NOTES**

APPROVALS and CERTIFICATIONS

Principal Investigator

(1) I certify the information submitted within the application is true, complete and accurate to the best of the PI's knowledge,
(2) that any false, ficticious, or fraudulent statements or claims may subject the PI to criminal, civil, or administrative penalties, and
(3) that the PI agrees to accept the responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of the application

Investigator(s)

The information provided on this form and in the accompanying proposal is correct to the best of my knowledge Equipment budgeted in this application is not otherwise available for use on this project from existing departmental or collegiate inventories In the event this application is awarded, I (we) agree to abide by all applicable institutional and sponsoring agency policies and procedures including the Intellectual Property Policy of the University of Minnesota and to follow commonly accepted scientific practices in recording and maintaining records of research I (we) certify that I (we) have read the University of Minnesota Code of Conduct policy and agree to abide by the rights and responsibilities as identified therein

Signature Fang Li **10-29-2015**
Fang Li (lifang) Principal Investigator **Date**

Department Head(s) and/or Division Head(s) Academic Dean(s), Chancellor or Provost.

We certify that the above statements are correct to the best of our knowledge The commitment of departmental and collegiate resources, if any, has been noted and approved We agree that the scientific objectives of this application are in keeping with departmental and collegiate goals We certify that the individuals from our unit(s) listed on this PRF have filed a current Report of External Professional Activities (REPA)

Signature DeJarlais, Carrie L **10-29-2015**
DeJarlais, Carrie L (dejar001) Dept Administrator/Representative **Date**

Signature Siegfried, Jill M **10-29-2015**
Siegfried, Jill M (jsiegfri) Dept Head/Delegate **Date**

Signature tmeddean **10-29-2015**
tmeddean (tmeddean) Dept Administrator/Representative **Date**

Signature Segal, Rosalyn A **10-29-2015**
Segal, Rosalyn A (rsegal) Research Associate Dean/Delegate **Date**

Subject: PRF# 835469 Fang Li NIH R01 Renewal
From: Lynn Weber <weber117@umn edu>
Date: 10/29/2015 12 13 PM
To: "proposal@umn edu" <proposal@umn edu>

--

Lynn Weber
Department of Pharmacology
University of Minnesota
Medical School
6-120 Jackson Hall
321 Church Street SE
Minneapolis, MN 55455
Phone 612-626-6902
Fax 612-625-8408
Email weber117@umn edu
http //www pharmacology med umn edu/

— Attachments

Fang Li Renew 3782 Recept Recog 11-5-15.pdf 7 7 MB

Subject: 835469LiFangNIH URGENT
From: Carrie DeJarlais <dejar001@umn.edu>
Date: 10/29/2015 1:20 PM
To: proposal@umn edu
CC: Lynn Weber <weber117@umn.edu>

Hello,

A proposal was submitted earlier today and the PI would like to make a few changes. Please do not submit to agency Abstract and research strategy will be updated Please let me know if you have any questions.

Thanks.

Carrie

--

Carrie DeJarlais
Administrator, Dept. of Pharmacology
Sr. Finance Manager, MNPI Center
University of Minnesota
6-120 Jackson Hall
612-625-4982

Subject: PRF# 835469 Fang Li NIH Renewal - FINAL
From: Lynn Weber <weber117@umn edu>
Date: 10/30/2015 8 08 AM
To: "proposal@umn edu" <proposal@umn edu>
CC: Erin Jondahl <jond0006@umn edu>

This is the FINAL version to submit to NIH
Please delete the version sent to SPA yesterday
Thanks!

--

Lynn Weber
Department of Pharmacology
University of Minnesota
Medical School
6-120 Jackson Hall
321 Church Street SE
Minneapolis, MN 55455
Phone 612-626-6902
Fax 612-625-8408
Email weber117@umn edu
http //www pharmacology med umn edu/

— Attachments

Fang Li Renew 3782 Recept Recog 11-5-15 pdf 7 7 MB

Department of Health and Human Services

Part 1. Overview Information

Participating Organization(s)	National Institutes of Health (NIH)
Components of Participating Organizations	National Cancer Institute (NCI) National Eye Institute (NEI) National Heart, Lung, and Blood Institute (NHLBI) National Human Genome Research Institute (NHGRI) National Institute on Aging (NIA) National Institute on Alcohol Abuse and Alcoholism (NIAAA) National Institute of Allergy and Infectious Diseases (NIAID) National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) National Institute of Biomedical Imaging and Bioengineering (NIBIB) *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) National Institute on Deafness and Other Communication Disorders (NIDCD) National Institute of Dental and Craniofacial Research (NIDCR) National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) National Institute on Drug Abuse (NIDA) National Institute of Environmental Health Sciences (NIEHS) National Institute of General Medical Sciences (NIGMS) National Institute of Mental Health (NIMH) National Institute on Minority Health and Health Disparities (NIMHD) National Institute of Neurological Disorders and Stroke (NINDS) National Institute of Nursing Research (NINR) National Library of Medicine (NLM) National Center for Complementary and Integrative Health (NCCIH formerly NCCAM) Division of Program Coordination, Planning and Strategic Initiatives, Office of Research Infrastructure Programs (ORIP)
Funding Opportunity Title	**Research Project Grant (Parent R01)**
Activity Code	R01 Research Project Grant
Announcement Type	Reissue of PA-11-260
Related Notices	• April 16, 2015 - Notice of Information NIMH High-Priority Areas for Research on Women's Mental Health During Pregnancy and the Postpartum Period See Notice NOT-MH-15-013 • September 25, 2014 - See Notice NOT-MH-14-033 Notice of Information on High-Priority Research Areas to Understand and Reduce Mental Health Disparities • September 4, 2014 - Notice Announcing Updates to NHLBI Topics of Special Interest (TOSI) See Notice NOT-HL-14-236 • August 29, 2014 - See Notice NOT-AR-14-021 NIAMS Policy for Submission of Applications Containing Clinical Trials • August 06, 2014 - See Notice NOT-MH-14-007 Notice of change to application requirements • July 10, 2014 - Notice of National Institute on Minority Health and Health Disparities (NIMHD) Participation in PA-13-302 See Notice

	NOT-MD-14-003 • June 10, 2014 - Notice of NICHD's Interest in Supporting Research on Contraception, Long-Term Outcomes of Assisted Reproductive Technologies, and Intrauterine Assessment of Placental and Fetal Function See Notice NOT-HD-14-020 • June 4, 2014 - Notice NOT-14-074 supersedes instructions in Section III 3 regarding applications that are essentially the same • November 25, 2013 - See Notice NOT-HL-13-201 Notice Announcing NHLBI Topics of Special Interest (TOSI) • August 21, 2013 Removed reference to ASSIST in section IV3, since ASSIST is currently only available for multi-project applications • NOT-DK-13-013 "Clarification of NIDDK Policy Investigator-Initiated Multi-Center Clinical Studies" • August 7, 2013 - Use this funding opportunity announcement for due dates of September 25, 2013 and beyond
Funding Opportunity Announcement (FOA) Number	**PA-13-302**
Companion Funding Opportunity	None
Number of Applications	See Section III 3 Additional Information on Eligibility
Catalog of Federal Domestic Assistance (CFDA) Number(s)	93 361, 93 233, 93 839, 93 838, 93 837, 93 113, 93 859, 93 879, 93 286, 93 273, 93 172, 93 173, 93 866, 93 853, 93 847, 93 856, 93 855, 93 121, 93 351, 93 867, 93 213, 93 242, 93 846, 93 865, 93 399, 93 307, 93 396, 93 395, 93 394, 93 393, 93 279
Funding Opportunity Purpose	The Research Project Grant (R01) supports a discrete, specified, circumscribed project to be performed by the named investigator(s) in areas representing the specific interests and competencies of the investigator(s) The proposed project must be related to the programmatic interests of one or more of the participating NIH Institutes and Centers (ICs) based on descriptions of their programs

Key Dates

Posted Date	August 2, 2013
Open Date (Earliest Submission Date)	August 7, 2013
Letter of Intent Due Date(s)	Not Applicable
Application Due Date(s)	Standard dates apply, by 5 00 PM local time of applicant organization Applicants are encouraged to apply early to allow adequate time to make any corrections to errors found in the application during the submission process by the due date
AIDS Application Due Date(s)	Standard AIDS dates apply, by 5 00 PM local time of applicant organization Applicants are encouraged to apply early to allow adequate time to make

	any corrections to errors found in the application during the submission process by the due date
Scientific Merit Review	Standard dates apply
Advisory Council Review	Standard dates apply
Earliest Start Date	Standard dates apply
Expiration Date	September 8, 2016
Due Dates for E.O. 12372	Not Applicable

Required Application Instructions

It is critical that applicants follow the instructions in the SF424 (R&R) Application Guide, except where instructed to do otherwise (in this FOA or in a Notice from the *NIH Guide for Grants and Contracts*) Conformance to all requirements (both in the Application Guide and the FOA) is required and strictly enforced Applicants must read and follow all application instructions in the Application Guide as well as any program-specific instructions noted in Section IV When the program-specific instructions deviate from those in the Application Guide, follow the program-specific instructions **Applications that do not comply with these instructions may be delayed or not accepted for review.**

There are several options to submit your application to the agency through Grants gov You can use the ASSIST system to prepare, submit and track your application online You can download an application package from Grants gov, complete the forms offline, submit the completed forms to Grants gov and track your application in eRA Commons Or, you can use other institutional system-to-system solutions to prepare and submit your application to Grants gov and track your application in eRA Commons Learn more

Apply Online Using ASSIST **Apply Using Downloadable Forms**

Problems accessing or using ASSIST should be directed to the eRA Service Desk
Problems downloading forms should be directed to Grants gov Customer Support

Table of Contents

Part 1 Overview Information
Part 2 Full Text of the Announcement
- Section I Funding Opportunity Description
- Section II Award Information
- Section III Eligibility Information
- Section IV Application and Submission Information
- Section V Application Review Information
- Section VI Award Administration Information
- Section VII Agency Contacts
- Section VIII Other Information

Part 2. Full Text of Announcement

Section I. Funding Opportunity Description

The Research Project Grant (R01) supports a discrete, specified, circumscribed project in scientific areas that represent the investigators' specific interests and competencies and that fall within the mission of the participating NIH Institutes and Centers (ICs) The R01 is the original, and historically the oldest, grant mechanism used by the NIH to support health-related research and development

Research grant applications are assigned to an NIH IC based on receipt and referral guidelines and many

applications are assigned to multiple ICs with related research interests

Each IC maintains a web site with funding opportunities and areas of interest Contacting an IC representative may help focus the proposed research based on an understanding of the mission of the IC For specific information about the mission of each NIH IC, see http //www nih gov/icd, which provides a brief summary of the research interests in each IC and access to individual IC websites

Section II. Award Information

Funding Instrument	Grant A support mechanism providing money, property, or both to an eligible entity to carry out an approved project or activity
Application Types Allowed	New Renewal Resubmission Revision The OER Glossary and the SF424 (R&R) Application Guide provide details on these application types
Funds Available and Anticipated Number of Awards	The number of awards is contingent upon NIH appropriations and the submission of a sufficient number of meritorious applications
Award Budget	Application budgets are not limited but need to reflect the actual needs of the proposed project
Award Project Period	The scope of the proposed project should determine the project period The maximum project period is 5 years

NIH grants policies as described in the *NIH Grants Policy Statement* will apply to the applications submitted and awards made in response to this FOA

Section III. Eligibility Information

1. Eligible Applicants

Eligible Organizations

Higher Education Institutions

- Public/State Controlled Institutions of Higher Education
- Private Institutions of Higher Education

The following types of Higher Education Institutions are always encouraged to apply for NIH support as Public or Private Institutions of Higher Education

- Hispanic-serving Institutions
- Historically Black Colleges and Universities (HBCUs)
- Tribally Controlled Colleges and Universities (TCCUs)
- Alaska Native and Native Hawaiian Serving Institutions
- Asian American Native American Pacific Islander Serving Institutions (AANAPISIs)

Nonprofits Other Than Institutions of Higher Education

- Nonprofits with 501(c)(3) IRS Status (Other than Institutions of Higher Education)
- Nonprofits without 501(c)(3) IRS Status (Other than Institutions of Higher Education)

For-Profit Organizations

- Small Businesses
- For-Profit Organizations (Other than Small Businesses)

Governments

- State Governments
- County Governments
- City or Township Governments
- Special District Governments
- Indian/Native American Tribal Governments (Federally Recognized)
- Indian/Native American Tribal Governments (Other than Federally Recognized)
- Eligible Agencies of the Federal Government
- U S Territory or Possession

Other

- Independent School Districts
- Public Housing Authorities/Indian Housing Authorities
- Native American Tribal Organizations (other than Federally recognized tribal governments)
- Faith-based or Community-based Organizations
- Regional Organizations
- Non-domestic (non-U S) Entities (Foreign Institutions)

Foreign Institutions

Non-domestic (non-U S) Entities (Foreign Institutions) **are** eligible to apply
Non-domestic (non-U S) components of U S Organizations **are** eligible to apply

Foreign components, as defined in the *NIH Grants Policy Statement*. **are** allowed

Required Registrations

Applicant Organizations

Applicant organizations must complete and maintain the following registrations as described in the SF 424 (R&R) Application Guide to be eligible to apply for or receive an award All registrations must be completed prior to the application being submitted Registration can take 6 weeks or more, so applicants should begin the registration process as soon as possible The NIH Policy on Late Submission of Grant Applications states that failure to complete registrations in advance of a due date is not a valid reason for a late submission

- Dun and Bradstreet Universal Numbering System (DUNS) - All registrations require that applicants be issued a DUNS number After obtaining a DUNS number, applicants can begin both SAM and eRA Commons registrations The same DUNS number must be used for all registrations, as well as on the grant application
- System for Award Management (SAM) (formerly CCR) – Applicants must complete and maintain an active registration, **which requires renewal at least annually** The renewal process may require as much time as the initial registration SAM registration includes the assignment of a Commercial and Government Entity (CAGE) Code for domestic organizations which have not already been assigned a CAGE Code
- NATO Commercial and Government Entity (NCAGE) Code – Foreign organizations must obtain an NCAGE code (in lieu of a CAGE code) in order to register in SAM
- eRA Commons - Applicants must have an active DUNS number and SAM registration in order to complete the eRA Commons registration Organizations can register with the eRA Commons as they are working through their SAM or Grants gov registration eRA Commons requires organizations to identify at least one Signing Official (SO) and at least one Program Director/Principal Investigator (PD/PI) account in order to submit an application
- Grants gov – Applicants must have an active DUNS number and SAM registration in order to complete the Grants gov registration

Program Directors/Principal Investigators (PD(s)/PI(s))

All PD(s)/PI(s) must have an eRA Commons account and should work with their organizational officials to either create a new account or to affiliate an existing account with the applicant organization's eRA Commons account If the PD/PI is also the organizational Signing Official, they must have two distinct eRA Commons accounts, one for each role Obtaining an eRA Commons account can take up to 2 weeks

Eligible Individuals (Program Director/Principal Investigator)

Any individual(s) with the skills, knowledge, and resources necessary to carry out the proposed research as the Program Director(s)/Principal Investigator(s) (PD(s)/PI(s)) is invited to work with his/her organization to develop an application for support Individuals from underrepresented racial and ethnic groups as well as individuals with disabilities are always encouraged to apply for NIH support

For institutions/organizations proposing multiple PDs/PIs, visit the Multiple Program Director/Principal Investigator Policy and submission details in the Senior/Key Person Profile (Expanded) Component of the SF424 (R&R) Application Guide

2. Cost Sharing

This FOA does not require cost sharing as defined in the *NIH Grants Policy Statement*

3. Additional Information on Eligibility

Number of Applications

Applicant organizations may submit more than one application, provided that each application is scientifically distinct

NIH will not accept any application that is essentially the same as one already reviewed within the past thirty-seven months (as described in the *NIH Grants Policy Statement*). except for submission

- To an RFA of an application that was submitted previously as an investigator-initiated application but not paid.
- Of an investigator-initiated application that was originally submitted to an RFA but not paid, or
- Of an application with a changed grant activity code

Section IV. Application and Submission Information

1. Requesting an Application Package

Applicants must download the SF424 (R&R) application package associated with this funding opportunity using the "Apply for Grant Electronically" button in this FOA or following the directions provided at Grants gov

2. Content and Form of Application Submission

It is critical that applicants follow the instructions in the SF424 (R&R) Application Guide, except where instructed in this funding opportunity announcement to do otherwise Conformance to the requirements in the Application Guide is required and strictly enforced Applications that are out of compliance with these instructions may be delayed or not accepted for review

For information on Application Submission and Receipt, visit Frequently Asked Questions – Application Guide, Electronic Submission of Grant Applications

Page Limitations

All page limitations described in the SF424 Application Guide and the Table of Page Limits must be followed

Required and Optional Components

The forms package associated with this FOA includes all applicable components, required and optional Please note that some components marked optional in the application package are required for submission of

applications for this FOA Follow all instructions in the SF424 (R&R) Application Guide to ensure you complete all appropriate "optional" components

Instructions for Application Submission

The following section supplements the instructions found in the SF424 (R&R) Application Guide and should be used for preparing an application to this FOA

SF424(R&R) Cover

All instructions in the SF424 (R&R) Application Guide must be followed

SF424(R&R) Project/Performance Site Locations

All instructions in the SF424 (R&R) Application Guide must be followed

SF424(R&R) Other Project Information

All instructions in the SF424 (R&R) Application Guide must be followed

SF424(R&R) Senior/Key Person Profile

All instructions in the SF424 (R&R) Application Guide must be followed

R&R or Modular Budget

All instructions in the SF424 (R&R) Application Guide must be followed

PHS 398 Cover Page Supplement

All instructions in the SF424 (R&R) Application Guide must be followed

PHS 398 Research Plan

All instructions in the SF424 (R&R) Application Guide must be followed, with the following additional instructions

Resource Sharing Plan Individuals are required to comply with the instructions for the Resource Sharing Plans (Data Sharing Plan, Sharing Model Organisms, and Genome Wide Association Studies (GWAS)) as provided in the SF424 (R&R) Application Guide

Appendix Do not use the Appendix to circumvent page limits Follow all instructions for the Appendix as described in the SF424 (R&R) Application Guide

Planned Enrollment Report

When conducting clinical research, follow all instructions for completing Planned Enrollment Reports as described in the SF424 (R&R) Application Guide

PHS 398 Cumulative Inclusion Enrollment Report

When conducting clinical research, follow all instructions for completing Cumulative Inclusion Enrollment Report as described in the SF424 (R&R) Application Guide

Foreign Institutions

Foreign (non-U S) institutions must follow policies described in the *NIH Grants Policy Statement*, and procedures for foreign institutions described throughout the SF424 (R&R) Application Guide

3. Submission Dates and Times

Part I Overview Information contains information about Key Dates Applicants are encouraged to submit applications before the due date to ensure they have time to make any application corrections that might be necessary for successful submission

Organizations must submit applications to Grants gov, the online portal to find and apply for grants across all Federal agencies Applicants must then complete the submission process by tracking the status of the application in the eRA Commons, NIH's electronic system for grants administration NIH and Grants gov systems check the application against many of the application instructions upon submission Errors must be corrected and a changed/corrected application must be submitted to Grants gov on or before the application due date If a Changed/Corrected application is submitted after the deadline, the application will be considered late

Applicants are responsible for viewing their application before the due date in the eRA Commons to ensure accurate and successful submission

Information on the submission process and a definition of on-time submission are provided in the SF424 (R&R) Application Guide

4. Intergovernmental Review (E.O. 12372)

This initiative is not subject to intergovernmental review

5. Funding Restrictions

All NIH awards are subject to the terms and conditions, cost principles, and other considerations described in the *NIH Grants Policy Statement*

Pre-award costs are allowable only as described in the *NIH Grants Policy Statement*

6. Other Submission Requirements and Information

Applications must be submitted electronically following the instructions described in the SF424 (R&R) Application Guide Paper applications will not be accepted

Applicants must complete all required registrations before the application due date. Section III Eligibility Information contains information about registration

For assistance with your electronic application or for more information on the electronic submission process, visit Applying Electronically

Important reminders:
All PD(s)/PI(s) must include their eRA Commons ID in the Credential field of the Senior/Key Person Profile Component of the SF424(R&R) Application Package. Failure to register in the Commons and to include a valid PD/PI Commons ID in the credential field will prevent the successful submission of an electronic application to NIH See Section III of this FOA for information on registration requirements

The applicant organization must ensure that the DUNS number it provides on the application is the same number used in the organization's profile in the eRA Commons and for the System for Award Management Additional information may be found in the SF424 (R&R) Application Guide

See more tips for avoiding common errors

Upon receipt, applications will be evaluated for completeness by the Center for Scientific Review, NIH Applications that are incomplete will not be reviewed

Requests of $500,000 or more for direct costs in any year

Applicants requesting $500,000 or more in direct costs in any year (excluding consortium F&A) must contact NIH program staff at least 6 weeks before submitting the application and follow the Policy on the Acceptance for Review of Unsolicited Applications that Request $500,000 or More in Direct Costs as described in the SF424 (R&R) Application Guide

Post-Submission Materials

Applicants are required to follow the instructions for post-submission materials, as described in NOT-OD-13-030

Section V. Application Review Information

1. Criteria

Only the review criteria described below will be considered in the review process As part of the NIH mission, all applications submitted to the NIH in support of biomedical and behavioral research are evaluated for scientific and technical merit through the NIH peer review system

Overall Impact

Reviewers will provide an overall impact score to reflect their assessment of the likelihood for the project to exert a sustained, powerful influence on the research field(s) involved. in consideration of the following review criteria and additional review criteria (as applicable for the project proposed)

Scored Review Criteria

Reviewers will consider each of the review criteria below in the determination of scientific merit, and give a separate score for each An application does not need to be strong in all categories to be judged likely to have major scientific impact For example, a project that by its nature is not innovative may be essential to advance a field

Significance

Does the project address an important problem or a critical barrier to progress in the field? If the aims of the project are achieved, how will scientific knowledge, technical capability, and/or clinical practice be improved? How will successful completion of the aims change the concepts. methods, technologies, treatments, services, or preventative interventions that drive this field?

Investigator(s)

Are the PD(s)/PI(s), collaborators, and other researchers well suited to the project? If Early Stage Investigators or New Investigators, or in the early stages of independent careers, do they have appropriate experience and training? If established, have they demonstrated an ongoing record of accomplishments that have advanced their field(s)? If the project is collaborative or multi-PD/PI, do the investigators have complementary and integrated expertise, are their leadership approach, governance and organizational structure appropriate for the project?

Innovation

Does the application challenge and seek to shift current research or clinical practice paradigms by utilizing novel theoretical concepts, approaches or methodologies, instrumentation, or interventions? Are the concepts, approaches or methodologies, instrumentation, or interventions novel to one field of research or novel in a broad sense? Is a refinement, improvement, or new application of theoretical concepts, approaches or methodologies, instrumentation, or interventions proposed?

Approach

Are the overall strategy, methodology. and analyses well-reasoned and appropriate to accomplish the specific aims of the project? Are potential problems, alternative strategies, and benchmarks for success presented? If the project is in the early stages of development, will the strategy establish feasibility and will particularly risky aspects be managed?

If the project involves humans subjects and/or NIH-defined clinical research. are the plans to address 1) the protection of human subjects from research risks and 2) the inclusion (or exclusion) of individuals on the basis of sex/gender, race, and ethnicity, as well as the inclusion (or exclusion) of children justified in terms of the scientific goals and research strategy proposed?

Environment

Will the scientific environment in which the work will be done contribute to the probability of success? Are the institutional support, equipment and other physical resources available to the investigators adequate for the project proposed? Will the project benefit from unique features of the scientific environment, subject populations, or collaborative arrangements?

Additional Review Criteria

As applicable for the project proposed, reviewers will evaluate the following additional items while determining scientific and technical merit, and in providing an overall impact score, but will not give separate scores for these items

Protections for Human Subjects

For research that involves human subjects but does not involve one of the six categories of research that are exempt under 45 CFR Part 46, the committee will evaluate the justification for involvement of human subjects and the proposed protections from research risk relating to their participation according to the following five review criteria 1) risk to subjects. 2) adequacy of protection against risks, 3) potential benefits to the subjects and others, 4) importance of the knowledge to be gained, and 5) data and safety monitoring for clinical trials

For research that involves human subjects and meets the criteria for one or more of the six categories of research that are exempt under 45 CFR Part 46, the committee will evaluate 1) the justification for the exemption, 2) human subjects involvement and characteristics, and 3) sources of materials For additional information, see the Human Subjects Protections Guidelines

Inclusion of Women, Minorities, and Children

When the proposed project involves human subjects and/or NIH-defined clinical research, the committee will evaluate the proposed plans for the inclusion (or exclusion) of individuals on the basis of sex/gender, race, and ethnicity, as well as the inclusion (or exclusion) of children to determine if it is justified in terms of the scientific goals and research strategy proposed For additional information, see the Human Subjects Inclusion Guidelines

Vertebrate Animals

The committee will evaluate the involvement of live vertebrate animals as part of the scientific assessment according to the following five points 1) proposed use of the animals, and species, strains, ages, sex, and numbers to be used, 2) justifications for the use of animals and for the appropriateness of the species and numbers proposed, 3) adequacy of veterinary care, 4) procedures for limiting discomfort, distress, pain and injury to that which is unavoidable in the conduct of scientifically sound research including the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices, and 5) methods of euthanasia and reason for selection if not consistent with the AVMA Guidelines on Euthanasia For additional information on review of the Vertebrate Animals section, please refer to the Worksheet for Review of the Vertebrate Animal Section

Biohazards

Reviewers will assess whether materials or procedures proposed are potentially hazardous to research personnel and/or the environment, and if needed, determine whether adequate protection is proposed

Resubmissions

For Resubmissions, the committee will evaluate the application as now presented, taking into consideration the responses to comments from the previous scientific review group and changes made to the project

Renewals

For Renewals, the committee will consider the progress made in the last funding period

Revisions

For Revisions, the committee will consider the appropriateness of the proposed expansion of the scope of the project If the Revision application relates to a specific line of investigation presented in the original application that was not recommended for approval by the committee, then the committee will consider whether the responses to comments from the previous scientific review group are adequate and whether substantial changes are clearly evident

Additional Review Considerations

As applicable for the project proposed, reviewers will consider each of the following items, but will not give scores for these items, and should not consider them in providing an overall impact score

Applications from Foreign Organizations

Reviewers will assess whether the project presents special opportunities for furthering research programs through the use of unusual talent, resources, populations, or environmental conditions that exist in other countries and either are not readily available in the United States or augment existing U S resources

Select Agent Research

Reviewers will assess the information provided in this section of the application, including 1) the Select Agent(s) to be used in the proposed research. 2) the registration status of all entities where Select Agent(s) will be used, 3) the procedures that will be used to monitor possession use and transfer of Select Agent(s), and 4) plans for appropriate biosafety, biocontainment, and security of the Select Agent(s)

Resource Sharing Plans

Reviewers will comment on whether the following Resource Sharing Plans, or the rationale for not sharing the following types of resources, are reasonable 1) Data Sharing Plan, 2) Sharing Model Organisms, and 3) Genome Wide Association Studies (GWAS)

Budget and Period of Support

Reviewers will consider whether the budget and the requested period of support are fully justified and reasonable in relation to the proposed research

2. Review and Selection Process

Applications will be evaluated for scientific and technical merit by (an) appropriate Scientific Review Group(s) convened by the Center for Scientific Review, in accordance with NIH peer review policy and procedures, using the stated review criteria Assignment to a Scientific Review Group will be shown in the eRA Commons

As part of the scientific peer review, all applications

- May undergo a selection process in which only those applications deemed to have the highest scientific and technical merit (generally the top half of applications under review) will be discussed and assigned an overall impact score
- Will receive a written critique

Applications will be assigned on the basis of established PHS referral guidelines to the appropriate NIH Institute or Center Applications will compete for available funds with all other recommended applications Following initial peer review, recommended applications will receive a second level of review by the appropriate national Advisory Council or Board The following will be considered in making funding decisions

- Scientific and technical merit of the proposed project as determined by scientific peer review
- Availability of funds
- Relevance of the proposed project to program priorities

3. Anticipated Announcement and Award Dates

After the peer review of the application is completed, the PD/PI will be able to access his or her Summary Statement (written critique) via the eRA Commons

Information regarding the disposition of applications is available in the *NIH Grants Policy Statement*

Section VI. Award Administration Information

1. Award Notices

If the application is under consideration for funding, NIH will request "just-in-time" information from the applicant as described in the *NIH Grants Policy Statement*

A formal notification in the form of a Notice of Award (NoA) will be provided to the applicant organization for successful applications The NoA signed by the grants management officer is the authorizing document and will be sent via email to the grantee's business official

Awardees must comply with any funding restrictions described in Section IV5 Funding Restrictions Selection of an application for award is not an authorization to begin performance Any costs incurred before receipt of the NoA are at the recipient's risk These costs may be reimbursed only to the extent considered allowable pre-award costs

Any application awarded in response to this FOA will be subject to the DUNS, SAM Registration, and Transparency Act requirements as noted on the Award Conditions and Information for NIH Grants website

2. Administrative and National Policy Requirements

All NIH grant and cooperative agreement awards include the *NIH Grants Policy Statement* as part of the NoA For these terms of award, see the *NIH Grants Policy Statement* Part II Terms and Conditions of NIH Grant Awards, Subpart A General and Part II Terms and Conditions of NIH Grant Awards, Subpart B Terms and Conditions for Specific Types of Grants, Grantees, and Activities More information is provided at Award Conditions and Information for NIH Grants

Cooperative Agreement Terms and Conditions of Award

Not Applicable

3. Reporting

When multiple years are involved, awardees will be required to submit the annual Non-Competing Progress Report (PHS 2590 or RPPR) and financial statements as required in the *NIH Grants Policy Statement*

A final progress report, invention statement, and the expenditure data portion of the Federal Financial Report are required for closeout of an award, as described in the *NIH Grants Policy Statement*

The Federal Funding Accountability and Transparency Act of 2006 (Transparency Act), includes a requirement for awardees of Federal grants to report information about first-tier subawards and executive compensation under Federal assistance awards issued in FY2011 or later All awardees of applicable NIH grants and cooperative agreements are required to report to the Federal Subaward Reporting System (FSRS) available at www fsrs gov on all subawards over $25,000 See the *NIH Grants Policy Statement* for additional information on this reporting requirement

Section VII. Agency Contacts

We encourage inquiries concerning this funding opportunity and welcome the opportunity to answer questions from potential applicants

Application Submission Contacts

eRA Service Desk (Questions regarding ASSIST, eRA Commons registration, submitting and tracking an application. documenting system problems that threaten submission by the due date, post submission issues)
Telephone 301-402-7469 or 866-504-9552 (Toll Free)

Web ticketing system https //public era nih gov/commonshelp
TTY 301-451-5939
Email commons@od nih gov

Grants gov Customer Support (Questions regarding Grants gov registration and submission, downloading forms and application packages)
Contact Center Telephone 800-518-4726

Web ticketing system https //grants-portal psc gov/ContactUs aspx
Email support@grants gov

GrantsInfo (Questions regarding application instructions and process, finding NIH grant resources)
Telephone 301-435-0714
TTY 301-451-5936
Email GrantsInfo@nih gov

Scientific/Research Contact(s)

Participating NIH Institutes and Centers are listed on the first page of this announcement ("Components of Participating Organizations") Scientific/Research Contact information is listed in the table at http //grants nih gov/grants/guide/contacts/parent_R01 html

Peer Review Contact(s)

Examine your eRA Commons account for review assignment and contact information (information appears two weeks after the submission due date)

Financial/Grants Management Contact(s)

Participating NIH Institutes and Centers are listed on the first page of this announcement ("Components of Participating Organizations") Financial/Grants Management Contact information is listed in the table at http //grants nih gov/grants/guide/contacts/parent_R01 html

Section VIII. Other Information

Recently issued trans-NIH policy notices may affect your application submission A full list of policy notices published by NIH is provided in the NIH Guide for Grants and Contracts All awards are subject to the terms and conditions, cost principles, and other considerations described in the *NIH Grants Policy Statement*

Authority and Regulations

Awards are made under the authorization of Sections 301 and 405 of the Public Health Service Act as amended (42 USC 241 and 284) and under Federal Regulations 42 CFR Part 52 and 45 CFR Parts 74 and 92

Weekly TOC for this Announcement
NIH Funding Opportunities and Notices

National Institutes of Health
Office of Extramural Research

Department of Health and Human Services (HHS)

USA.gov Government Made Easy

NIH Turning Discovery Into Health®

Note For help accessing PDF, RTF, MS Word, Excel, PowerPoint, Audio or Video files, see Help Downloading Files

Principal Investigator/Program Director *(Last,first, middle)* Li, Fang

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 07/01/2016 THROUGH 06/30/2017

PERSONNEL *(Applicant organization only)*		Months Devoted to Project				DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	Cal Mnths	Acad Mnths	Sum Mnths	INST BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Li, Fang	Prin Investigator	3 00	0 00	0 00	128,135	32,033	10,795	42,828
TO BE NAMED	Post Doc Researcher	12 00	0 00	0 00	40,000	40,000	8,959	48,959
TO BE NAMED	Post Doc Researcher	9 00	0 00	0 00	40,000	30,000	6,719	36,719
TO BE NAMED	Jr Scientist	6 00	0 00	0 00	32,000	16,000	4,383	20,383
SUBTOTALS →						118,033	30,856	148,889

CONSULTANT COSTS		0
EQUIPMENT *(Itemize)*		0
SUPPLIES *(Itemize by category)* Molecular biology supplies ($15,000), Cell culture supplies ($11,000), Protein purification supplies ($11,000), Protein crystallization supplies ($15,000), Plastic & glassware supplies ($8,611)		60,611
TRAVEL PI and post doc travel to meetings to present results ($3,000)		3,000
PATIENT CARE COSTS	INPATIENT	0
	OUTPATIENT	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		0
OTHER EXPENSES *(Itemize by category)* Publication and reprints ($2,200), Equipment repair & maintenance services ($2,500), Lab services ($2,500), Communications ($300)		7,500
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	80,000
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 300,000
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATION COSTS	41,600
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 341,600

245,000 IDC base 52% 127,400

469,000

Principal Investigator/Program Director *(Last,first. middle)* Li, Fang

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 07/01/2017 THROUGH 06/30/2018

PERSONNEL *(Applicant organization only)*		Months Devoted to Project				DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	Cal Mnths	Acad Mnths	Sum Mnths	INST BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Li, Fang	Prin Investigator	3 00	0 00	0 00	128,135	32,033	10,795	42,828
TO BE NAMED	Post Doc Researcher	12 00	0 00	0 00	40,000	40,000	8,959	48,959
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SUBTOTALS →						118,033	30,856	148,889

CONSULTANT COSTS		0
EQUIPMENT *(Itemize)*		0
SUPPLIES *(Itemize by category)* Molecular biology supplies ($15,000), Cell culture supplies ($11,000), Protein purification supplies ($11,000), Protein crystallization supplies ($15,000), Plastic & glassware supplies ($8,611)		60,611
TRAVEL PI and post doc travel to meetings to present results ($3,000)		3,000
PATIENT CARE COSTS	INPATIENT	0
	OUTPATIENT	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		0
OTHER EXPENSES *(Itemize by category)* Publication and reprints ($2,200), Equipment repair & maintenance services ($2,500), Lab services ($2,500), Communications ($300)		7,500
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	80,000
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 300,000
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATION COSTS	41,600
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 341,600

IDC Base 220,000 53% 116,600

458,200

Principal Investigator/Program Director *(Last,first, middle)* Li, Fang

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 07/01/2018 THROUGH 06/30/2019

PERSONNEL *(Applicant organization only)*		Months Devoted to Project				DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	Cal Mnths	Acad Mnths	Sum Mnths	INST BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Li, Fang	Prin Investigator	3 00	0 00	0 00	128,135	32,033	10,795	42,828
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CONSULTANT COSTS		0
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PATIENT CARE COSTS	INPATIENT	0
	OUTPATIENT	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		0
OTHER EXPENSES *(Itemize by category)* Publication and reprints ($2,200), Equipment repair & maintenance services ($2,500), Lab services ($2,500), Communications ($300)		7,500
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	80,000
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 300,000
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATION COSTS	41,600
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 341,600

54% 118,800
460,400
IDC Base 220,000

Principal Investigator/Program Director *(Last,first, middle)* Li, Fang

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 07/01/2019 THROUGH 06/30/2020

PERSONNEL *(Applicant organization only)*		Months Devoted to Project				DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	Cal Mnths	Acad Mnths	Sum Mnths	INST BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Li, Fang	Prin Investigator	3 00	0 00	0 00	128,135	32,033	10,795	42,828
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TRAVEL PI and post doc travel to meetings to present results ($3,000)		3,000
PATIENT CARE COSTS	INPATIENT	0
	OUTPATIENT	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		0
OTHER EXPENSES *(Itemize by category)* Publication and reprints ($2,200), Equipment repair & maintenance services ($2,500), Lab services ($2,500), Communications ($300)		7,500
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	80,000
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 300,000
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATION COSTS	41,600
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 341,600

220,000 IDC Base

54% 118,800

460,400

Principal Investigator/Program Director *(Last,first, middle)* Li, Fang

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 07/01/2020 THROUGH 06/30/2021

PERSONNEL *(Applicant organization only)*		Months Devoted to Project				DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	Cal Mnths	Acad Mnths	Sum Mnths	INST BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Li, Fang	Prin Investigator	3 00	0 00	0 00	128,135	32,033	10,795	42,828
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TO BE NAMED	Jr Scientist	6 00	0 00	0 00	32,000	16,000	4,383	20,383
SUBTOTALS →						118,033	30,856	148,889

CONSULTANT COSTS	0
EQUIPMENT *(Itemize)*	0
SUPPLIES *(Itemize by category)* Molecular biology supplies ($15,000), Cell culture supplies ($11,000), Protein purification supplies ($11,000), Protein crystallization supplies ($15,000), Plastic & glassware supplies ($8,611)	60,611
TRAVEL PI and post doc travel to meetings to present results ($3,000)	3,000
PATIENT CARE COSTS — INPATIENT	0
PATIENT CARE COSTS — OUTPATIENT	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	0
OTHER EXPENSES *(Itemize by category)* Publication and reprints ($2,200), Equipment repair & maintenance services ($2,500), Lab services ($2,500), Communications ($300)	7,500
CONSORTIUM/CONTRACTUAL COSTS — DIRECT COSTS	80,000
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*	**$ 300,000**
CONSORTIUM/CONTRACTUAL COSTS — FACILITIES AND ADMINISTRATION COSTS	41,600
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD	**$ 341,600**

IDC Base 54% 118,800
220,000 460,400

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL *Salary and fringe benefits Applicant organization only*		148,889	148,889	148,889	148,889	148,889
CONSULTANT COSTS		0	0	0	0	0
EQUIPMENT		0	0	0	0	0
SUPPLIES		60,611	60,611	60,611	60,611	60,611
TRAVEL		3,000	3,000	3,000	3,000	3,000
PATIENT CARE COSTS	INPATIENT	0	0	0	0	0
	OUTPATIENT	0	0	0	0	0
ALTERATIONS AND RENOVATIONS		0	0	0	0	0
OTHER EXPENSES		7,500	7,500	7,500	7,500	7,500
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	80,000	80,000	80,000	80,000	80,000
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*		300,000	300,000	300,000	300,000	300,000
CONSORTIUM/ CONTRACTUAL COSTS	F & A	41,600	41,600	41,600	41,600	41,600
TOTAL DIRECT COSTS		341,600	341,600	341,600	341,600	341,600
TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD						$ 1,708,000

JUSTIFICATION Follow the budget justification instructions exactly Use continuation pages as needed

***** BUDGET NUMBER JUSTIFICATIONS *****

Year 1 $245,000 x 52% = 127,400

Year 2 $220,000 x 53% = 116,600

Year 3 $220,000 x 54% = 118,800

Year 4 $220,000 x 54% = 118,800

Year 5 $220,000 x 54% = 118,800

Total To Apply F&A Rate = Total Direct Costs - Total Backed Out Costs

1,125,000 = 1,708,000 - 583,000

Total F&A Costs = 600,400

Total Costs = Total Direct Costs + Total F&A Costs

2,308,400 = 1,708,000 + 600,400

600,400
2,308,400

Future years recurring costs have been calculated using a 0% inflation rate, unless noted otherwise in the item justification

Base salaries listed are for the current University appointment Calculation of the amount of salary requested in each budget period includes inflation effective July 1 of each fiscal year, prorated to the budget period, and adjusted if appointment is less than 100% If this award is reduced, effort obligations and proposed cost sharing (matching or in-kind) will be reduced proportionately

FISCAL YEAR	ACADEMIC	CIVIL SERVICE	TRADES/TEMP
2014	33 60%	36 80%	N/A
2015	33 80%	26 30%	7 70%
2016	33 70%	27 40%	7 90%

FISCAL YEAR	POST DOCS	GRAD ASSIST	UNDERGRADS
2014	N/A	N/A	N/A
2015	21 40%	16 60%	0 0%
2016	22 40%	17 60%	0 0%

GRADUATE/PROFESSIONAL TRAINING STUDENTS FRINGE RATES
The tuition remission component of fringe varies by individual based on the number of credits

For more information about fringe rates, check the SPA webpage at www ospa edu

********** BUDGET SETUP JUSTIFICATION(S) **********
Future years recurring costs have been calculated using a 0% inflation rate, unless noted otherwise in the item justification

Justification -

********** BUDGET PERSONNEL JUSTIFICATION(S) **********
Li, Fang , Principal Investigator
- Fang Li, PhD, Principal Investigator (3 0 calendar months), 25% effort, will be responsible for laboratory management and supervision of the projects, including experimental design, execution, data analysis, and write-up

TO BE NAMED, Post Doc Researcher
- One Postdoctoral Scientist (To be named) (12 00 calendar months), 100% effort, will be responsible for conducting experiments on (i) crystallographic studies of coronavirus spike RBDs and their receptors, (ii) electron microscopic studies of coronavirus spike proteins

TO BE NAMED, Post Doc Researcher
- One Postdoctoral Scientist (To be named) (9 00 calendar months), 75% effort, will be responsible for conducting experiments on (i) biochemical studies of the interactions between coronavirus spike RBDs and their receptors, (ii) coronavirus-spike-mediated pseudovirus entry into cells

TO BE NAMED, Jr Scientist
- One Junior Scientist (To be named) (6 00 calendar months), 50% effort, will be responsible for conducting experiments on construction, expression, and purification of coronavirus spike RBDs and their receptors

********** SUPPLIES JUSTIFICATION(S) **********
Molecular biology supplies
- Research supplies will include reagents for molecular biology, insect and mammalian cell culture, protein expression and purification, protein-protein binding assays, pseudovirus entry assays, negative-stain electron microscopy, and protein crystallography More specifically, these reagents will include cloning enzymes, restriction enzymes, plasmid purification kits, DNA retraction kits, DNA transfection reagents, insect cells, insect cell culture media, mammalian cells, mammalian cell culture media, protein purification columns, Biacore chips, electron microscopy grids and tools, crystallization kits and plates, and crystal freezing tools

Cell culture supplies
- Research supplies will include reagents for molecular biology, insect and mammalian cell culture, protein expression and purification, protein-protein binding assays, pseudovirus entry assays, negative-stain electron microscopy, and protein crystallography More specifically, these reagents will include cloning enzymes, restriction enzymes, plasmid purification kits, DNA retraction kits, DNA transfection reagents, insect cells, insect cell culture media, mammalian cells, mammalian cell culture media, protein purification columns, Biacore chips, electron microscopy grids and tools, crystallization kits and plates, and crystal freezing tools

Protein purification supplies
- Research supplies will include reagents for molecular biology, insect and mammalian cell culture, protein expression and purification, protein-protein binding assays, pseudovirus entry assays, negative-stain electron microscopy, and protein crystallography More specifically, these reagents will include cloning enzymes, restriction enzymes, plasmid purification kits, DNA retraction kits, DNA transfection reagents, insect cells, insect cell culture media, mammalian cells, mammalian cell culture media, protein purification columns, Biacore chips, electron microscopy grids and tools, crystallization kits and plates, and crystal freezing tools

Protein crystallization supplies
- Research supplies will include reagents for molecular biology, insect and mammalian cell culture, protein expression and purification, protein-protein binding assays, pseudovirus entry assays, negative-stain electron microscopy, and protein crystallography More specifically, these reagents will include cloning enzymes, restriction enzymes, plasmid purification kits, DNA retraction kits, DNA transfection reagents, insect cells, insect cell culture media, mammalian cells, mammalian cell culture media, protein purification columns, Biacore chips, electron microscopy grids and tools, crystallization kits and plates, and crystal freezing tools

Plastic & glassware supplies
- Research supplies will include reagents for molecular biology, insect and mammalian cell culture, protein expression and purification, protein-protein binding assays, pseudovirus entry assays, negative-stain electron microscopy, and protein crystallography More specifically, these reagents will include cloning enzymes, restriction enzymes, plasmid purification kits, DNA retraction kits, DNA transfection reagents, insect cells,

insect cell culture media, mammalian cells, mammalian cell culture media, protein purification columns, Biacore chips, electron microscopy grids and tools, crystallization kits and plates, and crystal freezing tools

********** TRAVEL COSTS JUSTIFICATION(S) **********

PI and post doc travel to meetings to present results

- For the PI, the postdoctoral scientists and junior scientist, on average there will be two trips to National synchrotron laboratories for X-ray data collection, and one trip to a national conference per year

********** OTHER EXPENSES JUSTIFICATION(S) **********

Publication and reprints

- On average three publications will be expected per year

Equipment repair & maintenance services

- Purchase of service contracts for equipments such as AKTA purification system, centrifuges, plate readers, and insect cell shaker/incubators

Lab services

- Use of services from several on-campus labs, such as the electron microscopy lab, robot crystallization lab, mass spectrometry lab, and protein sequencing lab

Communications

- Fax, long distance phone call, and shipping of experimental materials to collaborators

********** CONTRACTOR COSTS JUSTIFICATION(S) **********

The University of North Carolina at Chapel Hill

Period 1	Direct Costs $80,000	Indirect Costs $41,600
Period 2	Direct Costs $80,000	Indirect Costs $41,600
Period 3	Direct Costs $80,000	Indirect Costs $41,600
Period 4	Direct Costs $80,000	Indirect Costs $41,600
Period 5	Direct Costs $80,000	Indirect Costs $41,600

- U of NC at Chapel Hill ICR rate at 52%

Erin Jondahl <jond0006@umn.edu>

IBC Approval Needed for PRF 835469

1 message

Erin Jondahl <jond0006@umn edu> Tue, Nov 3, 2015 at 11 10 AM
To Fang Li <lifang@umn edu>, Lynn Weber <weber117@umn edu>, ibc Institutional Biosafety Committee <ibc@umn edu>

Title Receptor recognition and cell entry of coronaviruses
PI· Fang Li
Sponsor NIH
PRF 835469

This email is to advise you that SPA was unable to locate an approved IBC protocol for this project during the proposal review process Should this proposal be funded, no funds may be expended for activities involving recombinant DNA, infectious agents or biological toxins until Institutional Biosafety Committee (IBC) approval has been obtained

The Principal Investigator should contact the Institutional Biosafety Committee at ibc@umn edu or call the RSPP office at (612) 626-5654 with any questions or concerns regarding the status of the IBC protocol for this proposal

--

Erin Jondahl
Grants & Contracts Administrator
Sponsored Project Administration
University of Minnesota
McNamara Alumni Center
200 Oak Street SE, Suite 450
Minneapolis, MN 55455

Phone 612 626-2724
Fax 612 624 4843
Email jond0006@umn edu

awardtrk Award Tracking <awardtrk@umn.edu>

Fwd: Auto verified electronic application AN # 3877580; PI: Li, Fang; Title: Receptor recognition and cell entry of coronaviruses; Grants.gov Tracking #: GRANT12031055

spamon <spamon@umn edu> Fri, Nov 6, 2015 at 4 47 AM
To Award Tracking <awardtrk@umn edu>

------ Original Message -------
Subject: Auto verified electronic application AN # 3877580, PI Li, Fang, Title Receptor recognition and cell entry of coronaviruses, Grants.gov Tracking # GRANT12031055
Date: Fri, 6 Nov 2015 00 03 32 -0500
From:<era-notify@mail nih gov>
Reply-To:<commons@od nih gov>
To:<awards@umn edu>

eRA has received grant application titled Receptor recognition and cell entry of coronaviruses, at least 2 business days have passed since application image was assembled and made available to you for viewing The grant application has now advanced to the next step - Referral

You can view and track the status of this grant application via the 'Status' module in eRA Commons (log into eRA Commons and select the 'Status' tab on the top blue menu bar)

If you have any questions about this email, please contact the eRA Help Desk via the web at http //era nih gov/help/, by phone 1-866-504-9552 (tty 301-451-5939) or commons@od nih gov

EnFILE

LI
835469
1055

GRANTS GOV

Home > Apply for Grants > Confirmation

Confirmation

Thank you for submitting your grant application package via Grants gov Your application is currently being processed by the Grants gov system Once your submission has been processed, Grants gov will send email messages to advise you of the progress of your application through the system Over the next 24 to 48 hours you should receive two emails The first will confirm receipt of your application by the Grants gov system and the second will indicate that the application has either been successfully validated by the system prior to transmission to the grantor agency or has been rejected due to errors

Please do not hit the back button on your browser

If your application is successfully validated and subsequently retrieved by the grantor agency from the Grants gov system. you will receive an additional email This email may be delivered several days or weeks from the date of submission depending on when the grantor agency retrieves it

You may also monitor the processing status of your submission within the Grants gov system by clicking on the Track My Application link listed at the end of this form

Note Once the grantor agency has retrieved your application from Grants gov, you will need to contact them directly for any subsequent status updates Grants gov does not participate in making any award decisions

IMPORTANT NOTICE If you do not receive a receipt confirmation and either a validation confirmation or a rejection email message within 48 hours, please contact us The Grants gov Contact Center can be reached by email at support@grants gov or by telephone at 1-800-518-4726 Always include your Grants gov tracking number in all correspondence The tracking numbers issued by Grants gov look like GRANTXXXXXXXX

If you have questions please contact the Grants gov Contact Center support@grants.gov
1-800-518-4726 24 hours a day, 7 days a week Closed on federal holidays

The following application tracking information was generated by the system

Grants gov Tracking Number	GRANT12031055
Applicant DUNS	55-59[illegible]-7996
Submitter's Name	Kevin McKoskey
CFDA Number	
CFDA Description	
Funding Opportunity Number	PA-13-302
Funding Opportunity Description	Research Project Grant (Parent R01)
Agency Name	National Institutes of Health
Application Name of this Submission	835469 Li VIH
Date/Time of Receipt	Nov 03 2015 05 09 03 PM EST

TRACK MY APPLICATION – To check the status of this application, please click the link below
http //www grants gov/web/grants/applicants/track-my-application html?tracking_num=GRANT12031055

It is suggested you Save and/or Print this response for your records

Grants. gov emails
1. 11.3 2. ___ 3. 11.3 4. 11.3 5. 11.3
ERA Commons emails
1. 11.3 2. ___ 3. ___ 4. ___
Notes: NIH due 11.5

7. 11.6.15

awardtrk Award Tracking <awardtrk@umn.edu>

GRANT12031055/Li, Fang: Check Assembled Application in eRA Commons

awardtrk Award Tracking <awardtrk@umn edu> Wed, Nov 4, 2015 at 8 47 AM
To Erin Jondahl <jond0006@umn edu>

GRANT12031055

No errors or warnings

-------- Forwarded message --------
From <era-notify@mail nih gov>
Date Tue, Nov 3, 2015 at 4 11 PM
Subject GRANT12031055/Li, Fang Check Assembled Application in eRA Commons
To lifang@umn edu, awards@umn edu

NIH has received the electronic grant application Grants gov Tracking # GRANT12031055 / PI Li, Fang and has placed the assembled application in the eRA Commons for your review **You have a viewing window of two business days (i.e., Monday - Friday, excluding federal holidays) to check the assembled application before it automatically moves forward to NIH staff for further processing and consideration.** Once the application has moved forward, no additional changes to the application will be accepted through Grants gov or eRA Commons

It is your responsibility to view the entire assembled application in eRA Commons and notify the eRA Commons Help Desk within this window if the assembled application does not correctly reflect the information submitted to Grants gov (e g , submitted information is missing in the image or a graph/chart appears upside-down) The eRA Commons Help Desk will provide guidance on appropriate corrective actions

Within the viewing window, signing officials have the authority to reject an application to stop it from completing the submission process After an application is rejected, a changed/corrected application can be submitted if it is still before the submission deadline However, changed/corrected applications submitted after the submission deadline will be subject to the NIH Late Policy and may not be accepted

View detailed steps for PI to track submission status
View detailed steps for AOR/SO to track submission status
View detailed steps for AOR/SO to reject an application
View detailed steps for submitting a changed/corrected application

If you still require support after taking full advantage of these resources, please contact the eRA Commons Help Desk

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number 4040-0001
Expiration Date 6/30/2016

ORGANIZATIONAL DUNS 5559179960000 **Enter name of Organization** Regents of the University of Minnesota

Budget Type: ☒ Project ☐ Subaward/Consortium **Budget Period 1** **Start Date.** 07/01/2016 **End Date** 06/30/2017

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Fang		Li	Ph D	128,135 00	3 00			32,033 00	10,795 00	42,828 00

Project Role PD/PI

Additional Senior Key Persons Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 42,828 00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	12 00			40,000 00	8,959 00	48,959 00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Postdoc Researcher	9 00			30,000 00	6,719 00	36,719 00
1	Jr Scientist	6 00			16,000 00	4,383 00	20,383 00

3 **Total Number Other Personnel**

Total Other Personnel 106,061 00

Total Salary, Wages and Fringe Benefits (A+B) 148,889 00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment | Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl Canada, Mexico and U S Possessions)	3,000 00
2. Foreign Travel Costs	
Total Travel Cost	3,000 00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5 Other	
Total Participant/Trainee Support Costs	

Number of Participants/Trainees

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	60,611 00
2.	Publication Costs	2,200 00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	121,600 00
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Equipment repair and maintenance services	2,500 00
9.	Lab services	2,500 00
10.	Communications	300 00
	Total Other Direct Costs	189,711 00

exclude 96,600

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	341,600 00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Modified Total Direct Costs	52 00	245,000 00	127,400 00

Total Indirect Costs 127,400 00

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Arif Karim (214) 767-3600

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	469,000 00

J. Fee

Funds Requested ($):

K. Budget Justification

(Only attach one file) R01-budget-justification-Li-2015.pdf [Add Attachment] [Delete Attachment] [View Attachment]

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number 4040-0001
Expiration Date 6/30/2016

ORGANIZATIONAL DUNS. 5559179960000 **Enter name of Organization:** Regents of the University of Minnesota

Budget Type: ☒ Project ☐ Subaward/Consortium **Budget Period 2** **Start Date** 07/01/2017 **End Date:** 06/30/2018 ✓

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal	Months Acad.	Months Sum	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Fang		Li	Ph D	128,135 00	3 00			32,033 00	10,795 00	42,828 00

Project Role PD/PI

Additional Senior Key Persons Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 42,828 00

B. Other Personnel

Number of Personnel	Project Role	Months Cal	Months Acad	Months Sum	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	12 00			40,000 00	8,959 00	48,959 00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Postdoc Researcher	9 00			30,000 00	6,719 00	36,719 00
1	Jr Scientist	6 00			16,000 00	4,383 00	20,383 00

3 **Total Number Other Personnel**

Total Other Personnel 106,061 00

Total Salary, Wages and Fringe Benefits (A+B) 148,889 00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment | Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl Canada, Mexico and U S Possessions)	3,000 00
2. Foreign Travel Costs	
Total Travel Cost	3,000 00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1 Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	

Number of Participants/Trainees | **Total Participant/Trainee Support Costs**

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	60,611 00
2	Publication Costs	2,200 00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	121,600 00
6	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Equipment repair and maintenance services	2,500 00
9.	Lab services	2,500 00
10.	Communications	300 00
	Total Other Direct Costs	189,711 00

exclude full amt.

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	341,600 00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Modified Total Direct Costs	53 00	220,000 00	116,600 00

Total Indirect Costs 116,600 00

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Arif Karim (214) 767-3600

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	458,200 00

J. Fee

Funds Requested ($):

K. Budget Justification

(Only attach one file) R01-budget-justification-L1-2015 pdf [Add Attachment] [Delete Attachment] [View Attachment]

RESEARCH & RELATED BUDGET - Budget Period 3

OMB Number 4040-0001
Expiration Date 6/30/2016

ORGANIZATIONAL DUNS 5559179960000 **Enter name of Organization.** Regents of the University of Minnesota

Budget Type ☒ Project ☐ Subaward/Consortium **Budget Period** 3 **Start Date.** 07/01/2018 **End Date.** 06/30/2019

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Fang		Li	Ph D	128,135 00	3 00			32,033 00	10,795 00	42,828 00

Project Role PD/PI

Additional Senior Key Persons Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 42,828 00

B. Other Personnel

Number of Personnel	Project Role	Cal	Acad.	Sum	Salary ($)	Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	12 00			40,000 00	8,959 00	48,959 00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Postdoc Researcher	9 00			30,000 00	6,719 00	36,719 00
1	Jr Scientist	6 00			16,000 00	4,383 00	20,383 00

3 **Total Number Other Personnel**

Total Other Personnel 106,061 00

Total Salary, Wages and Fringe Benefits (A+B) 148,889 00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment | Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl Canada, Mexico and U S Possessions)	3,000 00
2. Foreign Travel Costs	
Total Travel Cost	3,000 00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3 Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	60,611 00
2.	Publication Costs	2,200 00
3	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	121,600 00
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Equipment repair and maintenance services	2,500 00
9	Lab services	2,500 00
10.	Communications	300 00
	Total Other Direct Costs	189,711 00

exclude full amt

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	341,600 00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Modified Total Direct Costs	54 00	220,000 00	118,800 00

Total Indirect Costs 118,800 00

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Arif Karim (214) 767-3600

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	460,400 00

J. Fee

Funds Requested ($):

K. Budget Justification

(Only attach one file) R01-budget-justification-Li-2015.pdf [Add Attachment] [Delete Attachment] [View Attachment]

RESEARCH & RELATED BUDGET - Budget Period 4

OMB Number 4040-0001
Expiration Date 6/30/2016

ORGANIZATIONAL DUNS 5559179960000 **Enter name of Organization:** Regents of the University of Minnesota

Budget Type: ☒ Project ☐ Subaward/Consortium **Budget Period** 4 **Start Date** 07/01/2019 **End Date** 06/30/2020

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Fang		Li	Ph D	128,135 00	3 00			32,033 00	10,795 00	42,828 00

Project Role PD/PI

Additional Senior Key Persons Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 42,828 00

B. Other Personnel

Number of Personnel	Project Role	Months Cal	Months Acad.	Months Sum	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	12 00			40,000 00	8,959 00	48,959 00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Postdoc Researcher	9 00			30,000 00	6,719 00	36,719 00
1	Jr Scientist	6 00			16,000 00	4,383 00	20,383 00

3 **Total Number Other Personnel**

Total Other Personnel 106,061 00

Total Salary, Wages and Fringe Benefits (A+B) 148,889 00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment | Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

		Funds Requested ($)
1	Domestic Travel Costs (Incl Canada, Mexico and U S Possessions)	3,000 00
2.	Foreign Travel Costs	
	Total Travel Cost	3,000 00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Total Participant/Trainee Support Costs	

Number of Participants/Trainees

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	60,611 00
2.	Publication Costs	2,200 00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	121,600 00
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Equipment repair and maintenance services	2,500 00
9.	Lab services	2,500 00
10.	Communications	300 00
	Total Other Direct Costs	189,711 00

exclude full amt

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	341,600 00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Modified Total Direct Costs	54 00	220,000 00	118,800 00

Total Indirect Costs 118,800 00

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Arif Karim (214) 767-3600

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	460,400 00

J. Fee

Funds Requested ($):

K. Budget Justification

(Only attach one file) R01-budget-justification-Li-2015.pdf [Add Attachment] [Delete Attachment] [View Attachment]

RESEARCH & RELATED BUDGET - Budget Period 5

OMB Number 4040-0001
Expiration Date 6/30/2016

ORGANIZATIONAL DUNS 5559179960000 **Enter name of Organization.** Regents of the University of Minnesota

Budget Type ☒ Project ☐ Subaward/Consortium **Budget Period** 5 **Start Date** 07/01/2020 **End Date.** 06/30/2021

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal	Months Acad.	Months Sum	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Fang		Li	Ph D	128,135 00	3 00			32,033 00	10,795 00	42,828 00

Project Role PD/PI

Additional Senior Key Persons Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 42,828 00

B. Other Personnel

Number of Personnel	Project Role	Months Cal	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	12 00			40,000 00	8,959 00	48,959 00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Postdoc Researcher	9 00			30,000 00	6,719 00	36,719 00
1	Jr Scientist	6 00			16,000 00	4,383 00	20,383 00

3 **Total Number Other Personnel**

Total Other Personnel 106,061 00

Total Salary, Wages and Fringe Benefits (A+B) 148,889 00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment | Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl Canada, Mexico and U S Possessions)	3,000 00
2. Foreign Travel Costs	
Total Travel Cost	3,000 00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3 Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees / **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	60,611 00
2.	Publication Costs	2,200 00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	121,600 00
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Equipment repair and maintenance services	2,500 00
9.	Lab services	2,500 00
10.	Communications	300 00
	Total Other Direct Costs	189,711 00

exclude full amt

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	341,600 00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Modified Total Direct Costs	54 00	220,000 00	118,800 00

Total Indirect Costs 118,800 00

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Arif Karim (214) 767-3600

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	460,400 00

J. Fee

Funds Requested ($):

K. Budget Justification

(Only attach one file) R01-budget-justification-Li-2015 pdf [Add Attachment] [Delete Attachment] [View Attachment]

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		214,140 00
Section B, Other Personnel		530,305 00
Total Number Other Personnel	15	
Total Salary, Wages and Fringe Benefits (A+B)		744,445 00
Section C, Equipment		
Section D, Travel		15,000 00
1. Domestic	15,000 00	
2 Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3 Travel		
4. Subsistence		
5. Other		
6 Number of Participants/Trainees		
Section F, Other Direct Costs		948,555 00
1. Materials and Supplies	303,055 00	
2. Publication Costs	11,000 00	
3 Consultant Services		
4 ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	608,000 00	
6 Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	12,500 00	
9. Other 2	12,500 00	
10. Other 3	1,500 00	
Section G, Direct Costs (A thru F)		1,708,000 00
Section H, Indirect Costs		600,400 00
Section I, Total Direct and Indirect Costs (G + H)		2,308,400 00
Section J, Fee		

Budget justification

Personnel Justification

Fang Li, PhD, Principal Investigator (3 0 calendar months), 25% effort, will be responsible for laboratory management and supervision of the projects, including experimental design, execution, data analysis, and write-up

One Postdoctoral Scientist (To be named) (12 00 calendar months), 100% effort, will be responsible for conducting experiments on (i) crystallographic studies of coronavirus spike RBDs and their receptors, (ii) electron microscopic studies of coronavirus spike proteins

One Postdoctoral Scientist (To be named) (9 00 calendar months), 75% effort, will be responsible for conducting experiments on (i) biochemical studies of the interactions between coronavirus spike RBDs and their receptors, (ii) coronavirus-spike-mediated pseudovirus entry into cells

One Junior Scientist (To be named) (6 00 calendar months), 50% effort, will provide support in the expression and purification of coronavirus spike RBDs and their receptors

Research Supplies Justification

Research supplies will include reagents for molecular biology, insect and mammalian cell culture, protein expression and purification, protein-protein binding assays, pseudovirus entry assays, negative-stain electron microscopy, and protein crystallography More specifically, these reagents will include cloning enzymes, restriction enzymes, plasmid purification kits, DNA retraction kits, DNA transfection reagents, insect cells, insect cell culture media, mammalian cells, mammalian cell culture media, protein purification columns, Biacore chips, electron microscopy grids and tools, crystallization kits and plates, and crystal freezing tools

Travel

For the PI, the postdoctoral scientists and junior scientist, on average there will be two trips to National synchrotron laboratories for X-ray data collection, and one trip to a national conference per year

Publication

On average three publications will be expected per year

Communications

Fax, long distance phone call, and shipping of experimental materials to collaborators

Equipment maintenance

Purchase of service contracts for equipments such as AKTA purification system, centrifuges, plate readers, and insect cell shaker/incubators

Lab service fees

Use of services from several on-campus labs, such as the electron microscopy lab, robot crystallization lab, mass spectrometry lab, and protein sequencing lab

Form Approved Through 8/31/2015 OMB No 0925-0001

Department of Health and Human Services Public Health Services

SUBAWARD Grant Application

Do not exceed character length restrictions indicated

LEAVE BLANK—FOR PHS USE ONLY		
Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1 TITLE OF PROJECT *(Do not exceed 81 characters, including spaces and punctuation)*

Receptor recognition and cell entry of coronaviruses

2 RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☒ NO ☐ YES
(If 'Yes,' state number and title)

Number Title

3 SUBAWARD PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR

3a NAME (Last, first, middle)	3b DEGREE(S)	3h eRA Commons User Name
Baric, Ralph	PhD	ralph_baric

3c POSITION TITLE
Professor

3d **SUBAWARD** MAILING ADDRESS *(Street, city, state, zip code)*
Department of Epidemiology
University of North Carolina – Chapel Hill
CB# 7435, McGavran-Greenberg Hall
Chapel Hill, NC 27599-7435

3e DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Epidemiology/Microbiology/Immunology

3f MAJOR SUBDIVISION

3g TELEPHONE AND FAX *(Area code, number and extension)*
TEL 919-966-3895 FAX 919-966-2089

E-MAIL ADDRESS
rbaric@email unc edu

4 **SUBAWARD** HUMAN SUBJECTS RESEARCH	4a Research Exempt	If "Yes," Exemption No
☒ No ☐ Yes	☐ No ☐ Yes	

4b Federal-Wide Assurance No	4c Clinical Trial	4d NIH-defined Phase III Clinical Trial
	☐ No ☐ Yes	☐ No ☐ Yes

5 **SUBAWARD** VERTEBRATE ANIMALS ☒ No ☐ Yes 5a Animal Welfare Assurance No

6 **SUBAWARD** DATES OF PROPOSED PERIOD OF SUPPORT *(month day year—MM/DD/YY)*		7 **SUBAWARD** COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8 **SUBAWARD** COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a Direct Costs ($)	7b Total Costs ($)	8a Direct Costs ($)	8b Total Costs ($)
07/01/2016	06/30/2021	80000	121600	400000	608000

9 **SUBAWARD** APPLICANT ORGANIZATION

Name The University of North Carolina at Chapel Hill

Address Office of Sponsored Research (OSR)
Administrative Office Bldg , Suite 2200
104 Airport Drive, CB# 1350
Chapel Hill, NC 27599-1350

10 **SUBAWARD** TYPE OF ORGANIZATION
Public → ☐ Federal ☒ State ☐ Local
Private → ☐ Private Nonprofit
For-profit → ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11 **SUBAWARD** ENTITY IDENTIFICATION NUMBER
1566001393A1
DUNS NO 608195277 Cong District NC-004

12 **SUBAWARD** ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE

Name Robin Cyr

Title Director, Office of Sponsored Research

Address Office of Sponsored Research (OSR)
Administrative Office Bldg , Suite 2200
104 Airport Drive, CB# 1350
Chapel Hill. NC 27599-1350

Tel 919-966-3411 FAX 919-962-3352

E-Mail resadminosr@unc edu

13 **SUBAWARD** OFFICIAL SIGNING FOR APPLICANT ORGANIZATION

Name Barbara Entwisle. PhD

Title Vice Chancellor for Research

Address Office of Sponsored Research (OSR)
Administrative Office Bldg , Suite 2200
104 Airport Drive, CB# 1350
Chapel Hill NC 27599-1350

Tel 919-966-3411 FAX 919-962-3352

E-Mail resadminosr@unc edu

14 APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE I certify that the statements herein are true complete and accurate to the best of my knowledge and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application I am aware that any false fictitious or fraudulent statements or claims may subject me to criminal civil or administrative penalties

SIGNATURE OF SUBAWARD OFFICIAL NAMED IN 13 *(In ink Per signature not acceptable)*

Carol J. Burkhart

DATE 10/22/15

Barbara Entwisle
Vice Chancellor for Research

Updated 10/06/2015 Office of Sponsored Research Information Sheet

General Information		
Official Legal Name	The University of North Carolina at Chapel Hill	
Institutional Official	Barbara Entwisle Vice Chancellor for Research Office of Sponsored Research (OSR) 104 Airport Dr Ste 2200, CB #1350 Chapel Hill, NC 27599-1350 Phone - (919) 966-3411/3412 Fax - (919) 962-3352/5011	
Administrative Official (Contract & Grant Administrator)	Robin Cyr Associate Vice Chancellor for Research Director, Office of Sponsored Research Office of Sponsored Research (OSR) 104 Airport Dr Ste 2200, CB #1350 Chapel Hill, NC 27599-1350 Phone - (919) 966-3411 Fax - (919) 962-3352/5011 Email - resadminosr@unc edu	
Financial Officer	Vanessa Peoples Deputy Director, Office of Sponsored Research 104 Airport Dr Ste 2200, CB #1350 Chapel Hill, NC 27599-1350 Phone - (919) 966-3411/3412 Fax - (919) 962-3352/5011	
Email	resadminosr@unc edu	
Frequently Used Information for Sponsored Research		
NIH/PHS Animal Welfare Assurance Number:	A3410-01 (Expires 4/30/2017)	
Congressional District:	4th	
Dun & Bradstreet (DUNS) #	608195277	
Federal Tax (EIN) ID#	56-600-1393	
For Public Health Service (e g. NIH, HRSA, CDC, etc) use Entity Identification Number	1566001393A-1	
SAM (System for Award Management)	Expiration Date 07/26/2016	
Fiscal Year	July 1 to June 30	
Human Subjects DHHS #	FWA-4801	expires 6/30/2016
Institutional Profile No:	578206	
Mileage rate for private car	57 5 cents/mile	01/01/2015
NIH Salary Cap	$181,500	Effective 01/12/2014
	$183,300	Effective 01/11/2015
NSF Submitting Institutional Code	0029744000	
Responsible Conduct in Research	https //research unc edu/files/2012/11/research conduct pocket guide pdf	
Type of Organization	State Related Institution of Higher Education	
Miscellaneous Information for Sponsored Research		
AAALAC International Approval	#329 (date of accreditation 10/31/2014) https //research unc edu/files/2015/01/AAALAC-Approval-Letter-dated-103114 pdf	
Age Discrimination Policy	On file, signed 9/6/01	
Assurance of Compliance Title IX: (HHS 639-A)	On file, signed 9/27/76	
Board of Trustees member list	http //www unc edu/depts/trustees/	
Civil Rights Assurance (HHS 441) Reg. #	43-0520 Dated 1/11/65	
Certification of Liability Coverage	Dated 7/1/2014 – 6/30/2015 http //research unc edu/files/2012/11/UNCCH Certificate OfCoverage 2014-2015 pdf	
Cognizant Federal Agency for F&A Rate Agreement (For purposes of completing grant proposals, use this office's address on any applicable forms asking for info on F&A)	Department of Health and Human Services Division of Cost Allocation (202) 401-2808 Attn Darryl Mayes	
Commercial and Government Entity Code (CAGE)	4B856	
Conflict of Interest Info: **Individual Conflicts of Interest & Commitment** **Institutional Conflict of Interest**	http //policy sites unc edu/files/2013/04/Individual-COI-Policy pdf http //policy sites unc edu/files/2013/04/Institutional-COI-Policy pdf	

Export Control Policy page	http //policies unc edu/policies/export-controls/
Federal Interagency Committee on Education (FICE)	002974-00
Misconduct in Science Form PHS-6349	Dated 01/31/2014 Annual update
NAICS Code	611310
Number of UNC-CH Employees	11,964 (as of Fall 2014 - includes permanent full time and part time) http //oira unc edu/facts-and-figures/faculty-and-staff-data/employees-by-category-fall-2014/
Radioisotopes: NC License Number	068-214-1
Rehab. Act of 1973 (HHS 641)	On file, signed 6/22/77
Sex Discrimination Policy	On file, signed 9/6/01
Southern Association of Colleges and Schools Accreditation	Dated January 9, 2007 http //research unc edu/files/2013/03/CCM1_031561 pdf
Standard Industrial Code	8221
Student/Fellowship/Postdoctoral Link	http //research unc edu/offices/sponsored-research/resources/DATA_RES_OSR_STUDENTINFO
UNC-CH Institutional Info (Fact Book)	http //oira unc edu/facts-and-figures/

Institutional Financial Information

Include the UNC-CH account number and invoice number on all payments.
These addresses are for payments only. All other correspondence should be sent to:
Office of Sponsored Research at 104 Airport Drive, Suite 2200, Chapel Hill, NC 27599-1350

REMITTANCE ADDRESS (U S MAIL ONLY) University of North Carolina at Chapel Hill Office of Sponsored Research C/O Bank of America Lock Box Service P O Box 402420 Atlanta, GA 30384-2420	**COURIER ONLY:** Bank of America Lock Box Services Lock Box 402420 6000 Feldwood Road College Park, GA 30349

Institutional Banking Information	Contact Vanessa Peoples at vpeoples@email unc edu,(919) 962-3397
UNC-CH Single Audit web site (A-133) **UNC-CH Compliance Audit Procedures**	http //www ncauditor net/EPSWeb/Reports/Financial/FSA-2013-8730 pdf http //www ncauditor net/EPSWeb/Reports/Financial/FSA-2013-6020 pdf
UNC-CH Financial Reports	http //finance unc edu/reports-and-data/financial-statements-archive/
IRS 501-c (3) Tax Exemption	http //research unc edu/files/2013/03/CCM1_031605 pdf

Facilities and Administrative Cost Rates (F&A)

DHHS Agreement Date: May 16, 2012

Rates Effective: 7/1/12-6/30/16	On-Campus	Off-Campus* (Adjacent - within a 10-mile radius)	Off-Campus* (Remote)
Organized Research	52 00%	28 00%	26 00%
Instruction	50 00%	28 00%	26 00%
Other Sponsored Activities	36 00%	28 00%	26 00%
Clinical Trials (Federal)*	52 00%	28 00%	26 00%
Clinical Trials (non Federal)**	28 00%	28 00%	26 00%

* See OSR FAQs for an explanation of how to determine whether a sponsored research project is on or off campus http //research unc edu/offices/sponsored-research/faq/index htm

** F&A calculations for federally funded Clinical Trials are based on MTDC (see definition below)

*** F&A calculations for INDUSTRY funded Clinical Trials are based on TOTAL DIRECT COSTS with the exception of the Initial IRB review fee, which is excluded from F&A

F&A rates online	http //research unc edu/offices/sponsored-research/resources/DATA_RES_OSR_INFOSHEET#facilities

BASE: Modified total direct costs (MTDC), consisting of all salaries and wages, fringe benefits, materials and supplies, services, travel and sub grants and subcontracts up to $25,000 of each sub grant or subcontract (regardless of the period covered by the sub grant or subcontract) Modified total direct costs shall exclude equipment[1], capital expenditures, charges for patient care and tuition remission, long term rental costs of off-site facilities, scholarships, and fellowships as well as the portion of each sub grant and subcontract in excess of $25,000 (Reminder Mandatory in-state tuition for eligible GRAs on research grants must be excluded from the F&A cost base for each budget year) Note Equi*pment[1] is defined as an item costing $5,000 or greater*

Current F&A agreement:	http //research unc edu/files/2012/11/CCM3_036953 pdf

Fringe Benefits

http //research unc edu/offices/sponsored-research/resources/DATA_RES_OSR_INFOSHEET#fringe2

	Budget
For full-time permanent EPA/SPA employees	**22.741%**
Post Doc Appointees and Grad Students (Depends on type of Award)	**8 990%**

PLUS Health Insurance annual premium prorated to % of effort

	Monthly	Annually
Full-time Permanent EPA/SPA employees (ConnectCarolina Acct 515530) (e)	**$455.92**	**$5,471.00**
Post Doctoral Trainees (ConnectCarolina Acct 528150) (a)	**$364.44**	**$4,373.28**
Post Doctoral Research Associates (ConnectCarolina Acct 515510)(a)	**$364.44**	**$4,373.28**
Grad Student Health Insurance (ConnectCarolina Accts 515540, 569200)(a)(b)(c)(d)	**$269.50**	**$3,234.00**

(a) Post Doctoral rates include Student Health fee, but the Graduate Student rate does not

(b) See Business Manual for Student FICA Tax Exemption Policy	http //financepolicy unc edu/1111_1rd
(d) For information on Graduate Student Health Insurance eligibility	http //finance unc edu/news/ddd-graduate-student-health-insurance-program-enrollment-for-2015-2016-and-other-issues/
(e) For information on North Carolina Department of Health and Human Services (DHHS) approved fringe benefit rates	http //research unc edu/files/2014/09/FY-2016-UNC-CH-DHHS-approved-rates pdf

