DEFENSE INTELLIGENCE AGENCY

(b)(3):10 USC 424; (b)(6)

29 JUNE 2020

(b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

Overall Briefing: ~~TOP SECRET~~/

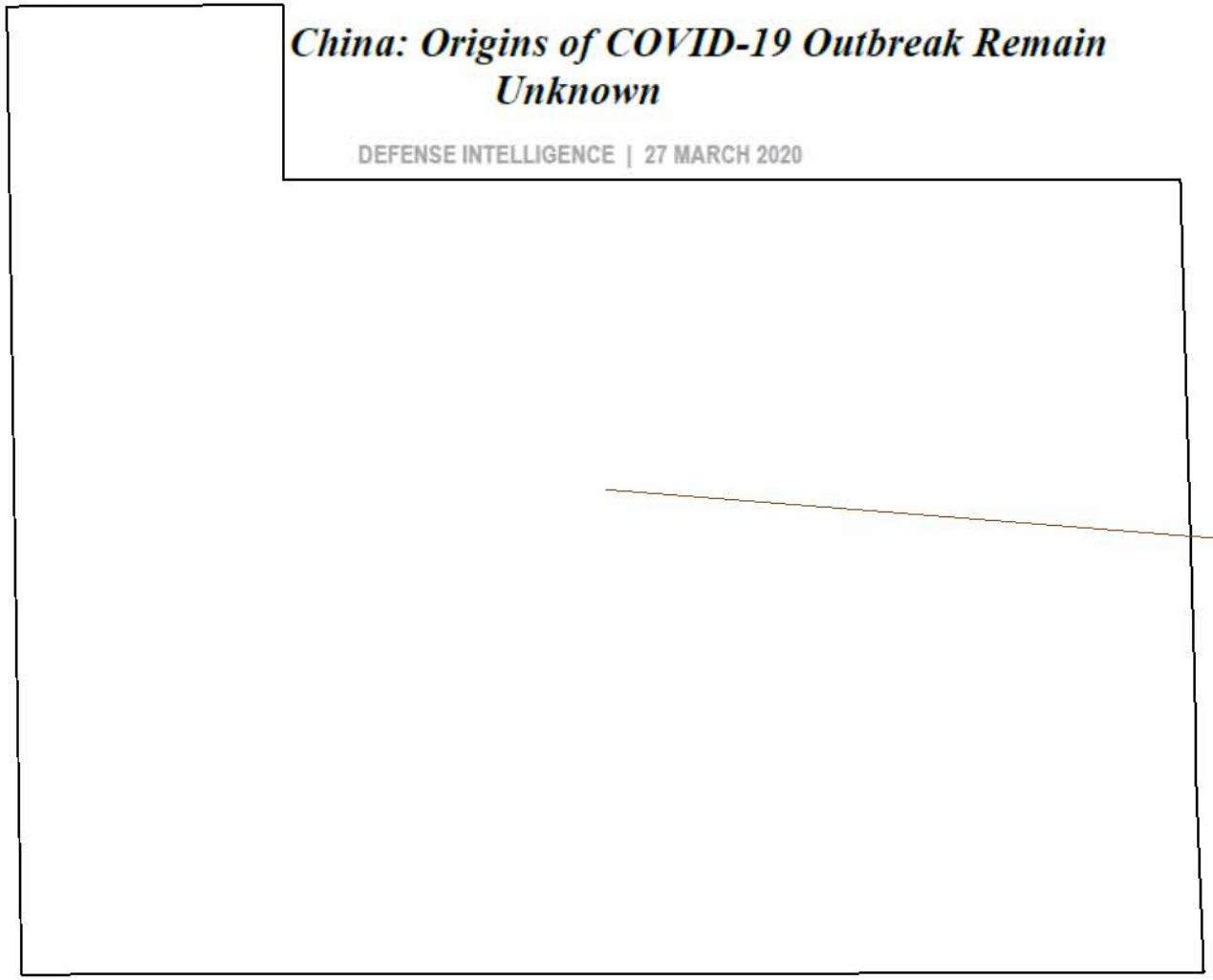
The next 2 pages are withheld in full citing (b)(1) and (b)(3) 50 USC 3024(i), and are not provided.

