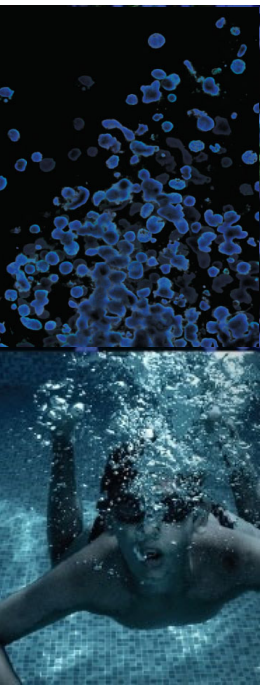
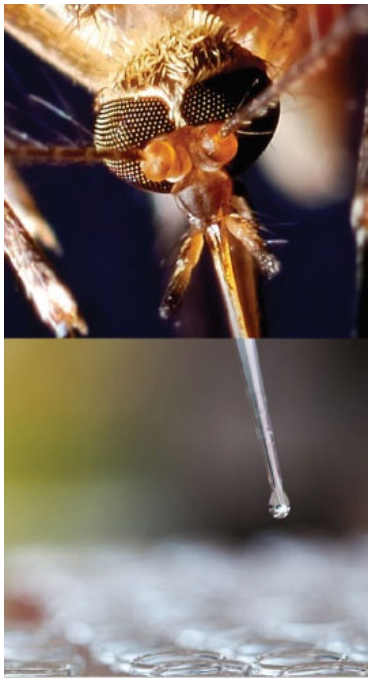
Variations of the SARS-CoV-2 Spike Protein – Challenges and Impact

Partha Mitra, PhD
Lead Biologist, ATCC

Credible Leads to Incredible™





About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for microbial culture – the "*gold standard*"
- Innovative R&D company featuring a novel genome portal, BSL-1 derivatives of infectious organisms, novel technologies for R_x and D_x development
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, and viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 500+ employees, over one-third with advanced degrees

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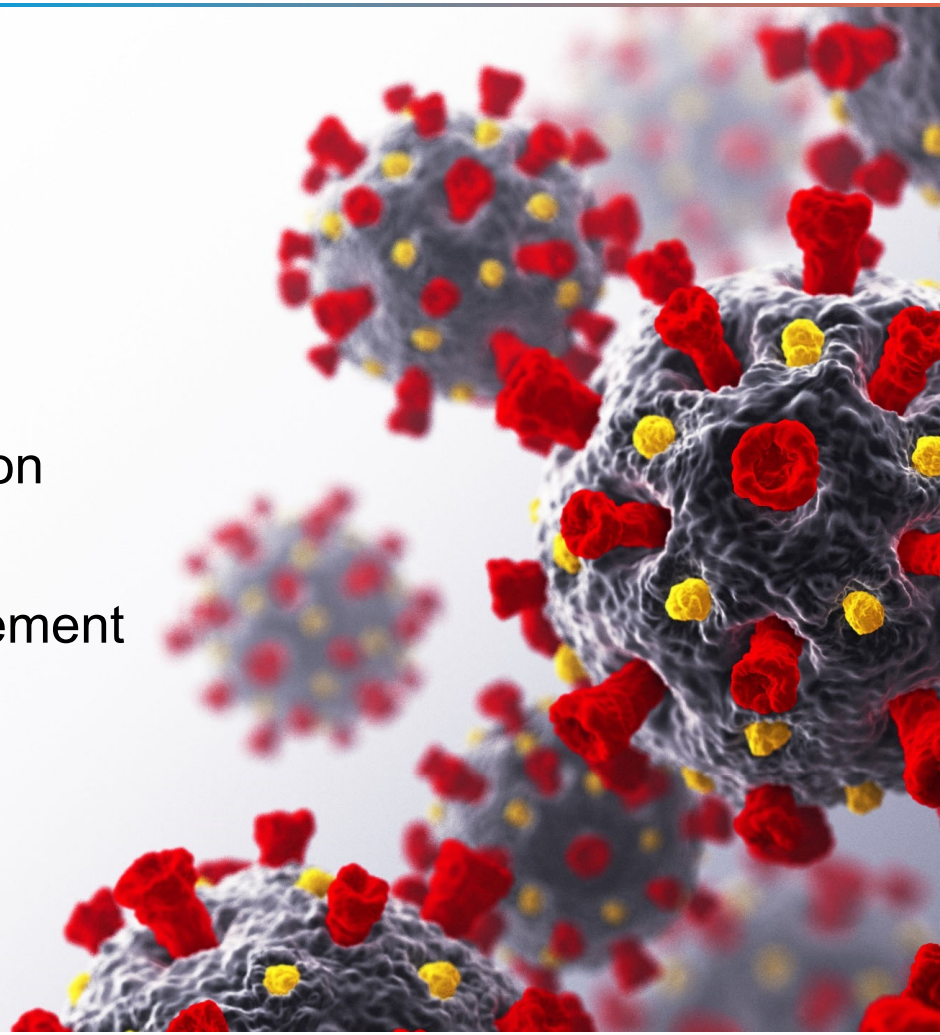
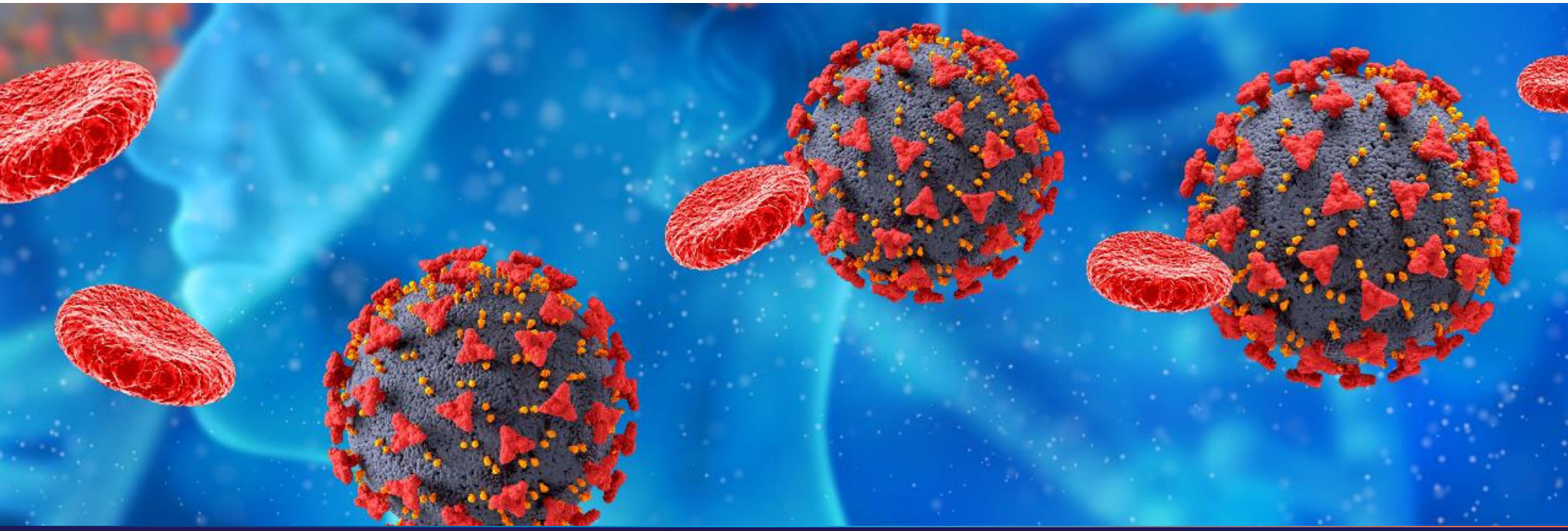
Agenda

- Coronavirus infection in humans
- Cellular entry of SARS-CoV-2
- The spike protein conformation
- SARS-CoV-2 variants: effect on conformation
- Conformation and vaccine design
- Impact of the variations on disease management
- Exploring additional avenues





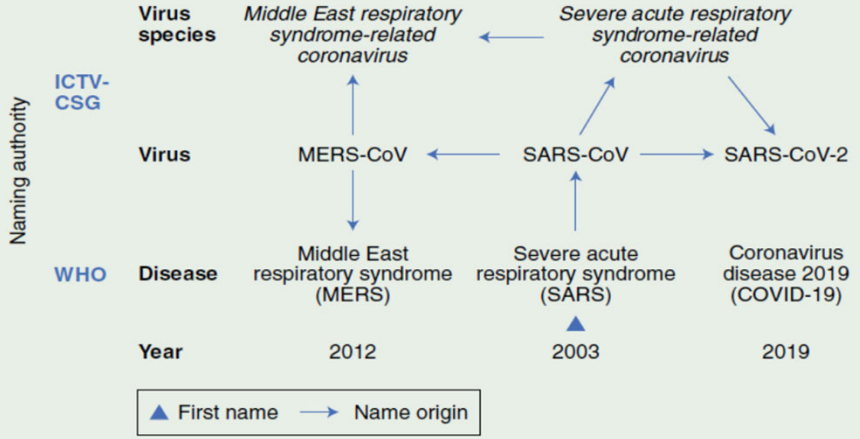
Coronavirus Infection In Human

SARS-CoV-2 – the Causative Agent of COVID-19

SARS-CoV-2
- **S** – Severe
- **A** – Acute
- **R** – Respiratory
- **S** – Syndrome
- **Co** – Corona
- **V** – Virus
- **2** – 2

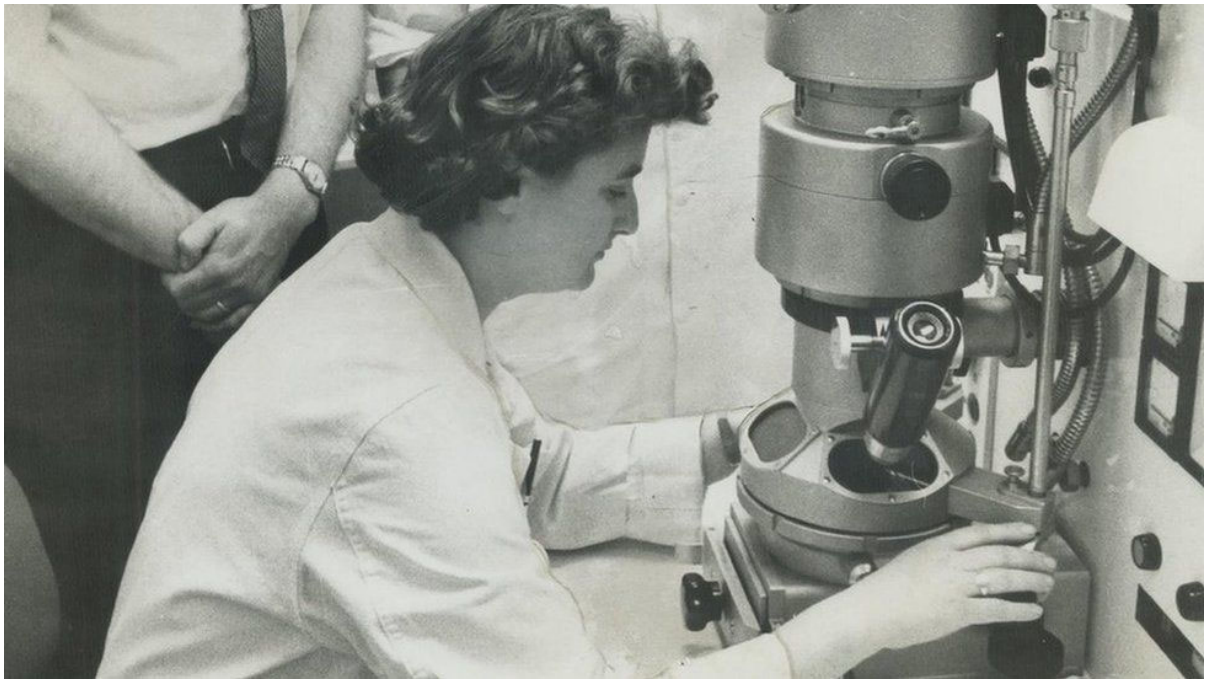
- Enveloped
- Positive-sense
- Single-stranded
- RNA virus

Coronavirus Nomenclature



https://www.news-medical.net/health/The-Naming-System-Behind-SARS-CoV-2.aspx ,Coronavirus, 2020: Illustration by David S. Goodsell, RCSB Protein Data Bank.

First Human Coronavirus



The woman who discovered the first coronavirus By Steven Brocklehurst BBC Scotland News https://www.bbc.com/news/uk-scotland-52278716

Dr. June Almeida (1930-2007)

- Identified a particle in nasal washings under an electron microscope
- Described it as like influenza viruses but not the same
- It became known as the first human coronavirus image

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First Coronavirus Electron Microscopy Picture

J. Gen. Virol. (1967), 1, 175-178
With 2 plates
Printed in Great Britain

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The Morphology of Three Previously Uncharacterized Human Respiratory Viruses that Grow in Organ Culture

By JUNE D. ALMEIDA
Department of Medical Microbiology, St Thomas's Hospital Medical School, London, S.E. 1
AND D. A. J. TYRRELL
Common Cold Research Unit, Medical Research Council, Salisbury, England

(Accepted 28 *November* 1966)

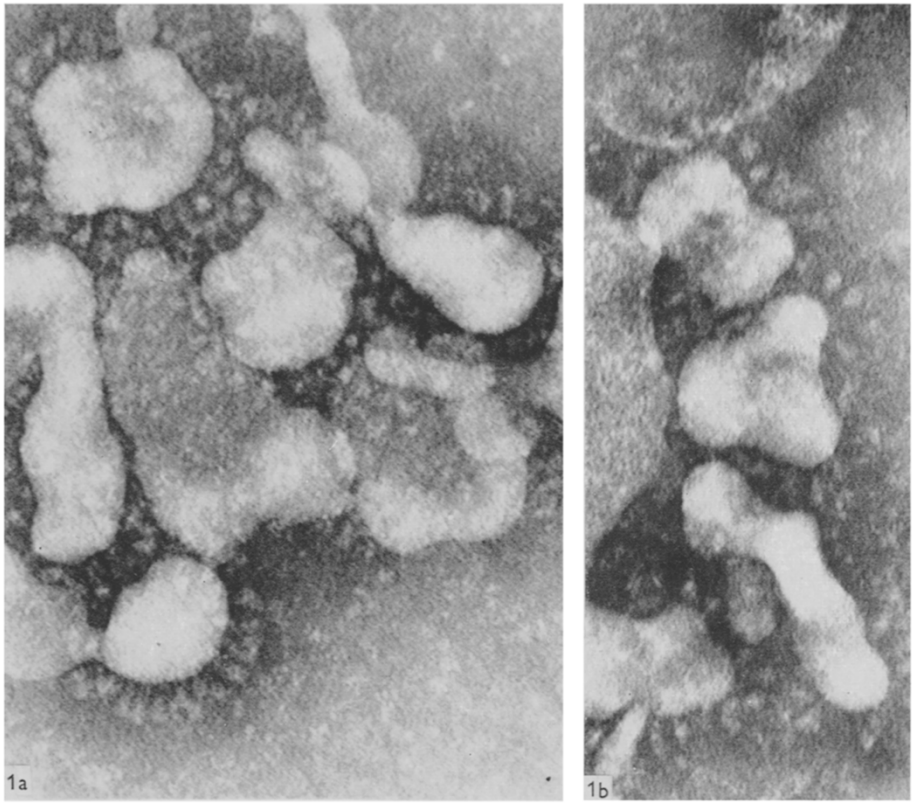

Fig. 1 a, b. This type of particle was seen when organ cultures infected with strain 229E were examined by the present technique. The particles are pleomorphic, in the size range 800 to 1200 A, and are surrounded by a distinct 200 A long fringe. They are indistinguishable from the particles of avian infectious bronchitis, the only virus previously known to have this morphology.

