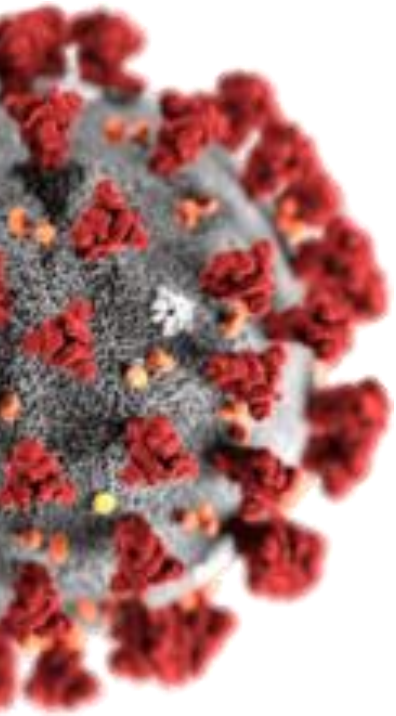
Co jste kdy (ne)chtěli vědět o strukturách SARS-CoV-2



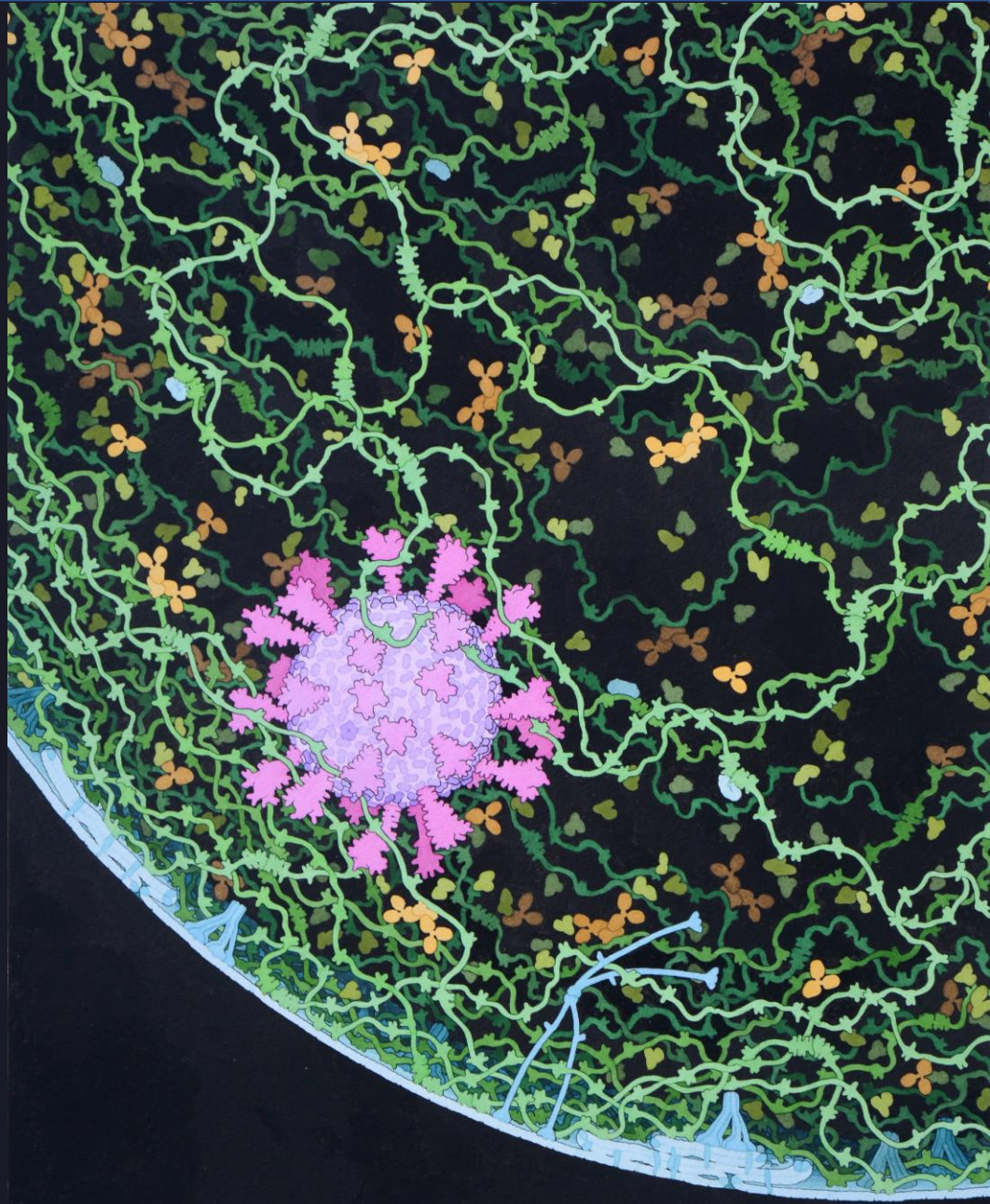
Karel Berka

KFC PřF UPOL

Osnova

SARS-CoV-2

- Genom
- Proteiny
  - Viru
  - Hostitele
- Virová kapsle
- Life cycle viru
- Léčba
- Vakcíny



http://pdb101.rcsb.org/sci-art/goodsell-gallery/respiratory-droplet

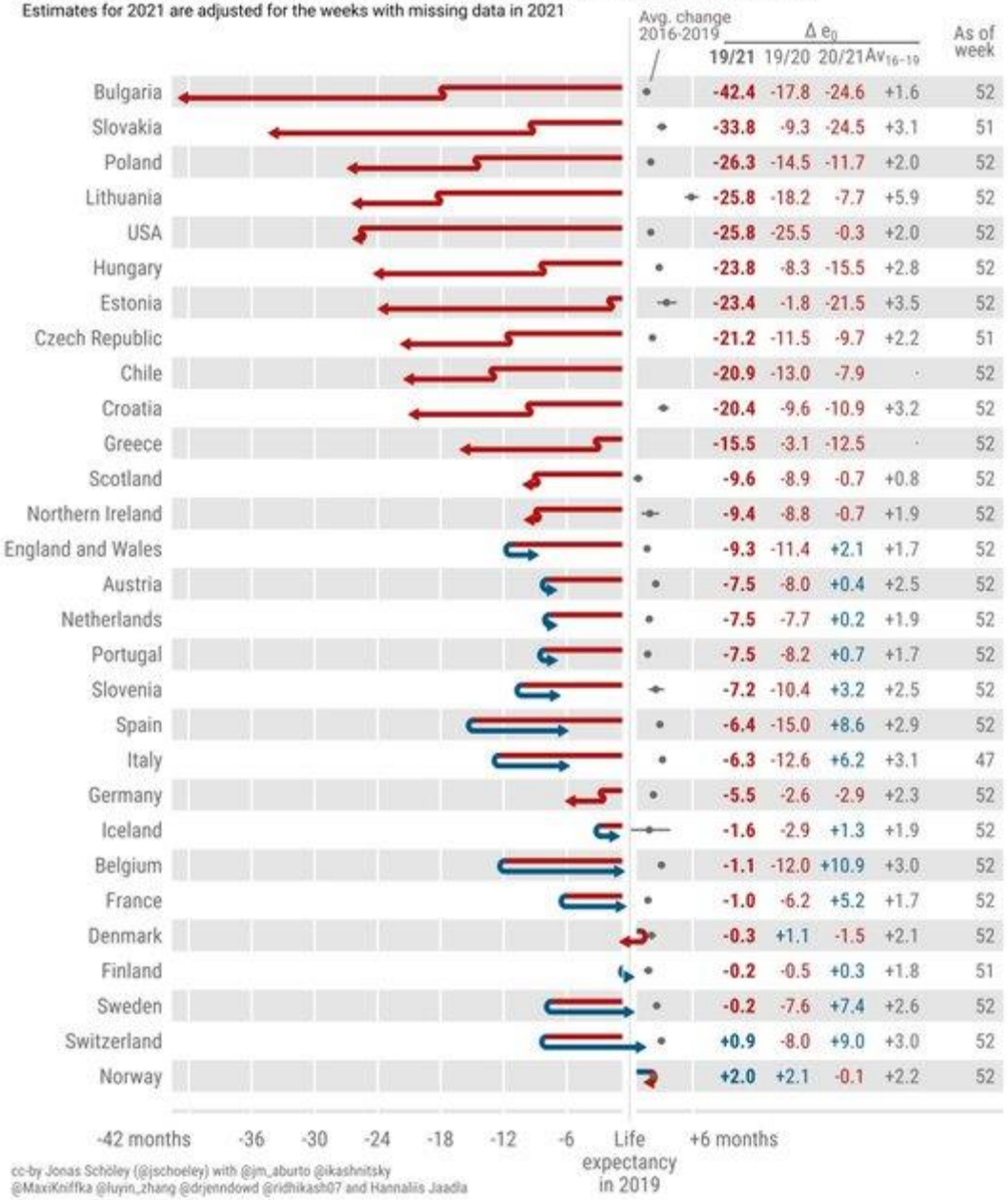
Illustration by David S. Goodsell, RCSB Protein Data Bank; doi: 10.2210/rcsb_pdb/goodsell-gallery-024

Life Cycle

Life expectancy bounce-backs amid continued losses

Life expectancy changes since the start of the COVID-19 pandemic

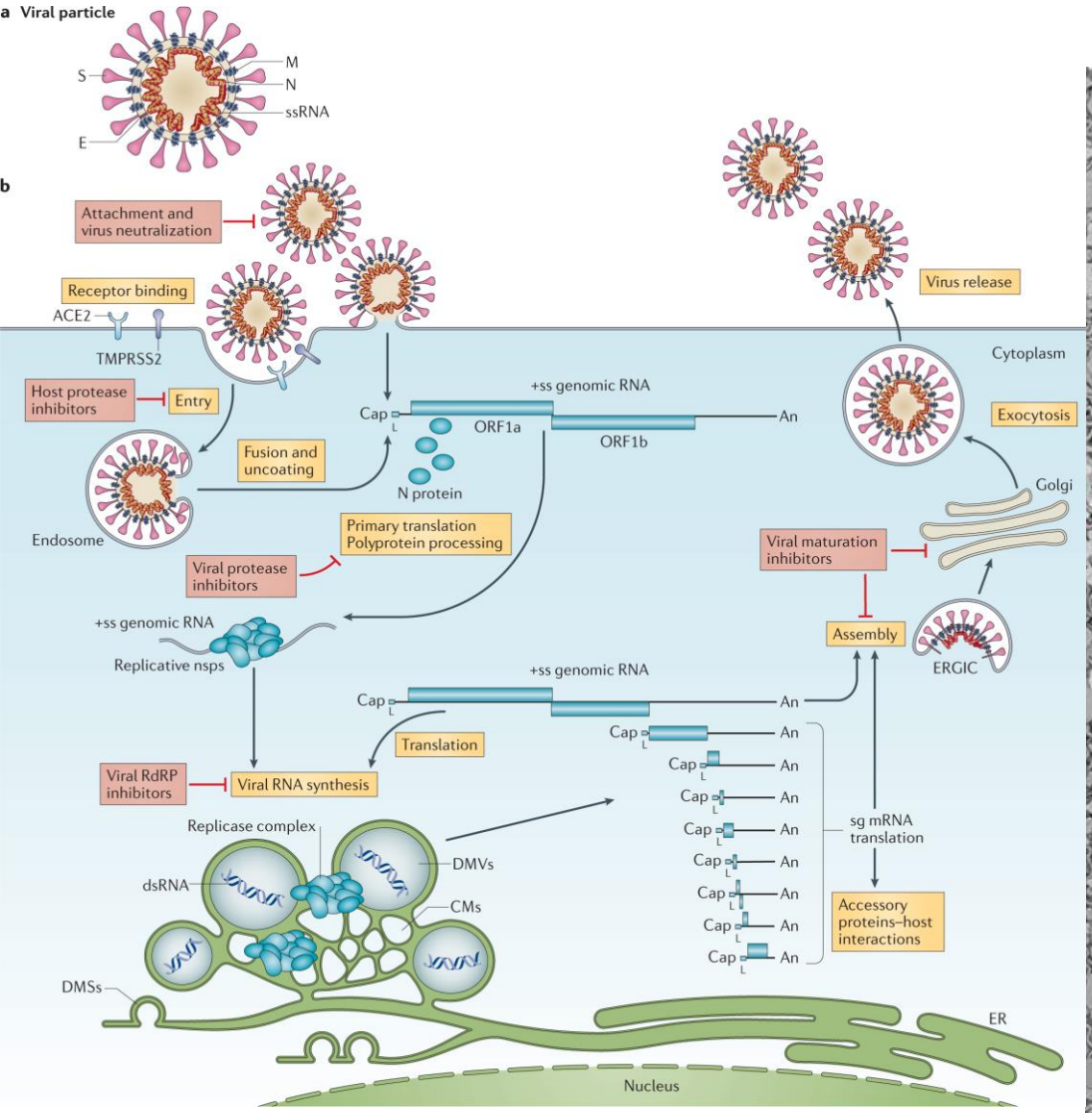
Estimates for 2021 are adjusted for the weeks with missing data in 2021