(b)(3):50 USC 3024(i)

Classified by: Derived from: Declassify on:

(U) DIA AUTHORITATIVE ASSESSMENT (27 MAR 2020)

The next page is withheld in full citing (b)(1) and (b)(3) 50 USC 3024(i), and are not provided.

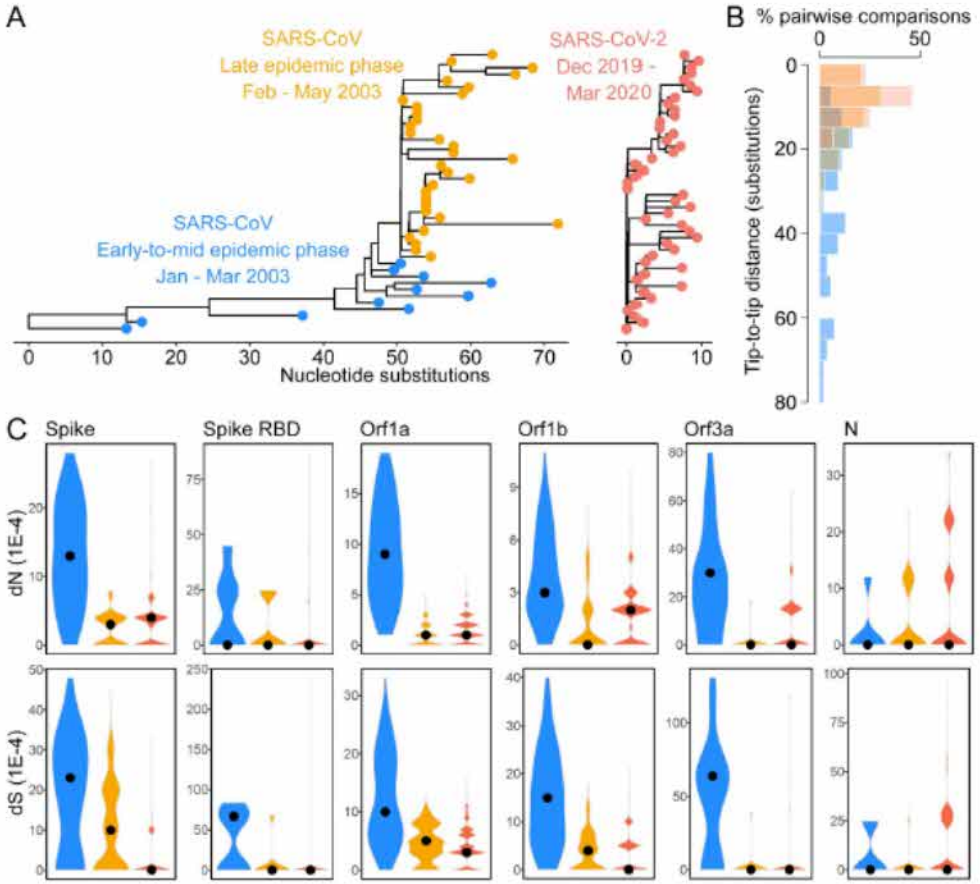


(b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

(U) **"Genetic engineering" here is**: using a set of biotechnologies to manipulate or "edit" an organism or virus's genome; it does not include directed evolution by other means such as the use of repeated passage through animals or cell culture.