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Coronaviruses that Infect Humans

- Human coronavirus 229E or HCoV-299E (α-coronavirus) – 1963
- Human coronavirus OC43 or HCoV-OC43 (β-coronavirus) – 1963
- SARS-CoV (β-coronavirus) – 2003
- Human coronavirus-NL63 or HCoV-NL63 (α-coronavirus) – 2004
- Human coronavirus HKU1 or HCoV-HKU1 (lineage A β-coronavirus) – 2004
- Middle East respiratory syndrome coronavirus or MERS-CoV (β-coronavirus) – 2012
- SARS-CoV-2 (β-coronavirus) – 2019

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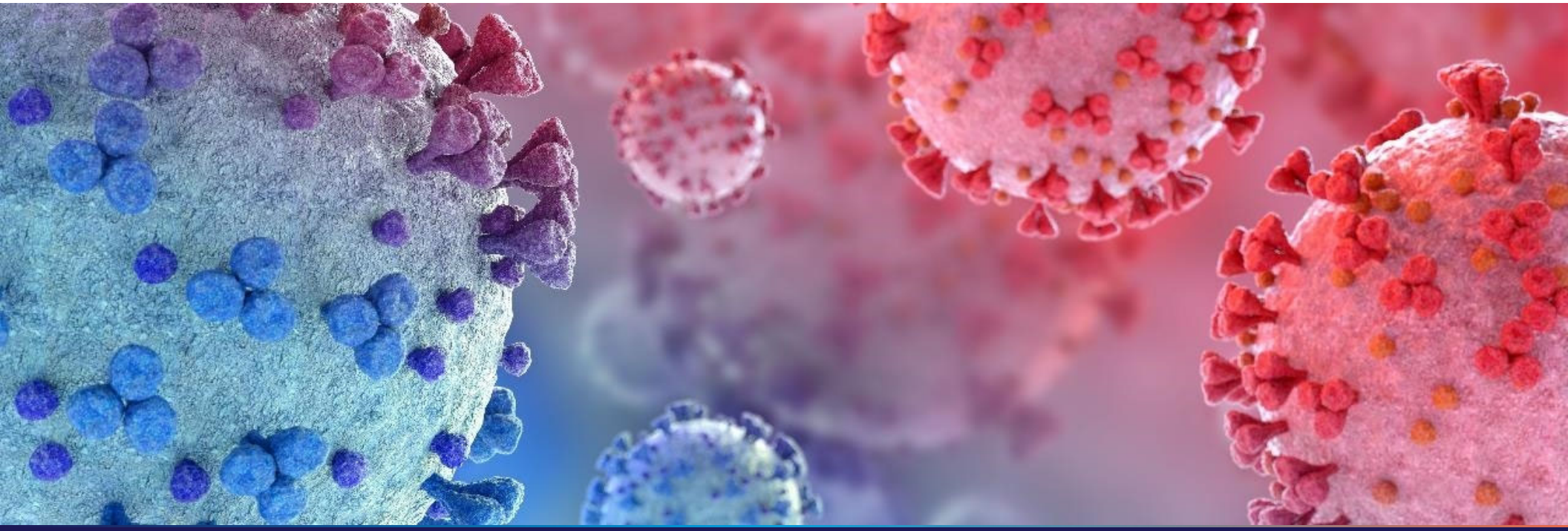

Cellular Entry of SARS-CoV-2

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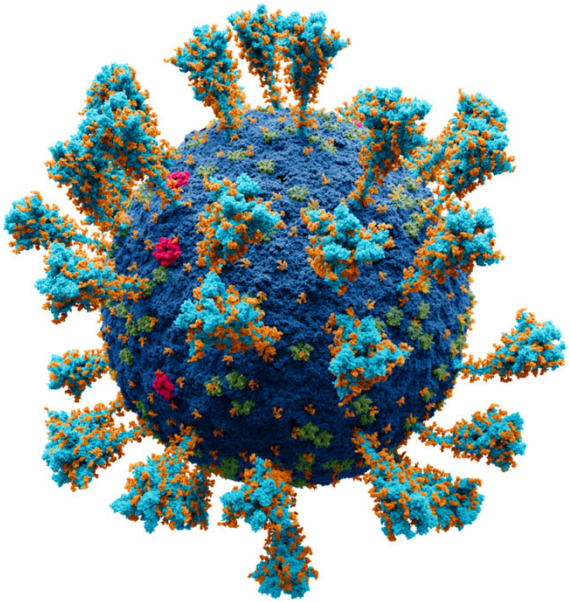
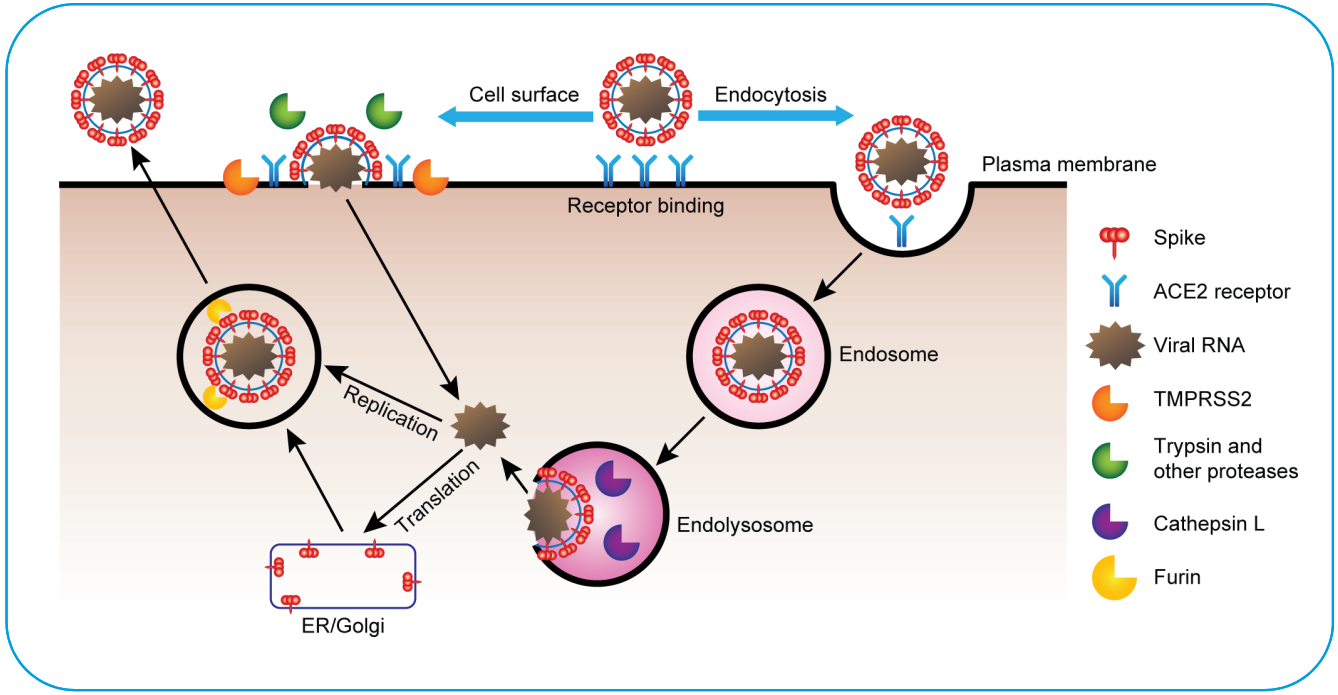
Cellular Entry of SARS-CoV-2



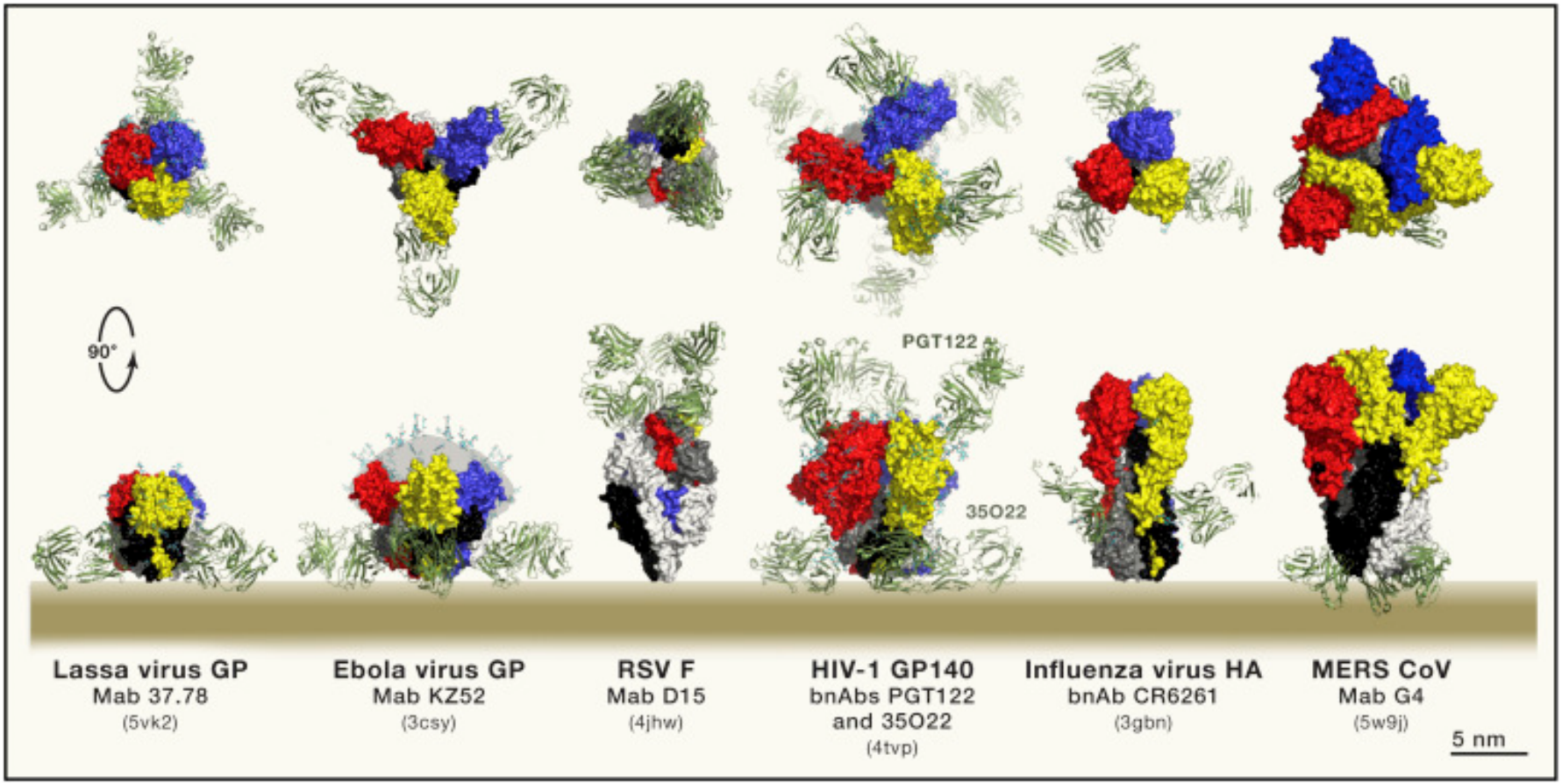
cobalt — membrana
turquoise — spike glycoprotein (S)
crimson — E protein
green — M protein.
orange — glucose

SARS-CoV-2 entry mechanisms



https://en.wikipedia.org/wiki/File:Novel_Coronavirus_SARS-CoV-2.jpg; SARS-CoV-2 tropism, entry, replication, and propagation: Considerations for drug discovery and development Murgolo N, Therien AG, Howell B, Klein D, Koeplinger K, et al. (2021) PLOS Pathogens 17(2) e1009225. https://doi.org/10.1371/journal.ppat.1009225

Cellular Entry of Enveloped Viruses



Rey FA, Lok SM. Common Features of Enveloped Viruses and Implications for Immunogen Design for Next-Generation Vaccines. Cell. 2018 Mar 8;172(6):1319-1334. doi: 10.1016/j.cell.2018.02.054. PMID: 29522750; PMCID: PMC7112304

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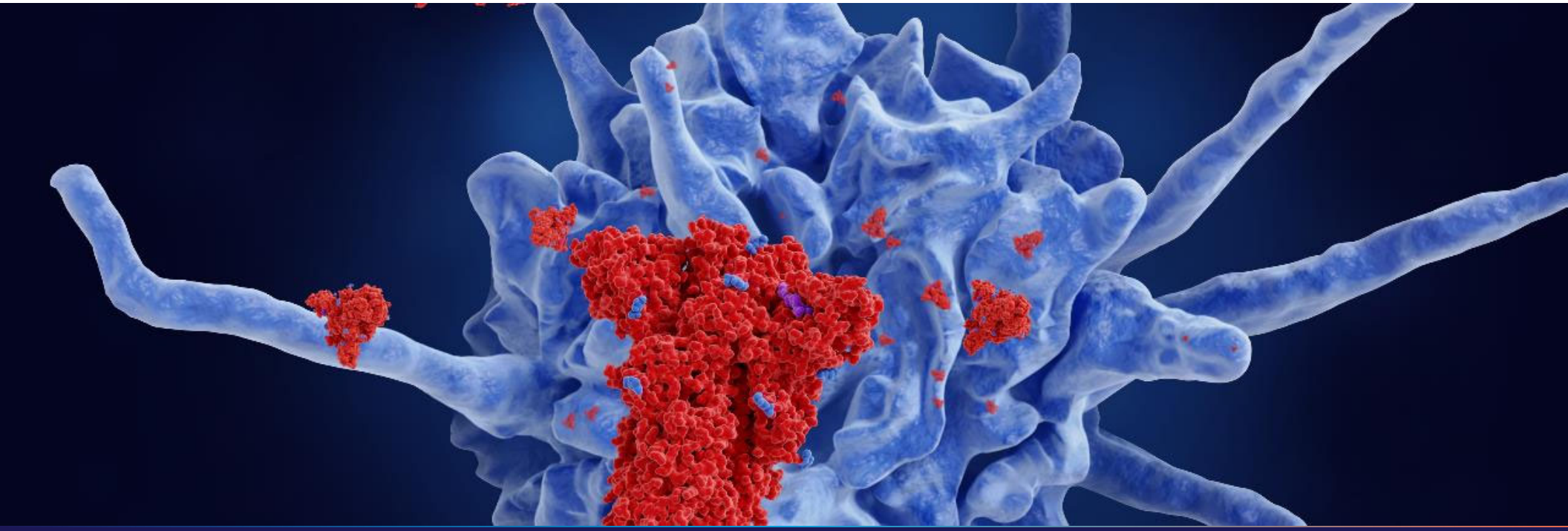