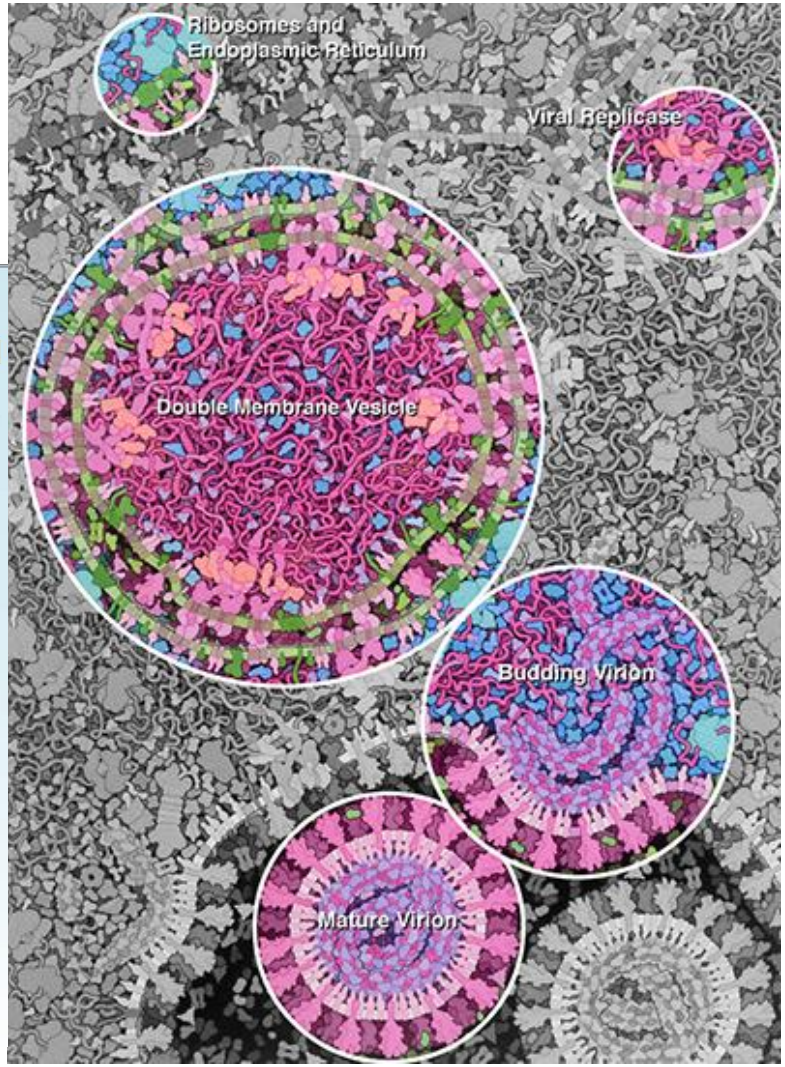


Life Cycle of SARS-CoV-2

https://www.youtube.com/watch?v=k2GlafQ9YhY

Life Cycle of SARS-CoV-2

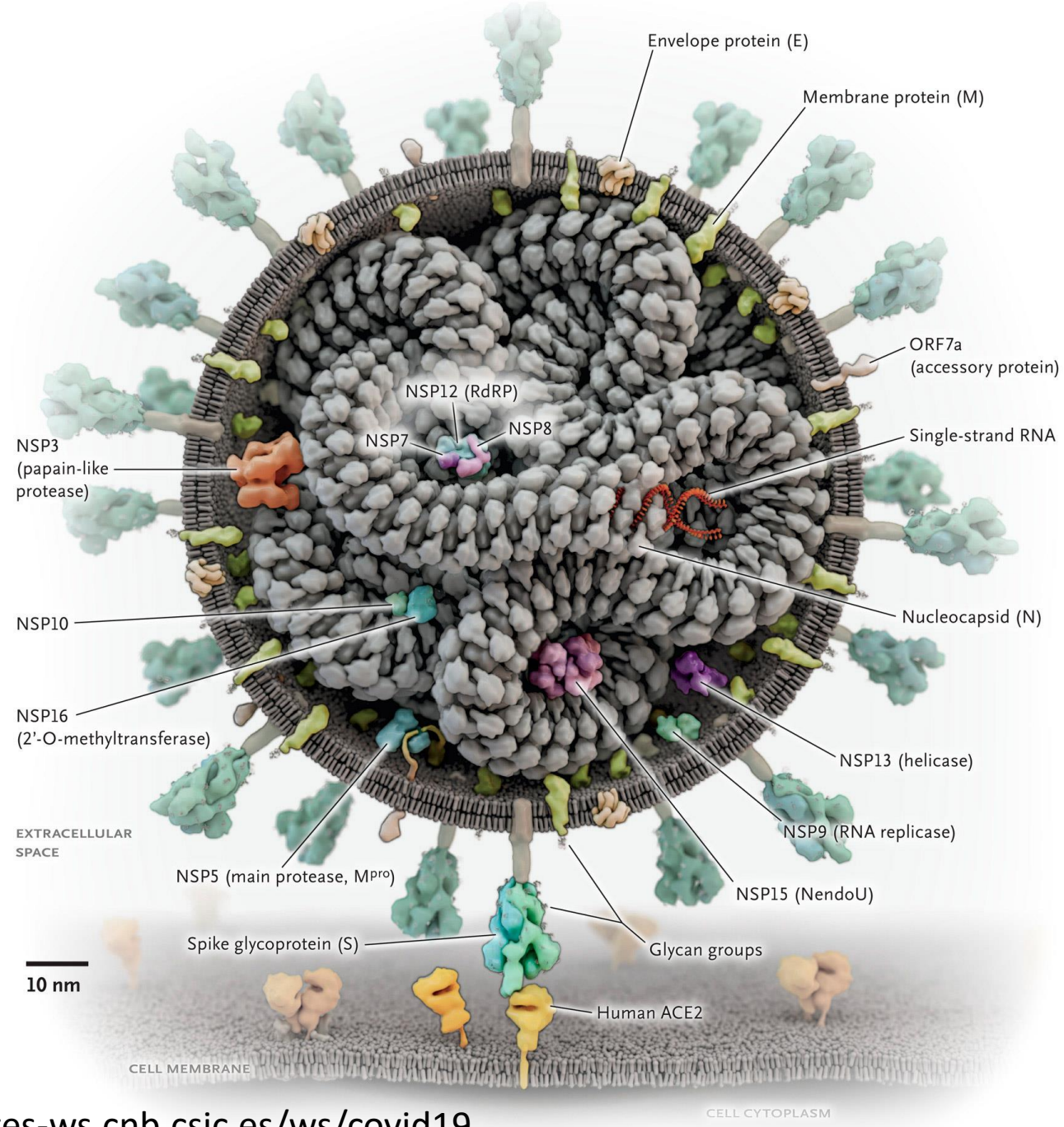




https://www.nature.com/articles/s41579-020-00468-6/figures/1

http://pdb101.rcsb.org/sci-art/goodsell-gallery/coronavirus-life-cycle

Virus Genome/Proteome



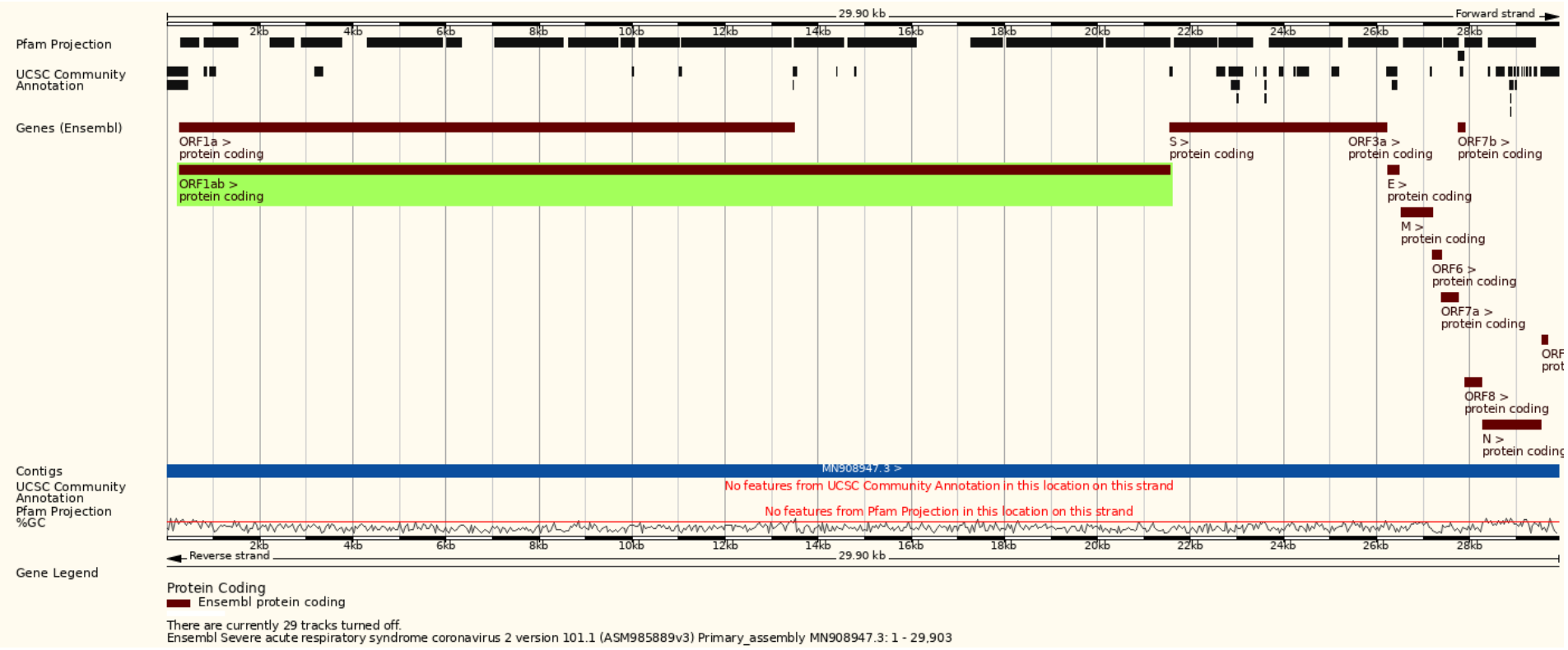
http://3dbionotes-ws.cnb.csic.es/ws/covid19