(U) NOTABLE VIRUS FEATURES

(U) Adaptation to humans early in the outbreak: genomic evolution study

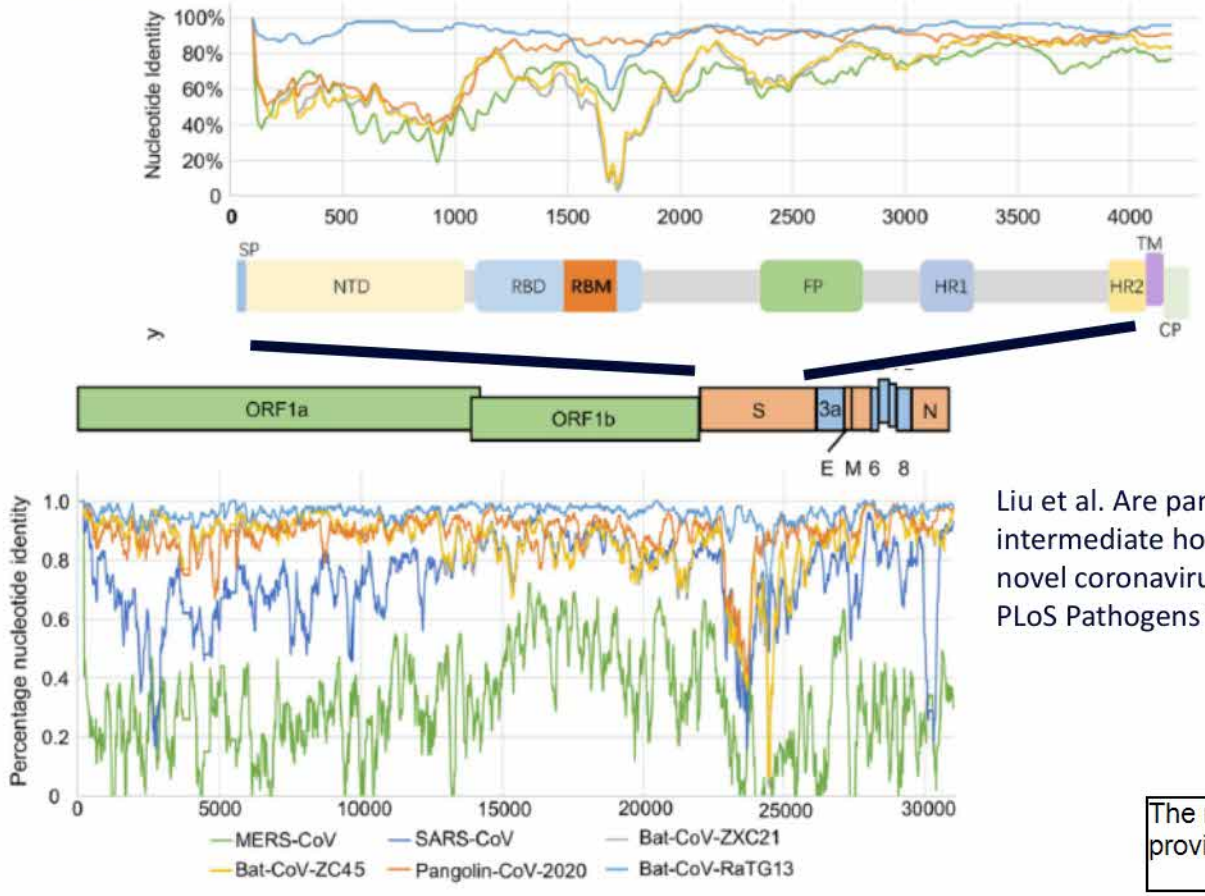


(U) "In a side-by-side comparison of evolutionary dynamics between the 2019/2020 SARS-CoV-2 and the 2003 SARS-CoV, we were surprised to find that SARS-CoV-2 resembles SARS-CoV in the late phase of the 2003 epidemic after SARS-CoV had developed several advantageous adaptations for human transmission. Our observations suggest that by the time SARS-CoV-2 was first detected in late 2019, it was already pre-adapted to human transmission to an extent similar to late epidemic SARS-CoV."

Zhan et al. SARS-CoV-2 is well adapted for humans. What does this mean for emergence? bioRxiv 2020.

(U) NOTABLE VIRUS FEATURES

(U) Similar to bat and pangolin coronaviruses in different regions



Liu et al. Are pangolins the intermediate host of the 2019 novel coronavirus (SARS-CoV-2)? PLoS Pathogens 2020.