The Spike Protein Conformation

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SARS-CoV-2 Spike(S) protein

- 1273 Amino Acid
- 180–200 kDa
- Glycosylated (22 sites/protomer)
- Homo-trimeric
- Transmembrane
- Class I fusion protein

The SARS-CoV-2 Spike(S) Protein Determines:

- Receptor binding and specificity
- Membrane fusion
- Virus neutralization by antibody

A Key Target for:

- Vaccine development
- Therapeutic antibody
- Diagnostic use

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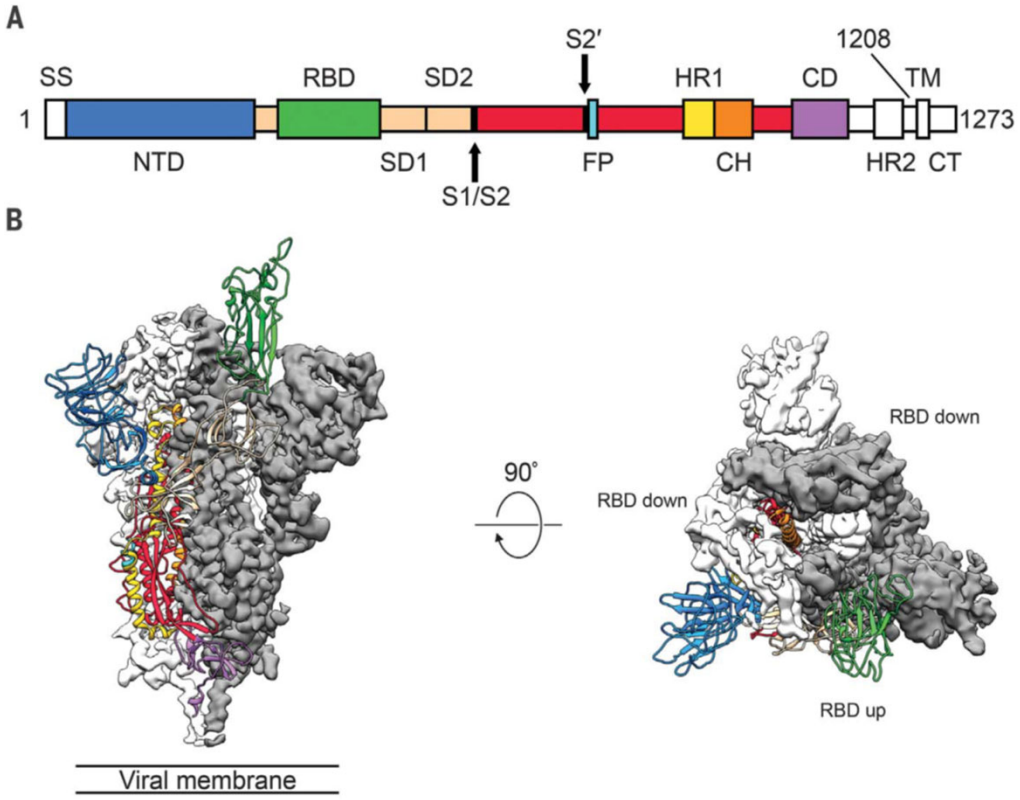
Structure of 2019-nCoV S



(A) Schematic of 2019-nCoV S primary structure colored by domain

SS, signal sequence
S2′, S2′ protease cleavage site
FP, fusion peptide
HR1, heptad repeat 1
CH, central helix
CD, connector domain
HR2, heptad repeat 2
TM, transmembrane domain
CT, cytoplasmic tail
Arrows denote protease cleavage sites

(B) Side and top views - single RBD in the up conformation

The two RBD down protomers are shown as cryo-EM density in either white or gray colored corresponding to the schematic in (A).
The sequences excluded from the ectodomain expression construct or could not be visualized in the final map are colored white

Wrapp et al., Science 367, 1260–1263 (2020) 13 March 2020

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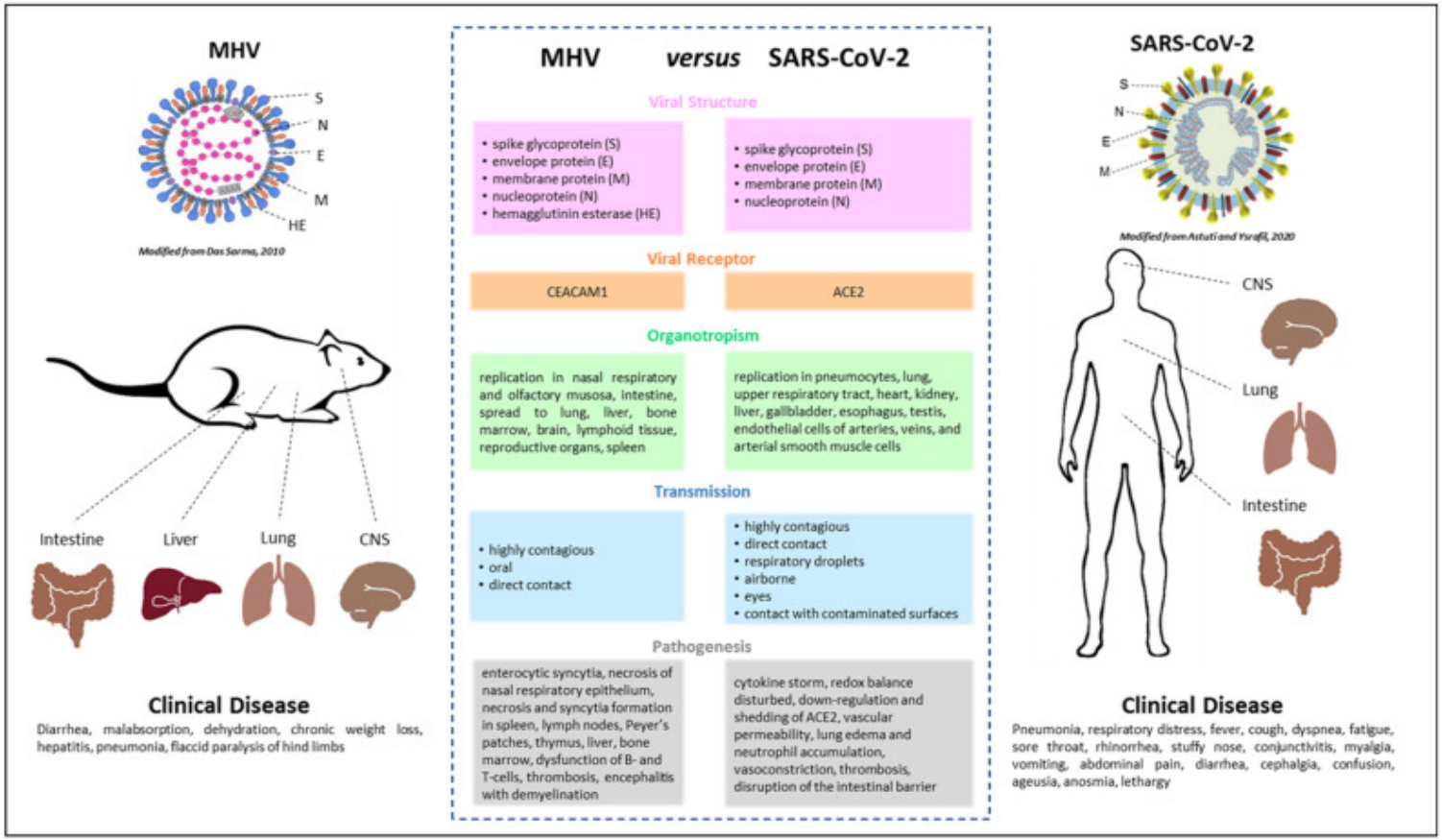
MHV-a model of Coronavirus Study



Körner RW, Majjouti M, Alcazar MAA, Mahabir E. Of Mice and Men: The Coronavirus MHV and Mouse Models as a Translational Approach to Understand SARS-CoV-2. Viruses. 2020 Aug 12;12(8):880. doi: 10.3390/v12080880. PMID: 32806708; PMCID: PMC7471983.

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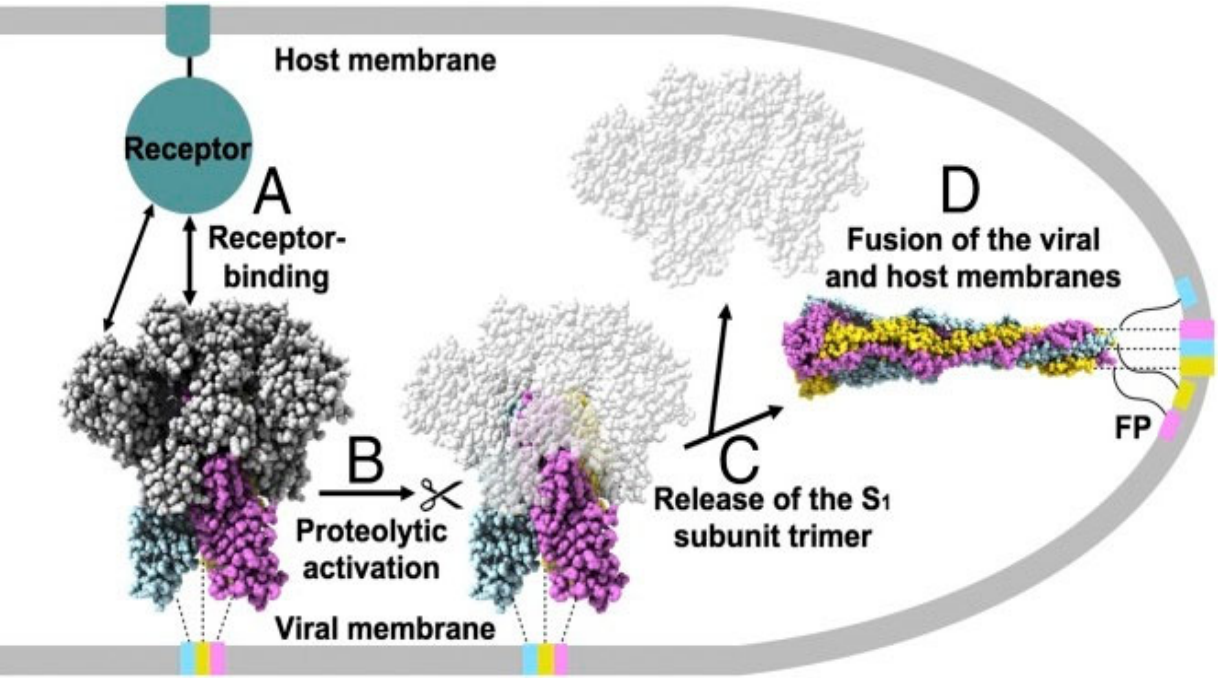
Cellular Entry of Coronavirus



Model of Coronavirus entry

A. S glycoprotein binds to transmembrane receptor
B. Activation of the S trimer occurs via protease cleavage
C. Shedding of the S_1 subunit trimer frees the fusion machinery
D. Conformational changes of the S glycoprotein result in fusion of the viral and host membranes

Walls AC, Tortorici MA, Snijder J, Xiong X, Bosch BJ, Rey FA, Veesler D. Tectonic conformational changes of a coronavirus spike glycoprotein promote membrane fusion. Proc Natl Acad Sci U S A. 2017 Oct 17;114(42):11157-11162. doi: 10.1073/pnas.1708727114. Epub 2017 Oct 3

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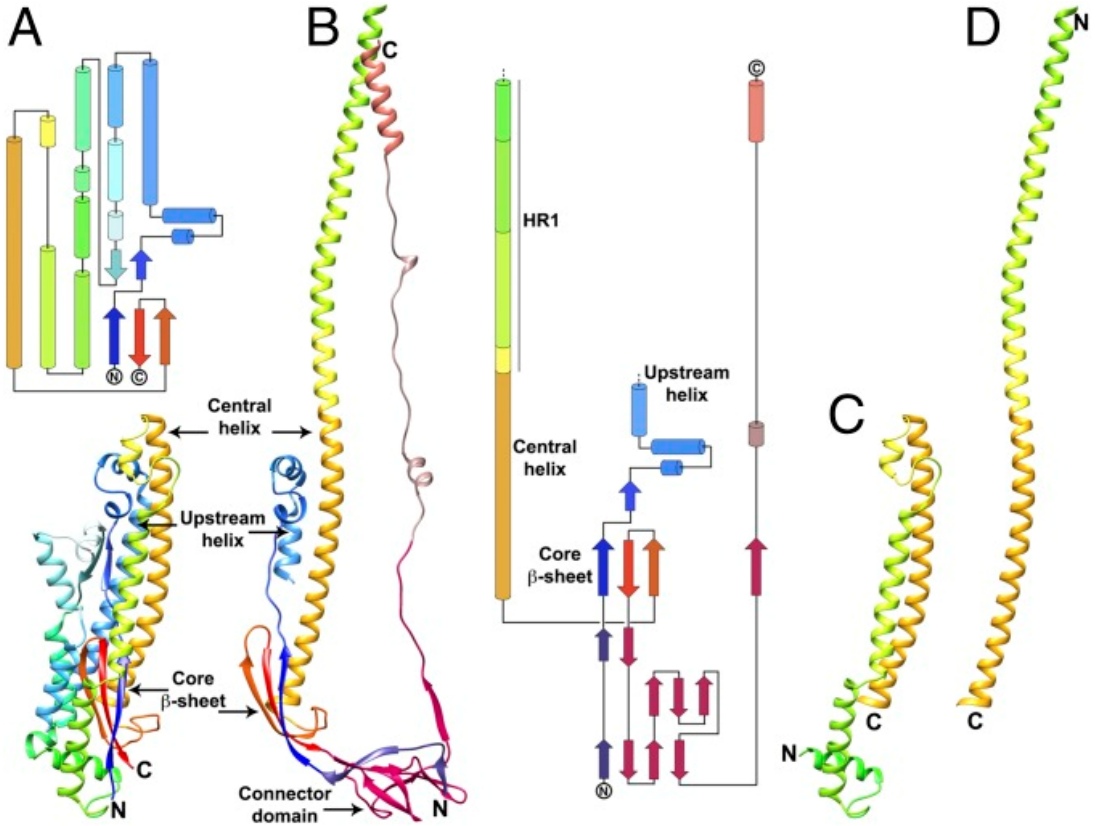
Conformational Change in Spike Protein Structure



Conformational changes associated with the fusion reaction

A. Ribbon and topology diagrams of the MHV S_2 subunit in the prefusion conformation
B. Ribbon and topology diagrams of the MHV S_2 subunit in the post-fusion conformation
C. Ribbon rendering of the MHV S central helix and HR1 in the prefusion
D. Postfusion states highlighting the jack-knife refolding of the four HR1 helices and intervening regions into a single continuous helix