UNC Physicians & Associates (P&A) Supplemental Fringe Benefits

Allowable Benefit	Annual Rate	Unallowable Benefit
Employee Life Insurance	0 6240%	Dependent Life Insurance
Employee Accidental Death & Dismemberment	0 1152%	Dependent Accidental Death & Dismemberment
Employee Long Term Disability	0 8400%	Professional Liability Insurance
Employee Retirement	3 5000%	
Employee Medical Insurance	$1331 52	Dependent Medical Insurance
Employee Dental Insurance	$600 36	Dependent Dental Insurance
Employee Vision Insurance	$95 28	Dependent Vision Insurance

Note As a general guideline budget 27 831% (22 741% + 5 09%) of annual salary plus $7,498 16 ($5,471 00 + $2,027 16) prorated to employee % effort

OMB Number 4040-0001
Expiration Date 6/30/2016

RESEARCH & RELATED BUDGET - Budget Period 1

ORGANIZATIONAL DUNS 6081952770000 **Enter name of Organization:** The University of North Carolina at Chapel Hill

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period** 1 **Start Date.** 07/01/2016 **End Date** 06/30/2017

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Ralph	S	Baric	PhD	183,300 00	0 36			5,499 00	1,415 00	6,914 00

Project Role PD/PI

Additional Senior Key Persons Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 6,914 00

B. Other Personnel

Number of Personnel	Project Role	Months Cal	Months Acad	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	4 80			17,822 00	3,351 00	21,173 00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician, Trevor Scobey	4 80			14,859 00	5,568 00	20,427 00

2 **Total Number Other Personnel**

Total Other Personnel 41,600 00

Total Salary, Wages and Fringe Benefits (A+B) 48,514 00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment | Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl Canada, Mexico and U S Possessions)	3,000 00
2. Foreign Travel Costs	
Total Travel Cost	3,000 00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4 Subsistence	
5. Other	
Total Participant/Trainee Support Costs	

Number of Participants/Trainees

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	23,486 00
2.	Publication Costs	2,000 00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Maintenance Contracts and repair	3,000 00
9.		
10.		
	Total Other Direct Costs	28,486 00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	80,000 00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
MTDC	52 00	80,000 00	41,600 00

Total Indirect Costs 41,600 00

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	121,600 00

J. Fee

Funds Requested ($):

K. Budget Justification

(Only attach one file) Baric-Budget Justification-rsb pdf [Add Attachment] [Delete Attachment] [View Attachment]

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number 4040-0001
Expiration Date 6/30/2016

ORGANIZATIONAL DUNS: 6081952770000 **Enter name of Organization:** The University of North Carolina at Chapel Hill

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period 2** **Start Date:** 07/01/2017 **End Date.** 06/30/2018

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal	Months Acad.	Months Sum	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Ralph	S	Baric	PhD	183,300 00	0 36			5,499 00	1,415 00	6,914 00

Project Role PD/PI

Additional Senior Key Persons Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 6,914 00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad	Months Sum	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	4 80			17,822 00	3,351 00	21,173 00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician, Trevor Scobey	4 80			14,859 00	5,568 00	20,427 00

2 **Total Number Other Personnel**

Total Other Personnel 41,600 00

Total Salary, Wages and Fringe Benefits (A+B) 48,514 00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment | Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl Canada, Mexico and U S Possessions)	3,000 00
2. Foreign Travel Costs	
Total Travel Cost	3,000 00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1 Tuition/Fees/Health Insurance	
2. Stipends	
3 Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	23,486 00
2.	Publication Costs	2,000 00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Maintenance Contracts and repair	3,000 00
9.		
10.		
	Total Other Direct Costs	28,486 00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	80,000 00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
MTDC	52 00	80,000 00	41,600 00

Total Indirect Costs 41,600 00

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	121,600 00

J. Fee

Funds Requested ($):

K. Budget Justification

(Only attach one file) Baric-Budget Justification-rsb pdf [Add Attachment] [Delete Attachment] [View Attachment]

RESEARCH & RELATED BUDGET - Budget Period 3

OMB Number 4040-0001
Expiration Date 6/30/2016

ORGANIZATIONAL DUNS: 6081952770000 **Enter name of Organization** The University of North Carolina at Chapel Hill

Budget Type ☐ Project ☒ Subaward/Consortium **Budget Period** 3 **Start Date.** 07/01/2018 **End Date.** 06/30/2019

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal	Months Acad	Months Sum	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Ralph	S	Baric	PhD	183,300 00	0 36			5,499 00	1,415 00	6,914 00

Project Role PD/PI

Additional Senior Key Persons Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 6,914 00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad	Months Sum	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	4 80			17,822 00	3,351 00	21,173 00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician, Trevor Scobey	4 80			14,859 00	5,568 00	20,427 00

2 **Total Number Other Personnel**

Total Other Personnel 41,600 00

Total Salary, Wages and Fringe Benefits (A+B) 48,514 00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment | Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl Canada, Mexico and U S Possessions)	3,000 00
2. Foreign Travel Costs	
Total Travel Cost	3,000 00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2 Stipends	
3. Travel	
4. Subsistence	
5. Other	
Total Participant/Trainee Support Costs	

Number of Participants/Trainees

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	23,486 00
2.	Publication Costs	2,000 00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Maintenance Contracts and repair	3,000 00
9.		
10.		
	Total Other Direct Costs	28,486 00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	80,000 00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
MTDC	52 00	80,000 00	41,600 00

Total Indirect Costs 41,600 00

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	121,600 00

J. Fee

Funds Requested ($):

K. Budget Justification

(Only attach one file) Baric-Budget Justification-rsb pdf [Add Attachment] [Delete Attachment] [View Attachment]

RESEARCH & RELATED BUDGET - Budget Period 4

OMB Number 4040-0001
Expiration Date 6/30/2016

ORGANIZATIONAL DUNS 6081952770000 **Enter name of Organization:** The University of North Carolina at Chapel Hill

Budget Type. ☐ Project ☒ Subaward/Consortium **Budget Period:** 4 **Start Date** 07/01/2019 **End Date** 06/30/2020

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal	Months Acad	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Ralph	S	Baric	PhD	183,300 00	0 36			5,499 00	1,415 00	6,914 00

Project Role PD/PI

Additional Senior Key Persons Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 6,914 00

B. Other Personnel

Number of Personnel	Project Role	Months Cal	Months Acad.	Months Sum	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	4 80			17,822 00	3,351 00	21,173 00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician, Trevor Scobey	4 80			14,859 00	5,568 00	20,427 00

2 **Total Number Other Personnel**

Total Other Personnel 41,600 00

Total Salary, Wages and Fringe Benefits (A+B) 48,514 00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment | Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl Canada, Mexico and U S Possessions)	3,000 00
2. Foreign Travel Costs	
Total Travel Cost	3,000 00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4 Subsistence	
5. Other	

Number of Participants/Trainees | **Total Participant/Trainee Support Costs**

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	23,486 00
2.	Publication Costs	2,000 00
3.	Consultant Services	
4	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7	Alterations and Renovations	
8.	Maintenance Contracts and repair	3,000 00
9.		
10		
	Total Other Direct Costs	28,486 00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	80,000 00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
MTDC	52 00	80,000 00	41,600 00

Total Indirect Costs 41,600 00

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	121,600 00

J. Fee

Funds Requested ($):

K. Budget Justification

(Only attach one file) Baric-Budget Justification-rsb pdf [Add Attachment] [Delete Attachment] [View Attachment]

RESEARCH & RELATED BUDGET - Budget Period 5

OMB Number 4040-0001
Expiration Date 6/30/2016

ORGANIZATIONAL DUNS 6081952770000 **Enter name of Organization:** The University of North Carolina at Chapel Hill

Budget Type ☐ Project ☒ Subaward/Consortium **Budget Period** 5 **Start Date** 07/01/2020 **End Date** 06/30/2021

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Ralph	S	Baric	PhD	183,300 00	0 36			5,499 00	1,415 00	6,914 00

Project Role PD/PI

Additional Senior Key Persons Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 6,914 00

B. Other Personnel

Number of Personnel	Project Role	Months Cal	Months Acad.	Months Sum	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	4 80			17,822 00	3,351 00	21,173 00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician, Trevor Scobey	4 80			14,859 00	5,568 00	20,427 00

2 **Total Number Other Personnel**

Total Other Personnel 41,600 00

Total Salary, Wages and Fringe Benefits (A+B) 48,514 00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment | Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl Canada, Mexico and U S Possessions)	3,000 00
2. Foreign Travel Costs	
Total Travel Cost	3,000 00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Total Participant/Trainee Support Costs	

Number of Participants/Trainees

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	23,486 00
2.	Publication Costs	2,000 00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Maintenance Contracts and repair	3,000 00
9.		
10.		
	Total Other Direct Costs	28,486 00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	80,000 00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
MTDC	52 00	80,000 00	41,600 00

Total Indirect Costs 41,600 00

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	121,600 00

J. Fee

Funds Requested ($):

K. Budget Justification

(Only attach one file) Baric-Budget Justification-rsb pdf [Add Attachment] [Delete Attachment] [View Attachment]

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		34,570 00
Section B, Other Personnel		208,000 00
Total Number Other Personnel	10	
Total Salary, Wages and Fringe Benefits (A+B)		242,570 00
Section C, Equipment		
Section D, Travel		15,000 00
1. Domestic	15,000 00	
2 Foreign		
Section E, Participant/Trainee Support Costs		
1 Tuition/Fees/Health Insurance		
2 Stipends		
3 Travel		
4. Subsistence		
5 Other		
6 Number of Participants/Trainees		
Section F, Other Direct Costs		142,430 00
1 Materials and Supplies	117,430 00	
2. Publication Costs	10,000 00	
3 Consultant Services		
4 ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6 Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8 Other 1	15,000 00	
9 Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		400,000 00
Section H, Indirect Costs		208,000 00
Section I, Total Direct and Indirect Costs (G + H)		608,000 00
Section J, Fee		

BUDGET JUSTIFICATION – BARIC UNC

PERSONNEL ($48,514):

Ralph S. Baric, PhD, Consortium PI (0 36 calendar months, 3% effort) Dr Baric is an expert in human emerging coronavirus (SARS-CoV/MERS-CoV) replication, cross species transmission, genetics and pathogenesis and his group is well positioned to conduct the studies proposed in the application His group has developed molecular clones to a large number of human and animal coronaviruses, including the strains germane to this proposal Dr Baric will work closely with Drs Li to conduct the basic research on virus-receptor interactions, protease usage and entry Within his own group, he will interact closely with Dr Kocher and Mr Scobey to ensure steady progress during the course of the proposal, evaluate results and propose alternative experiments Dr Baric will communicate his group's findings, share data, discuss and solve problems by interacting with the rest of the scientific team at monthly meetings and on at an ad hoc basis for specialized topics that are in need immediate solutions Drs Baric and Li have worked together over the past year, published papers on MERS-CoV RBD structure and function and in studying coronavirus cross species transmission Dr Baric and the members of his group will attend yearly meetings to discuss the progress of the project

Dr Baric's salary reflects the current NIH salary cap of $183,300 The remainder of his salary will be supported by other institutional funds

Jacob Kocher, PhD, Postdoctoral Research Associate (4 8 calendar months, 40% effort) Dr **Kocher** will assist in the generation of recombinant viruses including panels of mutants with defects in receptor binding and/or protease processing He has considerable expertise in working in a BSL3 facility, has considerable experience working with human and animal coronaviruses, appropriate cell culture systems and entry based assays He will work closely with Dr Baric, help direct and follow the progress of Mr Scobey to coordinate recombinant virus production, phenotypic characterization, sample collection and data interpretation He will assist in the experimental workflow He will attend lab meetings, present data and present findings/write papers, and participate in group meetings

Mr. Drew Scobey, Research Technician (4 8 calendar months, 40% effort) Mr Scobey is certified to work with high containment pathogens under BSL3 and has considerable experience in working with coronavirus under BSL2 conditions as well He is expert in recombinant virus recover, virus enumeration, cell fractionation, FACs based analyses, safety testing of samples, and in the preparation of RNA and protein samples He developed the MERS-CoV molecular clone in our laboratory Thus, he is well versed in molecular biology and virus reverse genetics and has isolated and characterized a large number of coronavirus mutants He will also assist and help Dr Kocher in the genetic and phenotypic characterization of mutants Finally, he will ensure that the BSL3 and BSL2 laboratory is well stocked and compliant in terms of regulations and day to day maintenance

Fringe Benefits Faculty/Staff 22 741% Social Security and Retirement, $5,471/FTE Health Insurance Post-doctoral Research Associates 8 99% Social Security and benefits, $4,373/FTE Health Insurance

EQUIPMENT: None

SUPPLIES ($23,486):

Synthetic DNAs and Sequencing Costs ($4,000/year) The Baric lab routinely uses synthetic genomics for the creation of virus mutants potentially used in validation studies, as it is cheaper and faster than using standard recombinant DNA approaches (considering personnel time, resequencing costs, time, materials, etc) Synthetic DNAs will be used to build new virologic reagents for measuring the impact of target mutations on receptor interactions, protease processing and entry Synthetic DNAs are primarily purchased from small biotec companies like Blue Heron or Bio Basic Inc at costs of about 0 30$/base, sufficient for our needs over the course of this project We will also require funds for sequence validation of recombinant viruses

Serum and Media ($5,500/year). A large amount of cell culture work is associated with the project, requiring media, serum and culturing flasks This includes virus tittering and neutralization assays Consequently, funds are requested for cell culture supplies to maintain Vero and related permissive cells for each coronavirus under study, and for measuring virus growth kinetics, evaluate entry kinetics and virus viability, titer virus stocks, and to isolate recombinant viruses for in vitro characterization or other phenotypes These monies support cell culture work in BSL2 and BSL3 facilities

Personnel Protective Gear ($3,000/year) PAPRs, tyvek suits and other protective gear Personnel wear powered air purifying HEPA filtered breathing apparatuses, wear tyvek suits, tyvek aprons, hoods, booties and are double gloved when entering the BSL3 facility These materials are expensive as the HEPA, organic chemical filters and even batteries must be replaced every ~6-12 months, and the tyvek suits are disposable Moreover, the PAPRs (powered air breathing apparatus) are expensive and must be replaced every ~2 years from normal wear and tear, and daily contact with EPA disinfectants Personnel also use high quantities of EPA approved disinfectants like ethanol alcohol, Clorox and other EPA approved disinfectants in maintaining a safe working environment in the BSL3 Personnel spray down tyvek suits, etc with alcohol or related disinfectants in the process of deconing and leaving the BSL3 facility All materials that leave the BSL3 must be disinfected, packaged in disinfected, sealed containers which are disinfected prior to removal from the BSL3 facility

Consumables ($4,500/year). A large amount of plastic ware will be consumed during the course of this project including eppendorf tubes, flasks, plates, pipettes, pipette tips, etc This includes plastic ware for virus growth kinetics, plaque assays, TCID50 quantization's, etc used to evaluate and compare virus growth efficiency and to evaluate neutralization titers following vaccination Small quantities of chemicals will be purchased, including Brefeldin-A and formalin for fixing cells

Enzymes, Kits and Reagents ($2,800/year) Oligodeoxynucleotide synthesis and kits for quantitative RT-PCR for measuring concentrations of viral and discrete cellular mRNAs will be used during the course of the project A variety of reagents needed for tissue fixation and immunohistochemistry A variety of chemicals will be purchased during the course of the project that will be used for making buffers, reagents, and other solutions These costs include reagents (e g , plasticware/EIA kits, etc) for EIA analyses A variety of antibodies and immunological reagents will be used during the course of the proposal to characterize S topology in the context of recombinant viruses

Miscellaneous ($2,186/yr). Funds are requested to purchase chemicals, reagents, paper products, gloves, micropipetors, autoclave supplies, plastic tips, waterbaths, and other small equipment items that typically have short half lives in laboratory settings

Computers ($1,500/year). Funds are requested to provide NoV specific computer hardware and software updates required over the course of the project Statistical data analysis and modeling of data require up to date equipment and software

TRAVEL ($3,000/year)
Scientific Meetings Travel funds are requested for the PI and fellow to attend the scientific meeting each year, such as ASV, during the course of the proposal and visit University of Minnesota This allows program faculty to communicate results, develop collaborations and share research interests

OTHER EXPENSES ($5,000)

Maintenance and Service Contracts ($3,000/year). Autoclave service contract and BSL3 maintenance costs for decontaminating/maintaining the facility are requested for each year of the program These dollars are critical for maintaining a safe environment for BSL3 research and for maintenance contracts for the BSL-2 equipment, like the autoclave in the BSL3

Publication Costs ($2,000/year). Funds are requested for publication of manuscripts associated with this research

F&A In an agreement with DHHS dated 5/16/2012 the indirect cost rate for The University of North Carolina is 52% of MTDC, excluding equipment and tuition

A. COVER PAGE

Project Title: Receptor recognition and cell entry of coronaviruses	
Grant Number: 5R01AI089728-07	**Project/Grant Period:** 06/07/2016 - 05/31/2021
Reporting Period: 06/07/2016 - 05/31/2017	**Requested Budget Period:** 06/01/2017 - 05/31/2018
Report Term Frequency: Annual	**Date Submitted:** 04/14/2017
Program Director/Principal Investigator Information: FANG LI , PHD **Phone number:** 612-625-6149 **Email:** lifang@umn.edu	**Recipient Organization:** UNIVERSITY OF MINNESOTA UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS, MN 554552070 **DUNS:** 555917996 **EIN:** 1416007513A1 **RECIPIENT ID:** 906521
Change of Contact PD/PI: N/A	
Administrative Official: ERIN E KNUDSEN 450 McNamara Alumni Center 200 Oak Street SE Minneapolis, MN 55455 **Phone number:** 612-624-5599 **Email:** jond0006@umn.edu	**Signing Official:** KEVIN MCKOSKEY 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS, MN 554552070 **Phone number:** 612-624-5599 **Email:** kevin@umn.edu
Human Subjects: No	**Vertebrate Animals:** No
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Specific Aims
Coronaviruses (CoVs) cause epidemic outbreaks of diseases in humans and other animals, symbolized by the recent emergence of SARS-CoV, MERS-CoV, and porcine epidemic diarrhea CoV (PEDV). Receptor recognition and cell entry are two essential steps in the CoV infection cycle and function as critical determinants of CoV host range and tissue tropism. They are also primary targets for antiviral intervention. An envelope-anchored CoV spike protein recognizes host receptors through its S1 subunit and fuses viral and host membranes through its S2 subunit. CoVs recognize a variety of host receptors through one or both of its spike S1 domains (S1-NTD and S1-CTD), and are also regulated for cell entry by host proteases that cleave at the S1/S2 boundary. In the previous funding cycle, we have determined the crystal structures of a number of CoV S1 domains alone or in complex with their receptor. We have also identified host receptors as well as the cell entry mechanisms for a number of different CoVs. Our studies have revealed how CoVs exploit host receptors and proteases to regulate their host range, tropism and pathogenesis, and how SARS-CoV and MERS-CoV transmit from animals to humans to cause epidemics. In this competitive grant renewal, we will continue to investigate fundamental questions regarding receptor recognition and cell entry of CoVs from different genera. The long-term goals of these studies are to develop global models of CoV-receptor interactions and protease processing in evolution, entry, tropism, pathogenesis and cross-species transmission of CoVs.

Aim 1: Receptor binding by CoV S1-NTDs. We previously determined the structures of beta-genus mouse CoV (MHV) S1-NTD complexed with its protein receptor CEACAM1 and of beta-genus bovine CoV (BCoV) S1-NTD by itself. We also showed that S1-NTDs of BCoV and alpha-genus PEDV both recognize sugar receptors. Our studies revealed that beta-CoV S1-NTDs have the same structural fold as human galectins (galactose-binding lectins), but bind either protein or non-galactose sugar receptors. This aim will investigate how S1-NTDs from different genera have evolved to bind sugar receptors. Our overall hypothesis is that S1-NTDs from different CoV genera originated from host galectins, but have evolved molecular mechanisms to recognize novel receptors. To test this hypothesis, we will determine the molecular and structural basis for sugar binding by BCoV and PEDV S1-NTDs. These studies will reveal evolution of CoV S1-NTDs, help evaluate sugar binding by other CoVs, and may facilitate future design of sugar analogue inhibitors and subunit vaccines against CoVs.

Aim 2: Receptor biding by CoV S1-CTDs. We determined the structures of several CoV S1-CTDs in complex with their protein receptors, including beta-genus SARS-CoV S1-CTD complexed with its protein receptor ACE2. Our research suggested that two mutations played critical roles in the transmission of SARS-CoV from palm civets to humans. However, recent studies isolated several bat SARS-like CoVs (SLCoVs) with potentials to infect humans directly. This aim will investigate the cross-species transmission pathway of bat SLCoVs by examining the interactions between their S1-CTDs and ACE2 from different species. Our overall hypothesis is that to transmit from bats to another species, bat SLCoVs need to adapt to ACE2 from that species through selective mutations in their S1-CTD. To test the hypothesis, we will (i) identify receptor and viral determinants for transmission of bat SLCoVs to other species (civet, human and mouse); (ii) determine the crystal structures of bat SLCoV S1-CTDs and ACE2 from other species. These studies will reveal the molecular mechanisms for cross-species transmission of bat SLCoVs, inform future bat SLCoV emergence pathways to other mammals, and facilitate epidemic monitoring and the development of broadly cross-protective intervention strategies.

Aim 3: Cell entry by CoVs. Previously we characterized the pre-fusion and post-fusion conformations of SARS-CoV spike, demonstrated that host proteases serve as a cross-species barrier for MERS-CoV, and showed that a glycosylated protease motif attenuates human cell entry of bat CoV HKU4. Our overall hypothesis is that CoV spikes have evolved a variety of mechanisms for protease activation that can meet their specific need for host range, tissue tropism, and pathogenesis. We will investigate the following questions regarding CoV entry. (i) How does neurotropic MHV enter neurons where receptor expression is low? (ii) How does HCoV-NL63 modulate its cell entry and pathogenesis? (iii) How does PEDV enter intestinal cells where extracellular proteases are abundant? These studies will reveal how cell entry regulates the host range, tropism and pathogenesis of CoVs, and may facilitate future design of protease inhibitors against CoVs.

Approaches: We take multi-disciplinary approaches including crystallographic studies of CoV S1 domains complexed with their receptor, biochemical assays on S1 domains/receptor binding, biochemical analysis of protease cleavages of CoV spikes, electron microscopic analysis of conformational states of CoV spikes, pseudovirus entry assays, reverse genetics, and live virus infection assays.

Impact: This research uses CoVs as model systems to establish novel principles in virology that govern viral evolution, receptor recognition, cell entry, cross-species infections and tropism, which may be extended to other virus families. These studies are also critical for evaluating the emerging disease potentials of CoVs and for preventing, controlling and treating the spread of CoVs in humans and other animals.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Fang_Li_NIH_Report_03272017.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

For the next funding year, we will focus on the following studies on the receptor recognition and cell entry of coronaviruses.

First, we will determine the cryo-EM structures of spike proteins from a number of coronaviruses including SARS coronavirus, avian infectious bronchitis virus, and porcine epidemic diarrhea coronavirus (PEDV). We have used a number of methods to stabilize and obtain distinctive conformations of these spike proteins, including pre-fusion state (state before membrane fusion), intermediate state (transitional state during membrane fusion), and post-fusion state (state after membrane fusion). We will determine the high-resolution cryo-EM structures at each of these conformations for different coronaviruses. These studies will (1) elucidate detailed membrane fusion mechanisms by coronavirus spike proteins, and thus cell entry mechanisms of coronaviruses; (2) provide insight into the evolution and receptor binding of coronaviruses; (3) establish a structural framework for vaccine and drug design.

Second, we will investigate sugar-binding mechanisms for a number of coronaviruses. So far we have determined the crystal structure of sugar-binding receptor-binding domain (RBD) of bovine coronavirus. We will further determine its structure complexed with its sugar receptor. We will also determine the structures of the sugar-binding RBDs from PEDV and porcine delta coronavirus as well as these structures in complex with their respective sugar receptor. These studies will (1) elucidate the structural basis for sugar receptor binding by coronaviruses; (2) establish a structural framework for design and development of sugar analogues as anti-coronavirus inhibitors.

Third, we will investigate the proteases that activate coronavirus entry into host cells. We will examine whether proteases from different host species or from the different tissues within the same host species activate coronavirus entry differently, thus serving as a critical determinant of the host range and tissue tropism of coronaviruses. The host species that we will study include humans, pigs, and bats. The tissues that we will study include cell lines from the lung, the GI tract, the kidney, and the liver. These studies will (1) provide novel insight into the host range and tissue tropism of coronaviruses; (2) establish the correlation between viral entry and viral tropism.

Progress report for AI089728

Studies and Results from Li lab

Du et al. (2016, Nature Communications) identified an intrinsic limitation associated with viral subunit vaccine design and found a way to overcome this limitation. More specifically, we found that when the receptor-binding domain (RBD) of MERS coronavirus (MERS-CoV) was taken out of the context of the full-length spike protein trimer, large surface areas on the RBD become artificially exposed and they contain immunodominant non-neutralizing epitopes that distract the host immune system from reacting to neutralizing epitopes, reducing vaccine efficacy. To over this limitation, we introduce a new concept 'neutralizing immunogenicity index' (NII) to evaluate individual epitopes' neutralizing immunogenicity. To determine the NII, we mask the epitope with a glycan probe and then assess the epitope's contribution to the vaccine's overall neutralizing immunogenicity. As proof-of-concept, we measure the NII for different epitopes on MERS-CoV RBD. Further, we design a variant form of this vaccine by masking an epitope that has a negative NII score. This engineered vaccine demonstrates significantly enhanced efficacy in protecting transgenic mice from lethal MERS-CoV challenge. Our study may guide the rational design of highly effective subunit vaccines to combat MERS-CoV and other life-threatening viruses.

Peng et al. (2017, Journal of Biological Chemistry) investigated the structural and molecular mechanism for coronavirus-driven evolution of host receptor. Hosts and pathogens are locked in an evolutionary arms race. To infect mice, mouse hepatitis coronavirus (MHV) has evolved to recognize mouse CEACAM1a (mCEACAM1a) as its receptor. To elude MHV infections, mice may have evolved a variant allele from the *Ceacam1a* gene, called *Ceacam1b*, producing mCEACAM1b, which is a much poorer MHV receptor than mCEACAM1a. Previous studies showed that sequence differences between mCEACAM1a and mCEACAM1b in a critical MHV-binding CC' loop partially account for the low receptor activity of mCEACAM1b, but detailed structural and molecular mechanisms for the differential MHV receptor activities of mCEACAM1a and mCEACAM1b remained elusive. Here we have determined the crystal structure of mCEACAM1b and identified the structural differences and additional residue differences between mCEACAM1a and mCEACAM1b that affect MHV binding and entry. These differences include conformational alterations of the CC' loop as well as residue variations in other MHV-binding regions, including β-strands C' and C'' and loop C'C''. Using pseudovirus entry and protein-protein binding assays, we show that substituting the structural and residue features from mCEACAM1b into mCEACAM1a reduced the viral receptor activity of mCEACAM1a, whereas substituting the reverse changes from mCEACAM1a into mCEACAM1b increased the viral receptor activity of mCEACAM1b. These results elucidate the detailed molecular mechanism for how mice may have kept pace in the evolutionary arms race with MHV by undergoing structural and residue changes in the MHV receptor, providing insight into this possible example of pathogen-driven evolution of a host receptor protein.

Liu et al. (2016, Journal of Biological Chemistry) examined proteases that activate the cell entry of porcine epidemic diarrhea coronavirus (PEDV). PEDV is currently devastating the United States pork industry by causing an 80-100% fatality rate in infected piglets. Coronavirus spike proteins mediate virus entry into cells, a process that requires the spike proteins to be proteolytically activated. It has been a conundrum which proteases activate PEDV entry. Here we systematically investigated the roles of different proteases in PEDV entry using pseudovirus entry, biochemical, and live virus infection assays. We found that the PEDV spike is activated by lysosomal cysteine proteases but not proprotein convertases or cell surface serine proteases. Extracellular trypsin activates PEDV entry when lysosomal cysteine proteases are inhibited. We further pinpointed cathepsin L and cathepsin B as the lysosomal cysteine proteases that activate the PEDV spike. These results advance our understanding of the molecular mechanism for PEDV entry and identify potential antiviral targets for curbing the spread of PEDV.

Li (2016, Annual Review of Virology) reviewed the current knowledge about the structures, functions, and evolution of the coronavirus spike protein, which is a multifunctional molecular machine that mediates coronavirus entry into host cells. During viral entry, the spike protein first binds to a receptor on the host cell surface through its S1 subunit and then fuses viral and host membranes through its S2 subunit. Two domains in S1 from different coronaviruses recognize a variety of host receptors, leading to viral attachment. The spike protein exists in two structurally distinct conformations, prefusion and postfusion. The transition from prefusion to postfusion conformation of the spike protein must be triggered, leading to membrane fusion. This article

discussed how the two S1 domains recognize different receptors and how the spike proteins are regulated to undergo conformational transitions. It further discussed the evolution of these two critical functions of coronavirus spike proteins, receptor recognition and membrane fusion, in the context of the corresponding functions from other viruses and host cells.

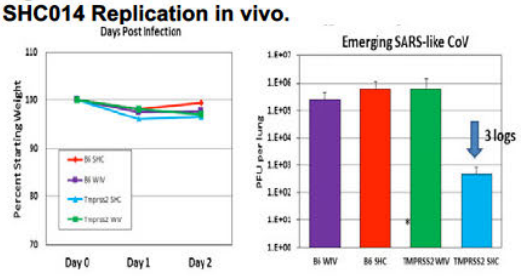
Tao et al. (2016, Journal of Virology) constructed four recombinant RBD (rRBD) proteins with single or multiple mutations detected in representative human MERS-CoV strains from the 2012, 2013, 2014, and 2015 outbreaks, respectively, and one rRBD protein with multiple changes derived from camel MERS-CoV strains. Like the RBD of prototype EMC2012 (EMC-RBD), all five RBDs maintained good antigenicity and functionality, the ability to bind RBD-specific neutralizing monoclonal antibodies (MAbs) and the DPP4 receptor, and high immunogenicity, able to elicit S-specific antibodies. They induced potent neutralizing antibodies cross-neutralizing 17 MERS pseudoviruses expressing S proteins of representative human and camel MERS-CoV strains identified during the 2012-2015 outbreaks, 5 MAb escape MERS-CoV mutants, and 2 live human MERS-CoV strains. We then constructed two RBDs mutated in multiple key residues in the receptor-binding motif (RBM) of RBD and demonstrated their strong cross-reactivity with anti-EMC-RBD antibodies. These RBD mutants with diminished DPP4 binding also led to virus attenuation, suggesting that immunoevasion after RBD immunization is accompanied by loss of viral fitness. Therefore, this study demonstrates that MERS-CoV RBD is an important vaccine target able to induce highly potent and broad-spectrum neutralizing antibodies against infection by divergent circulating human and camel MERS-CoV strains.

Du et al. (2016, Expert Opinion On Therapeutic Targets) reviewed MERS-CoV S protein's structure and function, particularly S1 receptor-binding domain (RBD) and S2 heptad repeat 1 (HR1) as therapeutic targets, and summarizes current advancement on developing anti-MERS-CoV therapeutics, focusing on neutralizing monoclonal antibodies (mAbs) and antiviral peptides.

Studies and Results from Baric lab

Introduction. CoVs recognize a variety of host receptors through one or both of its spike S1 domains (S1-NTD and S1-CTD), and are also regulated for cell entry by host proteases that cleave at the S1/S2 boundary. Understanding the receptor recognition and cell entry by CoVs has important implications for virology, medicine, and epidemics. Our studies have elucidated how CoVs explore different host receptors and host proteases to expand their host ranges, and how SARS-CoV and MERS-CoV transmit from animals to humans to cause epidemics. During the past year, the Baric laboratory has made considerable progress in two principle areas of Aims 1-3 of the grant; specifically, developing a molecular clone for porcine delta coronavirus and in designing and recovering synthetic bat SARS-like CoV.

Figure 1. TMPRSS2 KO Blocks SARS-like Bt CoV SHC014 Replication in vivo.



Porcine epidemic diarrhea virus. Previously, our group developed a molecular clone for porcine epidemic diarrhea virus. Several S glycoprotein deletions mutants have been described in the literature, including TC-PC177. To investigate whether 197 aa-deletion was the determinant for the attenuation of TC-PC177, we generated a mutant (icPC22A-S1Δ197) bearing the 197 aa-deletion based on an infectious cDNA clone of PC22A strain (icPC22A). In collaboration with the Wang laboratory at Ohio State University, the icPC22A-S1Δ197 virus caused mild to moderate diarrhea and no mortality, whereas the icPC22A virus caused severe diarrhea and death in all piglets. Our data indicate that the deletion of this 197 aa-fragment in spike protein can attenuate a highly virulent PEDV, but may lose important epitopes for inducing robust protective immunity. A manuscript detailing the construct of the mutant and its pathogenic outcomes in swine has been drafted and will soon be submitted for review.

Porcine delta coronavirus reverse genetic platforms. In addition to porcine epidemic diarrhea virus, porcine delta coronavirus was first reported in the United States in February 2014, causing severe disease outbreaks of lethal disease in piglets in swine herds. *Deltacoronavirus* genus is new, having only been recently defined by genomic sequence analysis from both pig and avian isolates. Since 2009, avian deltacoronaviruses have been

detected in a wide range of domestic and wild birds and then porcine deltacoronaviruses (PdCV) Hong Kong (HK) strains emerged suddenly in south east asia. To develop a PdCV molecular clone, we developed a collaboration with Dr. Linda Saif, who provided us with wildtype and tissue culture adapted strain OH-FD22. We have deep sequenced these isolates and have ordered a molecular clone from BioBasic. We anticipate having recombinant viruses within a few months of obtaining the reverse genetic platform. In parallel, we have dropped the PdCV S glycoprotein into Venezuelan equine encephalitis virus replicon particles (VRP-PdCV-S), and after VRP vaccination, we will obtain antisera against the S glycoprotein gene. The development of these two molecular clone reagent sets will be extremely valuable for downstream studies focusing on mechanisms of coronavirus entry.

SARS-like Bat Coronaviruses. We have synthetically reconstructed recombinant bat SARS-like viruses encoding the WIV-1 and SHC014 spike, which are about 10-15% distinct from the SARS-CoV epidemic S glycoprotein. While these strains are not pathogenic in mice, infection of TMPRS2 KO mice revealed that SHC014 S, but not isogenic backbone viruses encoding the SARS or WIV-1 S glycoproteins, cannot replicate in the absence of TMPRS2 in vivo (Fig 1). These data suggest that virus emergence may have been associated with adaptive changes that allowed for epidemic SARS-CoV strains to use the TMPRSS2 to replicate efficiently in the human lung. We are currently in the process of studying the exact role of specific mutations in SHC014 TMPRSS2 protease usage, as well as evaluating tropism differences in the lungs of infected control and KO animals.

New SARS-Like Bat Coronaviruses**.** The SARS-like bat coronavirus, WIV16 was isolated from a single fecal sample of *Rhinolophus sinicus*, which was collected in Kunming, Yunnan Province, in July 2013. The full genomic sequence of SL-CoV WIV16 (GenBank accession number KT444582) was determined (PMC4810638). The overall nucleotide sequence of WIV16 has 96% identity (higher than that of any previously reported bat SL-CoVs) to human and civet SARS-CoVs, including 97% amino acid identity in the S glycoprotein gene. Thus, WIV16 bridges the divide between zoonotic and epidemic SARS like viruses, but may retain mutation profiles in S2 that prevent TMPRSS2 proteolytic cleavage and efficient replication in vivo. To address this question (previously GOF requested and approved to move forward), we inserted the WIV16 S glycoprotein into the genome backbone of SARS-CoV and isolated recombinant viruses. Sequence analyses revealed the recombinant virus encoded with WIV16 S glycoprotein. In preliminary in vivo studies, the WIV16-S virus is attenuated as compared to the wildtype SARS-CoV MA strain. We are characterizing WIV16-S growth in Vero and primary human airway epithelial cells in vitro and for its ability to recognize the murine, human, civet and bat ACE2 receptors ectopically expressed in DBT cells for entry and to replicate in KO mice lacking the TMPRSS2, TMPRSS4 and TMPRSS2/4 genes.

PDF-2386 (SARS-Ubanda)**.** We have recently identified a new bat SARS like virus from Uganda (SARS-Uganda) in collaboration with Simon Anthony at EcoHealth. Analyses of the receptor binding domain suggests that it will not be able to recognize the human or mouse ACE2 receptor for entry (**Fig 2**), although it does retain some contact interface recognition sites with human (Y491, L472) or mouse (Y491, L472, L442) ACE2 receptors. The sequence comparisons predict, however, that incorporation of 5 residues *436Y, L442W, K479R, T484F, V487A from SCH014 into PDF-2386 (PDF-2386-SHC-RBD) or the 8 known mouse adapting mutations (K411E, *436H, L472F, K411E, G473N, K479N, T484Y and V487T) into the PDF-2386 Spike (PDF-2386-MA) which are predicted to confer replication of the PDF-2386 strain in mice, and perhaps, primate cells. As we are proposing to introduce the PDF-2386 S glycoprotein, with and without mouse ACE2 binding residues, into the bat SARS-like SHC014 coronavirus, we believe these experiments are exempt because neither virus has been

Strain	402	426	436	441	442	472	473	475	479	484	486	487	488	491
Urbani	T	R	Y	R	Y	L	N	Y	N	Y	T	T	G	Y
MA15	T	R	H	R	Y	L	N	Y	N	Y	T	T	G	Y
Rs3367	T	R	Y	R	S	F	N	Y	N	Y	T	N	G	Y
PDF-2386	T	N	*	R	L	L	G	Y	K	T	T	V	G	Y
RsSHC014	T	N	Y	R	W	P	N	Y	R	F	T	A	G	H
HKU3	T	A	*	R	S	*	N	V	K	N	N	V	G	Y
ACE2 interaction s	325				31*	79* 82*			34*	41 42 45		41 353*		37 353* 354

* indicates sequence differences between human and mouse ACE2 at this position

Figure 2. PDF-2386 (SARS-Uganda) Receptor Binding Domain Residues.

demonstrated to have pathogenic potential or be able to be transmitted in humans. Moreover, we are introducing SHC014 preferred and mouse adapted RBD residues into PDF-2386, hence, it is unlikely to be more dangerous than the original SHC014 strain. We have not started these experiments, as we are awaiting permission to proceed.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Li W, Li F. Cross-crystal averaging with search models to improve molecular replacement phases. Structure (London, England : 1993). 2011 February 9;19(2):155-61. PubMed PMID: 21300285; PubMed Central PMCID: PMC3037595.
N/A: Not Peer Reviewed	Li F. Structure, Function, and Evolution of Coronavirus Spike Proteins. Annual review of virology. 2016 September 29;3(1):237-261. PubMed PMID: 27578435.
Complete	Liu C, Ma Y, Yang Y, Zheng Y, Shang J, Zhou Y, Jiang S, Du L, Li J, Li F. Cell Entry of Porcine Epidemic Diarrhea Coronavirus Is Activated by Lysosomal Proteases. The Journal of biological chemistry. 2016 November 18;291(47):24779-24786. PubMed PMID: 27729455; PubMed Central PMCID: PMC5114425.
Complete	Du L, Tai W, Yang Y, Zhao G, Zhu Q, Sun S, Liu C, Tao X, Tseng CK, Perlman S, Jiang S, Zhou Y, Li F. Introduction of neutralizing immunogenicity index to the rational design of MERS coronavirus subunit vaccines. Nature communications. 2016 November 22;7:13473. PubMed PMID: 27874853; PubMed Central PMCID: PMC5121417.
Complete	Tai W, Wang Y, Fett CA, Zhao G, Li F, Perlman S, Jiang S, Zhou Y, Du L. Recombinant Receptor-Binding Domains of Multiple Middle East Respiratory Syndrome Coronaviruses (MERS-CoVs) Induce Cross-Neutralizing Antibodies against Divergent Human and Camel MERS-CoVs and Antibody Escape Mutants. Journal of virology. 2017 January 1;91(1). PubMed PMID: 27795425; PubMed Central PMCID: PMC5165220.
In Process at NIHMS	Du L, Yang Y, Zhou Y, Lu L, Li F, Jiang S. MERS-CoV spike protein: a key target for antivirals. Expert opinion on therapeutic targets. 2017 February;21(2):131-143. PubMed PMID: 27936982.
Complete	Peng G, Yang Y, Pasquarella JR, Xu L, Qian Z, Holmes KV, Li F. Structural and Molecular Evidence Suggesting Coronavirus-driven Evolution of Mouse Receptor. The Journal of biological chemistry. 2017 February 10;292(6):2174-2181. PubMed PMID: 28035001; PubMed Central PMCID: PMC5313091.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
FANGLI	Y	Li, Fang	PHD	PD/PI	3	0	0			NA
JIANSHANG	N	Shang, Jian	SCD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	4	0	0			NA
Minn Stat 13.32	N	Minn Stat 13.32	BS	Researcher 2	12	0	0			NA
RALPH_BARIC	Y	Baric, Ralph S	PHD,BS	Subaward PI	1	0	0			NA
Minn Stat 13.32	N	Minn Stat 13.32	MS	Researcher 2	12	0	0			NA
JKOCHER	N	Kocher, Jacob	BS,PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	5	0	0			NA
	N	Scobey, Drew T.		Technician	4	0	0			NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

File uploaded: Li and Baric Other Support 03-28-17.pdf

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

Fang Li Other Support

National Institutes of Health 04/01/15 – 03/31/20 4.8 cal months
R01AI110700 Annual Direct Costs $245,000
Role: Co-Principal Investigator (contact Co-PI: Ralph Baric, University of North Carolina)
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
Goal: This research investigates genetic pathways regulating MERS coronavirus cross species transmission and receptor homolog usage, establishes robust animal models of human disease, and discovers critical reagents for therapeutic and vaccine testing.

National Institutes of Health 06/07/16 – 05/31/21 3.0 cal months
R01AI089728 Annual Direct Costs $217,800
Role: Principal Investigator
Receptor recognition and cell entry of coronaviruses
Goal: This research investigates how coronaviruses recognize their receptors and how they interact with receptors from different hosts. It explores novel principles governing viral evolution, virus-receptor interactions, viral host ranges and cross-species infections, and may lead to new approaches in the prevention and treatment of coronavirus infections in humans and other animals.

AHC Faculty Research Development Grant 09/01/16 – 08/31/18 0.12 cal month
University of Minnesota Annual Direct Costs $30,000
Role: Co Principal Investigator (contact PI: Robert Geraghty, University of Minnesota)
Development of biological and structural approaches to Zika virus drug discovery
Goal: This research develops and implements the tools necessary to identify small molecule inhibitors of Zika virus, and also elucidates the structure/function of the viral RNA-dependent RNA polymerase.

OTHER SUPPORT

BARIC, RALPH S.

ACTIVE:

U19 AI 107810 (PI: Baric) 06/21/13-05/31/18 2.4 cal mos
NIH/NIAID $1,572,931
Characterization of novel genes encoded by RNA and DNA viruses
Using highly pathogenic human respiratory and systemic viruses which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and host responses to determine severe disease outcomes.

U19-AI100625 (PI: Baric/Heise-MPI) 08/05/12-07/31/17 1.44 cal mos
NIH/NIAID $3,580,599
Systems Immunogenetics of Biodefense Pathogens in the Collaborative Cross
Specific Aims: In this proposal, we are utilizing the Collaborative Cross (CC), a novel panel of reproducible, recombinant inbred (RI) mouse lines to identify genes and gene interactions which regulate the induction, kinetics, and magnitude of the innate, inflammatory and adaptive arms of the immune response following virus infection. Specifically, we will develop novel modeling algorithms to predict and validate the causal relationships between natural genetic variation and host signaling networks, immune cell recruitment, and immune function.

00008956 (PI: De Silva) 07/29/15-06/30/17 0.36 cal mos
UCB/NIH $279,165
Protective immunity following dengue virus natural infections and vaccination
We will perform studies to characterize the B-cell/ antibody (responses in people who receive dengue live attenuated virus vaccines (DLAV).
Role: Co-Investigator

R01 AI 107731 (PI: De Silva) 08/05/13-07/31/17 0.24 cal mos
NIH/NIAID $621,124
Molecular Basis of Dengue Virus Neutralization by Human Antibodies
These studies proposed here are directly relevant to developing simple assays to predict the performance of the leading dengue vaccine candidates and also for developing the next generation of safe and effective dengue vaccines.
Role: Co-Investigator

R01 AI108197 (MPI: Denison/Baric) 08/01/13-07/31/17 0.60 cal mos
Vanderbilt University/NIH/NIAID $187,635
Determinants of Coronavirus Fidelity in Replication and Pathogenesis
Experiments in this aim will test the hypothesis nsp1 functions in maintaining high replication fidelity and viral RNA synthesis are coupled and that targeted engineered mutations across nsp14 alter: a) RNA fidelity outcomes; b) sensitivity nucleoside mutagens, terminators and polymerase inhibitors; c) the kinetics and magnitude of positive, negative, genomic and subgenomic RNA synthesis; and d) RNA recombination frequencies.

U19-AI106772-01 (PI: Kawaoka) 06/01/13-05/31/17 0.12 cal mos
Univ of Wisconsin/NIH $55,729
MERS-CoV Supplement for OMICs Proposal
The proposed studies will provide a more detailed look at the intracellular environment by taking “snapshots” of the lipids, metabolytes, and proteins present during viral infection time courses. These assays will allow us to determine the innate immune response occurring immediately following virus infection and to determine how the virus and cell interact over a 72 hour window.
Role: Investigator

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19 1.08 cal mos
UAB/NIH/NIAID $304,371
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Co-Investigator

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19 1.2 cal mos
Columbia/NIH/NIAID $584,891
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Project Leader, Consortium PI

Not assigned (PI: deSilva) 11/05/14-09/30/17 0.36 cal mos
Johns Hopkins U/Gates Foundation $726,915
The dengue human infection model: Defining correlates of protection and advancing vaccine development
The goal of these studies conducted by the Baric laboratory are to use recombinant dengue viruses encoding multiple homotypic neutralizing sites from multiple strains, as well as a collection of null mutants, to characterize the homotypic immune response elicited in humans following natural infection and after challenge in GSK DENV tetravalent vaccinated individuals. This grant has been funded by Gates.
Role: Co-Investigator

R01 AI110700 (PI: Baric) 04/20/15-03/31/20 0.96 cal mos
NIH/NIAID $613,691
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.

Not Assigned (PI: Baric) 01/08/16-01/07/19 0.24 cal mos
Taked Vaccines, Inc $1,243,048
In Vitro and In Vivo Characterization of Bivalent DENV Live Virus Vaccines
To provide expertise in molecular virology required for creating recombinant dengue viruses for in vitro and in vivo testing.

R01-AI125198 (PI: deSilva) 05/04/16-04/30/21 0.60 cal mos
NIH/NIAID $1,153,997
Preclinical Assays To Predict Tetravalent Dengue Vaccine Efficacy
Dengue is the most significant mosquito transmitted viral infection of humans. Vaccination is a feasible solution to prevent and control dengue. Although dengue vaccines are under development, we do not know the specific properties of antibodies induced by vaccines that are likely to protect from infection. In this project investigators from the University of North Carolina and Sanofi Pasture, a leading dengue vaccine developer, will collaborate to define properties of antibodies induced by the Sanofi vaccine that correlate with protection. The main goal of the project is to develop new assays to support the current global effort to develop dengue virus vaccines. Role: Co-Investigator

60045042 (PI: Baric) 02/01/15-01/31/18 0.12 cal mos
Ohio State Univ/USDA $44,804
Molecular attenuation mechanisms of porcine epidemic diarrhea virus in pigs
Reverse genetic strategies are used to construct a panel of live attenuated porcine epidemic diarrhea recombinant viruses for in vivo pathogenesis studies and in vitro biological characterization. We test rationale vaccine strategies to protect new born piglets against this devastating porcine epidemic virus.

64807 (PI: Baric) 06/23/16-06/22/18 0.24 cal mos
Takeda Vaccines, Inc $1,066,500
Breadth of Blockade Antibody Responses Following Norovirus Vaccination
To conduct a project as an agreement in which Dr. Ralph Baric will test Takeda provided serum samples for cross-strain blockade antibody responses.

0258-3962 (PI: Lim) 09/30/11-02/28/17 0.60 cal mos
Mount Sinai/NIH $166,793
MERS-CoV Mouse Model for Vaccine and Therapeutic Testing
To: 1) confirm that the non-conserved region of mouse DPP4 is responsible for its inability to serve as a MERS-CoV entry receptor, 2) use a powerful new in vivo site directed mutagenesis approach to humanize the murine DPP4, 3) test whether mice carrying the humanized DPP4 receptor will support MERS-CoV replication and disease within the lungs. Role: Subcontract PI

N005402801 (PI: Li) 06/07/16-05/2831/17 0.36 cal mos
Univ Minn/NIH $120,384
Receptor recognition and cell entry of coronaviruses
To investigate how CoVs explore host receptors and host proteases for regulation of their host range, cross-species transmission, tissue tropism, and pathogenesis. Role: Subcontract PI

684K644 (PI: Sims) 06/01/16-05/31/17 0.12 cal mos
Univ of Wisconsin/NIH $200,000
Systems Virology of MERS-CoV in vivo
The major goal of this award is to define host cell gene networks and pathways that are modulated following infection in our newly developed lethal mouse model of MERS-CoV. Role: Investigator

PENDING: None

OVERLAP: None

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

No

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: Regents of the University of Minnesota	555917996	MN-005	3-284 Nils Hasselmo Hall 312 Church Street SE Minneapolis MN 554550215
University of North Carolina - Chapel Hill	608195277	NC-004	3109 Hooker Research Center 135 Dauer Drive Chapel Hill NC 275997435

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

UNIVERSITY OF MINNESOTA

Notice of Grant or Contract Award

Sponsored Projects Administration

(Online Page Navigation: Main Menu > Grants >Awards > UM_NOGA Report)

Award: CON000000058289
Primary Project: 00054028
PRF #: 835469

AWARD INFORMATION (Online Page Navigation: Main Menu > Grants > Awards >Award Profile)

Award Start Date:	06/07/2016	**Award End Date:**	05/31/2021
Funds Approved to Date:	$917,877.00		
Principal Investigator:	Li,Fang	**Award Type:**	Grant
Primary Project Dept:	PHCL Pharmacology Admin	**Purpose:**	Research
Sponsor:	NIH Nat'l Inst of Allergy & Infect Dis		
Sponsor Award #:	5R01AI089728-07		
Award Title #:	Receptor recognition and cell entry of coronaviruses		

ASSOCIATED PROJECTS (Online Page Navigation: Main Menu > Grants >Awards > Award Profile - Funding Tab)

Project #:	00054028	**Department:**	11718 - PHCL Pharmacology Admin
Project PI:	Li,Fang	**Dates:**	06/07/2016 - 05/31/2021
F&A:	54.00 MTDC	**EFFDT:**	07/01/2018
F&A:	53.00 MTDC	**EFFDT:**	07/01/2017
F&A:	52.00 MTDC	**EFFDT:**	06/07/2016

Project Title: Receptor recognition and cell

AWARD HISTORY (Online Page Navigation: Main Menu > Grants > Awards > Review Award Modifications)
(Displayed in action order ('Mod Issue Date', 'Period', 'Modification Sequence' in descending order))

Reference Award#: 5R01AI089728-07 **Amount:** $453,437.00
Period: 2 **Begin Date:** 06/01/2017 **End Date:** 05/31/2018
Modification Seq: 2 **Modification Type:** Continuation **Mod Issue Date:** 06/14/2017
Comments:
Continuation funding is provided in the amount of $453,437 for the period 6/1/17-5/31/18.

Reference Award#: 2R01AI089728-06 **Amount:** $464,440.00
Period: 1 **Begin Date:** 06/07/2016 **End Date:** 05/31/2017
Modification Seq: 1 **Modification Type:** Original **Mod Issue Date:** 06/24/2016
Comments:
$464,440 for 06/07/2016 - 05/31/2017

Subject to 45 CFR Part 75
An unobligated balance may be carried forward to the next budget period without prior, agency approval.

No funds are provided and no funds can be used to support gain-of-function research covered under the October 17, 2014 White House Announcement (NIH Guide Notice NOT-OD-15-011).

Direct costs awarded are limited to a 20% increase over the direct costs awarded in the last non-competing continuation (see NIH Guide, Vol. 26, No. 35, 10/17/97).

This award includes funds awarded for subrecipient activity with University of North Carolina, Chapel Hill in the amount of $120,384 ($79,200 direct costs + $41,184 facilities and administrative costs).

See NOA for information on Select and Highly Pathogenic Agents.

**Per the letter dated May 18, 2016 to Mr. McKoskey at the University of Minnesota, NIAID funding cannot be used to generate SARS-like viruses with enhanced affinity for human receptors (via either reverse genetics or serial passaging). NIAID funding will instead be used to pursue alternative loss-of-function strategies or mutation of the ACE2 receptor as described in the letter from the University of Minnesota dated April 15, 2016. If any of the alternative experiments proposed, or any experiments proposed under the other Aims, result in a virus with a phenotype of enhanced pathogenicity and/or transmissibility via the respiratory route, or enhanced growth by more than 1 log compared to wild type strains, you must immediately stop the work and notify the NIAID Program Officer and Grants Management Specialist.

ESTIMATED FUTURE FUNDING / ACTIONS (Online Page Navigation: Main Menu > Grants > Awards > Review Award Modifications)
(Displayed by 'Period' and 'Modification Sequence' in ascending order)

12
7-12
56

Reference Award#: 2R01AI089728-06 **Amount:** $455,615.00
Period: 3 **Begin Date:** 06/01/2018 **End Date:** 05/31/2019
Modification Seq: 3 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

Reference Award#: 2R01AI089728-06 **Amount:** $455,796.00
Period: 4 **Begin Date:** 06/01/2019 **End Date:** 05/31/2020
Modification Seq: 4 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

Reference Award#: 2R01AI089728-06 **Amount:** $455,796.00
Period: 5 **Begin Date:** 06/01/2020 **End Date:** 05/31/2021
Modification Seq: 5 **Modification Type:** Continuation **Mod Issue Date:**
Comments:

COMMENTS

Description: **Entered by:** SACHI003 **Date & Time:** 6/24/2016 6:49 PM
Date and Time Stamp indicates Proposal-to-Award Process

Description: **Entered by:** SACHI003 **Date & Time:** 6/24/2016 8:53 PM
NIH Contacts:
Program Official: Erik J. Stemmy, Email: erik.stemmy@nih.gov Phone: 240-627-3380
Grants Management Specialist: Devon R. Bumbray-quarles, Email: dbumbray@niaid.nih.gov Phone: 240-669-2950 Fax: 301-493-0597

SPECIAL REVIEWS (Online Page Navigation: Main Menu > Grants >Awards > Award Profile - Certifications Tab)

Project #: 00054028

Description: rDNA, InfectAgents, BioToxins
Approval Date: 01/01/1901 **Expiration Date:** 01/01/1901 **AssuranceNumber:**
Comments: No IBC costs can be incurred on this grant until all approvals are in place with the IBC Committee.

BUDGET INFORMATION (Online Page Navigation: Main Menu > Grants>Awards>Project Budget)

Please review online budget for valid account string combinations (ex. PCBU, Activity, Fund, Dept_Id, Project, Account)

Project #: 00054028 **Cost Share Budget Total:**
Project Start Date: 06/07/2016 **Project End Date:** 05/31/2021
ICR Sharing **Department:** PHCL Pharmacology Admin **Percentage:** 100.00

Period	Start Date	End Date	Direct Cost (Approved)	F & A Cost (Approved)	Direct Cost (Pending)	F & A Cost (Pending)	Total Period Cost
1	06/07/2016	05/31/2017	$339,894.00	$124,546.00	$0.00	$0.00	$464,440.00
2	06/01/2017	05/31/2018	$338,184.00	$115,253.00	$0.00	$0.00	$453,437.00
3	06/01/2018	05/31/2019	$0.00	$0.00	$455,615.00	$0.00	$455,615.00
4	06/01/2019	05/31/2020	$0.00	$0.00	$455,796.00	$0.00	$455,796.00
5	06/01/2020	05/31/2021	$0.00	$0.00	$455,796.00	$0.00	$455,796.00
TOTAL			$678,078.00	$239,799.00	$1,367,207.00	$0.00	$2,285,084.00

Budget Detail (all periods)

Fund Code	DeptID	Program Code	Chartfield 1	Chartfield 2	Fin EmplID	Activity	Analysis Type	Account	Cost Share	Budget Item	Amount	Hold
3002	11718					1	BUD	700100		FACULTY_SALARY	$61,791.00	
3002	11718					1	BUD	700210		POSTDOC_SALARY	$64,243.00	
3002	11718					1	BUD	700500		CS_BU_SALARY	$102,338.00	
3002	11718					1	BUD	710100		FACULTY_FRINGE	$20,722.00	
3002	11718					1	BUD	710210		POSTDOC_FRINGE	$14,232.00	
3002	11718					1	BUD	710500		CS_BU_FRINGE	$27,838.00	
3002	11718					1	BUD	720200		LABMED_SUPPLIES	$104,346.00	
3002	11718					1	BUD	720300		GEN_OP_SERVICES	$4,500.00	
3002	11718					1	BUD	720400		LABMED_SERVICES	$16,000.00	
3002	11718					1	BUD	720500		TELECOMM	$300.00	
3002	11718					1	BUD	720600		TRAVL_MILE_MOVE	$6,000.00	
3002	11718					1	BUD	750100		NON_CAP_EQUIP	$5,000.00	
3002	11718					1	BUD	780100		REPAIRS_MAINT	$5,000.00	
3002	11718					1	BUD	810300		SUB_NO_FACADM	$215,768.00	
3002	11718					1	BUD	810400		SUB_WITH_FACADM	$25,000.00	
3002	11718					1	BUD	810500		FACADM	$239,799.00	
3002	11718					1	BUD	850100		CAPITAL_EQ_5K	$5,000.00	

COST SHARE/MATCH/IN-KIND

Project #: 00054028

CSM - Cost Share, Matching and InKind

CSM05 - Departments must document all cost share/unpaid effort that is shown in the proposal.

PROGRAM INCOME INFORMATION

Project #: 00054028

PIN - Program Income

PIN03 - Reportable Program Income must be deposited by the department into the Sponsored Program Income Unapplied Cash account.

PIN04 - Additional - Program Income can be used in addition to sponsor funds to cover project costs that will further the objectives of the legislation under which the grant was made.

TERMS & CONDITIONS (Online Page Navigation: Main Menu > Grants >Awards > Award Profile - Terms Tab)

Project #: 00054028

APV - Additional Approval

APV10 - Prior sponsor approval required for a change in the scope or objective of the project or program (even if there is no associated budget revision requiring prior written approval).

APV17 - Prior sponsor approval required for patient care costs or rebudgeting in or out of this category.

APV27 - Prior sponsor approval required for PD/PI and other Senior/Key Personnel named in the NOA's reduction in effort of 25% or more.

APV28 - Prior sponsor approval required to replace PD/PI or other Senior/Key Personnel named in the NOA.

APV29 - Prior sponsor approval required for the absence of the PD/PI and/or other Senior/Key Personnel named in the NOA for 3 months or more.

APV30 - Prior sponsor approval required for a no cost extension that is submitted after the agency notification deadline.

APV31 - Prior sponsor approval required for the addition of a foreign component.

APV44 - Particpant costs are only allowable when identified in the funding opportunity announcement (FOA).

APV62 - Prior sponsor approval required for fixed amount subawards that exceed the Simplified Acquisition Threshold of $150,000.

APV74 - Prior sponsor approval required to charge admin and clerical costs that are not identified in the approved budget. Costs may be allowable if integral to project.

EQP - Equipment

EQP70 - Allocable computing devices deemed essential and with unit cost less than $5,000 may be charged.

FIS - Fiscal

FIS03 - Reversion of funds at termination / final

FIS08 - Deficits may not be carried forward.

FIS14 - Grant/contract funds may not be used to pay the salary of an individual at a rate in excess of Executive Level II of the Federal Executive Pay Scale

IPR - IP Rights

IPR02 - Sponsor support must be acknowledged in patent applications and patent issuing.

IPR04 - Title to U of M; non-exclusive, non-transferable, irrevocable, paid-up license to Federal Government.

IPR12 - PI obligation to report inventions (and other IP) to the sponsor (and to OTC per policy).

PRP - Property

PRP02 - U of M receives title to equipment purchased or fabricated under this award.

PRP03 - Sponsor may require transfer of equipment and its title upon award completion for those items costing in excess of $5000.

PUB - Publications

PUB02 - U of M will place an acknowledgement of sponsor support and a disclaimer, as appropriate, on any publication generated by this award or reporting on the award results and/or activity.

PUB07 - Peer-reviewed articles must be submitted to NIH Pubmed Central (PMC) upon acceptance for publication and made publicly available within 12 months. Cite PMC ID number in progress reports/proposals.

PUB70 - Costs for award publications or sharing of results may be charged and posted up to 60 days after the award end date. Page charges can only be charged if the journal's policy ensures that charges are calculated the same regardless of fund source.

RBD - Rebudgeting

RBD10 - Rebudgeting is allowed as long as there is no change in work scope

REG - Regulatory

REG09 - Subject to Streamlined Noncompeting Award Procedures (SNAP)

REG10 - Federal Demonstration Partnership (FDP) guidelines (www.thefdp.org)

REG16 - If applicable, no funds may be expended for human subject related activities until all appropriate human subject related approvals are in place

REG17 - If applicable, no funds may be expended for animal related activities until all appropriate animal related approvals are in place

REG18 - If applicable, any personnel involved in the design or conduct of human subject activity must satisfy the requirement for education on the protection of human subjects prior to participating in the study

REG19 - NIH Grants Policy Statement - 10/1/2013 (http://grants.nih.gov/grants/policy/policy.htm)

REG21 - If applicable, no funds may be expended for activities involving recombinant DNA, Infectious Agents or Biological Toxins until Institutional Biosafety Committee (IBC) approval has been obtained.

REG26 - Federal Funding Accountability and Transparency Act (FFATA)

REG41 - All University personnel meeting the FCOI definition of key personnel must maintain a current REPA and must have completed FCOI training.

REG42 - Final grant reports (Final FFR, Final Progress Report & Final Invention Statement) must be submitted within 120 days of the grant end date.

REG43 - NIH Interim Grant General Conditions located at (http://grants.nih.gov/grants/policy/policy.htm).

REG44 - 45 CFR Part 75 - Uniform Administrative Requirements, Cost Principles, and Audit Requirements for Federal Awards ("Uniform Guidance")

REG70 - UNIFORM GUIDANCE - 2 CFR 200 (http://www.ecfr.gov/cgi-bin/text-idx?SID=704835d27377ef5213a51c149de40cab&node=2:1.1.2.2.1&rgn=div5)

TRV - Travel Restrictions

TRV16 - Regulations implementing the Fly America Act provide that federal funds may be used to pay for air travel only on U.S. flag carriers, except in specified cases. 41 C.F.R. section 301-10.131, et. seq. For more about Fly America Act requirements and exceptions, see https://travel.umn.edu/flyamerica.html.

UMN - U of M Requirements

UMN01 - The U of M Effort Certification system must be used to reflect all U of M staff committed effort on this award, whether or not any salary is charged to the award.

UMN02 - Salary cost transfers cannot be made after effort is submitted.

UMN03 - All U of M Policy and Guidelines relating to research must be followed and can be read in detail on the web at http://www.ospa.umn.edu/policiesandprocedures/index.htm

UMN04 - U of M Cost Transfer guidelines state transfers of non-salary expenses on accounts be clearly documented & made within 30 days after the end of the accounting period the charge was processed.

NON-FINANCIAL REPORTING (Online Page Navigation: Main Menu > Grants >Awards > Award Profile - Milestones Tab)

Type: Final Report **Description:** Final Technical Report **Due Date:**
Comments: Due 90 days after termination/final or included in renewal proposal.

Type: Final Report **Description:** Final Invention Statement **Due Date:**
Comments: Due 90 days after termination/final or included in renewal proposal.

Type: Report **Description:** PHS 2590-noncompeting continua **Due Date:**
Comments: Progress Report - Noncompeting continuation must be submitted via RPPR

INVOICE/BILLING INFORMATION

Contract Type: COST_REIMBURSE
Invoicing Method: Cost Reimbursable
Invoice Form: GPC - GM_LOC
Interim Invoice Deadline: 30
Final Invoice Deadline: 90

Contact Name:
Contact Phone:
Contact Fax:
Contact Email:
Contact Address: PSC-FMS (301) 443-3020
PARKLAWN BUILDING ROOM 16A-12
5600 FISHERS LANE
ROCKVILLE MD 20857

FINANCIAL REPORTING

Report Form ID: R_FFR
Reporting Frequency: Termination/Final
Interim Reporting Deadline: 90
Final Reporting Deadline: 90

GENERAL PROVISIONS

This document is intended to provide guidance in managing this award, to the Principal Investigator, Academic Unit, and to those who are noted as receiving this document. Its intent is to provide general information regarding University and sponsor policies when given the award. In addition, it is intended to reference specific conditions related to this award. Specific questions should be addressed to the grant administrator from SPA noted below. By accepting this award, the Principal Investigator acknowledges and agrees to abide by all provisions of the sponsoring agency and the University of Minnesota policies related to sponsored projects management, including the Code of Conduct.

U OF M CONTACTS

Administrative questions to SPA staff member:	Erin Knudsen	612/626-2724 jond0006@umn.edu
Billing and financial questions to SFR:	Kristin Lien	612/624-0353 lienx110@umn.edu

Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 05/15/2017

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI089728-07
FAIN: R01AI089728

Principal Investigator(s):
Fang Li, PHD

Project Title: Receptor recognition and cell entry of coronaviruses

KEVIN MCKOSKEY
Grant Administrator
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 554552070

Award e-mailed to: awards@umn.edu

Period Of Performance:
Budget Period: 06/01/2017 – 05/31/2018
Project Period: 06/07/2016 – 05/31/2021

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $453,437 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MINNESOTA in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI089728. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Tina M. Carlisle
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI089728-07

Award Calculation (U.S. Dollars)	
Salaries and Wages	$116,853
Fringe Benefits	$30,547
Personnel Costs (Subtotal)	$147,400
Materials & Supplies	$60,005
Travel	$2,970
Other	$5,247
Subawards/Consortium/Contractual Costs	$120,384
Publication Costs	$2,178
Federal Direct Costs	$338,184
Federal F&A Costs	$115,253
Approved Budget	$453,437
Total Amount of Federal Funds Obligated (Federal Share)	$453,437
TOTAL FEDERAL AWARD AMOUNT	$453,437
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$453,437

SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
7	$453,437	$453,437
8	$455,615	$455,615
9	$455,796	$455,796
10	$455,796	$455,796

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name: Allergy and Infectious Diseases Research
CFDA Number: 93.855
EIN: 1416007513A1
Document Number: RAI089728B
PMS Account Type: P (Subaccount)
Fiscal Year: 2017

IC	CAN	2017	2018	2019	2020
AI	8472364	$453,437	$455,615	$455,796	$455,796

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C B / **OC**: 414E / **Released**: CARLISLET 05/12/2017
Award Processed: 05/15/2017 12:04:15 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI089728-07

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI089728-07

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.

c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI089728. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI089728-07

No funds are provided and no funds can be used to support gain-of-function research covered under the October 17, 2014 White House Announcement (NIH Guide Notice NOT-OD-15-011).

Per the letter dated May 18, 2016 to Mr. McKoskey at the University of Minnesota, NIAID funding cannot be used to generate SARS-like viruses with enhanced affinity for human receptors (via either reverse genetics or serial passaging). NIAID funding will instead be used to pursue alternative loss-of-function strategies or mutation of the ACE2 receptor as described in the letter from the University of Minnesota dated April 15, 2016. If any of the alternative experiments proposed, or any experiments proposed under the other Aims, result in a virus with a phenotype of enhanced pathogenicity and/or transmissibility via the respiratory route, or enhanced growth by more than 1 log compared to wild type strains, you must immediately stop the work and notify the NIAID Program Officer and Grants Management Specialist.
******

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- o A list of the new and/or additional Agent(s) that will be studied;
- o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;

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o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Madeline Elizabeth Clarke
Email: madeline.clarke@nih.gov **Phone**: 301-761-7794 **Fax**: 301-493-0597

Program Official: Erik J. Stemmy
Email: erik.stemmy@nih.gov **Phone**: 240-627-3380

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI089728-07

INSTITUTION: UNIVERSITY OF MINNESOTA

Budget	Year 7	Year 8	Year 9	Year 10
Salaries and Wages	$116,853	$116,853	$116,853	$116,853
Fringe Benefits	$30,547	$30,547	$30,547	$30,547
Personnel Costs (Subtotal)	$147,400	$147,400	$147,400	$147,400
Materials & Supplies	$60,005	$60,005	$60,005	$60,005
Travel	$2,970	$2,970	$2,970	$2,970
Other	$5,247	$5,247	$5,247	$5,247
Subawards/Consortium/Contractual Costs	$120,384	$120,384	$120,384	$120,384
Publication Costs	$2,178	$2,178	$2,178	$2,178
TOTAL FEDERAL DC	$338,184	$338,184	$338,184	$338,184
TOTAL FEDERAL F&A	$115,253	$117,431	$117,612	$117,612
TOTAL COST	$453,437	$455,615	$455,796	$455,796

Facilities and Administrative Costs	Year 7	Year 8	Year 9	Year 10
F&A Cost Rate 1	52%	53%	54%	54%
F&A Cost Base 1	$18,150	$18,150	$217,800	$217,800
F&A Costs 1	$9,438	$9,620	$117,612	$117,612
F&A Cost Rate 2	53%	54%		
F&A Cost Base 2	$199,650	$199,650		
F&A Costs 2	$105,815	$107,811		

GA: SL for ETC

Awarded CON #: 58289

(If Applicable) Related Proposal CON #: 67100

No FRED; ✓

Please Mail All Pages Prior To Page Separator

UNIVERSITY OF MINNESOTA

ReportID: UMFGM017F

PROPOSAL SUMMARY

Run Date: 05/15/2017
Run Time: 11:12:48 AM

Status **4 AwdB Award Sent to GA**

Grant Administrator: Knudsen,Erin Elizabeth

PRF #	906521	Proposal #/Version	CON000000067100/A101
EGMS Proposal #	489033	Sponsor Award #	5R01AI089728-07
Proposal Type	Non-Competing Continuation	Concurrent	N
Previous Award	CON000000058289	SubContract	N
Purpose			
Title	Receptor recognition and cell entry of coronaviruses		
Principal Investigator	Li,Fang	lifang@umn.edu	

Budget Summary

Start Date	06/01/2017	End Date	05/31/2018	
Direct Costs	$338,185.00	Indirect Costs	$115,253.00	Total Costs $453,438.00

Sponsor: NIH Nat'l Inst of Allergy & Infect Dis

Submission Information

Due Date	04/15/2017	Date Type	Receipt Date
Submission Method	Electronically Only	# of Copies Submitted	_____

Mailing Address

Comments/Corrections

REPA	__verified	__email sent for _____		
IRB	__verified	__pending	__email sent	__Not Applicable
IACUC	__verified	__pending	__email sent	__Not Applicable
IBC	__verified	__pending	__email sent	__Not Applicable
F&A rate	__at full or agency limit	__waiver on file	__waiver requested	
Cost share documented?		__yes	__no	__Not Applicable
ICR sharing?		__yes	__no	

RCR
MET
NOT MET _____

Subject: NoA: 5 R01 AI089728-07 PI: Li, Fang
From: era-notify@mail.nih.gov
Date: 5/14/2017 11:04 PM
To: awards@umn.edu

Grant Number: 5 R01 AI089728-07
Principal Investigator: Li, Fang
Project Title: Receptor recognition and cell entry of coronaviruses
Institution: UNIVERSITY OF MINNESOTA
CFDA: 93.855
OC: 414E
PCC: M51C B
Award Issue Date: 05/15/2017
Grants Management Officer: Carlisle, Tina
Program Official: Stemmy, Erik
Grants Specialist: Clarke, MADELINE

*** This is an automated notification - Please do not reply to this message. ***

Attachments:

NIH_NOA_5R01AI089728-07.PDF 27 bytes

Con - 67100/582859
PRF - 906521 4/19/17
Proj # - 00064059
PI - Li
GA: EK

Status Information

(CON-58289e)
CON-67100

Contacts

Administration: Grants Management Specialist(GMS)
Name: Clarke, MADELINE ELIZABETH
Phone: 301-761-7794
Email: madeline.clarke@nih.gov
Administration: Program Official(PO)
Name: Stemmy, Erik J.
Phone: 240-627-3380
Email: erik.stemmy@nih.gov

Grant Number: 5 R01 AI089728-07

906521

EK

Status

Status: Pending administrative review. Refer any questions to Program Official or Grants Management Specialist.
Last Status Update Date: 06/07/2016
PI Name: Li, Fang
Institution Name: UNIVERSITY OF MINNESOTA
School Name: MEDICAL SCHOOL
School Category: SCHOOLS OF MEDICINE
Division Name: NONE
Department Name: PHARMACOLOGY
NIH Appl. ID: 9291416

Proposal Receipt Date: 04/14/2017
Proposal Title: Receptor recognition and cell entry of coronaviruses
Budget Start Date: 06/01/2017
Budget End Date: 05/31/2018
Project Period Begin Date: 06/07/2016
Project Period End Date: 05/31/2021
Progress Report Due Date: 04/15/2017
Current Award Notice Date:
Application Source: RPPR
eApplication Status:
FOA: [PA13-302] - RESEARCH PROJECT GRANT (PARENT R01)

Other Relevant Documents

e-Application

Additions for Review

Review

Application	Study Section	Advisory Council (AC)
Award Document Number: RAI089728B	**Scientific Review Group:** VIRA	
FSR Accepted Code: N	**Council Meeting Date (YYYY/MM):** 2017/00	
Snap Indicator Code: Y		
Review Outcome Available: No		
Summary Statement Available: No		
Early Stage Investigator Eligible:		
New Investigator Eligible:		
Eligible for FFATA Reporting: No		

Institute/Center Assignment

Institute or Center	Assignment Date
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES (Primary)	06/07/2016

Status History

Effect Date	Status Message

Reference Letter(s)

This list shows Reference Letters associated with this particular Grant Application. Principal Investigator can see a list of all Reference Letters within Personal Profile - Reference Letters section on eRA Commons

UNIVERSITY OF MINNESOTA

ReportID: UMFGM017F

PROPOSAL SUMMARY

Page No: 1
Run Date: 04/03/2017
Run Time: 09:55:04 AM

Status 2 PropB Elect ~~Received~~ at SPA

Grant Administrator: ERIN K

PRF #	906521	Proposal #/Version	CON000000067100/V101
EGMS Proposal #		Sponsor Award #	
Proposal Type	Non-Competing Continuation	Concurrent	N
Previous Award		SubContract	N
Purpose			
Title	Receptor recognition and cell entry of coronaviruses		
Principal Investigator	Li,Fang	lifang@umn.edu	

Budget Summary

Start Date	06/07/2016	End Date	05/31/2018	
Direct Costs	~~$0.00~~ 338,185	Indirect Costs	~~$0.00~~ 115,253	Total Costs ~~$0.00~~ 453,438
Sponsor	NATIONAL INSTITUTES OF HEALTH (NIH) / NIAID			

Submission Information

Due Date	04/15/2017	Date Type	Receipt Date
Submission Method		# of Copies Submitted	

Mailing Address

RPPR

4/14/17 - 4:49 pm EDT via Commons - Kevin

Comments/Corrections

REPA	X verified	__email sent for		
IRB	__verified	__pending	__email sent	X Not Applicable
IACUC	__verified	__pending	__email sent	X Not Applicable
IBC	__verified	__pending	__email sent	__Not Applicable
F&A rate	52/54 X at full or agency limit	__waiver on file	__waiver requested	
Cost share documented?	54	__yes	X no	__Not Applicable
ICR sharing?		__yes	X no	

SUBAWARDS 1 #

For more than 4 subawards please record on separate page

	BUDGET	SCOPE	BUDGET & SCOPE	NOT APPLICABLE
✓ University of North Carolina - Chapel Hill Subrecipient	X		X	
Subrecipient				
Subrecipient				
Subrecipient				

4.17.17 logout NLB

☒ ELECTRONIC PROPOSAL
X FEDERAL _G-GOV.
_NON-FED.