(U) "In the region of nucleotides 1-914, Pangolin-CoV is more similar to Bat SARSr-CoV ZXC21 and Bat SARSr-CoV ZC45, while in the remaining part of the gene, Pangolin-CoV is more similar to SARS-CoV-2 and Bat-CoV-RaTG13 . . . In particular, the receptor-binding domain of the S protein of Pangolin-CoV has only one amino acid difference from that of SARS-CoV-2. Overall, these data indicate that SARS-CoV-2 might have originated as the recombination of a Pangolin-CoV-like virus with a Bat-CoV-RaTG13-like virus.

Xiao et al. Isolation of SARS-CoV-2-related coronavirus from Malayan pangolins. Nature 2020.

The next page is withheld in full citing (b)(3) 50 USC 3024(i), and is not provided.

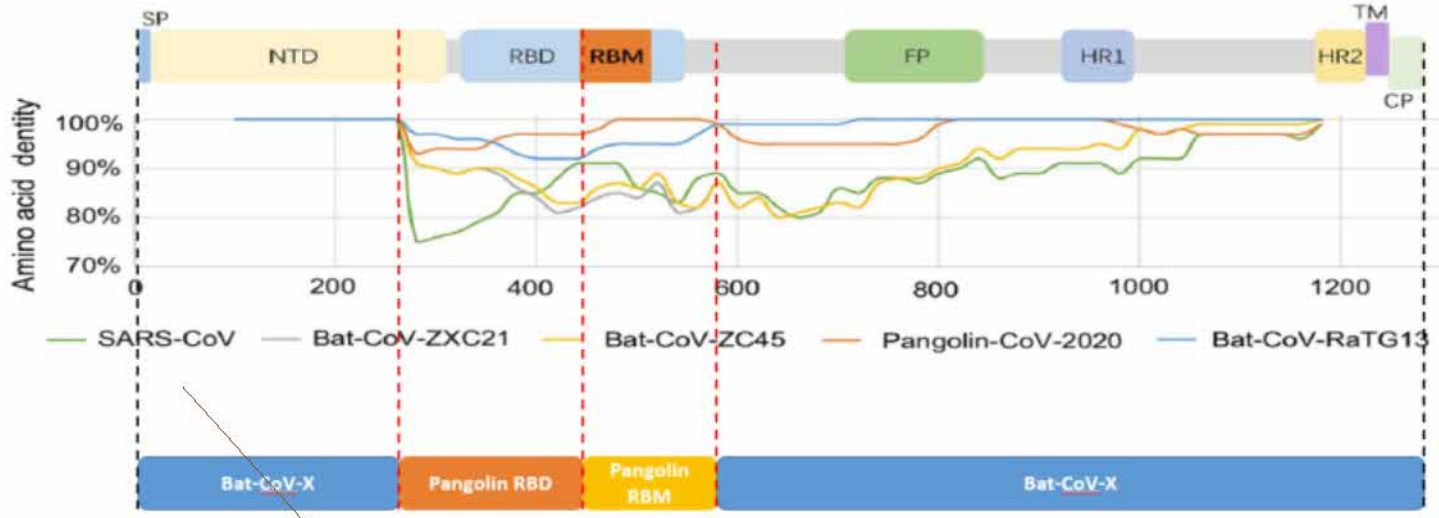
(U) COULD A LAB HAVE MADE THE VIRUS?

(U) “The Institute [Wuhan Institute of Virology] does not have the capability to design and synthesize a new coronavirus . . .”(U) China Ministry of Foreign Affairs, press release 5 May 2020

The next page is withheld in full citing (b)(1) and (b)(3) 50 USC 3024(i), and is not provided.