Genom SARS-Cov-2

RNA+ virus

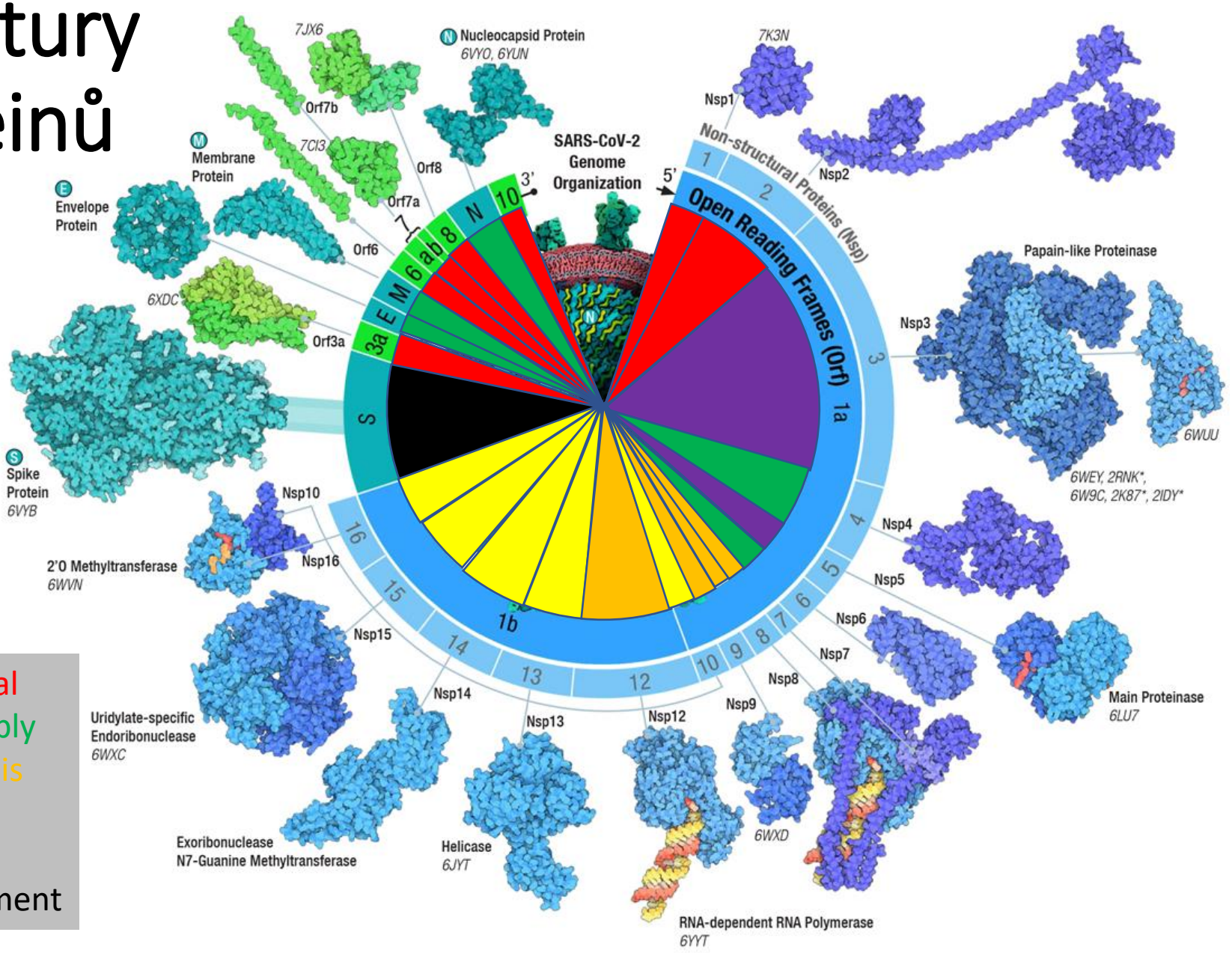
29,903 b

12 coding genes – ORF1ab v sobě obsahuje 16 nsp proteinů



https://covid-19.ensembl.org/Sars_cov_2/Location/View?r=MN908947.3:1-29903

Struktury proteinů

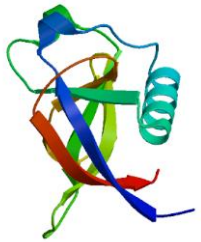


X Cell survival
Virus assembly
RNA synthesis
RNA editing
Proteases
Host attachment

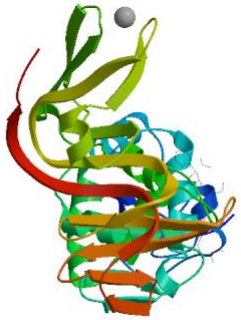
- https://cdn.rcsb.org/pdb101/learn/resources/flyers/covid-genome/covid-genome-prots.png

SARS-CoV-2 proteiny

- Replicase polyprotein 1ab
  - **Multifunctional protein involved in the transcription and replication** of viral RNAs. Contains the proteinases responsible for the cleavages of the polyprotein.
- Host translation inhibitor nsp1
  - Inhibits **host translation** by interacting with the 40S ribosomal subunit. The nsp1-40S ribosome complex further induces an endonucleolytic cleavage near the 5'UTR of host mRNAs, targeting them for degradation. Viral mRNAs are not susceptible to nsp1-mediated endonucleolytic RNA cleavage thanks to the presence of a 5'-end leader sequence and are therefore protected from degradation. By suppressing host gene expression, nsp1 facilitates efficient viral gene expression in infected cells and evasion from host immune response.



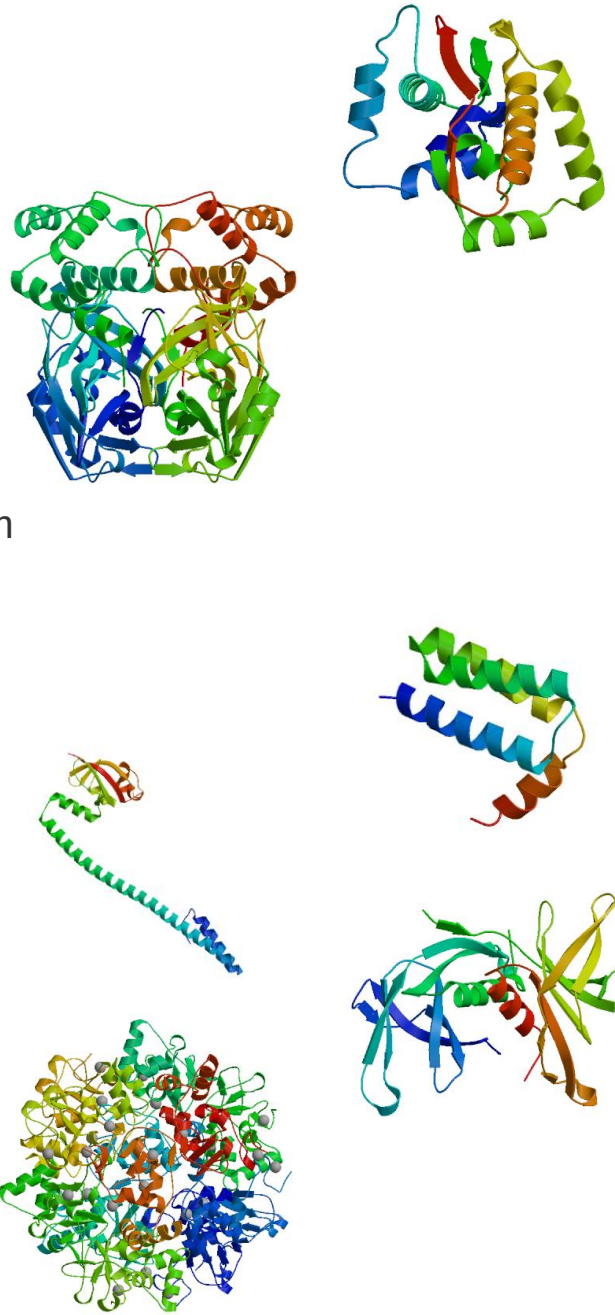
- Non-structural protein 2 (nsp2)
  - **modulation of host cell survival signaling pathway by interacting with host PHB and PHB2**. Indeed, these two proteins play a role in maintaining the functional integrity of the mitochondria and protecting cells from various stresses



- Non-structural protein 3 (nsp3) - PL-PRO
  - Responsible for the **cleavages of polyprotein**

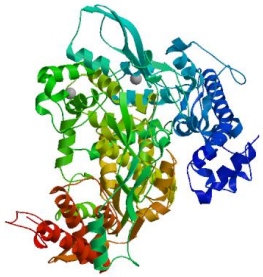
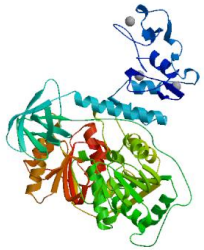
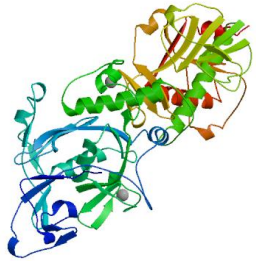
https://swissmodel.expasy.org/repository/species/2697049

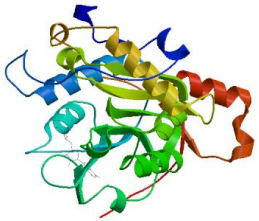
- Non-structural protein 4 (nsp4)
  - **assembly of** virally-induced cytoplasmic **double-membrane vesicles** necessary for viral replication.
- 3C-like proteinase (3CL-PRO)
  - **Cleaves** the C-terminus of **replicase polyprotein** at 11 sites
- Non-structural protein 6 (nsp6)
  - initial **induction of autophagosomes** from host reticulum endoplasmic. Later, limits the expansion of these phagosomes that are no longer able to deliver viral components to lysosomes
- Non-structural protein 7 (nsp7)
  - **viral RNA synthesis**
- Non-structural protein 8 (nsp8)
  - **viral RNA synthesis**
- Non-structural protein 9 (nsp9)
  - viral replication by acting as a **ssRNA-binding protein.**
- Non-structural protein 10 (nsp10)
  - viral mRNAs **cap methylation**



https://swissmodel.expasy.org/repository/species/2697049

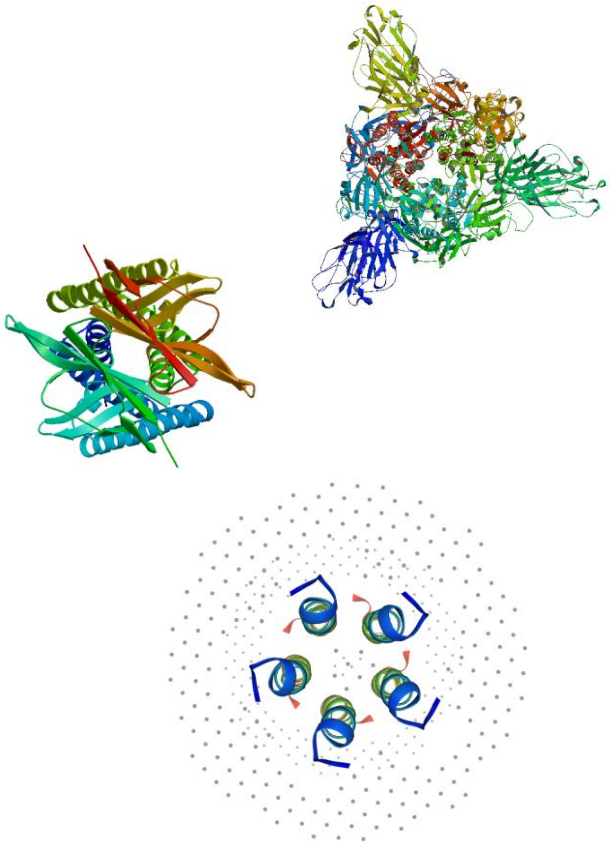
- RNA-directed RNA polymerase (Pol)
  - Responsible for **replication and transcription** of the viral RNA genome.
- Helicase (Hel)
  - RNA and DNA **duplex-unwinding** activities with 5' to 3' polarity
- Proofreading exoribonuclease (ExoN)
  - **exoribonuclease** activity acting on both ssRNA and dsRNA in a 3' to 5' direction and a N7-guanine methyltransferase activity.
- Uridylate-specific endoribonuclease
  - Mn(2+)-dependent, uridylate-specific enzyme, which leaves 2'-3'-cyclic phosphates 5' to the **cleaved** bond.
- 2'-O-methyltransferase
  - mRNA **cap 2'-O-ribose** methylation to the **5'-cap structure** of viral mRNAs. - to evade immune system