Walls AC, Tortorici MA, Snijder J, Xiong X, Bosch BJ, Rey FA, Veesler D. Tectonic conformational changes of a coronavirus spike glycoprotein promote membrane fusion. Proc Natl Acad Sci U S A. 2017 Oct 17;114(42):11157-11162. doi: 10.1073/pnas.1708727114. Epub 2017 Oct 3

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Comparison of Sequences Between Human Coronaviruses

Table 3 Summary of sequence identities and similarities between SARS CoV-2 (GenBank ID: BCA87361.1) and other human coronaviruses [50]. AA overlap: amino acid overlap

Entry	Name	GenBank ID	Sequence identity (%)	Sequence similarity (%)	AA overlap
1	SARS CoV-1	AAP13441.1	76.0	91.5	1277
2	HCoV 229-E	QOP39313.1	31.3	61.6	777
3	MERS CoV	ASU91305.1	34.8	65.6	1049
4	HCoV OC43	AAA03055.1	30.2	57.9	1344
5	HCoV HKU1	ADN03339.1	29.2	59.0	1358
6	HCoV NL63	AGT51394.1	29.8	60.0	861

*Yoshimoto, F.K. A Biochemical Perspective of the Nonstructural Proteins (NSPs) and the Spike Protein of SARS CoV-2. Protein J **40,** 260–295 (2021). https://doi.org/10.1007/s10930-021-09967-8*

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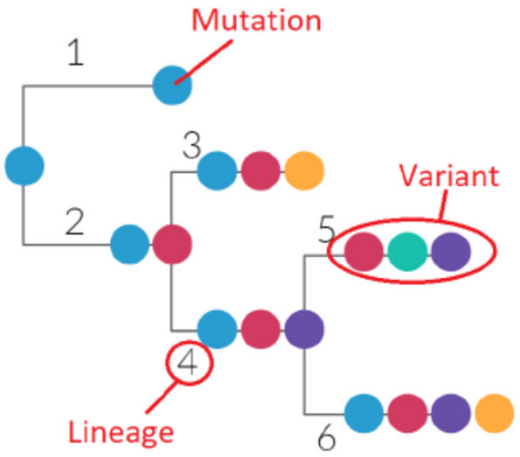
Terminology Related to Variation

Phylogenetic tree:
a "family tree" showing how mutations are related to each other



Mutation: an error introduced during viral replication
Substitution: one nucleotide is replaced with another
synonymous results in no change in amino acid
non-synonymous results in changes in amino acid
Deletion: one or more nucleotides are left out
frame-shifting /no frame shift/ termination
Insertion: one or more extra nucleotides are added
frame-shifting /no frame shift/ termination

Variant: A genome that contains a particular set of mutations

Lineage: All the descendants of a branch of a phylogenetic tree viz. PANGO lineages (Phylogenetic Assignment of Named Global Outbreak)

Convergent evolution: Selective evolutionary pressure

- Randomly, two variants of a virus pop up with the same mutation without the cases being connected
- This suggests selective pressure – the virus has mutated to improve biological fitness

outbreak.info https://outbreak.info/situation-reports/caveats

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Variation/mutation with Public Health Significance (Source: WHO)

Variant of Concern (VOC): A variant that has been associated with at least one of the following:

- Increase in transmissibility or detrimental change in COVID-19 epidemiology
- Increase in virulence or change in clinical disease presentation
- Decrease in effectiveness of public health and social measures or available diagnostics, vaccines, or therapeutics

Variant of Interest (VOI) A SARS-CoV-2 variant

- with genetic changes that are predicted or known to affect virus characteristics such as transmissibility, disease severity, immune escape, diagnostic or therapeutic escape; AND
- Identified to cause significant community transmission or multiple COVID-19 clusters, in multiple countries with increasing relative prevalence alongside increasing number of cases over time, or other apparent epidemiological impacts to suggest an emerging risk to global public health

Tracking SARS-CoV-2 variants (who.int) *https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/*

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Currently designated Variants of Concern

WHO label	Pango lineages	GISAID clade	Nextstrain clade	Additional amino acid changes monitored*	Earliest documented samples	Date of designation
Alpha	B.1.1.7	GRY	20I (V1)	+S:484K +S:452R	United Kingdom Sep-2020	18-Dec-2020
Beta	B.1.351 B.1.351.2 B.1.351.3	GH/501Y.V2	20H (V2)	+S:L18F	South Africa May-2020	18-Dec-2020
Gamma	P.1 P.1.1 P.1.2	GR/501Y.V3	20J (V3)	+S:681H	Brazil Nov-2020	11-Jan-2021
Delta	B.1.617.2 AY.1 AY.2 AY.3	G/478K.V1	21A	+S:417N	India Oct-2020	VOI: 4-Apr-2021 VOC: 11-May-2021

Tracking SARS-CoV-2 variants (who.int) *https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/*

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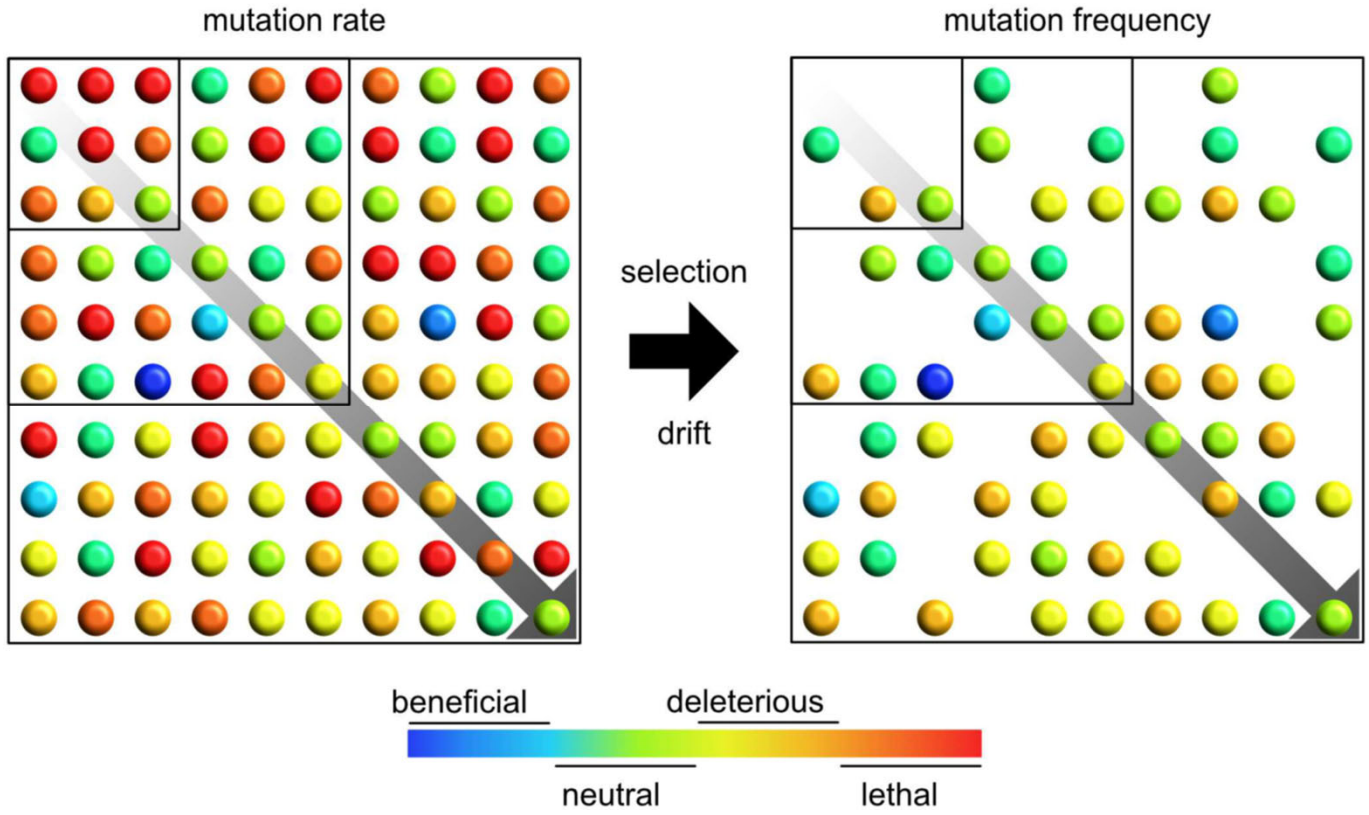
Mutations Instill Variation



Smith EC (2017) The not-so-infinite malleability of RNA viruses: Viral and cellular determinants of RNA virus mutation rates PLoS Pathog 13(4): e1006254

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Mutation Rate Modulators

- Polymerase fidelity
- Sequence context
- Template secondary structure
- Cellular microenvironment
- Replication mechanisms
- Proofreading mechanism

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Effects of Mutations on the Protein Level

- Amino acid substitutions
- Amino acid deletions and insertions
- Chain termination and truncation
- Changes in glycosylation
- Effects on allosteric structure
- Modified immunogenic property
- Altered receptor/ligand interaction
- Effect on protein function

Harvey, William T et al. "SARS-CoV-2 variants, spike mutations and immune escape." Nature reviews. Microbiology vol. 19,7 (2021): 409-424. doi:10.1038/s41579-021-00573-0

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The Evolution of SARS-CoV-2

- Approximately two mutations per month in the global population (from December 2019 to October 2020)
- Genomic analyses indicate a change in host environment and signatures of increased selective pressures
- The emergence of variants with higher numbers of mutations relative to previous circulating variants
- The increased transmissibility

Harvey, William T et al. "SARS-CoV-2 variants, spike mutations and immune escape." Nature reviews. Microbiology vol. 19,7 (2021): 409-424. doi:10.1038/s41579-021-00573-0

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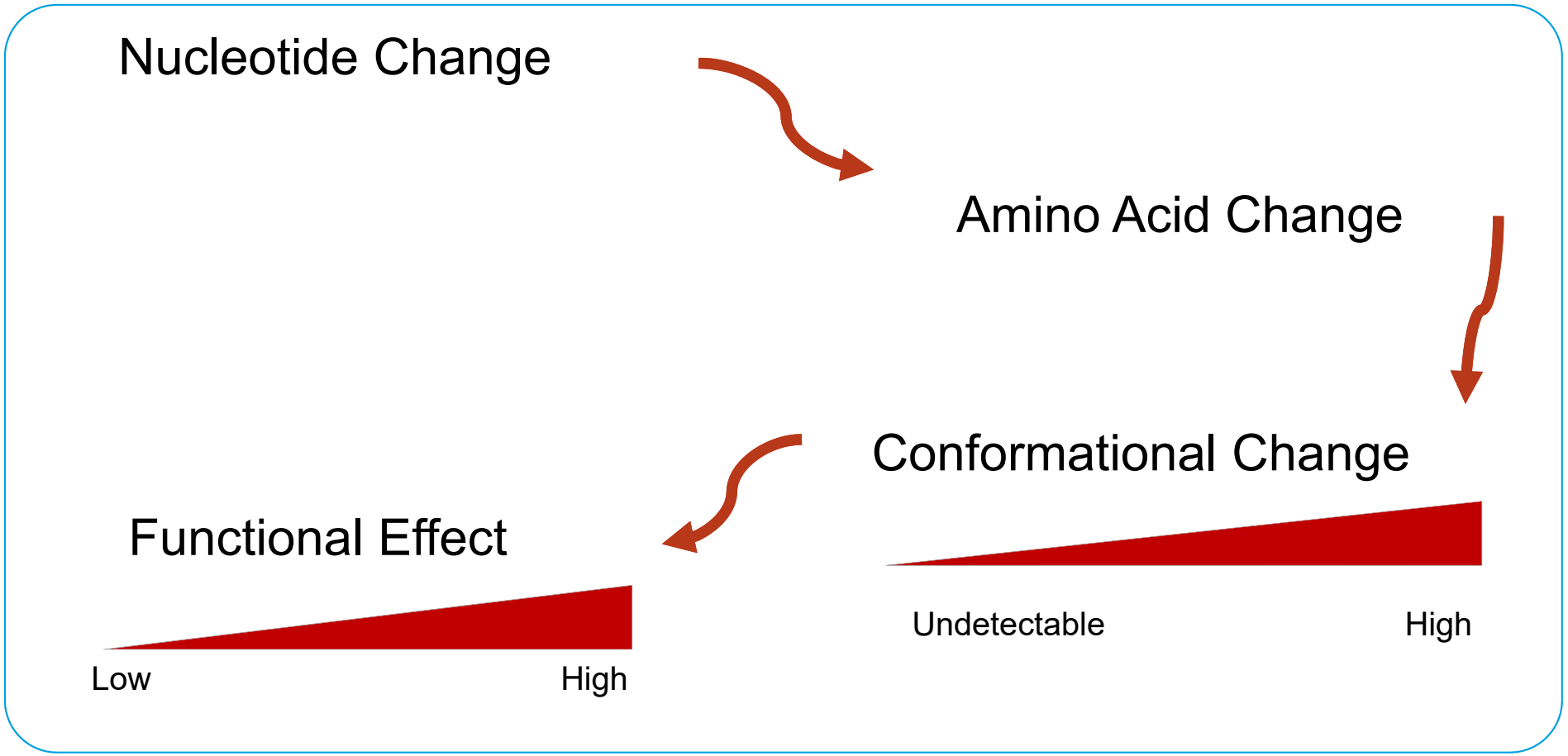
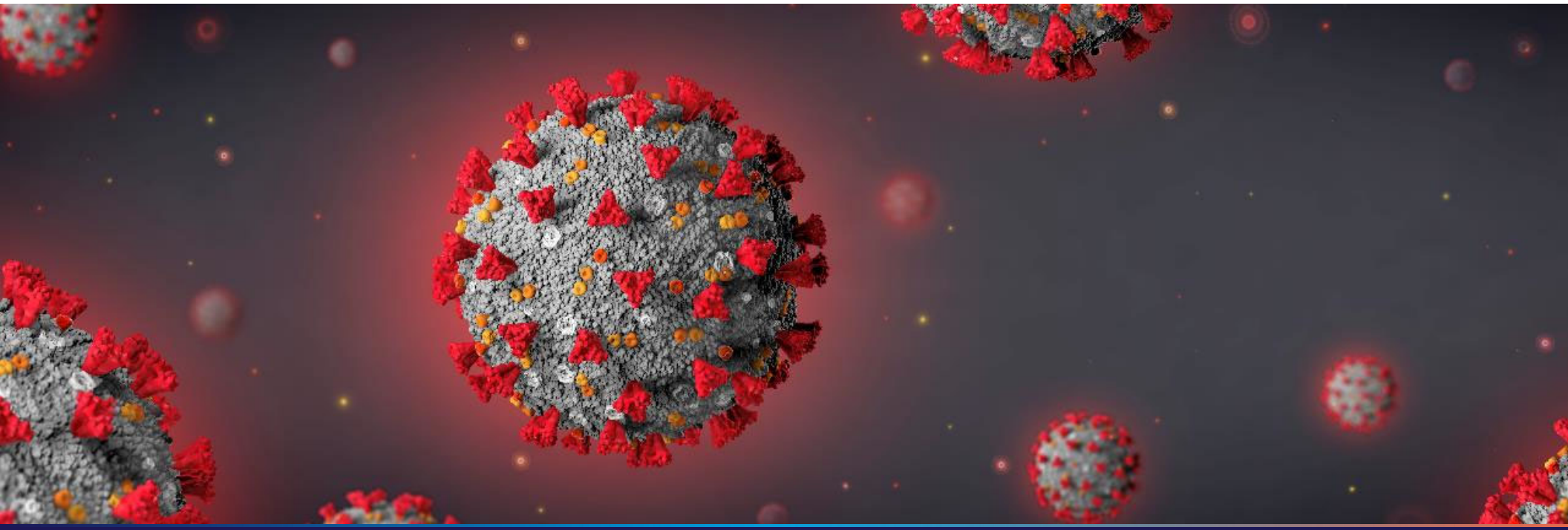
The Manifestation of Variation in SARS-CoV-2 Spike Protein