(U) PREVIOUS WIV RESEARCH IN RELATION TO SARS-COV-2

(U) Example: Identification of specific mutations that enable bat CoV to infect human cells (2008)

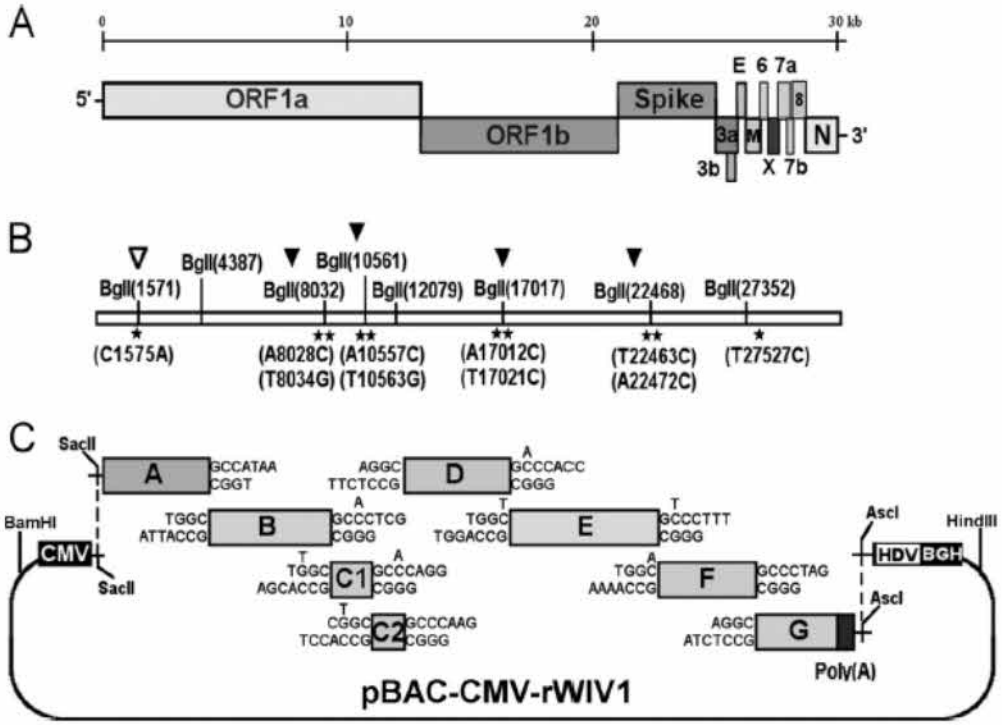


Ren et al. Difference in receptor usage between Severe Acute Respiratory Syndrome (SARS) Coronavirus and SARS-Like Coronavirus of bat origin. J Virol 2008.

(b)(3):10 USC 424; (b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e); (b)(6)

(U) PREVIOUS WIV RESEARCH IN RELATION TO SARS-COV-2

(U) Example: Method for synthesizing bat CoV WIV1 (reverse genetics system; 2016)



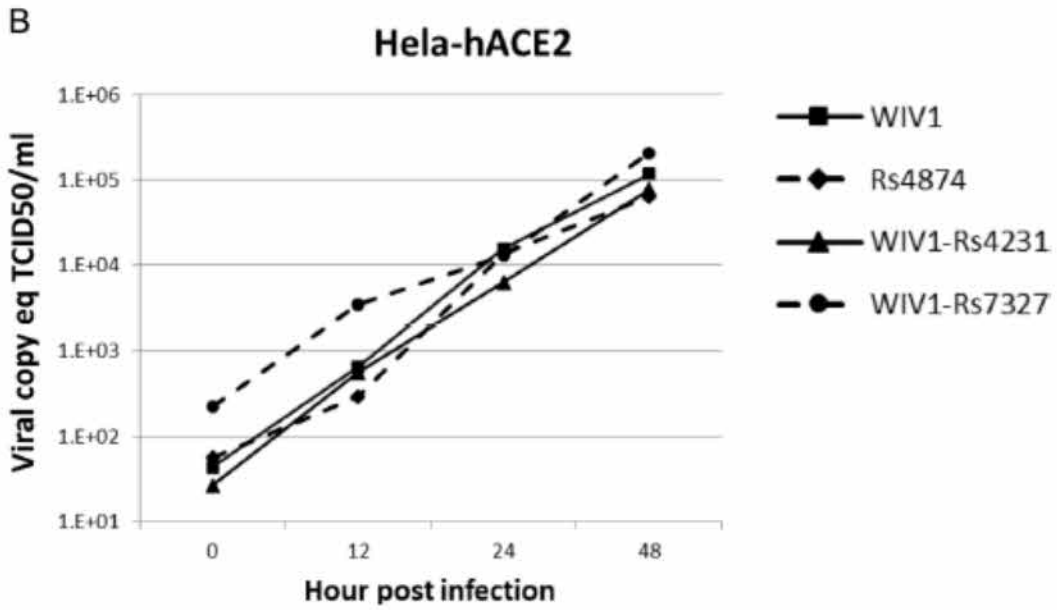
(U) "Strategy for construction of an infectious WIV1 BAC clone."

