PI: **Li, Fang**	Title: Receptor recognition and cell entry of coronaviruses	
Received: 11/03/2015	Opportunity: PA-13-302	Council: 05/2016
Competition ID: FORMS-C	FOA Title: RESEARCH PROJECT GRANT (PARENT R01)	
2R01AI089728-06	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

Redacted by agreement; Non Key Personnel

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

Redacted by agreement; Non Key Personnel

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*

Redacted by agreement; Non Key Personnel

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

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

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 Redacted by agreement 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, Redacted by agreement; Specific Animal Location 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 Redacted by agreement; Specific Animal Location; Square Footage of newly renovated or new BSL3 facilities with enhanced features including Redacted by agreement; Specific Animal Location; Facility Security Redacted by agreement; Specific Animal Location; Facility Security Redacted by agreement; Specific Animal Location; Facility Security PAPR and tyvek suits are worn at all times in the BSL3 facility. Redacted by agreement; Specific Animal Location Redacted by agreement; Specific Animal Location 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. Redacted by agreement; Specific Animal Location Redacted by agreement; Specific Animal Location

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. Redacted by agreement; Specific Animal Location

Redacted by agreement; Specific Animal Location

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^{o}C, Thermo Sci Isotemp –20^{o}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: eRA Commons User Name

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: eRA Commons User Name

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): eRA Commons User Name

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): eRA Commons User Name

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 Private Source **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	Institutional Base Salary	EFFORT			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	EFFORT			40,000.00	8,959.00	48,959.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Postdoc Researcher				30,000.00	6,719.00	36,719.00
1	Jr. Scientist				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	Institutional Base Salary	EFFORT			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	EFFORT			40,000.00	8,959.00	48,959.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Postdoc Researcher				30,000.00	6,719.00	36,719.00
1	Jr. Scientist				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)

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)

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	Institutional Base Salary	EFFORT			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	EFFORT			40,000.00	8,959.00	48,959.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Postdoc Researcher				30,000.00	6,719.00	36,719.00
1	Jr. Scientist				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)

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 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)

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 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	Institutional Base Salary	EFFORT			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	EFFORT			40,000.00	8,959.00	48,959.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Postdoc Researcher				30,000.00	6,719.00	36,719.00
1	Jr. Scientist				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)

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 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	Institutional Base Salary	EFFORT			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	EFFORT			40,000.00	8,959.00	48,959.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Postdoc Researcher				30,000.00	6,719.00	36,719.00
1	Jr. Scientist				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)

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 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) 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) 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) 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		

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	Institutional Base Salary	EFFORT			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	EFFORT			17,822.00	3,351.00	21,173.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician				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**

Redacted by agreement; Non Key Personnel

Redacted by agreement; Non Key Personnel

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	Institutional Base Salary	EFFORT			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	EFFORT			17,822.00	3,351.00	21,173.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician				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**

Redacted by agreement; Non Key Personnel

Redacted by agreement; Non Key Personnel

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)

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*: 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	Institutional Base Salary	EFFORT			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	EFFORT			17,822.00	3,351.00	21,173.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician				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**

Redacted by agreement; Non Key Personnel

Redacted by agreement; Non Key Personnel

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)

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*: 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	Institutional Base Salary	EFFORT			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	EFFORT			17,822.00	3,351.00	21,173.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician				14,859.00	5,568.00	20,427.00
	Redacted by agreement; Non Key Personnel						
2	**Total Number Other Personnel**					**Total Other Personnel**	**41,600.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**48,514.00**

Redacted by agreement; Non Key Personnel

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)

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*: 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)

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 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	Institutional Base Salary	EFFORT			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	EFFORT			17,822.00	3,351.00	21,173.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician, Redacted by agreement; Non Key Personnel				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**

Redacted by agreement; Non Key Personnel

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 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. Redacted and Redacted by agreement 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.

Institutional Base Salary

Redacted by agreement; Non Key Personnel

Redacted by agreement; Non Key Personnel **Postdoctoral Research Associate** EFFORT 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 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.

Redacted by agreement; Non Key Personnel

Redacted by agreement; Non Key Personnel

Redacted by agreement; Non Key Personnel **Research Technician** EFFORT 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 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.

Redacted by agreement; Non Key Personnel

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**

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

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*:

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	Redacted by agreement; Letters of Support
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.