SARS-CoV-2 Variants: Effect on Conformation

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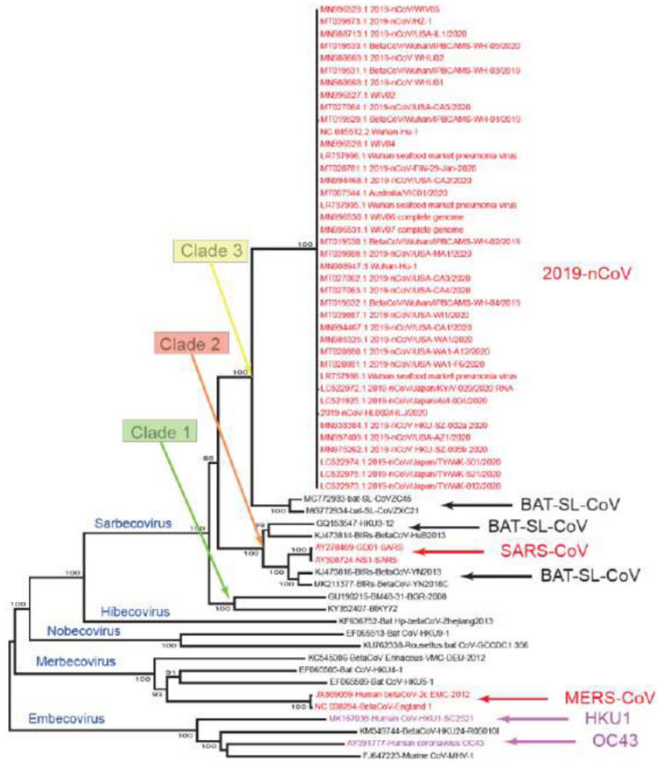
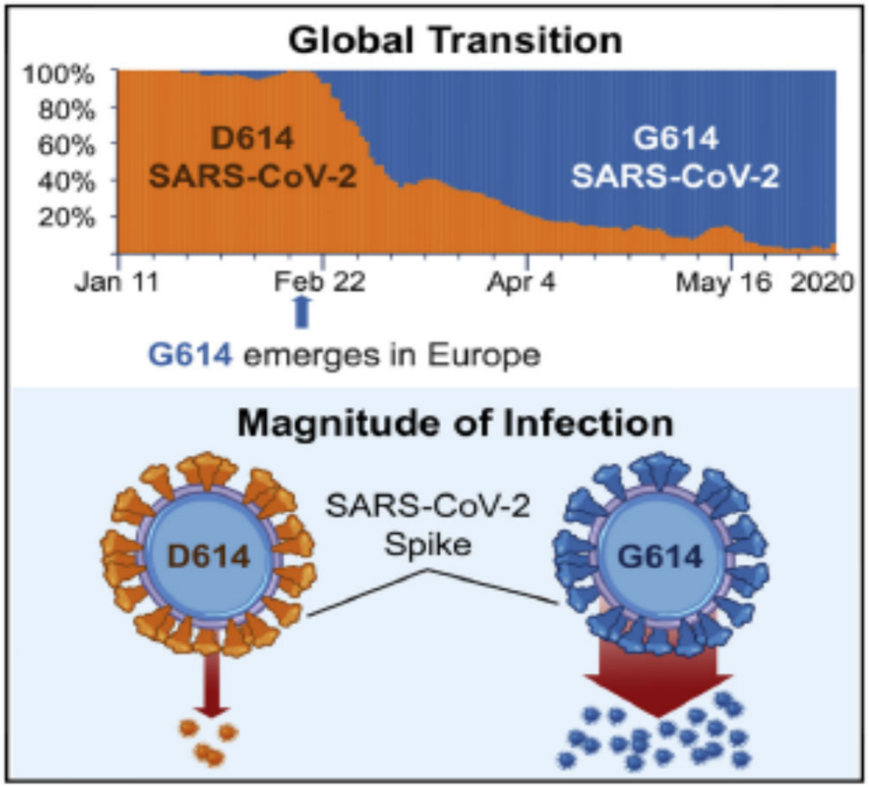
Genetic Evolution of SARS-CoV-2



Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19) 16-24 February 2020



Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. Korber B et al Cell. 2020 Aug 20;182(4):812-827

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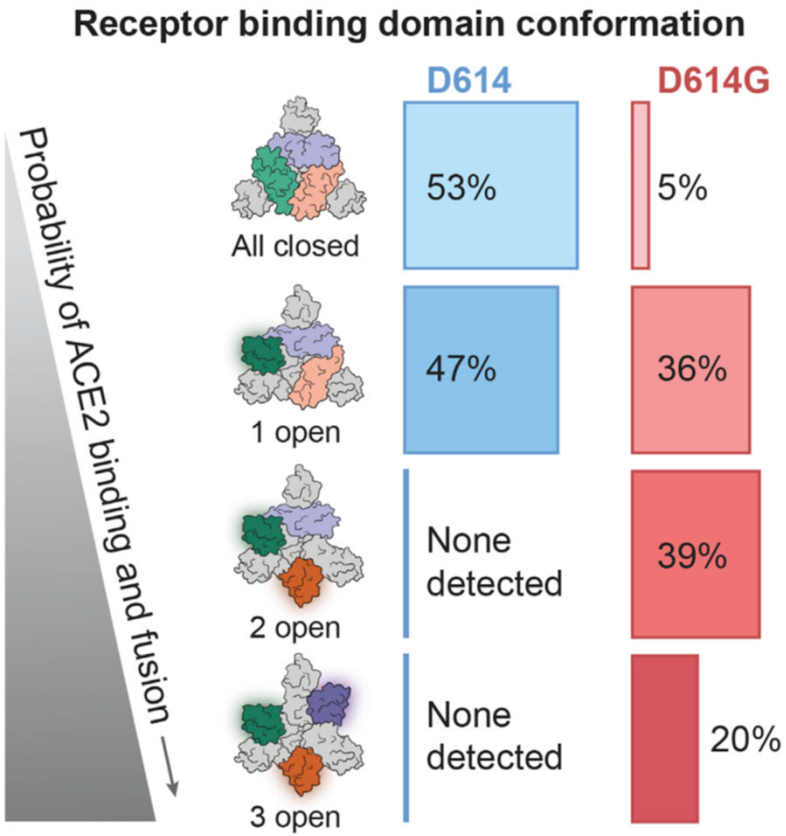
RBD conformation of the D614G Spike Protein Variant



- The SARS-CoV-2 D614G S protein variant supplanted the ancestral virus in people
- D614G increases infectivity on human lung cells or cells with bat or pangolin ACE2
- D614G is potently neutralized by antibodies targeting the receptor-binding domain
- D614G shifts S protein conformation toward an ACE2- binding fusion-competent state

Structural and Functional Analysis of the D614G SARS-CoV-2 Spike Protein Variant Yurkovetskiy et al., 2020, Cell 183, 739–751 October 29, 2020

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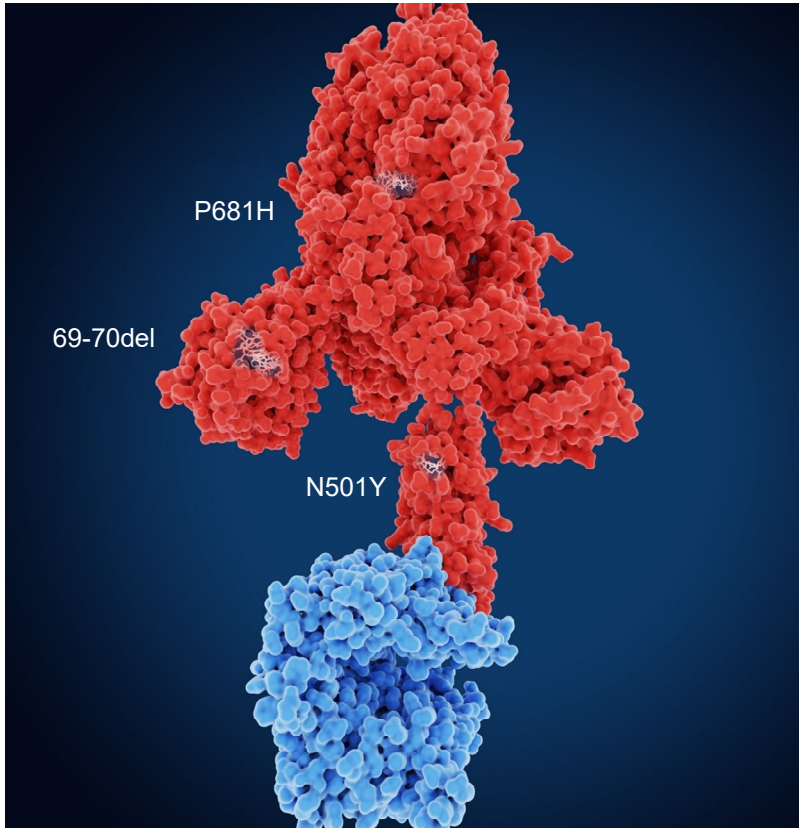
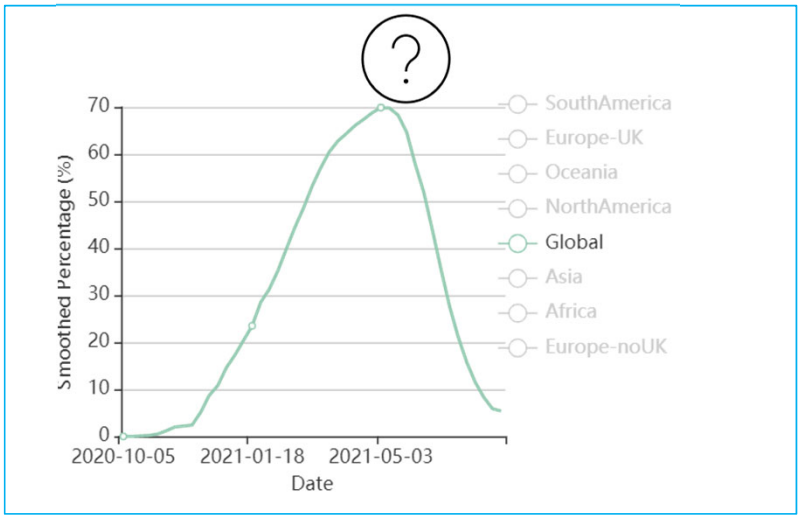
SARS-CoV-2 variant B.1.1.7 (UK variant)



The spike protein (red) and angiotensin converting enzyme 2 (ACE2; blue)

Identified in December 2020



Total #Alpha 202012/01 GRY (B.1.1.7)

https://www.gisaid.org/hcov19-variants/

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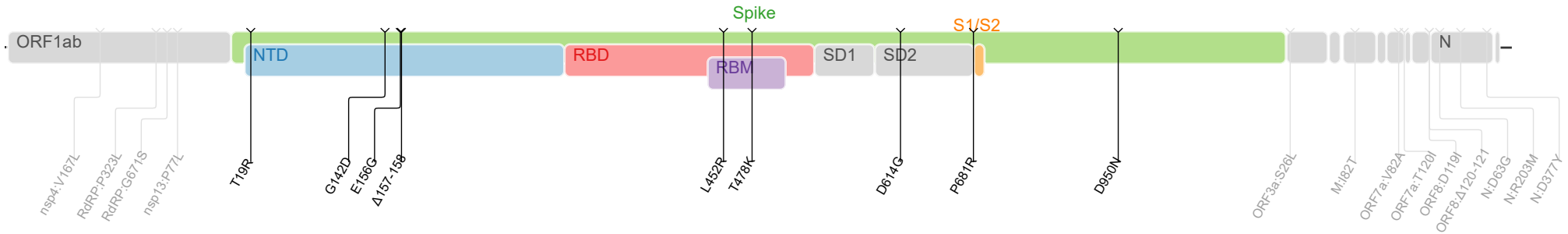
SARS-CoV-2 variant B.1.617.2



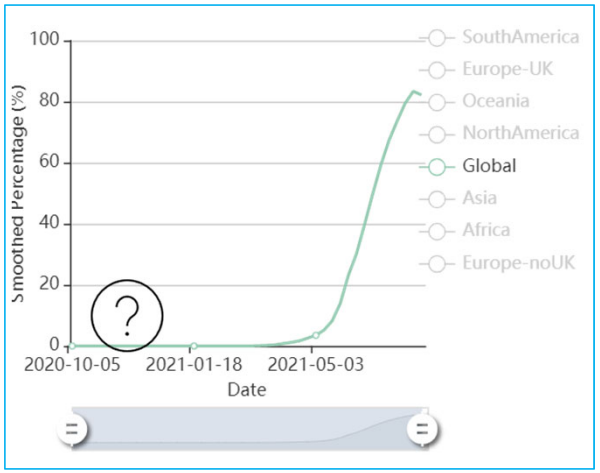
Spike Protein Substitutions:

T19R, (V70F*), T95I, G142D, E156-, F157-, R158G, (A222V*), (W258L*), (K417N*), **L452R, T478K,** D614G, P681R, D950N

Attributes:

- Increased transmissibility
- Potential reduction in neutralization by some EUA monoclonal antibody treatments
- Potential reduction in neutralization by post-vaccination sera