(U "In this study, we have developed a fast and cost-effective method for reverse genetics of coronaviruses by combining two approaches developed by others. Our method allows the genomes of coronaviruses to be split into multiple fragments and inserted into a BAC plasmid with a single step . . . As the genomes can be divided into multiple short fragments, mutations can be introduced into individualfragments easily."

Zeng et al. Bat Severe Acute Respiratory Syndrome-Like Coronavirus WIV1 encodes an extra accessory protein, ORFX, involved in modulation of the host immune response. J Virol 2016.

(U) PREVIOUS WIV RESEARCH IN RELATION TO SARS-COV-2

(U) Example: Construction of chimeras with spike from new bat CoVs on WIV1 backbone, infection studies (2017)



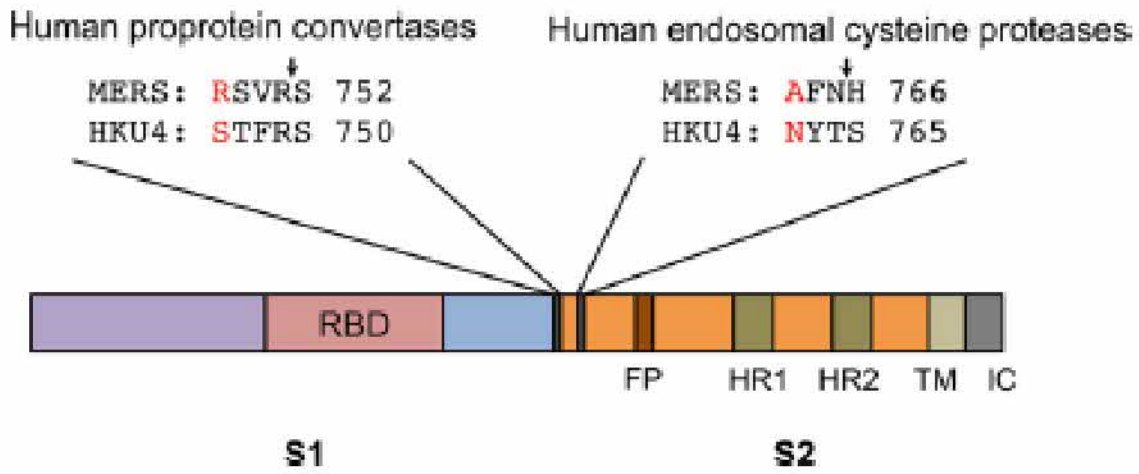
(U) Synthetic chimera infection of cells with human receptor.

(U "In this cave, we have now obtained full-length genome sequences of additional 11 novel SARSr-CoVs from bats . . . Using the reverse genetics technique we previously developed for WIV1, we constructed a group of infectious bacterial artificial chromosome (BAC) clones with the backbone of WIV1 and variants of S genes from 8 different bat SARSr-CoVs."

Hu et al. Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insight into the origin of SARS coronavirus. PLoS Pathogens 2017.