Green Slip

PRF: 906521

CON: 61100

☒ Not Late

☐ Late

☐ Late Emergency

☐ Missing signatures – Front Desk

☐ Missed deadline

☐ Reason/Other: _____

GA Initial: GK

17 APR 09:55A

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Welcome: Kevin McKoskey
ID: KEVINM
Institution: UNIVERSITY OF MINNESOTA
Roles: SO

Routing History

Reviewer Name	Action	Notification Sent	Date of Action	Next Reviewer Name	Comments
Weber, Lynn	Initiate	03-20-2017 09:43:57	03-20-2017 09:43:57	Weber, Lynn	
Weber, Lynn	Route	04-03-2017 10:28:39	04-03-2017 10:28:39	Knudsen, Erin	
Knudsen, Erin	Route	04-14-2017 03:26:30	04-14-2017 03:26:30	McKoskey, Kevin	
McKoskey, Kevin	Submit	04-14-2017 04:49:44	04-14-2017 04:49:44	Agency	

Back

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National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland 20892

Department of Health and Human Services

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Version: 3.30.01

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Subject: eRA Commons: RPPR for Grant R01AI089728-07 Submitted to Agency
From: <era-notify@mail.nih.gov>
Date: 4/14/2017 3:49 PM
To: <lifang@umn.edu>, <jond0006@umn.edu>, <kevin@umn.edu>

*** This is an automated notification - Please do not reply to this message. ***

McKoskey, Kevin has submitted RPPR for Grant R01AI089728-07 associated with Program Director/Principal Investigator Fang Li and may now be viewed in the eRA Commons by going to Status, executing a query for the grant and selecting link associated with the Grant Number.

For any further questions about this email, call the eRA Service Desk at 1-866-504-9552 or refer to http://grants.nih.gov/support for additional methods of contact. Please access Commons at http://public.era.nih.gov/commons/.
For more information please visit http://era.nih.gov/

E

UNIVERSITY OF MINNESOTA PRF

EGMS ID: 489033 PRF ID: 906521

PS Proposal ID:

Proposal Routing Form for External Research, Training or Public Service Support

1. Sponsor Name: **National Institutes of Health** **Primary Funding Source:**
2. Proposal Title: **Receptor recognition and cell entry of coronaviruses**
3. Sponsor Due Date/Time: **04/15/2017 - 5pm CST** **Receipt**
4. RFA/RFP/CFDA/FOA#:
5. Submission Medium: **Electronic [other than Grants.gov]** **eRA Commons submission of RPPR**
6. University Contact: **Weber, Lynn F** **(612) 626-6902** **weber117@umn.edu**
7. Administering Dept: **PHCL PHARMACOLOGY ADMIN** **Department ID: 11718**
8. Principal Investigator:

			Paid	Cost Share	PI	Key
Li, Fang						
lifang	**ID 3694715**	**(612) 625-6149**	**25**	**0**	**X**	**X**
Associate Professor [9402]	**PHCL PHARMACOLOGY ADMIN**	**DeptID 11718**				

FCOI ✓ OK ✓2017

9. Investigator(s):
10. Proposal is for:

	Basic %	Applied %	Development %	Total %
Research	**100**	**0**	**0**	**100**

11. Proposal is:

	Agency Award #	EFS Award #	Primary Project
Continuation	**5 R01 AI089728-7**	**30021171800054028**	

12. Proposed Duration & Amount Requested:

	Start	End	Direct $$	Indirect $$	F&A Rate	Total
Initial/Current Budget	**06/01/2017**	**05/31/2018**	**338,185**	**115,253**	**52% MTDC**	**453,438**
All Years	**06/07/2016**	**05/31/2018**	**338,185**	**115,253**	**52% MTDC**	**453,438**

[X] Departmental Directions to SPA for Award Set Up
Proposal budget has been established in EGMS: this budget should be used to set up the award; SPA may pro-rate at time of award if needed to accommodate award amount.

[X] **F&A Rate Used** **52% MTDC**
Full F&A Rate

F&A Comments

13. Project Involves:

[X] **Recombinant DNA, Infectious Agents or Biological Toxins** **There will be recombinant DNA, infectious agents or biological toxins used**

[X] **Application status of this project with the Institutional Biosafety Committee**

Status	Study Code/Protocol ID	Approval Date
Approved	**1512-3326H**	**05/03/2016**

[X] **Chemicals** **There will be chemicals used** **The appropriate chemical safety training and hazardous waste training records have been completed**

14. Subawards: [X] University of North Carolina - Chapel Hill

Outside Entities: [ ]

15. Conflict of Interest: [X]

a. There is not a significant financial interest, OR business interest in a business entity that could benefit from the results of this project

REPA #: **Approval Date:**

Comments:

[X]

b. There is not a familial connection OR financial or business interest (of any amount) with a proposed subrecipient or collaborator.

Comments:

16. Inventions: [ ]

17. Space: [X]

There is sufficient and suitable space to house this project presently assigned to the principal investigator's or other investigator's department or college.	**3-258**	**NHH**

18. Resources/Space/Staff:

19. Indirect Cost Recovery: [X]

ICR revenue will not be shared

20. Matching and Cost Sharing: [ ]

21. Program Income: [ ]

22. International Component: [ ]

23. Fairview Health Services: [ ]

24. PROPOSAL ABSTRACT or EXECUTIVE SUMMARY

25. NOTES

APPROVALS and CERTIFICATIONS

Principal Investigator:

(1) I certify the information submitted within the application is true, complete and accurate to the best of the PI's knowledge;
(2) that any false, ficticious, or fraudulent statements or claims may subject the PI to criminal, civil, or administrative penalties; and
(3) that the PI agrees to accept the responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of the application.

Investigator(s):

The information provided on this form and in the accompanying proposal is correct to the best of my knowledge. Equipment budgeted in this application is not otherwise available for use on this project from existing departmental or collegiate inventories. In the event this application is awarded, I (we) agree to abide by all applicable institutional and sponsoring agency policies and procedures including the Intellectual Property Policy of the University of Minnesota and to follow commonly accepted scientific practices in recording and maintaining records of research. I (we) certify that I (we) have read the University of Minnesota Code of Conduct policy and agree to abide by the rights and responsibilities as identified therein.

Signature Fang Li — **03-31-2017**
Fang Li (lifang) : Principal Investigator — **Date**

Department Head(s) and/or Division Head(s): Academic Dean(s), Chancellor or Provost:

We certify that the above statements are correct to the best of our knowledge. The commitment of departmental and collegiate resources, if any, has been noted and approved. We agree that the scientific objectives of this application are in keeping with departmental and collegiate goals. We certify that the individuals from our unit(s) listed on this PRF have filed a current Report of External Professional Activities (REPA).

Signature DeJarlais, Carrie L — **03-31-2017**
DeJarlais, Carrie L (dejar001) : Dept Administrator/Representative — **Date**

Signature Hendricksen, Kirsti — **03-31-2017**
Hendricksen, Kirsti (hendr056) : Dept Administrator/Representative — **Date**

Signature Siegfried, Jill M — **03-31-2017**
Siegfried, Jill M (jsiegfri) : Dept Head/Delegate — **Date**

Signature tmeddean — **03-31-2017**
tmeddean (tmeddean) : Dept Administrator/Representative — **Date**

Signature Segal, Rosalyn A — **03-31-2017**
Segal, Rosalyn A (rsegal) : Research Associate Dean/Delegate — **Date**

Subject: PRF# 906521 Fang Li NIH AI089728 RPPR
From: Lynn Weber <weber117@umn.edu>
Date: 4/3/2017 9:37 AM
To: "proposal@umn.edu" <proposal@umn.edu>
CC: Erin Knudsen <jond0006@umn.edu>

--

Lynn Weber
Department of Pharmacology
University of Minnesota
Medical School
6-120 Jackson Hall
321 Church Street SE
Minneapolis, MN 55455
Phone: 612-626-6902
Fax: 612-625-8408
Email: weber117@umn.edu
http://www.pharmacology.med.umn.edu/

Attachments:

Fang Li AI089728 RPPR PRF# 906521.pdf	21.0 KB
*Fang Li AI089728 Budget 489033 REV 3-29-17.pdf	561 KB
UNC Budget for NIH AI089728 Progress Report 3-28-17 REV.xlsx	14.4 KB
UNC Facepage signed REV 3-28-17.pdf	667 KB

Program Director/Principal Investigator (Last, First, Middle): Li, Fang

DETAILED BUDGET FOR NEXT BUDGET PERIOD – DIRECT COSTS ONLY	FROM	THROUGH	GRANT NUMBER
	06/01/2017	05/31/2018	AI089728

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Li, Fang	PD/PI	3.00	0.00	0.00	29758	9927	39685
Minn Stat 13.32	Researcher 2	12.00	0.00	0.00	33669	9124	42793
Shang, Jian	Post Doc	3.60	0.00	0.00	14243	3032	17275
SUBTOTALS					111339	31207	142546

CONSULTANT COSTS

EQUIPMENT *(Itemize)*

SUPPLIES *(Itemize by category)*
Molecular biology supplies ($15,000); Cell culture supplies ($11,000); Protein purification ($11,000); Protein crystallization supplies ($15,000); Plastic & glassware supplies ($12,755);

64755

TRAVEL
PI and post doc travel to meeting to present results ($3,000);

3000

INPATIENT CARE COSTS

OUTPATIENT CARE COSTS

ALTERATIONS AND RENOVATIONS *(Itemize by category)*

OTHER EXPENSES *(Itemize by category)*
Lab Services ($2,500); Publication costs ($2,200);

7500

SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD		**$** 217801
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	79200
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	41184
TOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD *(Item 8a, Face Page)*		**$** 338185

***** ADDITIONAL OTHER EXPENSES *****

Equipment repair & maintenance services ($2,500); Communications ($300);

Program Director/Principal Investigator (Last, First, Middle): Li, Fang

BUDGET JUSTIFICATION	GRANT NUMBER AI089728

Provide a detailed budget justification for those line items and amounts that represent a significant change from that previously recommended. Use continuation pages if necessary.

***** BUDGET NUMBER JUSTIFICATIONS *****

Year 1: ($18,150 x 52%) = $9,438 + ($199,651 x 53%) = $105,815 = $115,253

Total To Apply F&A Rate = Total Direct Costs - Total Backed Out Costs

217,801 = 338,185 - 120,384

Total F&A Costs = 115,253

Total Costs = Total Direct Costs + Total F&A Costs

453,438 = 338,185 + 115,253

Future years recurring costs have been calculated using a 0% inflation rate, unless noted otherwise in the item justification.

Base salaries listed are for the current University appointment. Calculation of the amount of salary requested in each budget period includes inflation effective July 1 of each fiscal year, prorated to the budget period, and adjusted if appointment is less than 100%. If this award is reduced, effort obligations and proposed cost sharing (matching or in-kind) will be reduced proportionately.

FISCAL YEAR	ACADEMIC	CIVIL SERVICE	TRADES/TEMP
2017	31.80%	26.00%	8.10%
2018	33.50%	27.20%	7.70%
FISCAL YEAR	POST DOCS	GRAD ASSIST	UNDERGRADS

CURRENT BUDGET PERIOD	FROM 06/01/17	THROUGH 05/31/18

Explain any estimated unobligated balance (including prior year carryover) that is greater than 25% of the current year's total budget.

********** ADDITIONAL BUDGET JUSTIFICATIONS **********

2017	20.10%	16.90%	0.00%
2018	21.40%	15.00%	0.00%

GRADUATE/PROFESSIONAL TRAINING STUDENTS FRINGE RATES:
The tuition remission component of fringe varies by individual based on the number of credits.

For more information about fringe rates, check the SPA webpage at www.ospa.edu.

********** BUDGET SETUP JUSTIFICATION(S) **********
Future years recurring costs have been calculated using a 0% inflation rate, unless noted otherwise in the item justification.

********** CONTRACTOR COSTS JUSTIFICATION(S) **********
University of North Carolina, Chapel Hill
Period 1: Direct Costs $79,200 Indirect Costs $41,184

Help

Prompt Path: Acctg Date→Award

Sponsored Award Overview

Report Run Date: Thursday,4/13/2017

Data as of Thursday,04/13/2017 01:21 AM

Accounting Date Through: 04/13/2017
Encumbered Facilities and Admin: Included

Award: CON000000058289 - Receptor recognition and cell entry of coronaviruses
Award Sponsor: NIH Nat'l Inst of Allergy & Infect Dis
Award Start Date: 06/07/2016
Award End Date: 05/31/2021
Award Time Elapsed: 17%
Award %Left: 12%
F&A: RSRCH MTDC 52%

Cost Share (Actuals):	$0.00
Program Income (Actuals):	$0.00
TIP Income (Actuals):	$0.00
Invoiced Amount ITD:	$302,161.46
Open AR Amount ITD:	$2,132.33

Project	PP	PI	Detail Account	A Finalized Budget Amount	B Pre-Enc & Enc (04/01/2017 - 04/13/2017)	C Expenses (04/01/2017 - 04/13/2017)	D Pre-Enc & Enc (INCEPTION THROUGH 04/13/2017)	E Expenses (INCEPTION THROUGH 04/13/2017)	F = A - D - E Available Balance
00054028	Y	Li,Fang	700100 - Salaries-Faculty-Fin Bdg Only	32,033.00	-1,232.01	1,232.01	5,297.64	25,872.21	863.15
00054028	Y	Li,Fang	700210 - Salaries-Post Doc-FinBd	50,000.00	0.00	0.00	0.00	0.00	50,000.00
00054028	Y	Li,Fang	700500 - Salaries-Civil Service-Fin Bdg	35,000.00	-2,412.24	2,412.24	10,372.63	41,325.48	-16,698.11
00054028	Y	Li,Fang	710100 - Fringe - Faculty-Fin Bdg Only	10,795.00	-391.78	391.78	1,684.65	8,227.38	882.97
00054028	Y	Li,Fang	710210 - Fringe-Post Doc-FinBd	11,200.00	0.00	0.00	0.00	0.00	11,200.00
00054028	Y	Li,Fang	710500 - Fringe-Civil Service-Fin Bdg	9,590.00	-627.19	627.19	2,696.92	10,744.75	-3,851.67
00054028	Y	Li,Fang	720200 - Lab/Med Supplies-FinBdg Only	50,592.00	1,107.68	838.17	4,718.85	48,924.20	-3,051.05
00054028	Y	Li,Fang	720300 - Gen Oper Services-Fin Bdg Only	2,000.00	0.00	161.63	0.00	161.63	1,838.37
00054028	Y	Li,Fang	720400 - Lab/Medical Svcs-FinBdg Only	2,500.00	0.00	0.00	0.00	35.00	2,465.00
00054028	Y	Li,Fang	720500 - Telecommunications-FinBdg Only	300.00	0.00	0.00	0.00	0.00	300.00
00054028	Y	Li,Fang	720600 - Travel/Mileage/Mov-FinBdg Only	3,000.00	0.00	0.00	0.00	0.00	3,000.00
00054028	Y	Li,Fang	750100 - NC Bldgs/Equip-Fin Bdg Only	5,000.00	0.00	0.00	0.00	1,626.63	3,373.37
00054028	Y	Li,Fang	780100 - Repairs/Maint-FinBdg Only	2,500.00	0.00	0.00	0.00	0.00	2,500.00
00054028	Y	Li,Fang	810300 - Sub-Cont Not Subj to ICR-FBOnl	95,384.00	0.00	0.00	32,157.37	63,226.63	0.00
00054028	Y	Li,Fang	810400 - Sub-Contr Subj to ICR-FB Only	25,000.00	0.00	0.00	0.00	25,000.00	0.00
00054028	Y	Li,Fang	850100 - Capital Equip - Fin Bdg Only	5,000.00	0.00	0.00	0.00	0.00	5,000.00
			Direct Cost Total:	**$339,894.00**	**-$3,555.54**	**$5,663.02**	**$56,928.06**	**$225,143.91**	**$57,822.03**
00054028	Y	Li,Fang	810500 - F/A Costs-Fin Bdg Only	124,546.00	0.00	519.90	42,773.68	81,772.32	0.00
			F&A Total:	**$124,546.00**	**$0.00**	**$519.90**	**$42,773.68**	**$81,772.32**	**$0.00**

CSR: $0.00 RFA: $0.00	**Project Total:**	**$464,440.00**	**-$3,555.54**	**$6,182.92**	**$99,701.74**	**$306,916.23**	**$57,822.03**
CSR: $0.00 RFA: $0.00	**Award Total:**	**$464,440.00**	**-$3,555.54**	**$6,182.92**	**$99,701.74**	**$306,916.23**	**$57,822.03**

Back to top

Encumbered Facilities and Admin is included on this report.

yr 6 464,440
yr 7 453,437 ✓

162,278.54
34.9%

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ID: JOND0006
Institution: UNIVERSITY OF MINNESOTA
Roles: SO
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Application Information

Grant Number:	5R01AI089728-07
Institution:	UNIVERSITY OF MINNESOTA
PD/PI Name:	Li, Fang
Project Title:	Receptor recognition and cell entry of coronaviruses
Due Date:	04/15/2017
Current Reviewer:	Knudsen, Erin E
Status:	Reviewer Work in Progress

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National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland 20892

Department of Health and Human Services

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A. COVER PAGE

Project Title: Receptor recognition and cell entry of coronaviruses	
Grant Number: 5R01AI089728-07	**Project/Grant Period:** 06/07/2016 - 05/31/2021
Reporting Period: 06/07/2016 - 05/31/2017	**Requested Budget Period:** 06/01/2017 - 05/31/2018
Report Term Frequency: Annual	**Date Submitted:**
Program Director/Principal Investigator Information: FANG LI , PHD **Phone number:** 612-625-6149 **Email:** lifang@umn.edu	**Recipient Organization:** UNIVERSITY OF MINNESOTA UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS, MN 554552070 **DUNS:** 555917996 **EIN:** 1416007513A1 **RECIPIENT ID:** 906521
Change of Contact PD/PI: N/A	
Administrative Official: ERIN E KNUDSEN 450 McNamara Alumni Center 200 Oak Street SE Minneapolis, MN 55455 **Phone number:** 612-624-5599 **Email:** jond0006@umn.edu	**Signing Official:** KEVIN MCKOSKEY 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS, MN 554552070 **Phone number:** 612-624-5599 **Email:** kevin@umn.edu
Human Subjects: No	**Vertebrate Animals:** No
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Specific Aims
Coronaviruses (CoVs) cause epidemic outbreaks of diseases in humans and other animals, symbolized by the recent emergence of SARS-CoV, MERS-CoV, and porcine epidemic diarrhea CoV (PEDV). Receptor recognition and cell entry are two essential steps in the CoV infection cycle and function as critical determinants of CoV host range and tissue tropism. They are also primary targets for antiviral intervention. An envelope-anchored CoV spike protein recognizes host receptors through its S1 subunit and fuses viral and host membranes through its S2 subunit. CoVs recognize a variety of host receptors through one or both of its spike S1 domains (S1-NTD and S1-CTD), and are also regulated for cell entry by host proteases that cleave at the S1/S2 boundary. In the previous funding cycle, we have determined the crystal structures of a number of CoV S1 domains alone or in complex with their receptor. We have also identified host receptors as well as the cell entry mechanisms for a number of different CoVs. Our studies have revealed how CoVs exploit host receptors and proteases to regulate their host range, tropism and pathogenesis, and how SARS-CoV and MERS-CoV transmit from animals to humans to cause epidemics. In this competitive grant renewal, we will continue to investigate fundamental questions regarding receptor recognition and cell entry of CoVs from different genera. The long-term goals of these studies are to develop global models of CoV-receptor interactions and protease processing in evolution, entry, tropism, pathogenesis and cross-species transmission of CoVs.

Aim 1: Receptor binding by CoV S1-NTDs. We previously determined the structures of beta-genus mouse CoV (MHV) S1-NTD complexed with its protein receptor CEACAM1 and of beta-genus bovine CoV (BCoV) S1-NTD by itself. We also showed that S1-NTDs of BCoV and alpha-genus PEDV both recognize sugar receptors. Our studies revealed that beta-CoV S1-NTDs have the same structural fold as human galectins (galactose-binding lectins), but bind either protein or non-galactose sugar receptors. This aim will investigate how S1-NTDs from different genera have evolved to bind sugar receptors. Our overall hypothesis is that S1-NTDs from different CoV genera originated from host galectins, but have evolved molecular mechanisms to recognize novel receptors. To test this hypothesis, we will determine the molecular and structural basis for sugar binding by BCoV and PEDV S1-NTDs. These studies will reveal evolution of CoV S1-NTDs, help evaluate sugar binding by other CoVs, and may facilitate future design of sugar analogue inhibitors and subunit vaccines against CoVs.

Aim 2: Receptor biding by CoV S1-CTDs. We determined the structures of several CoV S1-CTDs in complex with their protein receptors, including beta-genus SARS-CoV S1-CTD complexed with its protein receptor ACE2. Our research suggested that two mutations played critical roles in the transmission of SARS-CoV from palm civets to humans. However, recent studies isolated several bat SARS-like CoVs (SLCoVs) with potentials to infect humans directly. This aim will investigate the cross-species transmission pathway of bat SLCoVs by examining the interactions between their S1-CTDs and ACE2 from different species. Our overall hypothesis is that to transmit from bats to another species, bat SLCoVs need to adapt to ACE2 from that species through selective mutations in their S1-CTD. To test the hypothesis, we will (i) identify receptor and viral determinants for transmission of bat SLCoVs to other species (civet, human and mouse); (ii) determine the crystal structures of bat SLCoV S1-CTDs and ACE2 from other species. These studies will reveal the molecular mechanisms for cross-species transmission of bat SLCoVs, inform future bat SLCoV emergence pathways to other mammals, and facilitate epidemic monitoring and the development of broadly cross-protective intervention strategies.

Aim 3: Cell entry by CoVs. Previously we characterized the pre-fusion and post-fusion conformations of SARS-CoV spike, demonstrated that host proteases serve as a cross-species barrier for MERS-CoV, and showed that a glycosylated protease motif attenuates human cell entry of bat CoV HKU4. Our overall hypothesis is that CoV spikes have evolved a variety of mechanisms for protease activation that can meet their specific need for host range, tissue tropism, and pathogenesis. We will investigate the following questions regarding CoV entry. (i) How does neurotropic MHV enter neurons where receptor expression is low? (ii) How does HCoV-NL63 modulate its cell entry and pathogenesis? (iii) How does PEDV enter intestinal cells where extracellular proteases are abundant? These studies will reveal how cell entry regulates the host range, tropism and pathogenesis of CoVs, and may facilitate future design of protease inhibitors against CoVs.

Approaches: We take multi-disciplinary approaches including crystallographic studies of CoV S1 domains complexed with their receptor, biochemical assays on S1 domains/receptor binding, biochemical analysis of protease cleavages of CoV spikes, electron microscopic analysis of conformational states of CoV spikes, pseudovirus entry assays, reverse genetics, and live virus infection assays.

Impact: This research uses CoVs as model systems to establish novel principles in virology that govern viral evolution, receptor recognition, cell entry, cross-species infections and tropism, which may be extended to other virus families. These studies are also critical for evaluating the emerging disease potentials of CoVs and for preventing, controlling and treating the spread of CoVs in humans and other animals.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Fang_Li_NIH_Report_03272017.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

For the next funding year, we will focus on the following studies on the receptor recognition and cell entry of coronaviruses.

First, we will determine the cryo-EM structures of spike proteins from a number of coronaviruses including SARS coronavirus, avian infectious bronchitis virus, and porcine epidemic diarrhea coronavirus (PEDV). We have used a number of methods to stabilize and obtain distinctive conformations of these spike proteins, including pre-fusion state (state before membrane fusion), intermediate state (transitional state during membrane fusion), and post-fusion state (state after membrane fusion). We will determine the high-resolution cryo-EM structures at each of these conformations for different coronaviruses. These studies will (1) elucidate detailed membrane fusion mechanisms by coronavirus spike proteins, and thus cell entry mechanisms of coronaviruses; (2) provide insight into the evolution and receptor binding of coronaviruses; (3) establish a structural framework for vaccine and drug design.

Second, we will investigate sugar-binding mechanisms for a number of coronaviruses. So far we have determined the crystal structure of sugar-binding receptor-binding domain (RBD) of bovine coronavirus. We will further determine its structure complexed with its sugar receptor. We will also determine the structures of the sugar-binding RBDs from PEDV and porcine delta coronavirus as well as these structures in complex with their respective sugar receptor. These studies will (1) elucidate the structural basis for sugar receptor binding by coronaviruses; (2) establish a structural framework for design and development of sugar analogues as anti-coronavirus inhibitors.

Third, we will investigate the proteases that activate coronavirus entry into host cells. We will examine whether proteases from different host species or from the different tissues within the same host species activate coronavirus entry differently, thus serving as a critical determinant of the host range and tissue tropism of coronaviruses. The host species that we will study include humans, pigs, and bats. The tissues that we will study include cell lines from the lung, the GI tract, the kidney, and the liver. These studies will (1) provide novel insight into the host range and tissue tropism of coronaviruses; (2) establish the correlation between viral entry and viral tropism.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

Progress report for AI089728

Studies and Results from Li lab

Du et al. (2016, Nature Communications) identified an intrinsic limitation associated with viral subunit vaccine design and found a way to overcome this limitation. More specifically, we found that when the receptor-binding domain (RBD) of MERS coronavirus (MERS-CoV) was taken out of the context of the full-length spike protein trimer, large surface areas on the RBD become artificially exposed and they contain immunodominant non-neutralizing epitopes that distract the host immune system from reacting to neutralizing epitopes, reducing vaccine efficacy. To over this limitation, we introduce a new concept 'neutralizing immunogenicity index' (NII) to evaluate individual epitopes' neutralizing immunogenicity. To determine the NII, we mask the epitope with a glycan probe and then assess the epitope's contribution to the vaccine's overall neutralizing immunogenicity. As proof-of-concept, we measure the NII for different epitopes on MERS-CoV RBD. Further, we design a variant form of this vaccine by masking an epitope that has a negative NII score. This engineered vaccine demonstrates significantly enhanced efficacy in protecting transgenic mice from lethal MERS-CoV challenge. Our study may guide the rational design of highly effective subunit vaccines to combat MERS-CoV and other life-threatening viruses.

Peng et al. (2017, Journal of Biological Chemistry) investigated the structural and molecular mechanism for coronavirus-driven evolution of host receptor. Hosts and pathogens are locked in an evolutionary arms race. To infect mice, mouse hepatitis coronavirus (MHV) has evolved to recognize mouse CEACAM1a (mCEACAM1a) as its receptor. To elude MHV infections, mice may have evolved a variant allele from the *Ceacam1a* gene, called *Ceacam1b*, producing mCEACAM1b, which is a much poorer MHV receptor than mCEACAM1a. Previous studies showed that sequence differences between mCEACAM1a and mCEACAM1b in a critical MHV-binding CC' loop partially account for the low receptor activity of mCEACAM1b, but detailed structural and molecular mechanisms for the differential MHV receptor activities of mCEACAM1a and mCEACAM1b remained elusive. Here we have determined the crystal structure of mCEACAM1b and identified the structural differences and additional residue differences between mCEACAM1a and mCEACAM1b that affect MHV binding and entry. These differences include conformational alterations of the CC' loop as well as residue variations in other MHV-binding regions, including β-strands C' and C'' and loop C'C''. Using pseudovirus entry and protein-protein binding assays, we show that substituting the structural and residue features from mCEACAM1b into mCEACAM1a reduced the viral receptor activity of mCEACAM1a, whereas substituting the reverse changes from mCEACAM1a into mCEACAM1b increased the viral receptor activity of mCEACAM1b. These results elucidate the detailed molecular mechanism for how mice may have kept pace in the evolutionary arms race with MHV by undergoing structural and residue changes in the MHV receptor, providing insight into this possible example of pathogen-driven evolution of a host receptor protein.

Liu et al. (2016, Journal of Biological Chemistry) examined proteases that activate the cell entry of porcine epidemic diarrhea coronavirus (PEDV). PEDV is currently devastating the United States pork industry by causing an 80-100% fatality rate in infected piglets. Coronavirus spike proteins mediate virus entry into cells, a process that requires the spike proteins to be proteolytically activated. It has been a conundrum which proteases activate PEDV entry. Here we systematically investigated the roles of different proteases in PEDV entry using pseudovirus entry, biochemical, and live virus infection assays. We found that the PEDV spike is activated by lysosomal cysteine proteases but not proprotein convertases or cell surface serine proteases. Extracellular trypsin activates PEDV entry when lysosomal cysteine proteases are inhibited. We further pinpointed cathepsin L and cathepsin B as the lysosomal cysteine proteases that activate the PEDV spike. These results advance our understanding of the molecular mechanism for PEDV entry and identify potential antiviral targets for curbing the spread of PEDV.

Li (2016, Annual Review of Virology) reviewed the current knowledge about the structures, functions, and evolution of the coronavirus spike protein, which is a multifunctional molecular machine that mediates coronavirus entry into host cells. During viral entry, the spike protein first binds to a receptor on the host cell surface through its S1 subunit and then fuses viral and host membranes through its S2 subunit. Two domains in S1 from different coronaviruses recognize a variety of host receptors, leading to viral attachment. The spike protein exists in two structurally distinct conformations, prefusion and postfusion. The transition from prefusion to postfusion conformation of the spike protein must be triggered, leading to membrane fusion. This article

discussed how the two S1 domains recognize different receptors and how the spike proteins are regulated to undergo conformational transitions. It further discussed the evolution of these two critical functions of coronavirus spike proteins, receptor recognition and membrane fusion, in the context of the corresponding functions from other viruses and host cells.

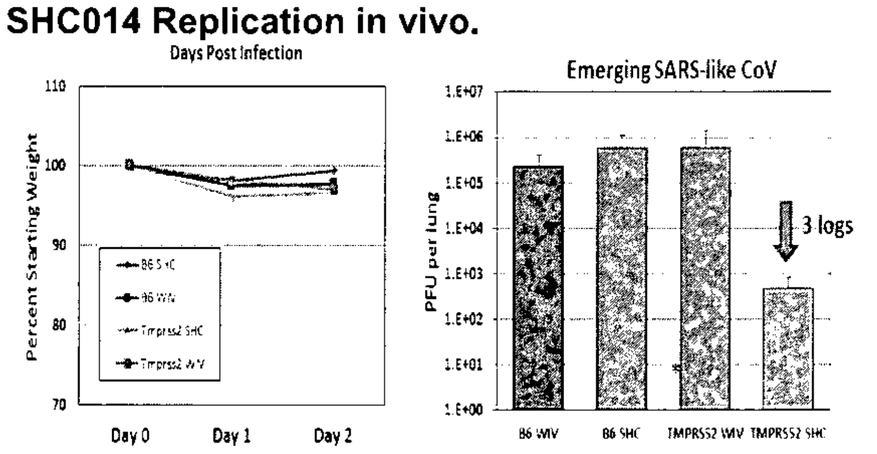
Tao et al. (2016, Journal of Virology) constructed four recombinant RBD (rRBD) proteins with single or multiple mutations detected in representative human MERS-CoV strains from the 2012, 2013, 2014, and 2015 outbreaks, respectively, and one rRBD protein with multiple changes derived from camel MERS-CoV strains. Like the RBD of prototype EMC2012 (EMC-RBD), all five RBDs maintained good antigenicity and functionality, the ability to bind RBD-specific neutralizing monoclonal antibodies (MAbs) and the DPP4 receptor, and high immunogenicity, able to elicit S-specific antibodies. They induced potent neutralizing antibodies cross-neutralizing 17 MERS pseudoviruses expressing S proteins of representative human and camel MERS-CoV strains identified during the 2012-2015 outbreaks, 5 MAb escape MERS-CoV mutants, and 2 live human MERS-CoV strains. We then constructed two RBDs mutated in multiple key residues in the receptor-binding motif (RBM) of RBD and demonstrated their strong cross-reactivity with anti-EMC-RBD antibodies. These RBD mutants with diminished DPP4 binding also led to virus attenuation, suggesting that immunoevasion after RBD immunization is accompanied by loss of viral fitness. Therefore, this study demonstrates that MERS-CoV RBD is an important vaccine target able to induce highly potent and broad-spectrum neutralizing antibodies against infection by divergent circulating human and camel MERS-CoV strains.

Du et al. (2016, Expert Opinion On Therapeutic Targets) reviewed MERS-CoV S protein's structure and function, particularly S1 receptor-binding domain (RBD) and S2 heptad repeat 1 (HR1) as therapeutic targets, and summarizes current advancement on developing anti-MERS-CoV therapeutics, focusing on neutralizing monoclonal antibodies (mAbs) and antiviral peptides.

Studies and Results from Baric lab

Introduction. CoVs recognize a variety of host receptors through one or both of its spike S1 domains (S1-NTD and S1-CTD), and are also regulated for cell entry by host proteases that cleave at the S1/S2 boundary. Understanding the receptor recognition and cell entry by CoVs has important implications for virology, medicine, and epidemics. Our studies have elucidated how CoVs explore different host receptors and host proteases to expand their host ranges, and how SARS-CoV and MERS-CoV transmit from animals to humans to cause epidemics. During the past year, the Baric laboratory has made considerable progress in two principle areas of Aims 1-3 of the grant; specifically, developing a molecular clone for porcine delta coronavirus and in designing and recovering synthetic bat SARS-like CoV.

Figure 1. TMPRSS2 KO Blocks SARS-like Bt CoV SHC014 Replication in vivo.



Porcine epidemic diarrhea virus. Previously, our group developed a molecular clone for porcine epidemic diarrhea virus. Several S glycoprotein deletions mutants have been described in the literature, including TC-PC177. To investigate whether 197 aa-deletion was the determinant for the attenuation of TC-PC177, we generated a mutant (icPC22A-S1Δ197) bearing the 197 aa-deletion based on an infectious cDNA clone of PC22A strain (icPC22A). In collaboration with the Wang laboratory at Ohio State University, the icPC22A-S1Δ197 virus caused mild to moderate diarrhea and no mortality, whereas the icPC22A virus caused severe diarrhea and death in all piglets. Our data indicate that the deletion of this 197 aa-fragment in spike protein can attenuate a highly virulent PEDV, but may lose important epitopes for inducing robust protective immunity. A manuscript detailing the construct of the mutant and its pathogenic outcomes in swine has been drafted and will soon be submitted for review.

Porcine delta coronavirus reverse genetic platforms. In addition to porcine epidemic diarrhea virus, porcine delta coronavirus was first reported in the United States in February 2014, causing severe disease outbreaks of lethal disease in piglets in swine herds. *Deltacoronavirus* genus is new, having only been recently defined by genomic sequence analysis from both pig and avian isolates. Since 2009, avian deltacoronaviruses have been

detected in a wide range of domestic and wild birds and then porcine deltacoronaviruses (PdCV) Hong Kong (HK) strains emerged suddenly in south east asia. To develop a PdCV molecular clone, we developed a collaboration with Dr. Linda Saif, who provided us with wildtype and tissue culture adapted strain OH-FD22. We have deep sequenced these isolates and have ordered a molecular clone from BioBasic. We anticipate having recombinant viruses within a few months of obtaining the reverse genetic platform. In parallel, we have dropped the PdCV S glycoprotein into Venezuelan equine encephalitis virus replicon particles (VRP-PdCV-S), and after VRP vaccination, we will obtain antisera against the S glycoprotein gene. The development of these two molecular clone reagent sets will be extremely valuable for downstream studies focusing on mechanisms of coronavirus entry.

SARS-like Bat Coronaviruses. We have synthetically reconstructed recombinant bat SARS-like viruses encoding the WIV-1 and SHC014 spike, which are about 10-15% distinct from the SARS-CoV epidemic S glycoprotein. While these strains are not pathogenic in mice, infection of TMPRS2 KO mice revealed that SHC014 S, but not isogenic backbone viruses encoding the SARS or WIV-1 S glycoproteins, cannot replicate in the absence of TMPRS2 in vivo (Fig 1). These data suggest that virus emergence may have been associated with adaptive changes that allowed for epidemic SARS-CoV strains to use the TMPRSS2 to replicate efficienly in the human lung. We are currently in the process of studying the exact role of specific mutations in SHC014 TMPRSS2 protease usage, as well as evaluating tropism differences in the lungs of infected control and KO animals.

New SARS-Like Bat Coronaviruses. The SARS-like bat coronavirus, WIV16 was isolated from a single fecal sample of *Rhinolophus sinicus*, which was collected in Kunming, Yunnan Province, in July 2013. The full genomic sequence of SL-CoV WIV16 (GenBank accession number KT444582) was determined (PMC4810638). The overall nucleotide sequence of WIV16 has 96% identity (higher than that of any previously reported bat SL-CoVs) to human and civet SARS-CoVs, including 97% amino acid identity in the S glycoprotein gene. Thus, WIV16 bridges the divide between zoonotic and epidemic SARS like viruses, but may retain mutation profiles in S2 that prevent TMPRSS2 proteolytic cleavage and efficient replication in vivo. To address this question (previously GOF requested and approved to move forward), we inserted the WIV16 S glycoprotein into the genome backbone of SARS-CoV and isolated recombinant viruses. Sequence analyses revealed the recombinant virus encoded with WIV16 S glycoprotein. In preliminary in vivo studies, the WIV16-S virus is attenuated as compared to the wildtype SARS-CoV MA strain. We are characterizing WIV16-S growth in Vero and primary human airway epithelial cells in vitro and for its ability to recognize the murine, human, civet and bat ACE2 receptors ectopically expressed in DBT cells for entry and to replicate in KO mice lacking the TMPRSS2, TMPRSS4 and TMPRSS2/4 genes.

PDF-2386 (SARS-Ubanda). We have recently identified a new bat SARS like virus from Uganda (SARS-Uganda) in collaboration with Simon Anthony at EcoHealth. Analyses of the receptor binding domain suggests that it will not be able to recognize the human or mouse ACE2 receptor for entry (**Fig 2**), although it does retain some contact interface recognition sites with human (Y491, L472) or mouse (Y491, L472, L442) ACE2 receptors. The sequence comparisons predict, however, that incorporation of 5 residues *436Y, L442W, K479R, T484F, V487A from SCH014 into PDF-2386 (PDF-2386-SHC-RBD) or the 8 known mouse adapting mutations (K411E, *436H, L472F, K411E, G473N, K479N, T484Y and V487T) into the PDF-2386 Spike (PDF-2386-MA) which are predicted to confer replication of the PDF-2386 strain in mice, and perhaps, primate cells. As we are proposing to introduce the PDF-2386 S glycoprotein, with and without mouse ACE2 binding residues, into the bat SARS-like SHC014 coronavirus, we believe these experiments are exempt because neither virus has been

Strain	402	426	436	441	442	472	473	475	479	484	486	487	488	491
Urbani	T	R	Y	R	Y	L	N	Y	N	Y	T	T	G	Y
MA15	T	R	H	R	Y	L	N	Y	N	Y	T	T	G	Y
Rs3367	T	R	Y	R	S	F	N	Y	N	Y	T	N	G	Y
PDF-2386	T	N	C	R	C	L	G	Y	K	T	T	V	G	Y
RsSHC014	T	N	Y	R	W	P	N	Y	R	F	T	A	G	H
HKU3	T	A	C	R	S	*	N	V	K	N	N	V	G	Y
ACE2 interaction s	325				31*	79*			34*	41		41		37
						82*				42		353*		353*
										45				354

* indicates sequence differences between human and mouse ACE2 at this position

Figure 2. PDF-2386 (SARS-Uganda) Receptor Binding Domain Residues.

demonstrated to have pathogenic potential or be able to be transmitted in humans. Moreover, we are introducing SHC014 preferred and mouse adapted RBD residues into PDF-2386, hence, it is unlikely to be more dangerous than the original SHC014 strain. We have not started these experiments, as we are awaiting permission to proceed.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Li W, Li F. Cross-crystal averaging with search models to improve molecular replacement phases. Structure (London, England : 1993). 2011 February 9;19(2):155-61. PubMed PMID: 21300285; PubMed Central PMCID: PMC3037595.
N/A: Not Peer Reviewed	Li F. Structure, Function, and Evolution of Coronavirus Spike Proteins. Annual review of virology. 2016 September 29;3(1):237-261. PubMed PMID: 27578435.
Complete	Liu C, Ma Y, Yang Y, Zheng Y, Shang J, Zhou Y, Jiang S, Du L, Li J, Li F. Cell Entry of Porcine Epidemic Diarrhea Coronavirus Is Activated by Lysosomal Proteases. The Journal of biological chemistry. 2016 November 18;291(47):24779-24786. PubMed PMID: 27729455; PubMed Central PMCID: PMC5114425.
Complete	Du L, Tai W, Yang Y, Zhao G, Zhu Q, Sun S, Liu C, Tao X, Tseng CK, Perlman S, Jiang S, Zhou Y, Li F. Introduction of neutralizing immunogenicity index to the rational design of MERS coronavirus subunit vaccines. Nature communications. 2016 November 22;7:13473. PubMed PMID: 27874853; PubMed Central PMCID: PMC5121417.
Complete	Tai W, Wang Y, Fett CA, Zhao G, Li F, Perlman S, Jiang S, Zhou Y, Du L. Recombinant Receptor-Binding Domains of Multiple Middle East Respiratory Syndrome Coronaviruses (MERS-CoVs) Induce Cross-Neutralizing Antibodies against Divergent Human and Camel MERS-CoVs and Antibody Escape Mutants. Journal of virology. 2017 January 1;91(1). PubMed PMID: 27795425; PubMed Central PMCID: PMC5165220.
In Process at NIHMS	Du L, Yang Y, Zhou Y, Lu L, Li F, Jiang S. MERS-CoV spike protein: a key target for antivirals. Expert opinion on therapeutic targets. 2017 February;21(2):131-143. PubMed PMID: 27936982.
Complete	Peng G, Yang Y, Pasquarella JR, Xu L, Qian Z, Holmes KV, Li F. Structural and Molecular Evidence Suggesting Coronavirus-driven Evolution of Mouse Receptor. The Journal of biological chemistry. 2017 February 10;292(6):2174-2181. PubMed PMID: 28035001; PubMed Central PMCID: PMC5313091.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
FANGLI	Y	Li, Fang	PHD	PD/PI	3	0	0			NA
JIANSHANG	N	Shang, Jian	SCD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	4	0	0			NA
Minn Stat 13.32	N	Minn Stat 13.32	BS	Researcher 2	12	0	0			NA
RALPH_BARIC	Y	Baric, Ralph S	PHD,BS	Subaward PI	1	0	0			NA
Minn Stat 13.32	N	Minn Stat 13.32	MS	Researcher 2	12	0	0			NA
JKOCHER	N	Kocher, Jacob	BS,PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	5	0	0			NA
	N	Scobey, Drew T.		Technician	4	0	0			NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)

Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

File uploaded: Li and Baric Other Support 03-28-17.pdf

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

Fang Li Other Support

National Institutes of Health 04/01/15 – 03/31/20 4.8 cal months
R01AI110700 Annual Direct Costs $245,000
Role: Co-Principal Investigator (contact Co-PI: Ralph Baric, University of North Carolina)
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
Goal: This research investigates genetic pathways regulating MERS coronavirus cross species transmission and receptor homolog usage, establishes robust animal models of human disease, and discovers critical reagents for therapeutic and vaccine testing.

National Institutes of Health 06/07/16 – 05/31/21 3.0 cal months
R01AI089728 Annual Direct Costs $217,800
Role: Principal Investigator
Receptor recognition and cell entry of coronaviruses
Goal: This research investigates how coronaviruses recognize their receptors and how they interact with receptors from different hosts. It explores novel principles governing viral evolution, virus-receptor interactions, viral host ranges and cross-species infections, and may lead to new approaches in the prevention and treatment of coronavirus infections in humans and other animals.

AHC Faculty Research Development Grant 09/01/16 – 08/31/18 0.12 cal month
University of Minnesota Annual Direct Costs $30,000
Role: Co Principal Investigator (contact PI: Robert Geraghty, University of Minnesota)
Development of biological and structural approaches to Zika virus drug discovery
Goal: This research develops and implements the tools necessary to identify small molecule inhibitors of Zika virus, and also elucidates the structure/function of the viral RNA-dependent RNA polymerase.

OTHER SUPPORT

BARIC, RALPH S.

ACTIVE:

U19 AI 107810 (PI: Baric) 06/21/13-05/31/18 2.4 cal mos
NIH/NIAID $1,572,931
Characterization of novel genes encoded by RNA and DNA viruses
Using highly pathogenic human respiratory and systemic viruses which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and host responses to determine severe disease outcomes.

U19-AI100625 (PI: Baric/Heise-MPI) 08/05/12-07/31/17 1.44 cal mos
NIH/NIAID $3,580,599
Systems Immunogenetics of Biodefense Pathogens in the Collaborative Cross
Specific Aims: In this proposal, we are utilizing the Collaborative Cross (CC), a novel panel of reproducible, recombinant inbred (RI) mouse lines to identify genes and gene interactions which regulate the induction, kinetics, and magnitude of the innate, inflammatory and adaptive arms of the immune response following virus infection. Specifically, we will develop novel modeling algorithms to predict and validate the causal relationships between natural genetic variation and host signaling networks, immune cell recruitment, and immune function.

00008956 (PI: De Silva) 07/29/15-06/30/17 0.36 cal mos
UCB/NIH $279,165
Protective immunity following dengue virus natural infections and vaccination
We will perform studies to characterize the B-cell/ antibody (responses in people who receive dengue live attenuated virus vaccines (DLAV).
Role: Co-Investigator

R01 AI 107731 (PI: De Silva) 08/05/13-07/31/17 0.24 cal mos
NIH/NIAID $621,124
Molecular Basis of Dengue Virus Neutralization by Human Antibodies
These studies proposed here are directly relevant to developing simple assays to predict the performance of the leading dengue vaccine candidates and also for developing the next generation of safe and effective dengue vaccines.
Role: Co-Investigator

R01 AI108197 (MPI: Denison/Baric) 08/01/13-07/31/17 0.60 cal mos
Vanderbilt University/NIH/NIAID $187,635
Determinants of Coronavirus Fidelity in Replication and Pathogenesis
Experiments in this aim will test the hypothesis nsp1 functions in maintaining high replication fidelity and viral RNA synthesis are coupled and that targeted engineered mutations across nsp14 alter: a) RNA fidelity outcomes; b) sensitivity nucleoside mutagens, terminators and polymerase inhibitors; c) the kinetics and magnitude of positive, negative, genomic and subgenomic RNA synthesis; and d) RNA recombination frequencies.

U19-AI106772-01 (PI: Kawaoka) 06/01/13-05/31/17 0.12 cal mos
Univ of Wisconsin/NIH $55,729
MERS-CoV Supplement for OMICs Proposal
The proposed studies will provide a more detailed look at the intracellular environment by taking "snapshots" of the lipids, metabolytes, and proteins present during viral infection time courses. These assays will allow us to determine the innate immune response occurring immediately following virus infection and to determine how the virus and cell interact over a 72 hour window.
Role: Investigator

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19 1.08 cal mos
UAB/NIH/NIAID $304,371
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Co-Investigator

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19 1.2 cal mos
Columbia/NIH/NIAID $584,891
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Project Leader, Consortium PI

Not assigned (PI: deSilva) 11/05/14-09/30/17 0.36 cal mos
Johns Hopkins U/Gates Foundation $726,915
The dengue human infection model: Defining correlates of protection and advancing vaccine development
The goal of these studies conducted by the Baric laboratory are to use recombinant dengue viruses encoding multiple homotypic neutralizing sites from multiple strains, as well as a collection of null mutants, to characterize the homotypic immune response elicited in humans following natural infection and after challenge in GSK DENV tetravalent vaccinated individuals. This grant has been funded by Gates.
Role: Co-Investigator

R01 AI110700 (PI: Baric) 04/20/15-03/31/20 0.96 cal mos
NIH/NIAID $613,691
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.

Not Assigned (PI: Baric) 01/08/16-01/07/19 0.24 cal mos
Taked Vaccines, Inc $1,243,048
In Vitro and In Vivo Characterization of Bivalent DENV Live Virus Vaccines
To provide expertise in molecular virology required for creating recombinant dengue viruses for in vitro and in vivo testing.

R01-AI125198 (PI: deSilva) 05/04/16-04/30/21 0.60 cal mos
NIH/NIAID $1,153,997
Preclinical Assays To Predict Tetravalent Dengue Vaccine Efficacy
Dengue is the most significant mosquito transmitted viral infection of humans. Vaccination is a feasible solution to prevent and control dengue. Although dengue vaccines are under development, we do not know the specific properties of antibodies induced by vaccines that are likely to protect from infection. In this project investigators from the University of North Carolina and Sanofi Pasture, a leading dengue vaccine developer, will collaborate to define properties of antibodies induced by the Sanofi vaccine that correlate with protection. The main goal of the project is to develop new assays to support the current global effort to develop dengue virus vaccines. Role: Co-Investigator

60045042 (PI: Baric) 02/01/15-01/31/18 0.12 cal mos
Ohio State Univ/USDA $44,804
Molecular attenuation mechanisms of porcine epidemic diarrhea virus in pigs
Reverse genetic strategies are used to construct a panel of live attenuated porcine epidemic diarrhea recombinant viruses for in vivo pathogenesis studies and in vitro biological characterization. We test rationale vaccine strategies to protect new born piglets against this devastating porcine epidemic virus.

64807 (PI: Baric) 06/23/16-06/22/18 0.24 cal mos
Takeda Vaccines, Inc $1,066,500
Breadth of Blockade Antibody Responses Following Norovirus Vaccination
To conduct a project as an agreement in which Dr. Ralph Baric will test Takeda provided serum samples for cross-strain blockade antibody responses.

0258-3962 (PI: Lim) 09/30/11-02/28/17 0.60 cal mos
Mount Sinai/NIH $166,793
MERS-CoV Mouse Model for Vaccine and Therapeutic Testing
To: 1) confirm that the non-conserved region of mouse DPP4 is responsible for its inability to serve as a MERS-CoV entry receptor, 2) use a powerful new in vivo site directed mutagenesis approach to humanize the murine DPP4, 3) test whether mice carrying the humanized DPP4 receptor will support MERS-CoV replication and disease within the lungs. Role: Subcontract PI

N005402801 (PI: Li) 06/07/16-05/2831/17 0.36 cal mos
Univ Minn/NIH $120,384
Receptor recognition and cell entry of coronaviruses
To investigate how CoVs explore host receptors and host proteases for regulation of their host range, cross-species transmission, tissue tropism, and pathogenesis. Role: Subcontract PI

684K644 (PI: Sims) 06/01/16-05/31/17 0.12 cal mos
Univ of Wisconsin/NIH $200,000
Systems Virology of MERS-CoV in vivo
The major goal of this award is to define host cell gene networks and pathways that are modulated following infection in our newly developed lethal mouse model of MERS-CoV. Role: Investigator

PENDING: None

OVERLAP: None

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

No

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: Regents of the University of Minnesota	555917996	MN-005	3-284 Nils Hasselmo Hall 312 Church Street SE Minneapolis MN 554550215
University of North Carolina - Chapel Hill	608195277	NC-004	3109 Hooker Research Center 135 Dauer Drive Chapel Hill NC 275997435

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

RESEARCH & RELATED BUDGET

ORGANIZATIONAL DUNS: 608195277 **Name of Organization:** The University of North Carolina at Chapel Hill

Budget Period: **Start Date:** 6/1/17 **End Date:** 5/31/18

A. Senior/Key Person

Project Role	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)
Consortium PI	Ralph	S	Baric	PhD	187,000	0.36			5,610	1,465

Total Senior/Key Person

B. Other Personnel

Project Role	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)
Postdoctoral Fellow	Jacob		Kocher	PhD	48,216	4.80			19,286	3,458
Technician	Drew	Trevor	Scobey		42,591	4.20			14,907	5,423
Total Number Other Personnel										

Total Other Personnel

TOTAL SALARY, WAGES AND FRINGE (A+B)

C. Equipment Description

Equipment item	Fund

Total Equipment

D. Travel

	Fund
1. Domestic Travel Costs (Includes Canada, Mexico and US Possessions	
2. Foreign Travel Costs	

Total Travel

E. Participant/Trainee Support Costs

	Fund
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other (Specify)	
Total Number Participants/Trainees	**Total Partipant/Trainee Costs**

F. Other Direct Costs

	Fund
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subaward/Consortium/Contractual Costs	
6. Equipment or Facility Rental/ User Fees	
7. Alterations and Renovations	
8. Maintenance Contracts	
9. Shipping	
10. Other (Specify)	
	Total Other Direct Costs

G. Direct Costs **Total Direct Costs (A thru F)**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate	Indirect Cost Base ($)	Fund
MTDC 52%	52.00%	79,200	
		Total Indirect costs	

Cognizant Federal Agency

Agency Name	
POC Name	
POC phone #	

I. Total Direct and Indirect Costs

	Fund
Total Direct and Institutional Costs (G+H)	

J. Fee

	Fund

K. Budget Justification

Send as a separate Word document. Do not include justification on this spreadsheet.
Do NOT send justification as a PDF.

Funds Requested ($)
7,075
0
0
0
0
0
0
0

7,075

Funds Requested ($)
22,744
20,330
0
0
0
0
0
0

43,074

50,149

s Requested ($)
0

s Requested ($)
2,000
2,000

s Requested ($)
0

s Requested ($)
22,051
2,000
3,000
27,051

79,200

s Requested ($)
41184
0
0

41,184

s Requested ($)

120,384

s Requested ($)

Form Approved Through 08/31/2015 OMB No. 0925-0002

Department of Health and Human Services
Public Health Services

Grant Progress Report

Review Group	Type	Activity	Grant Number

Total Project Period
From: Through:

Requested Budget Period
From: Through:

1. TITLE OF PROJECT
Receptor recognition and cell entry of coronaviruses

2a. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR
(Name and address, street, city, state, zip code)
Ralph S Baric, PhD
Department of Epidemiology
University of North Carolina – Chapel Hill
CB# 7435, McGavran-Greenberg Hall
Chapel Hill, NC 27599-7435

2b. E-MAIL ADDRESS
rbaric@email.unc.edu

2c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Epidemiology

2d. MAJOR SUBDIVISION
Public Health

2e. Tel: 919-966-3895 Fax:

3a. APPLICANT ORGANIZATION
(Name and address, street, city, state, zip code)
Office of Sponsored Research (OSR)
Administrative Office Bldg., Suite 2200
104 Ariport Drive, CB# 1350
Chapel Hill, NC 27599-1350

3b. Tel: 919-966-3411 Fax: 919-962-3352

3c. DUNS: 608195277

4. ENTITY IDENTIFICATION NUMBER
1566001393A1

6. HUMAN SUBJECTS ☒ No ☐ Yes

6a. Research Exempt	If Exempt ("Yes" in 6a): Exemption No.	If Not Exempt ("No" in 6a): IRB approval date
☐ No ☐ Yes		

6b. Federal Wide Assurance No.

6c. NIH-Defined Phase III Clinical Trial ☐ No ☐ Yes

5. NAME, TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL
Andy Johns, Assoc. Vice Chancellor for Research Suite
104 Ariport Drive, CB# 1350, Ste 2200
Chapel Hill, NC 27599-1350

Tel: 919-966-3411 Fax: 919-962-3352

E-MAIL: resadminosr@unc.edu

7. VERTEBRATE ANIMALS ☒ No ☐ Yes

7a. If "Yes," IACUC approval Date

7b. Animal Welfare Assurance No.

10. PROJECT/PERFORMANCE SITE(S)

Organizational Name: Same as above

DUNS:

8. COSTS REQUESTED FOR NEXT BUDGET PERIOD

8a. DIRECT $79,200 8b. TOTAL $120,384

Street 1:

Street 2:

City:	County:
State:	Province:
Country:	Zip/Postal Code:

9. INVENTIONS AND PATENTS ☒ No ☐ Yes

If "Yes, ☐ Previously Reported
☐ Not Previously Reported

Congressional Districts: NC-004

11. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION *(Item 13)*

Terry Magnuson, PhD Vice Chancellor for Research

TEL: 919-966-3411	FAX: 919-962-3352	E-MAIL: resadminosr@unc.edu

12. Corrections to Page 1 Face Page

13. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 11. *(In ink)*

on behalf of Terry Magnuson, PH.D.
Vice Chancellor for Research
University of North Carolina at Chapel Hill

DATE 3/28/17

A. COVER PAGE

Project Title: Receptor recognition and cell entry of coronaviruses	
Grant Number: 5R01AI089728-08	**Project/Grant Period:** 06/07/2016 - 05/31/2021
Reporting Period: 06/01/2017 - 05/31/2018	**Requested Budget Period:** 06/01/2018 - 05/31/2019
Report Term Frequency: Annual	**Date Submitted:** 04/13/2018
Program Director/Principal Investigator Information: FANG LI , PHD **Phone number:** 612-625-6149 **Email:** lifang@umn.edu	**Recipient Organization:** UNIVERSITY OF MINNESOTA UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS, MN 554552070 **DUNS:** 555917996 **EIN:** 1416007513A1 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: ERIN E KNUDSEN 450 McNamara Alumni Center 200 Oak Street SE Minneapolis, MN 55455 **Phone number:** 612-624-5599 **Email:** jond0006@umn.edu	**Signing Official:** KEVIN MCKOSKEY 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS, MN 554552070 **Phone number:** 612-624-5599 **Email:** kevin@umn.edu
Human Subjects: No	**Vertebrate Animals:** No
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Specific Aims
Coronaviruses (CoVs) cause epidemic outbreaks of diseases in humans and other animals, symbolized by the recent emergence of SARS-CoV, MERS-CoV, and porcine epidemic diarrhea CoV (PEDV). Receptor recognition and cell entry are two essential steps in the CoV infection cycle and function as critical determinants of CoV host range and tissue tropism. They are also primary targets for antiviral intervention. An envelope-anchored CoV spike protein recognizes host receptors through its S1 subunit and fuses viral and host membranes through its S2 subunit. CoVs recognize a variety of host receptors through one or both of its spike S1 domains (S1-NTD and S1-CTD), and are also regulated for cell entry by host proteases that cleave at the S1/S2 boundary. In the previous funding cycle, we have determined the crystal structures of a number of CoV S1 domains alone or in complex with their receptor. We have also identified host receptors as well as the cell entry mechanisms for a number of different CoVs. Our studies have revealed how CoVs exploit host receptors and proteases to regulate their host range, tropism and pathogenesis, and how SARS-CoV and MERS-CoV transmit from animals to humans to cause epidemics. In this competitive grant renewal, we will continue to investigate fundamental questions regarding receptor recognition and cell entry of CoVs from different genera. The long-term goals of these studies are to develop global models of CoV-receptor interactions and protease processing in evolution, entry, tropism, pathogenesis and cross-species transmission of CoVs.

Aim 1: Receptor binding by CoV S1-NTDs. We previously determined the structures of beta-genus mouse CoV (MHV) S1-NTD complexed with its protein receptor CEACAM1 and of beta-genus bovine CoV (BCoV) S1-NTD by itself. We also showed that S1-NTDs of BCoV and alpha-genus PEDV both recognize sugar receptors. Our studies revealed that beta-CoV S1-NTDs have the same structural fold as human galectins (galactose-binding lectins), but bind either protein or non-galactose sugar receptors. This aim will investigate how S1-NTDs from different genera have evolved to bind sugar receptors. Our overall hypothesis is that S1-NTDs from different CoV genera originated from host galectins, but have evolved molecular mechanisms to recognize novel receptors. To test this hypothesis, we will determine the molecular and structural basis for sugar binding by BCoV and PEDV S1-NTDs. These studies will reveal evolution of CoV S1-NTDs, help evaluate sugar binding by other CoVs, and may facilitate future design of sugar analogue inhibitors and subunit vaccines against CoVs.

Aim 2: Receptor biding by CoV S1-CTDs. We determined the structures of several CoV S1-CTDs in complex with their protein receptors, including beta-genus SARS-CoV S1-CTD complexed with its protein receptor ACE2. Our research suggested that two mutations played critical roles in the transmission of SARS-CoV from palm civets to humans. However, recent studies isolated several bat SARS-like CoVs (SLCoVs) with potentials to infect humans directly. This aim will investigate the cross-species transmission pathway of bat SLCoVs by examining the interactions between their S1-CTDs and ACE2 from different species. Our overall hypothesis is that to transmit from bats to another species, bat SLCoVs need to adapt to ACE2 from that species through selective mutations in their S1-CTD. To test the hypothesis, we will (i) identify receptor and viral determinants for transmission of bat SLCoVs to other species (civet, human and mouse); (ii) determine the crystal structures of bat SLCoV S1-CTDs and ACE2 from other species. These studies will reveal the molecular mechanisms for cross-species transmission of bat SLCoVs, inform future bat SLCoV emergence pathways to other mammals, and facilitate epidemic monitoring and the development of broadly cross-protective intervention strategies.

Aim 3: Cell entry by CoVs. Previously we characterized the pre-fusion and post-fusion conformations of SARS-CoV spike, demonstrated that host proteases serve as a cross-species barrier for MERS-CoV, and showed that a glycosylated protease motif attenuates human cell entry of bat CoV HKU4. Our overall hypothesis is that CoV spikes have evolved a variety of mechanisms for protease activation that can meet their specific need for host range, tissue tropism, and pathogenesis. We will investigate the following questions regarding CoV entry. (i) How does neurotropic MHV enter neurons where receptor expression is low? (ii) How does HCoV-NL63 modulate its cell entry and pathogenesis? (iii) How does PEDV enter intestinal cells where extracellular proteases are abundant? These studies will reveal how cell entry regulates the host range, tropism and pathogenesis of CoVs, and may facilitate future design of protease inhibitors against CoVs.

Approaches: We take multi-disciplinary approaches including crystallographic studies of CoV S1 domains complexed with their receptor, biochemical assays on S1 domains/receptor binding, biochemical analysis of protease cleavages of CoV spikes, electron microscopic analysis of conformational states of CoV spikes, pseudovirus entry assays, reverse genetics, and live virus infection assays.

Impact: This research uses CoVs as model systems to establish novel principles in virology that govern viral evolution, receptor recognition, cell entry, cross-species infections and tropism, which may be extended to other virus families. These studies are also critical for evaluating the emerging disease potentials of CoVs and for preventing, controlling and treating the spread of CoVs in humans and other animals.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Fang_Li_NIH_report_R01_CoV_04092018.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Plans

Plans for the Li Lab:

The most notable achievement from the last funding cycle was that we have learned and mastered the use of high-resolution cryo-EM in studying the structures and functions of coronavirus spike proteins (Shang et al., 2018, Journal of Virology; Shang et al. 2018, PLOS pathogens, in press). For the next funding cycle, we will apply cryo-EM to study the spike proteins of different coronaviruses such as porcine epidemic diarrhea coronavirus (PEDV), porcine transmissible gastroenteritis coronavirus (TGEV), human 229E coronavirus, bat HKU4 coronavirus, etc. Moreover, we will determine the cryo-EM structures of coronavirus spike proteins in complex with their host receptor or monoclonal antibodies; these spike proteins are from the aforementioned coronaviruses as well as mouse hepatitis coronavirus and MERS-CoV. These studies will elucidate receptor recognition, immune evasion, antigenicity of coronavirus spike proteins, and will facilitate structure-based design of coronavirus spike-based subunit vaccines.

The other focus of our current research is to study the conformational changes of coronavirus spike proteins that are associated with the membrane fusion process. To this end, we will apply different chemical triggers (e.g., low pH, receptor binding, proteolysis, etc.) to coronavirus spike proteins, and will identify, characterize, and determine the cryo-EM structures of different conformations of these spike proteins. These studies will elucidate the molecular mechanisms for the coronavirus spike-mediated membrane fusion process.

Plans for the Baric Lab:

Bat SARS-CoV WIV16. We have recovered and completely characterized recombinant chimeric viruses encoding the WIV16 spike. The virus grows efficiently in Vero E6 and primary human airway epithelial cultures, and uses the bat, human and civet ACE2 receptor for entry. The virus is attenuated in young and aged mice, even when encoding a mouse adapted mutation at positon 436 in the WIV16 spike glycoprotein. We have introduced additional mouse adapted mutations to enhance virulence in mice, and these recombinant clones have been sequence verified and recombinant viruses will be assembled shortly and then tested in young and aged mice. We have also isolated a full length molecular clone of WIV16 and are in the process of recovering full length recombinant viruses. These studies are nearing completion and we imagine writing a manuscript in the near future.

PDF-2386 (SARS-Ubanda). We have recently identified a new bat SARS like virus from Uganda (SARS-Uganda) in collaboration with Simon Anthony at EcoHealth. All attempts to get the NIH GOF and 3PCO review committee's to voice approval for these studies have not been fruitful, not because of lack of effort on our end, but rather an inability to get an answer from any of these committee's. As we have full approval at UNC to proceed with these experiments, as they will be performed using a bat coronavirus, SCH014 as the backbone genome, these experiments are not subject to 3PCO review as there is no evidence to indicate that bat coronavirus SCH014 has epidemic potential in humans.

Swine Coronavirus HKU2. We note the recent discovery of a new swine coronavirus that causes fatal acute diarrhea in swine. We will purchase this spike glycoprotein and express it from a full length replication competent hKU2 molecular clone, available in our laboratory. Zhou P et al., Nature. 2018 Apr 4. [Epub ahead of print]. Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin.

Progress report

Studies and Results

Shang et al., (2018, *Journal of Virology*, *92, e01556-17*) determined the cryo-EM structure of porcine deltacoronavirus spike protein at 3.3-Å resolution. The trimeric protein contains three receptor-binding S1 subunits that tightly pack into a crown-like structure and three membrane fusion S2 subunits that form a stalk. Each S1 subunit contains two domains, an N-terminal domain (S1-NTD) and C-terminal domain (S1-CTD). PdCoV S1-NTD has the same structural fold as alpha- and betacoronavirus S1-NTDs as well as host galectins, and it recognizes sugar as its potential receptor. PdCoV S1-CTD has the same structural fold as alphacoronavirus S1-CTDs, but its structure differs from that of betacoronavirus S1-CTDs. PdCoV S1-CTD binds to an unidentified receptor on host cell surfaces. PdCoV S2 is locked in the prefusion conformation by structural restraint of S1 from a different monomeric subunit. PdCoV spike possesses several structural features that may facilitate immune evasion by the virus, such as its compact structure, concealed receptor-binding sites, and shielded critical epitopes. Overall, this study reveals that deltacoronavirus spikes are structurally and evolutionally more closely related to alphacoronavirus spikes than to betacoronavirus spikes; it also has implications for the receptor recognition, membrane fusion, and immune evasion by deltacoronaviruses as well as coronaviruses in general.

Shang et al., (2018, *PLOS pathogens*, *in press*) determined the cryo-EM structure of avian infectious bronchitis coronavirus (IBV) spike protein from the γ-genus. The trimeric IBV spike ectodomain contains three receptor-binding S1 heads and a trimeric membrane-fusion S2 stalk. While IBV S2 is structurally similar to those from the other genera, IBV S1 possesses structural features that are unique to different other genera, thereby bridging these diverse spikes into an evolutionary spectrum. Specifically, among different genera, the two domains of S1, the N-terminal domain (S1-NTD) and C-terminal domain (S1-CTD), diverge from simpler tertiary structures and quaternary packing to more complex ones, leading to different functions of the spikes in receptor usage and membrane fusion. Based on the above structural and functional comparisons, we propose that the evolutionary spectrum of coronavirus spikes follows the order of α-, δ-, γ-, and β-genus. This study has provided insight into the evolutionary relationships among coronavirus spikes and deepened our understanding of their structural and functional diversity.

Zheng et al. (2018, *Journal of Virology*, under review) investigated the roles of lysosomal proteases in the cell tropism of SARS and MERS coronaviruses. Lysosomal proteases play critical roles in coronavirus entry by cleaving coronavirus-surface spike protein and activating the fusion of host and viral membranes. They also play important roles in cell physiology by digesting cellular wastes and providing nutrients to cell metabolism. Then how do differential lysosomal protease activities from different cells contribute to coronavirus entry? In this study, we carefully controlled the contributions from other factors in coronavirus entry including receptor binding and other host proteases, such that lysosomal protease activities became the only determinant of coronavirus entry. Using pseudovirus entry assay, cell-cell fusion assay and direct biochemical assay, we showed that lysosomal proteases from bat cells are more efficient in activating coronavirus-spike-mediated membrane fusion than their counterparts from human cells. To our knowledge, our study provides the first direct evidence supporting that lysosomal proteases are an important determinant of the cell tropism of coronaviruses.

Luo et al., (2018, *Journal of Virology*, *in press*) discovered novel bat coronaviruses in south China that use the same receptor as MERS-CoV. Previous studies suggested that the MERS coronavirus may have originated in bats. However, its evolutionary path from bats to humans remains unclear. In this study, we discovered 89 novel lineage C betacoronaviruses (BetaCoVs) in eight bat species. We provide the first evidence of a MERS-CoV-related (MERSr-CoV) virus derived from the great evening bat that uses the same host receptor as human MERS-CoV. This virus also provides evidence for a natural recombination event between the bat MERSr-CoV and another bat coronavirus HKU4. Our study expands the host ranges of MERSr-CoV and represents an important step toward establishing bats as the natural reservoir of MERS-CoV. These findings may lead to improved epidemiological surveillance of MERS-CoV and the prevention and control of the spread of MERS-CoV to humans.

Zhao et al., (2018, *Science Advances*, under review) discovered a novel nanobody that targets MERS-CoV receptor-binding domain (RBD) and demonstrates potent cross-neutralizing activity and protective efficacy against MERS-CoV infection. MERS-CoV has been circulating in both humans and camels, calling for cost- and therapeutically effective antiviral agents. Compared to traditional antibodies, nanobodies (also called single-domain antibodies) have both advantages and limitations as antiviral agents due to their small size. Here we developed a nanobody that specifically targets the RBD of MERS-CoV spike protein. It possesses the advantages common to all nanobodies such as its good expression yield and high environmental stability. It also overcomes the limitations of nanobodies by binding strongly to a conserved site on the MERS-CoV RBD, blocking the binding of the RBD to its receptor. The short half-lives of nanobodies can also be overcome through adding a C-terminal Fc dimerization tag. Indeed, the nanobody demonstrates cross-neutralizing activities against diverse human and camel MERS-CoV strains, and protects animal models from lethal MERS-CoV challenge. Therefore, our study has discovered a novel nanobody with great potentials to be developed into a cost-effective, highly potent, and wide-spectrum anti-MERS-CoV agent.

Hou et al. (2018, *Journal of Virology, 91*, e00227-17) found that Deletion of a 197-Amino-Acid Region in the N-Terminal Domain of Spike Protein Attenuates Porcine Epidemic Diarrhea Virus in Piglets. We previously isolated a porcine epidemic diarrhea virus (PEDV) strain, PC177, by blind serial passaging of the intestinal contents of a diarrheic piglet in Vero cell culture. Compared with the highly virulent U.S. PEDV strain PC21A, the tissue culture-adapted PC177 (TC-PC177) contains a 197-amino-acid (aa) deletion in the N-terminal domain of the spike (S) protein. We orally inoculated neonatal, conventional suckling piglets with TC-PC177 or PC21A to compare their pathogenicities. Within 7 days postinoculation, TC-PC177 caused mild diarrhea and lower fecal viral RNA shedding, with no mortality, whereas PC21A caused severe clinical signs and 55% mortality. To investigate whether infection with TC-PC177 can induce cross-protection against challenge with a highly virulent PEDV strain, all the surviving piglets were challenged with PC21A at 3 weeks postinoculation. Compared with 100% protection in piglets initially inoculated with PC21A, 88% and 100% TC-PC177- and mock-inoculated piglets had diarrhea following challenge, respectively, indicating incomplete cross-protection. To investigate whether this 197-aa deletion was the determinant for the attenuation of TC-PC177, we generated a mutant (icPC22A-S1Δ197) bearing the 197-aa deletion from an infectious cDNA clone of the highly virulent PEDV PC22A strain (infectious clone PC22A, icPC22A). In neonatal gnotobiotic pigs, the icPC22A-S1Δ197 virus caused mild to moderate diarrhea, lower titers of viral shedding, and no mortality, whereas the icPC22A virus caused severe diarrhea and 100% mortality. Our data indicate that deletion of this 197-aa fragment in the spike protein can attenuate a highly virulent PEDV, but the virus may lose important epitopes for inducing robust protective immunity.

Peck et al. (2017, *Journal of Virology, 91, e00534-17*) found that permissivity of Dipeptidyl Peptidase 4 Orthologs to Middle East Respiratory Syndrome Coronavirus Is Governed by Glycosylation and Other Complex Determinants. Middle East respiratory syndrome coronavirus (MERS-CoV) utilizes dipeptidyl peptidase 4 (DPP4) as an entry receptor. While bat, camel, and human DPP4 support MERS-CoV infection, several DPP4 orthologs, including mouse, ferret, hamster, and guinea pig DPP4, do not. Previous work revealed that glycosylation of mouse DPP4 plays a role in blocking MERS-CoV infection. Here, we tested whether glycosylation also acts as a determinant of permissivity for ferret, hamster, and guinea pig DPP4. We found that, while glycosylation plays an important role in these orthologs, additional sequence and structural determinants impact their ability to act as functional receptors for MERS-CoV. These results provide insight into DPP4 species-specific differences impacting MERS-CoV host range and better inform our understanding of virus-receptor interactions associated with disease emergence and host susceptibility. Here, we investigated whether glycosylation acts as a barrier to infection for other nonpermissive small-animal species, namely, ferret, guinea pig, and hamster. Understanding the virus-receptor interactions for these DPP4 orthologs will help in the development of additional animal models while also revealing species-specific differences impacting MERS-CoV host range.

Plans

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Publications funded by R01AI089728 in the current cycle

1. Shang, J., Zheng, Y., Yang, Y., Liu, C., Geng, Q., Tai, W., Du, L., Zhou, Y., Zhang, W., **Li, F**. (2018) Cryo-EM structure of porcine delta coronavirus spike protein in the pre-fusion state. *Journal of Virology*, *92, e01556-17* (PMID: 29070693)

2. Shang, J., Zheng, Y., Yang, Y., Liu, C., Geng, Q., Zhang, W., **Li, F. *** (* correspondence) (2018) Cryo-EM structure of infectious bronchitis coronavirus spike protein reveals structural and functional evolution of coronavirus spike proteins. *PLOS Pathogens, in press*

3. Luo, C., Wang, N., Yang, X., Liu, H., Zhang, W., Li, B., Hu, B., Peng, C., Geng, Q., Zhu, G., **Li, F. ***, Shi, Z. * (* co-correspondence) (2018) Discovery of novel bat coronaviruses in south China that use the same receptor as MERS coronavirus. *Journal of Virology, in press*

4. Zhao, G. He, L., Sun, S., Qiu, H., Tai, W., Chen, J., Li, J., Chen, Y., Guo, Y., Wang, Y., Ji, K., Fan, R., Du, E., Jiang, S., **Li, F. ***, Du, L. *, Zhou, Y. * (* co-correspondence) (2018) A novel nanobody targeting MERS coronavirus receptor-binding domain has potent cross-neutralizing activity and protective efficacy against MERS coronavirus infection. *Science Advances (under review)*

5. Zheng, Y., Shang, J., Yang, Y., Liu, C., Wan, Y., Wang, M., Baric, R., **Li, F. *** (* correspondence) (2018) Lysosomal proteases are a determinant of coronavirus tropism. *Journal of Virology (under review)*

6. Hou, Y., Lin, C.M., Yokoyama, M., Yount, B.L., Marthaler, D., Douglas, A.L. Ghimire, S., Qin, Y., Baric, R.S., Saif, L.J., Wang, Q. (2017) Deletion of a 197-Amino-Acid Region in the N-Terminal Domain of Spike Protein Attenuates Porcine Epidemic Diarrhea Virus in Piglets. *Journal of Virology, 91*, e00227-17 (PMC5487580).

7. Peck, K.M., Scobey, T., Swanstrom, J., Jensen, K.L., Burch, C.L., Baric, R.S., Heise, M.T. (2017) Permissivity of Dipeptidyl Peptidase 4 Orthologs to Middle East Respiratory Syndrome Coronavirus Is Governed by Glycosylation and Other Complex Determinants. *Journal of Virology 91*, e00534-17 (PMC5599747).