GISAID - hCov19 Variants https://www.gisaid.org/hcov19-variants/



https://covdb.stanford.edu/page/mutation-viewer/#sec_b-1-617-2,

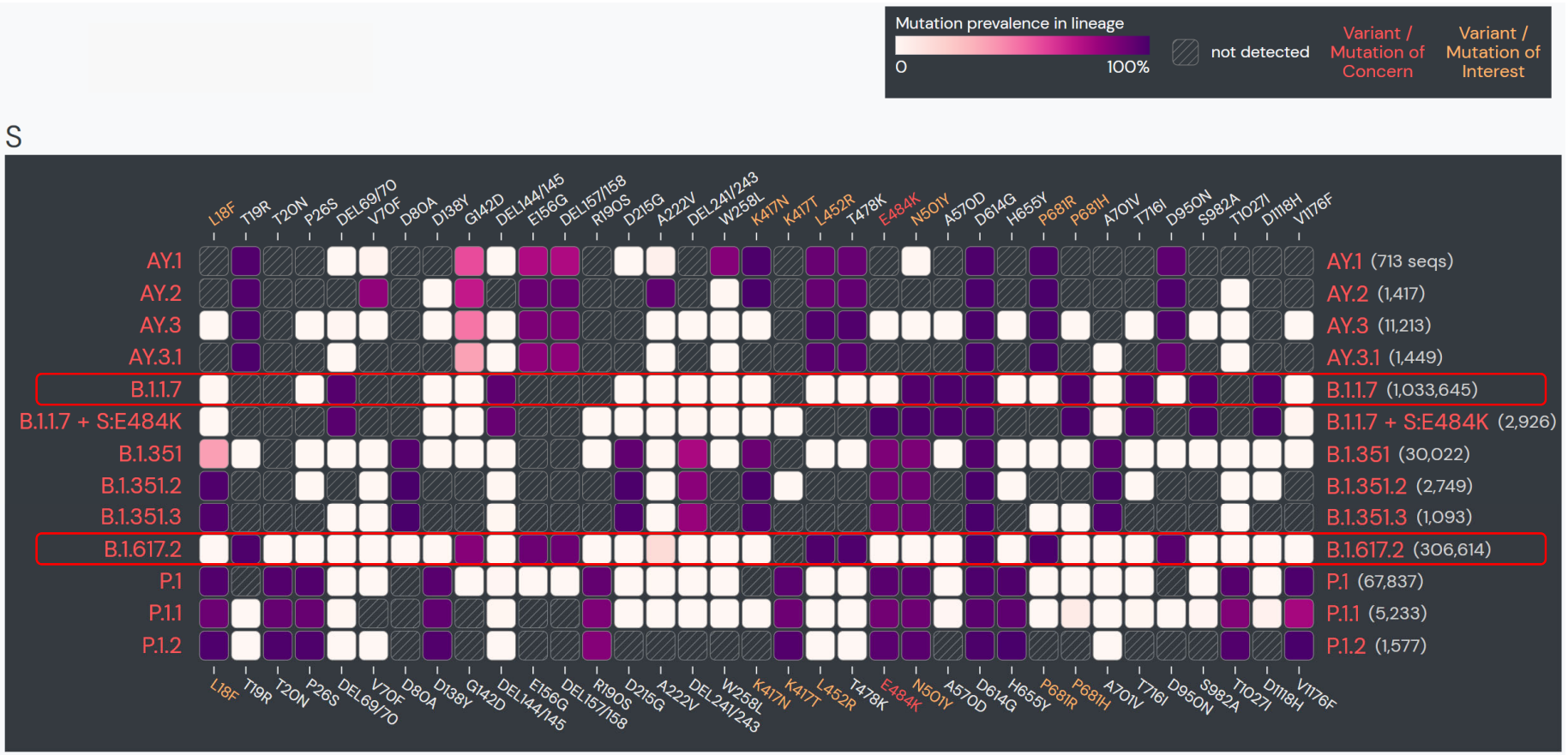
Identified in India Oct. 2020

VOI: 4-Apr-2021

VOC: 11-May-2021

Delta G/478K.V1 (B.1.617.2+AY.1+AY.2+AY.3+AY.3.1)

Mutation Prevalence Across Lineages



outbreak.inhttps://outbreak.info/compare-lineages?pango=AY.1&pango= AY.2&pango=AY.3&pango=AY.3.1&pango=B.1.1.7&pango=B.1.1.7%20%2B%20S%3AE484K&pango=B.1.351&pango=B.1.351.2&pango=B.1.351.3&pango=B.1.617.2&pango=P.1&pango=P.1.1&pango=P.1.2&gene= S&threshold=75&dark=truefo

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SARS-CoV-2 Vaccine Design

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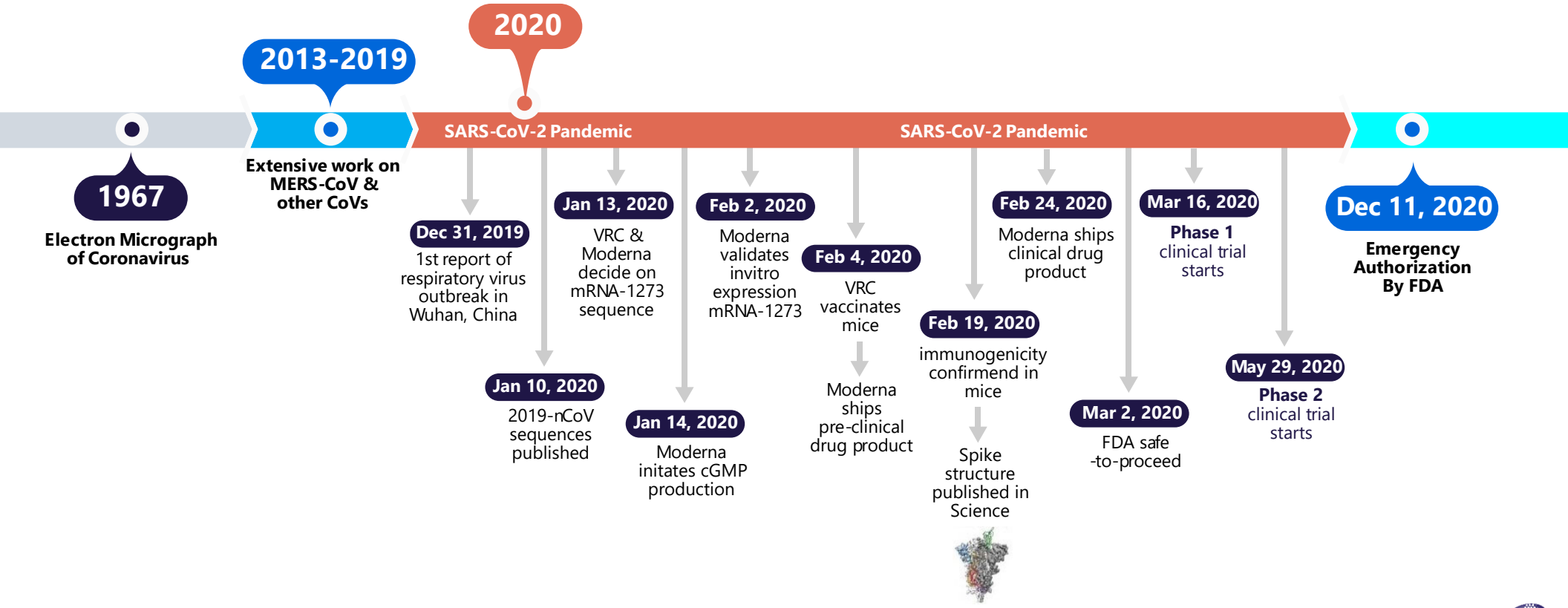
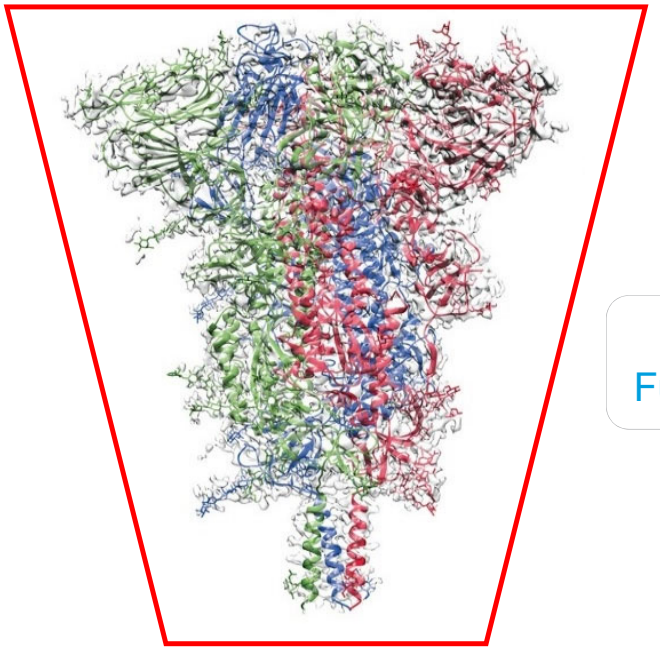
COVID-19 Pandemic and Vaccine Development



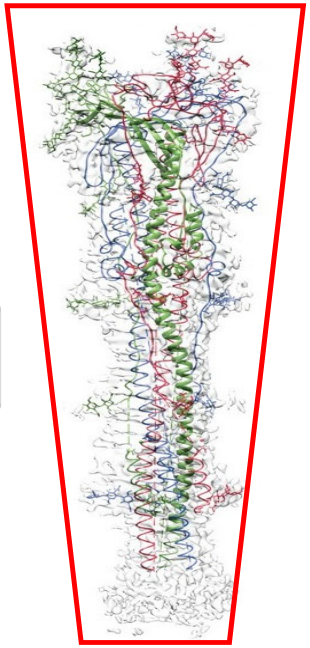
Conformational Variation of Spike Protein



Preferred Conformation for Antibody Design

Pre Fusion

Post Fusion



Adopted from The tiny tweak behind COVID-19 vaccines (acs.org) https://cen.acs.org/pharmaceuticals/vaccines/tiny-tweak-behind-COVID-19/98/i38

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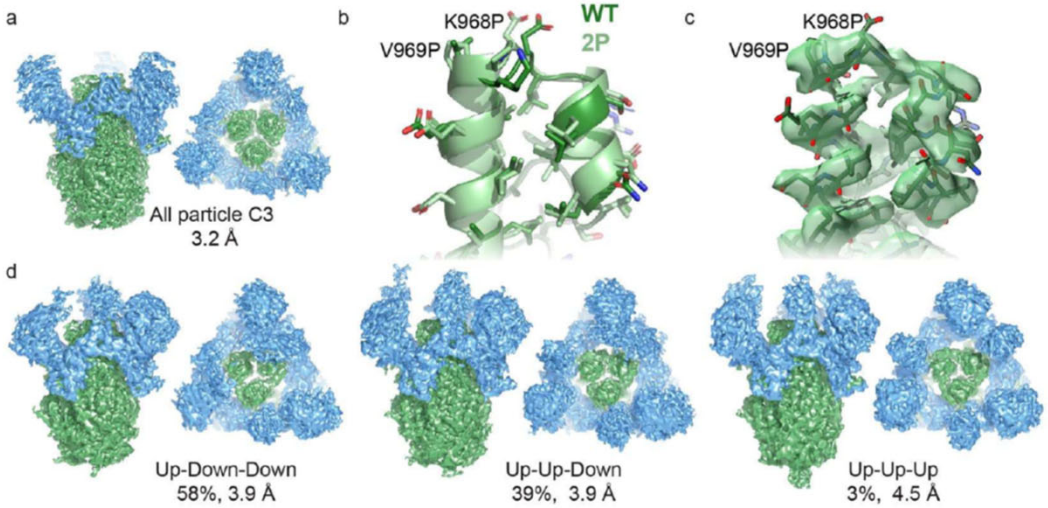
2P Mutation-Stabilized Conformation



Structure of the SARS-CoV S 2P ectodomain.

A. The C3 symmetrized reconstruction
B. Coordinate models derived from cryo-EM reconstructions of the wild-type SARS-CoV S ectodomain (5X58.pdb, dark green) and the prefusion stabilized SARS-CoV S 2P ectodomain (6CRV.pdb light green) adopt identical conformations near the 2P mutation site
C. There is highly featured density in the region containing the 2P mutation site
D. Classification of heterogeneity within the S1 RBD

S1 regions are shown in blue and S2 regions are shown in green

Adopted from Kirchdoerfer, R.N., Wang, N., Pallesen, J. et al. Stabilized coronavirus spikes are resistant to conformational changes induced by receptor recognition or proteolysis. Sci Rep 8, 15701 (2018). https://doi.org/10.1038/s41598-018-34171-7

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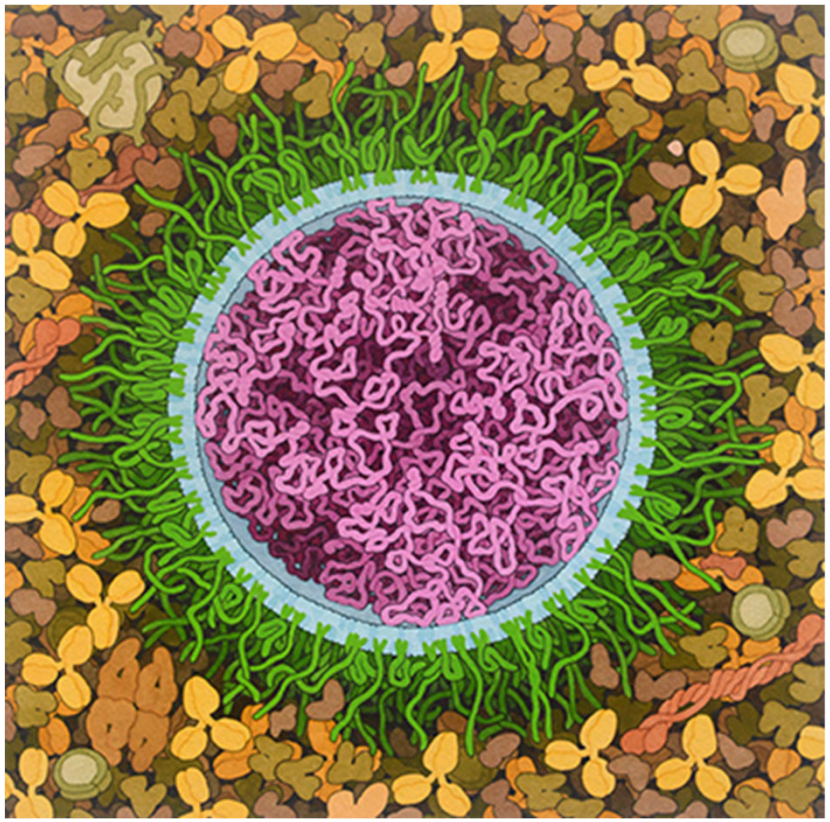
SARS-CoV-2 Messenger RNA (mRNA) Vaccines



https://pdb101.rcsb.org/sci-art/goodsell-gallery/sars-cov-2-mrna-vaccine

Messenger RNA (mRNA) vaccine particle

- RNA encoding the SARS-CoV-2 spike protein (magenta)
- Lipid bilayer of cholesterol and ionizable lipids
- Polyethylene glycol chains having both extended and folded conformations (green)