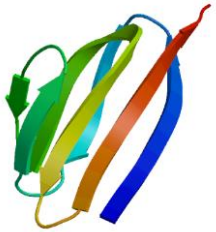
https://swissmodel.expasy.org/repository/species/2697049

- Spike glycoprotein (S protein)
  - **attaches** the virion to the cell membrane by binding to human **ACE2 receptor**
- ORF3a protein (ORF3a)
  - potassium sensitive ion channels (**viroporin**) and may modulate virus release. Up-regulates expression of fibrinogen subunits FGA, FGB and FGG in host lung epithelial cells. Induces apoptosis in cell culture
- Envelope small membrane protein (E)
  - central role in **virus morphogenesis and assembly**. Acts as a viroporin and self-assembles in host membranes forming pentameric protein-lipid pores that allow ion transport. Also plays a role in the induction of apoptosis
- Membrane protein (M)
  - viral envelope that plays a central role in **virus morphogenesis and assembly**
  - Regulates the **localization of S protein** at cis-Golgi, the place of virus budding.
- ORF6 protein (ORF6)
  - Disrupts cell nuclear import complex formation by tethering karyopherin alpha 2 and karyopherin beta 1 to the membrane. **blocking the expression of interferon** stimulated genes (ISGs) that display multiple antiviral activities



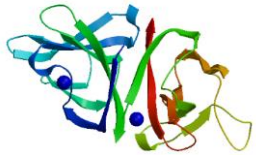
https://swissmodel.expasy.org/repository/species/2697049

- ORF7a protein (ORF7a)
  - **antagonist of host tetherin** (BST2), disrupting its antiviral effect. May suppress small interfering RNA (siRNA)

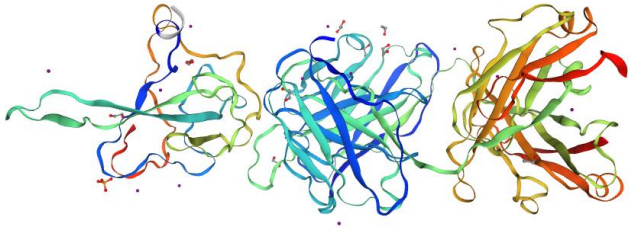


- ORF7b protein (ORF7b)
  - No known function – **locates to host Golgi and host endosome**

- ORF8 protein (ORF8)
  - Binds to IL17RA receptor, leading to IL17 pathway activation and an increased secretion of pro-inflammatory factors. **Contributes to cytokine storm during COVID-19 infection..**



- Nucleoprotein (N)
  - **Packages the positive strand viral genome RNA** into a helical ribonucleocapsid (RNP) and plays a fundamental role during viric assembly through its interactions with the viral genome and membrane protein M



- ORF9b protein (ORF9b)
  - **inhibition of host innate immune** response by targeting the mitochondrial-associated adapter MAVS

- ORF9c protein (ORF9c)
  - May play a role **in host-virus interaction**.
- ORF10 protein
  - No known function

https://swissmodel.expasy.org/repository/species/2697049

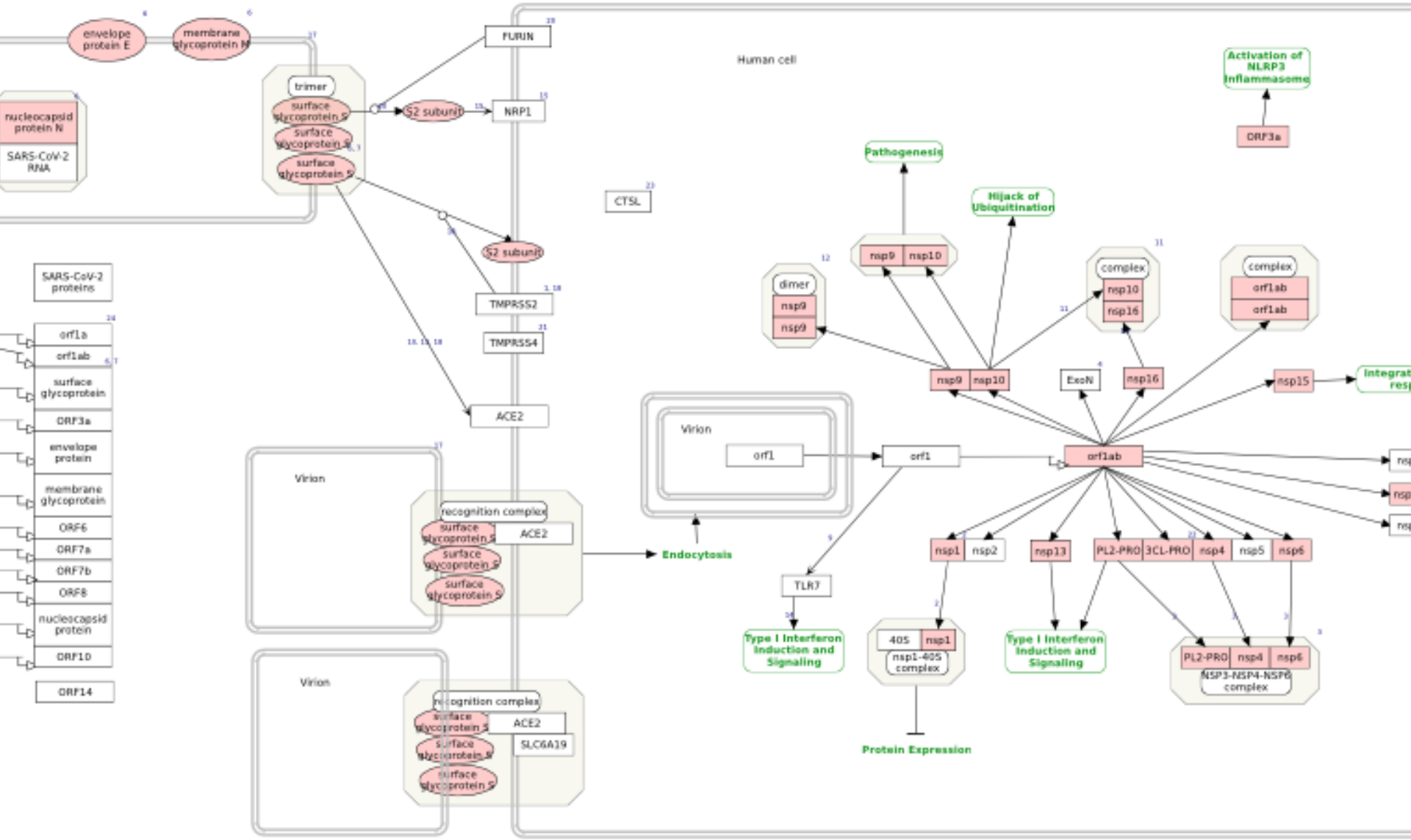
Interactions with Human cells

Reakce organismu na SARS-CoV-2

- **Replikace viru** - WP4846
- **Viral subversion of host defence:**
  - ER stress and unfolded protein response - **WP4861**
  - Autophagy and protein degradation **-**WP4860, WP4936, WP4863
  - Apoptosis - WP4864
- **Integrative stress response:**
  - Renin-angiotensin **-** WP4883, WP4965
  - Coagulopathy **-** WP4927
- **Innate Immune Response:**
  - PAMP signalling - WP4912
  - Induction of interferons and the cytokine storm - WP4868, WP4880, WP4876
  - Altered host metabolism - WP4853