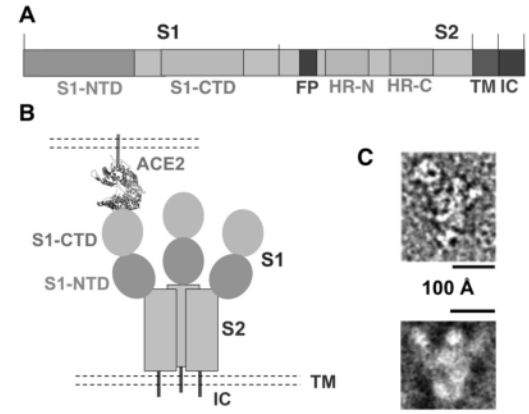


Figure 1. Overall structure of SARS-CoV spike revealed by Dr. Li's electron microscopic study. (A) Schematic structure of CoV spike. S1-NTD: N-terminal domain of S1. S1-CTD: C-terminal domain of S1. FP: fusion peptide. HR-N and HR-C: heptad repeat regions N and C. FP, HR-N and HR-C are required for membrane 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

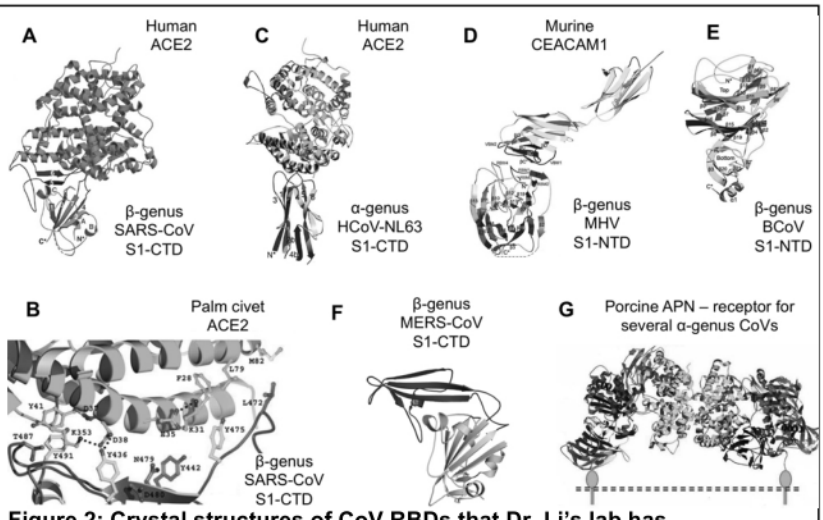


Figure 2: Crystal structures of CoV RBDs that Dr. Li's lab has determined. (A) SARS-CoV S1-CTD complexed with human ACE2 (2). (B) SARS-CoV S1-CTD complexed with palm civet ACE2 (1, 3); (C) Human coronavirus NL63 (HCoV-NL63) S1-CTD complexed with human ACE2 (3, 13); (D) Mouse CoV (MHV) S1-NTD complexed 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.

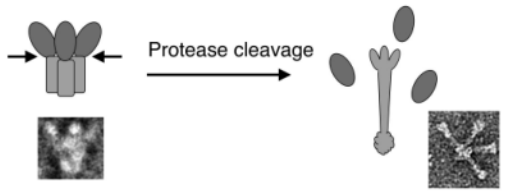
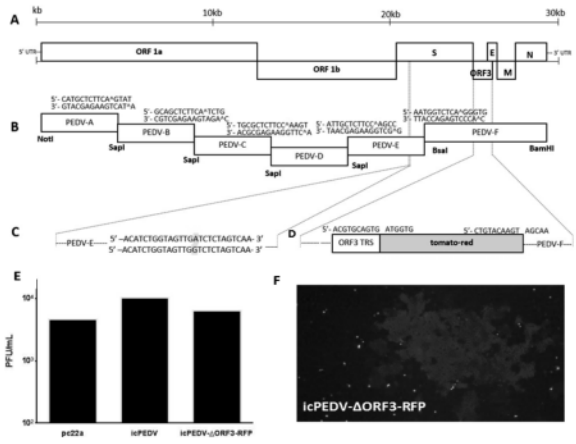


Figure 3. Conformational changes of SARS-CoV spike revealed by Dr. Li's electron microscopic study (10). Shown are schematic drawings of SARS-CoV spike in two different states and their corresponding electron microscopic images. The molecule in the pre-fusion state (left) is a clove-shaped trimer, with 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.