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Tai W, Zhao G, Sun S, Guo Y, Wang Y, Tao X, Tseng CK, Li F, Jiang S, Du L, Zhou Y. A recombinant receptor-binding domain of MERS-CoV in trimeric form protects human dipeptidyl peptidase 4 (hDPP4) transgenic mice from MERS-CoV infection. Virology. 2016 December;499:375-382. PubMed PMID: 27750111; PubMed Central PMCID: PMC5167628.
Complete	Shang J, Zheng Y, Yang Y, Liu C, Geng Q, Tai W, Du L, Zhou Y, Zhang W, Li F. Cryo-EM structure of porcine delta coronavirus spike protein in the pre-fusion state. Journal of virology. 2017 October 25. PubMed PMID: 29070693; PubMed Central PMCID: PMC5790952.
Complete	Douglas MG, Kocher JF, Scobey T, Baric RS, Cockrell AS. Adaptive evolution influences the infectious dose of MERS-CoV necessary to achieve severe respiratory disease. Virology. 2018 April;517:98-107. PubMed PMID: 29277291; PubMed Central PMCID: PMC5869108.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
FANGLI	Y	Li, Fang	PHD	PD/PI	3	0	0			NA
Minn Stat 13.32	N	Minn Stat 13.32	MS	Researcher 2	6	0	0			NA
RALPH_BARIC	N	Baric, Ralph S	PHD,BS	Subaward PI	1	0	0			NA
Minn Stat 13.32	N	Minn Stat 13.32	BS	Graduate Student (research assistant)	6	0	0			NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

No

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: Regents of the University of Minnesota	555917996	MN-005	3-284 Nils Hasselmo Hall 312 Church Street SE Minneapolis MN 554550215
University of North Carolina - Chapel Hill	608195277	NC-004	3109 Hooker Research Center 135 Dauer Drive Chapel Hill NC 275997435
UNIVERSITY OF MINNESOTA	555917996		UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER MINNEAPOLIS MN 554552070

University of North Carolina - Chapel Hill	608195277	NC-004	3109 Hooker Research Center 135 Dauer Drive Chapel Hill NC 275997435

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

A. COVER PAGE

Project Title: Receptor recognition and cell entry of coronaviruses	
Grant Number: 5R01AI089728-09	**Project/Grant Period:** 06/07/2016 - 05/31/2021
Reporting Period: 06/01/2018 - 05/31/2019	**Requested Budget Period:** 06/01/2019 - 05/31/2020
Report Term Frequency: Annual	**Date Submitted:** 04/11/2019
Program Director/Principal Investigator Information: FANG LI , PHD **Phone number:** 612-625-6149 **Email:** lifang@umn.edu	**Recipient Organization:** UNIVERSITY OF MINNESOTA UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER MINNEAPOLIS, MN 554552070 **DUNS:** 555917996 **EIN:** 1416007513A1 **RECIPIENT ID:**
Change of Contact PD/PI: No	
Administrative Official: BRETT CARLSON 200 Oak St SE Suite 450 Minneapolis, MN 554552070 **Phone number:** 612-624-5599 **Email:** carls234@umn.edu	**Signing Official:** BRETT CARLSON 200 Oak St SE Suite 450 Minneapolis, MN 554552070 **Phone number:** 612-624-5599 **Email:** carls234@umn.edu
Human Subjects: No	**Vertebrate Animals:** No
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Specific Aims
Coronaviruses (CoVs) cause epidemic outbreaks of diseases in humans and other animals, symbolized by the recent emergence of SARS-CoV, MERS-CoV, and porcine epidemic diarrhea CoV (PEDV). Receptor recognition and cell entry are two essential steps in the CoV infection cycle and function as critical determinants of CoV host range and tissue tropism. They are also primary targets for antiviral intervention. An envelope-anchored CoV spike protein recognizes host receptors through its S1 subunit and fuses viral and host membranes through its S2 subunit. CoVs recognize a variety of host receptors through one or both of its spike S1 domains (S1-NTD and S1-CTD), and are also regulated for cell entry by host proteases that cleave at the S1/S2 boundary. In the previous funding cycle, we have determined the crystal structures of a number of CoV S1 domains alone or in complex with their receptor. We have also identified host receptors as well as the cell entry mechanisms for a number of different CoVs. Our studies have revealed how CoVs exploit host receptors and proteases to regulate their host range, tropism and pathogenesis, and how SARS-CoV and MERS-CoV transmit from animals to humans to cause epidemics. In this competitive grant renewal, we will continue to investigate fundamental questions regarding receptor recognition and cell entry of CoVs from different genera. The long-term goals of these studies are to develop global models of CoV-receptor interactions and protease processing in evolution, entry, tropism, pathogenesis and cross-species transmission of CoVs.

Aim 1: Receptor binding by CoV S1-NTDs. We previously determined the structures of beta-genus mouse CoV (MHV) S1-NTD complexed with its protein receptor CEACAM1 and of beta-genus bovine CoV (BCoV) S1-NTD by itself. We also showed that S1-NTDs of BCoV and alpha-genus PEDV both recognize sugar receptors. Our studies revealed that beta-CoV S1-NTDs have the same structural fold as human galectins (galactose-binding lectins), but bind either protein or non-galactose sugar receptors. This aim will investigate how S1-NTDs from different genera have evolved to bind sugar receptors. Our overall hypothesis is that S1-NTDs from different CoV genera originated from host galectins, but have evolved molecular mechanisms to recognize novel receptors. To test this hypothesis, we will determine the molecular and structural basis for sugar binding by BCoV and PEDV S1-NTDs. These studies will reveal evolution of CoV S1-NTDs, help evaluate sugar binding by other CoVs, and may facilitate future design of sugar analogue inhibitors and subunit vaccines against CoVs.

Aim 2: Receptor biding by CoV S1-CTDs. We determined the structures of several CoV S1-CTDs in complex with their protein receptors, including beta-genus SARS-CoV S1-CTD complexed with its protein receptor ACE2. Our research suggested that two mutations played critical roles in the transmission of SARS-CoV from palm civets to humans. However, recent studies isolated several bat SARS-like CoVs (SLCoVs) with potentials to infect humans directly. This aim will investigate the cross-species transmission pathway of bat SLCoVs by examining the interactions between their S1-CTDs and ACE2 from different species. Our overall hypothesis is that to transmit from bats to another species, bat SLCoVs need to adapt to ACE2 from that species through selective mutations in their S1-CTD. To test the hypothesis, we will (i) identify receptor and viral determinants for transmission of bat SLCoVs to other species (civet, human and mouse); (ii) determine the crystal structures of bat SLCoV S1-CTDs and ACE2 from other species. These studies will reveal the molecular mechanisms for cross-species transmission of bat SLCoVs, inform future bat SLCoV emergence pathways to other mammals, and facilitate epidemic monitoring and the development of broadly cross-protective intervention strategies.

Aim 3: Cell entry by CoVs. Previously we characterized the pre-fusion and post-fusion conformations of SARS-CoV spike, demonstrated that host proteases serve as a cross-species barrier for MERS-CoV, and showed that a glycosylated protease motif attenuates human cell entry of bat CoV HKU4. Our overall hypothesis is that CoV spikes have evolved a variety of mechanisms for protease activation that can meet their specific need for host range, tissue tropism, and pathogenesis. We will investigate the following questions regarding CoV entry. (i) How does neurotropic MHV enter neurons where receptor expression is low? (ii) How does HCoV-NL63 modulate its cell entry and pathogenesis? (iii) How does PEDV enter intestinal cells where extracellular proteases are abundant? These studies will reveal how cell entry regulates the host range, tropism and pathogenesis of CoVs, and may facilitate future design of protease inhibitors against CoVs.

Approaches: We take multi-disciplinary approaches including crystallographic studies of CoV S1 domains complexed with their receptor, biochemical assays on S1 domains/receptor binding, biochemical analysis of protease cleavages of CoV spikes, electron microscopic analysis of conformational states of CoV spikes, pseudovirus entry assays, reverse genetics, and live virus infection assays.

Impact: This research uses CoVs as model systems to establish novel principles in virology that govern viral evolution, receptor recognition, cell entry, cross-species infections and tropism, which may be extended to other virus families. These studies are also critical for evaluating the emerging disease potentials of CoVs and for preventing, controlling and treating the spread of CoVs in humans and other animals.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Fang_Li_NIH_report_R01_CoV_03192019.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

We have been providing research materials (including DNA constructs and protein samples), which were generated from this project, to the scientific community when requested.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Plans for the Li Lab:
(1) The most notable achievement from the last funding cycle was that we kept learning and mastering the use of high-resolution cryo-EM in studying the structures and functions of coronavirus spike proteins (Shang et al. 2018, PLOS pathogens). For the next funding cycle, we will apply cryo-EM to study the spike proteins of different coronaviruses by themselves or in complex with their host receptor. Currently we are in the process of determining the cryo-EM structure of mouse coronavirus spike protein complexed with its receptor mouse CEACAM1a. We have already obtained a cryo-EM density map at 3.9 angstrom and built a structural model. Based on the structure, we have come up with several hypotheses on the cell mechanism of the virus and are currently doing biochemical assays to test these hypotheses. These studies have revealed detailed molecular mechanism of mouse coronavirus.
(2) We will determine the cryo-EM structures of coronavirus spike proteins in complex with monoclonal antibodies or nanobodies. We have observed the conformational changes of coronavirus spike proteins induced by antibodies, and are currently investigating antibody-enhanced entry of coronaviruses. We have obtained several lines of evidence revealing the molecular mechanisms of antibody-enhanced entry of coronaviruses. These studies will provide insight into antibody-enhanced entry of viruses and have important implications on vaccine designs and antibody-based drug therapies.
(3) Another focus of our current research is to receptor recognition mechanisms of coronaviruses, focusing on the interactions between the coronavirus spike protein receptor-binding domains (RBDs) and their receptor. Particularly APN is a receptor for numerous alpha- and delta-coronviruses. We are investigating the structural basis for these RBD/APN interactions.

Plans for the Baric Lab:

(1) Trypsin treatment reveals another barrier for zoonotic coronaviruses infection.

The new century has been marked by the emergence of several zoonotic viruses causing damage to both the global economy and public health. By exploiting viral metagenomics data, we have an opportunity to identify potential threats and define barriers to emergence. While receptor binding has been a primary focus, this study suggests that spike processing may be a major barrier to coronavirus emergence. By unlocking infection of two bat coronaviruses, HKU5 and SARS-Uganda (PDF-2386), this discovery provides an additional correlate to evaluate zoonosis potential and the opportunity to define the efficacy of targeted therapeutic treatments. It also indicates that factors beyond receptor binding govern coronavirus emergence in new hosts. (submission planned within next 30 days or so)

(2) Genetic Recovery of Severe Acute Diarrhea Swine Coronavirus (SADS-CoV).

The swine industry has suffered from the sequential emergence of a series of highly pathogenic respiratory and enteric Nidoviruses, including coronaviruses and arteriviruses. The Severe Acute Diarrhea Swine Coronavirus (SADS-CoV) emerged suddenly from bats in southern China and caused severe outbreaks with high mortality in swine herds. As outbreaks are still ongoing, global swine production and markets are endangered, highlighting a critical need for available recombinant viruses, in vitro culturing methods, and animal models for future pandemic preparedness. Using in silico sequence information and synthetic genome design, we recovered a wildtype recombinant and recombinant SADS-CoV encoding indicator genes. These viruses replicate efficiently in vitro and express a full repertoire of full length and subgenomic mRNAs, mapping the coding capacity of SADS-CoV using Minion deep sequencing approaches. Further, the host range of these viruses are evaluated using a panel of human, swine and primate cell lines. We also prepared Venezuelan equine encephalitis vaccines that encode the SADS-CoV S glycoprotein and N protein and after mouse vaccination, demonstrate that these sera detect viral proteins by Western blot analyses and anti S antisera neutralize live virus in vitro. The availability of recombinant SADS-CoV, candidate vaccines and diagnostic reagents promote a state of preparedness in the event of the global emergence of this critical pathogen of domesticated animals. (Anticipated submission date, within 60 days or so).

(3) Bat SARS-CoV WIV16. We have recovered and completely characterized recombinant chimeric viruses encoding the WIV16 spike. The virus grows efficiently in Vero E6 and primary human airway epithelial cultures, and uses the bat, human and civet ACE2 receptor for entry. The virus is attenuated in young and aged mice, even when encoding a mouse adapted mutation at positon 436 in the WIV16 spike glycoprotein. We have introduced additional mouse adapted mutations to enhance virulence in mice and isolated a full length molecular clone of WIV16, recovering full length recombinant viruses. These studies are nearing completion and we imagine writing a manuscript in the near future.

Progress report

Studies and Results

Shang et al., (2018, *PLOS pathogens*) determined the cryo-EM structure of avian infectious bronchitis coronavirus (IBV) spike protein from the γ-genus. The trimeric IBV spike ectodomain contains three receptor-binding S1 heads and a trimeric membrane-fusion S2 stalk. While IBV S2 is structurally similar to those from the other genera, IBV S1 possesses structural features that are unique to different other genera, thereby bridging these diverse spikes into an evolutionary spectrum. Specifically, among different genera, the two domains of S1, the N-terminal domain (S1-NTD) and C-terminal domain (S1-CTD), diverge from simpler tertiary structures and quaternary packing to more complex ones, leading to different functions of the spikes in receptor usage and membrane fusion. Based on the above structural and functional comparisons, we propose that the evolutionary spectrum of coronavirus spikes follows the order of α-, δ-, γ-, and β-genus. This study has provided insight into the evolutionary relationships among coronavirus spikes and deepened our understanding of their structural and functional diversity.

Zheng et al. (2018, *Journal of Virology*) investigated the roles of lysosomal proteases in the cell tropism of SARS and MERS coronaviruses. Lysosomal proteases play critical roles in coronavirus entry by cleaving coronavirus-surface spike protein and activating the fusion of host and viral membranes. They also play important roles in cell physiology by digesting cellular wastes and providing nutrients to cell metabolism. Then how do differential lysosomal protease activities from different cells contribute to coronavirus entry? In this study, we carefully controlled the contributions from other factors in coronavirus entry including receptor binding and other host proteases, such that lysosomal protease activities became the only determinant of coronavirus entry. Using pseudovirus entry assay, cell-cell fusion assay and direct biochemical assay, we showed that lysosomal proteases from bat cells are more efficient in activating coronavirus-spike-mediated membrane fusion than their counterparts from human cells. To our knowledge, our study provides the first direct evidence supporting that lysosomal proteases are an important determinant of the cell tropism of coronaviruses.

Luo et al., (2018, *Journal of Virology*) discovered novel bat coronaviruses in south China that use the same receptor as MERS-CoV. Previous studies suggested that the MERS coronavirus may have originated in bats. However, its evolutionary path from bats to humans remains unclear. In this study, we discovered 89 novel lineage C betacoronaviruses (BetaCoVs) in eight bat species. We provide the first evidence of a MERS-CoV-related (MERSr-CoV) virus derived from the great evening bat that uses the same host receptor as human MERS-CoV. This virus also provides evidence for a natural recombination event between the bat MERSr-CoV and another bat coronavirus HKU4. Our study expands the host ranges of MERSr-CoV and represents an important step toward establishing bats as the natural reservoir of MERS-CoV. These findings may lead to improved epidemiological surveillance of MERS-CoV and the prevention and control of the spread of MERS-CoV to humans.

Cui et al., (2019, *Nature Reviews Microbiology*) reviewed the origin and evolution of pathogenic coronaviruses. Severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) are two highly transmissible and pathogenic viruses that emerged in humans at the beginning of the 21st century. Both viruses likely originated in bats, and genetically diverse coronaviruses that are related to SARS-CoV and MERS-CoV were discovered in bats worldwide. In this Review, we summarize the current knowledge on the origin and evolution of these two pathogenic coronaviruses and discuss their receptor usage; we also highlight the diversity and potential of spillover of bat-borne coronaviruses, as evidenced by the recent spillover of swine acute diarrhoea syndrome coronavirus (SADS-CoV) to pigs.

Zhao et al., (2018, *Journal of Virology*) discovered a novel nanobody that targets MERS-CoV receptor-binding domain (RBD) and demonstrates potent cross-neutralizing activity and protective efficacy against MERS-CoV infection. MERS-CoV has been circulating in both humans and camels, calling for cost- and therapeutically effective antiviral agents. Compared to traditional antibodies, nanobodies (also called single-domain antibodies) have both advantages and limitations as antiviral agents due to their small size. Here we developed a nanobody that specifically targets the RBD of MERS-CoV spike protein. It possesses the advantages common to all nanobodies such as its good expression yield and high environmental stability. It also overcomes the limitations of nanobodies by binding strongly to a conserved site on the MERS-CoV RBD, blocking the binding of the RBD to its receptor. The short half-lives of nanobodies can also be overcome through adding a C-terminal Fc dimerization tag. Indeed, the nanobody demonstrates cross-neutralizing activities against diverse human and camel MERS-CoV strains, and protects animal models from lethal MERS-CoV challenge. Therefore, our study has discovered a novel nanobody with great potentials to be developed into a cost-effective, highly potent, and wide-spectrum anti-MERS-CoV agent.

Gunaratne et al. (2018, *Cell Calcium*) investigated how NAADP-dependent Ca^{2+} signaling regulates Middle East respiratory syndrome-coronavirus pseudovirus translocation through the endolysosomal system. Middle East Respiratory Syndrome coronavirus (MERS-CoV) infections are associated with a significant mortality rate, and existing drugs show poor efficacy. Identifying novel targets/pathways required for MERS infectivity is therefore important for developing novel therapeutics. As an enveloped virus, translocation through the endolysosomal system provides one pathway for cellular entry of MERS-CoV. In this context, Ca^{2+}-permeable channels within the endolysosomal system regulate both the luminal environment and trafficking events, meriting investigation of their role in regulating processing and trafficking of MERS-CoV. Knockdown of endogenous two-pore channels (TPCs), targets for the Ca^{2+} mobilizing second messenger NAADP, impaired infectivity in a MERS-CoV spike pseudovirus particle translocation assay. This effect was selective as knockdown of the lysosomal cation channel mucolipin-1 (TRPML1) was without effect. Pharmacological inhibition of NAADP-evoked Ca^{2+} release using several bisbenzylisoquinoline alkaloids also blocked MERS pseudovirus translocation. Knockdown of TPC1 (biased endosomally) or TPC2 (biased lysosomally) decreased the activity of furin, a protease which facilitates MERS fusion with cellular membranes. Pharmacological or genetic inhibition of TPC1 activity also inhibited endosomal motility impairing pseudovirus progression through the endolysosomal system. Overall, these data support a selective, spatially autonomous role for TPCs within acidic organelles to support MERS-CoV translocation.

Algaissi et al. (2019, *Journal of Infectious Diseases*) identified that elevated Human Dipeptidyl Peptidase 4 Expression Reduces the Susceptibility of hDPP4 Transgenic Mice to Middle East Respiratory Syndrome Coronavirus Infection and Disease. We used homozygous (+/+) and heterozygous (+/-) human dipeptidyl peptidase 4 (hDPP4) transgenic mice to study the effect of hDPP4 on MERS-CoV infection. Specifically, we determined values of 50% lethal dose (LD50) of MERS-CoV for the 2 strains of mice, compared and correlated their levels of soluble (s)hDPP4 expression to susceptibility, and explored recombinant (r)shDPP4 as an effective MCM for MERS infection. The results showed that hDPP4+/+ mice were unexpectedly more resistant than hDPP4+/- mice to MERS-CoV infection, as judged by increased LD50, reduced lung viral infection, attenuated morbidity and mortality, and reduced histopathology. Additionally, the resistance to MERS-CoV infection directly correlated with increased serum shDPP4 and serum virus neutralizing activity. Finally, administration of rshDPP4 led to reduced lung virus titer and histopathology. Overall, our studies suggest that the serum shDPP4 levels play a role in MERS pathogenesis and demonstrate a potential of rshDPP4 as a treatment option for MERS. Additionally, it offers a validated pair of Tg mice strains for characterizing the effect of shDPP4 on MERS pathogenesis.

van Doremalen et al. (Viruses, 2018) showed that SARS-Like Coronavirus WIV1-CoV Does Not Replicate in Egyptian Fruit Bats (*Rousettus aegyptiacus*). Severe acute respiratory syndrome (SARS)-like WIV1-

coronavirus (CoV) was first isolated from *Rhinolophus sinicus* bats and can use the human angiotensin converting enzyme 2 (ACE2) receptor. In the current study, we investigate the ability of WIV1-CoV to infect *Rousettus aegyptiacus* bats. No clinical signs were observed throughout the experiment. Furthermore, only four oropharyngeal swabs and two respiratory tissues, isolated on day 3 post inoculation, were found positive for viral RNA. Two out of twelve bats showed a modest increase in coronavirus specific antibodies post challenge. In conclusion, WIV1-CoV was unable to cause a robust infection in *Rousettus aegyptiacus* bats.

Sheahan TP and Baric RS. (Future Virol. 2018) analyzed whether regulation preventing the development of therapeutics may prevent future coronavirus pandemics. The recent surge of zoonotic EIDs in human populations is driven by a constellation of socioeconomic factors including human population growth, eroding public health infrastructures, changes in land use and agriculture and ease of global travel. HIV, Ebola virus, avian influenza (H5N1, H7N9, etc.), severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) are but a few recent examples of highly virulent, zoonotic viral EIDs that have catastrophically affected global economies and public health. Geopolitical flux since the 1990s and the potential for weaponizing EIDs provoked the creation of myriad policies aimed at protecting the USA from bioterrorist threats and the accidental release of potential pandemic pathogens from laboratories. Are these policies effective? Are they impacting countermeasure development for current and future EIDs? How are they shaping the direction of individual research programs, the recruitment of new investigators and the stability of impacted fields?

Cockrell AS et al. (Mamm Geome, 2018) investigated modeling pathogenesis of emergent and pre-emergent human coronaviruses in mice. The emergence of highly pathogenic human coronaviruses (hCoVs) in the last two decades has illuminated their potential to cause high morbidity and mortality in human populations and disrupt global economies. Global pandemic concerns stem from their high mortality rates, capacity for human-to-human spread by respiratory transmission, and complete lack of approved therapeutic countermeasures. Limiting disease may require the development of virus-directed and host-directed therapeutic strategies due to the acute etiology of hCoV infections. Therefore, understanding how hCoV-host interactions cause pathogenic outcomes relies upon mammalian models that closely recapitulate the pathogenesis of hCoVs in humans. Pragmatism has largely been the driving force underpinning mice as highly effective mammalian models for elucidating hCoV-host interactions that govern pathogenesis. Notably, tractable mouse genetics combined with hCoV reverse genetic systems has afforded the concomitant manipulation of virus and host genetics to evaluate virus-host interaction networks in disease. In addition to assessing etiologies of known hCoVs, mouse models have clinically predictive value as tools to appraise potential disease phenotypes associated with pre-emergent CoVs. Knowledge of CoV pathogenic potential before it crosses the species barrier into the human population provides a highly desirable preclinical platform for addressing global pathogen preparedness.

Douglas MG et al., (Virology, 2018) examined how adaptive evolution influences the infectious dose of MERS-CoV necessary to achieve severe respiratory disease. We recently established a mouse model (288-330$^{+/+}$) that developed acute respiratory disease resembling human pathology following infection with a high dose (5×10^6 PFU) of mouse-adapted MERS-CoV (icMERSma1). Although this high dose conferred fatal respiratory disease in mice, achieving similar pathology at lower viral doses may more closely reflect naturally acquired infections. Through continued adaptive evolution of icMERSma1 we generated a novel mouse-adapted MERS-CoV (maM35c4) capable of achieving severe respiratory disease at doses between 10^3 and 10^5 PFU. Novel mutations were identified in the maM35c4 genome that may be responsible for eliciting etiologies of acute respiratory distress syndrome at 10-1000 fold lower viral doses. Importantly, comparative genetics of the two mouse-adapted MERS strains allowed us to identify specific mutations that remained fixed through an additional 20 cycles of adaptive evolution. Our data indicate that the extent of MERS-CoV adaptation determines the minimal infectious dose required to achieve severe respiratory disease.
Addendum: A second manuscript is in progress where we have made derivative recombinant viruses designed to map the role of S mutations and mutations outside of the S gene in in vivo pathogenesis.

Plans

Plans for the Li Lab:

(1) The most notable achievement from the last funding cycle was that we kept learning and mastering the use of high-resolution cryo-EM in studying the structures and functions of coronavirus spike proteins (Shang et al. 2018, PLOS pathogens). For the next funding cycle, we will apply cryo-EM to study the spike proteins of different coronaviruses by themselves or in complex with their host receptor. Currently we are in the process of determining the cryo-EM structure of mouse coronavirus spike protein complexed with its receptor mouse CEACAM1a. We have already obtained a cryo-EM density map at 3.9 angstrom and built a structural model. Based on the structure, we have come up with several hypotheses on the cell mechanism of the virus and are currently doing biochemical assays to test these hypotheses. These studies have revealed detailed molecular mechanism of mouse coronavirus.

(2) We will determine the cryo-EM structures of coronavirus spike proteins in complex with monoclonal antibodies or nanobodies. We have observed the conformational changes of coronavirus spike proteins induced by antibodies, and are currently investigating antibody-enhanced entry of coronaviruses. We have obtained several lines of evidence revealing the molecular mechanisms of antibody-enhanced entry of coronaviruses. These studies will provide insight into antibody-enhanced entry of viruses and have important implications on vaccine designs and antibody-based drug therapies.

(3) Another focus of our current research is to receptor recognition mechanisms of coronaviruses, focusing on the interactions between the coronavirus spike protein receptor-binding domains (RBDs) and their receptor. Particularly APN is a receptor for numerous alpha- and delta-coronviruses. We are investigating the structural basis for these RBD/APN interactions.

Plans for the Baric Lab:

(1) Trypsin treatment reveals another barrier for zoonotic coronaviruses infection.

The new century has been marked by the emergence of several zoonotic viruses causing damage to both the global economy and public health. By exploiting viral metagenomics data, we have an opportunity to identify potential threats and define barriers to emergence. While receptor binding has been a primary focus, this study suggests that spike processing may be a major barrier to coronavirus emergence. By unlocking infection of two bat coronaviruses, HKU5 and SARS-Uganda (PDF-2386), this discovery provides an additional correlate to evaluate zoonosis potential and the opportunity to define the efficacy of targeted therapeutic treatments. It also indicates that factors beyond receptor binding govern coronavirus emergence in new hosts. (submission planned within next 30 days or so)

(2) Genetic Recovery of Severe Acute Diarrhea Swine Coronavirus (SADS-CoV).

The swine industry has suffered from the sequential emergence of a series of highly pathogenic respiratory and enteric Nidoviruses, including coronaviruses and arteriviruses. The Severe Acute Diarrhea Swine Coronavirus (SADS-CoV) emerged suddenly from bats in southern China and caused severe outbreaks with high mortality in swine herds. As outbreaks are still ongoing, global swine production and markets are endangered, highlighting a critical need for available recombinant viruses, in vitro culturing methods, and animal models for future pandemic preparedness. Using in silico sequence information and synthetic genome design, we recovered a wildtype recombinant and recombinant SADS-CoV encoding indicator genes. These viruses replicate efficiently in vitro and express a full repertoire of full length and subgenomic mRNAs, mapping the coding

capacity of SADS-CoV using Minion deep sequencing approaches. Further, the host range of these viruses are evaluated using a panel of human, swine and primate cell lines. We also prepared Venezuelan equine encephalitis vaccines that encode the SADS-CoV S glycoprotein and N protein and after mouse vaccination, demonstrate that these sera detect viral proteins by Western blot analyses and anti S antisera neutralize live virus in vitro. The availability of recombinant SADS-CoV, candidate vaccines and diagnostic reagents promote a state of preparedness in the event of the global emergence of this critical pathogen of domesticated animals. (Anticipated submission date, within 60 days or so).

(3) Bat SARS-CoV WIV16. We have recovered and completely characterized recombinant chimeric viruses encoding the WIV16 spike. The virus grows efficiently in Vero E6 and primary human airway epithelial cultures, and uses the bat, human and civet ACE2 receptor for entry. The virus is attenuated in young and aged mice, even when encoding a mouse adapted mutation at positon 436 in the WIV16 spike glycoprotein. We have introduced additional mouse adapted mutations to enhance virulence in mice and isolated a full length molecular clone of WIV16, recovering full length recombinant viruses. These studies are nearing completion and we imagine writing a manuscript in the near future.

Publications funded by R01AI089728 in the current cycle

1. Cui, J., **Li, F.**, Shi, Z.L. (2019) Origin and evolution of pathogenic coronaviruses. *Nature Reviews Microbiology*, 17, 181-192 (PMID: 30531947)

2. Zheng, Y., Shang, J., Yang, Y., Liu, C., Wan, Y., Geng, Q., Wang, M., **Baric, R**., **Li, F.** (2018) Lysosomal proteases are a determinant of coronavirus tropism. *Journal of Virology*, e01504-18 (PMID: 30258004)

3. Algaissi, A., Agrawal, A.S., Han, S., Peng, B.H., Luo, C., **Li, F.**, Chan, T.S., Couch, R.B., Tseng, C.K. (2018) Elevated Human Dipeptidyl Peptidase 4 Expression Reduces the Susceptibility of hDPP4 Transgenic Mice to Middle East Respiratory Syndrome Coronavirus Infection and Disease. *Journal of Infectious Diseases*, 219, 829-835 (PMID: 30256968)

4. Gunaratne, G.S., Yang, Y., **Li, F.**, Walseth, T.F., Marchant, J.S. (2018) NAADP-dependent Ca2+ signaling regulates Middle East respiratory syndrome-coronavirus pseudovirus translocation through the endolysosomal system. *Cell Calcium*, 75, 30-41 (PMID: 30121440)

5. Zhao, G., He, L., Sun, S., Qiu, H., Tai, W., Chen, J., Li, J., Chen, Y., Guo, Y., Wang, Y., Shang, J., Ji, K., Fan, R., Du, E., Jiang, S., **Li, F.**, Du, L., Zhou, Y. (2018) A novel nanobody targeting Middle East Respiratory Syndrome Coronavirus (MERS-CoV) receptor-binding domain has potent cross-neutralizing activity and protective efficacy against MERS-CoV. *Journal of Virology*, 92, e00116-18 (PMID: 29669833)

6. Shang, J., Zheng, Y., Yang, Y., Liu, C., Geng, Q., Luo, C., Zhang, W., **Li, F**. (2018) Cryo-EM structure of infectious bronchitis coronavirus spike protein reveals structural and functional evolution of coronavirus spike proteins. *PLOS Pathogens,* 14, e1007009 (PMID: 29684066)

7. Luo, C.M., Wang, N., Yang, X.L., Liu, H.Z., Zhang, W., Li, B., Hu, B., Peng, C., Geng, Q.B., Zhu, G.J., **Li, F. ***, Shi, Z.L. ***** (* co-correspondence) (2018) Discovery of novel bat coronaviruses in south China that use the same receptor as MERS coronavirus. *Journal of Virology*, 92, e00116-18 (PMID: 29669833)

8. van Doremalen N, Schäfer A, Menachery VD, Letko M, Bushmaker T, Fischer RJ, Figueroa DM, Hanley PW, Saturday G, **Baric RS**, Munster VJ. SARS-Like Coronavirus WIV1-CoV Does Not Replicate in Egyptian Fruit Bats (*Rousettus aegyptiacus*). Viruses. 2018 Dec 19;10(12) pii: E727. PMC6316779.

9. Sheahan TP, **Baric RS**. Is regulation preventing the development of therapeutics that may prevent future coronavirus pandemics? Future Virol. 2018 Mar;13(3):143-146. PMC6289267.

10. Cockrell AS, Leist SR, Douglas MG, **Baric RS**. Modeling pathogenesis of emergent and pre-emergent human coronaviruses in mice. Mamm Genome. 2018 Aug; 29(7-8):367-383. PMC6132729.

11. Douglas MG, Kocher JF, Scobey T, **Baric RS**, Cockrell AS. Adaptive evolution influences the infectious dose of MERS-CoV necessary to achieve severe respiratory disease. Virology. 2018 Apr;517:98-107. PMC5869108.

B.4 What opportunities for training and professional development has the project provided?

Currently the project is training one PhD student and one undergraduate student who works as a research scientist.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Gunaratne GS, Yang Y, Li F, Walseth TF, Marchant JS. NAADP-dependent Ca²⁺ signaling regulates Middle East respiratory syndrome-coronavirus pseudovirus translocation through the endolysosomal system. Cell calcium. 2018 November;75:30-41. PubMed PMID: 30121440; PubMed Central PMCID: PMC6251489.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
FANGLI	Y	Li, Fang	PHD	PD/PI	0.0	3.0	1.3			NA
	N	Scobey, Drew T		Technician	2.9	0.0	0.0			NA
Minn Stat 13.32	N	Minn Stat 13.32	MS	Researcher 2	6.5	0.0	0.0			NA
RALPH_BARIC	Y	Baric, Ralph S	PHD,BS	Co-Investigator	0.4	0.0	0.0			NA
SARAH_LEIST	N	Leist, Sarah R.	PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	3.3	0.0	0.0			NA
Minn Stat 13.32	N	Minn Stat 13.32	BS	Graduate Student (research assistant)	2.0	0.0	0.0			NA
	N	Auerbach, Ashley		Technician	8.3	0.0	0.0			NA
JKOCHER	N	Kocher, Jacob	BS,PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	2.5	0.0	0.0			NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

File uploaded: RPPR Other Support.pdf

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

No

Fang Li Other Support

Active

Overlap: There is no scientific overlap among the following grants.

National Institutes of Health 04/1/2015 – 03/31/2020
R01AI110700
Role: Co-Principal Investigator (The other Co-PI: Ralph Baric, University of North Carolina)
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
Goal: This research investigates genetic pathways regulating MERS coronavirus cross species transmission and receptor homolog usage, establishes robust animal models of human disease, and discovers critical reagents for therapeutic and vaccine testing.

National Institutes of Health 06/7/2016 – 05/31/2021
2R01AI089728
Receptor Recognition and Cell Entry of Coronaviruses
Role: Principal Investigator
Goal: This research investigates the molecular and structural mechanisms for the receptor recognition and cell entry of coronaviruses. It explores novel principles governing viral evolution, receptor recognition, cell entry, host ranges, cross-species infections, and tissue tropisms. These studies are critical for evaluating the emerging disease potentials of coronaviruses and for preventing, controlling and treating the spread of coronaviruses in humans or other animals.

National Institutes of Health 05/21/2018 – 04/30/2023
R01AI139092
Structure-based design of coronavirus subunit vaccines
Role: Co-Investigator (PI: Lanying Du, New York Blood Center)
Goal: This research establishes that artificially exposed unfavorable epitopes on viral subunit vaccines pose a severe intrinsic limitation to viral subunit vaccine designs. It further develops novel structure-based approaches to overcome this limitation in vaccine design. It produces highly effective subunit vaccines to combat the infections of highly pathogenic coronaviruses such as MERS and SARS coronaviruses, and also has implications for subunit vaccine design against other life-threatening viruses.

OTHER SUPPORT

BARIC, RALPH S.

ACTIVE:

U19 AI107810 (PI: Baric) 06/21/13-05/31/19 2.16 cal mos
NIH/NIAID $1,766,262
Characterization of novel genes encoded by RNA and DNA viruses
Using highly pathogenic human respiratory and systemic viruses which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and host responses to determine severe disease outcomes.

U19 AI100625 (PI: Baric/Heise-MPI) 08/05/12-08/31/22 1.20 cal mos
NIH/NIAID $2,662,979
Systems Immunogenetics of Biodefense Pathogens in the Collaborative Cross
Specific Aims: In this proposal, we are utilizing the Collaborative Cross (CC), a novel panel of reproducible, recombinant inbred (RI) mouse lines to identify genes and gene interactions which regulate the induction, kinetics, and magnitude of the innate, inflammatory and adaptive arms of the immune response following virus infection. Specifically, we will develop novel modeling algorithms to predict and validate the causal relationships between natural genetic variation and host signaling networks, immune cell recruitment, and immune function.

****U19 AI142759 CETR** (PI: Whitley) 03/07/19-02/29/24 1.12 cal mos
UAB/NIH/NIAID $374,220
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease. Role: Investigator

U19 AI109761 CETR (PI: Lipkin) 03/07/14-02/29/20 1.2 cal mos
Columbia/NIH/NIAID $584,891 (NCE)
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Project Leader, Consortium PI

P01 AI106695/00008956 (PI: Harris) 07/29/15-06/30/19 0.48 cal mos
UCB/NIH $279,680
Protective immunity following dengue virus natural infections and vaccination
We will perform studies to characterize the B-cell/ antibody (responses in people who receive dengue live attenuated virus vaccines (DLAV).
Role: Investigator

R01 AI125198 (PI: DeSilva) 05/08/16-04/30/21 0.60 cal mos
NIH/NIAID $848,808
Preclinical Assays To Predict Tetravalent Dengue Vaccine Efficacy
Dengue is the most significant mosquito transmitted viral infection of humans. Vaccination is a feasible solution to prevent and control dengue. Although dengue vaccines are under development, we do not know the specific properties of antibodies induced by vaccines that are likely to protect from infection. In this project investigators from the University of North Carolina and Sanofi Pasture, a leading dengue vaccine developer, will collaborate to define properties of antibodies induced by the Sanofi vaccine that correlate with protection. The main goal of the project is to develop new assays to support the current global effort to develop dengue virus vaccines. Role: Investigator

48415 (PI: Desilva) 06/30/16-12/31/19 0.12 cal mos
Takeda Vaccines, Inc $330,000
UNC-Takeda study to characterize human antibody response to DENVax
The deSilva and Baric laboratories will jointly characterize the properties of neutralizing antibodies in the serum samples using competition assays with monoclonal antibodies and neutralization assays with recombinant viruses. Role: Investigator

R01 AI110700 (PI: Baric) 04/20/15-03/31/20 0.96 cal mos
NIH/NIAID $605,933
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.

Not Assigned (PI: Baric) 01/08/16-07/31/19 0.36 cal mos
Takeda Vaccines, Inc $1,243,048
In Vitro and In Vivo Characterization of Bivalent DENV Live Virus Vaccines
To provide expertise in molecular virology required for creating recombinant dengue viruses for in vitro and in vivo testing.

R21 AI135682 (MPI: Geogiou/Baric) 02/01/18-1/31/20 0.36 cal mos.
NIH/NIAID $125,000
Molelcular Analysis of Serum Antibody Constituents in Zika Virus Infection
The goals of this project are to assess and quantify the persistence of specific and protective antibodies as well as cross-reactive (possible pathogenic) antibodies in Zika-infected patient blood. The antibodies developed in the project may lead to rapid development of new therapeutics and aid I n the design of future vaccine against Zika virus.

R01 AI108197 (MPI: Denison/Baric) 03/01/18-02/28/23 0.60 cal mos
Vanderbilt Univ/NIH $532,971
Determinants of Coronavirus Fidelity in Replication and Pathogenesis
To identify common and unique determinants of CoV nsp14-ExoN functions CoV replication, fidelity and IFN sensitivity across CoVs; To determine pathways of adaptation to loss of nsp14-ExoN activity in vitro and in vivo; and To define mechanisms of ExoN-regulated CoV sensitivity to the innate antiviral immune response.

R01 AI132178 (PI: Baric/Sheahan) 08/09/17-07/31/22 0.60 cal mos
NIH/NIAID $919,427
Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV
To focus on two areas: novel second generation compounds or compounds not previously provided by Gilead Sciences; and selecting and evaluating drug resistance profiles for SARS-CoV and MERS-CoV mutants in primary human lung cells.

R01 AI127845 (PI: Becker-Dreps) 09/27/16-08/31/21 0.12 cal mos
NIH/NIAID $500,513
Natural history, immunity, and transmission patterns of sapovirus in a Nicaraguan birth cohort
To characterize the natural history and risk factors for sapovirus gastroenteritis, elucidate the development of immunity to sapovirus in early childhood and the potential protective effect of maternal immunity, and apply novel genetic and analytic tools to characterize patterns of sapovirus transmission in households and communities. Role: Investigator

N005402801 (PI: Li) 06/07/16-05/31/21 0.36 cal mos
Univ Minn/NIH $120,384
Receptor recognition and cell entry of coronaviruses
To investigate how CoVs explore host receptors and host proteases for regulation of their host range, cross-species transmission, tissue tropism, and pathogenesis. Role: Subcontract PI

****D43 TW010923** (PI: Becker-Dreps/Meshnick) 05/10/18-02/28/23 0.24 cal mos.

NIH $230,000

Nicaraguan Emerging and Endemic Diseases (NEED)

The goals of this program are to 1) train young Nicaraguan scientists in Infectious Disease Epidemiology at the UNC, 2) create a sustainable supply of scientists in the region by establishing an accredited PhD program in Biomedical Sciences at the Universidad Nacional Autonoma de Nicaragua Leon and 3) foster professional growth and development among trainees and local faculty to ensure academic and research success. Role: Investigator

R21 AI137887 (MPI: Moorman/Heise) 02/05/18-01/31/20 0.12 cal mos
NIH/NIAID $125,000

Molecular Characterization of Functional RNA Structures in the ZikV genome

Zika virus is an emerging pathogen that is associated with severe congenital neurologic defects, such as microcephaly. The proposed studies will identify new viral virulence determinants that can be targeted to generate safer and more effective Zika virus vaccines and therapeutics. Role: Investigator

****R01AI 107731** (Desilva) 08/05/13-08/31/23 0.60 cal mos
NIH/NIAID $421,235

Molecular Basis of Dengue Virus Neutralization by Human Antibodies

The goal of this project is to determine the origin and properties of these useful cross protective antibodies. We will also study the properties of antibodies that neutralize Zika viruses. Role: Investigator

****K24AI141744** (Becker-Dreps) 12/06/18-11/30/23 0.12 cal mos
NIH/NIAID $157,100

The Development of Norovirus Immunity in Early Childhood and Implications for Norovirus Vaccines

To acquire new research skills and carry out a research plan that will allow guidance of the development of pediatric norovirus vaccines. Role: Investigator

Denotes new funding **OVERLAP: None

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

No

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: Regents of the University of Minnesota	555917996	MN-005	3-284 Nils Hasselmo Hall 312 Church Street SE Minneapolis MN 554550215
University of North Carolina - Chapel Hill	608195277	NC-004	3109 Hooker Research Center 135 Dauer Drive Chapel Hill NC 275997435
UNIVERSITY OF MINNESOTA	555917996	MN-005	UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER MINNEAPOLIS MN 554552070

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

SUBAWARD INFORMATION SHEET

Grant Administrator: ~~Erin Knudsen~~ Brett Carlson

EFS Award # CON: 58289

FCOI: FDP
(FDP, Form 1, Form 2)

Prime Sponsor: NIH

CFDA #: 93.855

Prime Award No.: R01AI089728

Period of Performance: 06/07/16 to 05/31/17 18 19 20

University Project Director: Fang Li

PO ID#: N005402801

Chart String (Fund–DeptID–PCBU–Proj-Acct): 3002 – 11718 – umspr - 00054028 - 810301 - ~~810401~~

11651 00866245

Subrecipient (Vendor Name): University of North Carolina, Chapel Hlll

Vendor ID: 0000012074 **Location:** ATLANTA **Vendor Address #** 5

Vendor Administrative Contact:

Rhonda Farrell
Office of Sponsored Research
104 Airport Road, Suite 2200, CB1350
Chapel Hill, NC 27599-1350

Phone: 919-962-4396
Fax:
Email: farrelrl@email.unc.edu

Vendor Payment Address:

Bank of America Lock Box
PO Box 402420
Atlanta, GA 30384-2420

Phone: 919-966-3411
Fax:
Email: resadminosr@unc.edu

Date	Amend No.	Summary of Changes	Total $ Amount +/-	New Total Subaward Amount	GA Initials
09/20/16		Original Setup		120,384	BEF
8/9/17	1	add $ & time	120,384	240,768	EK
9/5/18	2	adds funds	+120,384	361,152.00	KS
7/11/19	3	add funds & Extended	+120,384.00	481,536.00	LD

Vendor F&A Rate: 52%

Route copy of subaward and PO to ImageNow staff

DEPARTMENTAL CONTACT:

00054028

FDP Research Subaward Agreement
Amendment (Number 3)

UMN CON #: 58289

Pass-Through Entity (PTE)	Subrecipient
Regents of the University of Minnesota	Entity Name: University of North Carolina - Chapel Hill
awards@umn.edu	Email Address: resadminosr@unc.edu
Fang Li	Principal Investigator: Ralph Baric
Project Title: Receptor recognition and cell entry of coronaviruses	
PTE Federal Award No: R01AI089728	Federal Awarding Agency: NIH / NIAID
Subaward Period of Performance: Start Date: June 1, 2017 End Date: May 31, 2020	Amount Funded This Action: $ 120,384.00
Subaward No: N005402801	
Effective Date of Amendment: Jun 1, 2018	Total Amount of Federal Funds Obligated to Date: $ 481,536.00
Subject to FFATA: ○ Yes ◉ No	Automatic Carryover: ◉ Yes ○ No

Amendment(s) to Original Terms and Conditions

This Amendment revises the above-referenced Research Subaward Agreement as follows:

Amendment 3 awards $120,384 for the period of 6/1/19-5/31/20 as shown in Exhibit A-2. The total cumulative award amount is now $481,536. All other terms and conditions remain unchanged.

For clarity: all amounts stated in this amendment are in United States Dollars.

All other terms and conditions of this Subaward Agreement remain in full force and effect.

By an Authorized Official of PTE:

Name: Brett Carlson

Title: Sr. Grant & Contract Administrator

Date: 7-10-19

By an Authorized Official of Subrecipient:

Name:

Title: On behalf of Terry Magnuson, PH.D. Vice Chancellor for Research University of North Carolina at Chapel Hill

Date: 7/9/19

CONTINUATION YR09 NIH AWARD

Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 05/24/2019

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI089728-09
FAIN: R01AI089728

Principal Investigator(s):
Fang Li, PHD

Project Title: Receptor recognition and cell entry of coronaviruses

Brett Carlson
Grant Administrator
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 554552070

Award e-mailed to: awards@umn.edu

Period Of Performance:
Budget Period: 06/01/2019 – 05/31/2020
Project Period: 06/07/2016 – 05/31/2021

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $455,796 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MINNESOTA in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI089728. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health," Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

David Madoo
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI089728-09

Award Calculation (U.S. Dollars)	
Salaries and Wages	$116,853
Fringe Benefits	$30,547
Personnel Costs (Subtotal)	$147,400
Materials & Supplies	$60,005
Travel	$2,970
Other	$5,247
Subawards/Consortium/Contractual Costs	$120,384
Publication Costs	$2,178
	$338,184
Federal Direct Costs	$117,612
Federal F&A Costs	$455,796
Approved Budget	$455,796
Total Amount of Federal Funds Obligated (Federal Share)	$455,796
TOTAL FEDERAL AWARD AMOUNT	$455,796
AMOUNT OF THIS ACTION (FEDERAL SHARE)	

SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
9	$455,796	$455,796
10	$455,796	$455,796

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name: Allergy and Infectious Diseases Research
CFDA Number: 93.855
EIN: 1416007513A1
Document Number: RAI089728B
PMS Account Type: P (Subaccount)
Fiscal Year: 2019

IC	CAN	2019	2020
AI	8472364	$455,796	$455,796

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C B / **OC:** 414E / **Released:** MADOOD_614 05/23/2019
Award Processed: 05/24/2019 12:03:12 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI089728-09

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI089728-09

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants

Policy Statement, including addenda in effect as of the beginning date of the budget period.

e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI089728. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:

Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI089728-09

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

If any experiments proposed in this award result in a virus with enhanced growth by more than 1 log compared to wild type strains, you must notify the NIAID Program Officer and Grants Management Specialist immediately. Further research involving the resulting virus(es) may require review by the Department of Health and Human Services in accordance with the Framework for Guiding Funding Decisions about Proposed Research Involving Enhanced Potential Pandemic Pathogens.

This Notice of Award (NoA) includes funds for activity with **The University of North Carolina.**

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

o A list of the new and/or additional Agent(s) that will be studied;
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be

conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Jenna L. Briggs
Email: jenna.briggs@nih.gov **Phone:** 301-761-5137 **Fax:** 301-493-0597

Program Official: Erik J. Stemmy
Email: erik.stemmy@nih.gov **Phone:** 240-627-3380

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI089728-09

INSTITUTION: UNIVERSITY OF MINNESOTA

Budget	Year 9	Year 10
Salaries and Wages	$116,853	$116,853
Fringe Benefits	$30,547	$30,547
Personnel Costs (Subtotal)	$147,400	$147,400
Materials & Supplies	$60,005	$60,005
Travel	$2,970	$2,970
Other	$5,247	$5,247
Subawards/Consortium/Contractual Costs	$120,384	$120,384
Publication Costs	$2,178	$2,178
TOTAL FEDERAL DC	$338,184	$338,184
TOTAL FEDERAL F&A	$117,612	$117,612
TOTAL COST	$455,796	$455,796

Facilities and Administrative Costs	Year 9	Year 10
F&A Cost Rate 1	54%	54%
F&A Cost Base 1	$217,800	$217,800
F&A Costs 1	$117,612	$117,612

Subject: Re: Univ of MN Subaward N005402801 Amendment 3, Prime Award: R01AI089728
From: Brett Carlson <carls234@umn.edu>
Date: 7/10/2019, 1:58 PM
To: "Anthony, Lakeisha Melton" <keishaa@email.unc.edu>
CC: Anne M Mockovak-1 <a-mock@umn.edu>, Fang Li-5 <lifang@umn.edu>, Sponsored Projects Admin Subcontracts <subk@umn.edu>, ovpr-ffata@umn.edu, ovprffat OVPR SPA <ovprffat@umn.edu>

Hi Keisha,

Attached is the fully executed amendment.

P.O. Amend. Needed

Thanks,

Brett

Brett Carlson
Sr. Grant & Contract Administrator
University of Minnesota
Office of Sponsored Projects Administration
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 55455
Phone: 612-626-7441
Fax: 612-624-4843

On 7/10/2019 8:28 AM, Anthony, Lakeisha Melton wrote:

> Hello,
>
> Attached is a copy of the referenced Subaward N005402801 that has been partially executed. Please have the Authorized Official from your institution sign and remit one fully executed copy to our attention at your earliest convenience. Please inform us if additional information is required.
>
> Thanks
> Keisha
>
> Lakeisha Anthony, MBA, MHRM
> Sponsored Project Specialist, Research Administration
> Team 3
> Office of Sponsored Research
> The University of North Carolina at Chapel Hill
> 104 Airport Dr. CB 1350, Suite 2200
> Chapel Hill, N.C. 27599-1350
> E: keishaa@email.unc.edu | Main: (919) 966-3411 | Direct: (919) 962-2813
> **300.3 Deadlines for Proposal Submissions to University Offices** https://research.unc.edu/sponsored-

research/operating-standards/#300.3

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

From: Brett Carlson <carls234@umn.edu>
Sent: Thursday, June 27, 2019 11:16 AM
To: OSR Research Admin <resadminosr@unc.edu>; Farrell, Ronda Lee <farrelrl@email.unc.edu>
Cc: Anne M Mockovak-1 <a-mock@umn.edu>; Fang Li-5 <lifang@umn.edu>
Subject: Univ of MN Subaward N005402801 Amendment 3, Prime Award: R01AI089728

University of Minnesota PI:Fang Li
UNC PI: Ralph Baric
PO#: N005402801

Hello:

Attached is an amendment to subaward N005402801 between the University of Minnesota and University of North Carolina - Chapel Hill. Please have the amendment administratively reviewed and endorsed on behalf of your organization. A half-executed copy of the amendment should be returned to me via e-mail. I will return a fully-signed copy to you once final signature is obtained.

If at any time you have questions, please feel free to contact me at (612)-626-7441 or carls234@umn.edu. Questions regarding programmatic or scientific matters should be directed to the Principal Investigator, as indicated in Attachment 3A of the agreement.

Sincerely,

Brett

--
Brett Carlson
Sr. Grant & Contract Administrator
University of Minnesota
Office of Sponsored Projects Administration
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 55455
Phone: 612-626-7441
Fax: 612-624-4843

Attachments:

0957_001.pdf 27 bytes

Subject: RE: Univ of MN Subaward N005402801 Amendment 3, Prime Award: R01AI089728
From: "Anthony, Lakeisha Melton" <keishaa@email.unc.edu>
Date: 7/10/2019, 8:28 AM
To: Brett Carlson <carls234@umn.edu>
CC: Anne M Mockovak-1 <a-mock@umn.edu>, Fang Li-5 <lifang@umn.edu>

Hello,

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Thanks
Keisha

Lakeisha Anthony, MBA, MHRM
Sponsored Project Specialist, Research Administration
Team 3
Office of Sponsored Research
The University of North Carolina at Chapel Hill
104 Airport Dr. CB 1350, Suite 2200
Chapel Hill, N.C. 27599-1350
E: keishaa@email.unc.edu | Main: (919) 966-3411 | Direct: (919) 962-2813
300.3 Deadlines for Proposal Submissions to University Offices https://research.unc.edu/sponsored-research/operating-standards/#300.3

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

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Sent: Thursday, June 27, 2019 11:16 AM
To: OSR Research Admin <resadminosr@unc.edu>; Farrell, Ronda Lee <farrelrl@email.unc.edu>
Cc: Anne M Mockovak-1 <a-mock@umn.edu>; Fang Li-5 <lifang@umn.edu>
Subject: Univ of MN Subaward N005402801 Amendment 3, Prime Award: R01AI089728

University of Minnesota PI:Fang Li
UNC PI: Ralph Baric
PO#: N005402801

Hello:

Attached is an amendment to subaward N005402801 between the University of Minnesota and University of North Carolina - Chapel Hill. Please have the amendment administratively reviewed and endorsed on behalf of your organization. A half-executed copy of the amendment should be returned to me via e-mail. I will return a fully-signed copy to you once final signature is obtained.

If at any time you have questions, please feel free to contact me at (612)-626-7441 or carls234@umn.edu. Questions regarding programmatic or scientific matters should be directed to the Principal Investigator, as indicated in Attachment 3A of the agreement.

Sincerely,

Brett

--
Brett Carlson
Sr. Grant & Contract Administrator
University of Minnesota
Office of Sponsored Projects Administration
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 55455
Phone: 612-626-7441
Fax: 612-624-4843

Attachments:

Regents Amend 3.pdf 239 KB

Subject: Univ of MN Subaward N005402801 Amendment 3, Prime Award: R01AI089728
From: Brett Carlson <carls234@umn.edu>
Date: 6/27/2019, 10:16 AM
To: resadminosr@unc.edu, farrelrl@email.unc.edu
CC: Anne M Mockovak-1 <a-mock@umn.edu>, Fang Li-5 <lifang@umn.edu>

University of Minnesota PI:Fang Li
UNC PI: Ralph Baric
PO#: N005402801

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If at any time you have questions, please feel free to contact me at (612)-626-7441 or carls234@umn.edu. Questions regarding programmatic or scientific matters should be directed to the Principal Investigator, as indicated in Attachment 3A of the agreement.

Sincerely,

Brett

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Brett Carlson
Sr. Grant & Contract Administrator
University of Minnesota
Office of Sponsored Projects Administration
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 55455
Phone: 612-626-7441
Fax: 612-624-4843

--Attachments:

N005402801 Am3 with exhibits.pdf

27 bytes

FDP Research Subaward Agreement
Amendment (Number 3)

UMN CON #: 58289

Pass-Through Entity (PTE)		Subrecipient
Regents of the University of Minnesota	Entity Name	University of North Carolina - Chapel Hill
awards@umn.edu	Email Address	resadminosr@unc.edu
Fang Li	Principal Investigator	Ralph Baric

Project Title: Receptor recognition and cell entry of coronaviruses

PTE Federal Award No:	Federal Awarding Agency:
R01AI089728	NIH / NIAID

Subaward Period of Performance: Start Date / End Date	Amount Funded This Action:	Subaward No:
June 1, 2017 / May 31, 2020	$ 120,384.00	N005402801

Effective Date of Amendment:	Total Amount of Federal Funds Obligated to Date:	Subject to FFATA:	Automatic Carryover:
Jun 1, 2018	$ 481,536.00	○ Yes ◉ No	◉ Yes ○ No

Amendment(s) to Original Terms and Conditions

This Amendment revises the above-referenced Research Subaward Agreement as follows:

Amendment 3 awards $120,384 for the period of 6/1/19-5/31/20 as shown in Exhibit A-2. The total cumulative award amount is now $481,536. All other terms and conditions remain unchanged.

For clarity: all amounts stated in this amendment are in United States Dollars.

All other terms and conditions of this Subaward Agreement remain in full force and effect.

By an Authorized Official of PTE:		By an Authorized Official of Subrecipient:	
Name: Brett Carlson	Date	Name:	Date
Title: Sr. Grant & Contract Administrator		Title:	

EXHIBIT A-2

RESEARCH & RELATED BUDGET

ORGANIZATIONAL DUNS: 608195277 | Name of Organization: The University of North Carolina at Chapel Hill

Budget Period: Start Date: 6/1/18 End Date: 5/31/19

A. Senior/Key Person

Project Role	First	Middle	Last	Suffix	Base Salary ($)	Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested
Consortium PI	Ralph	S	Baric	PhD	189,600	0.96			5,888	1,578	7,266
											0
									Total Senior/Key Person		7,266

B. Other Personnel

Project Role	First	Middle	Last	Suffix	Base Salary ($)	Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested
Postdoctoral Fellow	Sarah		Leist	PhD	48,216	3.80			14,465	2,640	17,105
Technician	Drew	Trevor	Scobey		44,483	4.30			15,562	5,952	21,514
											0
									Total Other Personnel		38,619

Total Number Other Personnel

TOTAL SALARY, WAGES AND FRINGE (A- 45,885

C. Equipment Description

Equipment Item | Funds Requested ($)

Total Equipment 0

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Includes Canada, Mexico and US Possessions	2,000
2. Foreign Travel Costs	
Total Travel	2,000

E. Participant/Trainee Support

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other (Specify)	
Total Number Participants/Tra	
Total Partipant/Trainee Costs	0

F. Other Direct Costs

	Funds Requested ($)
1. Materials and Supplies	24,815
2. Publication Costs	2,500
3. Consultant Services	
4. ADP/Computer Services	
5. Subaward/Consortium/Contractual Costs	
6. Equipment or Facility Rental/ User Fees	
7. Alterations and Renovations	
8. Maintenance Contracts	4,000
9. Shipping	
10. Other (Specify)	
Total Other Direct Costs	31,315
Total Direct Costs (A thru F)	79,200
	79200

G. Direct Costs

H. Indirect Costs

Indirect Cost Type	Indirect Cost	Indirect Cost Base ($)	Funds Requested ($)
MTDC 52%	52.00%	79,200	41184
			0
			0
		Total Indirect costs	41,184

Cognizant Federal Agency

Agency Name: DHHS, Division of Cost alloc

POC Name: Darryl Mayes

POC phone #: 202-401-2808

I. Total Direct and Indirect Cost | Funds Requested ($)

Total Direct and Institutional Costs (G+I 120,384

CONTINUATION YR09 NIH AWARD

Notice of Award **Federal Award Date:** 05/24/2019

RESEARCH
Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI089728-09
FAIN: R01AI089728

Principal Investigator(s):
Fang Li, PHD

Project Title: Receptor recognition and cell entry of coronaviruses

Brett Carlson
Grant Administrator
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 554552070

Award e-mailed to: awards@umn.edu

Period Of Performance:
Budget Period: 06/01/2019 – 05/31/2020
Project Period: 06/07/2016 – 05/31/2021

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $455,796 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MINNESOTA in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI089728. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

David Madoo
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI089728-09

Award Calculation (U.S. Dollars)	
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Other	$5,247
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Publication Costs	$2,178
Federal Direct Costs	$338,184
Federal F&A Costs	$117,612
Approved Budget	$455,796
Total Amount of Federal Funds Obligated (Federal Share)	$455,796
TOTAL FEDERAL AWARD AMOUNT	$455,796

AMOUNT OF THIS ACTION (FEDERAL SHARE)

SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
9	$455,796	$455,796
10	$455,796	$455,796

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1416007513A1
Document Number:	RAI089728B
PMS Account Type:	P (Subaccount)
Fiscal Year:	2019

IC	CAN	2019	2020
AI	8472364	$455,796	$455,796

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C B / **OC**: 414E / **Released**: MADOOD_614 05/23/2019
Award Processed: 05/24/2019 12:03:12 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI089728-09

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI089728-09

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants

Subject: Re: CON-58289 Fang Li Sub amend with Univ of North Carolina
From: Anne Mockovak <a-mock@umn.edu>
Date: 6/27/2019, 9:59 AM
To: Brett Carlson <carls234@umn.edu>

Brett,
Please extend subcontract N5402801 on CON 58289 to University of North Carolina to May 31,2020 and provide additional funding of $120,384. The work scope has not changed. A budget is attached.

-Anne

Anne Mockovak
Accountant
Department of Veterinary and
Biomedical Sciences
612.301.2143

On Wed, Jun 26, 2019 at 2:47 PM Brett Carlson <carls234@umn.edu> wrote:

> Hi Anne,
>
> Please send the budget for this 09 year subaward. I'll also need to know if the workscope has changed. If it has, I'll need a new workscope document.
>
> Thanks,
>
> Brett
>
> --
> Brett Carlson
> Sr. Grant & Contract Administrator
> University of Minnesota
> Office of Sponsored Projects Administration
> 450 McNamara Alumni Center
> 200 Oak Street SE
> Minneapolis, MN 55455
> Phone: 612-626-7441
> Fax: 612-624-4843

--Attachments:

UNC Year 4 Budget.pdf

71.0 KB

Amd 3 - award is not subject to FFATA. Accidently reported 8/19/19.

updated for FFATA note ↑
RP 8/27/19

Step 4

Amendment #___3_____:

Audit Path (•) A-133 ( ) Financial Questionaire

Audit Weaknesses? (•) No ( ) Yes

Debarment: (•) No ( ) Yes

Initials: RP Date: 6/11/2019

F&A Rate: 52.00% Date: 6/11/2019

FFATA Required: (•) No ( ) Yes Date: 6/11/2019
Sent to ovprffat@umn.edu

FCOI: ( ) Sub policy ( ) UofM policy ( ) n/a Date: 6/11/2019
(•) FDP

AUDIT VALID THROUGH:_____3/31/20____

Step 4

Amendment #_____:

Audit Path ( ) A-133 ( ) Financial Questionaire

Audit Weaknesses? ( ) No ( ) Yes

Debarment: ( ) No ( ) Yes

Initials: Date:

F&A Rate: Date:

FFATA Required: ( ) No ( ) Yes Date:
Sent to ovprffat@umn.edu

FCOI: ( ) Sub policy ( ) UofM policy ( ) n/a Date:
( ) FDP

AUDIT VALID THROUGH:_____

Step 4

Amendment #_____:

Audit Path ( ) A-133 ( ) Financial Questionaire

Audit Weaknesses? ( ) No ( ) Yes

Debarment: ( ) No ( ) Yes

Initials: Date:

F&A Rate: Date:

FFATA Required: ( ) No ( ) Yes Date:
Sent to ovprffat@umn.edu

( ) UofM policy ( ) Sub policy ( ) n/a Date:
FCOI: ( ) FDP

AUDIT VALID THROUGH:_____

Attach page for multiple amendments

Subrecipient Risk Analysis and Compliance Record (06/10/2015)

Subrecipient Name:	University of Northa Carolina, Chapel Hill
Prime Sponsor:	NIH NIAID
CON#	58289
PO #:	N005402801
Date Completed & Entered into EFS:	8/1/2016
Grant Administrator:	Brett Carlson

Audit Path (•) A-133 ( ) Financial Questionaire — Step 1

Audit Weaknesses? ( ) No (•) Yes

Analysis Matrix — Step 2

Instructions: Mark yes or no.

Criteria		Wt.	Score
Is the entity located outside the U.S.?	( ) Y (•) N	6	0
Will work be done outside the U.S.?	( ) Y (•) N	3	0
Is entity non-profit or government?	(•) Y ( ) N	3	0
Is prime award a contract or subcontract (rather than grant)?	( ) Y (•) N	4	0
Is amount of Subgrant/Subcontract more than 500k?	(•) Y ( ) N	4	4
Is amount of subaward 50% or more of total award?	( ) Y (•) N	5	0
Are accounting systems established?	( ) Y (•) N	10	10
Do audit results indicate weaknesses in the entity's procurement system?	(•) Y ( ) N	3	3
Does entity have a negotiated indirect cost rate agreement?	(•) Y ( ) N	2	0
Is entity subject to A-133?	(•) Y ( ) N	2	0
Any prior negative experience with entity?	( ) Y (•) N	2	0
Is there ITAR/EAR type of work?	( ) Y (•) N	10	0
Is the entity using humans, animals, DNA, stem cells, etc. (compliance)?	( ) Y (•) N	2	0
Is entity mature?	(•) Y ( ) N	6	0
Is prime sponsor government owned & contractor operated?	( ) Y (•) N	10	0
Is there potential for COI regarding relationship of subrecipient and PI?	( ) Y (•) N	10	0
Are deliverables tangible products or pivotal to success?	(•) Y ( ) N	7	7
Is "Advantaged" status claimed?	( ) Y (•) N	1	0
Special Considerations? (provide details in notes)	( ) Y (•) N		
Total Risk Score (≥ than 15 is high and should be escalated)			24

Escalated to PGA? Y ☑ N ☐

Completed by: Lesley S Sindberg Date: 8/1/16

Debarment: (•) No ( ) Yes — Step 3

Initials: LSS — **Date:** 8/1/2016

F&A Rate: 52.00% — **Date:** 8/1/2016

FFATA Required: (•) No ( ) Yes — **Date:** 8/1/2016

Sent to ovprffat@umn.edu ☐

FCOI: ( ) Sub policy ( ) n/a ( ) UofM policy (•) FDP — **Date:** N/A

Date: 8/1/2016

Amendment #___1 & 2__: — Step 4

Audit Path (•) A-133 ( ) Financial Questionaire

Audit Weaknesses? (•) No ( ) Yes

Debarment: (•) No ( ) Yes

Initials: LSS — **Date:** 7/30/2018

F&A Rate: 52.00% — **Date:** 7/30/2018

FFATA Required: (•) No ( ) Yes — **Date:** 7/30/2018

Sent to ovprffat@umn.edu ☐

FCOI: ( ) Sub policy ( ) n/a ( ) UofM policy (•) FDP — **Date:** 7/30/2018

Attach page for multiple amendments

Notes: Recommend requiring detailed monthly invoices and preapproval for rebudgeting based on high risk anlaysis matrix score. PI and GA should closely monitor subrecipient progress and finanical reporting. SA for FY 2017 alleviate previously identified findings and helps mitigate potential risk.

AUDIT VALID THROUGH: 3/31/19

Closeout - Subaward Release Form: — Step 5

Received: ☐

Date:

Completion Process: ☐

By:

Date:

Step 4

Amendment #___3_____:

Audit Path (•) A-133 ( ) Financial Questionaire

Audit Weaknesses? (•) No ( ) Yes

Debarment: (•) No ( ) Yes

Initials: RP **Date:** 6/11/2019

F&A Rate: 52.00% **Date:** 6/11/2019

FFATA Required: (•) No ( ) Yes **Date:** 6/11/2019
Sent to ovprffat@umn.edu

FCOI: ( ) Sub policy ( ) UofM policy ( ) n/a **Date:** 6/11/2019
(•) FDP

AUDIT VALID THROUGH:_____3/31/20____

View assistance for SAM.gov

SAM

A NEW WAY TO SIGN IN - If you already have a SAM account, use your **SAM email** for login.gov. ▶ Log In

Login.gov FAQs

⚠ ALERT: June 11, 2018: Entities registering in SAM must submit a notarized letter appointing their authorized Entity Administrator. Read our updated FAQs to learn more about changes to the notarized letter review process and other system improvements.

⚠ ALERT: SAM.gov will be down for scheduled maintenance from Friday, June 14, 2019 at 9:00 AM until Monday, June 17, 2019 6:00 AM (EDT).

⚠ ALERT: CAGE is currently experiencing a high volume of registrations, and is working them in the order in which they are received. When your registration is assigned to a CAGE Technician, you will be contacted by CAGE, if necessary, for any additional information.

Search Results

Current Search Terms: ralph baric*

Save PDF Export Results Print

Total records: 0

Sort by Relevance Order by Descending

Result Page:

Your search for ralph baric* returned the following results...

No records found.

Save PDF Export Results Print

Result Page:

GSA

IBM P 20190315 1308

WWW8

Search Records
Data Access
Check Status
About
Help

Disclaimers
Accessibility
Privacy Policy

FAPIIS.gov
GSA.gov/TAF
GSA.gov
USA.gov

View assistance for SAM.gov

SAM

A NEW WAY TO SIGN IN - If you already have a SAM account, use your **SAM email** for login.gov. ▶ Log In

Login.gov FAQs

⚠ ALERT: June 11, 2018: Entities registering in SAM must submit a notarized letter appointing their authorized Entity Administrator. Read our updated FAQs to learn more about changes to the notarized letter review process and other system improvements.

⚠ ALERT: SAM.gov will be down for scheduled maintenance from Friday, June 14, 2019 at 9:00 AM until Monday, June 17, 2019 6:00 AM (EDT).

⚠ ALERT: CAGE is currently experiencing a high volume of registrations, and is working them in the order in which they are received. When your registration is assigned to a CAGE Technician, you will be contacted by CAGE, if necessary, for any additional information.

Search Results

Quick Search Results

Total records: 1

Save PDF | Export Results | Print

Result Page: 1

Sort by Relevance | Order by Descending

Your search returned the following results...

Entity **University of North Carolina at Chapel Hill**

Status: **Active**

DUNS: **608195277**

CAGE Code: **4B856**

View Details

Has Active Exclusion?: **No**

DoDAAC:

Expiration Date: **01/31/2020**

Debt Subject to Offset?: **No**

Purpose of Registration: **All Awards**

Result Page: 1

Save PDF | Export Results | Print

GSA

IBM-P-20190315-1318

WWW8

Search Records	Disclaimers	FAPIIS.gov
Data Access	Accessibility	GSA.gov/IAE
Check Status	Privacy Policy	GSA.gov
About		USA.gov
Help		

Thanks, U.S. General Services Administration [illegible]

Subject: CON-58289 Fang Li Sub amend with Univ of North Carolina
From: Brett Carlson <carls234@umn.edu>
Date: 6/26/2019, 2:47 PM
To: Anne M Mockovak-1 <a-mock@umn.edu>

Hi Anne,

Please send the budget for this 09 year subaward. I'll also need to know if the workscope has changed. If it has, I'll need a new workscope document.

Thanks,

Brett

--
Brett Carlson
Sr. Grant & Contract Administrator
University of Minnesota
Office of Sponsored Projects Administration
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 55455
Phone: 612-626-7441
Fax: 612-624-4843

00054028

FDP Research Subaward Agreement
Amendment (Number 2)

UMN CON #: 58289

Pass-Through Entity (PTE) — Subrecipient

Regents of the University of Minnesota	Entity Name: University of North Carolina - Chapel Hill
awards@umn.edu	Email Address: resadminosr@unc.edu
Fang Li	Principal Investigator: Ralph Baric

Project Title: Receptor recognition and cell entry of coronaviruses

PTE Federal Award No: R01AI089728	Federal Awarding Agency: NIH / NIAID
Subaward Period of Performance: Start Date: June 1, 2017 End Date: May 31, 2019	Amount Funded This Action: $ 120,384.00
Effective Date of Amendment: Jun 1, 2018	Total Amount of Federal Funds Obligated to Date: $ 361,152.00

Subaward No: N005402801

Subject to FFATA: ○ Yes ◉ No

Automatic Carryover: ◉ Yes ○ No

Amendment(s) to Original Terms and Conditions

This Amendment revises the above-referenced Research Subaward Agreement as follows

Amendment 2 awards $120,384 for the period of 6/1/18-5/31/19 as shown in Exhibit A-1. The total cumulative award amount is now $361,152. All other terms and conditions remain unchanged.

For clarity: all amounts stated in this amendment are in United States Dollars.

All other terms and conditions of this Subaward Agreement remain in full force and effect.

By an Authorized Official of PTE:	By an Authorized Official of Subrecipient:
Date: 8/9/18	Date: 8/3/18
Name: April Coon	Name: on behalf of Terry Magnuson, Ph.D., Vice Chancellor for Research
Title: Associate Director	Title: University of North Carolina at Chapel Hill

EXHIBIT A-1

Scope of Work-Baric Laboratory

Coronaviruses (CoVs) cause epidemic outbreaks of diseases in humans and animals, symbolized recently by the emergence of SARS-CoV, MERS-CoV, and porcine epidemic diarrhea CoV (PEDV). Receptor recognition and cell entry are two essential steps in the CoV infection cycle, critical determinants of CoV host range, cross species transmission and tissue tropism, and primary targets for antiviral intervention. In this competitive grant renewal, we will continue to investigate fundamental questions regarding receptor recognition and cell entry to CoVs from different genera. The long-term goals of the proposal are to develop global models of CoV-receptor interaction and protease processing in emerging coronavirus evolution, entry, tropism, pathogenesis and cross species transmission. The Baric laboratory will provide collaborative virological support including reverse genetics, evaluation of systematic mutation sets on emerging CoV receptor interactions and entry in vitro, mechanistic studies on viral gene function on receptor binding and entry, and experimental evolution in vitro.

Aim 1: Receptor binding by CoV S1-NTDs. The major goals of this aim are to determine the molecular mechanisms by which select subsets of emerging CoV recognize sugar receptors for entry and host range. Previous studies have shown that the S1-NTDs of β-genus BCoV and α-genus PEDV both recognize sugar receptors. In fact, the β-CoV S1-NTDs have the same structural fold as human galectins (galactose-binding lectins) that either function as novel protein receptor binding domains or non-galactose sugar receptors. This aim will investigate how CoV S1-NTDs from different genera have evolved to recognize sugar receptors. Our overall hypothesis is that S1-NTDs from all CoV genera originated from host galectins, but have evolved novel molecular mechanisms to recognize non-galactose sugar receptors. To test this hypothesis, the Li laboratory will determine the molecular and structural basis for sugar receptor recognition by S1-NTDs from (i) β-genus BCoV, (ii) α-genus PEDV, and (iii) δ-genus PdCoV. These studies will reveal sugar-binding mechanisms of CoV from different genera and provide insight into the evolutionary origins and pathways of CoV S1-NTDs.

Baric Laboratory. In parallel, the Baric laboratory will introduce select subsets of sugar enhancing or sugar-binding destroying mutations on recombinant MHV, HCoV OC43 and PEDV, produced by reverse genetics. In part 1, we will introduce mutation subsets into the existing molecular clones and recover recombinant viruses. In part 2, we will characterize virus growth kinetics on nonpermissive cell lines expressing target protein receptors (e.g., CEACAM1a, CEACAM1b, CEACAM2 for MHV and CD17 for PEDV). In part 3, we will evaluate virus binding kinetics by virus attachment assays.

Aim 2: Receptor biding by CoV S1-CTDs. Earlier work has solved the structure of several CoV S1-CTDs in complex with their protein receptors, including β-genus SARS-CoV S1-CTD complexed with its protein receptor ACE2. Our collaborator (Dr. Baric) and others have isolated several bat SARS-like CoVs (SLCoVs) that use human ACE2 and infect primary human airway cells. This aim will investigate the cross-species transmission pathway of bat SLCoVs by examining the interactions between their S1-CTDs and ACE2 from different species. Our overall hypothesis is that to transmit from bats to another species, bat SLCoVs need to adapt to ACE2 from that species through selective mutations in their S1-CTD. Dr. Li and his group will use biochemical and structural biology to elucidate the biochemical and molecular mechanisms for SLCoV S1-CTD binding to bat, civet and human ACE2, providing comparative metrics into strength of interaction and pandemic potential.

Baric Laboratory. In parallel, the Baric laboratory will introduce select subsets of mutations on recombinant SLCoV like WIV1, SHC014 and others, produced by reverse genetics. In part 1, we will introduce mutation subsets into the existing molecular clones and recover recombinant viruses. In part 2, we will characterize virus growth kinetics on nonpermissive cell lines expressing the civet, bat, mouse and human ACE2 receptors. In part 3, we will evaluate virus binding kinetics by virus attachment assays in these cells. In part 4, we will use experimental evolution to select for more efficient virus-receptor interactions in vitro using these cells.

Aim 3: Cell entry by CoVs. Previous studies have identified the host proteases that activate cell entry and serve as a cross-species barrier for some emerging CoV. Our overall hypothesis is that CoV spikes have evolved a variety of protease activation mechanisms for regulation of viral host range, tropism, and pathogenesis. Dr. Li and his group will investigate how the spike of neurotropic MHV strains evolved to be more prone to protease activation as adaptation to low receptor level in the brain and identify which proteases activate cell entry of PEDV. These studies will elucidate the molecular mechanisms for CoV entry and reveal how cell entry regulates CoV host range, tropism, and pathogenesis.

Baric Laboratory. In parallel, the Baric laboratory will introduce select subsets mutations in MHV and PEDV, designed to ablate or enhance protease interaction and cleavage during entry. In part 1, we will introduce mutation subsets into the existing molecular clones and recover recombinant viruses. In part 2, we will characterize virus growth kinetics on nonpermissive cell lines expressing CECAM1, CEACAM2, CD17 or sugar receptors, coupled with various surface and intracellular proteases. In part 3, we will evaluate virus penetration using escape from polyclonal antibody neutralization. In part 4, we will use experimental evolution to select for more efficient virus-proteolytic processing mutants for in vitro entry.

Our overall goal is to use CoVs as model systems and establish novel principles in virology that govern viral evolution, receptor recognition, cell entry, cross-species infections, and tropism. Due to our expertise and experience, we are in a unique position to perform the research and contribute to virology and human health.