https://covid.pages.uni.lu/map_contents

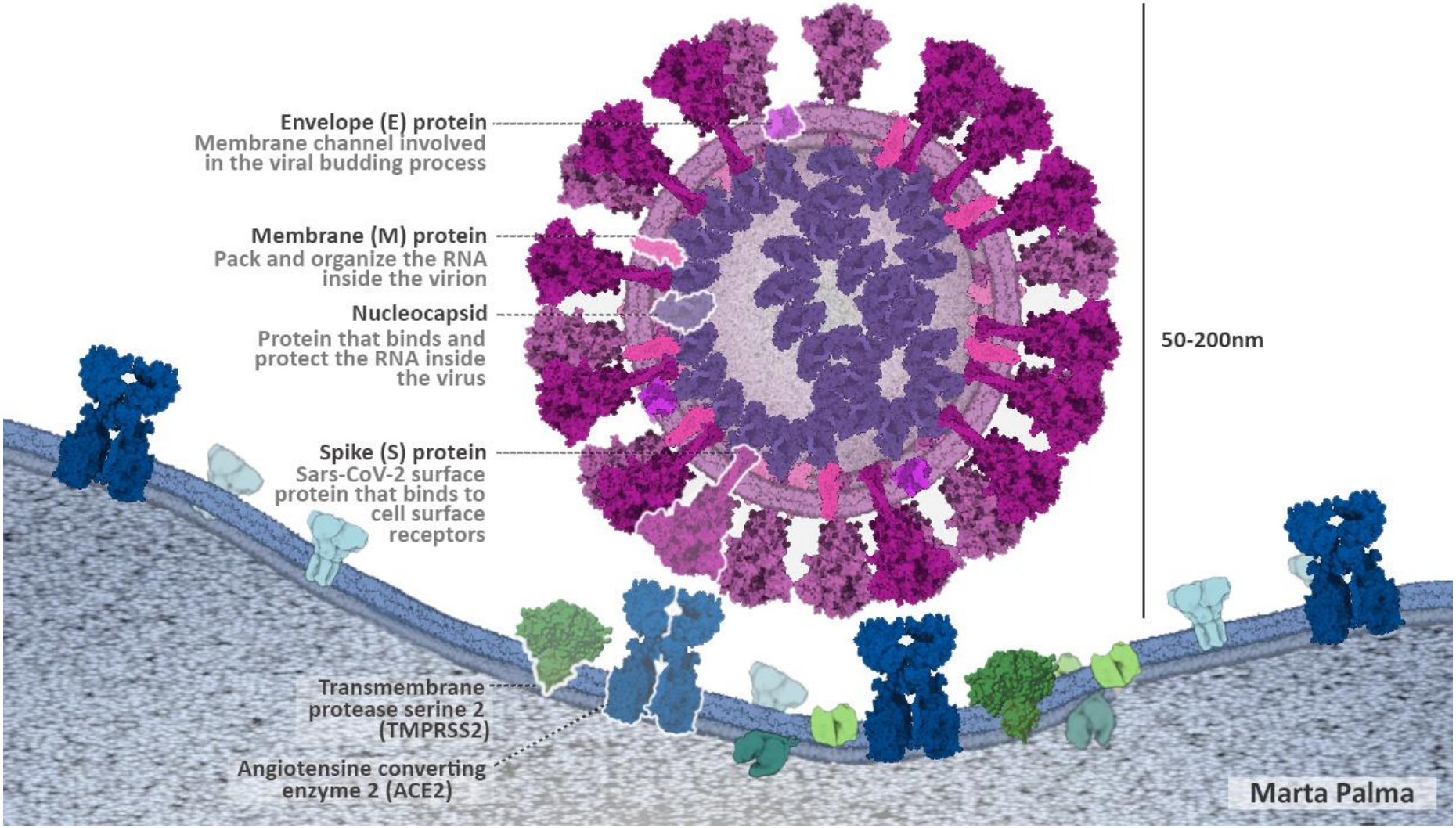
Wikipathways



https://www.wikipathways.org/index.php/Pathway:WP4846#nogo2

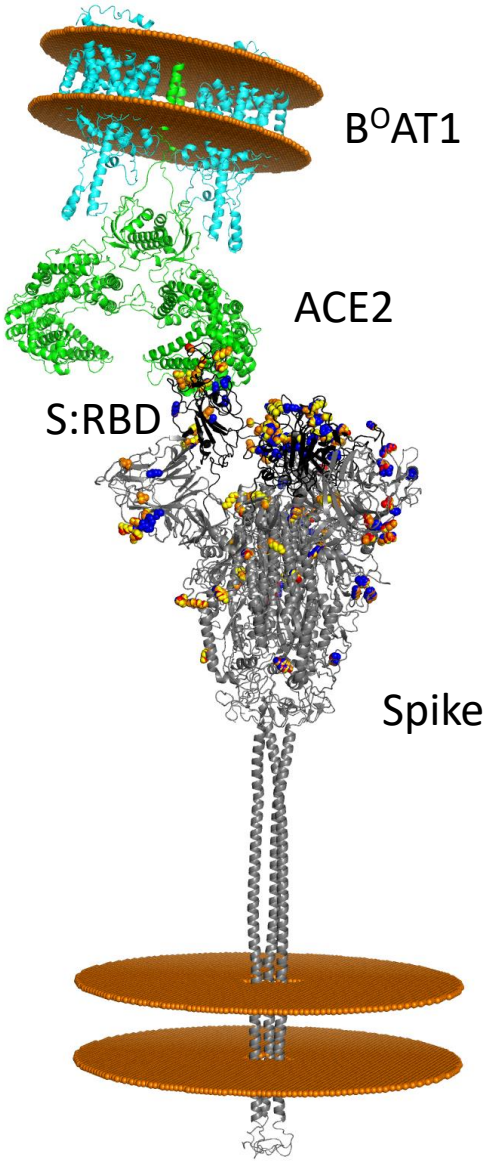
https://www.embopress.org/doi/full/10.15252/msb.202110387

Interacting proteins

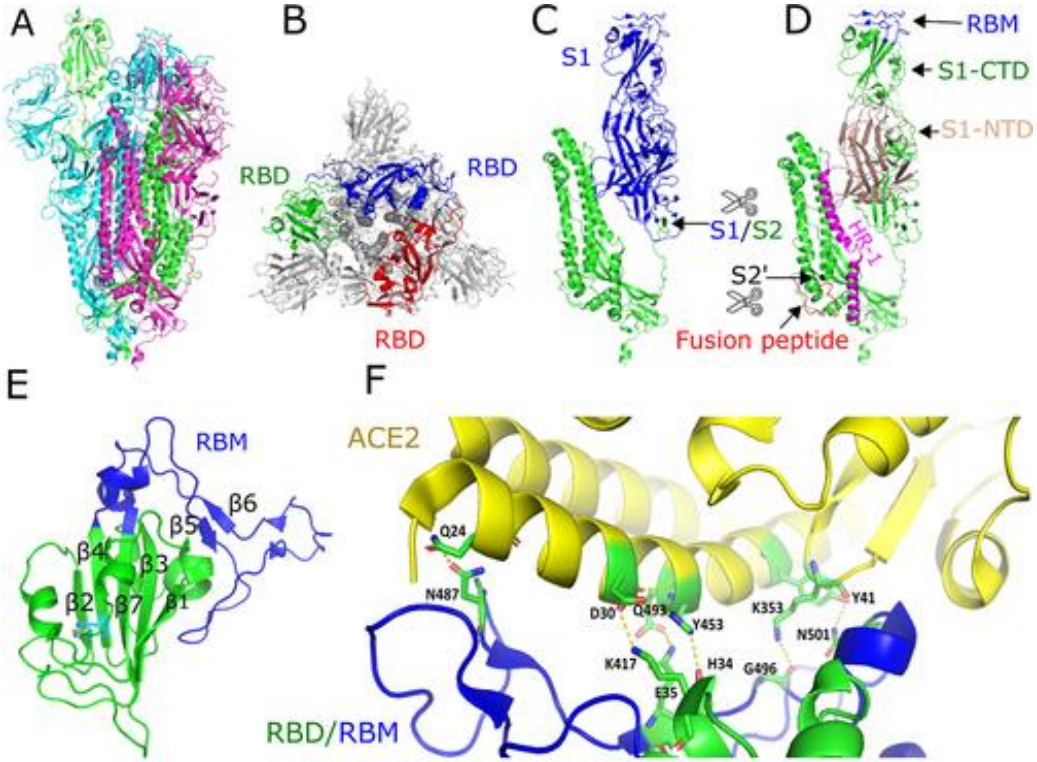


https://cdn.rcsb.org/news/2020/sars-ace2tmprs2-martapalma.png

SARS-CoV-2 S protein + ACE2



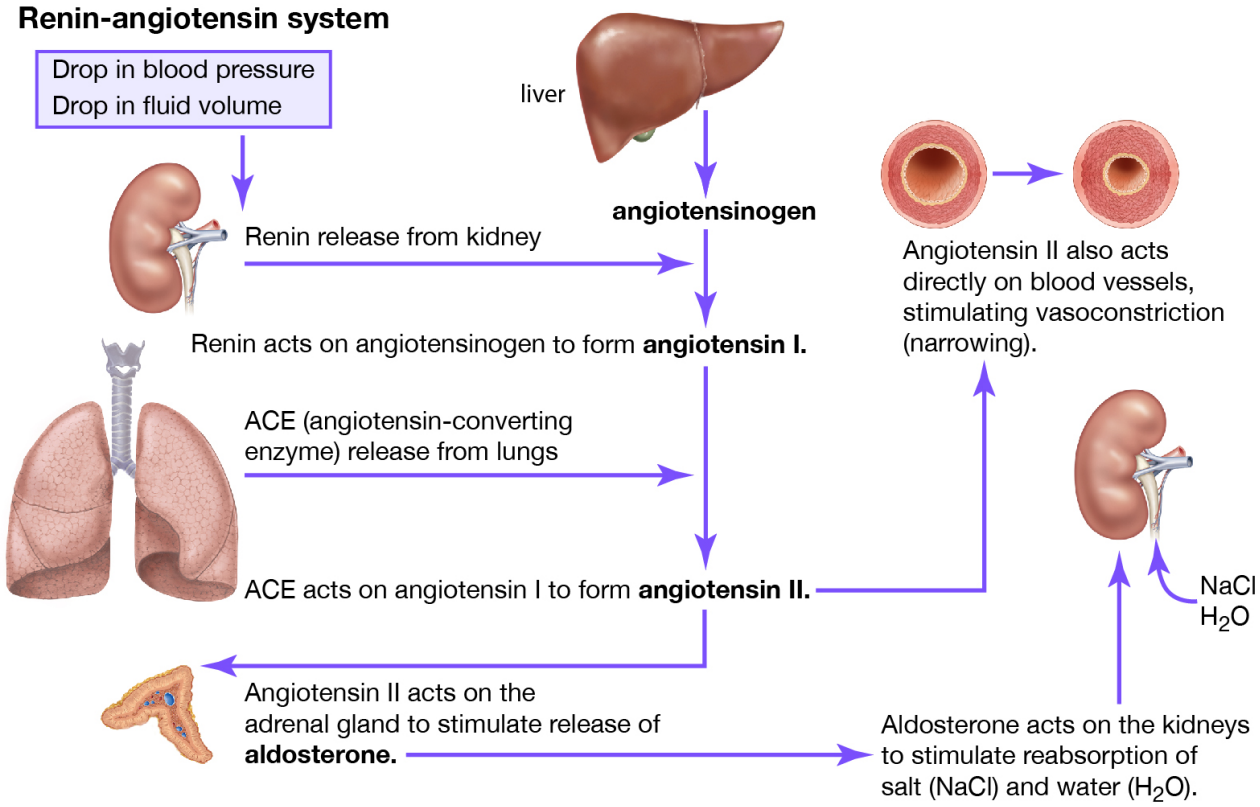
Berka K (2022) https://virus.img.cas.cz/

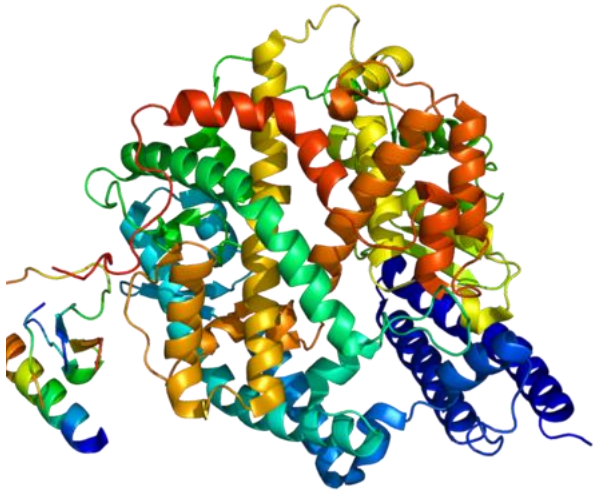


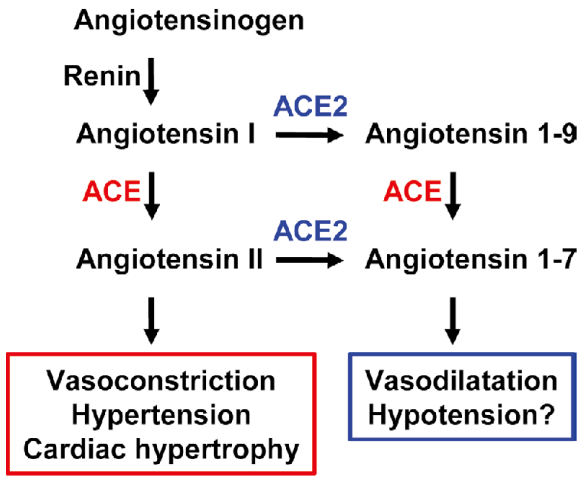
- Hlavní kontakt s buňkami

Mittal A, Manjunath K, Ranjan RK, Kaushik S, Kumar S, et al. (2020) COVID-19 pandemic: Insights into structure, function, and hACE2 receptor recognition by SARS-CoV-2. PLOS Pathogens 16(8): e1008762. https://doi.org/10.1371/journal.ppat.1008762 https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1008762

ACE2 – what is it? Why do we care?