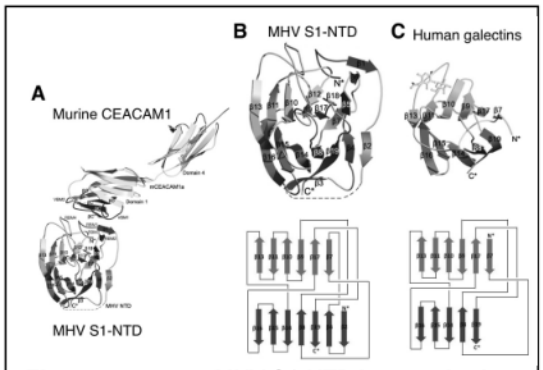


Figure 5. β-genus MHV S1-NTD has a galectin fold. (A) Crystal structure of MHV S1-NTD complexed with its receptor CEACAM1 (11). (B) Structure of MHV S1-NTD (top) and its schematic topology (bottom). (C) Structure of human galectins (top) and their schematic topology (bottom).

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. Redacted by agreement 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.

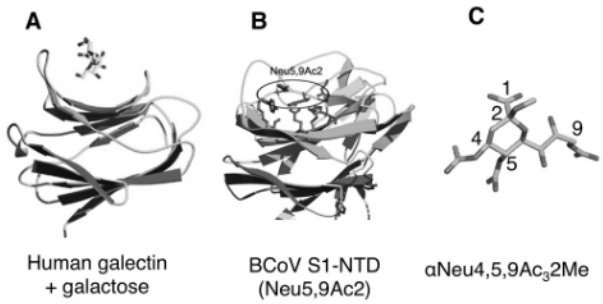


Figure 6. β-genus BCoV S1-NTD and human galectins share the same structural fold and use the same pocket to bind different types of sugar. (A) Structure of human galectin complexed with galactose. (B) Crystal structure of BCoV S1-NTD by itself (12). Residues in brown are sugar-binding residues as identified 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

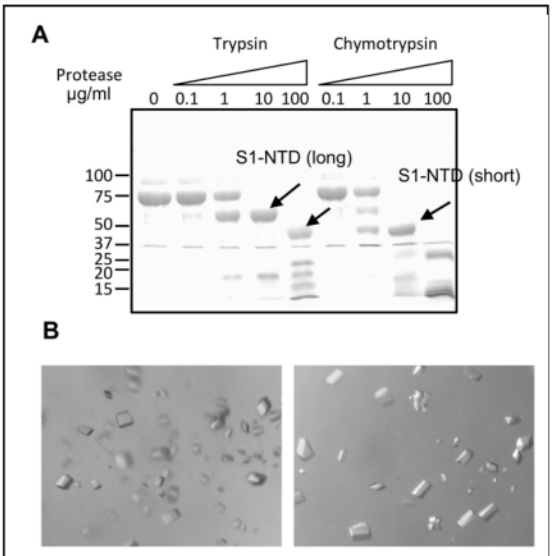


Figure 7. Characterization and crystallization of PEDV S1-NTD. (A) Limited proteolysis of PEDV S1-NTD-CTD produced two stable versions of S1-NTD, one from residues 14-490 and the other from residues 128-490. (B) The longer fragment has been crystallized under several conditions. The short one is being 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).

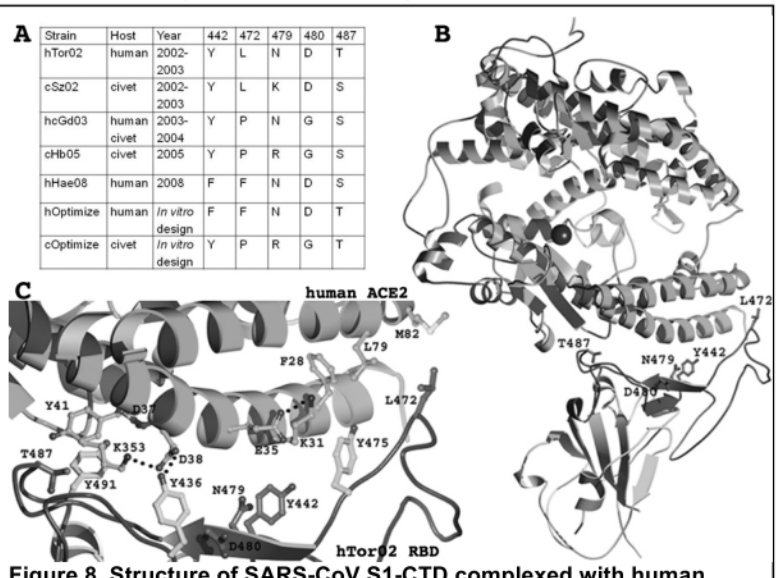
Potential pitfalls and alternative approaches: As an alternative approach to determining the co-crystal structure of PEDV S1-NTD and sugar, mutagenesis studies can be performed to identify sugar-binding residues in S1-NTD. Based on previous studies of protein/sugar interactions, three aromatic residues, 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
hOptimize	human	*In vitro* design	F	F	N	D	T
cOptimize	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.