YEAR 2 June 1, 2017-May 31, 2018

NAME	TITLE	SALARY	EFFORT	Cal Months	SALARY	FRINGE	TOTAL
Baric	PI	$189,600	3%	0.36	$5,688	$1,535	$7,223
Kocher, Jacob	Post Doc	$50,316	50%	6.00	$25,158	$4,421	$29,579
Scobey, Trevor	Research Tech	$43,591	15%	1.80	$6,539	$2,443	$8,982
				0.00	$0	$0	$0
				0.00	$0	$0	$0
TOTALS:			0.68		$37,385	$8,399	$45,784

CONSULTANTS			0
SUPPLIES	Synthetic DNA	2500	
	Cell Culture, Serum and Media	5000	
	DNA sequencing	3000	
	Plasticware	5000	
	Enymes, kits and reagents	2800	
	BL3 Protective Gear, etc.	3000	
	Misc.	2116	
	Computer Supplies	1500	
			24916
TOTAL SUPPLIES		24916	$0
EQUIPMENT (Itemize)			
OTHER (Itemize by category)		$2,500	
Publishing		$4,000	
Maint. Contracts		$0	
Histology		$0	
Flow cytometry		$0	
Animal Per Diem		$0	
Shipping		$6,500	$6,500
TOTAL OTHER:		$6,500	
TRAVEL	International		$2,000
	Domestic	$2,000	$79,200
TOTAL COST:			$79,200
TOTAL DIRECT COSTS		$79,200	$41,184
UNC-CH F & A @ 52% MOD			$120,384
TOTAL COST			

PRIME AWARD YR08

Subject: NoA: 5 R01 AI089728-08 PI: Li, Fang
From: era-notify@mail.nih.gov
Date: 5/22/2018 11:03 PM
To: awards@umn.edu

Grant Number: 5 R01 AI089728-08
Principal Investigator: Li, Fang
Project Title: Receptor recognition and cell entry of coronaviruses
Institution: UNIVERSITY OF MINNESOTA
CFDA: 93.855
OC: 414E
PCC: M51C B
Award Issue Date: 05/23/2018
Grants Management Officer: Machuca, Jorge
Program Official: Stemmy, Erik
Grants Specialist: Encarnacao, BRYAN

*** This is an automated notification - Please do not reply to this message. ***

--Attachments: 27 bytes

NIH_NOA_5R01AI089728-08.PDF

Notice of Award **Federal Award Date:** 05/23/2018

RESEARCH
Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI089728-08
FAIN: R01AI089728

Principal Investigator(s):
Fang Li, PHD

Project Title: Receptor recognition and cell entry of coronaviruses

KEVIN MCKOSKEY
Grant Administrator
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 554552070

Award e-mailed to: awards@umn.edu

Period Of Performance:
Budget Period: 06/01/2018 – 05/31/2019
Project Period: 06/07/2016 – 05/31/2021

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $455,615 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MINNESOTA in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI089728. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Jorge E. Machuca
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI089728-08

Award Calculation (U.S. Dollars)	
Salaries and Wages	$116,853
Fringe Benefits	$30,547
Personnel Costs (Subtotal)	$147,400
Materials & Supplies	$60,005
Travel	$2,970
Other	$5,247
Subawards/Consortium/Contractual Costs	$120,384
Publication Costs	$2,178
Federal Direct Costs	$338,184
Federal F&A Costs	$117,431
Approved Budget	$455,615
Total Amount of Federal Funds Obligated (Federal Share)	$455,615
TOTAL FEDERAL AWARD AMOUNT	$455,615

AMOUNT OF THIS ACTION (FEDERAL SHARE)

SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
8	$455,615	$455,615
9	$455,796	$455,796
10	$455,796	$455,796

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1416007513A1
Document Number:	RAI089728B
PMS Account Type:	P (Subaccount)
Fiscal Year:	2018

IC	CAN	2018	2019	2020
AI	8472364	$455,615	$455,796	$455,796

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C B / **OC:** 414E / **Released:** MACHUCAJ 05/21/2018
Award Processed: 05/23/2018 12:03:13 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI089728-08

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI089728-08

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.

d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI089728. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI089728-08

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

This Notice of Award (NoA) includes funds for activity with **The University of North Carolina at Chapel Hill** in the amount of **$120,384** (**$79,200** direct costs + **$41,184** F&A costs).
******

No funds are provided and no funds can be used to support gain-of-function research covered under the October 17, 2014 White House Announcement (NIH Guide Notice NOT-OD-15-011).

Per the letter dated May 18, 2016 to Mr. McKoskey at the University of Minnesota, NIAID funding cannot be used to generate SARS-like viruses with enhanced affinity for human receptors (via either reverse genetics or serial passaging). NIAID funding will instead be used to pursue alternative loss-of-function strategies or mutation of the ACE2 receptor as described in the letter from the University of Minnesota dated April 15, 2016. If any of the alternative experiments proposed, or any experiments proposed under the other Aims, result in a virus with a phenotype of enhanced pathogenicity and/or transmissibility via the respiratory route, or enhanced growth by more than 1 log compared to wild type strains, you must immediately stop the work and notify the NIAID Program Officer and Grants Management Specialist.
******

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL)
(http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level,

and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- A list of the new and/or additional Agent(s) that will be studied;
- A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Bryan Jose Encarnacao
Email: Bryan.Encarnacao@nih.gov **Phone**: 240-747-7367

Program Official: Erik J. Stemmy
Email: erik.stemmy@nih.gov **Phone**: 240-627-3380

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI089728-08

INSTITUTION: UNIVERSITY OF MINNESOTA

Budget	Year 8	Year 9	Year 10
Salaries and Wages	$116,853	$116,853	$116,853
Fringe Benefits	$30,547	$30,547	$30,547
Personnel Costs (Subtotal)	$147,400	$147,400	$147,400
Materials & Supplies	$60,005	$60,005	$60,005
Travel	$2,970	$2,970	$2,970
Other	$5,247	$5,247	$5,247
Subawards/Consortium/Contractual Costs	$120,384	$120,384	$120,384
Publication Costs	$2,178	$2,178	$2,178
TOTAL FEDERAL DC	$338,184	$338,184	$338,184
TOTAL FEDERAL F&A	$117,431	$117,612	$117,612
TOTAL COST	$455,615	$455,796	$455,796

Facilities and Administrative Costs	Year 8	Year 9	Year 10
F&A Cost Rate 1	53%	54%	54%
F&A Cost Base 1	$18,150	$217,800	$217,800
F&A Costs 1	$9,620	$117,612	$117,612
F&A Cost Rate 2	54%		
F&A Cost Base 2	$199,650		
F&A Costs 2	$107,811		

Subrecipient Risk Analysis and Compliance Record (06/10/201[illegible]

Subrecipient Name:	University of Northa Carolina, Chapel Hill	**PO #:**	N005402801
Prime Sponsor:	NIH NIAID	**Date Completed & Entered into EFS:**	8/1/2016
CON#	58289	**Grant Administrator:**	Brett Carlson

Step 1

Audit Path (•) A-133 ( ) Financial Questionaire

Audit Weaknesses? ( ) No (•) Yes

Step 2

Analysis Matrix

Instructions: Mark yes or no.

Criteria		Wt.	Score
Is the entity located outside the U.S.?	( ) Y (•) N	6	0
Will work be done outside the U.S.?	( ) Y (•) N	3	0
Is entity non-profit or government?	(•) Y ( ) N	3	0
Is prime award a contract or subcontract (rather than grant)?	( ) Y (•) N	4	0
Is amount of Subgrant/Subcontract more than 500k?	(•) Y ( ) N	4	4
Is amount of subaward 50% or more of total award?	( ) Y (•) N	5	0
Are accounting systems established?	( ) Y (•) N	10	10
Do audit results indicate weaknesses in the entity's procurement system?	(•) Y ( ) N	3	3
Does entity have a negotiated indirect cost rate agreement?	(•) Y ( ) N	2	0
Is entity subject to A-133?	(•) Y ( ) N	2	0
Any prior negative experience with entity?	( ) Y (•) N	2	0
Is there ITAR/EAR type of work?	( ) Y (•) N	10	0
Is the entity using humans, animals, DNA, stem cells, etc. (compliance)?	( ) Y (•) N	2	0
Is entity mature?	(•) Y ( ) N	6	0
Is prime sponsor government owned & contractor operated?	( ) Y (•) N	10	0
Is there potential for COI regarding relationship of subrecipient and PI?	( ) Y (•) N	10	0
Are deliverables tangible products or pivotal to success?	(•) Y ( ) N	7	7
Is "Advantaged" status claimed?	( ) Y (•) N	1	0
Special Considerations? (provide details in notes)	( ) Y (•) N		
Total Risk Score (≥ than 15 is high and should be escalated)			24

Escalated to PGA? Y ☑ N ☐

Completed by: Lesley S Sindberg Date: 8/1/16

Step 3

Debarment: (•) No ( ) Yes

Initials: LSS **Date:** 8/1/2016

F&A Rate: 52.00% **Date:** 8/1/2016

FFATA Required: (•) No ( ) Yes **Date:** 8/1/2016

Sent to ovprffat@umn.edu ☐ **Date:** N/A

FCOI: ( ) Sub policy ( ) n/a ( ) UofM policy (•) FDP **Date:** 8/1/2016

Step 4

Amendment #___1 & 2__:

Audit Path (•) A-133 ( ) Financial Questionaire

Audit Weaknesses? (•) No ( ) Yes

Debarment: (•) No ( ) Yes

Initials: LSS **Date:** 7/30/2018

F&A Rate: 52.00% **Date:** 7/30/2018

FFATA Required: (•) No ( ) Yes **Date:** 7/30/2018

Sent to ovprffat@umn.edu ☐

FCOI: ( ) Sub policy ( ) n/a ( ) UofM policy (•) FDP **Date:** 7/30/2018

Attach page for multiple amendments

Notes: Recommend requiring detailed monthly invoices and preapproval for rebudgeting based on high risk anlaysis matrix score. PI and GA should closely monitor subrecipient progress and finanical reporting. SA for FY 2017 alleviate previously identified findings and helps mitigate potential risk.

AUDIT VALID THROUGH:___3/31/19_____

Step 5

Closeout - Subaward Release Form: **Received:** ☐ **Date:**

Completion Process: ☐ **By:** **Date:**

A NEW WAY TO SIGN IN - If you already have a SAM account, use your **SAM email** for login.gov.

Log In

Login.gov FAQs

ALERT - June 11, 2018: Entities registering in SAM must submit a notarized letter appointing their authorized Entity Administrator. Read our updated FAQs to learn more about changes to the notarized letter review process and other system improvements.

Search Results

Current Search Terms: 608195277*

Your search for "608195277*" returned the following results...

Notice: This printed document represents only the first page of your SAM search results. More results may be available. To print your complete search results, you can download the PDF and print it.

Entity **University of North Carolina at Chapel Hill**

Status: **Active**

DUNS: 608195277

Has Active Exclusion?: No

Expiration Date: 02/21/2019

Purpose of Registration: All Awards

CAGE Code: 4B856

DoDAAC:

Debt Subject to Offset? No

View Details

A NEW WAY TO SIGN IN - If you already have a SAM account, use your **SAM email** for login.gov.

Login.gov FAQs

ALERT - June 11, 2018: Entities registering in SAM must submit a notarized letter appointing their authorized Entity Administrator. Read our updated FAQs to learn more about changes to the notarized letter review process and other system improvements.

Search Results

Current Search Terms: ralph* baric*

Your search for "ralph* baric*" returned the following results...

Notice: This printed document represents only the first page of your SAM search results. More results may be available. To print your complete search results, you can download the PDF and print it.

Status: **Active**

Entity **BARIC, RALPH S**

CAGE Code: 46S96 View Details

DUNS: 603707618

DoDAAC:

Has Active Exclusion?: No

Debt Subject to Offset? No

Expiration Date: 11/27/2018

Purpose of Registration: All Awards

Subject: Extend subcontract N005402801 CON - 58289
From: Anne Mockovak <a-mock@umn.edu>
Date: 7/12/2018 2:09 PM
To: Brett Carlson <carls234@umn.edu>
CC: "Sims, Amy C" <sims0018@email.unc.edu>, Fang Li <lifang@umn.edu>

Brett,

Please extend subcontract N005402801 with year 3 funding. I have attached the budget and work scope.

Extend to 5/31/2019
Additional funds = 120,384

Let me know if you need additional information.

Thanks,
Anne

Anne Mockovak
Accountant
Department of Veterinary and
Biomedical Sciences
612.301.2143

Attachments:

UNC Year 3 Subcontract.pdf 27 bytes

Subject: Re: PE Univ of MN Subaward N005402801 Amendment 2, Prime Award: R01AI089728
From: Brett Carlson <carls234@umn.edu>
Date: 8/9/2018 8:44 AM
To: "Lavrakas, Marybeth Therese" <lavrakas@unc.edu>
CC: Anne M Mockovak-1 <a-mock@umn.edu>, Sponsored Projects Admin Subcontracts <subk@umn.edu>

Hello,

Attached is the fully executed amendment for your records.

Thanks,

Brett

Brett Carlson
Sr. Grant & Contract Administrator
University of Minnesota
Office of Sponsored Projects Administration
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 55455
Phone: 612-626-7441
Fax: 612-624-4843

On 8/3/2018 1:56 PM, Lavrakas, Marybeth Therese wrote:

> Good day,
>
> I have attached a copy of the partially executed amendment. When the fully executed version is available, please send it to resadminosr@unc.edu.
>
> Best wishes,
>
> **Marybeth Lavrakas**
> Sponsored Projects Specialist, Team 5
> UNC Chapel Hill Office of Sponsored Research
> 919-962-7762 lavrakas@unc.edu
>
> *300.3 Deadlines for Proposal Submissions to University Offices*
> *http://research.unc.edu/offices/sponsored-research/policies-procedures/section-300/policy-3/*
>
> *OSR Standard Operating Procedure: Internal Deadlines for Proposal Submissions to University Offices*

http://research.unc.edu/files/2014/05/SOP-Internal-Deadlines-FINAL-5-7-2014.pdf

cid:d606e1d1-1a3d-4

Attachments:

2137_001.pdf 27 bytes

Subject: RE: PE Univ of MN Subaward N005402801 Amendment 2, Prime Award: R01AI089728
From: "Lavrakas, Marybeth Therese" <lavrakas@unc.edu>
Date: 8/3/2018 1:56 PM
To: "carls234@umn.edu" <carls234@umn.edu>

Good day,

I have attached a copy of the partially executed amendment. When the fully executed version is available, please send it to resadminosr@unc.edu.

Best wishes,

Marybeth Lavrakas
Sponsored Projects Specialist, Team 5
UNC Chapel Hill Office of Sponsored Research
919-962-7762 lavrakas@unc.edu

300.3 Deadlines for Proposal Submissions to University Offices
http://research.unc.edu/offices/sponsored-research/policies-procedures/section-300/policy-3/

OSR Standard Operating Procedure: Internal Deadlines for Proposal Submissions to University Offices
http://research.unc.edu/files/2014/05/SOP-Internal-Deadlines-FINAL-5-7-2014.pdf

UNC RESEARCH

Attachments:

PE N005402801 Amend2.pdf 27 bytes

Subject: Univ of MN Subaward N005402801 Amendment 2, Prime Award: R01AI089728
From: Brett Carlson <carls234@umn.edu>
Date: 7/31/2018 11:25 AM
To: resadminosr@unc.edu, farrelrl@email.unc.edu
CC: Anne M Mockovak-1 <a-mock@umn.edu>, Fang Li-5 <lifang@umn.edu>

University of Minnesota PI:Fang Li
UNC PI: Ralph Baric
PO#: N005402801

Hello:

Attached is an amendment to subaward N005402801 between the University of Minnesota and University of North Carolina - Chapel Hill. Please have the amendment administratively reviewed and endorsed on behalf of your organization. A half-executed copy of the amendment should be returned to me via e-mail. I will return a fully-signed copy to you once final signature is obtained.

If at any time you have questions, please feel free to contact me at (612)-626-7441 or carls234@umn.edu. Questions regarding programmatic or scientific matters should be directed to the Principal Investigator, as indicated in Attachment 3A of the agreement.

Sincerely,

Brett

--
Brett Carlson
Sr. Grant & Contract Administrator
University of Minnesota
Office of Sponsored Projects Administration

450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 55455
Phone: 612-626-7441
Fax: 612-624-4843

Attachments:

N005402801 Amend2.pdf 27 bytes

A

FDP Research Subaward Agreement
Amendment (Number 2)

UMN CON #: 58289

Pass-Through Entity (PTE) — Subrecipient

Regents of the University of Minnesota	Entity Name: University of North Carolina - Chapel Hill
awards@umn.edu	Email Address: resadminosr@unc.edu
Fang Li	Principal Investigator: Ralph Baric

Project Title: Receptor recognition and cell entry of coronaviruses

PTE Federal Award No: R01AI089728	Federal Awarding Agency: NIH / NIAID

Subaward Period of Performance: Start Date: June 1, 2017 End Date: May 31, 2019	Amount Funded This Action: $ 120,384.00	Subaward No: N005402801
Effective Date of Amendment: Jun 1, 2018	Total Amount of Federal Funds Obligated to Date: $ 361,152.00	Subject to FFATA: ○ Yes ◉ No — Automatic Carryover: ◉ Yes ○ No

Amendment(s) to Original Terms and Conditions

This Amendment revises the above-referenced Research Subaward Agreement as follows:

Amendment 2 awards $120,384 for the period of 6/1/18-5/31/19 as shown in Exhibit A-1. The total cumulative award amount is now $361,152. All other terms and conditions remain unchanged.

For clarity: all amounts stated in this amendment are in United States Dollars.

All other terms and conditions of this Subaward Agreement remain in full force and effect.

By an Authorized Official of PTE:	By an Authorized Official of Subrecipient:
Name: April Coon	Name:
Title: Associate Director	Title:
Date	Date

Scope of Work-Baric Laboratory

Coronaviruses (CoVs) cause epidemic outbreaks of diseases in humans and animals, symbolized recently by the emergence of SARS-CoV, MERS-CoV, and porcine epidemic diarrhea CoV (PEDV). Receptor recognition and cell entry are two essential steps in the CoV infection cycle, critical determinants of CoV host range, cross species transmission and tissue tropism, and primary targets for antiviral intervention. In this competitive grant renewal, we will continue to investigate fundamental questions regarding receptor recognition and cell entry to CoVs from different genera. The long-term goals of the proposal are to develop global models of CoV-receptor interaction and protease processing in emerging coronavirus evolution, entry, tropism, pathogenesis and cross species transmission. The Baric laboratory will provide collaborative virological support including reverse genetics, evaluation of systematic mutation sets on emerging CoV receptor interactions and entry in vitro, mechanistic studies on viral gene function on receptor binding and entry, and experimental evolution in vitro.

Aim 1: Receptor binding by CoV S1-NTDs. The major goals of this aim are to determine the molecular mechanisms by which select subsets of emerging CoV recognize sugar receptors for entry and host range. Previous studies have shown that the S1-NTDs of β-genus BCoV and α-genus PEDV both recognize sugar receptors. In fact, the β-CoV S1-NTDs have the same structural fold as human galectins (galactose-binding lectins) that either function as novel protein receptor binding domains or non-galactose sugar receptors. This aim will investigate how CoV S1-NTDs from different genera have evolved to recognize sugar receptors. Our overall hypothesis is that S1-NTDs from all CoV genera originated from host galectins, but have evolved novel molecular mechanisms to recognize non-galactose sugar receptors. To test this hypothesis, the Li laboratory will determine the molecular and structural basis for sugar receptor recognition by S1-NTDs from (i) β-genus BCoV, (ii) α-genus PEDV, and (iii) δ-genus PdCoV. These studies will reveal sugar-binding mechanisms of CoV from different genera and provide insight into the evolutionary origins and pathways of CoV S1-NTDs.

Baric Laboratory. In parallel, the Baric laboratory will introduce select subsets of sugar enhancing or sugar-binding destroying mutations on recombinant MHV, HCoV OC43 and PEDV, produced by reverse genetics. In part 1, we will introduce mutation subsets into the existing molecular clones and recover recombinant viruses. In part 2, we will characterize virus growth kinetics on nonpermissive cell lines expressing target protein receptors (e.g., CEACAM1a, CEACAM1b, CEACAM2 for MHV and CD17 for PEDV). In part 3, we will evaluate virus binding kinetics by virus attachment assays.

Aim 2: Receptor biding by CoV S1-CTDs. Earlier work has solved the structure of several CoV S1-CTDs in complex with their protein receptors, including β-genus SARS-CoV S1-CTD complexed with its protein receptor ACE2. Our collaborator (Dr. Baric) and others have isolated several bat SARS-like CoVs (SLCoVs) that use human ACE2 and infect primary human airway cells. This aim will investigate the cross-species transmission pathway of bat SLCoVs by examining the interactions between their S1-CTDs and ACE2 from different species. Our overall hypothesis is that to transmit from bats to another species, bat SLCoVs need to adapt to ACE2 from that species through selective mutations in their S1-CTD. Dr. Li and his group will use biochemical and structural biology to elucidate the biochemical and molecular mechanisms for SLCoV S1-CTD binding to bat, civet and human ACE2, providing comparative metrics into strength of interaction and pandemic potential.

Baric Laboratory. In parallel, the Baric laboratory will introduce select subsets of mutations on recombinant SLCoV like WIV1, SHC014 and others, produced by reverse genetics. In part 1, we will introduce mutation subsets into the existing molecular clones and recover recombinant viruses. In part 2, we will characterize virus growth kinetics on nonpermissive cell lines expressing the civet, bat, mouse and human ACE2 receptors. In part 3, we will evaluate virus binding kinetics by virus attachment assays in these cells. In part 4, we will use experimental evolution to select for more efficient virus-receptor interactions in vitro using these cells.

Aim 3: Cell entry by CoVs. Previous studies have identified the host proteases that activate cell entry and serve as a cross-species barrier for some emerging CoV. Our overall hypothesis is that CoV spikes have evolved a variety of protease activation mechanisms for regulation of viral host range, tropism, and pathogenesis. Dr. Li and his group will investigate how the spike of neurotropic MHV strains evolved to be more prone to protease activation as adaptation to low receptor level in the brain and identify which proteases activate cell entry of PEDV. These studies will elucidate the molecular mechanisms for CoV entry and reveal how cell entry regulates CoV host range, tropism, and pathogenesis.

Baric Laboratory. In parallel, the Baric laboratory will introduce select subsets mutations in MHV and PEDV, designed to ablate or enhance protease interaction and cleavage during entry. In part 1, we will introduce mutation subsets into the existing molecular clones and recover recombinant viruses. In part 2, we will characterize virus growth kinetics on nonpermissive cell lines expressing CECAM1, CEACAM2, CD17 or sugar receptors, coupled with various surface and intracellular proteases. In part 3, we will evaluate virus penetration using escape from polyclonal antibody neutralization. In part 4, we will use experimental evolution to select for more efficient virus-proteolytic processing mutants for in vitro entry.

Our overall goal is to use CoVs as model systems and establish novel principles in virology that govern viral evolution, receptor recognition, cell entry, cross-species infections, and tropism. Due to our expertise and experience, we are in a unique position to perform the research and contribute to virology and human health.

YEAR 2 June 1, 2017-May 31, 2018

NAME	TITLE	SALARY	EFFORT	Cal Months	SALARY	FRINGE	TOTAL
Baric	PI	$189,600	3%	0.36	$5,688	$1,535	$7,223
Kocher, Jacob	Post Doc	$50,316	50%	6.00	$25,158	$4,421	$29,579
Scobey, Trevor	Research Tech	$43,591	15%	1.80	$6,539	$2,443	$8,982
				0.00	$0	$0	$0
				0.00	$0	$0	$0

Subject: NoA: 5 R01 AI089728-08 PI: Li, Fang
From: era-notify@mail.nih.gov
Date: 5/22/2018 11:03 PM
To: awards@umn.edu

Grant Number: 5 R01 AI089728-08
Principal Investigator: Li, Fang
Project Title: Receptor recognition and cell entry of coronaviruses
Institution: UNIVERSITY OF MINNESOTA
CFDA: 93.855
OC: 414E
PCC: M51C B
Award Issue Date: 05/23/2018
Grants Management Officer: Machuca, Jorge
Program Official: Stemmy, Erik
Grants Specialist: Encarnacao, BRYAN

*** This is an automated notification - Please do not reply to this message. ***

Attachments:

NIH_NOA_5R01AI089728-08.PDF 27 bytes

Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 05/23/2018

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI089728-08
FAIN: R01AI089728

Principal Investigator(s):
Fang Li, PHD

Project Title: Receptor recognition and cell entry of coronaviruses

KEVIN MCKOSKEY
Grant Administrator
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 554552070

Award e-mailed to: awards@umn.edu

Period Of Performance:
Budget Period: 06/01/2018 – 05/31/2019
Project Period: 06/07/2016 – 05/31/2021

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $455,615 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MINNESOTA in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI089728. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Jorge E. Machuca
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI089728-08

Award Calculation (U.S. Dollars)	
Salaries and Wages	$116,853
Fringe Benefits	$30,547
Personnel Costs (Subtotal)	$147,400
Materials & Supplies	$60,005
Travel	$2,970
Other	$5,247
Subawards/Consortium/Contractual Costs	$120,384
Publication Costs	$2,178
	$338,184
Federal Direct Costs	$117,431
Federal F&A Costs	$455,615
Approved Budget	$455,615
Total Amount of Federal Funds Obligated (Federal Share)	$455,615
TOTAL FEDERAL AWARD AMOUNT	$455,615
AMOUNT OF THIS ACTION (FEDERAL SHARE)	

SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
8	$455,615	$455,615
9	$455,796	$455,796
10	$455,796	$455,796

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1416007513A1
Document Number:	RAI089728B
PMS Account Type:	P (Subaccount)
Fiscal Year:	2018

IC	CAN	2018	2019	2020
AI	8472364	$455,615	$455,796	$455,796

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C B / **OC**: 414E / **Released**: MACHUCAJ 05/21/2018
Award Processed: 05/23/2018 12:03:13 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI089728-08

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI089728-08

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.

d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI089728. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI089728-08

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

This Notice of Award (NoA) includes funds for activity with **The University of North Carolina at Chapel Hill** in the amount of **$120,384** (**$79,200** direct costs + **$41,184** F&A costs).
******

No funds are provided and no funds can be used to support gain-of-function research covered under the October 17, 2014 White House Announcement (NIH Guide Notice NOT-OD-15-011).

Per the letter dated May 18, 2016 to Mr. McKoskey at the University of Minnesota, NIAID funding cannot be used to generate SARS-like viruses with enhanced affinity for human receptors (via either reverse genetics or serial passaging). NIAID funding will instead be used to pursue alternative loss-of-function strategies or mutation of the ACE2 receptor as described in the letter from the University of Minnesota dated April 15, 2016. If any of the alternative experiments proposed, or any experiments proposed under the other Aims, result in a virus with a phenotype of enhanced pathogenicity and/or transmissibility via the respiratory route, or enhanced growth by more than 1 log compared to wild type strains, you must immediately stop the work and notify the NIAID Program Officer and Grants Management Specialist.
******

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL)
(http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level,

and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- A list of the new and/or additional Agent(s) that will be studied;
- A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Bryan Jose Encarnacao
Email: Bryan.Encarnacao@nih.gov **Phone**: 240-747-7367

Program Official: Erik J. Stemmy
Email: erik.stemmy@nih.gov **Phone**: 240-627-3380

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI089728-08

INSTITUTION: UNIVERSITY OF MINNESOTA

Budget	Year 8	Year 9	Year 10
Salaries and Wages	$116,853	$116,853	$116,853
Fringe Benefits	$30,547	$30,547	$30,547
Personnel Costs (Subtotal)	$147,400	$147,400	$147,400
Materials & Supplies	$60,005	$60,005	$60,005
Travel	$2,970	$2,970	$2,970
Other	$5,247	$5,247	$5,247
Subawards/Consortium/Contractual Costs	$120,384	$120,384	$120,384
Publication Costs	$2,178	$2,178	$2,178
TOTAL FEDERAL DC	$338,184	$338,184	$338,184
TOTAL FEDERAL F&A	$117,431	$117,612	$117,612
TOTAL COST	$455,615	$455,796	$455,796

Facilities and Administrative Costs	Year 8	Year 9	Year 10
F&A Cost Rate 1	53%	54%	54%
F&A Cost Base 1	$18,150	$217,800	$217,800
F&A Costs 1	$9,620	$117,612	$117,612
F&A Cost Rate 2	54%		
F&A Cost Base 2	$199,650		
F&A Costs 2	$107,811		

MEMORIAL [illegible]

Subject: Subcontract project change
From: Anne Mockovak <a-mock@umn.edu>
Date: 9/13/2017 2:07 PM
To: Brett Carlson <carls234@umn.edu>, Erin Knudsen <jond0006@umn.edu>

Brett and Erin,

The PI on CON 58289 moved to VBS. The subcontract needs to be transferred to the new project number. PO N005402801

Old project 00054028, New project 00066245

Thanks for your help,
Anne

Anne Mockovak
Accountant
Department of Veterinary and Biomedical Sciences
612.301.2143

approved 8/9/17

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Name of Sponsor:	University of Minnesota Tonya Knutson 450 McNamara Alumni Center 200 Oak Street SE. Minneapolis MN 55455-2070 United States	Invoice #: GM00130311 ○ Interim Billing ● Final Billing	Award Amount $120,384.00
Sponsor Ref No.:	N005402801		
UNC Award No.:	A17-0239-001	Budget Period:	
Award Title:	Receptor recognition and cell entry of coronaviruses	From: 06/07/2016	To: 05/31/2017
Project ID:	5105169		
knuts055@umn.edu, sub-inv@umn.edu.			

DESCRIPTION OF SERVICES	EXPENDITURES Current 05/01/2017 - 05/31/2017	Cumulative 05/31/2017
Salaries	$2,240.65	$35,053.25
Fringe Benefits	$588.80	$8,895.83
Supplies	$1,362.78	$33,976.65
Travel-Domestic	$0.00	$1,276.80
		$79,196.33
Total Direct	$4,192.23	$41,182.26
Indirect Cost	$2,179.96	$120,378.59
TOTAL	$6,372.19	

Payment due upon receipt of invoice

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	Signature	Date
Jenifer Womble Gwaltney		07/14/2017

For questions regarding invoice, please contact Julie Kadow
Phone: 919/962-4692 FAX: 919/962-5011
OSRBILLING@unc.edu

JK

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

From: psoftfs@unc.edu
Subject: Approved. eProcurement Order: UNCCH 1000196304
Date: October 11, 2016 at 1:11 PM
To: sims0018@email.unc.edu

eProcurement

No action required.

Order Details

Requester:	Amy Sims
Order ID:	UNCCH 1000196304
Vendor ID:	0000000009 FISHER SCIENTIFIC COMPANY LLC
Entered Date:	10/11/2016
Total Order Amount:	$1,362.78

Distributions

GL-BU Fund	Source	Account	Dept	PC-BU Project	Program	Amount
UNCCH 25210	49000	537110	463501	CHOSR 5105169		1,362.78

Line Items

Item ID	Description	Category	Quantity	Price	Amount
19268000	TR-602N VERSAFLO PAP46182002		3	454.26	1,362.78

If you have questions, please contact Lawson Oldenburg at 919- 962-3773 or Lawsonri@email.unc.edu
You can navigate directly to the approval page by clicking the link below.

Review Requisition

all, two more

PACKING SLIP

CUSTOMER SERVICE OFFICE	PRINT DATE	RPT NAME	SHIP WHSE	ORDER NO.	P/L	PAGE
[redacted]	10/27/16		RMI	[redacted]	1	1

CUSTOMER ACCOUNT NO.	CUSTOMER PURCHASE ORDER NUMBER	RELEASE NUMBER
[redacted]	[redacted]	

S H I P T O [redacted]

S O L D T O UNIVERSITY OF NO CAROLINA
ENVIRONMENT HEALTH
CAMPUS BOX
[redacted]
CHAPEL HILL NC 27599-1230

FISHER SCIENTIFIC COMPANY

Visit the Fisher Scientific Website:
fishersci.com

DOCUMENT TYPE: [redacted]
CALLER: [redacted]
PHONE NUMBER: [redacted]

INSTRUCTIONS

NOTE. QUANTITY INDICATED IN THIS COLUMN IS BEING SHIPPED DIRECT TO YOU FROM INDICATED ALTERNATE WAREHOUSE. SEE REVERSE SIDE FOR ABBREVIATIONS.

LINE	QUANTITY ORDERED	QUANTITY SHIPPED	BACK ORDERED	ALT. WHSE	UNIT	CATALOG NUMBER	SUPPLIER CAT. NO / DESCRIPTION	UNIT PRICE	AMOUNT
[redacted]							PHONE NUMBER		
1	3	3			CS	19 268 000	COAST LINE SERV GROUP TP-602N PERCHLOR PAPR 1/EA TP-602N		
[redacted]									
[redacted]									
[redacted]									
[redacted]									
[redacted]									
[redacted]									
[redacted]									
[redacted]									
[redacted]									
[redacted]									
[redacted]							TOTAL TRANSPORTATION CHARGE		

Rec'd
11/2/16
ACS

PROD. NBR	ORDER NO.	SEQ
M	CHP/1001	/
I	CHP/1001	/
N	CHP/1001	/
H	CHP/1001	/
P	CHP/1001	/
S	/	/
S	/	/

OPEN AND INSPECT IMMEDIATELY
QUESTIONS REGARDING THIS SHIPMENT SHOULD BE DIRECTED TO YOUR CUSTOMER SERVICE OFFICE WITHIN TEN (10) DAYS
AT: [redacted]

PLEASE REFERENCE THE ORDER NUMBER SHOWN ABOVE IN ALL CORRESPONDENCE
RETURNS WILL NOT BE ACCEPTED WITHOUT PRIOR AUTHORIZATION.

*V62855150001 *

UNCCH.200016986

CUST. ACCOUNT NO.	ORDER NO.	[redacted]	PCS.	CARRIER	SHIP DATE	WEIGHT	ASSEM. BY	PACKED BY	SHIPPED BY
[redacted]	[redacted]		1	[redacted]	10-27-16				

Approved for use through 11/30/2010

P.I.: Ralph Baric UNC No.: 5105169 OMB No. 0925-0001

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FINAL INVENTION STATEMENT AND CERTIFICATION

(FOR GRANT OR AWARD)

DHHS GRANT OR AWARD NO.
N005402801

A. We hereby certify that to the best of our knowledge and belief, all inventions are listed below which were conceived and/or first actually reduced to practice during the course of work under the above-referenced DHHS grant or award for the period.

6/7/2016 through **5/31/2017**
Original effective date — date of termination

B. INVENTIONS (Note: If no inventions have been made under the grant or award, insert the word "*NONE*" under Title below.)

NAME OF INVENTOR	TITLE OF INVENTION	DATE REPORTED TO DHHS
	NONE	

(Use continuation sheet if necessary)

C. SIGNATURE - - This block *must* be signed by an official authorized to sign on behalf of the institution.

TITLE	NAME AND MAILING ADDRESS OF INSTITUTION
Director of Licensing Office of Commercialization and Economic Development	The Univ. of North Carolina at Chapel Hill Office of Commercialization and Economic Development 100 Europa Drive, Suite #430, CB #4105 Chapel Hill, NC 27599-4105
TYPED NAME Jacqueline Quay, J.D.	
SIGNATURE [signature]	DATE July 5, 2017

HHS 568 (Rev. 11/07)

Subaward Release of Claims

University of Minnesota – Office of Sponsored Projects Administration

Subaward #: N005402801

Subrecipient Name: The University of North Carolina @ Chapel Hill UNC # 5105169

Section I – Financial Information

The total amount of $ 114,006.40 has been received under this subaward.

☐ There are NO outstanding claims against this subaward. The University of Minnesota is not obligated to honor claims made after this block is checked and this form is signed and returned.

☒ Only the amount included in the Final Claims Voucher/Invoice totaling $ 6372.19 is due. When the Final Claims Voucher/Invoice is paid by University of Minnesota, there will be no further claims against this subaward.

Section II – Patents

☒ There are no inventions to be reported under this award.

☐ Attached is a list of inventions required to be reported under this subaward. Please note that a complete invention disclosure must accompany this form, if one has not been previously provided.

Name of Inventor(s):

Site(s) of Invention:

Section III – Federal Government Equipment

☒ There is neither government furnished equipment nor equipment purchased with money from this subaward which needs to be reported to the sponsor under this subaward. (Please review subaward Terms and Conditions)

☐ All government furnished equipment and reportable equipment purchased with money from this subaward has been delivered to the US Government or is awaiting disposition instructions.

Date: 7/31/17

Signature (Authorized Signatory): FOR

Printed Name: Jenifer Gwaltney

Title: Assistant Director, Financial Reporting & Cash Management

Complete all sections, check boxes as appropriate, and return to
Office of Sponsored Project Administration
Fax #612.624.4843, Phone #612.624.5599
Email: sub-inv@umn.edu Please reference subaward number in email subject line

Approved for use through 11/30/2010

P.I.: Ralph Baric UNC No.: 5105169 OMB No. 0925-0001

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FINAL INVENTION STATEMENT AND CERTIFICATION

(FOR GRANT OR AWARD)

DHHS GRANT OR AWARD NO.
N005402801

A. We hereby certify that to the best of our knowledge and belief, all inventions are listed below which were conceived and/or first actually reduced to practice during the course of work under the above-referenced DHHS grant or award for the period.

6/7/2016 (Original effective date) through 5/31/2017 (date of termination)

B. **INVENTIONS** (Note: If no inventions have been made under the grant or award, insert the word "*NONE*" under Title below.)

NAME OF INVENTOR	TITLE OF INVENTION	DATE REPORTED TO DHHS
	NONE	

(Use continuation sheet if necessary)

C. **SIGNATURE** - - This block *must* be signed by an official authorized to sign on behalf of the institution.

TITLE
Director of Licensing
Office of Commercialization and Economic Development

TYPED NAME
Jacqueline Quay, J.D.

SIGNATURE

DATE
July 5, 2017

NAME AND MAILING ADDRESS OF INSTITUTION

The Univ. of North Carolina at Chapel Hill
Office of Commercialization and Economic Development
100 Europa Drive, Suite #430, CB #4105
Chapel Hill, NC 27599-4105

Subrecipient Risk Analysis and Compliance Record (06/10/2015)

Subrecipient Name:	University of Northa Carolina, Chapel Hill	**PO #:**	N005402801
Prime Sponsor:	NIH NIAID	**Date Completed & Entered into EFS:**	8/1/2016
CON#	58289	**Grant Administrator:**	Erin Knudsen

Step 1

Audit Path (•) A-133 ( ) Financial Questionaire

Audit Weaknesses? ( ) No (•) Yes

Analysis Matrix

Step 2

Instructions: Mark yes or no.

Criteria		Wt.	Score
Is the entity located outside the U.S.?	( ) Y (•) N	6	0
Will work be done outside the U.S.?	( ) Y (•) N	3	0
Is entity non-profit or government?	(•) Y ( ) N	3	0
Is prime award a contract or subcontract (rather than grant)?	( ) Y (•) N	4	0
Is amount of Subgrant/Subcontract more than 500k?	(•) Y ( ) N	4	4
Is amount of subaward 50% or more of total award?	( ) Y (•) N	5	0
Are accounting systems established?	( ) Y (•) N	10	10
Do audit results indicate weaknesses in the entity's procurement system?	(•) Y ( ) N	3	3
Does entity have a negotiated indirect cost rate agreement?	(•) Y ( ) N	2	0
Is entity subject to A-133?	(•) Y ( ) N	2	0
Any prior negative experience with entity?	( ) Y (•) N	2	0
Is there ITAR/EAR type of work?	( ) Y (•) N	10	0
Is the entity using humans, animals, DNA, stem cells, etc. (compliance)?	( ) Y (•) N	2	0
Is entity mature?	(•) Y ( ) N	6	0
Is prime sponsor government owned & contractor operated?	( ) Y (•) N	10	0
Is there potential for COI regarding relationship of subrecipient and PI?	( ) Y (•) N	10	0
Are deliverables tangible products or pivotal to success?	(•) Y ( ) N	7	7
Is "Advantaged" status claimed?	( ) Y (•) N	1	0
Special Considerations? (provide details in notes)	( ) Y (•) N		
Total Risk Score (≥ than 15 is high and should be escalated)			24

Escalated to PGA? Y ☑ N ☐

Completed by: Lesley S Sindberg Date: 8/1/16

Step 3

Debarment: (•) No ( ) Yes

Initials:	LSS	**Date:**	8/1/2016
F&A Rate:	52.00%	**Date:**	8/1/2016
FFATA Required: (•) No ( ) Yes		**Date:**	8/1/2016
Sent to ovprffat@umn.edu ☐		**Date:**	N/A
FCOI: ( ) Sub policy ( ) n/a ( ) UofM policy (•) FDP		**Date:**	8/1/2016

Step 4

Amendment #_____:

Audit Path ( ) A-133 ( ) Financial Questionaire

Audit Weaknesses? ( ) No ( ) Yes

Debarment: ( ) No ( ) Yes

Initials:		**Date:**	
F&A Rate:		**Date:**	
FFATA Required: ( ) No ( ) Yes		**Date:**	
Sent to ovprffat@umn.edu ☐			
FCOI: ( ) Sub policy ( ) n/a ( ) UofM policy ( ) FDP		**Date:**	

Attach page for multiple amendments

Notes: Recommend requiring detailed monthly invoices and preapproval for rebudgeting based on high risk anlaysis matrix score. PI and GA should closely monitor subrecipient progress and finanical reporting.

AUDIT VALID THROUGH:___3/31/17_____

Step 5

Closeout - Subaward Release Form: **Received:** ☐

Date:

Completion Process: ☐

By: **Date:**

USER NAME PASSWORD LOGIN

Forgot Username? Forgot Password?

Create an Account

Entity Dashboard

- Entity Overview
- Entity Registration
  - Core Data
  - Assertions
  - Reps & Certs
  - POCs
- Exclusions
  - Active Exclusions
  - Inactive Exclusions
  - Excluded Family Members

RETURN TO SEARCH

University of North Carolina at Chapel Hill

DUNS: 608195277 CAGE Code: 46856

Status: Active

Expiration Date: 07/07/2017

Purpose of Registration: All Awards

Entity Overview

Entity Registration Summary

Name: University of North Carolina at Chapel Hill
Doing Business As: Office Of Contracts & Grants
Business Type: Business or Organization
Last Updated By: Brian Collier
Registration Status: Active
Activation Date: 07/07/2016
Expiration Date: 07/07/2017

Exclusion Summary

Active Exclusion Records? No

Note to all Users: This is a Federal Government computer system. Use of this system constitutes consent to monitoring at all times.

USER NAME PASSWORD LOGIN

Forgot Username? Forgot Password?

Create an Account

Search Results

Current Search Terms: ralph* baric*

Your search for "ralph* baric*" returned the following results...

Notice: This printed document represents only the first page of your SAM search results. More results may be available. To print your complete search results, you can download the PDF and print it.

Glossary

Search

Results

Entity

Exclusion

Search

Filters

By Record Status

By Record Type

Entity **BARIC, RALPH S**

Status: **Active**

DUNS: 603707618

Has Active Exclusion?: No

Expiration Date: 01/12/2017

Purpose of Registration: All Awards

CAGE Code: 46S96

DoDAAC:

Delinquent Federal Debt? No

View Details

SAM | System for Award Management 1.0

IBM v1.P.48.20160624-1124

WWW1

Note to all Users: This is a Federal Government computer system. Use of this system constitutes consent to monitoring at all times.

USER NAME PASSWORD LOGIN

Forgot Username? Forgot Password?

Create an Account

Entity Dashboard

- Entity Overview
- Entity Registration
  - Core Data
  - Assertions
  - Reps & Certs
  - POCs
- Exclusions
  - Active Exclusions
  - Inactive Exclusions
  - Excluded Family Members

RETURN TO SEARCH

BARIC, RALPH S

[illegible]

Status: Active

[illegible]

Expiration Date: [illegible]

Purpose of Registration: All Awards

Entity Overview

Entity Registration Summary

Name: BARIC, RALPH S
Business Type: Business or Organization
Last Updated By: Ralph Baric
Registration Status: Active
Activation Date: 01/13/2016
Expiration Date: 01/12/2017

Exclusion Summary

Active Exclusion Records? No

Note to all Users: This is a Federal Government computer system. Use of this system constitutes consent to monitoring at all times.

Research Subaward Agreement
Amendment

UMN CON # 58289

Pass-Through Entity (PTE)		Subrecipient
Regents of the University of Minnesota	Entity Name	University of North Carolina-Chapel Hill
Sponsored Projects Administration 200 Oak Street SE, Suite 450	Address including City, State, Zip+4 (Country, if non-US)	Office of Sponsored Research 104 Airport Road, Suite 2200, CB1350 Chapel Hill, NC 27599-1350
Minneapolis, MN 55455-2070, USA	Principal Investigator	Ralph Baric
PTE Federal Award No: R01AI089728	Amendment No: 1	Federal Awarding Agency: NIH NIAID

Project Title: Receptor recognition and cell entry of coronaviruses

Subaward Period of Performance:
Start Date: Jun 1, 2017 End Date: May 31, 2018

Amount Funded This Action: $ 120,384.00

Subaward No (PO#): N005402801

Effective Date of Amendment: Jun 1, 2017

Total Amount of Federal Funds Obligated to Date: $ 240,768.00

Subject to FFATA: ☐ Yes ☑ No

Amendment(s) to Original Terms and Conditions
This Amendment revises the above-referenced Research Subaward Agreement as follows:

Action:

Amendment #1 awards $120,384 for the period of 06/01/2017 - 05/31/2018.
The total amount of the award is $240,768.

All other terms and conditions remain the same.

Please note - All invoices shall now be submitted in electronic format to sub-inv@umn.edu.

Except as modified by this amendment, all other terms and conditions of this Subaward Agreement remain in full force and effect.

By an Authorized Official of University of Minnesota:

Name: Kevin McKoskey, Director
Title: Sponsored Projects Administration
Date: 8/1/17

By an Authorized Official of Subrecipient:

Name: on behalf of Terry Magnuson, Ph.D.
Title: Vice Chancellor for Research
University of North Carolina at Chapel Hill
Date: 7-26-17

Erin Knudsen <jond0006@umn.edu>

Fwd: N005402801 UNC # 5105169 PI Ralph Baric

Erin Knudsen <jond0006@umn.edu> Wed, Aug 9, 2017 at 12:34 PM
To: "Farrell, Ronda Lee" <farrelrl@email.unc.edu>
Cc: Fang Li <lifang@umn.edu>, subk Sponsored Projects Admin Subcontracts <subk@umn.edu>, ovprffat OVPR SPA <ovprffat@umn.edu>, Lesley Schmidt Sindberg <schm1421@umn.edu>

Afternoon-

Attached is a fully executed copy of Amendment #1 for Subaward N005402801. Let me know if you have any questions.

Erin Knudsen

--

Erin Knudsen
Grants & Contracts Administrator
Sponsored Project Administration
University of Minnesota
McNamara Alumni Center
200 Oak Street SE, Suite 450
Minneapolis, MN 55455

Phone: 612.626-2724
Fax: 612.624.4843
Email: jond0006@umn.edu

[Quoted text hidden]

N005402801 Amendment #1.pdf
113K

Erin Knudsen <jond0006@umn.edu>

Amendment Subaward N005402801 UNC PI: Ralph Baric

1 message

Erin Knudsen <jond0006@umn.edu> Thu, Jun 29, 2017 at 9:39 AM
To: sims0018@email.unc.edu
Cc: Fang Li <lifang@umn.edu>, Lynn Weber <weber117@umn.edu>, Carrie DeJarlais <dejar001@umn.edu>, subk Sponsored Projects Admin Subcontracts <subk@umn.edu>, Lesley Schmidt Sindberg <schm1421@umn.edu>

University of Minnesota PI: Fang Li
University of North Carolina - Chapel Hill PI: Ralph Baric
PO#: N005402801

Attached is Amendment 1 for the subaward between the University of Minnesota and University of North Carolina- Chapel Hill for the period 06/01/2017 through 05/31/2018. Please review and endorse the amendment on behalf of your organization. One (1) half-executed copy of the agreement should be returned to me. I will return a fully-signed copy to you once final signature is obtained.

If at any time you have questions, please feel free to contact me at (612)-626-2724 or jond0006@umn.edu. Questions regarding programmatic or scientific matters should be directed to the Principal Investigator, as indicated in Attachment 3A of the agreement.

Sincerely,

--

Erin Knudsen
Grants & Contracts Administrator
Sponsored Project Administration
University of Minnesota
McNamara Alumni Center
200 Oak Street SE, Suite 450
Minneapolis, MN 55455

Phone: 612.626-2724
Fax: 612.624.4843
Email: jond0006@umn.edu

N005402801 Amd 1.pdf

175K

Research Subaward Agreement
Amendment

UMN CON # 58289

Pass-Through Entity (PTE)		Subrecipient
Regents of the University of Minnesota	Entity Name	University of North Carolina-Chapel Hill
Sponsored Projects Administration 200 Oak Street SE, Suite 450	Address including City, State, Zip+4 (Country, if non-US)	Office of Sponsored Research 104 Airport Road, Suite 2200, CB1350 Chapel Hill, NC 27599-1350
Minneapolis, MN 55455-2070, USA	Principal Investigator	Ralph Baric
PTE Federal Award No: R01AI089728	Amendment No: 1	Federal Awarding Agency: NIH NIAID

Project Title: Receptor recognition and cell entry of coronaviruses

Subaward Period of Performance: Start Date: Jun 1, 2017 End Date: May 31, 2018	Amount Funded This Action: $ 120,384.00	Subaward No (PO#): N005402801
Effective Date of Amendment: Jun 1, 2017	Total Amount of Federal Funds Obligated to Date: $ 240,768.00	Subject to FFATA: ☐ Yes ☑ No

Amendment(s) to Original Terms and Conditions

This Amendment revises the above-referenced Research Subaward Agreement as follows:

Action:

Amendment #1 awards $120,384 for the period of 06/01/2017 - 05/31/2018.
The total amount of the award is $240,768.

All other terms and conditions remain the same.

Please note - All invoices shall now be submitted in electronic format to sub-inv@umn.edu.

Except as modified by this amendment, all other terms and conditions of this Subaward Agreement remain in full force and effect.

By an Authorized Official of University of Minnesota:	By an Authorized Official of Subrecipient:
Name:	Name:
Title:	Title:
Date	Date

Erin Knudsen <jond0006@umn.edu>

Subaward N005402801 (University of NC-Chapel Hill) Amendment 1

Tue, Jun 27, 2017 at 10:09 AM

Fang Li <lifang@umn.edu>
To: Yang Chong <chong001@umn.edu>
Cc: Erin Jondahl <jond0006@umn.edu>

No changes. Same as last year. Thanks.

On Tue, Jun 27, 2017 at 10:08 AM, Yang Chong <chong001@umn.edu> wrote:

Hi, Fang:

Erin our GA wants to double check if there is any change in the following aspects with the NCU subaward on your R01 before she sets up the year 2 contract with them. Please respond to her ASAP so that the year 2 funding can be set up soon. Thanks a lot.

Yang

1.) Workscope narrative (if different than proposal)

2.) New technical/financial reports for year 2

3.) Approved budget (if different than proposal)

4.) Subaward performance period (if different than prime award)

5.) Your prime award allows for automatic carry forward of unspent funds from one budget period to the next; do you **STILL** want to provide this same process for the Collaborator?

---------- Forwarded message ----------
From: **Erin Knudsen** <jond0006@umn.edu>
Date: Tue, Jun 27, 2017 at 9:56 AM
Subject: Re: Subaward N005402801 (University of NC-Chapel Hill) Amendment 1
To: Yang Chong <chong001@umn.edu>

I have the information from the proposal/RPPR. I just want to know if the PI wants to change dates, reports or anything else before I write the sub. Sometimes they have issue with their partner PI and want to change things.

Erin

--

Erin Knudsen
Grants & Contracts Administrator
Sponsored Project Administration
University of Minnesota
McNamara Alumni Center
200 Oak Street SE, Suite 450
Minneapolis, MN 55455

Phone: 612.626-2724
Fax: 612.624.4843
Email: jond0006@umn.edu

On Tue, Jun 27, 2017 at 7:02 AM, Yang Chong <chong001@umn.edu> wrote:
Hi, Erin:

Am I supposed to provide with the sub info or have you requested the sub to provide the info? Please let me know ASAP so that I know what to do about your request? Thanks.

Yang

On Mon, Jun 26, 2017 at 4:02 PM, Erin Knudsen <jond0006@umn.edu> wrote:

Afternoon-

Please provide the following information to allow SPA to proceed with the required paper work for the subaward.

1.) Workscope narrative (if different than proposal)

2.) New technical/financial reports for year 2

3.) Approved budget (if different than proposal)

4.) Subaward performance period (if different than prime award)

5.) Your prime award allows for automatic carry forward of unspent funds from one budget period to the next; do you **STILL** want to provide this same process for the Collaborator?

Upon receipt of the information above we will initiate the formal award document and upon acceptance by the collaborating institution, SPA will create a formal purchase order for the Amendment. Should you have any questions, do not hesitate to contact

me.

--

Erin Knudsen
Grants & Contracts Administrator
Sponsored Project Administration
University of Minnesota
McNamara Alumni Center
200 Oak Street SE, Suite 450
Minneapolis, MN 55455

Phone: 612.626-2724
Fax: 612.624.4843
Email: jond0006@umn.edu



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Yang Chong
MNPI Center Accounting
IB-Physiology/ Pharmacology/ Neuroscience
6-120Jackson Hall
Mail delivery code: 1216
Phone#: 612-625-2970
Normal Working hours: 7 am to 3:30 pm
Monday through Friday

--

Yang Chong
MNPI Center Accounting
IB-Physiology/ Pharmacology/ Neuroscience
6-120Jackson Hall
Mail delivery code: 1216
Phone#: 612-625-2970
Normal Working hours: 7 am to 3:30 pm
Monday through Friday

--

****

Fang Li, Ph.D.
Associate Professor
Department of Pharmacology

University of Minnesota Twin Cities
612-625-6149, lifang@umn.edu
http://www.msi.umn.edu/~lifang
****

Erin Knudsen <jond0006@umn.edu>

Subaward N005402801 (University of NC-Chapel Hill) Amendment 1

1 message

Erin Knudsen <jond0006@umn.edu> Mon, Jun 26, 2017 at 4:02 PM
To: Yang Chong <chong001@umn.edu>

Afternoon-

Please provide the following information to allow SPA to proceed with the required paper work for the subaward.

1.) Workscope narrative (if different than proposal)

2.) New technical/financial reports for year 2

3.) Approved budget (if different than proposal)

4.) Subaward performance period (if different than prime award)

5.) Your prime award allows for automatic carry forward of unspent funds from one budget period to the next; do you **STILL** want to provide this same process for the Collaborator?

Upon receipt of the information above we will initiate the formal award document and upon acceptance by the collaborating institution, SPA will create a formal purchase order for the Amendment. Should you have any questions, do not hesitate to contact me.

--

Erin Knudsen
Grants & Contracts Administrator
Sponsored Project Administration
University of Minnesota
McNamara Alumni Center
200 Oak Street SE, Suite 450
Minneapolis, MN 55455

Phone: 612.626-2724
Fax: 612.624.4843
Email: jond0006@umn.edu



Notice of Award
Federal Award Date: 05/15/2017

RESEARCH
Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI089728-07
FAIN: R01AI089728

Principal Investigator(s):
Fang Li, PHD

Project Title: Receptor recognition and cell entry of coronaviruses

KEVIN MCKOSKEY
Grant Administrator
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 554552070

Award e-mailed to: awards@umn.edu

Period Of Performance:
Budget Period: 06/01/2017 – 05/31/2018
Project Period: 06/07/2016 – 05/31/2021

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $453,437 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MINNESOTA in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI089728. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Tina M. Carlisle
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI089728-07

Award Calculation (U.S. Dollars)	
Salaries and Wages	$116,853
Fringe Benefits	$30,547
Personnel Costs (Subtotal)	$147,400
Materials & Supplies	$60,005
Travel	$2,970
Other	$5,247
Subawards/Consortium/Contractual Costs	$120,384
Publication Costs	$2,178
	$338,184
Federal Direct Costs	$115,253
Federal F&A Costs	$453,437
Approved Budget	$453,437
Total Amount of Federal Funds Obligated (Federal Share)	$453,437
TOTAL FEDERAL AWARD AMOUNT	$453,437

AMOUNT OF THIS ACTION (FEDERAL SHARE)

SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
7	$453,437	$453,437
8	$455,615	$455,615
9	$455,796	$455,796
10	$455,796	$455,796

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name: Allergy and Infectious Diseases Research
CFDA Number: 93.855
EIN: 1416007513A1
Document Number: RAI089728B
PMS Account Type: P (Subaccount)
Fiscal Year: 2017

IC	CAN	2017	2018	2019	2020
AI	8472364	$453,437	$455,615	$455,796	$455,796

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C B / **OC**: 414E / **Released**: CARLISLET 05/12/2017
Award Processed: 05/15/2017 12:04:15 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI089728-07

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI089728-07

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.

c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI089728. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI089728-07

No funds are provided and no funds can be used to support gain-of-function research covered under the October 17, 2014 White House Announcement (NIH Guide Notice NOT-OD-15-011).

Per the letter dated May 18, 2016 to Mr. McKoskey at the University of Minnesota, NIAID funding cannot be used to generate SARS-like viruses with enhanced affinity for human receptors (via either reverse genetics or serial passaging). NIAID funding will instead be used to pursue alternative loss-of-function strategies or mutation of the ACE2 receptor as described in the letter from the University of Minnesota dated April 15, 2016. If any of the alternative experiments proposed, or any experiments proposed under the other Aims, result in a virus with a phenotype of enhanced pathogenicity and/or transmissibility via the respiratory route, or enhanced growth by more than 1 log compared to wild type strains, you must immediately stop the work and notify the NIAID Program Officer and Grants Management Specialist.
******

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- A list of the new and/or additional Agent(s) that will be studied;
- A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;

o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Madeline Elizabeth Clarke
Email: madeline.clarke@nih.gov **Phone**: 301-761-7794 **Fax**: 301-493-0597

Program Official: Erik J. Stemmy
Email: erik.stemmy@nih.gov **Phone**: 240-627-3380

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI089728-07

INSTITUTION: UNIVERSITY OF MINNESOTA

Budget	Year 7	Year 8	Year 9	Year 10
Salaries and Wages	$116,853	$116,853	$116,853	$116,853
Fringe Benefits	$30,547	$30,547	$30,547	$30,547
Personnel Costs (Subtotal)	$147,400	$147,400	$147,400	$147,400
Materials & Supplies	$60,005	$60,005	$60,005	$60,005
Travel	$2,970	$2,970	$2,970	$2,970
Other	$5,247	$5,247	$5,247	$5,247
Subawards/Consortium/Contractual Costs	$120,384	$120,384	$120,384	$120,384
Publication Costs	$2,178	$2,178	$2,178	$2,178
TOTAL FEDERAL DC	$338,184	$338,184	$338,184	$338,184
TOTAL FEDERAL F&A	$115,253	$117,431	$117,612	$117,612
TOTAL COST	$453,437	$455,615	$455,796	$455,796

Facilities and Administrative Costs	Year 7	Year 8	Year 9	Year 10
F&A Cost Rate 1	52%	53%	54%	54%
F&A Cost Base 1	$18,150	$18,150	$217,800	$217,800
F&A Costs 1	$9,438	$9,620	$117,612	$117,612
F&A Cost Rate 2	53%	54%		
F&A Cost Base 2	$199,650	$199,650		
F&A Costs 2	$105,815	$107,811		

RESEARCH & RELATED BUDGET

ORGANIZATIONAL DUNS: 608195277 **Name of Organization:** The University of North Carolina at Chapel Hill

Budget Period: **Start Date:** 6/1/17 **End Date:** 5/31/18

A. Senior/Key Person

Project Role	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)
Consortium PI	Ralph	S	Baric	PhD	187,000	0.36			5,610	1,465

Total Senior/Key Person

B. Other Personnel

Project Role	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)
Postdoctoral Fellow	Jacob		Kocher	PhD	48,216	4.80			19,286	3,458
Technician	Drew	Trevor	Scobey		42,591	4.20			14,907	5,423

Total Number Other Personnel

Total Other Personnel

TOTAL SALARY, WAGES AND FRINGE (A+B)

C. Equipment Description

Equipment item | **Fund**

Total Equipment

D. Travel

1. Domestic Travel Costs (Includes Canada, Mexico and US Possessions | **Fund**
2. Foreign Travel Costs

Total Travel

E. Participant/Trainee Support Costs

1. Tuition/Fees/Health Insurance | **Fund**
2. Stipends
3. Travel
4. Subsistence
5. Other (Specify)
Total Number Participants/Trainees

Total Partipant/Trainee Costs

F. Other Direct Costs

1. Materials and Supplies | **Fund**
2. Publication Costs
3. Consultant Services
4. ADP/Computer Services
5. Subaward/Consortium/Contractual Costs
6. Equipment or Facility Rental/ User Fees
7. Alterations and Renovations
8. Maintenance Contracts
9. Shipping
10. Other (Specify)

Total Other Direct Costs

G. Direct Costs

Total Direct Costs (A thru F)

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate	Indirect Cost Base ($)	Fund
MTDC 52%	52.00%	79,200	

Total Indirect costs

Cognizant Federal Agency

Agency Name

POC Name

POC phone #

I. Total Direct and Indirect Costs

	Fund
Total Direct and Institutional Costs (G+H)	

J. Fee

	Fund

K. Budget Justification

Send as a separate Word document. Do not include justification on this spreadsheet.

Do NOT send justification as a PDF.

Funds Requested ($)
7,075
0
0
0
0
0
0
0
7,075

Funds Requested ($)
22,744
20,330
0
0
0
0
0
0
43,074

50,149

ls Requested ($)
0

ls Requested ($)
2,000
2,000

ls Requested ($)
0

ls Requested ($)
22,051
2,000
3,000
27,051

79,200

s Requested ($)
41184
0
0
41,184

s Requested ($)
120,384

s Requested ($)

Form Approved Through 08/31/2015

OMB No. 0925-0002

Department of Health and Human Services
Public Health Services

Grant Progress Report

Review Group	Type	Activity	Grant Number

Total Project Period
From: Through:

Requested Budget Period
From: Through:

1. TITLE OF PROJECT
Receptor recognition and cell entry of coronaviruses

2a. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR
(Name and address, street, city, state, zip code)
Ralph S Baric, PhD
Department of Epidemiology
University of North Carolina – Chapel Hill
CB# 7435, McGavran-Greenberg Hall
Chapel Hill, NC 27599-7435

2b. E-MAIL ADDRESS
rbaric@email.unc.edu

2c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Epidemiology

2d. MAJOR SUBDIVISION
Public Health

2e. Tel: 919-966-3895 Fax:

3a. APPLICANT ORGANIZATION
(Name and address, street, city, state, zip code)
Office of Sponsored Research (OSR)
Administrative Office Bldg., Suite 2200
104 Ariport Drive, CB# 1350
Chapel Hill, NC 27599-1350

3b. Tel: 919-966-3411 Fax: 919-962-3352

3c. DUNS: 608195277

4. ENTITY IDENTIFICATION NUMBER
1566001393A1

5. NAME, TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL
Andy Johns, Assoc. Vice Chancellor for Research Suite
104 Ariport Drive, CB# 1350, Ste 2200
Chapel Hill, NC 27599-1350
Tel: 919-966-3411 Fax: 919-962-3352
E-MAIL: resadminosr@unc.edu

6. HUMAN SUBJECTS ☒ No ☐ Yes

6a. Research Exempt	If Exempt ("Yes" in 6a): Exemption No.	If Not Exempt ("No" in 6a): IRB approval date
☐ No ☐ Yes		

6b. Federal Wide Assurance No.

6c. NIH-Defined Phase III Clinical Trial ☐ No ☐ Yes

7. VERTEBRATE ANIMALS ☒ No ☐ Yes

7a. If "Yes," IACUC approval Date

7b. Animal Welfare Assurance No.

8. COSTS REQUESTED FOR NEXT BUDGET PERIOD

8a. DIRECT $79,200 8b. TOTAL $120,384

9. INVENTIONS AND PATENTS ☒ No ☐ Yes

If "Yes, ☐ Previously Reported
☐ Not Previously Reported

10. PROJECT/PERFORMANCE SITE(S)

Organizational Name: Same as above

DUNS:

Street 1:

Street 2:

City: County:

State: Province:

Country: Zip/Postal Code:

Congressional Districts: NC-004

11. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION *(Item 13)*
Terry Magnuson, PhD Vice Chancellor for Research

TEL: 919-966-3411 FAX: 919-962-3352 E-MAIL: resadminosr@unc.edu

12. Corrections to Page 1 Face Page

13. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 11. *(In ink)*

DATE 3/28/17

on behalf of Terry Magnuson, PH.D.
Vice Chancellor for Research
University of North Carolina at Chapel Hill

FDP Cost Reimbursement Research Subaward Agreement

UMN CON# 58269

Pass-through Entity (PTE): **Regents of the University of Minnesota**	Subrecipient: **University of North Carolina-Chapel Hill**
PTE Principal Investigator (PI): Fang Li	Subrecipient Principal Investigator (PI): Ralph Baric
PTE Federal Award No: 2R01AI089728-06 / FAIN: **R01AI089728**	Federal Awarding Agency: NIH NIAID
Federal Award Issue Date: 06/07/2016 / Total Amount of Federal Award to PTE: $464,440	CFDA No: 93.855 / CFDA Title: Allergy, Immunology and Transplantation Research
Project Title: Receptor recognition and cell entry of coronaviruses	
Subaward Period of Performance: Start: 06/07/2016 End: 05/31/2017	**Amount Funded This Action:** $120,384 / **Subaward No.** N005402801
Estimated Project Period (if incrementally funded): Start: End:	Incrementally *Estimated* Total: / Is this Award R & D [X] Yes or [ ] No
Check all that apply [X] Reporting Requirements (Attachment 4) [ ] Subject to FFATA (Attachment 3B) [ ] Cost Sharing (Attachment 5)	

Terms and Conditions

1) PTE hereby awards a cost reimbursable subaward, as described above, to Subrecipient. The statement of work and budget for this subaward are (check one) [ ] as specified in Subrecipient's proposal dated or [X] as shown in Attachment 5. In its performance of subaward work, Subrecipient shall be an independent entity and not an employee or agent of PTE.

2) PTE shall reimburse Subrecipient not more often than monthly for allowable costs. All invoices shall be submitted using Subrecipient's standard invoice, but at a minimum shall include current and cumulative costs (including cost sharing), subaward number, and certification, as required in 2 CFR 200.415 (a). Invoices that do not reference PTE Subaward number shall be returned to Subrecipient. Invoices and questions concerning invoice receipt or payments should be directed to sub-inv@umn.edu.

3) A final statement of cumulative costs incurred, including cost sharing, marked "FINAL" must be submitted to sub-inv@umn.edu NOT LATER THAN **60 days** after subaward end date. The final statement of costs shall constitute Subrecipient's final financial report.

4) All payments shall be considered provisional and subject to adjustment within the total estimated cost in the event such adjustment is necessary as a result of an adverse audit finding against the Subrecipient. PTE reserves the right to reject an invoice, in accordance with 2 CFR 200.305.

5) Matters concerning the technical performance of this subaward should be directed to the appropriate party's Principal Investigator as shown in Attachments 3A and 3B. Technical reports are required as shown above, "Reporting Requirements."

6) Matters concerning the request or negotiation of any changes in the terms, conditions, or amounts cited in this subaward agreement, and any changes requiring prior approval, should be directed to the appropriate party's AdministrativeContact, as shown in Attachments 3A and 3B. Any such changes made to this subaward agreement require the written approval of each party's Authorized Official, as shown in Attachments 3A and 3B.

7) Substantive changes made to this subaward agreement require the written approval of each party's Authorized Official as shown in Attachments 3A and 3B. The PTE may issue non-substantive changes to the Period of Performance (check one) [X] Bilaterally, or [ ] Unilaterally. Unilateral modifications shall be considered valid 14 days after receipt unless otherwise indicated by Subrecipient.

8) Each party shall be responsible for its negligent acts or omissions and the negligent acts or omissions of its employees, officers, or directors, to the extent allowed by law.

9) Either party may terminate this subaward with thirty days written notice to the appropriate party's AdministrativeContact, as shown in Attachments 3A and 3B. PTE shall pay Subrecipient for termination costs as allowable under Uniform Guidance, 2 CFR 200, or 45 CFR Part 74 Appendix E, "Principles for Determining Costs Applicable to Research & Development under Grants and Contracts with Hospitals, as applicable.

10) No-cost extensions require the approval of the PTE. Any requests for a no-cost extension should be addressed to and received by the Administrative Contact, as shown in Attachments 3A, not less than 30 days prior to the desired effective date of the requested change.

11) The Subaward is subject to the terms and conditions of the PTE Award and other special terms and conditions, as identified in Attachment 2.

12) By signing this Research Subaward Agreement Subrecipient makes the certifications and assurances shown in Attachments 1 and 2.

13) Research Terms & Conditions – RESERVED

By an Authorized Official of Regents of the University of Minnesota:	By an Authorized Official of Subrecipient:
Name: Kevin McKoskey, Director / Date: 8/8/16	On behalf of Terry Magnuson, PH.D. / Name: Vice Chancellor for Research / Date: 8/4/16
Title: Sponsor Projects Administration	Title: University of North Carolina at Chapel Hill

Attachment 1
Research Subaward Agreement
Certifications and Assurances

By signing the Subaward Agreement, the Authorized Official of Subrecipient certifies, to the best of his/her knowledge and belief, that:

Certification Regarding Lobbying

1) No Federal appropriated funds have been paid or will be paid, by or on behalf of the Subrecipient, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any Federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or intending to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the Subrecipient shall complete and submit Standard Form -LLL, "Disclosure Form to Report Lobbying," to the Pass-through Entity.

3) The Subrecipient shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements) and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by section 1352, title 31, U. S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

Debarment, Suspension, and Other Responsibility Matters

Subrecipient certifies by signing this Subaward Agreement that neither it nor its principals are presently debarred, suspended, proposed for debarment, declared ineligible or voluntarily excluded from participation in this transaction by any federal department or agency.

Audit and Access to Records

Subrecipient certifies by signing this Subaward Agreement that it complies with the Uniform Guidance, will provide notice of the completion of required audits and any adverse findings which impact this subaward as required by parts 200.501-200.521, and will provide access to records as required by parts 200.336, 200.337, and 200.201 as applicable.

Attachment 2
Research Subaward Agreement
Copy of Prime Award Terms and Conditions

Copy of Prime Award Notice (see attached [X])

Special terms and conditions:

1. **Copyrights**
   Subrecipient [X] grants / [ ] shall grant (check one) to Prime Recipient an irrevocable, royalty-free, non-transferable, non-exclusive right and license to use, reproduce, make derivative works, display, and perform publicly any copyrights or copyrighted material (including any computer software and its documentation and/or databases) first developed and delivered under this Subaward Agreement solely for the purpose of and only to the extent required to meet Prime Recipient's obligations to the Federal Government under its Prime Award.

2. **Data Rights**
   Subrecipient grants to Prime Recipient the right to use data created in the performance of this Subaward Agreement solely for the purpose of and only to the extent required to meet Prime Recipient's obligations to the Federal Government under its Prime Award.

3. **Automatic Carry Forward: [X] Yes [ ] No**
   (If No, Carry Forward requests must be sent to Prime Recipient's Administrative contact, as shown in Attachment 3).

[X] Additional Special Terms:

N/A

Attachment 3A

Research Subaward Agreement

Subaward Number: N005402801

Pass-through Entity (PTE) Contacts

Pass-through Entity (PTE)

Name: Regents of the University of Minnesota

Address: Office of Sponsored Projects Administration

450 McNamara Alumni Center, 200 Oak Street SE

City: Minneapolis State: Minnesota Zip Code: 55455-2070

Zip Code Look-Up

PTE Administrative Contact

Name: Erin Knudsen

Address: Office of Sponsored Projects Administration, 450 McNamara Alumni Center, 200 Oak Street SE

City: Minneapolis State: Minnesota Zip Code: 55455-2070

Telephone: 612-626-2724 E-mail: jond0006@umn.edu

PTE Principal Investigator (PI)

Name: Fang Li

Address: 3-106 Nils Hasselmo Hall, 312 Church St SE, Minneapolis, MN 5

City: Minneapolis State: Minnesota Zip Code: 55455

Telephone: 612-625-6149 E-mail: lifang@umn.edu

PTE Financial Contact

Name: Tonya Knutson

Address: 450 McNamara Alumni Center, 200 Oak Street SE

City: Minneapolis State: Minnesota Zip Code: 55455-2070

Telephone: (612) 624-7850 E-mail: knuts055@umn.edu

Emailed invoices preferred? ☒ Yes or ☐ No

Invoice E-mail (if different to financial contact): sub-inv@umn.edu

If invoices should not be e-mailed: send invoices to address above? ☐ Yes or ☐ No If no, enter address below.

Invoice Address (if different to Financial Contact):

PTE Authorized Official

Name: Pamela Webb, Kevin McKoskey, Judy Krzyzek, David Hagen, April Coon

Address: Office of Sponsored Projects Administration

450 McNamara Alumni Center, 200 Oak Street SE

City: Minneapolis State: Minnesota Zip Code: 55455-2070

Telephone: 612-624-5599 E-mail: awards@umn.edu

Central E-mail: awards@umn.edu

Attachment 3B
Research Subaward Agreement
Subrecipient Contacts

Subaward Number: N005402801

Subrecipient Place of Performance

Name: The University of North Carolina at Chapel Hill

Address: Office of Sponsored Research
104 Airport Road, Ste. 2200, CB 1350

City: Chapel Hill State: NC Zip Code + 4: 27599-1350 Look-Up

EIN No.: 56-600-1393 Institution Type: Public/State Controlled Inst. of Higher Education

Is Subrecipient currently registered in SAM.gov? (•) Yes ( ) No

Is Subrecipient exempt from reporting compensation? (•) Yes ( ) No

If no , please complete 3B page 2

DUNS No.: 608195277 Parent DUNS No.:

Congressional District: 4th Congressional District:

Subrecipient Administrative Contact

Name: Rhonda Farrell, Program Administrator

Address: Office of Sponsored Research, 104 Airport Road, Ste. 2200, CB 1350

City: Chapel Hill State: NC Zip Code: 27599-1350

Telephone: (919) 962-4396 Fax: (919) 962-3352

E-mail: farrelrl@email.unc.edu

Subrecipient Principal Investigator (PI)

Name: Ralph Baric

Address: Epidemiology Operations
3304 Michael Hooker Res Bldg , CB 7435

City: Chapel Hill State: NC Zip Code + 4: 27599-7435

Telephone: (919) 966-3895 Fax:

E-mail: rbaric@email.unc.edu

Subrecipient Financial Contact

Name: Jenifer Gwaltney

Address: Office of Sponsored Research, 104 Airport Road, Ste. 2200, CB 1350

City: Chapel Hill State: NC Zip Code: 27599-1350

Is this the remittance address? ( ) Yes or (X) No If no, enter address below.

Remit to address: (If different than above.) c/o Bank of America Lockbox Services, PO Box 402420, Atlanta, GA 30384-2420

Telephone: 919-966-3411 Fax: 919-962-5011

E-mail: resadminosr@unc.edu Other:

Subrecipient Authorized Official

Name: Terry Magnuson, Ph.D., Vice Chancellor for Research

Address: Office of Sponsored Research, 104 Airport Road, Ste. 2200, CB 1350

City: Chapel Hill State: NC Zip Code: 27599-1350

Telephone: 919-966-3411 Fax: 919-962-5011

E-mail: resadminosr@unc.edu

Central E-mail: resadminosr@unc.edu

Attachment 4
Research Subaward Agreement
Reporting Requirements

Subrecipient agrees to the following:

☑ Invoices shall reference the subaward number as shown on the face page of this agreement and be submitted in electronic format to sub-inv@umn.edu

☑ A Final technical/progress report will be submitted to the Pass-through Entity's Administrative Contact identified in Attachment 3 within 60 days after the end of the period of performance.

☐ Monthly technical/progress reports will be submitted to the Pass-through Entity's Administrative Contact identified in Attachment 3, within 90 days of the end of the month.

☐ Quarterly technical/progress reports will be submitted within thirty (30) days after the end of each project quarter to the Pass-through Entity's Administrative Contact identified in Attachment 3.

☐ Technical/progress reports on the project as may be required by Pass-through Entity's Administrative Contact in order that Pass-through Entity may be able to satisfy its reporting obligations to the Federal Awarding Agency.

☑ Annual technical /progress reports will be submitted within 60 days prior to the end of each project period to the Pass-through Entity's Administrative Contact identified in Attachment 3. Such report shall also include a detailed budget for the next budget period, updated Other Support for key personnel, certification of appropriate education in the conduct of human subject research of any new key personnel, and annual IRB or IACUC approval, if applicable.

☑ In accordance with 37 CFR 401.14, Subrecipient agrees to notify PTE's Administrative Contact identified in Attachment 3A within 60 days after Subrecipient's inventor discloses invention(s) in writing to Subrecipient's personnel responsible for patent matters. The Subrecipient will submit a final invention report using Awarding Agency specific forms to the PTE's Administrative Contact identified in Attachment 3A within 60 days of the end of the period of performance so that it may be included with the PTE's final invention report to the Awarding Agency. A negative report ☑ is ☐ is not required.