Receptor binding domain (RBD) antibodies contribute more to SARS-CoV-2 neutralization when target cells express high levels of ACE2

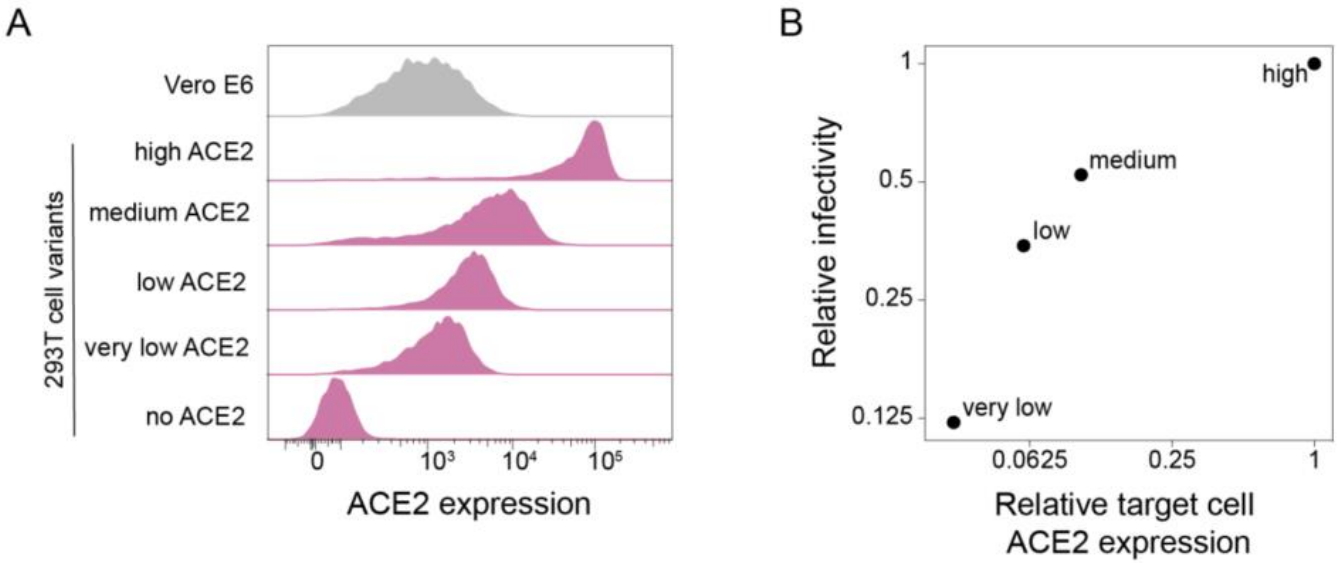
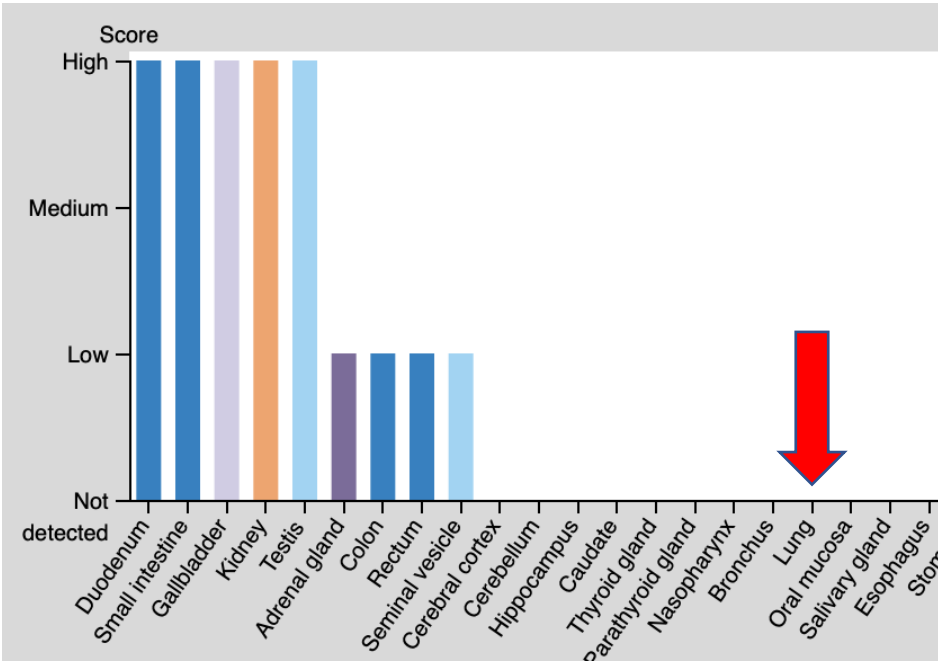
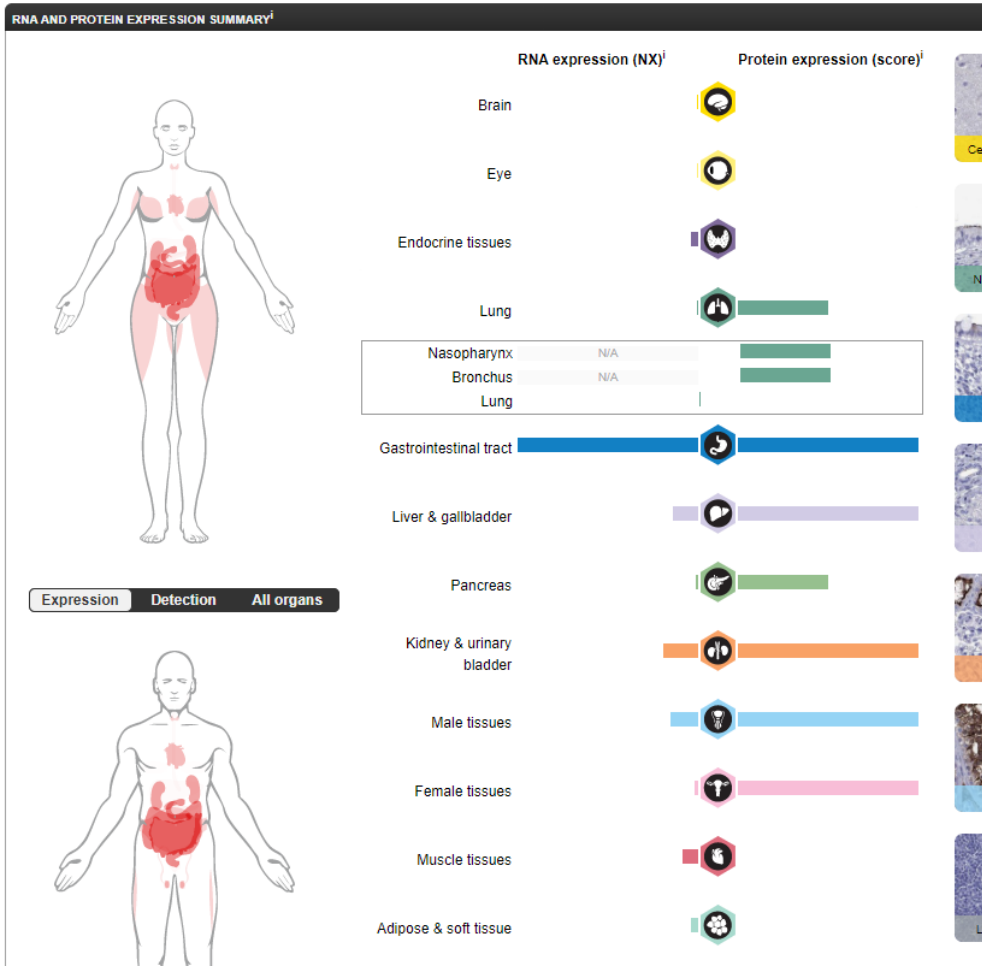


Fig. 1. 293T cell clones expressing ACE2 at different levels. (A) ACE2 expression in 293T cells engineered to express different levels of ACE2. ACE2 surface expression was measured by flow cytometry, and the histograms show the distribution of expression levels over a population of cells. Vero E6 cells are included for comparison. **(B)** Relationship between ACE2 expression in the four 293T target cell clones and infection by lentiviral particles pseudotyped with the SARS-CoV-2 D614G spike.