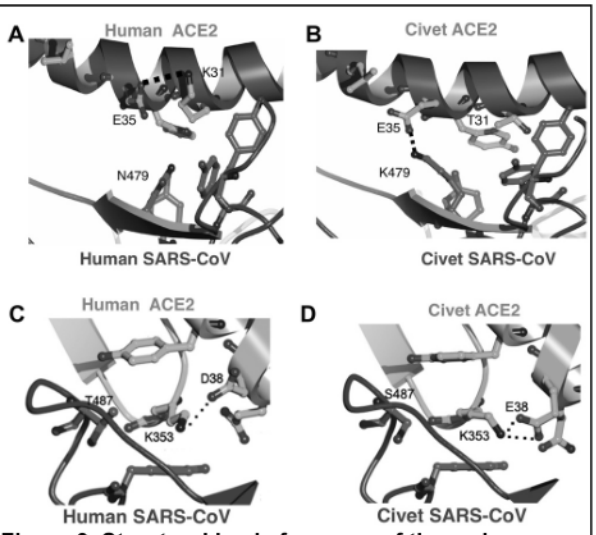


Figure 9. Structural basis for some of the major species barriers between human and civet for SARS-CoV infection (1, 2). Show are interfaces between human or civet SARS-CoV and human or civet ACE2. Asn479, but not Lys479, on SARS-CoV S1-CTD is compatible with human ACE2. Both Asn479 and Lys479 are compatible 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.

Overall hypotheses of Aim 2: To transmit from bats to another species, bat SLCoVs needed to adapt to ACE2 from that species through selective mutations in their S1-CTD.

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

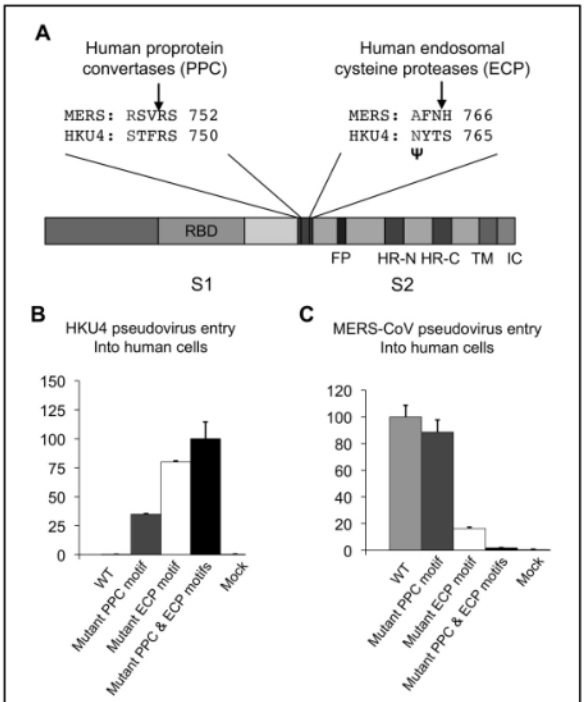

Figure 11. Host proteases as a cross-species barrier for MERS-CoV and bat CoV HKU4 (4, 5). (A) Domain structure of MERS-CoV and HKU4 spikes. MERS-CoV spike contains a PPC and an ECP motif. Arrows indicate the protease cleavage sites. The corresponding PPC motif in HKU4 spike contains mutations, and the 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.

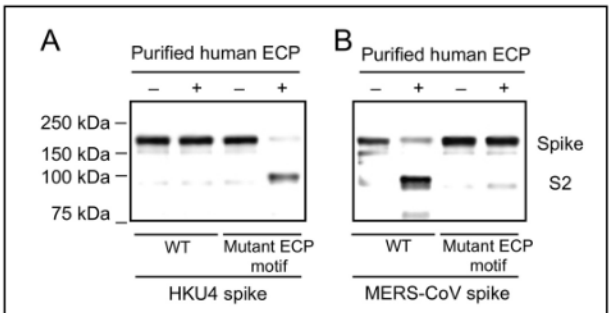

Figure 12. Cleavage of MERS-CoV and HKU4 spikes by purified human total ECPs (unpublished data from Dr. Li's lab). (A) Western blot analysis showed that purified human ECPs do not cleave HKU4 spike containing a glycosylated ECP motif, but cleave mutant HKU4 spike with the glycosylation removed. (B) Purified 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.

(ii) To examine whether the enhanced PPC motif contributes to the enhanced entry efficiency of MHV-JHM, we will mutate the enhanced PPC

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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).

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^{o}C both spikes are in the prefusion state, but after incubation at 37^{o}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 Redacted by agreement; Letters of Support Redacted by agreement; Letters of Support 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.