(U) PREVIOUS WIV RESEARCH IN RELATION TO SARS-COV-2

(U) Example: Insertion of furin cleavage site enabling bat CoV (MERS-CoV progenitor) to infect human cells (2015)



(U) MERS-CoV and bat CoV HKU4 spike proteins.

(U) " . . . the two mutations adaptive tohuman cellular proteases transformed MERS-CoV spike from completely lacking to fully possessing the capacity to mediate viral entry into human cells, and thus they likely played the most criticalrole in the bat-to-human transmission of MERS-CoV, either directly or through intermediate hosts."

Yang et al. Two mutations were critical for bat-to-human transmission of Middle East Respiratory Syndrome coronavirus. J Virol 2015.

The next 4 pages are withheld in full citing (b)(1) and (b)(3) 50 USC 3024(i), and are not provided.

DEFENSE INTELLIGENCE AGENCY

COMMITTED TO EXCELLENCE IN DEFENSE OF THE NATION

• (U) NOTABLE VIRUS FEATURES

- (U) Adaptation to humans early in the outbreak: computational receptor binding study

Table 4. Binding energies of SARS-CoV-2 spike protein to ACE2 of selected species and potential species susceptibilities from other studies

Species	Binding energy kcal/mol	MMPBSA energy kcal/mol	COVID infectivity n/a = not assessed
Homo sapiens (human)	-52.8	-57.6	Permissive, high infectivity, severe disease in 5-10%,
Manis javanica (pangolin)	-52.0	-56.5	Permissive
Canis luparis (dog)	-50.8	-49.5	Permissive, low infectivity, no overt disease
Macaca fascicularis (monkey)	-50.4	-50.8	Permissive, medium infectivity, lung disease
Mesocricetus auratus (hamster)	-49.7	-50.0	Permissive, high infectivity, lung disease
Mustela putorius furo (ferret)	-48.6	-49.2	Permissive, medium infectivity, mild disease
Felis catus (cat)	-47.6	-48.9	Permissive, high infectivity, lung disease
Panthera tigris (tiger)	-47.3	-42.5	Permissive, overt respiratory symptoms
Rhinolophus sinicus (bat)	-46.9	-49.6	n/a
Paguma larvata (civet)	-45.1	-46.1	n/a
Equus ferus caballus (horse)	-44.1	-49.2	Permissive, low infectivity, no overt disease
Bos taurus (cow)	-43.6	-42.5	n/a
Ophiophagus hannah (snake)	-39.5	-52.5	n/a
Mus musculus (mouse)	-38.8	-39.4	n/a

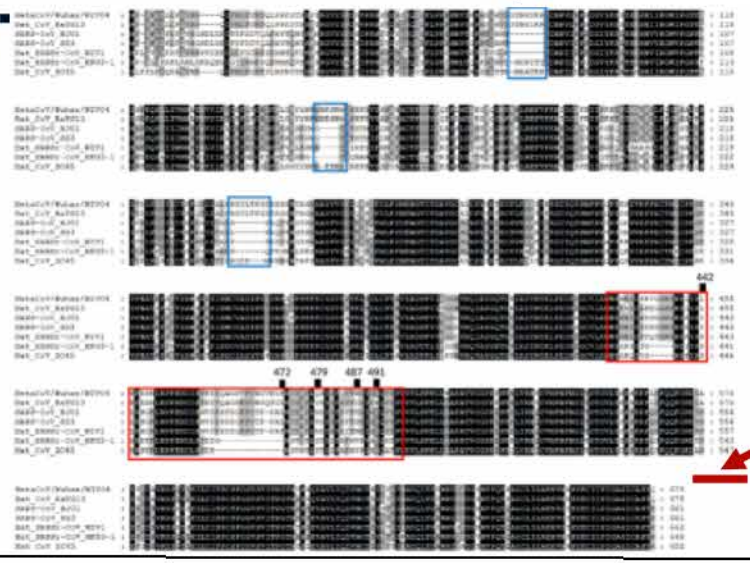
(b)(3):50 USC 3024(i)

Piplani et al. In silico comparison of spike protein-ACE2 binding affinities across species; significance for the possible origin of the SARS-CoV-2 virus. arXiv 2020.