https://www.biorxiv.org/content/10.1101/2022.08.29.505713v1

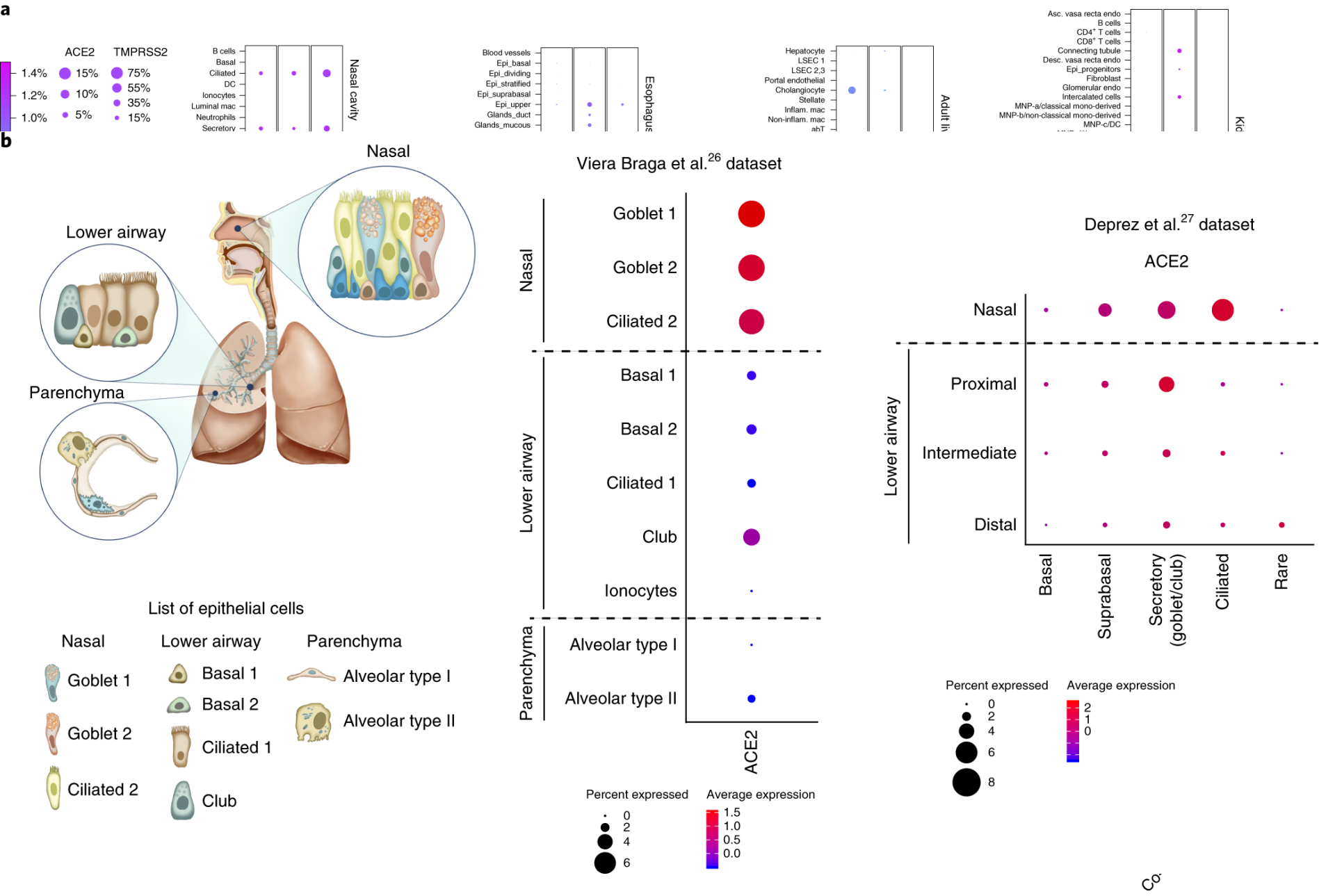
Expression of ACE2





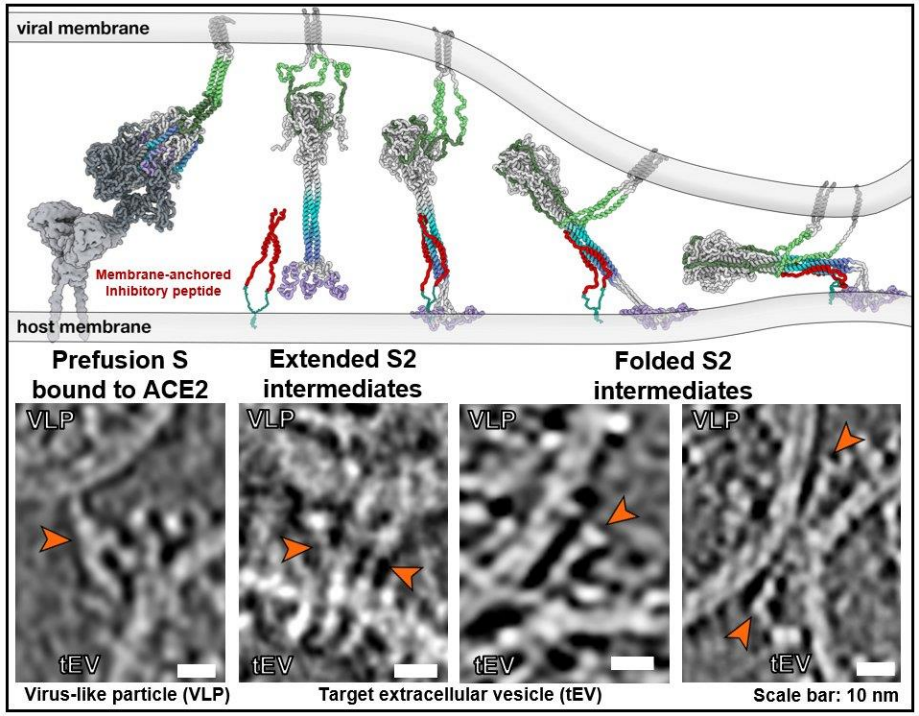
Weird, right?

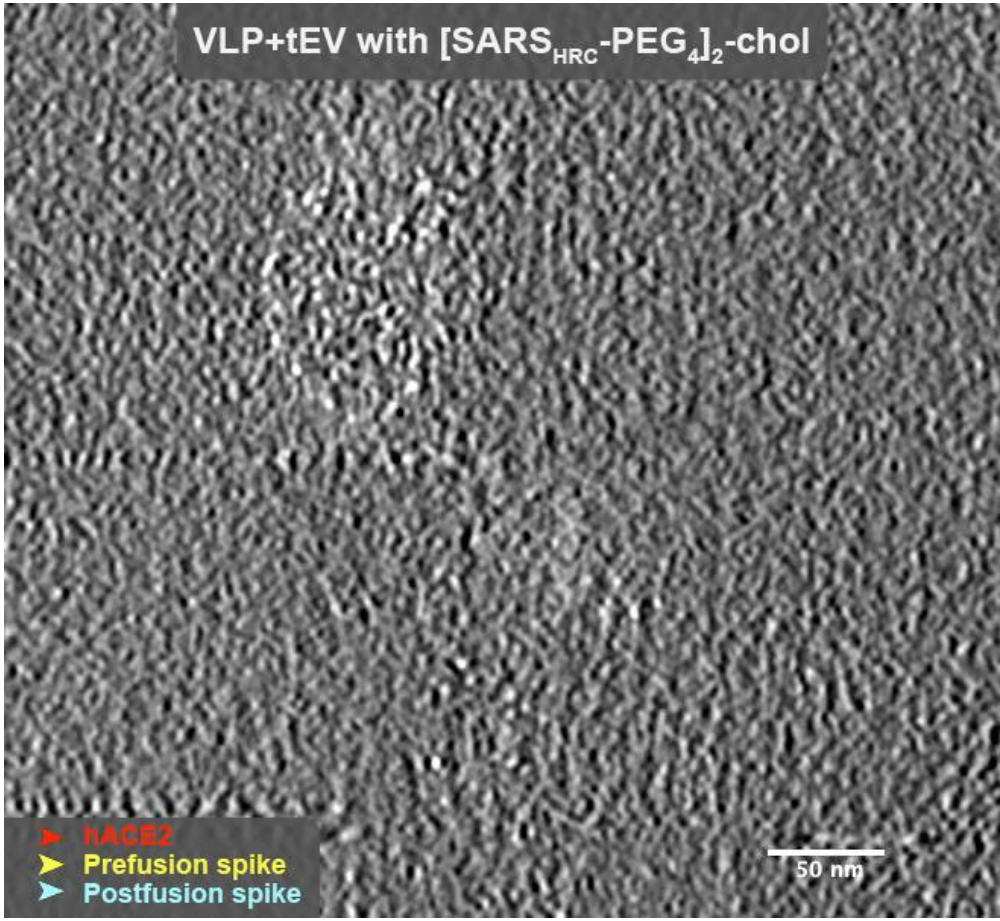
https://www.proteinatlas.org/ENSG00000130234-ACE2/tissue