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.

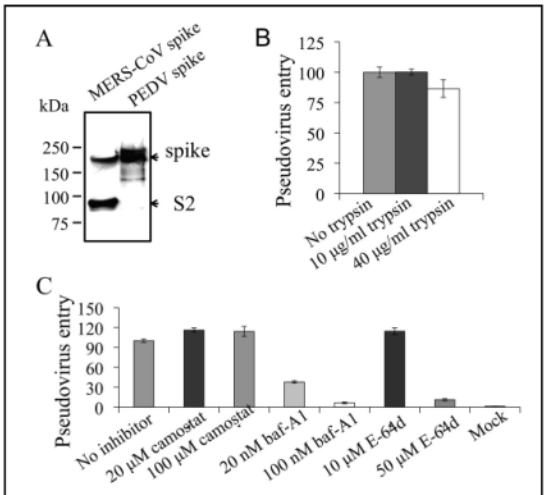

Figure 14. Endosomal cysteine proteases activate PEDV entry. (A) Western blot analysis of PEDV spike in pseudovirus particles. MERS-CoV pseudovirus was used as a control. During the viral maturation process, PEDV spike remained intact, while MERS-CoV spike underwent significant cleavage. (B) PEDV pseudovirus 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).

(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 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)**.

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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

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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	eRA Commons User Name

3c. POSITION TITLE
Professor

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Epidemiology/Microbiology/immunology

3f. MAJOR SUBDIVISION

3d. **SUBAWARD** MAILING ADDRESS *(Street, city, state, zip code)*
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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: Redacted by agreement; Non Key Personnel
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13. **SUBAWARD** OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: Redacted by agreement; Non Key Personnel
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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

Signature: Barbara Entwisle
Vice Chancellor for Research

DATE: 10/22/15

Redacted by agreement ; Letters of Support

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.

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: Redacted by agreement; Non Key Personnel 450 McNamara Alumni Center 200 Oak Street SE Minneapolis, MN 55455 **Phone number:** 612-624-5599 **Email:** Redacted by agreement; Non Key Personnel	**Signing Official:** Redacted by agreement; Non Key Personnel 450 MCNAMARA ALUMNI CENTER 200 OAK STREET SE MINNEAPOLIS, MN 554552070 **Phone number:** 612-624-5599 **Email:** Redacted by agreement; Non Key Personnel
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?

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.

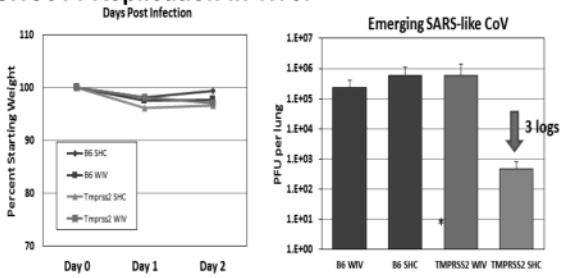
Tao et al. (2016, Journal of Virology) constructed four recombinant RBD (rRBD) proteins with single or multiple mutations detected in representative human MERS-CoV strains from the 2012, 2013, 2014, and 2015 outbreaks, respectively, and one rRBD protein with multiple changes derived from camel MERS-CoV 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 [redacted] 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.

Redacted by agreement

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 Redacted by agreement; Non Key Personnel 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 Redacted by agreement; Non Key Personnel 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*			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
eRA Commons User Name	Y	Li, Fang	PHD	PD/PI	EFFORT					NA
	N	Redacted by agreement; Non Key Personnel	SCD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA
	N		BS	Researcher 2						NA
	Y	Baric, Ralph S	PHD,BS	Subaward PI						NA
	N	Redacted by agreement; Non Key Personnel	MS	Researcher 2						NA
	N		BS,PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA
	N			Technician						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 EFFORT
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 EFFORT
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 EFFORT
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 EFFORT
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 EFFORT
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 EFFORT
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 EFFORT
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 EFFORT
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 EFFORT
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 EFFORT
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 EFFORT
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 EFFORT
Johns Hopkins U Private Source $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 Private Source
Role: Co-Investigator

R01 AI110700 (PI: Baric) 04/20/15-03/31/20 EFFORT
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 EFFORT
Private Source $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 EFFORT
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 EFFORT
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.

Private Source (PI: Baric) 06/23/16-06/22/18 EFFORT
$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 Private Source provided serum samples for cross-strain blockade antibody responses.

0258-3962 (PI: Lim) 09/30/11-02/28/17 EFFORT
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 EFFORT
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 EFFORT
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	Redacted by agreement 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