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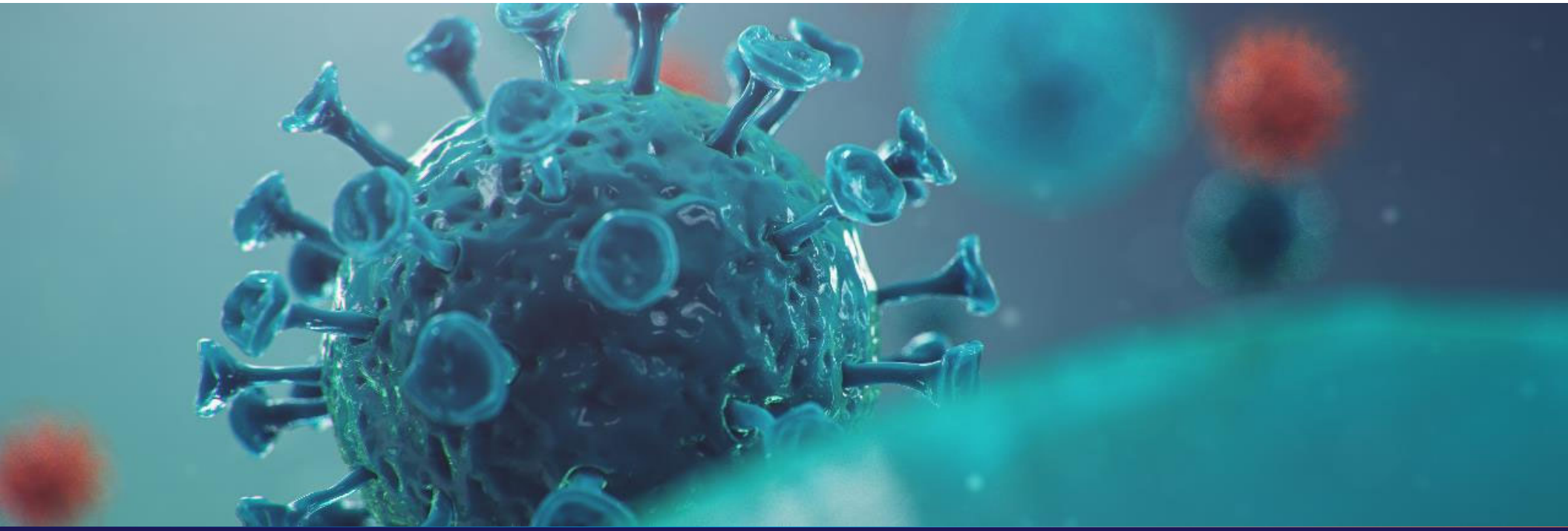

Impact of the Variations on Disease Management

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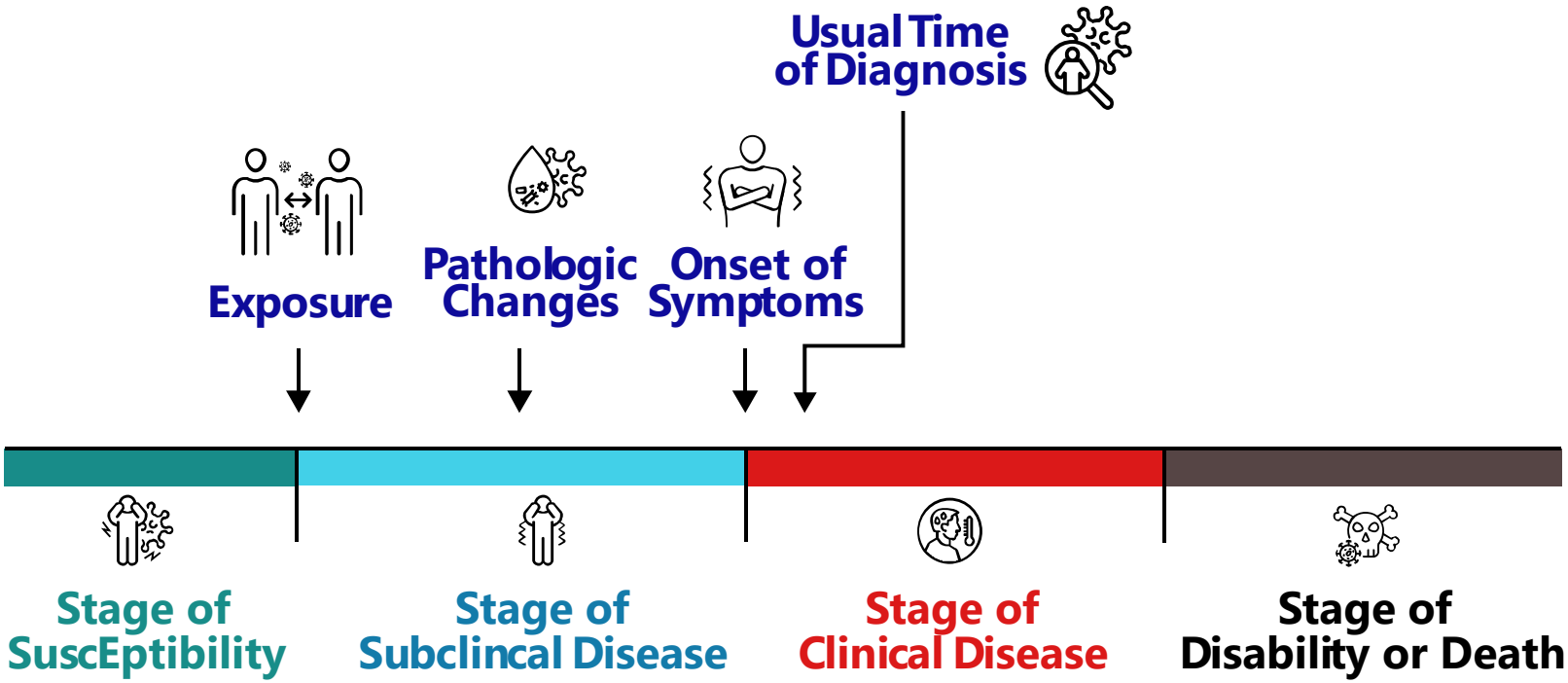
Natural History of Disease Timeline



Adopted from *Source: Centers for Disease Control and Prevention. Principles of epidemiology, 2nd ed. Atlanta: U.S. Department of Health and Human Services;1992.*

Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19) 16-24 February 2020

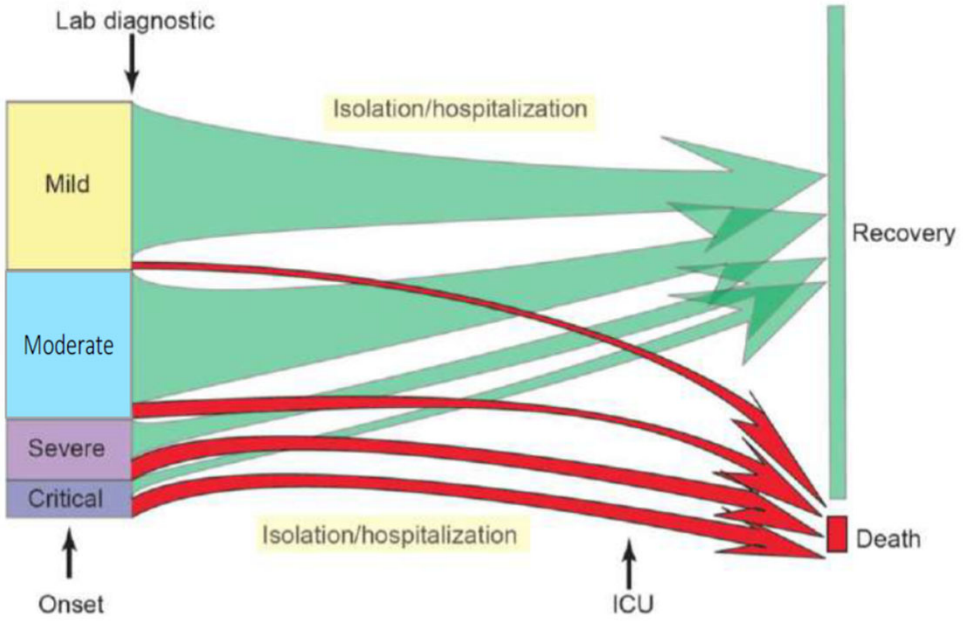


Figure 5. Pattern of disease progression for COVID-19 in China

Note: the relative size of the boxes for disease severity and outcome reflect the proportion of cases reported as of 20 February 2020. The size of the arrows indicates the proportion of cases who recovered or died. Disease definitions are described above. Moderate cases have a mild form of pneumonia.

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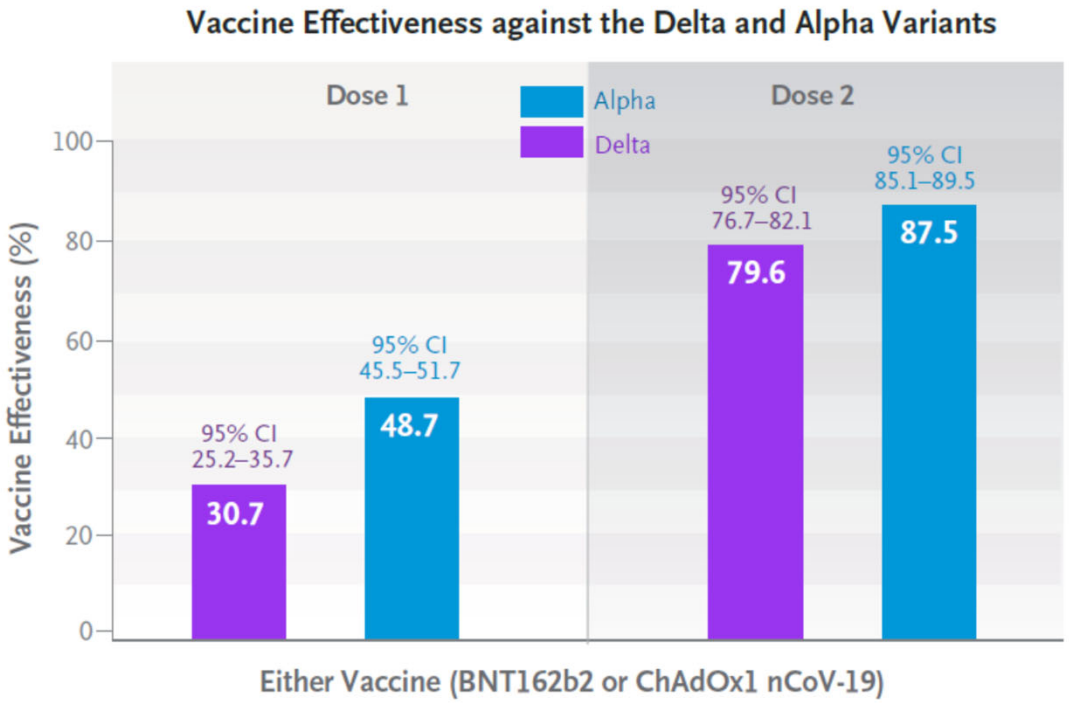
Vaccine Effectiveness on Alpha and Delta Variants



https://www.nejm.org/doi/full/10.1056/NEJMoa2108891

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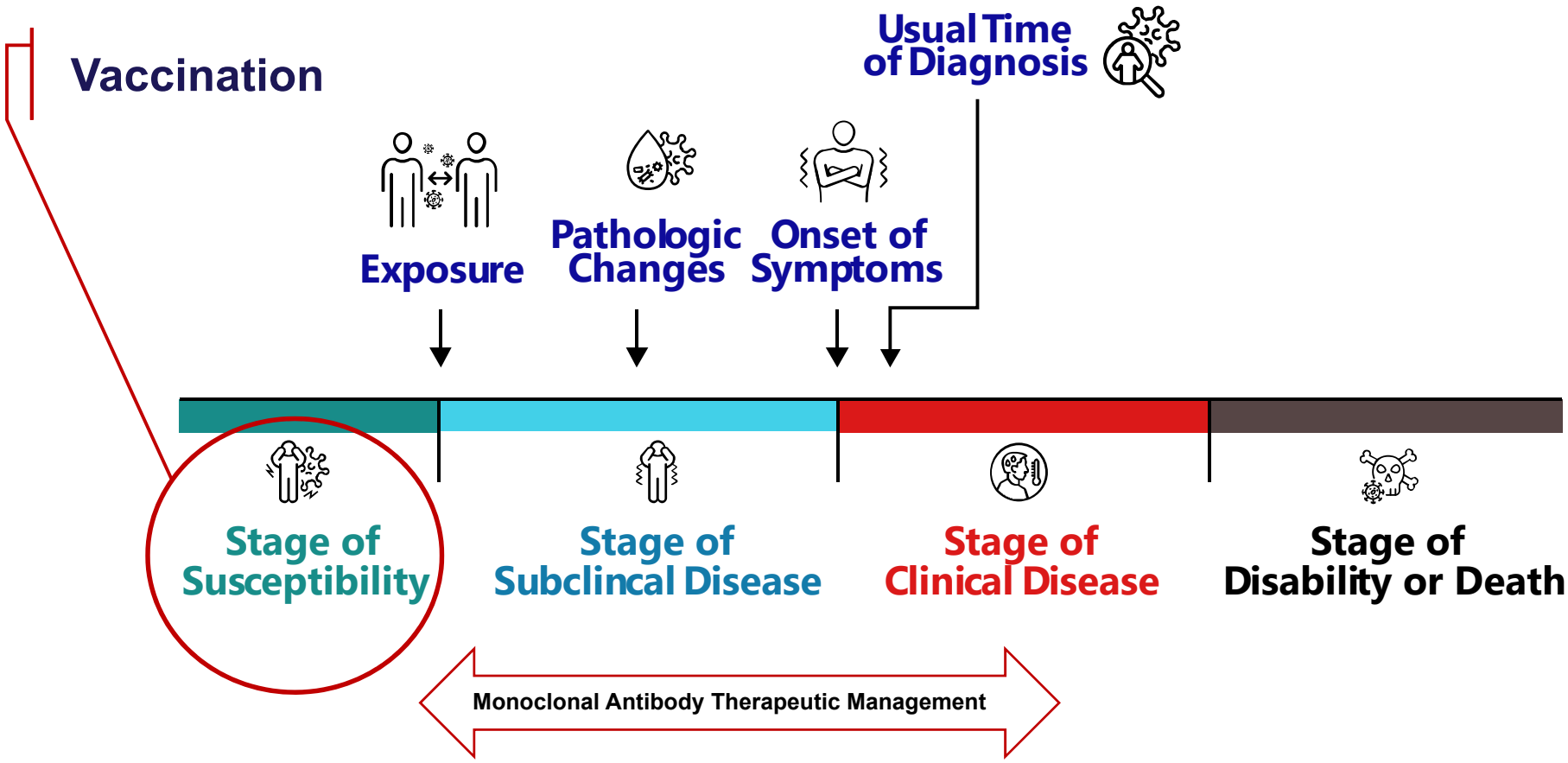
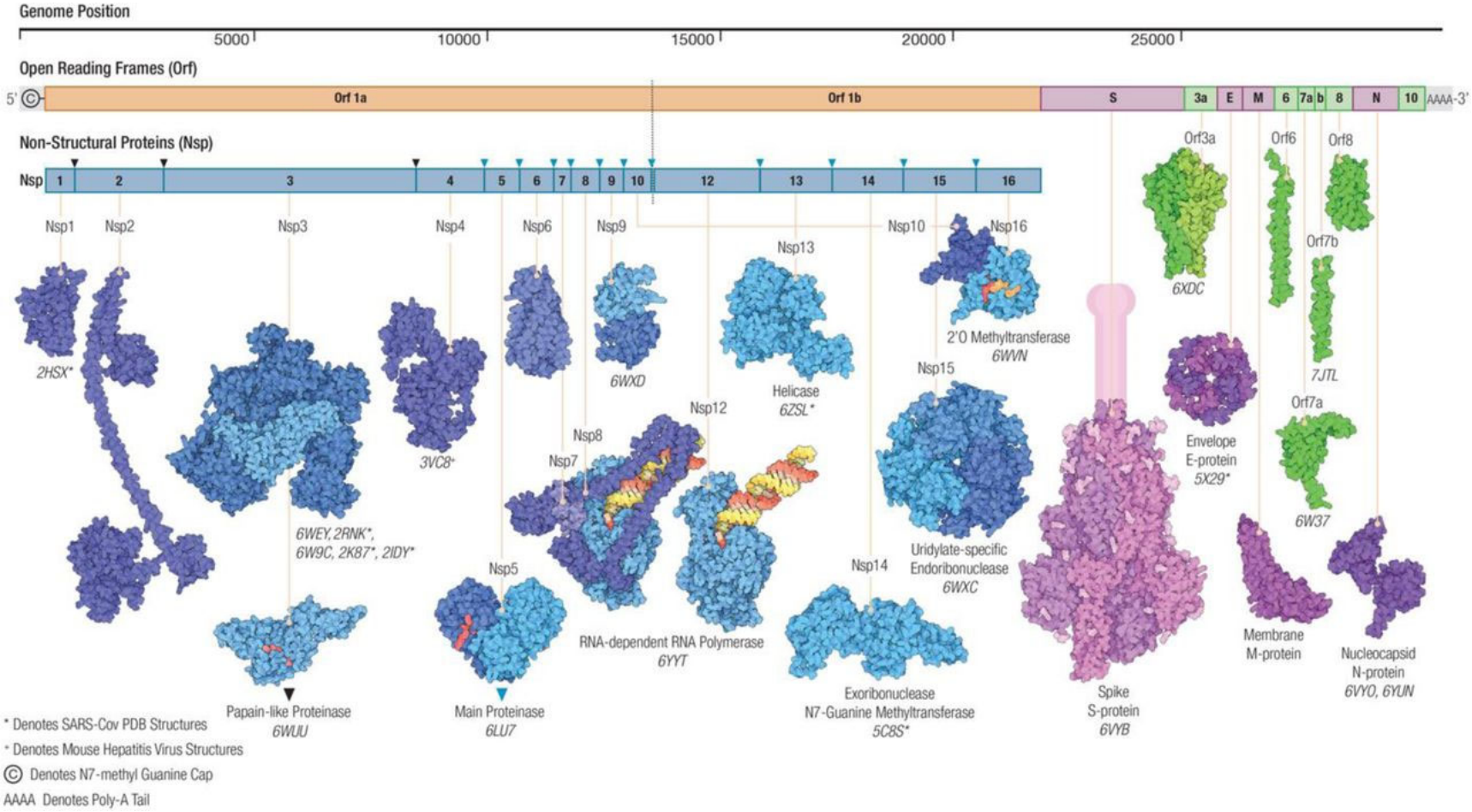
Natural History of Disease Timeline – Intervention