☐ A Certification of Completion, in accordance with 2 CFR 200.201(b)(3), will be submitted within 90 days after the end of the project period to the Pass Through Entity 's Administrative Contact identified in Attachment 3 (for Fixed Price subawards only.)

☐ Property Inventory Report; frequency, type, and submission instructions listed here and only to be used when required by PTE Federal Award

☑ Subaward Release Form shall be submitted to the University of Minnesota Office of Sponsored Projects Administration, along with the final invoice, no later than 60 days after Subaward ending date. Payment of Final Invoice shall be withheld pending receipt and acceptance of all closeout documents.

X Detailed monthly invoices and pre-approval for rebudgeting is required.

Attachment 5

Cost Reimbursement Research Subaward Agreement

Statement of Work(SOW) | Cost Sharing | Budget

Statement of Work

Below ☐ or ☑ Attached pages

If award is FFATA eligible and SOW exceeds 4000 characters, include a *Subrecipient Federal Award Project Description*

Cost Sharing:	☐ Yes, Amount $	☐ No

Budget Information

Below ☑ or ☐ Attached pages

Direct Costs	$ 79,200
Indirect Costs	$ 41,184
Total Costs	$ 120,384

Indirect Cost Rate (IDC) Applied 52% on ☐ TDC, ☑ MTDC , or ☐ other

☐ Check here if using the de minimis rate of **10** %

Subaward Release Form

University of Minnesota – Office of Sponsored Projects Administration

Subaward #: N005402801

Subrecipient Name: University of North Carolina -Chapel Hill

Section I – Financial Information

The total amount of $ _____ has been received under this subaward.

☐ There are NO outstanding claims against this subcontract. The University of Minnesota is not obligated to honor claims made after this block is checked and this form signed and returned.

☐ Only the amount included in the Final Claims Voucher/Invoice estimated to be $ _____ is due. When the Final Claims Voucher/Invoice is paid by University of Minnesota, there will be no further claims against this subcontract.

Section II – Patents

☐ There are no inventions to be reported under this award.

☐ Listed below are all inventions required to be reported under this award.

Name of Inventor: _____

Site(s) of Invention: _____

Please note that if an invention has resulted from this project, a complete invention disclosure must accompany this form, if one has not been previously provided.

Section III – Federal Government Equipment

Was any equipment provided by the Federal Government, or was any equipment purchased with federal funds provided under this subagreement?

☐ No ☐ Yes (Please see FAR 52.245-1 (j) (4) Submission requirements and (j) (8) Disposition Instructions)

☐ All equipment either provided, or purchased with funds, under this subagreement has been delivered to the US Government or is awaiting disposition instruction

Date: _____

Signature (Authorized Signatory): _____

Printed Name: _____

Title: _____

Complete all sections, check boxes as appropriate, and return to:
Office of Sponsored Projects Administration, 450 McNamara Alumni Center,
200 Oak Street SE, Minneapolis, MN 55455-2003
Fax #612.624.4843, Phone #612.624.5599, Email: awards@umn.edu

Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 06/07/2016

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 2R01AI089728-06
FAIN: R01AI089728

Principal Investigator(s):
Fang Li, PHD

Project Title: Receptor recognition and cell entry of coronaviruses

Ms Jondahl, Enn
Grant Administrator
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 554552070

Award e-mailed to: awards@umn edu

Period Of Performance:
Budget Period: 06/07/2016 – 05/31/2017
Project Period: 06/07/2016 – 05/31/2021

Dear Business Official

The National Institutes of Health hereby awards a grant in the amount of $464,440 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MINNESOTA in support of the above referenced project This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI089728 The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health " Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI). In accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards Consult the NIH website http //grants nih gov/grants/policy/coi/ for a link to the regulation and additional important information

If you have any questions about this award, please contact the individual(s) referenced in Section IV

Sincerely yours.

Devon R Bumbray-Quarles
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 2R01AI089728-06

Award Calculation (U.S. Dollars)	
Salaries and Wages	$116,853
Fringe Benefits	$30,547
Materials & Supplies	$60,005
Travel	$2,970
Other	$5,247
Subawards/Consortium/Contractual Costs	$120,384
Publication Costs	$2,178
	$338,184
Federal Direct Costs	$126,256
Federal F&A Costs	$464,440
Approved Budget	$464,440
Total Amount of Federal Funds Obligated (Federal Share)	$464,440
TOTAL FEDERAL AWARD AMOUNT	$464,440
AMOUNT OF THIS ACTION (FEDERAL SHARE)	

SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
6	$464,440	$464,440
7	$453,437	$453,437
8	$455,615	$455,615
9	$455,796	$455,796
10	$455,796	$455,796

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information.

CFDA Name.	Allergy, Immunology and Transplantation Research
CFDA Number:	93 855
EIN.	1416007513A1
Document Number:	RAI089728B
PMS Account Type:	P (Subaccount)
Fiscal Year:	2016

IC	CAN	2016	2017	2018	2019	2020
AI	8472364	$464,440	$453,437	$455,615	$455,796	$455,796

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC M51C B / **OC** 414B / **Released** BUMBRAYD 05/31/2016
Award Processed: 06/07/2016 12 02 20 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 2R01AI089728-06

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http //grants nih gov/grants/policy/awardconditions htm

SECTION III – TERMS AND CONDITIONS – 2R01AI089728-06

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following

a The grant program legislation and program regulation cited in this Notice of Award
b Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts

c 45 CFR Part 75
d National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period
e Federal Award Performance Goals As required by the periodic report in the RPPR or in the final progress report when applicable
f This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW

(See NIH Home Page at http //grants nih gov/grants/policy/awardconditions htm for certain references cited above)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75 2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA) The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs NIH recognizes that some awards may have another classification for purposes of indirect costs The auditor is not required to report the disconnect (i e , the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s)

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP)

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM) Should a consortium/subaward be issued under this award, a DUNS requirement must be included See http //grants nih gov/grants/policy/awardconditions htm for the full NIH award term implementing this requirement and other additional information

This award has been assigned the Federal Award Identification Number (FAIN) R01AI089728 Recipients must document the assigned FAIN on each consortium/subaward issued under this award

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170

In accordance with P L 110-161, compliance with the NIH Public Access Policy is now mandatory For more information, see NOT-OD-08-033 and the Public Access website http //publicaccess nih gov/

In accordance with the regulatory requirements provided at 45 CFR 75 113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period The recipient must also make semiannual disclosures regarding such proceedings Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)) Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75 This term does not apply to NIH fellowships

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 2R01AI089728-06

No funds are provided and no funds can be used to support gain-of-function research covered under the October 17, 2014 White House Announcement (NIH Guide Notice NOT-OD-15-011)

Per the letter dated May 18, 2016 to Mr McKoskey at the University of Minnesota, NIAID funding cannot be used to generate SARS-like viruses with enhanced affinity for human receptors (via either reverse genetics or serial passaging) NIAID funding will instead be used to pursue alternative loss-of-function strategies or mutation of the ACE2 receptor as described in the letter from the University of Minnesota dated April 15, 2016 If any of the alternative experiments proposed, or any experiments proposed under the other Aims, result in a virus with a phenotype of enhanced pathogenicity and/or transmissibility via the respiratory route, or enhanced growth by more than 1 log compared to wild type strains, you must immediately stop the work and notify the NIAID Program Officer and Grants Management Specialist
*****

Direct costs awarded are limited to a 20% increase over the direct costs awarded in the last non-competing continuation (see NIH Guide, Vol 26, No 35, 10/17/97)
*****

This award includes funds awarded for subrecipient activity with ***University of North Carolina, Chapel Hill*** in the amount of **$120,384** (**$79,200** direct costs + **$41,184** facilities and administrative costs)

Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS) The referenced section of the NIH Grants Policy Statement is available at http //grants nih gov/grants/policy/nihgps/HTML5/section_15/15_consortium_agreements htm
*****

Select Agents
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13 b (http //www selectagents gov/Regulations html)

Highly Pathogenic Agent
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http //www cdc gov/OD/ohs/biosfty/bmbl5/bmbl5toc htm) Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used
When submitting future Progress Reports indicate at the beginning of the report

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide

o A list of the new and/or additional Agent(s) that will be studied,
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment,
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official It is important to note if the work is being done in a new location

*****

The budget period anniversary start date for future year(s) will be **June** 1

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions The Program Official is responsible for the scientific, programmatic and technical aspects of this project These individuals work together in overall project administration Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist Requests may be made via e-mail

Grants Management Specialist Devon R Bumbray-quarles
Email dbumbray@niaid nih gov **Phone** 240-669-2950 **Fax** 301-493-0597

Program Official: Erik J Stemmy
Email erik stemmy@nih gov **Phone** 240-627-3380

SPREADSHEET SUMMARY
GRANT NUMBER: 2R01AI089728-06

INSTITUTION: UNIVERSITY OF MINNESOTA

Budget	Year 6	Year 7	Year 8	Year 9	Year 10
Salaries and Wages	$116,853	$116,853	$116,853	$116,853	$116,853
Fringe Benefits	$30,547	$30,547	$30,547	$30,547	$30,547
Materials & Supplies	$60,005	$60,005	$60,005	$60,005	$60,005
Travel	$2,970	$2,970	$2,970	$2,970	$2,970
Other	$5,247	$5,247	$5,247	$5,247	$5,247
Subawards/Consortium/Contractual Costs	$120,384	$120,384	$120,384	$120,384	$120,384
Publication Costs	$2,178	$2,178	$2,178	$2,178	$2,178
TOTAL FEDERAL DC	$338,184	$338,184	$338,184	$338,184	$338,184
TOTAL FEDERAL F&A	$126,256	$115,253	$117,431	$117,612	$117,612
TOTAL COST	$464,440	$453,437	$455,615	$455,796	$455,796

Facilities and Administrative Costs	Year 6	Year 7	Year 8	Year 9	Year 10
F&A Cost Rate 1	52%	52%	53%	54%	54%
F&A Cost Base 1	$242,800	$18,150	$18,150	$217,800	$217,800
F&A Costs 1	$126,256	$9,438	$9,620	$117,612	$117,612
F&A Cost Rate 2		53%	54%		
F&A Cost Base 2		$199,650	$199,650		
F&A Costs 2		$105,815	$107,811		

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Institute of Allergy
and Infectious Diseases
Bethesda, Maryland 20892

May 18, 2016

Mr Kevin McKoskey
Director, Sponsored Projects
University of Minnesota
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 55455-2070

RE. 2 R01AI089728-06

Dear Mr McKoskey:

Thank you for your correspondence of April 15, 2016, regarding the October 17, 2014 White House announcement of a U S. Government-wide pause on certain gain-of-function (GoF) experiments and its potential impact on your research (http //www whitehouse.gov/blog/2014/10/17/doing-diligence-assess-risks-and-benefits-life-sciences-gain-function-research). The research funding pause pertains to GoF research projects that may be reasonably anticipated to confer attributes to Influenza, MERS, or SARS viruses such that the resulting virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route

NIAID reviewed the original grant application, and the additional information provided by you, and made the following assessments regarding Subaim 2 1 of the above-referenced grant:

- NIAID is in agreement that the work you proposed under Experiments 1, 2, and 3 is not subject to the GoF research funding pause This determination is based on the fact that the work will be carried out using either recombinant proteins or replication-deficient pseudoviruses, and will not involve the generation of a replicating SARS-CoV variant with enhanced pathogenicity and/or transmissibility via the respiratory route.
- NIAID's determination is that the work proposed under Experiment 4 to generate SARS-Like viruses with enhanced affinity for human receptors (via both reverse genetics and serial passaging) is subject to the GoF research funding pause, and therefore may not be conducted under this grant. Given the lack of empiric evidence that increased receptor binding alone is insufficient to increase pathogenicity, NIAID has determined that the proposed work is reasonably anticipated to result in a SARS-Like virus with enhanced pathogenicity. NIAID acknowledges that in lieu of generating SARS-Like viruses with enhanced affinity for human receptors you will pursue alternative strategies using *loss-of-function* mutations, or mutations targeting the ACE2 receptor. NIAID also acknowledges your statement that if any mutant

viruses you generate demonstrate either enhanced virus growth >1 log compared to the wildtype parental backbone strain or more efficient growth in primary human airway epithelial cells, you will immediately stop all experiments with the mutant and notify NIAID and the IBCs at the University of Minnesota and the University of North Carolina at Chapel Hill of these results

Please remember that the institution must comply in full with all terms and conditions placed on this grant. If your research evolves to include experiments that may be subject to the GoF research funding pause or you observe enhanced pathogenicity and/or transmissibility of SARS or SARS-Like viruses in mammals via the respiratory route at any time during the course of conducting these experiments, you must immediately stop these research activities and provide the NIAID Program Officer and Grants Management Specialist with the relevant data and information related to these unanticipated outcomes

As indicated above, NIAID determinations are based on information from multiple sources, but primarily on our communication with you about the details of your proposed experiments and your research results Should NIAID's determination change based on information obtained through the U S Government GoF deliberative process, described here http://www.phe.gov/s3/dualuse/Documents/gain-of-function.pdf, you will be notified, however, until such time, or until the GoF research funding pause is lifted, NIAID's determination, indicated above, is final

Please let us know if you have any questions, or if you require additional information

Sincerely,

Devon Bumbray-Quarles
Grants Management Specialist
NIAID/NIH/DHHS

Erik J. Stemmy, Ph D.
Program Officer
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS

CC. Dr. Fang Li
Ms Erin Knudsen
Ms. Mary Kirker
Dr Irene Glowinski
Dr Andrew Ford
Dr Ralph Baric

BUDGET JUSTIFICATION – BARIC UNC

PERSONNEL ($48,514):

Ralph S. Baric, PhD, Consortium PI (0 36 calendar months, 3% effort) Dr Baric is an expert in human emerging coronavirus (SARS-CoV/MERS-CoV) replication, cross species transmission, genetics and pathogenesis and his group is well positioned to conduct the studies proposed in the application His group has developed molecular clones to a large number of human and animal coronaviruses, including the strains germane to this proposal Dr Baric will work closely with Drs Li to conduct the basic research on virus-receptor interactions, protease usage and entry Within his own group, he will interact closely with Dr Kocher and Mr Scobey to ensure steady progress during the course of the proposal, evaluate results and propose alternative experiments Dr Baric will communicate his group's findings, share data, discuss and solve problems by interacting with the rest of the scientific team at monthly meetings and on at an ad hoc basis for specialized topics that are in need immediate solutions Drs Baric and Li have worked together over the past year, published papers on MERS-CoV RBD structure and function and in studying coronavirus cross species transmission Dr Baric and the members of his group will attend yearly meetings to discuss the progress of the project

Dr Baric's salary reflects the current NIH salary cap of $183,300 The remainder of his salary will be supported by other institutional funds

Jacob Kocher, PhD, Postdoctoral Research Associate (4 8 calendar months, 40% effort) Dr Kocher will assist in the generation of recombinant viruses including panels of mutants with defects in receptor binding and/or protease processing He has considerable expertise in working in a BSL3 facility, has considerable experience working with human and animal coronaviruses, appropriate cell culture systems and entry based assays He will work closely with Dr Baric, help direct and follow the progress of Mr Scobey to coordinate recombinant virus production, phenotypic characterization, sample collection and data interpretation He will assist in the experimental workflow He will attend lab meetings, present data and present findings/write papers, and participate in group meetings

Mr. Drew Scobey, Research Technician (4 8 calendar months, 40% effort) Mr Scobey is certified to work with high containment pathogens under BSL3 and has considerable experience in working with coronavirus under BSL2 conditions as well He is expert in recombinant virus recover, virus enumeration, cell fractionation, FACs based analyses, safety testing of samples, and in the preparation of RNA and protein samples He developed the MERS-CoV molecular clone in our laboratory Thus, he is well versed in molecular biology and virus reverse genetics and has isolated and characterized a large number of coronavirus mutants He will also assist and help Dr Kocher in the genetic and phenotypic characterization of mutants Finally, he will ensure that the BSL3 and BSL2 laboratory is well stocked and compliant in terms of regulations and day to day maintenance

Fringe Benefits Faculty/Staff 22 741% Social Security and Retirement, $5,471/FTE Health Insurance Post-doctoral Research Associates 8 99% Social Security and benefits, $4,373/FTE Health Insurance

EQUIPMENT: None

SUPPLIES ($23,486):

Synthetic DNAs and Sequencing Costs **($4,000/year)** The Baric lab routinely uses synthetic genomics for the creation of virus mutants potentially used in validation studies, as it is cheaper and faster than using standard recombinant DNA approaches (considering personnel time, resequencing costs, time, materials, etc) Synthetic DNAs will be used to build new virologic reagents for measuring the impact of target mutations on receptor interactions, protease processing and entry Synthetic DNAs are primarily purchased from small biotec companies like Blue Heron or Bio Basic Inc at costs of about 0 30$/base, sufficient for our needs over the course of this project We will also require funds for sequence validation of recombinant viruses

Serum and Media ($5,500/year). A large amount of cell culture work is associated with the project, requiring media, serum and culturing flasks This includes virus tittering and neutralization assays Consequently, funds are requested for cell culture supplies to maintain Vero and related permissive cells for each coronavirus under study, and for measuring virus growth kinetics, evaluate entry kinetics and virus viability, titer virus stocks, and to isolate recombinant viruses for in vitro characterization or other phenotypes These monies support cell culture work in BSL2 and BSL3 facilities

Personnel Protective Gear ($3,000/year) PAPRs, tyvek suits and other protective gear Personnel wear powered air purifying HEPA filtered breathing apparatuses, wear tyvek suits, tyvek aprons, hoods, booties and are double gloved when entering the BSL3 facility These materials are expensive as the HEPA, organic chemical filters and even batteries must be replaced every ~6-12 months, and the tyvek suits are disposable Moreover, the PAPRs (powered air breathing apparatus) are expensive and must be replaced every ~2 years from normal wear and tear, and daily contact with EPA disinfectants Personnel also use high quantities of EPA approved disinfectants like ethanol alcohol, Clorox and other EPA approved disinfectants in maintaining a safe working environment in the BSL3 Personnel spray down tyvek suits, etc with alcohol or related disinfectants in the process of deconing and leaving the BSL3 facility All materials that leave the BSL3 must be disinfected, packaged in disinfected, sealed containers which are disinfected prior to removal from the BSL3 facility

Consumables ($4,500/year). A large amount of plastic ware will be consumed during the course of this project including eppendorf tubes, flasks, plates, pipettes, pipette tips, etc This includes plastic ware for virus growth kinetics, plaque assays, TCID50 quantization's, etc used to evaluate and compare virus growth efficiency and to evaluate neutralization titers following vaccination Small quantities of chemicals will be purchased, including Brefeldin-A and formalin for fixing cells

Enzymes, Kits and Reagents ($2,800/year) Oligodeoxynucleotide synthesis and kits for quantitative RT-PCR for measuring concentrations of viral and discrete cellular mRNAs will be used during the course of the project A variety of reagents needed for tissue fixation and immunohistochemistry A variety of chemicals will be purchased during the course of the project that will be used for making buffers, reagents, and other solutions These costs include reagents (e g , plasticware/EIA kits, etc) for EIA analyses A variety of antibodies and immunological reagents will be used during the course of the proposal to characterize S topology in the context of recombinant viruses

Miscellaneous ($2,186/yr). Funds are requested to purchase chemicals, reagents, paper products, gloves, micropipetors, autoclave supplies, plastic tips, waterbaths, and other small equipment items that typically have short half lives in laboratory settings

Computers ($1,500/year). Funds are requested to provide NoV specific computer hardware and software updates required over the course of the project Statistical data analysis and modeling of data require up to date equipment and software

TRAVEL ($3,000/year)

Scientific Meetings Travel funds are requested for the PI and fellow to attend the scientific meeting each year, such as ASV, during the course of the proposal and visit University of Minnesota This allows program faculty to communicate results, develop collaborations and share research interests

OTHER EXPENSES ($5,000)

Maintenance and Service Contracts ($3,000/year). Autoclave service contract and BSL3 maintenance costs for decontaminating/maintaining the facility are requested for each year of the program These dollars are critical for maintaining a safe environment for BSL3 research and for maintenance contracts for the BSL-2 equipment, like the autoclave in the BSL3

Publication Costs ($2,000/year). Funds are requested for publication of manuscripts associated with this research

F&A In an agreement with DHHS dated 5/16/2012 the indirect cost rate for The University of North Carolina is 52% of MTDC, excluding equipment and tuition

PS / SPONSOR PROPOSAL BUDGET WORKSHEET

6/7/16-5/31/17

Please complete the cost share and F&A distribution information at the bottom of the page, if applicable

Award R01AI089728-06 Project #, if known 0 Should this be the Primary Project? X Yes ___ No

Project DeptID 11718 Project PI Li, Fang **PRF#835469**

LINK TO CHART OF ACCOUNT **Includes new FY15 personnel budget accounts**

ITEM		Sponsor Funding	Cost Share or Match*	TOTAL PROJECT BUDGET
Personnel				
Salary				
700100	Salary - Faculty	32,033		
700200	Salary - Professional/Admin/Police	0		
700210	Salary - Post Doc/Research Spc	50,000		
700300	Salary - Grad Research Asst (with tuition)	0		
700310	Salary - Grad Research Asst (no tuition)			
700320	Salary - Salary - Residents/Fellows			
700400	Salary - Undergrad Student	0		
700500	Salary - Civil Service	35,000		
700510	Salary - Labor Represented			
700520	Salary - Trades			
700530	Salary - Temp/Casual			
Total Salary		117,033	0	117,033
Fringe				
710100	Fringe - Faculty	10,795		
710200	Fringe - Professional/Admin/Police	0		
710210	Fringe - Post Doc/Research Spc	11,200		
710300	Fringe - Grad Research Asst (with tuition)	0		
710310	Fringe - Grad Research Asst (no tuition)			
710320	Fringe - Residents/Fellows			
710400	Fringe - Undergrad Student			
710500	Fringe - Civil Service	9,590		
710510	Fringe - Labor Represented			
710520	Fringe - Trades			
710530	Fringe - Temp/Casual			
710600	Fringe - Direct Charge	31,585	0	31,585
Total Fringe		148,618	0	148,618
Total Personnel				
Consulting / Professional Services				
730100	Consulting Services			
730200	Professional Services	0	0	0
Total Consulting / Professional Services				
Equipment				
850100	Equipment	5,000		5,000
Supplies				
720100	General Operating Supplies	50,566		
720200	Lab & Medical Supplies	5,000		
750100	Non-Capitalized Equipment			
Total Supplies		55,566	0	55,566
Travel				
720600	Travel / Mileage / Moving	3,000		3,000
Other Direct Costs				
720300	General Operating Services	2,000		
720400	Lab & Medical Services	2,500		
720500	Telecommunications	300		
770100	Long Term Rents & Leases			
770150	Long Term Rents & Leases (bldg/facility)			
770200	Short Term Rents & Leases			
770250	Short Term Rents & Leases (bldg/facility)			
780100	Repairs & Maintenance	2,500		
810100	Research Subject Payments			
810200	Participant Payments			
Total Other Direct Costs		7,300	0	7,300
Subawards / Subcontracts				
810300	Subcontracts not subject to F&A	95,384		
810400	Subcontracts subject to F&A	25,000		
Total Subawards / Subcontracts		120,384	0	120,384
Additional Accounts (show budget account and a description, please)				
___	___			0
___	___			0
___	___			0
TOTAL DIRECT COSTS		339,868	0	339,868
810500	Facilities and Administrative Expenses	124,532		124,532
TOTAL COSTS		464,400	0	464,400

Principal Investigator/Program Director (Last,first, middle) Li, Fang

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 07/01/2016 THROUGH 06/30/2017

PERSONNEL (Applicant organization only) NAME	ROLE ON PROJECT	Months Devoted to Project: Cal Mnths	Acad Mnths	Sum Mnths	INST BASE SALARY	DOLLAR AMOUNT REQUESTED (omit cents): SALARY REQUESTED	FRINGE BENEFITS	TOTAL	
Li, Fang	Prin Investigator	3 00	0 00	0 00	128,135	32,033	10,795	42,828	33.7
TO BE NAMED	Post Doc Researcher	12 00	0 00	0 00	40,000	40,000	8,959	48,959	22.4
TO BE NAMED	Post Doc Researcher	9 00	0 00	0 00	40,000	30,000	6,719	36,719	22.4
TO BE NAMED	Jr Scientist	6 00	0 00	0 00	32,000	16,000	4,383	20,383	27.4
SUBTOTALS →						118,033	30,856	148,889	✓
CONSULTANT COSTS								0	
EQUIPMENT (Itemize)								0	
SUPPLIES (Itemize by category) Molecular biology supplies ($15,000), Cell culture supplies ($11,000), Protein purification supplies ($11,000), Protein crystallization supplies ($15,000), Plastic & glassware supplies ($8,611)								60,611	✓
TRAVEL PI and post doc travel to meetings to present results ($3,000)								3,000	✓
PATIENT CARE COSTS	INPATIENT							0	
	OUTPATIENT							0	
ALTERATIONS AND RENOVATIONS (Itemize by category)								0	
OTHER EXPENSES (Itemize by category) Publication and reprints ($2,200)✓, Equipment repair & maintenance services ($2,500)✓, Lab services ($2,500)✓, Communications ($300)✓								7,500	
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS							80,000	✓
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD (Item 7a, Face Page)								$ 300,000	
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATION COSTS							41,600	✓
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD								$ 341,600	

Form Page 4

245,000 IDC base 53% 127,400

469,000

OMB No. 0925-0001

Form Approved Through 8/31/2015

Department of Health and Human Services Public Health Services

SUBAWARD Grant Application

Do not exceed character length restrictions indicated

LEAVE BLANK—FOR PHS USE ONLY		
Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1 TITLE OF PROJECT *(Do not exceed 81 characters, including spaces and punctuation.)*

Receptor recognition and cell entry of coronaviruses

2 RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☒ NO ☐ YES

(If "Yes," state number and title)

Number Title

3 SUBAWARD PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR

3a NAME (Last, first, middle)	3b DEGREE(S)	3h eRA Commons User Name
Baric, Ralph	PhD	ralph_baric

3c POSITION TITLE
Professor

3d SUBAWARD MAILING ADDRESS *(Street, city, state, zip code)*
Department of Epidemiology
University of North Carolina – Chapel Hill
CB# 7435, McGavran-Greenberg Hall
Chapel Hill, NC 27599-7435

3e DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Epidemiology/Microbiology/Immunology

3f MAJOR SUBDIVISION

3g TELEPHONE AND FAX *(Area code, number and extension)*
TEL 919-966-3895 FAX 919-966-2089

E-MAIL ADDRESS
rbaric@email.unc.edu

4 SUBAWARD HUMAN SUBJECTS RESEARCH ☒ No ☐ Yes

4a Research Exempt ☐ No ☐ Yes If "Yes," Exemption No

4b Federal-Wide Assurance No

4c Clinical Trial ☐ No ☐ Yes

4d NIH-defined Phase III Clinical Trial ☐ No ☐ Yes

5 SUBAWARD VERTEBRATE ANIMALS ☒ No ☐ Yes

5a Animal Welfare Assurance No

6 SUBAWARD DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*

From: 07/01/2016 Through: 06/30/2021

7 SUBAWARD COSTS REQUESTED FOR INITIAL BUDGET PERIOD

7a Direct Costs ($): 80000

7b Total Costs ($): 121600

8 SUBAWARD COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT

8a Direct Costs ($): 400000

8b Total Costs ($): 608000

9 SUBAWARD APPLICANT ORGANIZATION

Name: The University of North Carolina at Chapel Hill

Address: Office of Sponsored Research (OSR)
Administrative Office Bldg., Suite 2200
104 Airport Drive, CB# 1350
Chapel Hill, NC 27599-1350

10 SUBAWARD TYPE OF ORGANIZATION

Public → ☐ Federal ☒ State ☐ Local

Private → ☐ Private Nonprofit

For-profit → ☐ General ☐ Small Business

☐ Woman-owned ☐ Socially and Economically Disadvantaged

11 SUBAWARD ENTITY IDENTIFICATION NUMBER
1566001393A1

DUNS NO: 608195277 Cong. District: NC-004

12 SUBAWARD ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE

Name: Robin Cyr

Title: Director, Office of Sponsored Research

Address: Office of Sponsored Research (OSR)
Administrative Office Bldg., Suite 2200
104 Airport Drive, CB# 1350
Chapel Hill, NC 27599-1350

Tel: 919-966-3411 FAX: 919-962-3352

E-Mail: resadminosr@unc.edu

13 SUBAWARD OFFICIAL SIGNING FOR APPLICANT ORGANIZATION

Name: Barbara Entwisle, PhD

Title: Vice Chancellor for Research

Address: Office of Sponsored Research (OSR)
Administrative Office Bldg., Suite 2200
104 Airport Drive, CB# 1350
Chapel Hill, NC 27599-1350

Tel: 919-966-3411 FAX: 919-962-3352

E-Mail: resadminosr@unc.edu

14 APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE. I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF SUBAWARD OFFICIAL NAMED IN 13 *(In ink. Per signature not acceptable.)*

Carol J. Burkhart

DATE: 10/22/15

Barbara Entwisle
Vice Chancellor for Research

Face Page

Form Page 1

PHS 398 (Rev. 09/12)

Updated 10/06/2015

Office of Sponsored Research Information Sheet

General Information	The University of North Carolina at Chapel Hill
Official Legal Name	
Institutional Official	Barbara Entwisle Vice Chancellor for Research Office of Sponsored Research (OSR) 104 Airport Dr Ste 2200, CB #1350 Chapel Hill, NC 27599-1350 Phone - (919) 966-3411/3412 Fax - (919) 962-3352/5011
Administrative Official (Contract & Grant Administrator)	Robin Cyr Associate Vice Chancellor for Research Director, Office of Sponsored Research Office of Sponsored Research (OSR) 104 Airport Dr Ste 2200, CB #1350 Chapel Hill, NC 27599-1350 Phone - (919) 966-3411 Fax - (919) 962-3352/5011 Email - resadminosr@unc edu
Financial Officer	Vanessa Peoples Deputy Director, Office of Sponsored Research 104 Airport Dr Ste 2200, CB #1350 Chapel Hill, NC 27599-1350 Phone - (919) 966-3411/3412 Fax - (919) 962-3352/5011
Email	resadminosr@unc edu

Frequently Used Information for Sponsored Research		
NIH/PHS Animal Welfare Assurance Number:	A3410-01 (Expires 4/30/2017)	
Congressional District:	4th	
Dun & Bradstreet (DUNS) #	608195277	
Federal Tax (EIN) ID#	56-600-1393	
For Public Health Service (e g. NIH, HRSA, CDC, etc) use Entity Identification Number	1566001393A-1	
SAM (System for Award Management)	Expiration Date 07/26/2016	
Fiscal Year	July 1 to June 30	
Human Subjects DHHS #	FWA-4801	expires 6/30/2016
Institutional Profile No:	578206	
Mileage rate for private car	57 5 cents/mile	01/01/2015
NIH Salary Cap	$181,500	Effective 01/12/2014
	$183,300	Effective 01/11/2015
NSF Submitting Institutional Code	0029744000	
Responsible Conduct in Research	https //research unc edu/files/2012/11/research_conduct pocket_guide pdf	
Type of Organization	State Related Institution of Higher Education	

Miscellaneous Information for Sponsored Research	
AAALAC International Approval	#329 (date of accreditation 10/31/2014) https //research unc edu/files/2015/01/AAALAC-Approval-Letter-dated-103114 pdf
Age Discrimination Policy	On file, signed 9/6/01
Assurance of Compliance Title IX: (HHS 639-A)	On file, signed 9/27/76
Board of Trustees member list	http //www unc edu/depts/trustees/
Civil Rights Assurance (HHS 441) Reg. #	43-0520 Dated 1/11/65
Certification of Liability Coverage	Dated 7/1/2014 – 6/30/2015 http //research unc edu/files/2012/11/UNCCH_Certificate OfCoverage_2014-2015 pdf
Cognizant Federal Agency for F&A Rate Agreement (For purposes of completing grant proposals, use this office's address on any applicable forms asking for info on F&A)	Department of Health and Human Services Division of Cost Allocation (202) 401-2808 Attn Darryl Mayes
Commercial and Government Entity Code (CAGE)	4B856
Conflict of Interest Info: **Individual Conflicts of Interest & Commitment** **Institutional Conflict of Interest**	http //policy sites unc edu/files/2013/04/Individual-COI-Policy pdf http //policy sites unc edu/files/2013/04/Institutional-COI-Policy pdf

Updated 10/06/2015 — Office of Sponsored Research Information Sheet

Export Control Policy page	http //policies unc edu/policies/export-controls/
Federal Interagency Committee on Education (FICE)	002974-00
Misconduct in Science Form PHS-6349	Dated 01/31/2014 Annual update
NAICS Code	611310
Number of UNC-CH Employees	11,964 (as of Fall 2014 - includes permanent full time and part time) http //oira unc edu/facts-and-figures/faculty-and-staff-data/employees-by-category-fall-2014/
Radioisotopes: NC License Number	068-214-1
Rehab. Act of 1973 (HHS 641)	On file, signed 6/22/77
Sex Discrimination Policy	On file, signed 9/6/01
Southern Association of Colleges and Schools Accreditation	Dated January 9, 2007 http //research unc edu/files/2013/03/CCM1_031561 pdf
Standard Industrial Code	8221
Student/Fellowship/Postdoctoral Link	http //research unc edu/offices/sponsored-research/resources/DATA_RES_OSR_STUDENTINFO
UNC-CH Institutional Info (Fact Book)	http //oira unc edu/facts-and-figures/

Institutional Financial Information

Include the UNC-CH account number and invoice number on all payments.
These addresses are for payments only. All other correspondence should be sent to:
Office of Sponsored Research at 104 Airport Drive, Suite 2200, Chapel Hill, NC 27599-1350

REMITTANCE ADDRESS (U S MAIL ONLY)
University of North Carolina at Chapel Hill
Office of Sponsored Research
C/O Bank of America Lock Box Service
P O Box 402420
Atlanta, GA 30384-2420

COURIER ONLY:
Bank of Amenca Lock Box Services
Lock Box 402420
6000 Feldwood Road
College Park, GA 30349

Institutional Banking Information	Contact Vanessa Peoples at vpeoples@email unc edu,(919) 962-3397
UNC-CH Single Audit web site (A-133)	http //www ncauditor net/EPSWeb/Reports/Financial/FSA-2013-8730 pdf
UNC-CH Compliance Audit Procedures	http //www ncauditor net/EPSWeb/Reports/Financial/FSA-2013-6020 pdf
UNC-CH Financial Reports	http //finance unc edu/reports-and-data/financial-statements-archive/
IRS 501-c (3) Tax Exemption	http //research unc edu/files/2013/03/CCM1_031605 pdf

Facilities and Administrative Cost Rates (F&A)

DHHS Agreement Date: May 16, 2012

Rates Effective: 7/1/12-6/30/16	**On-Campus**	**Off-Campus* (Adjacent - within a 10-mile radius)**	**Off-Campus* (Remote)**
Organized Research	52 00%	28 00%	26 00%
Instruction	50 00%	28 00%	26 00%
Other Sponsored Activities	36 00%	28 00%	26 00%
Clinical Trials (Federal)*	52 00%	28 00%	26 00%
Clinical Trials (non Federal)**	28 00%	28 00%	26 00%

* See OSR FAQs for an explanation of how to determine whether a sponsored research project is on or off campus http //research unc edu/offices/sponsored-research/faq/index htm

** F&A calculations for federally funded Clinical Trials are based on MTDC (see definition below)

*** F&A calculations for INDUSTRY funded Clinical Trials are based on TOTAL DIRECT COSTS with the exception of the Initial IRB review fee, which is excluded from F&A

F&A rates online	http //research unc edu/offices/sponsored-research/resources/DATA_RES_OSR_INFOSHEET#facilities

BASE: Modified total direct costs (MTDC), consisting of all salaries and wages, fringe benefits, materials and supplies, services, travel and sub grants and subcontracts up to $25,000 of each sub grant or subcontract (regardless of the period covered by the sub grant or subcontract) Modified total direct costs shall exclude equipment[1], capital expenditures, charges for patient care and tuition remission, long term rental costs of off-site facilities, scholarships, and fellowships as well as the portion of each sub grant and subcontract in excess of $25,000 (Reminder Mandatory in-state tuition for eligible GRAs on research grants must be excluded from the F&A cost base for each budget year) Note *Equipment' is defined as an item costing $5,000 or greater*

Current F&A agreement:	http //research unc edu/files/2012/11/CCM3_036953 pdf

Fringe Benefits

http //research unc edu/offices/sponsored-research/resources/DATA_RES_OSR_INFOSHEET#fringe2

	Budget	
For full-time permanent EPA/SPA employees	22.741%	
Post Doc Appointees and Grad Students (Depends on type of Award)	8 990%	
PLUS Health Insurance annual premium prorated to % of effort		
	Monthly	**Annually**
Full-time Permanent EPA/SPA employees (ConnectCarolina Acct 515530) (e)	**$455.92**	**$5,471.00**
Post Doctoral Trainees (ConnectCarolina Acct 528150) (a)	**$364.44**	**$4,373.28**
Post Doctoral Research Associates (ConnectCarolina Acct 515510)(a)	**$364.44**	**$4,373.28**
Grad Student Health Insurance (ConnectCarolina Accts 515540, 569200)(a)(b)(c)(d)	**$269.50**	**$3,234.00**

(a) Post Doctoral rates include Student Health fee, but the Graduate Student rate does not	http //financepolicy unc edu/1111_1rd
(b) See Business Manual for Student FICA Tax Exemption Policy	http //finance unc edu/news/ddd-graduate-student-health-insurance-program-enrollment-for-2015-2016-and-other-issues/
(d) For information on Graduate Student Health Insurance eligibility	
(e) For information on North Carolina Department of Health and Human Services (DHHS) approved fringe benefit rates	http //research unc edu/files/2014/09/FY-2016-UNC-CH-DHHS-approved-rates pdf

UNC Physicians & Associates (P&A) Supplemental Fringe Benefits

Allowable Benefit	**Annual Rate**	**Unallowable Benefit**
Employee Life Insurance	0 6240%	Dependent Life Insurance
Employee Accidental Death & Dismemberment	0 1152%	Dependent Accidental Death & Dismemberment
Employee Long Term Disability	0 8400%	Professional Liability Insurance
Employee Retirement	3 5000%	
Employee Medical Insurance	$1331 52	Dependent Medical Insurance
Employee Dental Insurance	$600 36	Dependent Dental Insurance
Employee Vision Insurance	$95 28	Dependent Vision Insurance

Note As a general guideline budget 27 831% (22 741% + 5 09%) of annual salary plus $7,498 16 ($5,471 00 + $2,027 16) prorated to employee % effort

Process checklist:

Grant Administrator

FCOI: FCOI Job Aid

- Add subrecipient in EFS and complete information in FCOI hyperlink
  - If Subrecipient policy: Check "subrecipient policy" box and enter dates of project.
  - If UMN policy, Check "UMN policy" and enter dates of project.
    - Enter ALL subrecipient Key Personnel, Mark if positive or negative disclosure and Disclosure date (date Form 2 signed)
    - Verify training is complete (must be renewed every 4 years)

EMAIL PI/DEPARTMENT FOR SUBAWARD INFORMATION – sample template for PI request

FFATA: Not applicable since prime award is not subject to FFATA.

Subaward Compliance Officer

RISK ANALYSIS: Risk Analysis Job Aid

- Complete Risk Analysis Form

Grant Administrator

PREPARE SUBAWARD

- Fill out Subaward Information Sheet
- Fill out Balance Sheet
- Fill out appropriate template
- Send subaward (NOT PRESIGNED) to subrecipient for signature
- Return fully executed copy to:
  - Subrecipient
  - Subaward Compliance Officer
  - PI
  - Department
- Set up PO PO Setup Flow/PO Express Job Aid
- Print copy of PO for file
- Provide copy of subaward and PO to Imagenow staff (for coordinating campus send complete subaward file to Nancy Benson, SPA)
- File Subaward

Subaward Balance Sheet

1 - Line PO

☐ Not Subject to F&A 8103_ _

☐ Subject to F&A 8104_ _

Purchase Order ID: N00540280l **End Date:** 5/31/19

Old Purchase Order Number:

Date	Action (Circle One)	Voucher ID / Invoice Number	$ Amount +/-	Balance	Initials
To check the converted total, find Amount Ordered at Purchasing/PO/Rev PO Info/Activity Sum, and enter the amount in the box to the right:				54,292.01	KG
4/23/19	PO/Voucher/Mod	Voucher: 05d6099 Invoice: GM00146919	-7,305.78	46,986.23	KG
5/20/19	PO/Voucher/Mod	Voucher: 05d60929 Invoice: GM00147944	-5,878.65	41,107.58	KG
7/11/19	PO/Voucher/Mod	Voucher: Invoice: Amd 3	+120,384.00	161,491.58	KG
7/15/19	PO/Voucher/Mod	Voucher: 05745303 Invoice: GM00149489	-32,273.41	129,218.17	KG
9/17/19	PO/Voucher/Mod	Voucher: 05853906 Invoice: GM00152069	-16,402.92	112,815,25	KG
10/4/19	PO/Voucher/Mod	Voucher: 05904082 Invoice: GM00152878	-10,884.05	101,931.20	KG
11/13/19	PO/Voucher/Mod	Voucher: 05960044 Invoice: GM00153986	-5,528.03	96,403.17	KG
12/9/19	PO/Voucher/Mod	Voucher: 06019613 Invoice: GM00157744	-4,162.53	92,240.64	KG
1/15/20	PO/Voucher/Mod	Voucher: 06053402 Invoice: GM00155588	-4,955.68	87,284.96	KG
2/17/20	PO/Voucher/Mod	Voucher: 06105497 Invoice: GM00158043	-17,083.35	70,201.61	KG
	PO/Voucher/Mod	Voucher: Invoice:			
	PO/Voucher/Mod	Voucher: Invoice:			
	PO/Voucher/Mod	Voucher: Invoice:			
	PO/Voucher/Mod	Voucher: Invoice:			
	PO/Voucher/Mod	Voucher: Invoice:			
	PO/Voucher/Mod	Voucher: Invoice:			
	PO/Voucher/Mod	Voucher: Invoice:			

Comments: Use project 00066245 for Payment

Subaward Balance Sheet

1 - Line PO

☐ Not Subject to F&A 810301

☐ Subject to F&A 8104__ --

Purchase Order ID: N005402801 **End Date:** 5/31/2018

Old Purchase Order Number:

Date	Action (Circle One)	Voucher ID / Invoice Number	$ Amount +/-	Balance	Initials
To check the converted total, find Amount Ordered at Purchasing/PO/Rev PO Info/Activity Sum, and enter the amount in the box to the right:				120,389.41	AC
12/4/17	PO/Voucher/Mod	Voucher: 04992784 Invoice: GM00133033	-23,919.28	96,470.13	KJ
12/4/17	PO/Voucher/Mod	Voucher: 04912792 Invoice: GM00132495	-8,580.48	87,889.65	KJ
12/12/17	PO/Voucher/Mod	Voucher: 04923427 Invoice: GM00131386	-6,738.34	81,151.31	KJ
2/5/18	PO/Voucher/Mod	Voucher: 04975156 Invoice: GM00135295	-5,667.72	75,483.59	KJ
3/7/18	PO/Voucher/Mod	Voucher: 05022920 Invoice: GM00136055	-5,710.10	69,773.49	KJ
3/29/18	PO/Voucher/Mod	Voucher: 05052703 Invoice: GM00136726	-5,710.10	64,063.40	KJ
5/2/18	PO/Voucher/Mod	Voucher: 05102123 Invoice: GM00137964	-23,764.81	40,298.59	KJ
5/14/18	PO/Voucher/Mod	Voucher: 05119445 Invoice: GM00137837	-32,741.06	7,557.53	KJ
7/18/18	PO/Voucher/Mod	Voucher: 05209215 Invoice: GM00134649	-7,543.02	14.51	BD
9/5/18	PO/Voucher/Mod	Voucher: Invoice: Amd 2	+120,383.00	120,398.51	KJ
9/21/18	PO/Voucher/Mod	Voucher: 05306244 Invoice: GM0014245	-17,032.18	103,366.33	KJ
10/25/18	PO/Voucher/Mod	Voucher: 05358081 Invoice: GM00142038	-8,374.72	94,991.61	KJ
11/21/18	PO/Voucher/Mod	Voucher: 05399084 Invoice: GM00142806	-7,365.87	87,625.74	KJ
12/20/18	PO/Voucher/Mod	Voucher: 05438575 Invoice: GM00143641	-11,750.77	75,874.97	KJ
2/1/19	PO/Voucher/Mod	Voucher: 05491463 Invoice: GM00902501	-5,573.63	70,301.34	KJ
2/14/19	PO/Voucher/Mod	Voucher: 05510535 Invoice: GM00145176	-10,292.07	60,009.27	KJ
4/3/19	PO/Voucher/Mod	Voucher: 05563713 Invoice: GM00146247	-5,717.26	54,292.01	KJ

Comments: Use project 00046245 for payment

Subaward Balance Sheet
2 - Line PO

Not Subject to F&A 810301
Subject to F&A 810401

Purchase Order ID: N005402801 **End Date:** 05/31/2017 18

Old Purchase Order Number:

Date	Action (Circle One)	Voucher ID / Invoice Number	$ Amount +/-	Balance	Initials
To check the converted total, find Amount Ordered at Purchasing/PO/Rev PO Info/Activity Sum, and enter the amount in the box to the right:					
9/20/16	PO/Voucher/Mod	Voucher: / Invoice:	8103__ / 8104__ / Total:	8103__ 95,384 / 8104__ 25,000 / Total: 120,384	BEF
12/7/16	PO/Voucher/Mod 9/30/16	Voucher: 04391910 / Invoice: GM00122576	8103__ -14,439.65 / 8104__ -25,000 / Total: -39,439.65	8103_ 80,944.35 / 8104__ ¢ / Total: 80,944.35	JH
12/15/16	PO/Voucher/Mod OCT 16	Voucher: 04403995 / Invoice: 122986	8103__ -9010.46 / 8104__ / Total:	8103__ 71,853.89 / 8104__ / Total:	EG
12/20/16	PO/Voucher/Mod NOV	Voucher: 04410333 / Invoice: GM00123498	8103__ -6,650.08 / 8104__ / Total:	8103__ 65,203.81 / 8104__ / Total:	KJ
1/27/17	PO/Voucher/Mod DEC 16	Voucher: 04457506 / Invoice: GM00124208	8103__ -15,127.25 / 8104__ / Total:	8103__ 50,076.56 / 8104__ / Total:	KJ
2/27/17	PO/Voucher/Mod JAN 17	Voucher: 04500200 / Invoice: GM00125520	8103__ -6,921.69 / 8104__ / Total:	8103__ 43,154.87 / 8104__ / Total:	KJ
3/21/17	PO/Voucher/Mod FEB 17	Voucher: 04533000 / Invoice: GM00126142	8103__ -10,997.50 / 8104__ / Total:	8103__ 32,157.37 / 8104__ / Total:	W
4/18/17	PO/Voucher/Mod MAR	Voucher: 04572342 / Invoice: GM00126864	8103__ -9,942.50 / 8104__ / Total:	8103__ 22,214.87 / 8104__ / Total: 22,214.87	KJ
6/3/17	PO/Voucher/Mod APR.	Voucher: 04638483 / Invoice: GM00128869	8103__ -8,552.57 / 8104__ / Total:	8103__ 13,662.30 / 8104__ / Total: 13,662.30	KJ
6/20/17	PO/Voucher/Mod MAY	Voucher: [illegible] / Invoice: GM00[illegible]82	8103__ [illegible] / 8104__ / Total:	8103__ 6,317.66 / 8104__ — / Total: 6,317.66	KJ
8/9/17	PO/Voucher/Mod	Voucher: / Invoice no: Amend #1	8103__ 120384 / 8104__ / Total:	8103__ 126,761.60 / 8104__ / Total: 126,761.60	EK
8/15/17	PO/Voucher/Mod	Voucher: 04736399 / Invoice: GM00130311	8103__ -6,372.19 / 8104__ / Total:	8103__ 120,389.41 / 8104__ — / Total: 120,389.41	KJ

Comments:

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	2/12/2020 6:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	2/12/2020 2:49:18 PM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	2/13/2020 10:10:18 AM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$17083.35
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	2/12/2020 4:06:58 PM
PI Notes:	

INVOICE

The University of North Carolina at Chapel Hill

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Name of Sponsor:	University of Minnesota Tonya Knutson 450 McNamara Alumni Center 200 Oak Street SE. Minneapolis MN 55455-2070 United States	Invoice #: GM00156643 •Interim Billing ○ Final Billing	Award Amount $481,536.00
Sponsor Ref No.:	N005402801		
UNC Award No.:	A17-0239-001	Budget Period:	
Award Title:	Receptor recognition and cell entry of coronaviruses	From: 06/07/2016	To: 05/31/2020
Project ID:	5105169		

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	EXPENDITURES Current 01/01/2020 - 01/31/2020	Cumulative 01/31/2020
Salaries	$2,721.26	$132,952.38
Fringe Benefits	$913.79	$34,699.52
Supplies	$6,712.60	$99,124.65
Travel-Domestic	$0.00	$2,127.96
Freight & Expres	$0.00	$21.48
Miscellaneous Expense	$891.50	$1,946.50
Total Direct	$11,239.05	$270,872.49
Indirect Cost	$5,844.30	$140,461.92
TOTAL	$17,083.35	$411,334.41

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	Signature	Date
Colleen A Williams		02/07/2020

For questions regarding invoice, please contact Ryan Hermreck
Phone: 919/962-4688 FAX:
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	1/13/2020 6:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	1/13/2020 1:58:29 PM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	1/13/2020 3:30:17 PM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$4955.68
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	1/14/2020 2:29:31 PM
PI Notes:	

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Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: **GM00155588**

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**•Interim Billing** **○ Final Billing**	**Award Amount** **$481,536.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2020**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** Current 12/01/2019 - 12/31/2019	Cumulative 12/31/2019
Salaries	**$2,483.87**	**$130,231.12**
Fringe Benefits	**$766.19**	**$33,785.73**
Supplies	**$11.25**	**$92,412.15**
Travel-Domestic	**$0.00**	**$2,127.96**
Freight & Express	**$0.00**	**$21.48**
Miscellaneous Expense	**$0.00**	**$1,055.00**
Total Direct	**$3,260.31**	**$259,633.44**
Indirect Cost	**$1,695.37**	**$134,617.62**
TOTAL	**$4,955.68**	**$394,251.06**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Colleen A Williams		**01/10/2020**

For questions regarding invoice, please contact Ryan Hermreck
Phone: 919/962-4688 FAX:
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	12/17/2019 6:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	12/17/2019 1:35:26 PM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	12/18/2019 2:56:22 PM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$4162.53
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	12/17/2019 7:33:51 PM
PI Notes:	

INVOICE

The University of North Carolina at Chapel Hill

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PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**Invoice #:** GM00154744 •Interim Billing ○ Final Billing	**Award Amount** **$481,536.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**	**Budget Period:**	
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**From: 06/07/2016**	**To: 05/31/2020**
Project ID:	**5105169**		

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** Current 11/01/2019 - 11/30/2019	Cumulative 11/30/2019
Salaries	**$2,048.83**	**$127,747.25**
Fringe Benefits	**$689.67**	**$33,020.54**
Supplies	**$0.00**	**$92,400.90**
Travel-Domestic	**$0.00**	**$2,127.96**
Freight & Express	**$0.00**	**$21.48**
Miscellaneous Expense	**$0.00**	**$1,055.00**
Total Direct	**$2,738.50**	**$256,373.13**
Indirect Cost	**$1,424.03**	**$132,922.25**
TOTAL	**$4,162.53**	**$389,295.38**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Colleen A Williams		**12/10/2019**

For questions regarding invoice, please contact Ryan Hermreck
Phone: 919/962-4688 FAX:
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	11/11/2019 6:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	11/11/2019 2:12:43 PM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	11/13/2019 10:00:02 AM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$5528.03
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	11/12/2019 10:18:12 AM
PI Notes:	

INVOICE

The University of North Carolina at Chapel Hill

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

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PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00153936

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**•Interim Billing** **○ Final Billing**	**Award Amount** **$481,536.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2020**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	EXPENDITURES Current 10/01/2019 - 10/31/2019	Cumulative 10/31/2019
Salaries	**$2,011.21**	**$125,698.42**
Fringe Benefits	**$715.15**	**$32,330.87**
Supplies	**$910.50**	**$92,400.90**
Travel-Domestic	**$0.00**	**$2,127.96**
Freight & Express	**$0.00**	**$21.48**
Miscellaneous Expense	**$0.00**	**$1,055.00**
Total Direct	**$3,636.86**	**$253,634.63**
Indirect Cost	**$1,891.17**	**$131,498.22**
TOTAL	**$5,628.03**	**$385,132.85**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	Signature	Date
Colleen A Williams		**11/08/2019**

For questions regarding invoice, please contact Ryan Hermreck
Phone: 919/962-4688 FAX:
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	10/10/2019 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	10/10/2019 9:47:08 AM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	10/10/2019 9:54:16 AM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$10884.05
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	10/10/2019 1:15:27 PM
PI Notes:	



INVOICE

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Remit Payment To:

Office of Sponsored Research
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PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00152878

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**•Interim Billing** **o Final Billing**	**Award Amount** **$481,536.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:** **From: 06/07/2016**	**To: 05/31/2020**
Project ID:	**5105169**		

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** Current 09/01/2019 - 09/30/2019	Cumulative 09/30/2019
Salaries	**$4,822.42**	**$123,687.21**
Fringe Benefits	**$1,330.75**	**$31,615.72**
Supplies	**$516.91**	**$91,490.40**
Travel-Domestic	**$0.00**	**$2,127.96**
Freight & Express	**$0.00**	**$21.48**
Miscellaneous Expense	**$745.50**	**$1,055.00**
Total Direct	**$7,415.58**	**$249,997.77**
Indirect Cost	**$3,468.47**	**$129,607.05**
TOTAL	**$10,884.05**	**$379,604.82**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Colleen A Williams	Colleen A Williams	**10/08/2019** (RJ)

For questions regarding invoice, please contact Ryan Johnson
Phone: 919/962-4662 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	9/13/2019 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	9/13/2019 11:56:40 AM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	9/16/2019 3:30:29 PM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$16402.92
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	9/14/2019 2:00:46 PM
PI Notes:	

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00152069

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	•Interim Billing ○ Final Billing	**Award Amount** **$481,536.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:** **From: 06/07/2016**	**To: 05/31/2020**
Project ID:	**5105169**		

✓ Email: knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES**	
	Current **06/01/2019 - 08/31/2019**	**Cumulative** **08/31/2019**
Salaries	**$5,936.18**	**$118,864.79**
Fringe Benefits	**$2,128.57**	**$30,284.97**
Supplies	**$2,726.64**	**$90,973.49**
Travel-Domestic	**$0.00**	**$2,127.96**
Freight & Express	**$0.00**	**$21.48**
Miscellaneous Expense	**$0.00**	**$309.50**
Total Direct	**$10,791.39**	**$242,582.19**
Indirect Cost	**$5,611.55**	**$126,138.58**
TOTAL	**$16,402.94**	**$368,720.77**

Payment due upon receipt of invoice

****

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By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Colleen A Williams	Colleen	**09/10/2019**

For questions regarding invoice, please contact Ryan Johnson
Phone: 919/962-4662 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	7/8/2019 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	7/8/2019 2:04:46 PM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	7/10/2019 2:01:29 PM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$32273.41
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	7/8/2019 3:05:08 PM
PI Notes:	

WORKFLOWED

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00149489

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	•Interim Billing ○ Final Billing	**Award Amount** $361,152.00
Sponsor Ref No.:	N005402801		
UNC Award No.:	A17-0239-001		
Award Title:	Receptor recognition and cell entry of coronaviruses	**Budget Period:**	
Project ID:	5105169	From: 06/07/2016	To: 05/31/2019

Email: knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	EXPENDITURES Current 05/01/2019 - 05/31/2019	EXPENDITURES Cumulative 05/31/2019
Salaries	$2,546.21	$112,928.61
Fringe Benefits	$840.80	$28,156.40
Supplies	$17,839.25	$88,246.85
Travel-Domestic	$0.00	$2,127.96
Freight & Express	$0.00	$21.48
Miscellaneous Expense	$9.50	$309.50
Total Direct	$21,235.76	$231,790.80
Indirect Cost	$11,037.65	$120,527.03
TOTAL	$32,273.41	$352,317.83

Payment due upon receipt of invoice

****

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By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	Signature	Date
Colleen A Williams	Colleen A.	07/02/2019

For questions regarding invoice, please contact Ryan Johnson
Phone: 919/962-4662 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

UNIVERSITY OF MINNESOTA

Purchase Order - Change Order

CHANGE ORDER # 5 TO PO # N005402801

(Include all digits on submitted invoice)

Supplier #: 0000012074 UNIV OF NORTH CAROLINA OFFICE OF SPONSORED RESEARCH PO BOX 402420 ATLANTA GA 30384-2420	**Date** 9/20/2016	**Tax Exempt #** 8029894	
	Payment Terms Net 30	**Freight Terms** FOB: DESTINATION- PLUS FREIGHT	**Ship Via** SHIP MOST ECONOMICAL
	Requestor		**Phone**

Federal Contract Funding? No

Bill To:	**Ship To:**
SPONSORED PROJECTS ADMIN SPONSORED PROJ ADMIN-P107220001 RM450 MCNAMARA ALUMNI CTR 200 OAK ST SE MINNEAPOLIS MN 55455	See Detail Below

Supplier: Review this document thoroughly to identify changes. Refer to original order for entire order.

Terms and Conditions of original order apply.

COPY

UNIVERSITY OF MINNESOTA

Purchase Order - Change Order

CHANGE ORDER # 5 TO PO # N005402801

(Include all digits on submitted invoice)

06/07/16-05/31/17

Change Order#1
Add $120,384 for a new total of $240,768. New end date 05/31/2018

PI changed departments and new project number was established. PO now under project 00066245

Change Order 2: AMD 2 (funds added to distribution line 5) adds $120,384.00 making the cumulative award amount to $361,152.00. All other terms and conditions remain unchanged

Change Order 4: AMD 3: Funds added in the amount of $120,384.00 and period extended to 5/31/2020. All other terms and conditions remain unchanged. Automatic carryforward allowed. Funds added to project 00066245 (distribution line 7). Change order 3 corrected chartfield string from previous project number.

Line	Item / Description	Quantity	UOM	Unit Price	Extended Amt
1	receptor Recognition and Cell Entry of Coronaviruses	1.00	LOT	$481,536.00	$481,536.00

Ship To:
SPONSORED PROJ ADMIN-P107220001
RM450 MCNAMARA ALUMNI CTR
200 OAK ST SE
MINNEAPOLIS MN 55455

PO TOTAL AMOUNT	$481,536.00
PO TOTAL AMOUNT BEFORE CHANGE	$361,152.00

ABOVE PURCHASE ORDER IS ELECTRONICALLY APPROVED BY UNIVERSITY OF MINNESOTA.

COPY

UNIVERSITY OF MINNESOTA

Purchase Order - Change Order

CHANGE ORDER # 5 TO PO # N005402801

(Include all digits on submitted invoice)

Line	Sched	Dist	Amount
1	1	1	89,006.40

Fund	Deptid	Program	PC BU	Project	Act	Account	Fin EmplID	Chartfield1	Chartfield2	CS
3002	11718		UMSPR	00054028	1	810301				

Line	Sched	Dist	Amount
1	1	2	25,000.00

Fund	Deptid	Program	PC BU	Project	Act	Account	Fin EmplID	Chartfield1	Chartfield2	CS
3002	11718		UMSPR	00054028	1	810401				

Line	Sched	Dist	Amount
1	1	3	6,372.19

Fund	Deptid	Program	PC BU	Project	Act	Account	Fin EmplID	Chartfield1	Chartfield2	CS
3002	11718		UMSPR	00054028	1	810301				

Line	Sched	Dist	Amount
1	1	4	112,831.88

Fund	Deptid	Program	PC BU	Project	Act	Account	Fin EmplID	Chartfield1	Chartfield2	CS
3002	11651		UMSPR	00066245	1	810301				

Line	Sched	Dist	Amount
1	1	5	0.00

Fund	Deptid	Program	PC BU	Project	Act	Account	Fin EmplID	Chartfield1	Chartfield2	CS
3002	11718		UMSPR	00054028	1	810301				

Line	Sched	Dist	Amount
1	1	6	7,552.12

Fund	Deptid	Program	PC BU	Project	Act	Account	Fin EmplID	Chartfield1	Chartfield2	CS
3002	11651		UMSPR	00066245	1	810301				

Line	Sched	Dist	Amount
1	1	7	240,773.41

Fund	Deptid	Program	PC BU	Project	Act	Account	Fin EmplID	Chartfield1	Chartfield2	CS
3002	11651		UMSPR	00066245	1	810301				

COPY

Summary Related Documents Invoice Information Payments Voucher Attributes Error Summary

Business Unit UMN01
Voucher ID 05660929
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
CB# 3480 MOREHEAD PLANETARIUM
SCIENCE CENTER
250 E FRANKLIN ST
CHAPEL HILL, NC 27514-3618

Entry Status Postable
Match Status Matched
Approval Status Approved
Post Status Posted

Approval History

Invoice Date 05/13/2019
Invoice No GM00147944
Invoice Total 5,878.65 USD
Pay Terms Due Now
Voucher Source Online

Origin 235
Created On 05/20/2019 12:24PM
Created By MOYER012
Last Update 05/20/2019 12:27PM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Valid
Budget Misc Status Valid
***View Related** Payment Inquiry Go

Return to Search Notify Refresh Add Update/Display

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	5/15/2019 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	5/15/2019 2:31:55 PM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	5/15/2019 3:37:22 PM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$5878.65
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	5/15/2019 2:58:17 PM
PI Notes:	

WORKFLOW

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**Invoice #:** GM00147944 •Interim Billing ○ Final Billing	**Award Amount** **$361,152.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**	**Budget Period:**	
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**From: 06/07/2016**	**To: 05/31/2019**
Project ID:	**5105169**		

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** **Current** **04/01/2019 - 04/30/2019**	**Cumulative** **04/30/2019**
Salaries	**$2,957.02**	**$110,382.40**
Fringe Benefits	**$910.49**	**$27,315.60**
Supplies	**$0.00**	**$70,407.60**
Travel-Domestic	**$0.00**	**$2,127.96**
Freight & Express	**$0.00**	**$21.48**
Miscellaneous Expense	**$0.00**	**$300.00**
Total Direct	**$3,867.51**	**$210,555.04**
Indirect Cost	**$2,011.14**	**$109,489.38**
TOTAL	**$5,878.65**	**$320,044.42**

Payment due upon receipt of invoice

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By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Colleen A Williams	Colleen	**05/13/2019**

For questions regarding invoice, please contact Ryan Johnson
Phone: 919/962-4662 **FAX: 919/962-5011**
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Business Unit UMN01
Voucher ID 05616099
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
CB# 3480 MOREHEAD PLANETARIUM
SCIENCE CENTER
250 E FRANKLIN ST
CHAPEL HILL, NC 27514-3618
Entry Status Postable
Match Status Ready
Approval Status Approved
Post Status Unposted

Approval History

Invoice Date 04/12/2019
Invoice No GM00146919
Invoice Total 7,305.78 USD
Pay Terms Due Now
Voucher Source Online

Origin 235
Created On 04/23/2019 2:20PM
Created By MOYER012
Last Update 04/23/2019 2:24PM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Not Chk'd
Budget Misc Status Valid
***View Related** Payment Inquiry Go

Return to Search | Notify | Refresh | Add | Update/Display

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	4/15/2019 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	4/16/2019 8:52:23 AM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	4/16/2019 2:35:07 PM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$7305.78
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	4/22/2019 11:10:38 AM
PI Notes:	

INVOICE

The University of North Carolina at Chapel Hill

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Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**Invoice #:** GM00146919 •Interim Billing ○ Final Billing	**Award Amount** **$361,152.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**	**Budget Period:**	
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**From: 06/07/2016**	**To: 05/31/2019**
Project ID:	**5105169**		

✓ knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** Current 03/01/2019 - 03/31/2019	Cumulative 03/31/2019
Salaries	**$3,693.17**	**$107,425.38**
Fringe Benefits	**$1,113.25**	**$26,405.11**
Supplies	**$0.00**	**$70,407.60**
Travel-Domestic	**$0.00**	**$2,127.96**
Freight & Express	**$0.00**	**$21.48**
Miscellaneous Expense		**$300.00**
Total Direct	**$4,806.42**	**$206,687.53**
Indirect Cost	**$2,499.36**	**$107,478.24**
TOTAL	**$7,305.78**	**$314,165.77**

Payment due upon receipt of invoice

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By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Colleen A Williams	Colleen	**04/12/2019** (RJ)

For questions regarding invoice, please contact Ryan Johnson
Phone: 919/962-4662 **FAX: 919/962-5011**
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

Business Unit UMN01
Voucher ID 05583773
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
CB# 3480 MOREHEAD PLANETARIUM
SCIENCE CENTER
250 E FRANKLIN ST
CHAPEL HILL, NC 27514-3618
Entry Status Postable
Match Status Matched
Approval Status Approved
Post Status Posted

Approval History

Invoice Date 03/19/2019
Invoice No GM00146247
Invoice Total 5,717.26 USD
Pay Terms Due Now
Voucher Source Online

Origin 235
Created On 04/03/2019 12:32PM
Created By MOYER012
Last Update 04/03/2019 1:01PM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Valid
Budget Misc Status Valid
***View Related** Payment Inquiry Go

Return to Search Notify Refresh Add Update/Display

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	3/28/2019 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	3/28/2019 8:35:27 AM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	3/28/2019 1:42:02 PM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$5717.26
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	3/29/2019 10:30:52 AM
PI Notes:	

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**Invoice #:** **GM00146247** **eInterim Billing** **o Final Billing**	**Award Amount** **$361,152.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**	**Budget Period:**	
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**From: 06/07/2016**	**To: 05/31/2019**
Project ID:	**5105169**		

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** **Current** **02/01/2019 - 02/28/2019**	**Cumulative** **02/28/2019**
Salaries	**$2,859.53**	**$103,732.21**
Fringe Benefits	**$901.80**	**$25,291.86**
Supplies	**$0.00**	**$70,407.60**
Travel-Domestic	**$0.00**	**$2,127.96**
Freight & Express	**$0.00**	**$21.48**
Miscellaneous Expense	**$0.00**	**$300.00**
Total Direct	**$3,761.33**	**$201,881.11**
Indirect Cost	**$1,955.93**	**$104,978.88**
TOTAL	**$5,717.26**	**$306,859.99**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
David Morgan		**03/19/2019**

For questions regarding invoice, please contact Ryan Johnson
Phone: 919/962-4662 **FAX: 919/962-5011**
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Business Unit UMN01
Voucher ID 05510535
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
CB# 3480 MOREHEAD PLANETARIUM
SCIENCE CENTER
250 E FRANKLIN ST
CHAPEL HILL, NC 27514-3618
Entry Status Postable
Match Status Matched
Approval Status Approved
Post Status Posted

Approval History

Invoice Date 02/12/2019
Invoice No GM00145176
Invoice Total 10,292.07 USD
Pay Terms Due Now
Voucher Source Online

Origin 235
Created On 02/14/2019 1:58PM
Created By MOYER012
Last Update 02/14/2019 1:59PM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Valid
Budget Misc Status Valid
View Related Payment Inquiry Go

Return to Search | Notify | Refresh | Add | Update/Display

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	2/13/2019 6:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	2/14/2019 9:41:27 AM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	2/14/2019 9:54:55 AM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$10292.07
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	2/14/2019 10:31:43 AM
PI Notes:	

WORKFLOWED

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00145176

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	•Interim Billing o Final Billing	**Award Amount** $361,152.00
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2019**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	EXPENDITURES Current 01/01/2019 - 01/31/2019	Cumulative 01/31/2019
Salaries	$2,859.52	$100,872.68
Fringe Benefits	$901.70	$24,390.06
Supplies	$3,009.86	$70,407.60
Travel-Domestic	$0.00	$2,127.96
Freight & Express	$0.00	$21.48
Miscellaneous Expense	$0.00	$300.00
Total Direct	$6,771.08	$198,119.78
Indirect Cost	$3,520.99	$103,022.95
TOTAL	$10,292.07	$301,142.73

Payment due upon receipt of Invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	Signature	Date
David Morgan		02/12/2019

For questions regarding invoice, please contact Ryan Johnson
Phone: 919/962-4662 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

UNIVERSITY OF MINNESOTA FSPRD

Summary Related Documents Invoice Information Payments Voucher Attributes Error Summary

Business Unit UMN01
Voucher ID 05491463
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
CB# 3480 MOREHEAD PLANETARIUM
SCIENCE CENTER
250 E FRANKLIN ST
CHAPEL HILL NC 27514-3618

Entry Status Postable
Match Status Ready
Approval Status Approved
Post Status Unposted

Approval History

Budget Status Not Chkd
Budget Misc Status Valid
View Related Payment Inquiry Go

Invoice Date 01/24/2019
Invoice No GA005402801
Invoice Total 6,573.63 USD
Pay Terms Due Now
Voucher Source Online

Origin 235
Created On 02/01/2019 2:45PM
Created By MOYER012
Last Update 02/01/2019 2:47PM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Return to Search Notify Refresh

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	1/28/2019 6:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	1/28/2019 4:05:35 PM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	1/31/2019 3:42:26 PM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$5573.63
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	1/28/2019 5:03:48 PM
PI Notes:	

...IED

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: **GM00144597**

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**•Interim Billing** **○ Final Billing**	**Award Amount** **$361,152.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2019**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	EXPENDITURES Current 12/01/2018 - 12/31/2018	Cumulative 12/31/2018
Salaries	**$2,427.72**	**$96,013.16**
Fringe Benefits	**$684.54**	**$23,488.36**
Supplies	**$554.57**	**$67,397.74**
Travel-Domestic	**$0.00**	**$2,127.96**
Freight & Express	**$0.00**	**$21.48**
Miscellaneous Expense	**$0.00**	**$300.00**
Total Direct	**$3,666.83**	**$191,348.70**
Indirect Cost	**$1,906.80**	**$99,501.96**
TOTAL	**$5,573.63**	**$290,850.66**

Payment due upon receipt of invoice

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	Signature	Date
David Morgan		**01/24/2019**

For questions regarding invoice, please contact Ryan Johnson
Phone: 919/962-4662 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

UNIVERSITY OF MINNESOTA **FSPRD**

Business Unit UMN01
Voucher ID 05438575
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN SUSAN THOMAS
CHAPEL HILL NC 27599-7555

Entry Status Postable
Match Status Matched
Approval Status Approved
Post Status Posted

Approval History

Invoice Date 12/17/2018
Invoice No CM00143641
Invoice Total 11,750.77 USD
Pay Terms Due Now
Voucher Source Online

Origin 235
Created On 12/20/2018 1:38PM
Created By MOYER012
Last Update 12/20/2018 1:39PM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Valid
Budget Misc Status Valid
View Related Payment Inquiry

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	12/18/2018 6:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	12/19/2018 11:05:11 AM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	12/19/2018 11:06:52 AM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$11750.77
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	12/19/2018 1:48:41 PM
PI Notes:	

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**Invoice #:** GM00143641 **Interim Billing** **Final Billing**	**Award Amount** **$361,152.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**	**Budget Period:**	
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**From: 06/07/2016**	**To: 05/31/2019**
Project ID:	**5105169**		

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** **Current** **11/01/2018 - 11/30/2018**	**Cumulative** **11/30/2018**
Salaries	**$6,293.47**	**$95,585.44**
Fringe Benefits	**$1,437.25**	**$22,803.82**
Supplies	**$0.00**	**$66,843.17**
Travel-Domestic	**$0.00**	**$2,127.96**
Freight & Express	**$0.00**	**$21.48**
Miscellaneous Expense	**$0.00**	**$300.00**
Total Direct	**$7,730.72**	**$187,681.87**
Indirect Cost	**$4,020.05**	**$97,595.16**
TOTAL	**$11,750.77**	**$285,277.03**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Bettina Lampkin		**12/17/2018**

For questions regarding invoice, please contact Bowen Zhu
Phone: 469/237-5060 **FAX: 919/962-5011**
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

UNIVERSITY OF MINNESOTA **FSPRD**

Summary Related Documents Invoice Information Payments Voucher Attributes Error Summary

Business Unit UMN01
Voucher ID 05399054
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN SUSAN THOMAS
CHAPEL HILL NC 27599-7555
Entry Status Postable
Match Status Ready
Approval Status Approved
Post Status Unposted

Approval History

Invoice Date 11/15/2018
Invoice No GM00142806
Invoice Total 7,365.87 USD
Pay Terms Due Now
Voucher Source Online

Origin 235
Created On 11/21/2018 12:05PM
Created By MOYER012
Last Update 11/21/2018 12:07PM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Not Chkd
Budget Misc Status Valid
View Related Payment Inquiry

Return to Search Notify Refresh

Summary Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	11/19/2018 6:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	11/19/2018 11:32:19 AM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	11/19/2018 3:26:00 PM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$7365.87
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	11/20/2018 9:24:59 AM
PI Notes:	

WORKFLOWED

INVOICE

The University of North Carolina at Chapel Hill

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00142806

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **United States**	•Interim Billing ○ Final Billing	**Award Amount** **$361,152.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:** **From: 06/07/2016**	**To: 05/31/2019**
Project ID:	**5105169**		

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** **Current** **10/01/2018 - 10/31/2018**	**Cumulative** **10/31/2018**
Salaries	**$3,087.94**	**$89,291.97**
Fringe Benefits	**$683.89**	**$21,366.57**
Supplies	**$1,074.14**	**$66,843.17**
Travel-Domestic	**$0.00**	**$2,127.96**
Freight & Express	**$0.00**	**$21.48**
Miscellaneous Expense		**$300.00**
Total Direct	**$4,845.97**	**$179,951.15**
Indirect Cost	**$2,519.90**	**$93,575.11**
TOTAL	**$7,365.87**	**$273,526.26**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Bettina Lampkin	BL	**11/15/2018** BZ

For questions regarding invoice, please contact Bowen Zhu
Phone: 469/237-5060 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

UNIVERSITY OF MINNESOTA FSPRD

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Business Unit UMN01
Voucher ID 05358061
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN SUSAN THOMAS
CHAPEL HILL NC 27599 7555

Entry Status Postable
Match Status Ready
Approval Status Approved
Post Status Unposted

Approval Hold(s)

Invoice Date 10/15/2018
Invoice No GM00142038
Invoice Total 8,374.72 USD
Pay Terms Due Now
Voucher Source Online

Origin 235
Created On 10/25/2018 9:04AM
Created By MOYER012
Last Update 10/25/2018 9:08AM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Not Chk'd
Budget Misc Status Valid
View Related Payment Inquiry

Return to Search | Notify | Refresh

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	10/17/2018 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	10/17/2018 2:05:13 PM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	10/23/2018 10:10:26 AM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$8374.72
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	10/17/2018 2:10:50 PM
PI Notes:	

INVOICE

The University of North Carolina at Chapel Hill

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00142038

Name of Sponsor:	University of Minnesota Tonya Knutson 450 McNamara Alumni Center 200 Oak Street SE. United States	•Interim Billing o Final Billing	Award Amount $361,152.00
Sponsor Ref No.: UNC Award No.: Award Title: Project ID:	N005402801 A17-0239-001 Receptor recognition and cell entry of coronaviruses 5105169	Budget Period: From: 06/07/2016	To: 05/31/2019

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	EXPENDITURES Current 09/01/2018 - 09/30/2018	Cumulative 09/30/2018
Salaries	$3,105.71	$86,204.03
Fringe Benefits	$889.00	$20,682.68
Supplies	$1,163.61	$65,769.03
Travel-Domestic	$251.36	$2,127.96
Freight & Express	$0.00	$21.48
Miscellaneous Expense	$300.00	$300.00
Total Direct	$5,509.68	$175,105.18
Indirect Cost	$2,865.04	$91,055.21
TOTAL	$8,374.72	$266,160.39

Payment due upon receipt of invoice

****

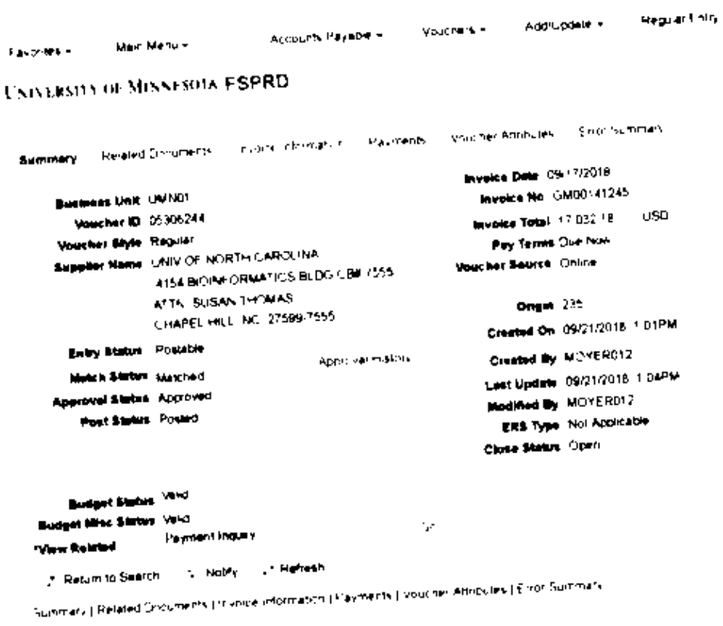
PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	Signature	Date
Bettina Lampkin		10/15/2018 BZ

For questions regarding invoice, please contact Bowen Zhu
Phone: 469/237-5060 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532


Favorites ▾ Main Menu ▾ Accounts Payable ▾ Vouchers ▾ Add/Update ▾ Regular Entry

UNIVERSITY OF MINNESOTA FSPRD

Summary Related Documents Invoice Information Payments Voucher Attributes Error Summary

Business Unit UMN01
Voucher ID 05306244
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN: SUSAN THOMAS
CHAPEL HILL NC 27599-7555

Entry Status Postable
Match Status Matched
Approval Status Approved
Post Status Posted

Invoice Date 09/17/2018
Invoice No GM00141245
Invoice Total 17,032.18 USD
Pay Terms Due Now
Voucher Source Online

Origin 235
Created On 09/21/2018 1:01PM
Created By MOYER012
Last Update 09/21/2018 1:04PM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Valid
Budget Misc Status Valid
View Related Payment Inquiry

Return to Search Notify Refresh

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	9/20/2018 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	9/20/2018 9:33:28 AM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	9/20/2018 9:35:26 AM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$17032.18
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	9/20/2018 9:53:16 AM
PI Notes:	

WORKFLOWED

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00141245

Name of Sponsor:	University of Minnesota Tonya Knutson 450 McNamara Alumni Center 200 Oak Street SE. United States	•Interim Billing Final Billing	Award Amount $361,152.00
Sponsor Ref No.:	N005402801		
UNC Award No.:	A17-0239-001	Budget Period:	
Award Title:	Receptor recognition and cell entry of coronaviruses	From: 06/07/2016	To: 05/31/2019
Project ID:	5105169		

knuts055@umn edu, sub-inv@umn edu

DESCRIPTION OF SERVICES	EXPENDITURES Current 06/01/2018 - 08/31/2018	Cumulative 08/31/2018
Salaries	$9,133.00	$83,098.32
Fringe Benefits	$1,979.36	$19,993.68
Supplies	$92.99	$64,605.42
Travel-Domestic	$0.00	$1,876.60
Freight & Express	$0.00	$21.48
Total Direct	$11,205.35	$169,595.50
Indirect Cost	$5,826.83	$88,190.17
TOTAL	$17,032.18	$257,785.67

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	Signature	Date
Bettina Lampkin	BL	09/17/2018 BZ

For questions regarding invoice, please contact Bowen Zhu
Phone: 469/237-5060 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

UNIVERSITY OF MINNESOTA

Purchase Order - Change Order

CHANGE ORDER # 3 TO PO # N005402801

(Include all digits on submitted invoice)

Supplier #: 0000012074 UNIV OF NORTH CAROLINA OFFICE OF SPONSORED RESEARCH PO BOX 402420 ATLANTA GA 30384-2420	**Date** 9/20/2016	**Tax Exempt #** 8029894	
	Payment Terms Net 30	**Freight Terms** FOB: DESTINATION- PLUS FREIGHT	**Ship Via** SHIP MOST ECONOMICAL
	Requestor		**Phone**

Federal Contract Funding? No

Bill To:	**Ship To:**
SPONSORED PROJECTS ADMIN SPONSORED PROJ ADMIN-P107220001 RM450 MCNAMARA ALUMNI CTR 200 OAK ST SE MINNEAPOLIS MN 55455	See Detail Below

Supplier: Review this document thoroughly to identify changes. Refer to original order for entire order.