(U) CHINESE GENOME REPOR

(U) We have questions

- (U) No mention of furin cleavage site in original report.(U) Reference to BatCoV RaTG13 in original report does not mention a virus reported by WIV in 2016 BatCoV/4991, with identical RdRp sequence (i.e., RaTG13=4991?).(U) WIV identified BatCoV/4991 in cave expedition following fatal pneumonia outbreak among miners in 2012 (Ge et al., 2016); no scientific reports of cause of the outbreak.



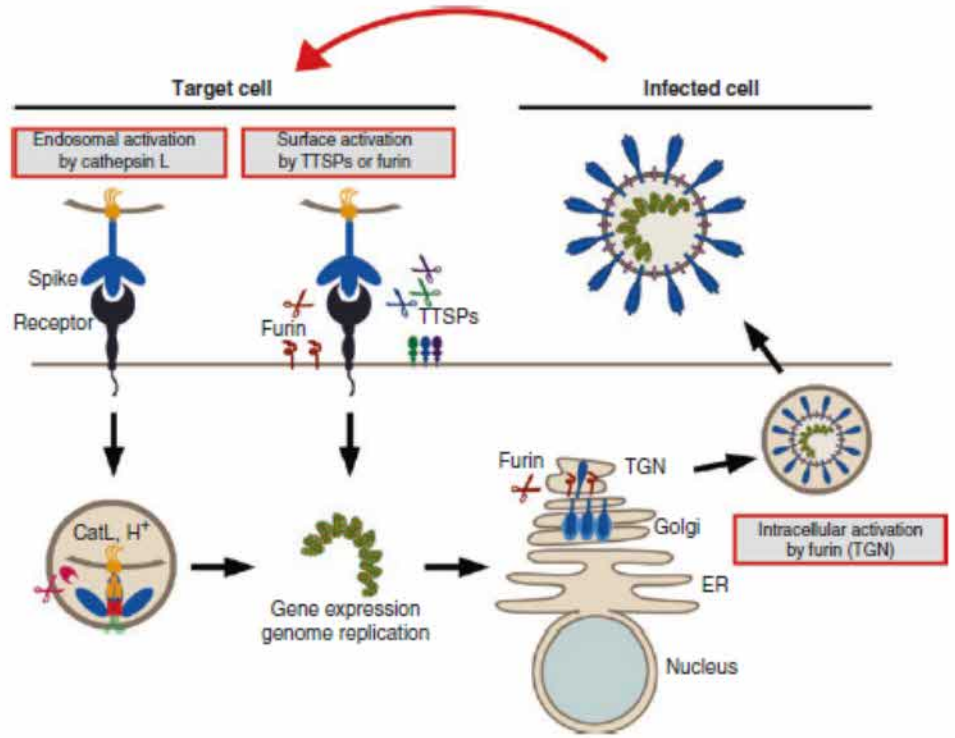
(b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

Live samples of RaTG13 or 4991 not known to exist.(U) Large-scale contamination evident in pangolin sequences (Zhang et al., 2020).

Zhou et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020.

(U) NOTABLE VIRUS FEATURES

Ploybasic furin cleavage site



(b)(3):50 USC 3024(i)

Hoffmann et al. Priming time: how cellular proteases arm coronavirus spike proteins. Activation of Viruses by Host Proteases 2018.

Piplani et al. In silico comparison of spike protein-ACE2 binding affinities across species; significance for the possible origin of the SARS-CoV-2 virus. arXiv 2020.

(U) COULD A LAB HAVE CONSTRUCTED THE VIRUS?

(U) "The Institute [Wuhan Institute of Virology] does not have the capability to design and synthesize a new coronavirus . . ."(U) China Ministry of Foreign Affairs, press release 5 May 2020

(b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

The final 5 pages are withheld in full citing (b)(1), (b)(3) 10 USC 424 + 50 USC 3024(i), and (b)(6) and are not provided.