Source: Centers for Disease Control and Prevention. Principles of epidemiology, 2nd ed. Atlanta: U.S. Department of Health and Human Services;1992. (Adopted)

Exploring Additional Avenues

Disease Pathology and Viral Factors



https://www.biorxiv.org/content/10.1101/2020.12.01.406637v1.full.pdf+html

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Prioritizing Diseases for Research and Development in Emergency Contexts

A WHO tool distinguishes which diseases pose the greatest public health risk due to their epidemic potential and/or whether there is no or insufficient countermeasures

At present, the priority diseases are:

- **COVID-19**
- Crimean-Congo haemorrhagic fever
- Ebola virus disease and Marburg virus disease
- Lassa fever
- Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome (SARS)
- Nipah and henipaviral diseases
- Rift Valley fever
- Zika
- **“Disease X”***

This is not an exhaustive list, nor does it indicate the most likely causes of the next epidemic. WHO reviews and updates this list as needs arise, and methodologies change. Based on the priority diseases, WHO then works to develop R&D roadmaps for each one.

**** Disease X represents the knowledge that a serious international epidemic could be caused by a pathogen currently unknown to cause human disease. The R&D Blueprint explicitly seeks to enable early cross-cutting R&D preparedness that is also relevant for an unknown “Disease X”.***

https://www.who.int/activities/prioritizing-diseases-for-research-and-development-in-emergency-contexts

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A Few Areas of Interest

- Repurposing of established medication
- Studies on comparative pathogenesis
- Pathway specific therapeutics
- dsRNA induced innate immune responses
- Peptide blocker for fusion protein
- Detectability enhancement diagnostics

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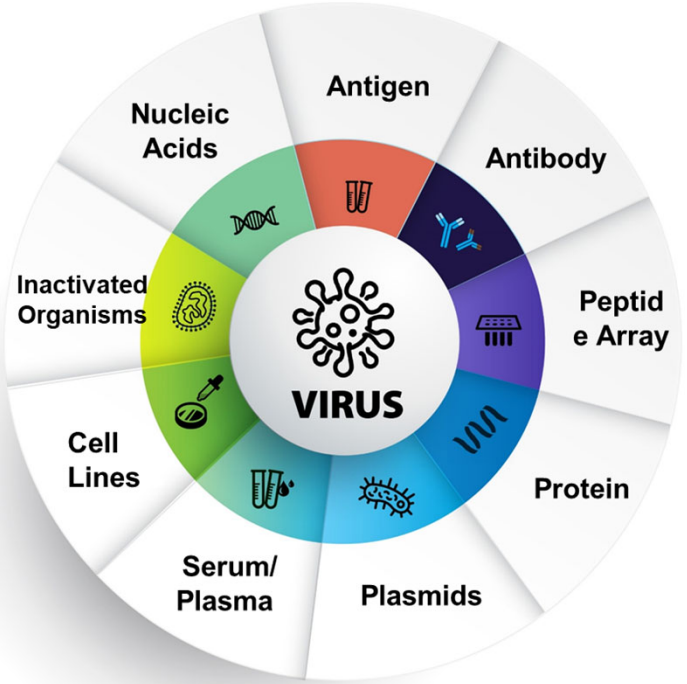
ATCC Coronavirus Reagents

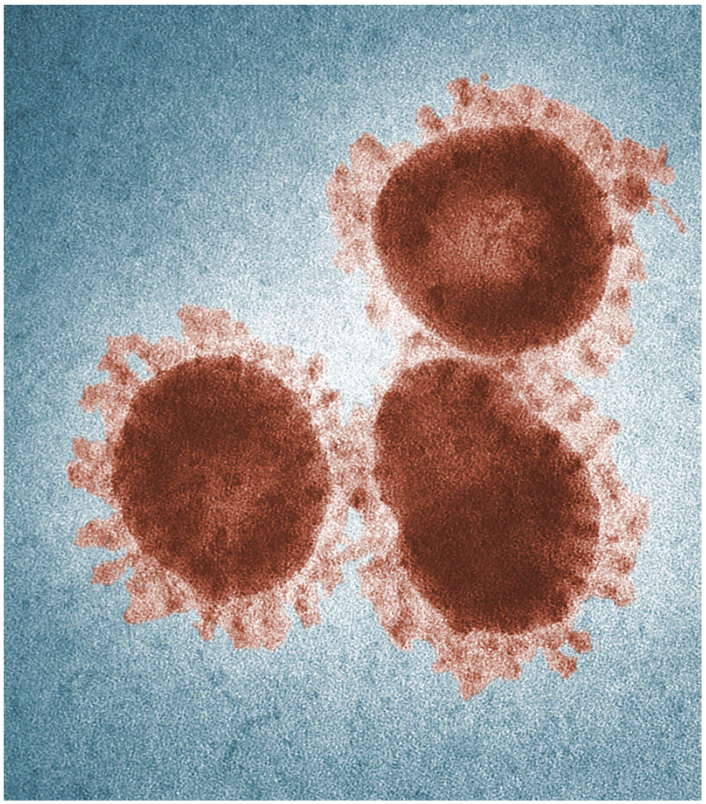
ATCC® No.	Product Description
VR-1986HK™	Heat-inactivated SARS-CoV-2, WA1/2020
VR-1986D™	Genomic SARS-CoV-2 RNA, WA1/2020
VR-3276SD™	Quantitative Synthetic SARS-CoV-2: ORF, E, N
VR-1991D™	Genomic SARS-CoV-2 RNA, Hong Kong
VR-3277SD™	Quantitative Synthetic SARS-CoV-2 RNA: Spike 5'
VR-3278SD™	Quantitative Synthetic SARS-CoV-2 RNA: Spike 3'
VR-1992D™	Genomic SARS-CoV-2 RNA, Italy
VR-1994D™	Genomic SARS-CoV-2 RNA, Germany
VR-3279SD™	Quantitative Synthetic SARS-CoV-2: nsp9, nsp12 (RdRp)
VR-1997™	Anti-SARS-CoV-2 Spike Rabbit Polyclonal Antibody
VR-3326D™	Genomic SARS-CoV-2 RNA, B.1.1.7
VR-3326HK™	Heat-inactivated SARS-CoV-2, B.1.1.7
VR-3327D™	Genomic SARS-CoV-2 RNA, B.1.351
VR-3327HK™	Heat-inactivated SARS-CoV-2, B.1.351
VR-3280SD™	Quantitative Synthetic SARS-CoV [2003] RNA
VR-740™	Human coronavirus Strain: 229E
VR-740D™	Genomic RNA from HCoV 229E
VR-740DQ™	Quantitative Genomic RNA from HCov 229E
VR-1558™	Human coronavirus Strain: OC43
VR-1558D™	Genomic RNA from HCoV OC43
VR-1558DQ™	Quantitative Genomic RNA from HCov OC43
VR-3262SD™	Quantitative Synthetic HCoV Strain: HKU1 RNA
VR-3263SD™	Quantitative Synthetic HCoV Strain: NL63 RNA
VR-3248SD™	Quantitative Synthetic MERS RNA



www.atcc.org/coronavirus

Summary

- Human tropism of coronaviruses
- Viral infection mechanism and membrane interaction
- Relationship of mutation and variation
- SARS-CoV-2 variations and effect on conformation
- Cumulative effect on variations and functional variants
- Spatial projection and vaccine design
- Escape variants and disease development
- Impact of variation on disease management
- Future of pandemic management
- Newer and/or revised information is generating and advancing the field every day



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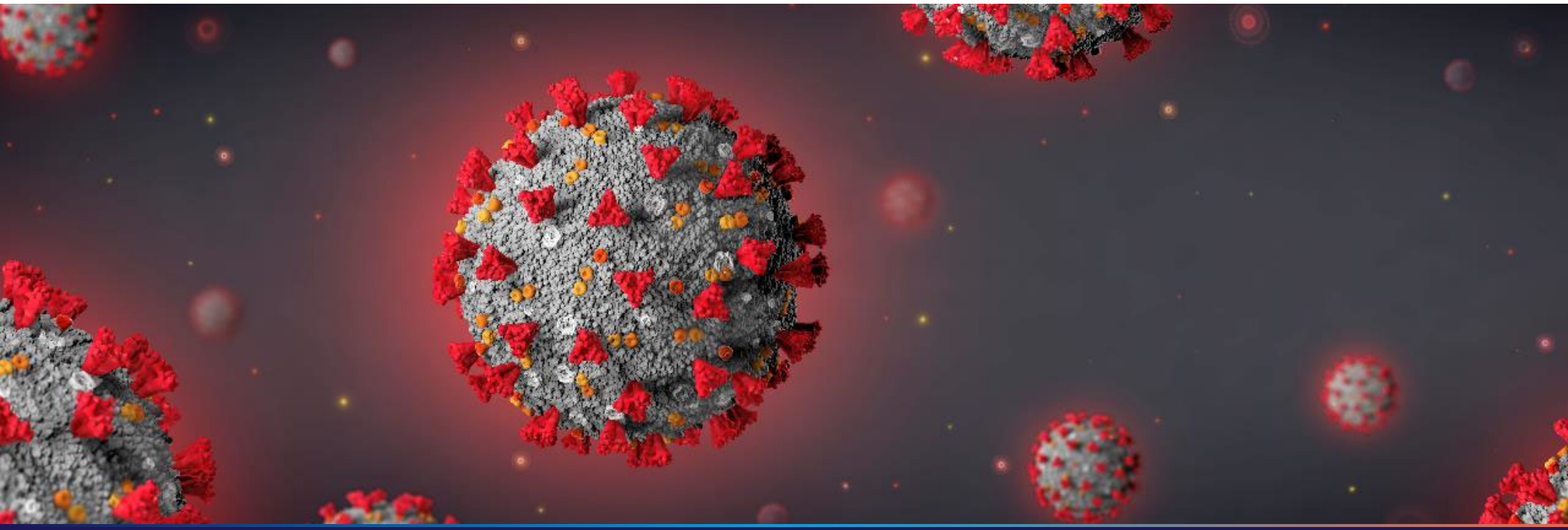


Thank you!

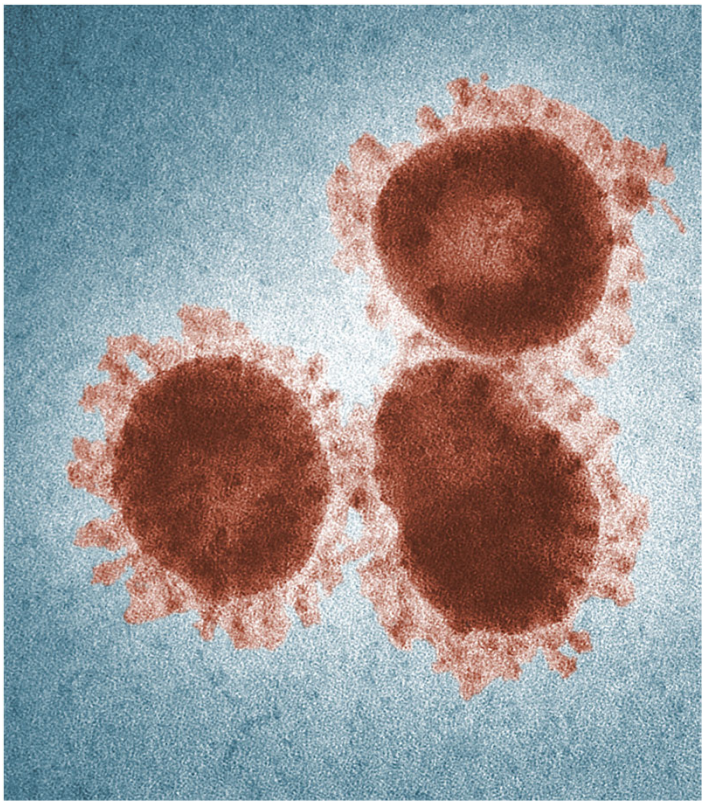
More ATCC Resources

For more coronavirus resources:

www.atcc.org/coronavirus

Upcoming webinar: Cell Culture Fundamentals

- Presented by Steve Budd, MS, MBA
- October 14, 2021, at 12:00 PM ET



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