Terms and Conditions of original order apply.

COPY

UNIVERSITY OF MINNESOTA

Purchase Order - Change Order

CHANGE ORDER # 3 TO PO # N005402801

(Include all digits on submitted invoice)

06/07/16-05/31/17

Change Order#1
Add $120,384 for a new total of $240,768. New end date 05/31/2018

PI changed departments and new project number was established. PO now under project 00066245

Change Order 2: AMD 2 (funds added to distribution line 5) adds $120,384.00 making the cumulative award amount to $361,152.00. All other terms and conditions remain unchanged

Line	Item / Description	Quantity	UOM	Unit Price	Extended Amt
1	receptor Recognition and Cell Entry of Coronaviruses	1.00	LOT	$361,152.00	$361,152.00

Ship To:
SPONSORED PROJ ADMIN-P107220001
RM450 MCNAMARA ALUMNI CTR
200 OAK ST SE
MINNEAPOLIS MN 55455

PO TOTAL AMOUNT $361,152.00

PO TOTAL AMOUNT BEFORE CHANGE $240,768.00

ABOVE PURCHASE ORDER IS ELECTRONICALLY APPROVED BY UNIVERSITY OF MINNESOTA.

COPY

UNIVERSITY OF MINNESOTA

Purchase Order - Change Order

CHANGE ORDER # 3 TO PO # N005402801

(Include all digits on submitted invoice)

Line	Sched	Dist	Amount
1	1	1	89,006.40

Fund	Deptid	Program	PC BU	Project	Act	Account	Fin EmplID	Chartfield1	Chartfield2	CS
3002	11718		UMSPR	00054028	1	810301				

Line	Sched	Dist	Amount
1	1	2	25,000.00

Fund	Deptid	Program	PC BU	Project	Act	Account	Fin EmplID	Chartfield1	Chartfield2	CS
3002	11718		UMSPR	00054028	1	810401				

Line	Sched	Dist	Amount
1	1	3	6,372.19

Fund	Deptid	Program	PC BU	Project	Act	Account	Fin EmplID	Chartfield1	Chartfield2	CS
3002	11718		UMSPR	00054028	1	810301				

Line	Sched	Dist	Amount
1	1	4	112,831.88

Fund	Deptid	Program	PC BU	Project	Act	Account	Fin EmplID	Chartfield1	Chartfield2	CS
3002	11651		UMSPR	00066245	1	810301				

Line	Sched	Dist	Amount
1	1	5	120,389.41

Fund	Deptid	Program	PC BU	Project	Act	Account	Fin EmplID	Chartfield1	Chartfield2	CS
3002	11718		UMSPR	00054028	1	810301				

Line	Sched	Dist	Amount
1	1	6	7,552.12

Fund	Deptid	Program	PC BU	Project	Act	Account	Fin EmplID	Chartfield1	Chartfield2	CS
3002	11651		UMSPR	00066245	1	810301				

COPY

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Business Unit UMN01
Voucher ID 05209215
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN SUSAN THOMAS
CHAPEL HILL, NC 27599-7555

Entry Status Postable
Match Status Ready
Approval Status Approved
Post Status Unposted

Approval History

Invoice Date 07/12/2018
Invoice No GM00139649
Invoice Total 7,543.02 USD
Pay Terms Net 30
Voucher Source Online

Origin 235
Created On 07/18/2018 2:09PM
Created By BWICHTER
Last Update 07/18/2018 2:13PM
Modified By BWICHTER
ERS Type Not Applicable
Close Status Open

Budget Status Not Chk'd
Budget Misc Status Valid
***View Related** Payment Inquiry Go

Return to Search | Notify | Refresh | Add | Update/Display

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	7/17/2018 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	7/17/2018 1:36:27 PM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	7/17/2018 2:42:03 PM
Wire Transfer:	N
Final Invoice:	N
Amount to be Paid:	$7543.02
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	7/17/2018 4:24:59 PM
PI Notes:	

WORKFLOWED

INVOICE

The University of North Carolina at Chapel Hill

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00139649

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **United States**	○Interim Billing • Final Billing	**Award Amount** **$240,768.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**	**Budget Period:**	
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**From: 06/07/2016**	**To: 05/31/2018**
Project ID:	**5105169**		

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES**	
	Current **05/01/2018 - 05/31/2018**	**Cumulative** **05/31/2018**
Salaries	**$2,684.02**	**$73,965.32**
Fringe Benefits	**$699.64**	**$18,014.32**
Supplies	**$1,578.83**	**$64,512.43**
Travel-Domestic	**$0.00**	**$1,876.60**
Freight & Express	**$0.00**	**$21.48**
Total Direct	**$4,962.49**	**$158,390.15**
Indirect Cost	**$2,580.53**	**$82,363.34**
TOTAL	**$7,543.02**	**$240,753.49**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Jenifer Womble Gwaltney		**07/12/2018**

For questions regarding invoice, please contact Bowen Zhu
Phone: 469/237-5060 **FAX: 919/962-5011**
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

UNIVERSITY OF MINNESOTA **FSPRD**

Summary Related Documents Invoice Information Payments Voucher Attributes Error Summary

Business Unit UMN01
Voucher ID 05119445
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN SUSAN THOMAS
CHAPEL HILL NC 27599-7555

Entry Status Postable
Match Status Ready
Approval Status Approved
Post Status Unposted

Approve History

Invoice Date 05/08/2018
Invoice No GM00137837
Invoice Total 32,741.06 USD
Pay Terms Net 30
Voucher Source Online

Origin 235
Created On 05/14/2018 7:48AM
Created By MOYER012
Last Update 05/14/2018 7:50AM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Not Chkd
Budget Misc Status Valid
View Related Payment Inquiry Go

Return to Search Notify Refresh

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	5/9/2018 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	5/9/2018 1:59:26 PM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	5/9/2018 2:11:29 PM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	5/10/2018 11:42:27 AM
PI Notes:	

INVOICE

The University of North Carolina at Chapel Hill

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00137837

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**•Interim Billing** **○ Final Billing**	**Award Amount** **$240,768.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2018**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** Current 04/01/2018 - 04/30/2018	Cumulative 04/30/2018
Salaries	**$3,078.42**	**$71,281.30**
Fringe Benefits	**$687.22**	**$17,314.68**
Supplies	**$17,753.03**	**$62,933.60**
Travel-Domestic	**$0.00**	**$1,876.60**
Freight & Express	**$21.48**	**$21.48**
Total Direct	**$21,540.15**	**$153,427.66**
Indirect Cost	**$11,200.91**	**$79,782.81**
TOTAL	**$32,741.06**	**$233,210.47**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Bettina Lampkin		**05/08/2018**

For questions regarding invoice, please contact Julie Kadow
Phone: 919/962-4692 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

ar Entry

UNIVERSITY OF MINNESOTA FSPRD

Business Unit UMN01
Voucher ID 05102123
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN SUSAN THOMAS
CHAPEL HILL NC 27599-7555
Entry Status Postable
Match Status Ready
Approval Status Approved
Post Status Unposted

Approval History

Invoice Date 04/26/2018
Invoice No GM00137564
Invoice Total 23,764.81 USD
Pay Terms Due Now
Voucher Source Online

Origin 235
Created On 05/02/2018 11:17AM
Created By MOYER012
Last Update 05/02/2018 11:20AM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Not Chkd
Budget Hdr Status Valid
View Related Payment Inquiry Go

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	4/30/2018 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	4/30/2018 12:18:59 PM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	4/30/2018 2:45:36 PM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	5/1/2018 10:30:46 AM
PI Notes:	

WORKFLOWED

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: **GM00137564**

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**•Interim Billing** **○ Final Billing**	**Award Amount** **$240,768.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2018**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** **Current** **03/01/2018 - 03/31/2018**	**Cumulative** **03/31/2018**
Salaries	**$3,324.91**	**$68,202.88**
Fringe Benefits	**$751.36**	**$16,627.46**
Supplies	**$10,958.44**	**$45,180.57**
Travel-Domestic	**$600.00**	**$1,876.60**
Total Direct	**$15,634.71**	**$131,887.51**
Indirect Cost	**$8,130.10**	**$68,581.90**
TOTAL	**$23,764.81**	**$200,469.41**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Bettina Lampkin		**04/26/2018**

For questions regarding invoice, please contact Julie Kadow
Phone: 919/962-4692 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Business Unit UMN01
Voucher ID 05052703
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN SUSAN THOMAS
CHAPEL HILL, NC 27599-7555

Entry Status Postable
Match Status Matched
Approval Status Approved
Post Status Posted

Approval History

Invoice Date 03/23/2018
Invoice No GM00136726
Invoice Total 5,710.09 USD
Pay Terms Due Now
Voucher Source Online

Origin 235
Created On 03/29/2018 11:39AM
Created By MOYER012
Last Update 03/29/2018 11:49AM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Valid
Budget Misc Status Valid
View Related Payment Inquiry Go

Return to Search | Notify | Refresh | Add | Update/Display

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	3/27/2018 5:00:00 AM
Department:	Veterinary Biomedical Science
Date Sent for Approval:	3/28/2018 9:31:05 AM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	3/28/2018 9:59:17 AM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	3/28/2018 3:11:27 PM
PI Notes:	

WITHDRAWN

INVOICE

The University of North Carolina at Chapel Hill

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: **GM00136726**

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	•Interim Billing ○ Final Billing	**Award Amount** **$240,768.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:** **From: 06/07/2016** **To: 05/31/2018**	
Project ID:	**5105169**		

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** **Current** **02/01/2018 - 02/28/2018**	**Cumulative** **02/28/2018**
Salaries	**$3,070.93**	**$64,877.97**
Fringe Benefits	**$685.69**	**$15,876.10**
Supplies	**$0.00**	**$34,222.13**
Travel-Domestic	**$0.00**	**$1,276.60**
Total Direct	**$3,756.62**	**$116,252.80**
Indirect Cost	**$1,953.47**	**$60,451.80**
TOTAL	**$5,710.09**	**$176,704.60**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Bettina Lampkin		**03/23/2018**

For questions regarding invoice, please contact Julie Kadow
Phone: 919/962-4692 **FAX: 919/962-5011**
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

Summary Related Documents Invoice Information Payments Voucher Attributes Error Summary

Business Unit UMN01
Voucher ID 05022920
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN: SUSAN THOMAS
CHAPEL HILL, NC 27599-7555
Entry Status Postable
Match Status Matched
Approval Status Approved
Post Status Posted

Approval History

Invoice Date 02/26/2018
Invoice No GM00136055
Invoice Total 5,710.10 USD
Pay Terms Due Now
Voucher Source Online

Origin 235
Created On 03/07/2018 2:16PM
Created By MOYER012
Last Update 03/07/2018 2:18PM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Valid
Budget Misc Status Valid
***View Related** Payment Inquiry Go

Return to Search Notify Refresh Add Update/Display

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	3/5/2018 6:00:00 AM
Department:	Veterinary Biomedical Science
Date Sent for Approval:	3/5/2018 2:18:14 PM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	3/7/2018 8:41:57 AM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	3/6/2018 2:51:38 PM
PI Notes:	

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INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00136055

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**•Interim Billing** **○ Final Billing**	**Award Amount** **$240,768.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2018**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES**	
	Current **01/01/2018 - 01/31/2018**	**Cumulative** **01/31/2018**
Salaries	**$3,070.92**	**$61,807.04**
Fringe Benefits	**$685.71**	**$15,190.41**
Supplies	**$0.00**	**$34,222.13**
Travel-Domestic	**$0.00**	**$1,276.60**
Total Direct	**$3,756.63**	**$112,496.18**
Indirect Cost	**$1,953.47**	**$58,498.33**
TOTAL	**$5,710.10**	**$170,994.51**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Bettina Lampkin		**02/26/2018**

For questions regarding invoice, please contact Julie Kadow
Phone: 919/962-4692 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

Summary Related Documents Invoice Information Payments Voucher Attributes Error Summary

Business Unit UMN01
Voucher ID 04975456
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN: SUSAN THOMAS
CHAPEL HILL, NC 27599-7555

Entry Status Postable
Match Status Ready
Approval Status Approved
Post Status Unposted

Approval History

Invoice Date 01/29/2018
Invoice No GM00135295
Invoice Total 5,667.72 USD
Pay Terms Net 30
Voucher Source Online

Origin 235
Created On 02/05/2018 11:20AM
Created By MOYER012
Last Update 02/05/2018 11:22AM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Not Chk'd
Budget Misc Status Valid
View Related Payment Inquiry Go

Return to Search Notify Refresh Add Update/Display

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	1/30/2018 6:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	1/31/2018 8:05:06 AM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	1/31/2018 3:00:17 PM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	1/31/2018 10:42:47 AM
PI Notes:	

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INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00135295

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**•Interim Billing** **○ Final Billing**	**Award Amount** **$240,768.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:** **From: 06/07/2016**	**To: 05/31/2018**
Project ID:	**5105169**		

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** **Current** **12/01/2017 - 12/31/2017**	**Cumulative** **12/31/2017**
Salaries	**$3,070.92**	**$58,736.12**
Fringe Benefits	**$657.83**	**$14,504.70**
Supplies	**$0.00**	**$34,222.13**
Travel-Domestic	**$0.00**	**$1,276.60**
Total Direct	**$3,728.75**	**$108,739.55**
Indirect Cost	**$1,938.97**	**$56,544.86**
TOTAL	**$5,667.72**	**$165,284.41**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Bettina Lampkin		**01/29/2018**

For questions regarding invoice, please contact Julie Kadow
Phone: 919/962-4692 **FAX: 919/962-5011**
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

Summary Related Documents Invoice Information Payments Voucher Attributes Error Summary

Business Unit UMN01
Voucher ID 04923427
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN: SUSAN THOMAS
CHAPEL HILL, NC 27599-7555
Entry Status Postable
Match Status Matched
Approval Status Approved
Post Status Posted

Approval History

Invoice Date 12/14/2017
Invoice No GM00134386
Invoice Total 6,738.34 USD
Pay Terms Due Now
Voucher Source Online

Origin 235
Created On 12/22/2017 9.44AM
Created By MOYER012
Last Update 12/22/2017 9.46AM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Valid
Budget Misc Status Valid
***View Related** Payment Inquiry Go

Return to Search Notify Refresh Add Update/Display

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	12/18/2017 6:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	12/18/2017 9:32:38 AM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	12/18/2017 9:33:31 AM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	12/19/2017 9:24:46 AM
PI Notes:	

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: **GM00134386**

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**•Interim Billing** **○ Final Billing**	**Award Amount** **$240,768.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2018**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES**	
	Current **11/01/2017 - 11/30/2017**	**Cumulative** **11/30/2017**
Salaries	**$3,450.92**	**$55,665.20**
Fringe Benefits	**$730.70**	**$13,846.87**
Supplies	**$251.48**	**$34,222.13**
Travel-Domestic	**$0.00**	**$1,276.60**
Total Direct	**$4,433.10**	**$105,010.80**
Indirect Cost	**$2,305.24**	**$54,605.89**
TOTAL	**$6,738.34**	**$159,616.69**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Bettina Lampkin		**12/14/2017**

For questions regarding invoice, please contact Julie Kadow
Phone: 919/962-4692 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

Summary Related Documents Invoice Information Payments Voucher Attributes Error Summary

Business Unit UMN01
Voucher ID 04892792
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN: SUSAN THOMAS
CHAPEL HILL, NC 27599-7555

Entry Status Postable
Match Status Matched
Approval Status Approved
Post Status Posted

Approval History

Invoice Date 11/12/2017
Invoice No GM00133495
Invoice Total 8,580.48 USD
Pay Terms Due Now
Voucher Source Online

Origin 235
Created On 12/04/2017 10:05AM
Created By MOYER012
Last Update 12/04/2017 10:07AM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Valid
Budget Misc Status Valid
***View Related** Payment Inquiry Go

Return to Search Notify Refresh Add Update/Display

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

2 of 2

PO#:	N005402801
Invoice Received:	11/14/2017 6:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	11/14/2017 12:50:34 PM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	11/30/2017 1:42:37 PM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	11/14/2017 3:04:01 PM
PI Notes:	

INVOICE

The University of North Carolina at Chapel Hill

THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA 30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00133495

Name of Sponsor:	**University of Minnesota** **Tonya Knutsen** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**Interim Billing** **Final Billing**	**Award Amount** **$240,768.00**
Sponsor Ref No.:	**N66546201**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5106199**	**From: 06/07/2016**	**To: 05/31/2018**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	EXPENDITURES Current 10/01/2017 - 10/31/2017	Cumulative 10/31/2017
[redacted]	$4,570.34	$52,214.28
	$1,074.72	$13,116.17
	$0.00	$33,970.65
	$0.00	$1,276.60
Total Direct	$5,645.06	$100,577.70
Indirect Cost	$2,935.42	$52,300.65
TOTAL	$8,580.48	$152,878.35

Payment due upon receipt of invoice

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	Signature	Date
Bettina Lamptin		**11/12/2017**

For questions regarding invoice, please contact Julie Kadow
Phone: 919/962-4692 FAX: 919/962-5811
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025387532

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Business Unit UMN01
Voucher ID 04892784
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN: SUSAN THOMAS
CHAPEL HILL, NC 27599-7555
Entry Status Postable
Match Status Matched
Approval Status Approved
Post Status Posted

Approval History

Invoice Date 10/24/2017
Invoice No GM00133033
Invoice Total 23,919.28 USD
Pay Terms Due Now
Voucher Source Online

Origin 235
Created On 12/04/2017 10:00AM
Created By MOYER012
Last Update 12/04/2017 10:03AM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Valid
Budget Misc Status Valid
***View Related** Payment Inquiry Go

Return to Search | Notify | Refresh | Add | Update/Display

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	10/27/2017 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	10/27/2017 1:05:41 PM
Invoice Coordinator Notes:	
Grant Administrator:	Brett Carlson
Date Invoice Approved by GA:	11/30/2017 1:41:17 PM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	10/28/2017 2:00:54 PM
PI Notes:	(1) I have changed department. So in the future, please process it through Department of Veterinary and Biomedical Sciences (2) Is the total of ~$240,000 the sum of direct and indirect costs for 2 years subcontracted to UNC?

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: **GM00133033**

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**•Interim Billing** **○ Final Billing**	**Award Amount** **$240,768.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2018**

knuts055@umn.edu, & sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** Current 06/01/2017 - 09/30/2017	Cumulative 09/30/2017
Salaries	**$12,590.69**	**$47,643.94**
Fringe Benefits	**$3,145.62**	**$12,041.45**
Supplies	**$0.00**	**$33,970.65**
Travel-Domestic	**$0.00**	**$1,276.60**
Total Direct	**$15,736.31**	**$94,932.64**
Indirect Cost	**$8,182.97**	**$49,365.23**
TOTAL	**$23,919.28**	**$144,297.87**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Bettina Lampkin		10/24/2017

For questions regarding invoice, please contact Julie Kadow
Phone: 919/962-4682 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

UNIVERSITY OF MINNESOTA

Purchase Order - Change Order

CHANGE ORDER # 2 TO PO # N005402801

(Include all digits on submitted invoice)

06/07/16-05/31/17

Change Order#1
Add $120,384 for a new total of $240,768. New end date 05/31/2018

PI changed departments and new project number was established. PO now under project 00066245

Line	Item / Description	Quantity	UOM	Unit Price	Extended Amt
1	receptor Recognition and Cell Entry of Coronaviruses	1.00	LOT	$240,768.00	$240,768.00

Ship To:
SPONSORED PROJ ADMIN-P107220001
RM450 MCNAMARA ALUMNI CTR
200 OAK ST SE
MINNEAPOLIS MN 55455

PO TOTAL AMOUNT	$240,768.00
PO TOTAL AMOUNT BEFORE CHANGE	$240,768.00

ABOVE PURCHASE ORDER IS ELECTRONICALLY APPROVED BY UNIVERSITY OF MINNESOTA.

COPY

UNIVERSITY OF MINNESOTA

Purchase Order - Change Order

CHANGE ORDER # 2 TO PO # N005402801

(Include all digits on submitted invoice)

Supplier #: 0000012074 UNIV OF NORTH CAROLINA OFFICE OF SPONSORED RESEARCH PO BOX 402420 ATLANTA GA 30384-2420	**Date** 9/20/2016	**Tax Exempt #** 8029894	
	Payment Terms Net 30	**Freight Terms** FOB: DESTINATION- PLUS FREIGHT	**Ship Via** MOST ECON
	Requestor		**Phone**

Federal Contract Funding? No

Bill To:	**Ship To:**
SPONSORED PROJECTS ADMIN SPONSORED PROJ ADMIN-P107220001 RM450 MCNAMARA ALUMNI CTR 200 OAK ST SE MINNEAPOLIS MN 55455	See Detail Below

Supplier: Review this document thoroughly to identify changes. Refer to original order for entire order.

Terms and Conditions of original order apply.

COPY

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Business Unit UMN01
Voucher ID 04736399
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN. SUSAN THOMAS
CHAPEL HILL NC 27599-7555

Entry Status Postable
Match Status Ready
Approval Status Approved
Post Status Unposted

Approval History

Invoice Date 07/14/2017
Invoice No GM00130311
Invoice Total 6,372.19 USD
Pay Terms Net 30
Voucher Source Online

Origin 235
Created On 08/15/2017 1:22PM
Created By MOYER012
Last Update 08/15/2017 1:23PM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Not Chk'd
Budget Misc Status Valid
***View Related** Payment Inquiry Go

Return to Search Notify Refresh Add

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	7/24/2017 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	7/24/2017 10:50:22 AM
Invoice Coordinator Notes:	Marked as final invoice. Release form requested.
Grant Administrator:	Erin Knudsen
Date Invoice Approved by GA:	8/9/2017 12:41:02 PM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	7/24/2017 5:40:03 PM
PI Notes:	

WORKFLOWED

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: **GM00130311**

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	○Interim Billing • Final Billing	**Award Amount** **$120,384.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2017**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** Current 05/01/2017 - 05/31/2017	Cumulative 05/31/2017
Salaries	**$2,240.65**	**$35,053.25**
Fringe Benefits	**$588.80**	**$8,895.83**
Supplies	**$1,362.78**	**$33,970.65**
Travel-Domestic	**$0.00**	**$1,276.60**
Total Direct	**$4,192.23**	**$79,196.33**
Indirect Cost	**$2,179.96**	**$41,182.26**
TOTAL	**$6,372.19**	**$120,378.59**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Jenifer Womble Gwaltney		**07/14/2017**

For questions regarding invoice, please contact Julie Kadow
Phone: 919/962-4692 FAX: 919/962-5011
OSRBILLING@unc.edu

JK

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

pc

From: psoftfs@unc.edu
Subject: Approved: eProcurement Order: UNCCH 1000196304
Date: October 11, 2016 at 1:11 PM
To: sims0018@email.unc.edu

eProcurement

No action required.

Order Details

Requester:	Amy Sims
Order ID:	UNCCH 1000196304
Vendor ID:	0000000009 FISHER SCIENTIFIC COMPANY LLC
Entered Date:	10/11/2016
Total Order Amount:	$1,362.78

Distributions

GL-BU	Fund	Source	Account	Dept	PC-BU	Project	Program	Amount
UNCCH	25210	49000	537110	463501	CHOSR	5105169		1,362.78

Line Items

Item ID	Description	Category	Quantity	Price	Amount
19268000	TR-602N VERSAFLO	PAP46182002	3	454.26	1,362.78

If you have questions, please contact Lawson Oldenburg at 919- 962-3773 or Lawsonri@email.unc.edu
You can navigate directly to the approval page by clicking the link below.

Review Requisition

PACKING SLIP

CUSTOMER SERVICE OFFICE		PRINT DATE	RPT NAME	SHIP WHSE	ORDER NO.	P/L	PAGE
FNC NORTHEAST REGION	[redacted]	10/27/16		RAL	V62855150-01	1	1
CUSTOMER ACCOUNT NO.	**CUSTOMER PURCHASE ORDER NUMBER**				**RELEASE NUMBER**		
577670-003	UNCCH.200016986						

FISHER SCIENTIFIC COMPANY

Visit the Fisher Scientific Website:
fishersci.com

S H I P T O
UNIV OF NORTH CAROLINA
RM 3105 HOWELL MCGAUGH
[illegible] ROBERT RESEARCH
[illegible] CAMP DR
CHAPEL HILL [illegible] NC 27514
AMY SIMS / AMY SIMS

S O L D T O
UNIVERSITY OF NO CAROLINA
DISBURSEMENT OFFICE
CB# 1220
104 AIRPORT DR
CHAPEL HILL NC 27599-1220

DOCUMENT TYPE: UNDER SUMMARY DOCUMENT
CALLER: AMY SIMS / AMY SIMS
PHONE NUMBER:

INSTRUCTIONS

NOTE: QUANTITY INDICATED IN THIS COLUMN IS BEING SHIPPED DIRECT TO YOU FROM INDICATED ALTERNATE WAREHOUSE. SEE REVERSE SIDE FOR ABBREVIATIONS.

9194 126

LINE	QUANTITY ORDERED	QUANTITY SHIPPED	BACK ORDERED	ALT. WHSE.	UNIT	CATALOG NUMBER	SUPPLIER CAT. NO. / DESCRIPTION	UNIT PRICE	AMOUNT
							PHONE NUMBER [illegible]		
001	3	3			CS	19 268 000	CUST LINE NBR: 000001 TR-602N VERSAFLO PAPR. 1/CS TR-602N		

Rec'd 11/2/16 ACS

TOTAL TRANSPORTATION PREPAID

CHG.	RHE	CARRIER	REF
M	CHP/1001	/	
I	CHP/1001	/	
W	CHP/1001	/	
H	CHP/1001	/	
F	CHP/1001	/	
S	/	/	
S	/	/	

*V62855150001 *

UNCCH.200016986

OPEN AND INSPECT IMMEDIATELY
QUESTIONS REGARDING THIS SHIPMENT SHOULD BE DIRECTED TO YOUR CUSTOMER SERVICE OFFICE WITHIN TEN (10) DAYS
AT: 800 766-7000

PLEASE REFERENCE THE ORDER NUMBER SHOWN ABOVE IN ALL CORRESPONDENCE.
RETURNS WILL NOT BE ACCEPTED WITHOUT PRIOR AUTHORIZATION.

CUST. ACCOUNT NO.	ORDER NO.	[redacted]	PCS.	CARRIER	SHIP DATE	WEIGHT	ASSEM. BY	PACKED BY	SHIPPED BY
577670-003	V62855150-01		1	1 &	10-27				

Approved for use through 11/30/2010
OMB No. 0925-0001

P.I.: Ralph Baric UNC No.: 5105169

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FINAL INVENTION STATEMENT AND CERTIFICATION

(FOR GRANT OR AWARD)

DHHS GRANT OR AWARD NO.
N005402801

A. We hereby certify that to the best of our knowledge and belief, all inventions are listed below which were conceived and/or first actually reduced to practice during the course of work under the above-referenced DHHS grant or award for the period.

6/7/2016 (Original effective date) through 5/31/2017 (date of termination)

B. INVENTIONS (Note: If no inventions have been made under the grant or award, insert the word "*NONE*" under Title below.)

NAME OF INVENTOR	TITLE OF INVENTION	DATE REPORTED TO DHHS
	NONE	

(Use continuation sheet if necessary)

C. SIGNATURE - - This block *must* be signed by an official authorized to sign on behalf of the institution.

TITLE
Director of Licensing
Office of Commercialization and Economic Development

TYPED NAME
Jacqueline Quay, J.D.

SIGNATURE
Jacqueline Quay

DATE
July 5, 2017

NAME AND MAILING ADDRESS OF INSTITUTION

The Univ. of North Carolina at Chapel Hill
Office of Commercialization and Economic Development
100 Europa Drive, Suite #430, CB #4105
Chapel Hill, NC 27599-4105

HHS 568 (Rev. 11/07)

UNIVERSITY OF MINNESOTA

Purchase Order - Change Order

CHANGE ORDER # 1 TO PO # N005402801

(Include all digits on submitted invoice)

Supplier #: 0000012074 UNIV OF NORTH CAROLINA OFFICE OF SPONSORED RESEARCH PO BOX 402420 ATLANTA GA 30384-2420	**Date** 9/20/2016	**Tax Exempt #** 8029894	
	Payment Terms Net 30	**Freight Terms** FOB: DESTINATION- PLUS FREIGHT	**Ship Via** MOST ECON
	Requestor		**Phone**

Federal Contract Funding? No

Bill To:	**Ship To:**
SPONSORED PROJECTS ADMIN SPONSORED PROJ ADMIN-P107220001 RM450 MCNAMARA ALUMNI CTR 200 OAK ST SE MINNEAPOLIS MN 55455	See Detail Below

Supplier: Review this document thoroughly to identify changes. Refer to original order for entire order.

Terms and Conditions of original order apply.

COPY

UNIVERSITY OF MINNESOTA

Purchase Order - Change Order

CHANGE ORDER # 1 TO PO # N005402801

(Include all digits on submitted invoice)

06/07/16-05/31/17

Change Order#1
Add $120,384 for a new total of $240,768. New end date 05/31/2018

Line	Item / Description	Quantity	UOM	Unit Price	Extended Amt
1	receptor Recognition and Cell Entry of Coronaviruses	1.00	LOT	$240,768.00	$240,768.00

Ship To:
SPONSORED PROJ ADMIN-P107220001
RM450 MCNAMARA ALUMNI CTR
200 OAK ST SE
MINNEAPOLIS MN 55455

PO TOTAL AMOUNT	$240,768.00
PO TOTAL AMOUNT BEFORE CHANGE	$120,384.00

ABOVE PURCHASE ORDER IS ELECTRONICALLY APPROVED BY UNIVERSITY OF MINNESOTA.

COPY

UNIVERSITY OF MINNESOTA

Purchase Order - Change Order

CHANGE ORDER # 1 TO PO # N005402801

(Include all digits on submitted invoice)

Line	Sched	Dist	Amount
1	1	1	89,006.40

Fund	Deptid	Program	PC BU	Project	Act	Account	Fin EmplID	Chartfield1	Chartfield2	CS
3002	11718		UMSPR	00054028	1	810301				

Line	Sched	Dist	Amount
1	1	2	25,000.00

Fund	Deptid	Program	PC BU	Project	Act	Account	Fin EmplID	Chartfield1	Chartfield2	CS
3002	11718		UMSPR	00054028	1	810401				

Line	Sched	Dist	Amount
1	1	3	126,761.60

Fund	Deptid	Program	PC BU	Project	Act	Account	Fin EmplID	Chartfield1	Chartfield2	CS
3002	11718		UMSPR	00054028	1	810301				

COPY

Business Unit UMN01
Voucher ID 04663764
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN: SUSAN THOMAS
CHAPEL HILL, NC 27599-7555

Entry Status Postable
Match Status Ready
Approval Status Approved
Post Status Unposted

Approval History

Invoice Date 06/08/2017
Invoice No GM00129282
Invoice Total 7,284.70 USD
Pay Terms Due Now
Voucher Source Online

Origin 235
Created On 06/20/2017 9:14AM
Created By MOYER012
Last Update 06/20/2017 9:16AM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Not Chk'd
Budget Misc Status Valid
***View Related** Payment Inquiry ▼ Go

Return to Search Notify Refresh Add Update/Display

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	6/15/2017 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	6/16/2017 9:02:58 AM
Invoice Coordinator Notes:	
Grant Administrator:	Erin Knudsen
Date Invoice Approved by GA:	6/16/2017 2:32:33 PM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	6/19/2017 9:49:26 AM
PI Notes:	

WORKFLOWED

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00129282

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	**•Interim Billing** **·· Final Billing**	**Award Amount** **$120,384.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:** **From: 06/07/2016**	**To: 05/31/2017**
Project ID:	**5105169**		

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** **Current** **05/01/2017 - 05/31/2017**	**Cumulative** **05/31/2017**
Salaries	**$3,795.11**	**$32,812.60**
Fringe Benefits	**$997.44**	**$8,307.03**
Supplies	**$0.00**	**$32,607.87**
Travel-Domestic	**$0.00**	**$1,276.60**
Total Direct	**$4,792.55**	**$75,004.10**
Indirect Cost	**$2,492.15**	**$39,002.30**
TOTAL	**$7,284.70**	**$114,006.40**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Bettina Lampkin		**06/08/2017**

For questions regarding invoice, please contact Julie Kadow
Phone: 919/962-4692 **FAX: 919/962-5011**
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

approved
Edited

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: **GM00129282**

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070** **United States**	•**Interim Billing** **Final Billing**	**Award Amount** **$120,384.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2017**

knuts055@umn.edu, sub-inv@umn.edu.

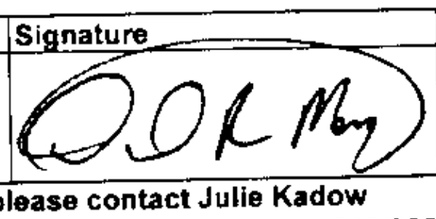
DESCRIPTION OF SERVICES	**EXPENDITURES** Current 05/01/2017 - 05/31/2017	Cumulative 05/31/2017 ✓
Salaries	**$3,795.11**	**$32,812.80**
Fringe Benefits	**$997.44**	**$8,187.80**
Supplies	**$0.00**	**$32,987.27**
Travel-Domestic	**$0.00**	**$1,276.50**
Total Direct	$4,792.55	$75,004.10
Indirect Cost	$2,492.15 ✓	$39,002.30
TOTAL	$7,284.70 ✓	$114,006.40 ✓

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	Signature	Date
Bettina Lampkin		06/08/2017

For questions regarding invoice, please contact Julie Kadow
Phone: 919/962-4692 FAX: 919/962-5011
OSRBILLING@unc.edu

JK

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Business Unit UMN01
Voucher ID 04638483
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN: SUSAN THOMAS
CHAPEL HILL, NC 27599-7555
Entry Status Postable
Match Status Ready
Approval Status Approved
Post Status Unposted

Approval History

Invoice Date 05/19/2017
Invoice No GM00128869
Invoice Total 8,552 57 USD
Pay Terms Net 30
Voucher Source Online

Origin 235
Created On 06/03/2017 11 12AM
Created By MOYER012
Last Update 06/03/2017 11 15AM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Not Chk'd
Budget Misc Status Valid
***View Related** Payment Inquiry Go

Notify Refresh Add Update/Display

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	5/24/2017 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	5/24/2017 3:48:56 PM
Invoice Coordinator Notes:	
Grant Administrator:	Erin Knudsen
Date Invoice Approved by GA:	5/31/2017 2:53:36 PM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	5/25/2017 8:57:20 PM
PI Notes:	

WORKFLOWED

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00128869

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070**	**Interim Billing** **Final Billing**	**Award Amount** **$120,384.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2017**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES**	
	Current **04/01/2017 - 04/30/2017**	**Cumulative** **04/30/2017**
Salaries	**$3,790.37**	**$29,017.49**
Fringe Benefits	**$996.31**	**$7,309.59**
Supplies	**$840.00**	**$32,607.87**
Travel-Domestic	**$0.00**	**$1,276.60**
Total Direct	**$5,626.68**	**$70,211.55**
Indirect Cost	**$2,925.89**	**$36,510.15**
TOTAL	**$8,552.57**	**$106,721.70**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Typed Name of Fiscal Officer	**Signature**	**Date**
Bettina Lampkin		**05/19/2017**

For questions regarding invoice, please contact Julie Kadow
Phone: 919/962-4692 **FAX: 919/962-5011**
OSRBILLING@unc.edu

JK

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

approved
5/31/17

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00128869

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070**	**•Interim Billing** **Final Billing**	**Award Amount** **$120,384.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2017**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	EXPENDITURES Current 04/01/2017 - 04/30/2017	Cumulative 04/30/2017
Salaries	**$3,790.37**	**$29,017.49**
Fringe Benefits	**$996.31**	**$7,309.59**
Supplies	**$840.00**	**$32,607.87**
Travel-Domestic	**$0.00**	**$1,276.60**
Total Direct	**$5,626.68**	**$70,211.55**
Indirect Cost	**$2,925.89**	**$36,510.15**
TOTAL	**$8,552.57**	**$106,721.70**

Payment due upon receipt of invoice

****

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Typed Name of Fiscal Officer	Signature	Date
Bettina Lampkin		05/19/2017

For questions regarding invoice, please contact Julie Kadow
Phone: 919/962-4692 FAX: 919/962-5011
OSRBILLING@unc.edu

JK

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

Summary Related Documents Invoice Information Payments Voucher Attributes Error Summary

Business Unit UMN01
Voucher ID 04572342
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN: SUSAN THOMAS
CHAPEL HILL, NC 27599-7555

Entry Status Postable
Match Status Ready
Approval Status Approved
Post Status Unposted

Approval History

Invoice Date 04/11/2017
Invoice No GM00126864
Invoice Total 9,942.50 USD
Pay Terms Net 30
Voucher Source Online

Origin 235
Created On 04/18/2017 11:20AM
Created By MOYER012
Last Update 04/18/2017 11:22AM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Budget Status Not Chk'd
Budget Misc Status Valid
View Related Payment Inquiry Go

Notify Refresh Add Update/Display

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	4/11/2017 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	4/11/2017 3:54:51 PM
Invoice Coordinator Notes:	
Grant Administrator:	Erin Knudsen
Date Invoice Approved by GA:	4/17/2017 1:39:09 PM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	4/11/2017 5:42:46 PM
PI Notes:	

WORKFLOWED

INVOICE

The University of North Carolina at Chapel Hill

THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00126884

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070**	•Interim Billing o Final Billing	**Award Amount** **$120,384.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2017**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	EXPENDITURES Current 03/01/2017 - 03/31/2017	EXPENDITURES Cumulative 03/31/2017
Salaries	**$4,171.73**	**$25,227.12**
Fringe Benefits	**$1,092.77**	**$6,313.28**
Supplies	**$0.00**	**$31,767.87**
Travel-Domestic	**$1,276.60**	**$1,276.60**
Total Direct	**$6,541.10**	**$64,584.87**
Indirect Cost	**$3,401.40**	**$33,584.26**
TOTAL	**$9,942.50**	**$98,169.13**

Payment due upon receipt of invoice

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

I hereby certify that the foregoing report is true in all respects and that the expenditures have been made solely for the purposes set forth in the application for the grant as approved and that I am authorized to sign this report for the institution named.

Typed Name of Fiscal Officer	Signature	Date
Bettina Lampkin		**04/11/2017**

For questions regarding invoice, please contact Julie Kadow
Phone: 919/962-4692 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursement and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil, or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001, and Title 31, Sections 3729-3730 and 3801-3812).

Approved
4/17/17

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: **GM00126864**

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070**	•Interim Billing ○ Final Billing	**Award Amount** **$120,384.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2017**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** Current 03/01/2017 - 03/31/2017	Cumulative 03/31/2017
Salaries	**$4,171.73**	**$25,227.12**
Fringe Benefits	**$1,092.77**	**$6,313.28**
Supplies	**$0.00**	**$31,767.87**
Travel-Domestic	**$1,276.60**	**$1,276.60**
Total Direct	**$6,541.10**	**$64,584.87**
Indirect Cost	**$3,401.40**	**$33,584.26**
TOTAL	**$9,942.50**	**$98,169.13**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

I hereby certify that the foregoing report is true in all respects and that the expenditures have been made solely for the purposes set forth in the application for the grant as approved and that I am authorized to sign this report for the institution named.

Typed Name of Fiscal Officer	**Signature**	**Date**
Bettina Lampkin		**04/11/2017**

For questions regarding invoice, please contact Julie Kadow
Phone: 919/962-4692 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursement and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil, or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001, and Title 31, Sections 3729-3730 and 3801-3812).

UNIVERSITY OF MINNESOTA FSPRD

Business Unit UMN01
Voucher ID 04533000
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN: SUSAN THOMAS
CHAPEL HILL, NC 27599-7555

Entry Status Postable
Match Status Ready
Approval Status Approved
Post Status Unposted

Approval History

Invoice Date 03/14/2017
Invoice No GM00126142
Invoice Total 10,997.50 USD
Pay Terms Net 30
Voucher Source Online

Origin 235
Created On 03/21/2017 10:28AM
Created By WVANG
Last Update 03/21/2017 10:33AM
Modified By WVANG
ERS Type Not Applicable
Close Status Open

Budget Status Not Chk'd
Budget Misc Status Valid
***View Related** Payment Inquiry Go

Notify Refresh Add Update/Display

INVOICE

The University of North Carolina at Chapel Hill

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: **GM00126142**

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070**	**•Interim Billing** **○ Final Billing**	**Award Amount** **$120,384.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2017**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** Current 02/01/2017 - 02/28/2017	Cumulative 02/28/2017
Salaries	**$3,605.10**	**$21,055.39**
Fringe Benefits	**$949.53**	**$5,220.51**
Supplies	**$2,680.56**	**$31,767.87**
Total Direct	**$7,235.19**	**$58,043.77**
Indirect Cost	**$3,762.31**	**$30,182.86**
TOTAL	**$10,997.50**	**$88,226.63**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

I hereby certify that the foregoing report is true in all respects and that the expenditures have been made solely for the purposes set forth in the application for the grant as approved and that I am authorized to sign this report for the institution named.

Typed Name of Fiscal Officer	**Signature**	**Date**
Jenifer Womble Gwaltney	Jenifer Womble Gwaltney	**03/14/2017**

For questions regarding invoice, please contact OSR
Phone: 919/966-3411 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursement and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil, or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001, and Title 31, Sections 3729-3730 and 3801-3812).

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	3/15/2017 5:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	3/15/2017 11:36:21 AM
Invoice Coordinator Notes:	
Grant Administrator:	Erin Knudsen
Date Invoice Approved by GA:	3/15/2017 2:05:29 PM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	3/16/2017 3:28:00 PM
PI Notes:	

WORKFLOWED

approved 3/15/17

INVOICE

The University of North Carolina at Chapel Hill

THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: **GM00126142**

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070**	**•Interim Billing** **○ Final Billing**	**Award Amount** **$120,384.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**		
Project ID:	**5105169**	**Budget Period:** **From: 06/07/2016** **To: 05/31/2017**	

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** **Current** **02/01/2017 - 02/28/2017**	**Cumulative** **02/28/2017**
Salaries	**$3,605.10**	**$21,055.39**
Fringe Benefits	**$949.53**	**$5,220.51**
Supplies	**$2,680.56**	**$31,767.87**
Total Direct	**$7,235.19**	**$58,043.77**
Indirect Cost	**$3,762.31**	**$30,182.86**
TOTAL	**$10,997.50**	**$88,226.63**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

I hereby certify that the foregoing report is true in all respects and that the expenditures have been made solely for the purposes set forth in the application for the grant as approved and that I am authorized to sign this report for the institution named.

Typed Name of Fiscal Officer	**Signature**	**Date**
Jenifer Womble Gwaltney	Beth Gwaltney	**03/14/2017**

For questions regarding invoice, please contact OSR
Phone: 919/966-3411 **FAX: 919/962-5011**
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursement and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil, or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001, and Title 31, Sections 3729-3730 and 3801-3812).

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Business Unit UMN01
Voucher ID 04500200
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN: SUSAN THOMAS
CHAPEL HILL, NC 27599-7555

Entry Status Postable
Match Status Ready
Approval Status Approved
Post Status Unposted

Approval History

Budget Status Not Chk'd
Budget Misc Status Valid
***View Related** Payment Inquiry Go

Invoice Date 02/22/2017
Invoice No GM00125520
Invoice Total 6,921.69 USD
Pay Terms Net 30
Voucher Source Online

Origin 235
Created On 02/27/2017 12:41PM
Created By MOYER012
Last Update 02/27/2017 12:45PM
Modified By MOYER012
ERS Type Not Applicable
Close Status Open

Notify Refresh Add Update/Display

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	2/24/2017 6:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	2/24/2017 3:57:53 PM
Invoice Coordinator Notes:	
Grant Administrator:	Erin Knudsen
Date Invoice Approved by GA:	2/24/2017 4:21:37 PM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	2/25/2017 10:58:21 PM
PI Notes:	

WORKFLOWED

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: **GM00125520**

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070**	**•Interim Billing** **○ Final Billing**	**Award Amount** **$120,384.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2017**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** Current 01/01/2017 - 01/31/2017	Cumulative 01/31/2017
Salaries	**$3,604.42**	**$17,450.29**
Fringe Benefits	**$949.33**	**$4,270.98**
Supplies	**$0.00**	**$29,087.31**
Total Direct	**$4,553.75**	**$50,808.58**
Indirect Cost	**$2,367.94**	**$26,420.55**
TOTAL	**$6,921.69**	**$77,229.13**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

I hereby certify that the foregoing report is true in all respects and that the expenditures have been made solely for the purposes set forth in the application for the grant as approved and that I am authorized to sign this report for the institution named.

Typed Name of Fiscal Officer	**Signature**	**Date**
Jenifer Womble Gwaltney	J.W.M.G.	**02/22/2017** EZ

For questions regarding invoice, please contact OSR
Phone: 919/966-3411 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursement and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil, or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001, and Title 31, Sections 3729-3730 and 3801-3812).

approved
2/24/17

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00125520

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070**	•Interim Billing ○ Final Billing	**Award Amount** **$120,384.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2017**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** Current 01/01/2017 - 01/31/2017	Cumulative 01/31/2017
Salaries	**$3,604.42**	**$17,450.29**
Fringe Benefits	**$949.33**	**$4,270.98**
Supplies	**$0.00**	**$29,087.31**
Total Direct	**$4,553.75**	**$50,808.58**
Indirect Cost	**$2,367.94**	**$26,420.55**
TOTAL	**$6,921.69**	**$77,229.13**

Payment due upon receipt of invoice

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

I hereby certify that the foregoing report is true in all respects and that the expenditures have been made solely for the purposes set forth in the application for the grant as approved and that I am authorized to sign this report for the institution named.

Typed Name of Fiscal Officer	Signature	Date
Jenifer Womble Gwaltney	J. M. McGwain	**02/22/2017** EZ

For questions regarding invoice, please contact OSR
Phone: 919/966-3411 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursement and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil, or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001, and Title 31, Sections 3729-3730 and 3801-3812).

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Business Unit	UMN01	**Invoice Date**	01/13/2017
Voucher ID	04457506	**Invoice No**	GM00124208
Voucher Style	Regular	**Invoice Total**	15 127 25 USD
Supplier Name	UNIV OF NORTH CAROLINA	**Pay Terms**	Net 30
	4154 BIOINFORMATICS BLDG CB# 7555	**Voucher Source**	Online
	ATTN SUSAN THOMAS		
	CHAPEL HILL, NC 27599-7555	**Origin**	235
Entry Status	Postable	**Created On**	01/27/2017 10:43AM
Match Status	Ready	**Created By**	MOYER012
Approval Status	Approved	**Last Update**	01/27/2017 10:47AM
Post Status	Unposted	**Modified By**	MOYER012
		ERS Type	Not Applicable
		Close Status	Open
Budget Status	Not Chk'd		
Budget Misc Status	Valid		

Approval History

*View Related: Payment Inquiry Go

Notify Refresh Add Update/Display

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	1/19/2017 6:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	1/19/2017 4:22:26 PM
Invoice Coordinator Notes:	
Grant Administrator:	Erin Knudsen
Date Invoice Approved by GA:	1/25/2017 2:56:33 PM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	1/24/2017 11:19:53 AM
PI Notes:	

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: **GM00124208**

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070**	**•Interim Billing** **o Final Billing**	**Award Amount** **$120,384.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2017**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** **Current** **12/01/2016 - 12/31/2016**	**Cumulative** **12/31/2016**
Salaries	**$6,360.63**	**$13,845.87**
Fringe Benefits	**$1,430.06**	**$3,321.65**
Supplies	**$2,161.44**	**$29,087.31**
Total Direct	**$9,952.13**	**$46,254.83**
Indirect Cost	**$5,175.12**	**$24,052.61**
TOTAL	**$15,127.25**	**$70,307.44**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

I hereby certify that the foregoing report is true in all respects and that the expenditures have been made solely for the purposes set forth in the application for the grant as approved and that I am authorized to sign this report for the institution named.

Typed Name of Fiscal Officer	**Signature**	**Date**
Jenifer Womble Gwaltney	J.M.M.G.	**01/13/2017** NK

For questions regarding invoice, please contact OSR
Phone: 919/966-3411 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Prime award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil, or administrative penalties for fraud, false statements, false claims, or otherwise. (US Code Title 18, Section 1001, and Title 31, Sections 3729-3730 and 3801-3812)".

approved
1/25/17

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00124208

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070**	**•Interim Billing** **○ Final Billing**	**Award Amount** **$120,384.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2017** ✓

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES** Current 12/01/2016 - 12/31/2016 ✓	Cumulative 12/31/2016
Salaries	**$6,360.63**	**$13,845.87**
Fringe Benefits	**$1,430.06**	**$3,321.65**
Supplies	**$2,161.44**	**$29,087.31**
Total Direct	**$9,952.13**	**$46,254.83**
Indirect Cost	**$5,175.12**	**$24,052.61**
TOTAL	**$15,127.25** ✓	**$70,307.44** ✓

Payment due upon receipt of invoice

****

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I hereby certify that the foregoing report is true in all respects and that the expenditures have been made solely for the purposes set forth in the application for the grant as approved and that I am authorized to sign this report for the institution named.

Typed Name of Fiscal Officer	**Signature**	**Date**
Jenifer Womble Gwaltney	J.M.McGlynn	**01/13/2017** NK

For questions regarding invoice, please contact OSR
Phone: 919/966-3411 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Prime award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil, or administrative penalties for fraud, false statements, false claims, or otherwise. (US Code Title 18, Section 1001, and Title 31, Sections 3729-3730 and 3801-3812)".

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Business Unit	UMN01	**Invoice Date**	12/13/2016
Voucher ID	04410333	**Invoice No**	GM00123498
Voucher Style	Regular	**Invoice Total**	6,650.08 USD
Supplier Name	UNIV OF NORTH CAROLINA	**Pay Terms**	Net 30
	4154 BIOINFORMATICS BLDG CB# 7555	**Voucher Source**	Online
	ATTN: SUSAN THOMAS		
	CHAPEL HILL, NC 27599-7555	**Origin**	235
Entry Status	Postable	**Created On**	12/20/2016 10:12AM
Match Status	Ready Approval History	**Created By**	MOYER012
Approval Status	Approved	**Last Update**	12/20/2016 10:16AM
Post Status	Unposted	**Modified By**	MOYER012
		ERS Type	Not Applicable
		Close Status	Open
Budget Status	Not Chk'd		
Budget Misc Status	Valid		

*View Related Payment Inquiry Go

Notify Refresh Add Update/Display

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	12/16/2016 6:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	12/16/2016 3:51:36 PM
Invoice Coordinator Notes:	
Grant Administrator:	Erin Knudsen
Date Invoice Approved by GA:	12/19/2016 12:02:46 PM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	12/16/2016 4:13:34 PM
PI Notes:	

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: **GM00123498**

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070**	**•Interim Billing** **○ Final Billing**	**Award Amount** **$120,384.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2017**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	EXPENDITURES Current 11/01/2016 - 11/30/2016	 Cumulative 11/30/2016
Salaries	**$3,280.92**	**$7,485.24**
Fringe Benefits	**$946.36**	**$1,891.59**
Supplies	**$147.74**	**$26,925.87**
Total Direct	**$4,375.02**	**$36,302.70**
Indirect Cost	**$2,275.06**	**$18,877.49**
TOTAL	**$6,650.08**	**$55,180.19**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

I hereby certify that the foregoing report is true in all respects and that the expenditures have been made solely for the purposes set forth in the application for the grant as approved and that I am authorized to sign this report for the Institution named.

Typed Name of Fiscal Officer	Signature	Date
Jenifer Womble Gwaltney	J.W.M.G.	**12/13/2016** NK

For questions regarding Invoice, please contact OSR
Phone: 919/966-3411 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Approved
12/19/16

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00123498

Name of Sponsor:	University of Minnesota Tonya Knutson 450 McNamara Alumni Center 200 Oak Street SE. Minneapolis MN 55455-2070	•Interim Billing ○ Final Billing	Award Amount $120,384.00
Sponsor Ref No.:	N005402801		
UNC Award No.:	A17-0239-001		
Award Title:	Receptor recognition and cell entry of coronaviruses	Budget Period:	
Project ID:	5105169	From: 06/07/2016	To: 05/31/2017
knuts055@umn.edu, sub-inv@umn.edu.			

DESCRIPTION OF SERVICES	EXPENDITURES Current 11/01/2016 - 11/30/2016	Cumulative 11/30/2016
Salaries	$3,280.92	$7,485.24
Fringe Benefits	$946.36	$1,891.59
Supplies	$147.74	$26,925.87
Total Direct	$4,375.02	$36,302.70
Indirect Cost	$2,275.06	$18,877.49
TOTAL	$6,650.08	$55,180.19

Payment due upon receipt of invoice

****

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I hereby certify that the foregoing report is true in all respects and that the expenditures have been made solely for the purposes set forth in the application for the grant as approved and that I am authorized to sign this report for the institution named.

Typed Name of Fiscal Officer	Signature	Date
Jenifer Womble Gwaltney	J. M. McGwa	12/13/2016 NK

For questions regarding invoice, please contact OSR
Phone: 919/966-3411 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

UNIVERSITY OF MINNESOTA FSPRD

Business Unit UMN01
Voucher ID 04403995
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN: SUSAN THOMAS
CHAPEL HILL, NC 27599-7555
Entry Status Postable
Match Status Ready
Approval Status Approved
Post Status Unposted

Approval History

Budget Status Not Chk'd
Budget Misc Status Valid
***View Related** Payment Inquiry Go

Invoice Date 11/22/2016
Invoice No GM00122986
Invoice Total 9,090.46 USD
Pay Terms Net 30
Voucher Source Online

Origin 235
Created On 12/15/2016 12:44PM
Created By GENOX003
Last Update 12/15/2016 12:46PM
Modified By GENOX003
ERS Type Not Applicable
Close Status Open

Notify Refresh Add Update

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: **GM00122986**

Name of Sponsor:	**University of Minnesota** **Tonya Knutson** **450 McNamara Alumni Center** **200 Oak Street SE.** **Minneapolis MN 55455-2070**	**•Interim Billing** **o Final Billing**	**Award Amount** **$120,384.00**
Sponsor Ref No.:	**N005402801**		
UNC Award No.:	**A17-0239-001**		
Award Title:	**Receptor recognition and cell entry of coronaviruses**	**Budget Period:**	
Project ID:	**5105169**	**From: 06/07/2016**	**To: 05/31/2017**

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	**EXPENDITURES**	
	Current **10/01/2016 - 10/31/2016**	**Cumulative** **10/31/2016**
Salaries	**$2,361.53**	**$4,204.32**
Fringe Benefits	**$565.96**	**$945.23**
Supplies	**$3,053.07**	**$26,778.13**
Total Direct	**$5,980.56**	**$31,927.68**
Indirect Cost	**$3,109.90**	**$16,602.43**
TOTAL	**$9,090.46**	**$48,530.11**

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

I hereby certify that the foregoing report is true in all respects and that the expenditures have been made solely for the purposes set forth in the application for the grant as approved and that I am authorized to sign this report for the Institution named.

Typed Name of Fiscal Officer	**Signature**	**Date**
Jenifer Womble Gwaltney	J. Womble Gwaltney	**11/22/2016** CC

For questions regarding invoice, please contact OSR
Phone: 919/966-3411 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	11/30/2016 6:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	11/30/2016 10:10:44 AM
Invoice Coordinator Notes:	
Grant Administrator:	Erin Knudsen
Date Invoice Approved by GA:	12/8/2016 10:16:40 AM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	12/15/2016 10:14:04 AM
PI Notes:	

approved
12/8/16

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA,30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00122986

Name of Sponsor:	University of Minnesota Tonya Knutson 450 McNamara Alumni Center 200 Oak Street SE. Minneapolis MN 55455-2070	•Interim Billing ○ Final Billing	Award Amount $120,384.00
Sponsor Ref No.:	N005402801		
UNC Award No.:	A17-0239-001		
Award Title:	Receptor recognition and cell entry of coronaviruses		
Project ID:	5105169	Budget Period: From: 06/07/2016	To: 05/31/2017

knuts055@umn.edu, sub-inv@umn.edu.

DESCRIPTION OF SERVICES	EXPENDITURES Current 10/01/2016 - 10/31/2016 ✓	 Cumulative 10/31/2016
Salaries	$2,361.53	$4,204.32
Fringe Benefits	$565.96	$945.23
Supplies	$3,053.07	$26,778.13
Total Direct	$5,980.56	$31,927.68
Indirect Cost	$3,109.90 ✓	$16,602.43
TOTAL	$9,090.46	$48,530.11 ✓

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

I hereby certify that the foregoing report is true in all respects and that the expenditures have been made solely for the purposes set forth in the application for the grant as approved and that I am authorized to sign this report for the institution named.

Typed Name of Fiscal Officer	Signature	Date
Jenifer Womble Gwaltney	J. Womble Gwaltney	11/22/2016 CC

For questions regarding invoice, please contact OSR
Phone: 919/966-3411 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Summary | Related Documents | Invoice Information | Payments | Voucher Attributes | Error Summary

Business Unit UMN01
Voucher ID 04391910
Voucher Style Regular
Supplier Name UNIV OF NORTH CAROLINA
4154 BIOINFORMATICS BLDG CB# 7555
ATTN: SUSAN THOMAS
CHAPEL HILL, NC 27599-7555
Entry Status Postable
Match Status Ready
Approval Status Approved
Post Status Unposted

Approval History

Invoice Date 11/08/2016
Invoice No GM00122576
Invoice Total 39,439.65 USD
Pay Terms Net 30
Voucher Source Online
Origin 235
Created On 12/07/2016 11:12AM
Created By JKHEU
Last Update 12/07/2016 11:21AM
Modified By JKHEU
ERS Type Not Applicable
Close Status Open

Budget Status Not Chk'd
Budget Misc Status Valid
***View Related** Payment Inquiry Go

Notify Refresh Add Update/Display

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA 30384-2420

Federal Tax ID # 56-6001393
Duns # 608195277

Invoice #: GM00122576

Name of Sponsor:	University of Minnesota Tonya Knutson 450 McNamara Alumni Center 200 Oak Street SE. Minneapolis MN 55455-2070	•Interim Billing Final Billing	Award Amount $120,384.00
Sponsor Ref No.:	N005402801		
UNC Award No.:	A17-0239-001		
Award Title:	Receptor recognition and cell entry of coronaviruses	Budget Period:	
Project ID:	5105169	From: 06/07/2016	To: 05/31/2017

sub-inv@umn.edu ; knuts055@umn.edu

DESCRIPTION OF SERVICES	EXPENDITURES Current 06/07/2016 - 09/30/2016	Cumulative 09/30/2016
Salaries	$1,842.79	$1,842.79
Fringe Benefits	$379.27	$379.27
Supplies	$23,725.06	$23,725.06
Total Direct	$25,947.12	$25,947.12
Indirect Cost	$13,492.53	$13,492.53
TOTAL	$39,439.65	$39,439.65

Payment due upon receipt of invoice

****

PLEASE RETURN A COPY OF THIS INVOICE WITH YOUR PAYMENT

I hereby certify that the foregoing report is true in all respects and that the expenditures have been made solely for the purposes set forth in the application for the grant as approved and that I am authorized to sign this report for the Institution named.

Typed Name of Fiscal Officer	Signature	Date
Office of Sponsored Research		11/08/2016

For questions regarding invoice, please contact Office of Sposnored Research
Phone: 919/966-3411 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
Financial Institution: Bank of America, Chapel Hill, NC
ABA or Routing Number: 053000196
Account Number: 237025397532

By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact may subject me to criminal, civil, or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730).

Subaward Invoice Processing Data

PO#:	N005402801
Invoice Received:	11/14/2016 6:00:00 AM
Department:	PHCL Pharmacology Admin
Date Sent for Approval:	11/15/2016 8:41:51 AM
Invoice Coordinator Notes:	
Grant Administrator:	Erin Knudsen
Date Invoice Approved by GA:	11/17/2016 12:10:01 PM
GA Notes:	
Principal Investigator:	Fang Li
Date Invoice Approved by PI:	12/1/2016 9:38:40 AM
PI Notes:	

approved
11/17/16

INVOICE

The University of North Carolina at Chapel Hill

Remit Payment To:

Office of Sponsored Research
The University of North Carolina at Chapel Hill
C/O Bank of America Lockbox Services
PO Box 402420
Atlanta GA 30384-2420

Federal Tax ID = 56-6001393
Duns = 608195277

Invoice #: GM00122576

Name of Sponsor:	University of Minnesota Tonya Knutson 450 McNamara Alumni Center 200 Oak Street SE. Minneapolis MN 55455-2070	•Interim Billing Final Billing	Award Amount $120,384.00
Sponsor Ref No.:	N005402801		
UNC Award No.:	A17-0239-G01		
Award Title:	Receptor recognition and cell entry of coronaviruses	Budget Period:	
Project ID:	5105169	From: 06/07/2016	To: 05/31/2017

sub-inv@umn.edu ; knuts0b5@umn.edu

DESCRIPTION OF SERVICES	EXPENDITURES Current 06/07/2016 - 09/30/2016	 Cumulative 09/30/2016
Salaries	$1,842.79	$1,842.79
Fringe Benefits	$379.27	$379.27
Supplies	$23,725.06	$23,725.06
Total Direct	$25,947.12	$25,947.12
Indirect Cost	$13,492.53	$13,492.53
TOTAL	$39,439.65	$39,439.65

Payment due upon receipt of invoice

****

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I hereby certify that the foregoing report is true in all respects and that the expenditures have been made solely for the purposes set forth in the application for the grant as approved and that I am authorized to sign this report for the institution named.

Typed Name of Fiscal Officer	Signature	Date
Office of Sponsored Research		11/08/2016

For questions regarding invoice, please contact Office of Sposnored Research
Phone: 919/966-3411 FAX: 919/962-5011
OSRBILLING@unc.edu

ACH Payments
Account Name: NC State Treasurer
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[illegible] I hereby certify to the best of my knowledge and belief that the report is true, complete and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Section 3729-3730 and 3801-3812)

UNIVERSITY OF MINNESOTA

Purchase Order

PO # N005402801

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Federal Contract Funding? No

Bill To:	**Ship To:**
SPONSORED PROJECTS ADMIN SPONSORED PROJ ADMIN-P107220001 RM450 MCNAMARA ALUMNI CTR 200 OAK ST SE MINNEAPOLIS MN 55455	SPONSORED PROJ ADMIN-P107220001 RM450 MCNAMARA ALUMNI CTR 200 OAK ST SE MINNEAPOLIS MN 55455

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If Yes appears after the Federal Contract Funding section on this purchase order, the Seller will be contacted and required to provide additional reporting information to fulfill the Federal Acquisition Regulations (FARs).

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UNIVERSITY OF MINNESOTA

Purchase Order

PO # N005402801

(Include all digits on submitted invoice)

06/07/16-05/31/17

Line	Item / Description	Quantity	UOM	Unit Price	Extended Amt
1	receptor Recognition and Cell Entry of Coronaviruses	1.00	LOT	$120,384.00	$120,384.00

PO TOTAL AMOUNT $120,384.00

ABOVE PURCHASE ORDER IS ELECTRONICALLY APPROVED BY UNIVERSITY OF MINNESOTA.

A. COVER PAGE

Project Title: Receptor recognition and cell entry of coronaviruses	
Grant Number: 5R01AI089728-10	**Project/Grant Period:** 06/07/2016 - 05/31/2021
Reporting Period: 06/01/2019 - 05/31/2020	**Requested Budget Period:** 06/01/2020 - 05/31/2021
Report Term Frequency: Annual	**Date Submitted:** 04/13/2020
Program Director/Principal Investigator Information: FANG LI , PHD **Phone number:** 612-625-6149 **Email:** lifang@umn.edu	**Recipient Organization:** UNIVERSITY OF MINNESOTA UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER MINNEAPOLIS, MN 554552070 **DUNS:** 555917996 **EIN:** 1416007513A1 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: BRETT CARLSON 200 Oak St SE Suite 450 Minneapolis, MN 554552070 **Phone number:** 612-624-5599 **Email:** carls234@umn.edu	**Signing Official:** BRETT CARLSON 200 Oak St SE Suite 450 Minneapolis, MN 554552070 **Phone number:** 612-624-5599 **Email:** carls234@umn.edu
Human Subjects: No	**Vertebrate Animals:** No
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Specific Aims
Coronaviruses (CoVs) cause epidemic outbreaks of diseases in humans and other animals, symbolized by the recent emergence of SARS-CoV, MERS-CoV, and porcine epidemic diarrhea CoV (PEDV). Receptor recognition and cell entry are two essential steps in the CoV infection cycle and function as critical determinants of CoV host range and tissue tropism. They are also primary targets for antiviral intervention. An envelope-anchored CoV spike protein recognizes host receptors through its S1 subunit and fuses viral and host membranes through its S2 subunit. CoVs recognize a variety of host receptors through one or both of its spike S1 domains (S1-NTD and S1-CTD), and are also regulated for cell entry by host proteases that cleave at the S1/S2 boundary. In the previous funding cycle, we have determined the crystal structures of a number of CoV S1 domains alone or in complex with their receptor. We have also identified host receptors as well as the cell entry mechanisms for a number of different CoVs. Our studies have revealed how CoVs exploit host receptors and proteases to regulate their host range, tropism and pathogenesis, and how SARS-CoV and MERS-CoV transmit from animals to humans to cause epidemics. In this competitive grant renewal, we will continue to investigate fundamental questions regarding receptor recognition and cell entry of CoVs from different genera. The long-term goals of these studies are to develop global models of CoV-receptor interactions and protease processing in evolution, entry, tropism, pathogenesis and cross-species transmission of CoVs.

Aim 1: Receptor binding by CoV S1-NTDs. We previously determined the structures of beta-genus mouse CoV (MHV) S1-NTD complexed with its protein receptor CEACAM1 and of beta-genus bovine CoV (BCoV) S1-NTD by itself. We also showed that S1-NTDs of BCoV and alpha-genus PEDV both recognize sugar receptors. Our studies revealed that beta-CoV S1-NTDs have the same structural fold as human galectins (galactose-binding lectins), but bind either protein or non-galactose sugar receptors. This aim will investigate how S1-NTDs from different genera have evolved to bind sugar receptors. Our overall hypothesis is that S1-NTDs from different CoV genera originated from host galectins, but have evolved molecular mechanisms to recognize novel receptors. To test this hypothesis, we will determine the molecular and structural basis for sugar binding by BCoV and PEDV S1-NTDs. These studies will reveal evolution of CoV S1-NTDs, help evaluate sugar binding by other CoVs, and may facilitate future design of sugar analogue inhibitors and subunit vaccines against CoVs.

Aim 2: Receptor biding by CoV S1-CTDs. We determined the structures of several CoV S1-CTDs in complex with their protein receptors, including beta-genus SARS-CoV S1-CTD complexed with its protein receptor ACE2. Our research suggested that two mutations played critical roles in the transmission of SARS-CoV from palm civets to humans. However, recent studies isolated several bat SARS-like CoVs (SLCoVs) with potentials to infect humans directly. This aim will investigate the cross-species transmission pathway of bat SLCoVs by examining the interactions between their S1-CTDs and ACE2 from different species. Our overall hypothesis is that to transmit from bats to another species, bat SLCoVs need to adapt to ACE2 from that species through selective mutations in their S1-CTD. To test the hypothesis, we will (i) identify receptor and viral determinants for transmission of bat SLCoVs to other species (civet, human and mouse); (ii) determine the crystal structures of bat SLCoV S1-CTDs and ACE2 from other species. These studies will reveal the molecular mechanisms for cross-species transmission of bat SLCoVs, inform future bat SLCoV emergence pathways to other mammals, and facilitate epidemic monitoring and the development of broadly cross-protective intervention strategies.

Aim 3: Cell entry by CoVs. Previously we characterized the pre-fusion and post-fusion conformations of SARS-CoV spike, demonstrated that host proteases serve as a cross-species barrier for MERS-CoV, and showed that a glycosylated protease motif attenuates human cell entry of bat CoV HKU4. Our overall hypothesis is that CoV spikes have evolved a variety of mechanisms for protease activation that can meet their specific need for host range, tissue tropism, and pathogenesis. We will investigate the following questions regarding CoV entry. (i) How does neurotropic MHV enter neurons where receptor expression is low? (ii) How does HCoV-NL63 modulate its cell entry and pathogenesis? (iii) How does PEDV enter intestinal cells where extracellular proteases are abundant? These studies will reveal how cell entry regulates the host range, tropism and pathogenesis of CoVs, and may facilitate future design of protease inhibitors against CoVs.

Approaches: We take multi-disciplinary approaches including crystallographic studies of CoV S1 domains complexed with their receptor, biochemical assays on S1 domains/receptor binding, biochemical analysis of protease cleavages of CoV spikes, electron microscopic analysis of conformational states of CoV spikes, pseudovirus entry assays, reverse genetics, and live virus infection assays.

Impact: This research uses CoVs as model systems to establish novel principles in virology that govern viral evolution, receptor recognition, cell entry, cross-species infections and tropism, which may be extended to other virus families. These studies are also critical for evaluating the emerging disease potentials of CoVs and for preventing, controlling and treating the spread of CoVs in humans and other animals.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Plans for the Li Lab:
(1) We will determine the cell entry mechanism of SARS-CoV-2. In preliminary studies, we found that furin cleavage of SARS-CoV-2 spike is important for cell entry of SARS-CoV-2. Our previous studies also showed that lysosomal proteases and trypsin play critical roles in coronavirus entry and tropism. Moreover, for coronavirus spikes to undergo final structural changes, the spikes need to be cleaved at two sites, one between S1 and S2 (i.e., S1/S2 site) and within S2 (i.e., S2' site). The formation of S2' fragments is strongly associated with the final structural changes of coronavirus spikes. We have recently performed in-depth analysis of the triggers and structural changes of mouse coronavirus (MHV) spike protein. Here we will systematically examine the roles of proteases and receptor binding in human cell entry to SARS-CoV-2 using pseudovirus entry assay and protease sensitivity assay.
(2) We will directly view the conformational changes of SARS-CoV-2 spike using negative-stain EM and cryo-EM. Moreover, although the cryo-EM structures of pre-fusion SARS-CoV-2 spike alone or in complex of hACE2 have been determined, the cryo-EM structures of SARS-CoV-2 spike in the post-fusion conformation is not known. We will determine this unknown structure.
(3) We will determine the cell entry mechanism of a MERS-related bat coronavirus 422-CoV. We have previously shown that 422-CoV uses the same entry receptor DPP4 as MERS-CoV. In preliminary studies, we have determined the cryo-EM structure of 422-CoV spike protein in the pre-fusion conformation. This structure has revealed the unique features of 422-CoV that facilitate 422-CoV entry.
(4) We will determine the cell entry mechanism of a neurotropic strain JHM of mouse hepatitis coronavirus (MHV). In the last funding cycle, we determined the cell entry mechanism of the hepatotropic strain A59 of MHV. Compared to A59, JHM spike protein has several unique features in mediating cell entry, most notably its less dependence on receptor and its proneness to undergo conformational changes spontaneously. We will elucidate the molecular mechanism for JHM entry.

Plans for Baric Laboratory
1)We have produced a full length molecular clone for SARS-CoV-2 and are writing up the manuscript. In parallel, we will remove the furin cleavage site, isolate live recombinant viruses and valuate the impact on virus replication both in vitro and in vivo.
2)We have constructed a full length recombinant virus for MERS like BtCoV 422, recovered viruses with indicator genes. The in vitro and in vivo characteristics of this virus are being studied. We are evaluating the range of animal DPP4 proteins that can serve as a receptor for this virus.
3)We are working on the design and recovery of mouse adapted variants of the SARS-CoV-2 strain, using structure-guided mutations.

Progress report

Studies and Results

Shang et al., (2020, *Nature*) determined the crystal structure of the SARS-CoV-2 receptor-binding domain (RBD) (engineered to facilitate crystallization) in complex with hACE2. Compared with the SARS-CoV RBD, a hACE2-binding ridge in SARS-CoV-2 RBD takes a more compact conformation; moreover, several residue changes in SARS-CoV-2 RBD stabilize two virus-binding hotspots at the RBD/hACE2 interface. These structural features of SARS-CoV-2 RBD enhance its hACE2-binding affinity. Additionally, we show that RaTG13, a bat coronavirus closely related to SARS-CoV-2, also uses hACE2 as its receptor. The differences among SARS-CoV-2, SARS-CoV and RaTG13 in hACE2 recognition shed light on potential animal-to-human transmission of SARS-CoV-2. This study provides guidance for intervention strategies targeting receptor recognition by SARS-CoV-2.

Shang et al., (2020, *PLOS Pathogens*) the cryo-EM structure of MHV spike complexed with mouse CEACAM1a. The trimeric spike contains three receptor-binding S1 heads sitting on top of a trimeric membrane-fusion S2 stalk. Three receptor molecules bind to the sides of the spike trimer, where three NTDs are located. Receptor binding induces structural changes in the spike, weakening the interactions between S1 and S2. Using protease sensitivity and negative-stain EM analyses, we further showed that after protease treatment of the spike, receptor binding facilitated the dissociation of S1 from S2, allowing S2 to transition from pre-fusion to post-fusion conformation. Together these results reveal a new role of receptor binding in MHV entry: in addition to its well-characterized role in viral attachment to host cells, receptor binding also induces the conformational change of the spike and hence the fusion of viral and host membranes. Our study provides new mechanistic insight into coronavirus entry and highlights the diverse entry mechanisms used by different viruses.

Wan et al., (2020, *Journal of Virology*) analyzed the potential receptor usage by 2019-nCoV, based on the rich knowledge about SARS-CoV and the newly released sequence of 2019-nCoV. First, the sequence of 2019-nCoV RBD, including its receptor-binding motif (RBM) that directly contacts ACE2, is similar to that of SARS-CoV, strongly suggesting that 2019-nCoV uses ACE2 as its receptor. Second, several critical residues in 2019-nCoV RBM (particularly Gln493) provide favorable interactions with human ACE2, consistent with 2019-nCoV's capacity for human cell infection. Third, several other critical residues in 2019-nCoV RBM (particularly Asn501) are compatible with, but not ideal for, binding human ACE2, suggesting that 2019-nCoV has acquired some capacity for human-to-human transmission. Last, while phylogenetic analysis indicates a bat origin of 2019-nCoV, 2019-nCoV also potentially recognizes ACE2 from a diversity of animal species (except mice and rats), implicating these animal species as possible intermediate hosts or animal models for 2019-nCoV infections. These analyses provide insights into the receptor usage, cell entry, host cell infectivity and animal origin of 2019-nCoV and may help epidemic surveillance and preventive measures against 2019-nCoV.

Wan et al., (2020, *Journal of Virology*) investigated how a neutralizing monoclonal antibody (MAb), which targets the receptor-binding domain (RBD) of Middle East respiratory syndrome (MERS) coronavirus spike, mediates viral entry using pseudovirus entry and biochemical assays. Our results showed that MAb binds to the virus surface spike, allowing it to undergo conformational changes and become prone to proteolytic activation. Meanwhile, MAb binds to cell surface IgG Fc receptor, guiding viral entry through canonical viral-receptor-dependent pathways. Our data suggest that the antibody/Fc-receptor complex functionally mimics viral receptor in mediating viral entry. Moreover, we characterized MAb dosages in viral-receptor-dependent, Fc-receptor-dependent, and both-receptors-dependent viral entry pathways, delineating guidelines on MAb usages in treating viral infections. Our study reveals a novel molecular mechanism for antibody-enhanced viral entry and can guide future vaccination and antiviral strategies.

Hou et al., manuscript in preparation. We have developed a SARS-CoV-2 molecular clone, built reporter viruses and are characterizing the cells in the lung that are susceptible to infection. The manuscript in essence,

captures an atlas of the primary cells in the lung that express hACE2, TMPRSS2, TMPRSS4 and that are susceptible to SARS-CoV infection. We have also evaluated SARS-CoV-2 GFP growth in cells overexpressing furin and TMPRSS2, the latter stimulating virus growth by 1-2 logs in select cells in vitro.

Hou et al., manuscript in preparation. We have developed a full length molecular clone for the MERS-like bat coronavirus designated 422 (MERS-422). The virus replicates efficiently on cells expressing the hDPP4 receptor, can replicate in primary human airway epithelial cells, however, it doesn't replicate in the mouse. We are mapping the mDPP4/hDPP4 interaction residues that can program a productive infection, and will be testing MERS 422 pathogenesis in transgenic mice expressing the human DPP4 receptor.

Hou et al., manuscript in preparation. We are isolating recombinant SARS-CoV-2 viruses that lack the furin cleavage site, designed to evaluate the role of this sequence in virus replication in cells in vitro and in mice in vivo. We will evaluate virus growth in cells overexpressing TMPRSS2 and furin and in mice expressing the hACE2 receptor.

Plans for the Li Lab:

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3) We are working on the design and recovery of mouse adapted variants of the SARS-CoV-2 strain, using structure-guided mutations.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Zhao G, He L, Sun S, Qiu H, Tai W, Chen J, Li J, Chen Y, Guo Y, Wang Y, Shang J, Ji K, Fan R, Du E, Jiang S, Li F, Du L, Zhou Y. A Novel Nanobody Targeting Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Receptor-Binding Domain Has Potent Cross-Neutralizing Activity and Protective Efficacy against MERS-CoV. Journal of virology. 2018 September 15;92(18). PubMed PMID: 29950421; PubMed Central PMCID: PMC6146697; DOI: 10.1128/JVI.00837-18.
Complete	van Doremalen N, Schäfer A, Menachery VD, Letko M, Bushmaker T, Fischer RJ, Figueroa DM, Hanley PW, Saturday G, Baric RS, Munster VJ. SARS-Like Coronavirus WIV1-CoV Does Not Replicate in Egyptian Fruit Bats (Rousettus aegyptiacus). Viruses. 2018 December 19;10(12). PubMed PMID: 30572566; PubMed Central PMCID: PMC6316779; DOI: 10.3390/v10120727.
Complete	Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nature reviews. Microbiology. 2019 March;17(3):181-192. PubMed PMID: 30531947; PubMed Central PMCID: PMC7097006; DOI: 10.1038/s41579-018-0118-9.
Complete	Li F, Du L. MERS Coronavirus: An Emerging Zoonotic Virus. Viruses. 2019 July 19;11(7). PubMed PMID: 31331035; PubMed Central PMCID: PMC6669680; DOI: 10.3390/v11070663.
Complete	Wan Y, Shang J, Sun S, Tai W, Chen J, Geng Q, He L, Chen Y, Wu J, Shi Z, Zhou Y, Du L, Li F. Molecular Mechanism for Antibody-Dependent Enhancement of Coronavirus Entry. Journal of virology. 2020 February 14;94(5). PubMed PMID: 31826992; PubMed Central PMCID: PMC7022351; DOI: 10.1128/JVI.02015-19.
Complete	Shang J, Wan Y, Liu C, Yount B, Gully K, Yang Y, Auerbach A, Peng G, Baric R, Li F. Structure of mouse coronavirus spike protein complexed with receptor reveals mechanism for viral entry. PLoS pathogens. 2020 March;16(3):e1008392. PubMed PMID: 32150576; PubMed Central PMCID: PMC7082060; DOI: 10.1371/journal.ppat.1008392.
Complete	Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. Journal of virology. 2020 March 17;94(7). PubMed PMID: 31996437; PubMed Central PMCID: PMC7081895; DOI: 10.1128/JVI.00127-20.
PMC Journal - In process	Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, Geng Q, Auerbach A, Li F. Structural basis of receptor recognition by SARS-CoV-2. Nature. 2020 March 30. PubMed PMID: 32225175; DOI: 10.1038/s41586-020-2179-y.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
FANGLI	Y	Li, Fang	PHD	PD/PI	0.0	4.0	1.0			NA
	N	Scobey, Drew T		Technician	3.0	0.0	0.0			NA
SARAH_LEIST	N	Leist, Sarah R.	PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	1.0	0.0	0.0			NA
Minn Stat 13.32	N	Minn Stat 13.32	MS	Graduate Student (research assistant)	6.0	0.0	0.0			NA
RALPH_BARIC	Y	Baric, Ralph S	PHD,BS	Co-Investigator	1.0	0.0	0.0			NA
	N	Auerbach, Ashley		Technician	12.0	0.0	0.0			NA
Minn Stat 13.32	N	Minn Stat 13.32	BS	Graduate Student (research assistant)	1.0	0.0	0.0			NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

File uploaded: Other Support.pdf

D.2.d New Other Significant Contributors Are there, or will there be, new other significant contributors? No
D.2.e Multi-PI (MPI) Leadership Plan Will there be a change in the MPI Leadership Plan for the next budget period? NA

Fang Li Other Support

Active

Overlap: There is no scientific overlap among the following grants.

National Institutes of Health 04/1/2015 – 03/31/2020
R01AI110700 $1,862,000
Role: Co-Principal Investigator (Co-PI: Ralph Baric, Univ of North Carolina) 5.0 calendar months
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
Goal: This research investigates genetic pathways regulating MERS coronavirus cross species transmission and receptor homolog usage, establishes robust animal models of human disease, and discovers critical reagents for therapeutic and vaccine testing.

National Institutes of Health 06/7/2016 – 05/31/2021
2R01AI089728 $2,285,084
Role: Principal Investigator 3.0 calendar months
Receptor Recognition and Cell Entry of Coronaviruses
Goal: This research investigates the molecular and structural mechanisms for the receptor recognition and cell entry of coronaviruses. It explores novel principles governing viral evolution, receptor recognition, cell entry, host ranges, cross-species infections, and tissue tropisms. These studies are critical for evaluating the emerging disease potentials of coronaviruses and for preventing, controlling and treating the spread of coronaviruses in humans or other animals.

National Institutes of Health 05/21/2018 – 04/30/2023
R01AI139092 $923,000
Role: Co-Investigator (PI: Lanying Du, New York Blood Center) 1.2 calendar months
Structure-based design of coronavirus subunit vaccines
Goal: This research establishes that artificially exposed unfavorable epitopes on viral subunit vaccines pose a severe intrinsic limitation to viral subunit vaccine designs. It further develops novel structure-based approaches to overcome this limitation in vaccine design. It produces highly effective subunit vaccines to combat the infections of highly pathogenic coronaviruses such as MERS and SARS coronaviruses, and also has implications for subunit vaccine design against other life-threatening viruses.
10% effort for Fang Li

National Institutes of Health 01/01/2020 – 12/31/2024
R01CA245922 $110,880 ($554,400-total)
Role: Co-Investigator (Aaron LeBeau, University of Minnesota) 0.9 calendar months
Cyclic Peptide Protease Inhibitors for the Treatment of Prostate Cancer
Goal: This research is to develop clinically relevant therapeutics by targeting a cell surface protease overexpressed in aggressive variant prostate carcinoma. This approach has significant clinical potential to result in an effective treatment modality for a form of prostate cancer that is currently incurable.

University of Minnesota 10/01/2019-9/30/2022
Academic Investment Research Program $125,000
Role: Co-Investigator (PI: Alon Herschhorn, University of Minnesota) 0.2 calendar months
Bioengineering conformation-stabilized HIV-1 Env immunogens
Goal: This research builds a new collaborative platform for the development of a conformation-defined HIV Envelope protein vaccine.

Pending

National Institutes of Health 10/01/2020 – 09/30/2025
Antibody-dependent enhancement of coronavirus entry: molecular mechanism and clinical implications
Role: Principal Investigator
1.2 calendar months
$3,996,113

National Institutes of Health 12/01/2020 – 11/30/2025
Cell entry, cross-species transmission and pathogenesis of novel coronavirus from Wuhan
Role: Co-Investigator (PI: Ralph Baric, University of North Carolina)
2.4 calendar months
$1,897,327 (Li subaward)

National Institutes of Health 4/01/2020 – 3/31/2022
Structure-based design of RBD-based vaccines against 2019 novel coronavirus
Role: Co-Investigator (PI: Lanying Du, New York Blood Center)
1.2 calendar months
$ 432,251 (Li subaward supplement)

National Institutes of Health 9/01/2020 – 8/31/2025
Novel nanobodies to prevent and treat SARS-CoV-2 and other pathogenic human coronaviruses
Role: Co-Investigator (PI: Lanying Du, New York Blood Center)
.72 calendar months
$ 1,424,340 (Li subaward)

OTHER SUPPORT

BARIC, RALPH S. **Active Total: 11.16 cal months**

ACTIVE

R01 AI110700 (PI: Baric) 04/20/15-03/31/21 1.08 cal mos
NIH/NIAID $601,878

Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis

The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.

Foreign component funded by grant: No

There are no Domestic/Foreign gifts, appointments, lab or office space, scientific materials, affiliations, and/or foreign faculty/scholars/scientists/post-docs for this grant.

U19AI142759 CETR (PI: Whitley) 03/01/14-02/28/24 1.20 cal mos
UAB/NIH/NIAID $375,233

Antiviral Drug Discovery and Development Center (AD3C)

The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.

Role: Investigator

Foreign component funded by grant: No

There are no Domestic/Foreign gifts, appointments, lab or office space, scientific materials, affiliations, and/or foreign faculty/scholars/scientists/post-docs for this grant.

U19-AI100625 (PI: Baric/Heise-MPI) 08/05/12-08/31/22 1.80 cal mos
NIH/NIAID $2,160,886

Systems Immunogenetics of Biodefense and Emerging Pathogens in the Collaborative Cross

Specific Aims: In this proposal, we are utilizing the Collaborative Cross (CC), a novel panel of reproducible, recombinant inbred (RI) mouse lines to identify genes and gene interactions which regulate the induction, kinetics, and magnitude of the innate, inflammatory and adaptive arms of the immune response following virus infection. Specifically, we will develop novel modeling algorithms to predict and validate the causal relationships between natural genetic variation and host signaling networks, immune cell recruitment, and immune function.

Foreign component funded by grant: No

There are no Domestic/Foreign gifts, appointments, lab or office space, scientific materials, affiliations, and/or foreign faculty/scholars/scientists/post-docs for this grant.

R01AI108197 (MPI: Denison/Baric) 03/01/18-02/28/23 0.60 cal mos
Vanderbilt Univ/NIH $189,141

Determinants of Coronavirus Fidelity in Replication and Pathogenesis

To identify common and unique determinants of CoV nsp14-ExoN functions CoV replication, fidelity and IFN sensitivity across CoVs; To determine pathways of adaptation to loss of nsp14-ExoN activity in vitro and in vivo; and To define mechanisms of ExoN-regulated CoV sensitivity to the innate antiviral immune response.

Foreign component funded by grant: No

There are no Domestic/Foreign gifts, appointments, lab or office space, scientific materials, affiliations, and/or foreign faculty/scholars/scientists/post-docs for this grant.

P01 AI 106695 (PI: Harris) 07/29/15-06/30/20 0.36 cal mos
UCB/NIH $186,002

Protective immunity following dengue virus natural infections and vaccination

We will perform studies to characterize the B-cell/ antibody (responses in people who receive dengue live attenuated virus vaccines (DLAV).

Role: Investigator

Foreign component funded by grant: No

There are no Domestic/Foreign gifts, appointments, lab or office space, scientific materials, affiliations, and/or foreign faculty/scholars/scientists/post-docs for this grant.

R01AI107731 (Desilva) 08/05/13-08/31/23 0.72 cal mos
NIH/NIAID $416,379

Molecular Basis of Dengue Virus Neutralization by Human Antibodies

To determine the origin and properties of these useful cross protective antibodies. We will also study the properties of antibodies that neutralize Zika viruses.

Role: Investigator

Foreign component funded by grant: No

There are no Domestic/Foreign gifts, appointments, lab or office space, scientific materials, affiliations, and/or foreign faculty/scholars/scientists/post-docs for this grant.

K24AI141744 (Becker-Dreps) 12/06/18-11/30/23 0.12 cal mos
NIH/NIAID $157,100

The Development of Norovirus Immunity in Early Childhood and Implications for Norovirus Vaccines

To acquire new research skills and carry out a research plan that will allow guidance of the development of pediatric norovirus vaccines.

Role: Investigator

Foreign component funded by grant: Yes

Foreign investigators: Dr. Samuel Vilchez (Universidad Nacional Autonoma de Nicaragua, Leon)
Dr. Filemon Bucardo (Universidad Nacional Autonoma de Nicaragua, Leon)

In Kind Lab: Universidad Nacional Autonoma de Nicaragua, LeonCenter for Infectious Disease Research (CEI) Lab

Technology transfer to foreign site: No

U01 AI149644 (PI: Baric) 04/19/19-03/31/24 1.20 cal mos
NIH/NIAID $644,071

Respiratory Virus Vaccine and Adjuvant Exploration

This project takes advantage of expertise in adjuvant development, vaccinology, and complex trait genetics, proposes to use advanced Systems Vaccinology and Genetics approaches to define the polymorphic genes/gene networks that regulate the immune response to select respiratory virus adjuvanted immunogens.

Foreign component funded by grant: No

There are no Domestic/Foreign gifts, appointments, lab or office space, scientific materials, affiliations, and/or foreign faculty/scholars/scientists/post-docs for this grant.

R01AI089728 (PI: Li) 06/07/16-05/31/20 0.36 cal mos
Univ Minn/NIH $120,384

Receptor recognition and cell entry of coronaviruses

To investigate how CoVs explore host receptors and host proteases for regulation of their host range, cross-species transmission, tissue tropism, and pathogenesis.

Role: Subcontract PI

Foreign component funded by grant: No

There are no Domestic/Foreign gifts, appointments, lab or office space, scientific materials, affiliations, and/or foreign faculty/scholars/scientists/post-docs for this grant.

R01AI127845 (PI: Becker-Dreps) 09/27/16-08/31/21 0.12 cal mos
NIH $498,959

Natural history, immunity, and transmission patterns of sapovirus in a Nicaraguan birth cohort

To characterize the natural history and risk factors for sapovirus gastroenteritis, elucidate the development of immunity to sapovirus in early childhood and the potential protective effect of maternal immunity, and apply novel genetic and analytic tools to characterize patterns of sapovirus transmission in households and communities.

Role: Investigator

Foreign component funded by grant: Yes

Foreign investigators: Dr. Samuel Vilchez (Universidad Nacional Autonoma de Nicaragua, Leon)
Dr. Filemon Bucardo (Universidad Nacional Autonoma de Nicaragua, Leon)
In Kind Lab: Universidad Nacional Autonoma de Nicaragua, LeonCenter for Infectious Disease Research (CEI) Lab
Technology transfer to foreign site: No

R01-AI125198 (PI: DeSilva) 05/04/16-04/30/21 0.60 cal mos
NIH/NIAID $755,289

Preclinical Assays To Predict Tetravalent Dengue Vaccine Efficacy

Dengue is the most significant mosquito transmitted viral infection of humans. Vaccination is a feasible solution to prevent and control dengue. Although dengue vaccines are under development, we do not know the specific properties of antibodies induced by vaccines that are likely to protect from infection. In this project investigators from the University of North Carolina and Sanofi Pasture, a leading dengue vaccine developer, will collaborate to define properties of antibodies induced by the Sanofi vaccine that correlate with protection. The main goal of the project is to develop new assays to support the current global effort to develop dengue virus vaccines.
Role: Investigator
Foreign component funded by grant: No
There are no Domestic/Foreign gifts, appointments, lab or office space, scientific materials, affiliations, and/or foreign faculty/scholars/scientists/post-docs for this grant.

R01AI132178 (PI: Baric/Sheahan) 08/09/17-07/31/22 0.60 cal mos
NIH $919,427

Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV

To focus on two areas: novel second generation compounds or compounds not previously provided by Gilead Sciences; and selecting and evaluating drug resistance profiles for SARS-CoV and MERS-CoV mutants in primary human lung cells.
Foreign component funded by grant: No
There are no Domestic/Foreign gifts, appointments, lab or office space, scientific materials, affiliations, and/or foreign faculty/scholars/scientists/post-docs for this grant.

D43 TW010923 (PI: Becker-Dreps/Meshnick) 05/10/18-02/28/23 0.24 cal mos.
NIH $230,000

Nicaraguan Emerging and Endemic Diseases (NEED)

The goals of this program are to 1) train young Nicaraguan scientists in Infectious Disease Epidemiology at the UNC, 2) create a sustainable supply of scientists in the region by establishing an accredited PhD program in Biomedical Sciences at the Universidad Nacional Autonoma de Nicaragua Leon and 3) foster professional growth and development among trainees and local faculty to ensure academic and research success.
Role: Investigator
Foreign component funded by grant: Yes
Foreign investigators: Dr. Samuel Vilchez (Universidad Nacional Autonoma de Nicaragua, Leon)
Dr. Filemon Bucardo (Universidad Nacional Autonoma de Nicaragua, Leon)
In Kind Lab: Universidad Nacional Autonoma de Nicaragua, LeonCenter for Infectious Disease Research (CEI) Lab
Technology transfer to foreign site: Training in epidemiologic research methods, basic molecular and immunological laboratory methods

U01 AI141997 (PI: Kirkpatrick) 02/01/19-01/31/24 0.24 cal mos
Univ Vermont/NIH $64,128

Mechanisms of Protection and Durability for a Live Attenuated Tetravalent Dengue Vaccine

Tetravalent dengue vaccination must offer safe and durable protection against all four serotypes of dengue viruses. We leverage four vaccine trials and viral challenges evaluating the NIH dengue vaccine to explore and confirm immune mechanisms associated with protection.
Role: Investigator
Foreign component funded by grant: No

There are no Domestic/Foreign gifts, appointments, lab or office space, scientific materials, affiliations, and/or foreign faculty/scholars/scientists/post-docs for this grant.

R21 AI137887 (MPI: Moorman/Heise) 02/05/18-01/31/21 0.12 cal mos
NIH/NIAID $125,000
Molecular Characterization of Functional RNA Structures in the ZikV genome
Zika virus is an emerging pathogen that is associated with severe congenital neurologic defects, such as microcephaly. The proposed studies will identify new viral virulence determinants that can be targeted to generate safer and more effective Zika virus vaccines and therapeutics. Role: Investigator
Foreign component funded by grant: No
There are no Domestic/Foreign gifts, appointments, lab or office space, scientific materials, affiliations, and/or foreign faculty/scholars/scientists/post-docs for this grant.

HHSN272201700036I (PI: Baric) 07/15/19-07/14/24 0.60 cal mos
NIH/NIAID $442,129
Task Order A24 - Establishment of Chronic Bacterial Infection Models in Mouse Models of Cystic Fibrosis with Pseudomonas aeruginosa and Staphylococcus aureus
To test and refine currently available mouse models of cystic fibrosis (CF) chronic lung infections. Key determinants for this refinement include the ability to screen existing clinical isolate collections (both P. aeruginosa and S. aureus) to identify bacterial strains with increased likelihood of establishing chronic lung infections in wild-type (WT) mice using an established protocol for embedding bacteria in agar beads and the availability of Cftr-deficient mice to test the outcome of chronic infection with novel bacterial strains.
Foreign component funded by grant: No
There are no Domestic/Foreign gifts, appointments, lab or office space, scientific materials, affiliations, and/or foreign faculty/scholars/scientists/post-docs for this grant.

HHSN272201700036I (PI: Baric) 09/15/19-03/14/21 0.60 cal mos
NIH/NIAID $271,640
Task Order A36 - Efficacy of Coronavirus Vaccines and Monoclonal Antibodies in Mouse Models
To use our novel panel of recombinant virus challenge strains to assess the efficacy of candidate vaccine and therapeutic monoclonal antibodies in murine coronavirus challenge models and to understand the immunological mechanisms and correlates of protection.
Foreign component funded by grant: No
There are no Domestic/Foreign gifts, appointments, lab or office space, scientific materials, affiliations, and/or foreign faculty/scholars/scientists/post-docs for this grant.

R21AI151231 (PI: Koller) 03/03/20-02/28/22 0.60 cal mos
NIH/NIAID $150,000
Genetically humanized mice for modeling human Fc-receptor interaction during influenza infection
The goal of this proposal is to develop and refine tools for the study of human antibody receptor interactions in vivo. Role: Investigator
Foreign component funded by grant: No
There are no Domestic/Foreign gifts, appointments, lab or office space, scientific materials, affiliations, and/or foreign faculty/scholars/scientists/post-docs for this grant.

PENDING:

R01AI110964-06 (PI: Daszak) 07/01/19-06/30/24 0.24 cal mos
EcoHealth Alliance/NIH $50,000
Understanding the Risk of Bat Coronavirus Emergence
The program articulates new strategies to identify high risk locations for virus emergence and epidemic spread and applies new methods to limit the spread to high risk human populations.

R01 AI148260 (PI: Baric) 09/01/19-08/31/24 1.2 cal mos
NIH/NIAID $619,713
Antibody Landscape following Human Norovirus Infection and Vaccination
These studies will reveal important new insights into the virus epitopes that elicit type specific and broadly neutralizing antibody (bnAB), reveal mechanisms of cross protection, antibody neutralization and escape, and provide an atomic level understanding of HuNoV neutralization and pandemic strain emergence mechanisms, leading to improved 2nd generation vaccines for in vivo testing.

OVERLAP: None

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

No

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: Regents of the University of Minnesota	555917996	MN-005	3-284 Nils Hasselmo Hall 312 Church Street SE Minneapolis MN 554550215
University of North Carolina - Chapel Hill	608195277	NC-004	3109 Hooker Research Center 135 Dauer Drive Chapel Hill NC 275997435
UNIVERSITY OF MINNESOTA	555917996	MN-005	UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER MINNEAPOLIS MN 554552070
UNIVERSITY OF MINNESOTA	555917996		UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER MINNEAPOLIS MN 554552070
Regents of the University of Minnesota	555917996	MN-005	3-284 Nils Hasselmo Hall 312 Church Street SE Minneapolis MN 554550215
University of North Carolina - Chapel Hill	608195277	NC-004	3109 Hooker Research Center 135 Dauer Drive

			Chapel Hill NC 275997435
UNIVERSITY OF MINNESOTA	555917996	MN-005	UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER MINNEAPOLIS MN 554552070

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

A. COVER PAGE

Project Title: Receptor recognition and cell entry of coronaviruses	
Grant Number: 5R01AI089728-10	**Project/Grant Period:** 05/15/2010 - 05/31/2021
Reporting Period: 06/01/2020 - 05/31/2021	**Requested Budget Period:** 06/01/2020 - 05/31/2021
Report Term Frequency: Annual	**Date Submitted:** 09/15/2021
Program Director/Principal Investigator Information: FANG LI , PHD **Phone Number:** 612-625-6149 **Email:** lifang@umn.edu	**Recipient Organization:** UNIVERSITY OF MINNESOTA UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER MINNEAPOLIS, MN 554552070 **DUNS:** 555917996 **EIN:** 1416007513A1 **RECIPIENT ID:**
Change of Contact PD/PI: NA	
Administrative Official: BRETT CARLSON 200 Oak St SE Suite 450 Minneapolis, MN 554552070 **Phone number:** 612-624-5599 **Email:** carls234@umn.edu	**Signing Official:** BRETT CARLSON 200 Oak St SE Suite 450 Minneapolis, MN 554552070 **Phone number:** 612-624-5599 **Email:** carls234@umn.edu
Human Subjects: No	**Vertebrate Animals:** NA
hESC: No	**Inventions/Patents:** Yes **If yes, previously reported:** Yes

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Specific Aims
Coronaviruses (CoVs) cause epidemic outbreaks of diseases in humans and other animals, symbolized by the recent emergence of SARS-CoV, MERS-CoV, and porcine epidemic diarrhea CoV (PEDV). Receptor recognition and cell entry are two essential steps in the CoV infection cycle and function as critical determinants of CoV host range and tissue tropism. They are also primary targets for antiviral intervention. An envelope-anchored CoV spike protein recognizes host receptors through its S1 subunit and fuses viral and host membranes through its S2 subunit. CoVs recognize a variety of host receptors through one or both of its spike S1 domains (S1-NTD and S1-CTD), and are also regulated for cell entry by host proteases that cleave at the S1/S2 boundary. In the previous funding cycle, we have determined the crystal structures of a number of CoV S1 domains alone or in complex with their receptor. We have also identified host receptors as well as the cell entry mechanisms for a number of different CoVs. Our studies have revealed how CoVs exploit host receptors and proteases to regulate their host range, tropism and pathogenesis, and how SARS-CoV and MERS-CoV transmit from animals to humans to cause epidemics. In this competitive grant renewal, we will continue to investigate fundamental questions regarding receptor recognition and cell entry of CoVs from different genera. The long-term goals of these studies are to develop global models of CoV-receptor interactions and protease processing in evolution, entry, tropism, pathogenesis and cross-species transmission of CoVs.

Aim 1: Receptor binding by CoV S1-NTDs. We previously determined the structures of beta-genus mouse CoV (MHV) S1-NTD complexed with its protein receptor CEACAM1 and of beta-genus bovine CoV (BCoV) S1-NTD by itself. We also showed that S1-NTDs of BCoV and alpha-genus PEDV both recognize sugar receptors. Our studies revealed that beta-CoV S1-NTDs have the same structural fold as human galectins (galactose-binding lectins), but bind either protein or non-galactose sugar receptors. This aim will investigate how S1-NTDs from different genera have evolved to bind sugar receptors. Our overall hypothesis is that S1-NTDs from different CoV genera originated from host galectins, but have evolved molecular mechanisms to recognize novel receptors. To test this hypothesis, we will determine the molecular and structural basis for sugar binding by BCoV and PEDV S1-NTDs. These studies will reveal evolution of CoV S1-NTDs, help evaluate sugar binding by other CoVs, and may facilitate future design of sugar analogue inhibitors and subunit vaccines against CoVs.

Aim 2: Receptor biding by CoV S1-CTDs. We determined the structures of several CoV S1-CTDs in complex with their protein receptors, including beta-genus SARS-CoV S1-CTD complexed with its protein receptor ACE2. Our research suggested that two mutations played critical roles in the transmission of SARS-CoV from palm civets to humans. However, recent studies isolated several bat SARS-like CoVs (SLCoVs) with potentials to infect humans directly. This aim will investigate the cross-species transmission pathway of bat SLCoVs by examining the interactions between their S1-CTDs and ACE2 from different species. Our overall hypothesis is that to transmit from bats to another species, bat SLCoVs need to adapt to ACE2 from that species through selective mutations in their S1-CTD. To test the hypothesis, we will (i) identify receptor and viral determinants for transmission of bat SLCoVs to other species (civet, human and mouse); (ii) determine the crystal structures of bat SLCoV S1-CTDs and ACE2 from other species. These studies will reveal the molecular mechanisms for cross-species transmission of bat SLCoVs, inform future bat SLCoV emergence pathways to other mammals, and facilitate epidemic monitoring and the development of broadly cross-protective intervention strategies.

Aim 3: Cell entry by CoVs. Previously we characterized the pre-fusion and post-fusion conformations of SARS-CoV spike, demonstrated that host proteases serve as a cross-species barrier for MERS-CoV, and showed that a glycosylated protease motif attenuates human cell entry of bat CoV HKU4. Our overall hypothesis is that CoV spikes have evolved a variety of mechanisms for protease activation that can meet their specific need for host range, tissue tropism, and pathogenesis. We will investigate the following questions regarding CoV entry. (i) How does neurotropic MHV enter neurons where receptor expression is low? (ii) How does HCoV-NL63 modulate its cell entry and pathogenesis? (iii) How does PEDV enter intestinal cells where extracellular proteases are abundant? These studies will reveal how cell entry regulates the host range, tropism and pathogenesis of CoVs, and may facilitate future design of protease inhibitors against CoVs.

Approaches: We take multi-disciplinary approaches including crystallographic studies of CoV S1 domains complexed with their receptor, biochemical assays on S1 domains/receptor binding, biochemical analysis of protease cleavages of CoV spikes,

electron microscopic analysis of conformational states of CoV spikes, pseudovirus entry assays, reverse genetics, and live virus infection assays.

Impact: This research uses CoVs as model systems to establish novel principles in virology that govern viral evolution, receptor recognition, cell entry, cross-species infections and tropism, which may be extended to other virus families. These studies are also critical for evaluating the emerging disease potentials of CoVs and for preventing, controlling and treating the spread of CoVs in humans and other animals.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

Using funding from this R01 grant in the past funding cycle (2016-2021), Dr. Li has published 21 peer-reviewed papers.

He was the corresponding author in 17 of these publications, including 1 in Nature, 1 in PNAS, 1 in Nature Communications, 1 in eLife, 3 in PLOS Pathogens, 7 in Journal of Virology, 1 in Journal of Biological Chemistry, 1 in Viruses, and 1 in Annual Review of Virology.

He also published 4 other papers as a middle author in Nature Review Microbiology (1), Expert Opinion On Therapeutic Targets (1), and Journal of Virology (2).

Over the past year Dr. Li and Dr. Baric and their groups have given numerous invited talks at ASV, Cell and Host Meeting, Scripps and at numerous academic institutions, worldwide.

In 2021, Dr. Baric presented results at the International Nidovirales Conference in June 2021, the World Microbiology Forum in June 202, the Innovative Mouse Model Workshop (IMM2021 workshop) held in June 2021, and the international cytokine conference, 2020, focused on emerging coronaviruses, mouse models, pathogenesis and entry/host range mechanisms.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Not Applicable

B.2 (Accomplishments.pdf)

B. Accomplishments

We made the following discoveries during the funding cycle of this R01 grant:

B1. Receptor recognition and cell entry mechanisms of MERS-CoV and intervention strategies

My lab examined the cell entry mechanisms of MERS-CoV. We discovered that two mutations in the protease cleavage sites of MERS-CoV spike allowed MERS-CoV to transmit from bats to humans, either directly or through intermediate hosts (**Yang, Y., et al., *Journal of Virology*, 2015)**. My lab further showed that different lysosomal protease activities from different host species and tissue cells are an important determinant of the species and tissue tropism of MERS-CoV and SARS-CoV-1 (**Zheng, Y. el al., *Journal of Virology*, 2018)**. My lab also revealed a novel molecular mechanism for antibody-dependent enhancement (ADE) of MERS-CoV entry, where antibody/Fc-receptor complex functionally mimics viral receptor in mediating coronavirus entry; we also characterized antibody dosages in ADE of coronavirus entry, delineating guidelines on antibody usages in treating viral infections (**Wan, Y., et al., *Journal of Virology*, 2020)**. Moreover, my lab showed that a recently discovered bat coronavirus uses the same DPP4 receptor as MERS-CoV, providing insight into the animal origin of MERS-CoV (**Luo, C., et al., *Journal of Virology*, 2018).**

Based on our understanding of the structure and function of coronavirus spike proteins, my lab developed a glycan probe-based strategy to improve the efficacy of RBD subunit vaccines against MERS-CoV infection (**Du, L., et al., *Nature Communications*, 2016**). In collaboration with other groups, my lab participated in the development of other intervention strategies against MERS-CoV, including vaccines and therapeutic antibodies **(Tai, W. et al., *Journal of Virology*, 2016; Zhao, G. et al., *Journal of Virology*, 2018)**.

In addition to the above research articles, I also led or co-authored in several review articles on MERS-CoV **(Li, F. and Du, L., *Viruses*, 2019; Cui, J. et al., *Nature Reviews Microbiology*, 2019; Du, L., et al., *Expert Opinion On Therapeutic Targets,* 2017)**.

B2. Receptor recognition and cell entry mechanisms of animal coronaviruses

My lab determined three novel cryo-EM structures of the spike proteins from animal coronaviruses. First, the cryo-EM structure of porcine deltacoronavirus (PdCoV) spike protein in the prefusion state revealed that δ-coronavirus spikes are structurally and evolutionally more closely related to α-coronavirus spikes than to β-coronavirus spikes; it also has implications for the receptor recognition, membrane fusion, and immune evasion by deltacoronaviruses as well as coronaviruses in general **(Shang, J., et al., *Journal of Virology*, 2018)**. Second, the cryo-EM structure of infectious bronchitis coronavirus (IBV) spike protein reveals structural and functional evolution of coronavirus spike proteins, suggesting that the evolutionary spectrum of coronavirus spikes follows the order of α-, δ-, γ-, and β-genus **(Shang, J., et al., *PLOS Pathogens*, 2018)**. Third, the cryo-EM structure of mouse coronavirus (MHV) spike protein complexed with its mouse receptor revealed a new role of receptor binding in mouse coronavirus entry: in addition to its well-characterized role in viral attachment to host cells, receptor binding also induces the conformational change of the spike protein and hence the fusion of viral and host membranes; this study provides new mechanistic insight into

coronavirus entry and highlights the diverse entry mechanisms used by different viruses **(Shang, J., et al., *PLOS Pathogens*, 2020).**

Besides above cryo-EM structures, my lab determined the crystal structure of a variant of mouse receptor for mouse coronavirus (MHV); this study elucidated the detailed molecular mechanism for how mice may have kept pace in the evolutionary arms race with MHV by undergoing structural and residue changes in the MHV receptor, providing insight into this possible example of pathogen-driven evolution of a host receptor protein **(Peng, G., et al., *Journal of Biological Chemistry*, 2017).** My lab also determined the cellular proteases that activate the entry of porcine epidemic diarrhea coronavirus (PEDV); this study advanced our understanding of the molecular mechanism for PEDV entry and identified potential antiviral targets for curbing the spread of PEDV **(Liu, C., et al., *Journal of Biological Chemistry*, 2016)**.

In addition to the above research articles, I also wrote on a highly influential review article on the structure, function and evolution of coronavirus spike proteins **(Li, F., *Annual Review of Virology,* 2016),** which was the most downloaded article among all *Annual Reviews* articles in 2020.

B3. Molecular mechanisms of COVID-19 and intervention strategies

Using funding of this R01, my lab has led several milestone studies on the molecular mechanisms of COVID-19 and intervention strategies.

First, one of the goals of our coronavirus research was to build an atomic-level iterative framework of virus-receptor interactions to facilitate epidemic surveillance, predict species-specific receptor usage, and identify potential animal hosts and animal models of viruses. Based on the sequence of SARS-CoV-2 spike protein, we applied this predictive framework to provide novel insights into the receptor usage and likely host range of SARS-CoV-2. This study provides a robust test of this reiterative framework, providing the basic, translational, and public health research communities with predictive insights that may help study and battle this novel SARS-CoV-2 **(Wan, Y., et al., *Journal of Virology*, 2020).**

Second, we determined the crystal structure of the receptor-binding domain (RBD) of the spike protein of SARS-CoV-2 in complex with ACE2. We identified structural features of SARS-CoV-2 RBD that increase its ACE2-binding affinity. Additionally, we showed that RaTG13, a bat coronavirus that is closely related to SARS-CoV-2, also uses human ACE2 as its receptor. The differences among SARS-CoV-2, SARS-CoV-1 and RaTG13 in ACE2 recognition shed light on the potential animal-to-human transmission of SARS-CoV-2. This study provides guidance for intervention strategies that target receptor recognition by SARS-CoV-2 **(Shang, J., et al., *Nature*, 2020).**

Third, we investigated receptor binding and protease activation of SARS-CoV-2 spike using biochemical and pseudovirus entry assays. Our findings have identified key cell entry mechanisms of SARS-CoV-2: the high hACE2 binding affinity of the RBD, furin preactivation of the spike, and hidden RBD in the spike potentially allow SARS-CoV-2 to maintain efficient cell entry while evading immune surveillance. These features may contribute to the wide spread of the virus **(Shang, J., et al., PNAS, 2020)**.

Fourth, we identified a series of single-domain antibodies (i.e., nanobody), Nanosota-1, from a camelid nanobody phage display library. Our lead nanobody Nanosota-1C-Fc bound to SARS-CoV-2 RBD ~3000 times more tightly than ACE2 did and inhibited SARS-CoV-2

pseudovirus ~160 times more efficiently than ACE2 did. Administered at a single dose, Nanosota-1C-Fc demonstrated preventive and therapeutic efficacy against live SARS-CoV-2 infection in both hamster and mouse models. Unlike conventional antibodies, Nanosota-1C-Fc was produced at high yields in bacteria and had exceptional thermostability. Pharmacokinetic analysis of Nanosota-1C-Fc documented an excellent in vivo stability and a high tissue bioavailability. As effective and inexpensive drug candidates, Nanosota-1 may contribute to the battle against COVID-19 **(Gang, Y., et al., *eLife*, 2021).**

Last, we developed a novel virus-like nanoparticle (VLP) vaccine that displays 120 copies of SARS-CoV-2 RBD on its surface. This VLP-RBD vaccine mimics virus-based vaccines in immunogen display, which boosts its efficacy, while maintaining the safety of protein-based subunit vaccines. Compared to the RBD vaccine, the VLP-RBD vaccine induced five times more neutralizing antibodies in mice that efficiently blocked SARS-CoV-2 from attaching to its host receptor and potently neutralized the cell entry of variant SARS-CoV-2 strains, SARS-CoV-1, and SARS-CoV-1-related bat coronavirus. These neutralizing immune responses induced by the VLP-RBD vaccine did not wane during the two-month study period. Furthermore, the VLP-RBD vaccine effectively protected mice from SARS-CoV-2 challenge, dramatically reducing the development of clinical signs and pathological changes in immunized mice. The VLP-RBD vaccine provides one potentially effective solution to controlling the spread of SARS-CoV-2 **(Geng, Q., et al., *PLOS Pathogens*, 2021).**

B.4 (B4.pdf)

B.4 What opportunities for training and professional development has the project provided?

The research program graduate students and postdocs and provides these trainees with training in virus reverse genetics, SARS-CoV2 pathogenesis, viral immunology, structure function studies and animal model development. Each trainee receives one on one instruction from the PIs on experimental design and interpretation and learns specific in viral genetics, virology, and immunology techniques. Each trainee receives extensive training in scientific communication through preparing manuscripts and presenting their work at national and international meetings. Each student also creates an Individual Development Plan (IDP). To develop their IDPs, the trainees have access to a number of resources that are provided by the university, primarily through the Office of Graduate Education that are designed to help students prepare their IDPs and to help P.I.s mentor and guide their trainees in this process. These resources include websites with links to useful programs, webinars, and face-to-face workshops. Drs. Li and Baric also actively work with each trainee as they develop their IDP, and they have regular meetings with each trainee throughout their tenure in the lab to discuss progress, determine if the goals of the IDP are met, and to modify the IDP, if necessary.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Yang Y, Liu C, Du L, Jiang S, Shi Z, Baric RS, Li F. Two Mutations Were Critical for Bat-to-Human Transmission of Middle East Respiratory Syndrome Coronavirus. Journal of virology. 2015 September;89(17):9119-23. PubMed PMID: 26063432; PubMed Central PMCID: PMC4524054; DOI: 10.1128/JVI.01279-15.
Complete	Shang J, Zheng Y, Yang Y, Liu C, Geng Q, Luo C, Zhang W, Li F. Cryo-EM structure of infectious bronchitis coronavirus spike protein reveals structural and functional evolution of coronavirus spike proteins. PLoS pathogens. 2018 April;14(4):e1007009. PubMed PMID: 29684066; PubMed Central PMCID: PMC5933801; DOI: 10.1371/journal.ppat.1007009.
Complete	Luo CM, Wang N, Yang XL, Liu HZ, Zhang W, Li B, Hu B, Peng C, Geng QB, Zhu GJ, Li F, Shi ZL. Discovery of Novel Bat Coronaviruses in South China That Use the Same Receptor as Middle East Respiratory Syndrome Coronavirus. Journal of virology. 2018 July 1;92(13). PubMed PMID: 29669833; PubMed Central PMCID: PMC6002729; DOI: 10.1128/JVI.00116-18.
Complete	Zheng Y, Shang J, Yang Y, Liu C, Wan Y, Geng Q, Wang M, Baric R, Li F. Lysosomal Proteases Are a Determinant of Coronavirus Tropism. Journal of virology. 2018 December 15;92(24). PubMed PMID: 30258004; PubMed Central PMCID: PMC6258935; DOI: 10.1128/JVI.01504-18.
Complete	Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, Li F. Cell entry mechanisms of SARS-CoV-2. Proceedings of the National Academy of Sciences of the United States of America. 2020 May 26;117(21):11727-11734. PubMed PMID: 32376634; PubMed Central PMCID: PMC7260975; DOI: 10.1073/pnas.2003138117.
Complete	Hou YJ, Okuda K, Edwards CE, Martinez DR, Asakura T, Dinnon KH 3rd, Kato T, Lee RE, Yount BL, Mascenik TM, Chen G, Olivier KN, Ghio A, Tse LV, Leist SR, Gralinski LE, Schäfer A, Dang H, Gilmore R, Nakano S, Sun L, Fulcher ML, Livraghi-Butrico A, Nicely NI, Cameron M, Cameron C, Kelvin DJ, de Silva A, Margolis DM, Markmann A, Bartelt L, Zumwalt R, Martinez FJ, Salvatore SP, Borczuk A, Tata PR, Sontake V, Kimple A, Jaspers I, O'Neal WK, Randell SH, Boucher RC, Baric RS. SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract. Cell. 2020 July 23;182(2):429-446.e14. PubMed PMID: 32526206; PubMed Central PMCID: PMC7250779; DOI: 10.1016/j.cell.2020.05.042.
Complete	Ye G, Gallant J, Zheng J, Massey C, Shi K, Tai W, Odle A, Vickers M, Shang J, Wan Y, Du L, Aihara H, Perlman S, LeBeau A, Li F. The development of *Nanosota-1* as anti-SARS-CoV-2 nanobody drug candidates. eLife. 2021 August 2;10. PubMed PMID: 34338634; PubMed Central PMCID: PMC8354634; DOI: 10.7554/eLife.64815.
PMC Journal - In process	Geng Q, Tai W, Baxter VK, Shi J, Wan Y, Zhang X, Montgomery SA, Taft-Benz SA, Anderson EJ, Knight AC, Dinnon KH 3rd, Leist SR, Baric RS, Shang J, Hong SW, Drelich A, Tseng CK, Jenkins M, Heise M, Du L, Li F. Novel virus-like nanoparticle vaccine effectively protects

	animal model from SARS-CoV-2 infection. PLoS pathogens. 2021 September 7;17(9):e1009897. PubMed PMID: 34492082; DOI: 10.1371/journal.ppat.1009897.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? Yes

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? Yes

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Category	Explanation
Other	This research did not generate DNA probes, animal models, protocols, or software. Generated cell lines and raw data from the published experiments are available to anyone upon written request to the PI. All protein structures determined during the past project period are being or have been entered in public databases (PDB or EMDB). Peer-reviewed publications accepted or published during the past project period are being or have been entered in NIHMS and PubMed Central.

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
FANGLI	Y	Li, Fang	PHD	PD/PI	5.2	0.0	0.0			NA
Minn Stat 13.32	N	Minn Stat 13.32	BS	Graduate Student (research assistant)	3.0	0.0	0.0			NA
Minn Stat 13.32	N	Minn Stat 13.32	MS	Graduate Student (research assistant)	2.0	0.0	0.0			NA
RALPH_BARIC	N	Baric, Ralph S	PHD,BS	Co-Investigator	0.4	0.0	0.0			NA
	N	May, Samantha		Technician	6.0	0.0	0.0			NA
WEI-ZHANG	N	Zhang, Wei	PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	7.0	0.0	0.0			NA
	N	Huang, Linfen		Technician	5.6	0.0	0.0			NA
	N	Scobey, Drew T		Technician	1.8	0.0	0.0			NA
SARAH_LEIST	N	Leist, Sarah R.	PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	3.0	0.0	0.0			NA

Glossary of acronyms:

S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Not Applicable

D.2.b New Senior/Key Personnel

Not Applicable

D.2.c Changes in Other Support

Not Applicable

D.2.d New Other Significant Contributors

Not Applicable

D.2.e Multi-PI (MPI) Leadership Plan

Not Applicable

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

G. SPECIAL REPORTING REQUIREMENTS SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

NOT APPLICABLE

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

I. OUTCOMES

I.1 What were the outcomes of the award?

In the past funding cycle of this grant, we have successfully achieved our goals. (i) We continued our pioneering work on the receptor recognition and cell entry of MERS-CoV, and also performed milestone studies on the molecular mechanisms of COVID-19. (ii) We determined more novel structures of coronavirus spike proteins, including cryo-EM structures of porcine deltacoronavirus (PdCoV) spike protein, cryo-EM structure of infectious bronchitis coronavirus (IBV) spike protin, cryo-EM structure of mouse coronavirus (MHV) spike protein complexed with its mouse receptor, and crystal structure of a variant of mouse receptor for mouse coronavirus. We also determined the cellular proteases that activate the entry of PEDV. (iii) We designed and developed vaccines and therapeutic antibodies against SARS-CoV-2 and MERS-CoV.
Overall, using the funds of this R01, my group has done pioneering work on the cell entry mechanisms of SARS-CoV-2 (which causes COVID-19), SARS-CoV-1 (which caused the 2002-2003 SARS epidemic), MERS-CoV, and many other coronaviruses. More specifically, my group has determined many structures of coronavirus invasion proteins and discovered the molecular events that lead to coronavirus entry into host cells. My research has been one of the major driving forces in shaping up what we now know about receptor recognition and cell entry of coronaviruses. Moreover, we have developed structure-based strategies for vaccine design and drug development. Our research provides foundational knowledge for the field of coronaviruses.

As a structural and molecular biologist, I use in my research multidisciplinary techniques such as cutting-edge X-ray crystallography, cryo-electron microscopy, protein biochemistry, molecular biology, vaccine design and drug development. My long-term goals are to (i) identify novel principles in virology that govern receptor recognition, cell entry, and epidemics of viruses, (ii) understand the structure and function of proteins that are critical for virus infections, and (iii) develop novel intervention strategies to prevent and treat viral infections.

spaimaging SPA IMAGING <spaimaging@umn.edu>

Request for Imaging

1 message

Brett Carlson <carls234@umn.edu> Wed, Sep 15, 2021 at 3:51 PM
To: "SPA IMAGING (spaimaging)" <spaimaging@umn.edu>

DOCUMENT TYPE:

CORRESPONDENCE A

CON NUMBER:58289
PROJECT NUMBER:00054028

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Brett Carlson
Sr. Grant & Contract Officer
University of Minnesota
Office of Sponsored Projects Administration
450 McNamara Alumni Center
200 Oak Street SE
Minneapolis, MN 55455

3 attachments

eRA Commons_ The Final RPPR for Award R01AI089728-10 has been submitted to the NIH.pdf
147K

FRPPR9934969.pdf
445K

Fwd_ Closeout of Award 5R01AI089728-10.pdf
158K

Subject: eRA Commons: The Final RPPR for Award R01AI089728-10 has been submitted to the NIH
From: era-notify@mail.nih.gov
Date: 9/15/2021, 3:46 PM
To: lifang@umn.edu, carls234@umn.edu
CC: aieranotifications1@mail.nih.gov, awards@umn.edu, awards@umn.edu

*** This is an automated notification - Please do not reply to this message. ***

The Final RPPR has been submitted to the NIH for Award R01AI089728-10 in the eRA Commons by: Brett Carlson. You may access this documentation in eRA Commons by going to Status, executing a query for the grant and selecting link associated with the Award Number.

For any further questions about this email, call the eRA Service Desk at 1-866-504-9552 or refer to http://grants.nih.gov/support for additional methods of contact.
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A. COVER PAGE

Project Title: Receptor recognition and cell entry of coronaviruses	
Grant Number: 5R01AI089728-10	**Project/Grant Period:** 05/15/2010 - 05/31/2021
Reporting Period: 06/01/2020 - 05/31/2021	**Requested Budget Period:** 06/01/2020 - 05/31/2021
Report Term Frequency: Annual	**Date Submitted:**
Program Director/Principal Investigator Information: FANG LI , PHD **Phone Number:** 612-625-6149 **Email:** lifang@umn.edu	**Recipient Organization:** UNIVERSITY OF MINNESOTA UNIVERSITY OF MINNESOTA TWIN CITIES 450 MCNAMARA ALUMNI CENTER MINNEAPOLIS, MN 554552070 **DUNS:** 555917996 **EIN:** 1416007513A1 **RECIPIENT ID:**
Change of Contact PD/PI: NA	
Administrative Official: BRETT CARLSON 200 Oak St SE Suite 450 Minneapolis, MN 554552070 **Phone number:** 612-624-5599 **Email:** carls234@umn.edu	**Signing Official:** BRETT CARLSON 200 Oak St SE Suite 450 Minneapolis, MN 554552070 **Phone number:** 612-624-5599 **Email:** carls234@umn.edu
Human Subjects: No	**Vertebrate Animals:** NA
hESC: No	**Inventions/Patents:** Yes **If yes, previously reported:** Yes

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Specific Aims
Coronaviruses (CoVs) cause epidemic outbreaks of diseases in humans and other animals, symbolized by the recent emergence of SARS-CoV, MERS-CoV, and porcine epidemic diarrhea CoV (PEDV). Receptor recognition and cell entry are two essential steps in the CoV infection cycle and function as critical determinants of CoV host range and tissue tropism. They are also primary targets for antiviral intervention. An envelope-anchored CoV spike protein recognizes host receptors through its S1 subunit and fuses viral and host membranes through its S2 subunit. CoVs recognize a variety of host receptors through one or both of its spike S1 domains (S1-NTD and S1-CTD), and are also regulated for cell entry by host proteases that cleave at the S1/S2 boundary. In the previous funding cycle, we have determined the crystal structures of a number of CoV S1 domains alone or in complex with their receptor. We have also identified host receptors as well as the cell entry mechanisms for a number of different CoVs. Our studies have revealed how CoVs exploit host receptors and proteases to regulate their host range, tropism and pathogenesis, and how SARS-CoV and MERS-CoV transmit from animals to humans to cause epidemics. In this competitive grant renewal, we will continue to investigate fundamental questions regarding receptor recognition and cell entry of CoVs from different genera. The long-term goals of these studies are to develop global models of CoV-receptor interactions and protease processing in evolution, entry, tropism, pathogenesis and cross-species transmission of CoVs.

Aim 1: Receptor binding by CoV S1-NTDs. We previously determined the structures of beta-genus mouse CoV (MHV) S1-NTD complexed with its protein receptor CEACAM1 and of beta-genus bovine CoV (BCoV) S1-NTD by itself. We also showed that S1-NTDs of BCoV and alpha-genus PEDV both recognize sugar receptors. Our studies revealed that beta-CoV S1-NTDs have the same structural fold as human galectins (galactose-binding lectins), but bind either protein or non-galactose sugar receptors. This aim will investigate how S1-NTDs from different genera have evolved to bind sugar receptors. Our overall hypothesis is that S1-NTDs from different CoV genera originated from host galectins, but have evolved molecular mechanisms to recognize novel receptors. To test this hypothesis, we will determine the molecular and structural basis for sugar binding by BCoV and PEDV S1-NTDs. These studies will reveal evolution of CoV S1-NTDs, help evaluate sugar binding by other CoVs, and may facilitate future design of sugar analogue inhibitors and subunit vaccines against CoVs.

Aim 2: Receptor biding by CoV S1-CTDs. We determined the structures of several CoV S1-CTDs in complex with their protein receptors, including beta-genus SARS-CoV S1-CTD complexed with its protein receptor ACE2. Our research suggested that two mutations played critical roles in the transmission of SARS-CoV from palm civets to humans. However, recent studies isolated several bat SARS-like CoVs (SLCoVs) with potentials to infect humans directly. This aim will investigate the cross-species transmission pathway of bat SLCoVs by examining the interactions between their S1-CTDs and ACE2 from different species. Our overall hypothesis is that to transmit from bats to another species, bat SLCoVs need to adapt to ACE2 from that species through selective mutations in their S1-CTD. To test the hypothesis, we will (i) identify receptor and viral determinants for transmission of bat SLCoVs to other species (civet, human and mouse); (ii) determine the crystal structures of bat SLCoV S1-CTDs and ACE2 from other species. These studies will reveal the molecular mechanisms for cross-species transmission of bat SLCoVs, inform future bat SLCoV emergence pathways to other mammals, and facilitate epidemic monitoring and the development of broadly cross-protective intervention strategies.

Aim 3: Cell entry by CoVs. Previously we characterized the pre-fusion and post-fusion conformations of SARS-CoV spike, demonstrated that host proteases serve as a cross-species barrier for MERS-CoV, and showed that a glycosylated protease motif attenuates human cell entry of bat CoV HKU4. Our overall hypothesis is that CoV spikes have evolved a variety of mechanisms for protease activation that can meet their specific need for host range, tissue tropism, and pathogenesis. We will investigate the following questions regarding CoV entry. (i) How does neurotropic MHV enter neurons where receptor expression is low? (ii) How does HCoV-NL63 modulate its cell entry and pathogenesis? (iii) How does PEDV enter intestinal cells where extracellular proteases are abundant? These studies will reveal how cell entry regulates the host range, tropism and pathogenesis of CoVs, and may facilitate future design of protease inhibitors against CoVs.

Approaches: We take multi-disciplinary approaches including crystallographic studies of CoV S1 domains complexed with their receptor, biochemical assays on S1 domains/receptor binding, biochemical analysis of protease cleavages of CoV spikes,

electron microscopic analysis of conformational states of CoV spikes, pseudovirus entry assays, reverse genetics, and live virus infection assays.

Impact: This research uses CoVs as model systems to establish novel principles in virology that govern viral evolution, receptor recognition, cell entry, cross-species infections and tropism, which may be extended to other virus families. These studies are also critical for evaluating the emerging disease potentials of CoVs and for preventing, controlling and treating the spread of CoVs in humans and other animals.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

Using funding from this R01 grant in the past funding cycle (2016-2021), Dr. Li has published 21 peer-reviewed papers.

He was the corresponding author in 17 of these publications, including 1 in Nature, 1 in PNAS, 1 in Nature Communications, 1 in eLife, 3 in PLOS Pathogens, 7 in Journal of Virology, 1 in Journal of Biological Chemistry, 1 in Viruses, and 1 in Annual Review of Virology.

He also published 4 other papers as a middle author in Nature Review Microbiology (1), Expert Opinion On Therapeutic Targets (1), and Journal of Virology (2).

Over the past year Dr. Li and Dr. Baric and their groups have given numerous invited talks at ASV, Cell and Host Meeting, Scripps and at numerous academic institutions, worldwide.

In 2021, Dr. Baric presented results at the International Nidovirales Conference in June 2021, the World Microbiology Forum in June 202, the Innovative Mouse Model Workshop (IMM2021 workshop) held in June 2021, and the international cytokine conference, 2020, focused on emerging coronaviruses, mouse models, pathogenesis and entry/host range mechanisms.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Not Applicable

B.2 (Accomplishments.pdf)

B. Accomplishments

We made the following discoveries during the funding cycle of this R01 grant:

B1. Receptor recognition and cell entry mechanisms of MERS-CoV and intervention strategies

My lab examined the cell entry mechanisms of MERS-CoV. We discovered that two mutations in the protease cleavage sites of MERS-CoV spike allowed MERS-CoV to transmit from bats to humans, either directly or through intermediate hosts (**Yang, Y., et al., *Journal of Virology*, 2015)**. My lab further showed that different lysosomal protease activities from different host species and tissue cells are an important determinant of the species and tissue tropism of MERS-CoV and SARS-CoV-1 (**Zheng, Y. el al., *Journal of Virology*, 2018)**. My lab also revealed a novel molecular mechanism for antibody-dependent enhancement (ADE) of MERS-CoV entry, where antibody/Fc-receptor complex functionally mimics viral receptor in mediating coronavirus entry; we also characterized antibody dosages in ADE of coronavirus entry, delineating guidelines on antibody usages in treating viral infections (**Wan, Y., et al., *Journal of Virology*, 2020)**. Moreover, my lab showed that a recently discovered bat coronavirus uses the same DPP4 receptor as MERS-CoV, providing insight into the animal origin of MERS-CoV (**Luo, C., et al., *Journal of Virology*, 2018).**

Based on our understanding of the structure and function of coronavirus spike proteins, my lab developed a glycan probe-based strategy to improve the efficacy of RBD subunit vaccines against MERS-CoV infection (**Du, L., et al., *Nature Communications*, 2016**). In collaboration with other groups, my lab participated in the development of other intervention strategies against MERS-CoV, including vaccines and therapeutic antibodies **(Tai, W. et al., *Journal of Virology*, 2016; Zhao, G. et al., *Journal of Virology*, 2018)**.

In addition to the above research articles, I also led or co-authored in several review articles on MERS-CoV **(Li, F. and Du, L., *Viruses*, 2019; Cui, J. et al., *Nature Reviews Microbiology*, 2019; Du, L., et al., *Expert Opinion On Therapeutic Targets*, 2017)**.

B2. Receptor recognition and cell entry mechanisms of animal coronaviruses

My lab determined three novel cryo-EM structures of the spike proteins from animal coronaviruses. First, the cryo-EM structure of porcine deltacoronavirus (PdCoV) spike protein in the prefusion state revealed that δ-coronavirus spikes are structurally and evolutionally more closely related to α-coronavirus spikes than to β-coronavirus spikes; it also has implications for the receptor recognition, membrane fusion, and immune evasion by deltacoronaviruses as well as coronaviruses in general **(Shang, J., et al., *Journal of Virology*, 2018)**. Second, the cryo-EM structure of infectious bronchitis coronavirus (IBV) spike protein reveals structural and functional evolution of coronavirus spike proteins, suggesting that the evolutionary spectrum of coronavirus spikes follows the order of α-, δ-, γ-, and β-genus **(Shang, J., et al., *PLOS Pathogens*, 2018)**. Third, the cryo-EM structure of mouse coronavirus (MHV) spike protein complexed with its mouse receptor revealed a new role of receptor binding in mouse coronavirus entry: in addition to its well-characterized role in viral attachment to host cells, receptor binding also induces the conformational change of the spike protein and hence the fusion of viral and host membranes; this study provides new mechanistic insight into

coronavirus entry and highlights the diverse entry mechanisms used by different viruses **(Shang, J., et al., *PLOS Pathogens*, 2020).**

Besides above cryo-EM structures, my lab determined the crystal structure of a variant of mouse receptor for mouse coronavirus (MHV); this study elucidated the detailed molecular mechanism for how mice may have kept pace in the evolutionary arms race with MHV by undergoing structural and residue changes in the MHV receptor, providing insight into this possible example of pathogen-driven evolution of a host receptor protein **(Peng, G., et al., *Journal of Biological Chemistry*, 2017).** My lab also determined the cellular proteases that activate the entry of porcine epidemic diarrhea coronavirus (PEDV); this study advanced our understanding of the molecular mechanism for PEDV entry and identified potential antiviral targets for curbing the spread of PEDV **(Liu, C., et al., *Journal of Biological Chemistry*, 2016)**.

In addition to the above research articles, I also wrote on a highly influential review article on the structure, function and evolution of coronavirus spike proteins **(Li, F., *Annual Review of Virology,* 2016),** which was the most downloaded article among all *Annual Reviews* articles in 2020.

B3. Molecular mechanisms of COVID-19 and intervention strategies

Using funding of this R01, my lab has led several milestone studies on the molecular mechanisms of COVID-19 and intervention strategies.

First, one of the goals of our coronavirus research was to build an atomic-level iterative framework of virus-receptor interactions to facilitate epidemic surveillance, predict species-specific receptor usage, and identify potential animal hosts and animal models of viruses. Based on the sequence of SARS-CoV-2 spike protein, we applied this predictive framework to provide novel insights into the receptor usage and likely host range of SARS-CoV-2. This study provides a robust test of this reiterative framework, providing the basic, translational, and public health research communities with predictive insights that may help study and battle this novel SARS-CoV-2 **(Wan, Y., et al., *Journal of Virology*, 2020).**

Second, we determined the crystal structure of the receptor-binding domain (RBD) of the spike protein of SARS-CoV-2 in complex with ACE2. We identified structural features of SARS-CoV-2 RBD that increase its ACE2-binding affinity. Additionally, we showed that RaTG13, a bat coronavirus that is closely related to SARS-CoV-2, also uses human ACE2 as its receptor. The differences among SARS-CoV-2, SARS-CoV-1 and RaTG13 in ACE2 recognition shed light on the potential animal-to-human transmission of SARS-CoV-2. This study provides guidance for intervention strategies that target receptor recognition by SARS-CoV-2 **(Shang, J., et al., *Nature*, 2020).**

Third, we investigated receptor binding and protease activation of SARS-CoV-2 spike using biochemical and pseudovirus entry assays. Our findings have identified key cell entry mechanisms of SARS-CoV-2: the high hACE2 binding affinity of the RBD, furin preactivation of the spike, and hidden RBD in the spike potentially allow SARS-CoV-2 to maintain efficient cell entry while evading immune surveillance. These features may contribute to the wide spread of the virus **(Shang, J., et al., PNAS, 2020)**.

Fourth, we identified a series of single-domain antibodies (i.e., nanobody), Nanosota-1, from a camelid nanobody phage display library. Our lead nanobody Nanosota-1C-Fc bound to SARS-CoV-2 RBD ~3000 times more tightly than ACE2 did and inhibited SARS-CoV-2

pseudovirus ~160 times more efficiently than ACE2 did. Administered at a single dose, Nanosota-1C-Fc demonstrated preventive and therapeutic efficacy against live SARS-CoV-2 infection in both hamster and mouse models. Unlike conventional antibodies, Nanosota-1C-Fc was produced at high yields in bacteria and had exceptional thermostability. Pharmacokinetic analysis of Nanosota-1C-Fc documented an excellent in vivo stability and a high tissue bioavailability. As effective and inexpensive drug candidates, Nanosota-1 may contribute to the battle against COVID-19 **(Gang, Y., et al., *eLife*, 2021).**

Last, we developed a novel virus-like nanoparticle (VLP) vaccine that displays 120 copies of SARS-CoV-2 RBD on its surface. This VLP-RBD vaccine mimics virus-based vaccines in immunogen display, which boosts its efficacy, while maintaining the safety of protein-based subunit vaccines. Compared to the RBD vaccine, the VLP-RBD vaccine induced five times more neutralizing antibodies in mice that efficiently blocked SARS-CoV-2 from attaching to its host receptor and potently neutralized the cell entry of variant SARS-CoV-2 strains, SARS-CoV-1, and SARS-CoV-1-related bat coronavirus. These neutralizing immune responses induced by the VLP-RBD vaccine did not wane during the two-month study period. Furthermore, the VLP-RBD vaccine effectively protected mice from SARS-CoV-2 challenge, dramatically reducing the development of clinical signs and pathological changes in immunized mice. The VLP-RBD vaccine provides one potentially effective solution to controlling the spread of SARS-CoV-2 **(Geng, Q., et al., *PLOS Pathogens*, 2021).**

B.4 (B4.pdf)

B.4 What opportunities for training and professional development has the project provided?

The research program graduate students and postdocs and provides these trainees with training in virus reverse genetics, SARS-CoV2 pathogenesis, viral immunology, structure function studies and animal model development. Each trainee receives one on one instruction from the PIs on experimental design and interpretation and learns specific in viral genetics, virology, and immunology techniques. Each trainee receives extensive training in scientific communication through preparing manuscripts and presenting their work at national and international meetings. Each student also creates an Individual Development Plan (IDP). To develop their IDPs, the trainees have access to a number of resources that are provided by the university, primarily through the Office of Graduate Education that are designed to help students prepare their IDPs and to help P.I.s mentor and guide their trainees in this process. These resources include websites with links to useful programs, webinars, and face-to-face workshops. Drs. Li and Baric also actively work with each trainee as they develop their IDP, and they have regular meetings with each trainee throughout their tenure in the lab to discuss progress, determine if the goals of the IDP are met, and to modify the IDP, if necessary.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Yang Y, Liu C, Du L, Jiang S, Shi Z, Baric RS, Li F. Two Mutations Were Critical for Bat-to-Human Transmission of Middle East Respiratory Syndrome Coronavirus. Journal of virology. 2015 September;89(17):9119-23. PubMed PMID: 26063432; PubMed Central PMCID: PMC4524054; DOI: 10.1128/JVI.01279-15.
Complete	Shang J, Zheng Y, Yang Y, Liu C, Geng Q, Luo C, Zhang W, Li F. Cryo-EM structure of infectious bronchitis coronavirus spike protein reveals structural and functional evolution of coronavirus spike proteins. PLoS pathogens. 2018 April;14(4):e1007009. PubMed PMID: 29684066; PubMed Central PMCID: PMC5933801; DOI: 10.1371/journal.ppat.1007009.
Complete	Luo CM, Wang N, Yang XL, Liu HZ, Zhang W, Li B, Hu B, Peng C, Geng QB, Zhu GJ, Li F, Shi ZL. Discovery of Novel Bat Coronaviruses in South China That Use the Same Receptor as Middle East Respiratory Syndrome Coronavirus. Journal of virology. 2018 July 1;92(13). PubMed PMID: 29669833; PubMed Central PMCID: PMC6002729; DOI: 10.1128/JVI.00116-18.
Complete	Zheng Y, Shang J, Yang Y, Liu C, Wan Y, Geng Q, Wang M, Baric R, Li F. Lysosomal Proteases Are a Determinant of Coronavirus Tropism. Journal of virology. 2018 December 15;92(24). PubMed PMID: 30258004; PubMed Central PMCID: PMC6258935; DOI: 10.1128/JVI.01504-18.
Complete	Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, Li F. Cell entry mechanisms of SARS-CoV-2. Proceedings of the National Academy of Sciences of the United States of America. 2020 May 26;117(21):11727-11734. PubMed PMID: 32376634; PubMed Central PMCID: PMC7260975; DOI: 10.1073/pnas.2003138117.
Complete	Hou YJ, Okuda K, Edwards CE, Martinez DR, Asakura T, Dinnon KH 3rd, Kato T, Lee RE, Yount BL, Mascenik TM, Chen G, Olivier KN, Ghio A, Tse LV, Leist SR, Gralinski LE, Schäfer A, Dang H, Gilmore R, Nakano S, Sun L, Fulcher ML, Livraghi-Butrico A, Nicely NI, Cameron M, Cameron C, Kelvin DJ, de Silva A, Margolis DM, Markmann A, Bartelt L, Zumwalt R, Martinez FJ, Salvatore SP, Borczuk A, Tata PR, Sontake V, Kimple A, Jaspers I, O'Neal WK, Randell SH, Boucher RC, Baric RS. SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract. Cell. 2020 July 23;182(2):429-446.e14. PubMed PMID: 32526206; PubMed Central PMCID: PMC7250779; DOI: 10.1016/j.cell.2020.05.042.
Complete	Ye G, Gallant J, Zheng J, Massey C, Shi K, Tai W, Odle A, Vickers M, Shang J, Wan Y, Du L, Aihara H, Perlman S, LeBeau A, Li F. The development of *Nanosota-1* as anti-SARS-CoV-2 nanobody drug candidates. eLife. 2021 August 2;10. PubMed PMID: 34338634; PubMed Central PMCID: PMC8354634; DOI: 10.7554/eLife.64815.
PMC Journal - In process	Geng Q, Tai W, Baxter VK, Shi J, Wan Y, Zhang X, Montgomery SA, Taft-Benz SA, Anderson EJ, Knight AC, Dinnon KH 3rd, Leist SR, Baric RS, Shang J, Hong SW, Drelich A, Tseng CK, Jenkins M, Heise M, Du L, Li F. Novel virus-like nanoparticle vaccine effectively protects

	animal model from SARS-CoV-2 infection. PLoS pathogens. 2021 September 7;17(9):e1009897. PubMed PMID: 34492082; DOI: 10.1371/journal.ppat.1009897.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? Yes

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? Yes

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Category	Explanation
Other	This research did not generate DNA probes, animal models, protocols, or software. Generated cell lines and raw data from the published experiments are available to anyone upon written request to the PI. All protein structures determined during the past project period are being or have been entered in public databases (PDB or EMDB). Peer-reviewed publications accepted or published during the past project period are being or have been entered in NIHMS and PubMed Central.

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
FANGLI	Y	Li, Fang	PHD	PD/PI	5.2	0.0	0.0			NA
Minn Stat 13.32	N	Minn Stat 13.32	BS	Graduate Student (research assistant)	3.0	0.0	0.0			NA
Minn Stat 13.32	N	Minn Stat 13.32	MS	Graduate Student (research assistant)	2.0	0.0	0.0			NA
RALPH_BARIC	N	Baric, Ralph S	PHD,BS	Co-Investigator	0.4	0.0	0.0			NA
	N	May, Samantha		Technician	6.0	0.0	0.0			NA
WEI-ZHANG	N	Zhang, Wei	PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	7.0	0.0	0.0			NA
	N	Huang, Linfen		Technician	5.6	0.0	0.0			NA
	N	Scobey, Drew T		Technician	1.8	0.0	0.0			NA
SARAH_LEIST	N	Leist, Sarah R.	PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	3.0	0.0	0.0			NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Not Applicable

D.2.b New Senior/Key Personnel

Not Applicable

D.2.c Changes in Other Support

Not Applicable
D.2.d New Other Significant Contributors Not Applicable
D.2.e Multi-PI (MPI) Leadership Plan Not Applicable

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES? Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE? NOTHING TO REPORT
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER? Not Applicable
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)? NOTHING TO REPORT

G. SPECIAL REPORTING REQUIREMENTS SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

NOT APPLICABLE

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable
G.12 F&A COSTS Not Applicable

I. OUTCOMES

I.1 What were the outcomes of the award?

In the past funding cycle of this grant, we have successfully achieved our goals. (i) We continued our pioneering work on the receptor recognition and cell entry of MERS-CoV, and also performed milestone studies on the molecular mechanisms of COVID-19. (ii) We determined more novel structures of coronavirus spike proteins, including cryo-EM structures of porcine deltacoronavirus (PdCoV) spike protein, cryo-EM structure of infectious bronchitis coronavirus (IBV) spike protin, cryo-EM structure of mouse coronavirus (MHV) spike protein complexed with its mouse receptor, and crystal structure of a variant of mouse receptor for mouse coronavirus. We also determined the cellular proteases that activate the entry of PEDV. (iii) We designed and developed vaccines and therapeutic antibodies against SARS-CoV-2 and MERS-CoV.
Overall, using the funds of this R01, my group has done pioneering work on the cell entry mechanisms of SARS-CoV-2 (which causes COVID-19), SARS-CoV-1 (which caused the 2002-2003 SARS epidemic), MERS-CoV, and many other coronaviruses. More specifically, my group has determined many structures of coronavirus invasion proteins and discovered the molecular events that lead to coronavirus entry into host cells. My research has been one of the major driving forces in shaping up what we now know about receptor recognition and cell entry of coronaviruses. Moreover, we have developed structure-based strategies for vaccine design and drug development. Our research provides foundational knowledge for the field of coronaviruses.

As a structural and molecular biologist, I use in my research multidisciplinary techniques such as cutting-edge X-ray crystallography, cryo-electron microscopy, protein biochemistry, molecular biology, vaccine design and drug development. My long-term goals are to (i) identify novel principles in virology that govern receptor recognition, cell entry, and epidemics of viruses, (ii) understand the structure and function of proteins that are critical for virus infections, and (iii) develop novel intervention strategies to prevent and treat viral infections.

Subject: Fwd: Closeout of Award 5R01AI089728-10
From: Lesley Schmidt Sindberg <schm1421@umn.edu>
Date: 9/15/2021, 12:07 PM
To: Brett Carlson <carls234@umn.edu>
CC: lifang University of Minnesota <lifang@umn.edu>

Hi Brett,

The final RPPR for Dr. Fang Li's NIH award 5R01AI089728-10 is ready to submit and has been routed to you in the Commons. Please let us know if you have any questions.

Thanks,
Lesley

---------- Forwarded message ---------
From: **lifang University of Minnesota** <lifang@umn.edu>
Date: Wed, Jun 23, 2021 at 9:20 PM
Subject: Fwd: Closeout of Award 5R01AI089728-10
To: Anne Mockovak <a-mock@umn.edu>, Lesley Schmidt Sindberg <schm1421@umn.edu>

---------- Forwarded message ---------
From: <desireej@mail.nih.gov>
Date: Mon, Jun 7, 2021 at 3:08 PM
Subject: Closeout of Award 5R01AI089728-10
To: <carls234@umn.edu>, <awards@umn.edu>
Cc: <lifang@umn.edu>, <desireej@mail.nih.gov>

Dear Signing Official:

Title of Project: Receptor recognition and cell entry of coronaviruses

The above referenced grant ended on 05/31/2021. As reflected in the terms and conditions in the final Notice of Award, NIH grant closeout policy requires the submission of three final reports no later than 120 calendar days after termination of the grant. The following documents must be submitted no later than 09/28/2021.

Final Federal Financial Report (Final FFR) - The recipient organization business office is responsible for the completion and submission of this document. NIH requires submission of the Final FFR via the Payment Management System (PMS) at https://pms.psc.gov. The Final FFR must indicate the exact balance of unobligated funds and may not reflect unliquidated obligations. There must be no discrepancies between the Final FFR expenditure data and FFR cash transaction data in PMS. It is the recipient's responsibility to reconcile reports submitted to PMS and to the NIH awarding IC. NIH cannot close the grant if the Federal share of expenditures reported on the Final expenditure FFR do not match the net cash disbursements reported in the last PMS quarterly cash disbursement report.

Final Research Performance Progress Report (FRPPR): An FRPPR is required, per section 8.6.2 of the NIH Grants Policy Statement. NIH requires submission of the FRPPR via the eRA Commons at https://commons.era.nih.gov/commons. Information on the FRPPR, including screenshots and an instruction guide, may be found at: http://grants.nih.gov/grants/rppr/index.htm

Final Invention Statement (HHS 568) - The Final Invention Statement form is located at http://grants.nih.gov/grants/forms.htm

All final Federal Financial Report expenditure data, if applicable, must be submitted through PMS at https://pms.psc.gov. All non-financial closeout documents (the Final Progress Report and the Final Invention Statement, if applicable) must be submitted through the eRA Commons at https://commons.era.nih.gov/commons or at the address below.

Thank you for your attention in fulfilling your organization's grant closeout requirements. Should you have any questions, please contact me at the address below.

Sincerely,

Campbell, Desiree
Grants Closeout Specialist
National Institutes of Health
Office of Extramural Research
Division of Central Grants Processing
Grants Closeout Center
6705 Rockledge Drive
Room 5016, MSC 7986
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