https://www.nature.com/articles/s41591-020-0868-6

Intermediates in SARS-CoV-2 spike–mediated cell entry

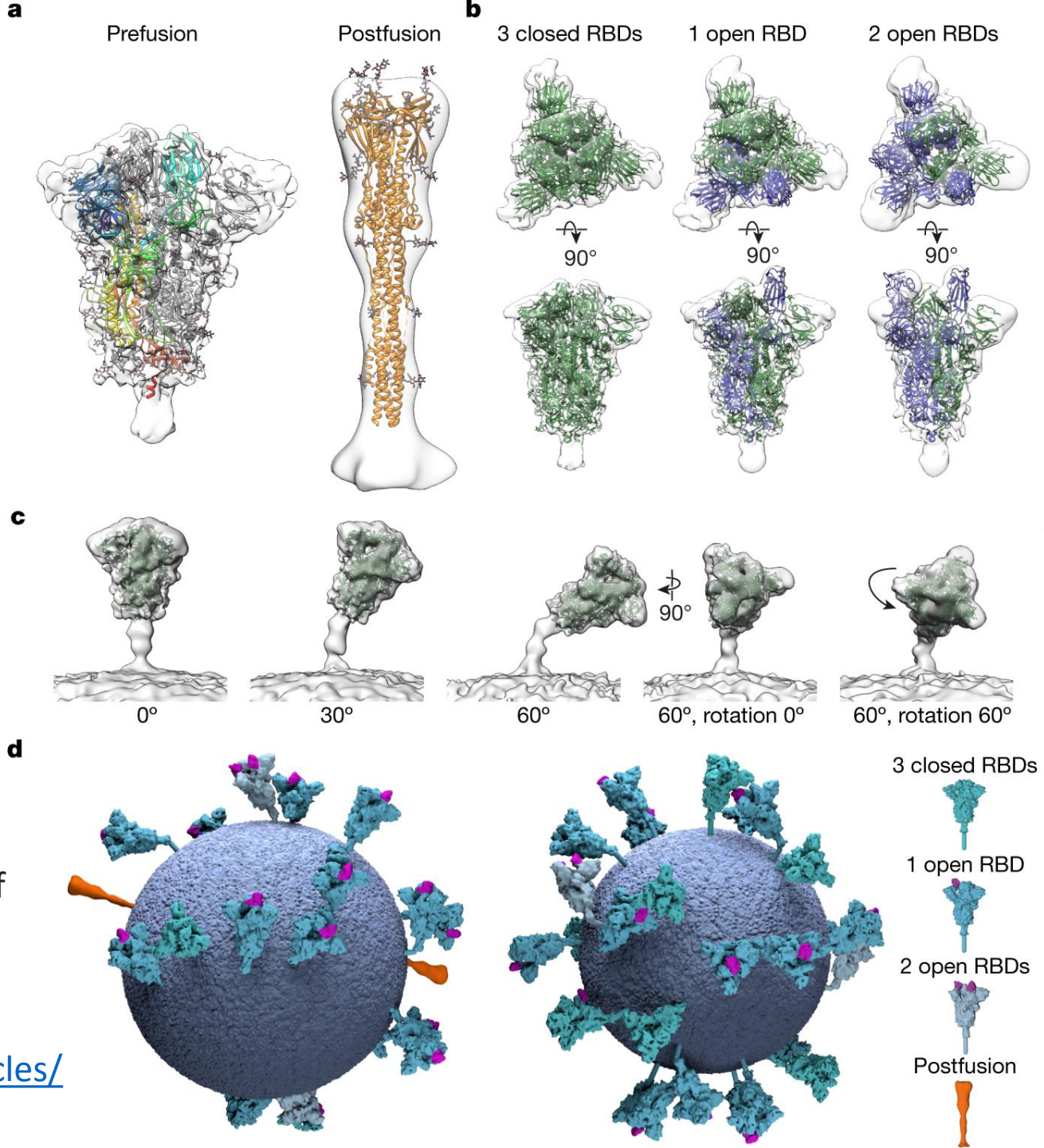




https://www.science.org/doi/10.1126/sciadv.abo3153

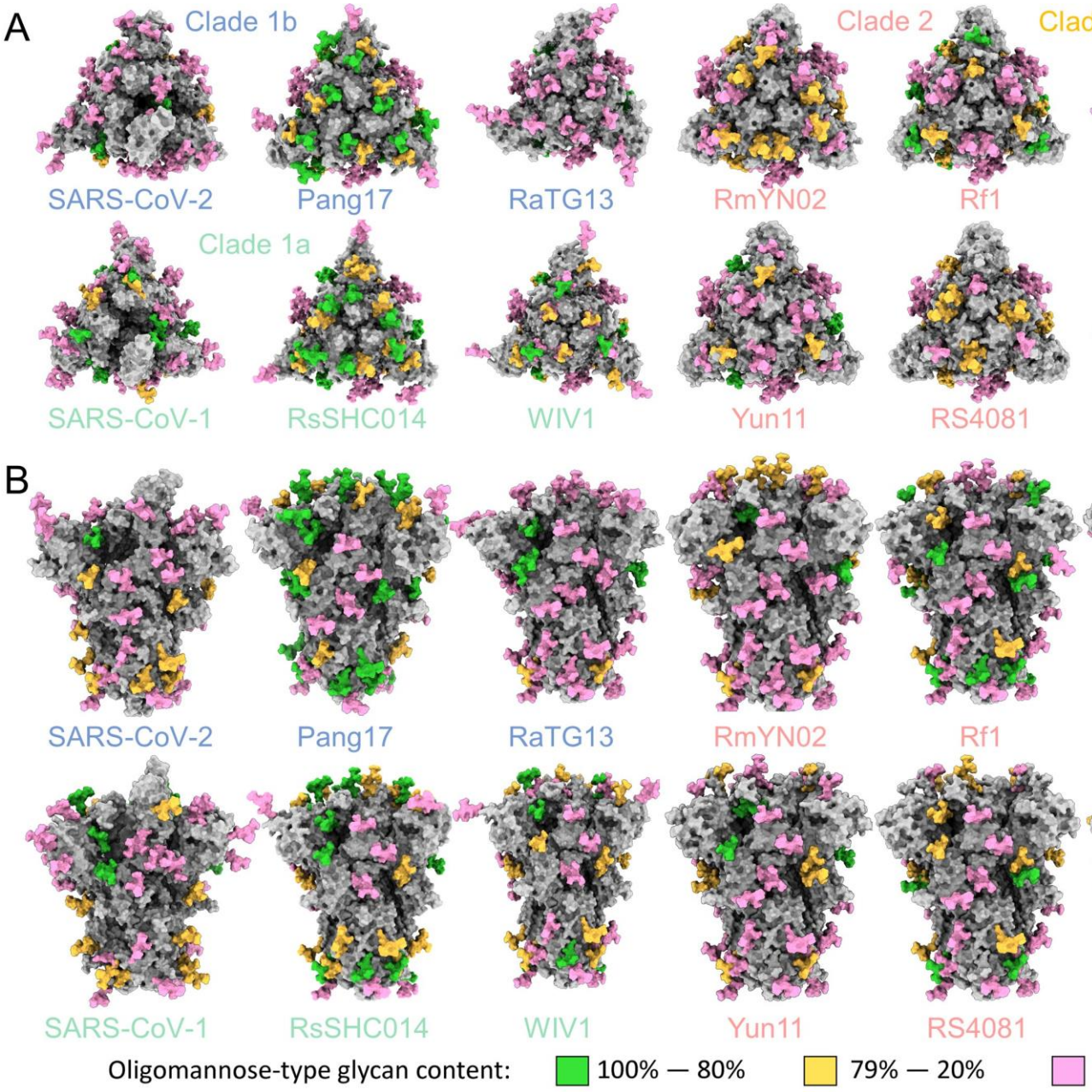
Spike protein in detail

Conformers S proteins



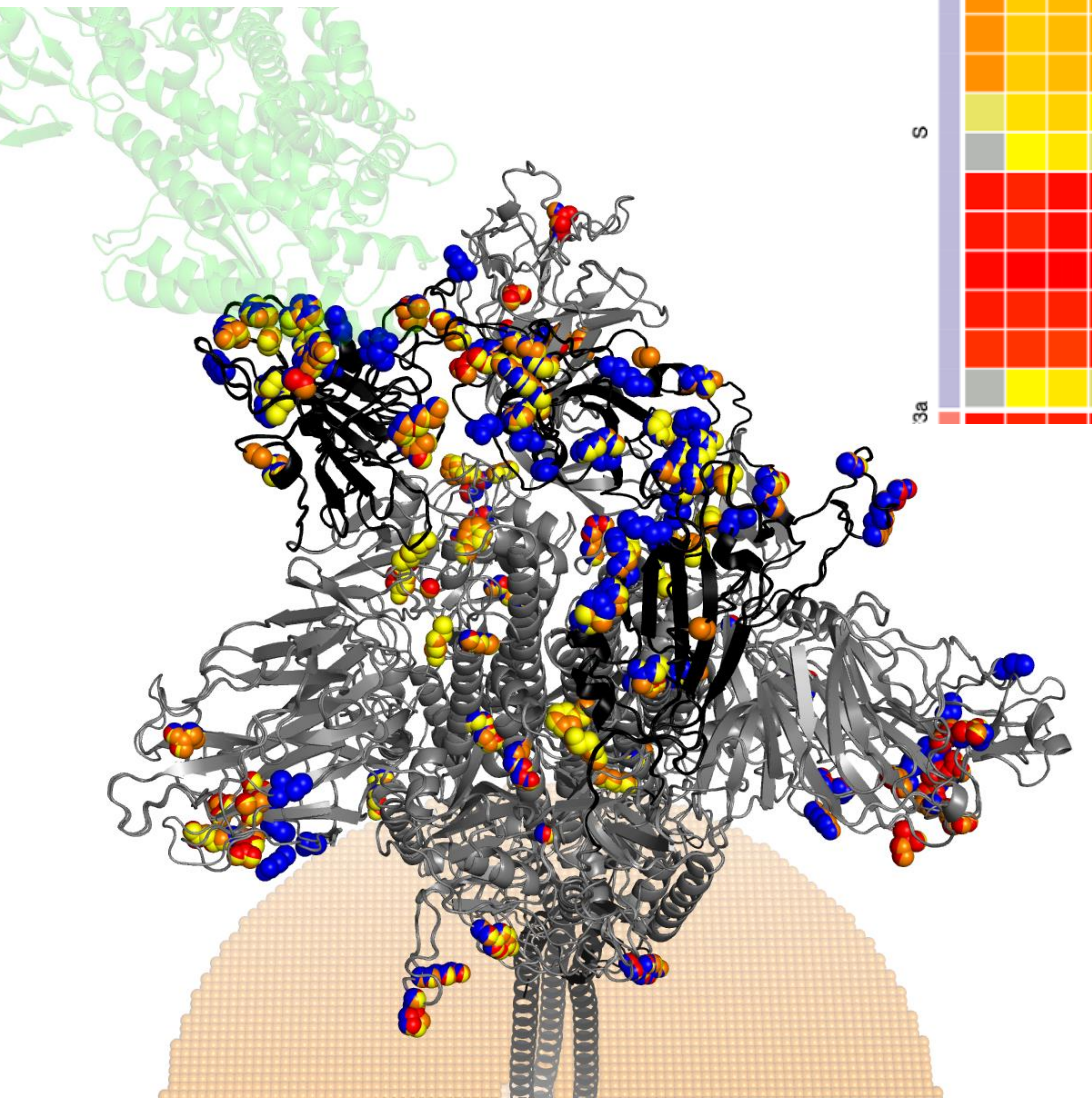
Ke, Z., Oton, J., Qu, K. *et al.* Structures and distributions of SARS-CoV-2 spike proteins on intact virions. *Nature* **588**, 498–502 (2020). https://www.nature.com/articles/s41586-020-2665-2/figures/2

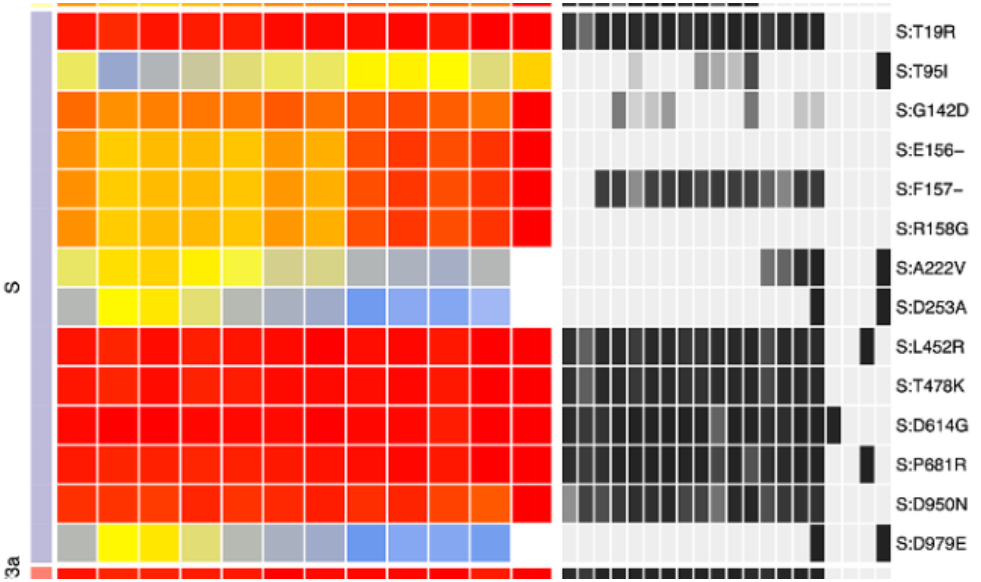
Glycosylation



https://www.biorxiv.org/content/10.1101/2022.08.24.505118v1

Mutace S proteinu



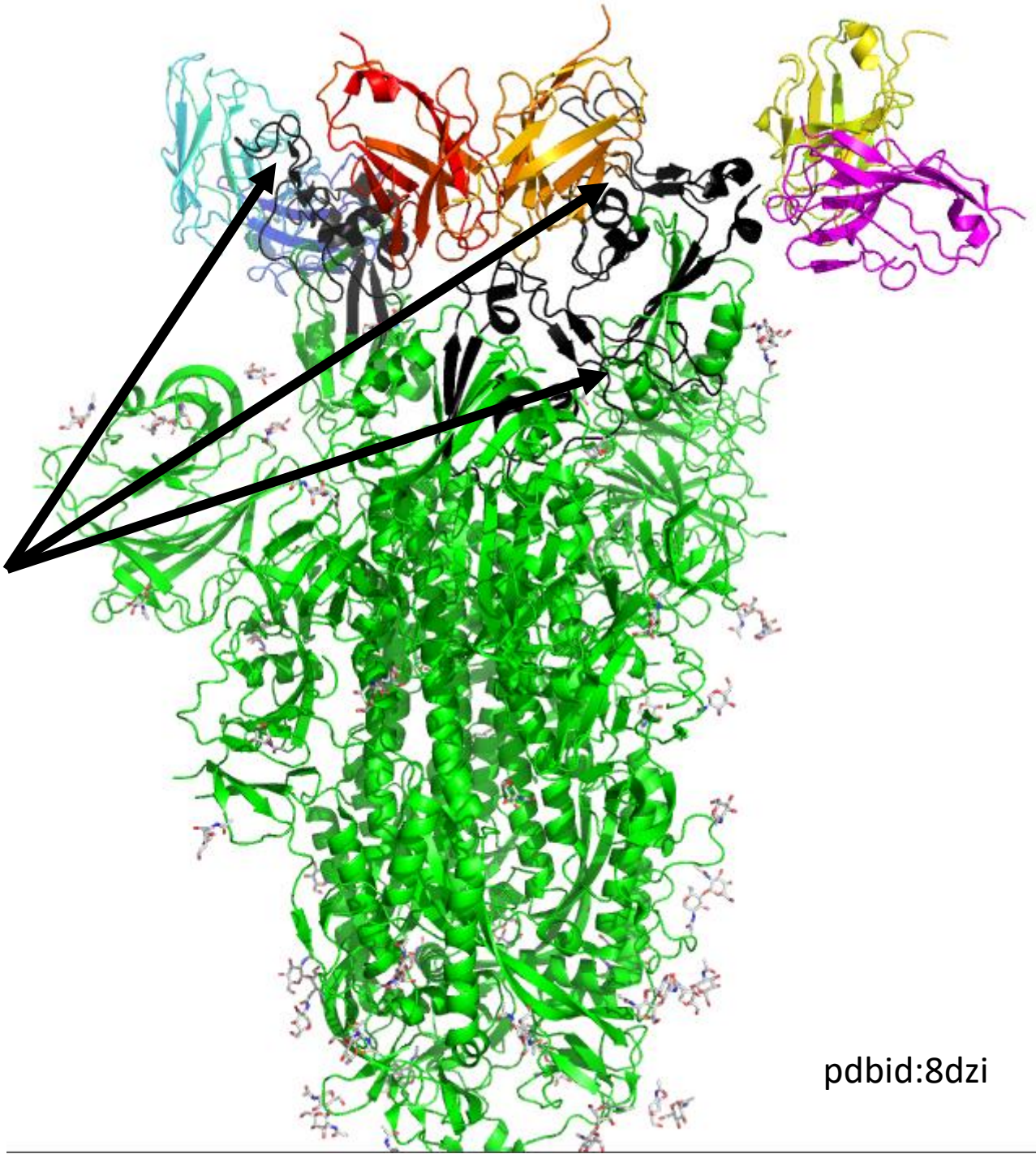


https://virus.img.cas.cz/

Antibodies

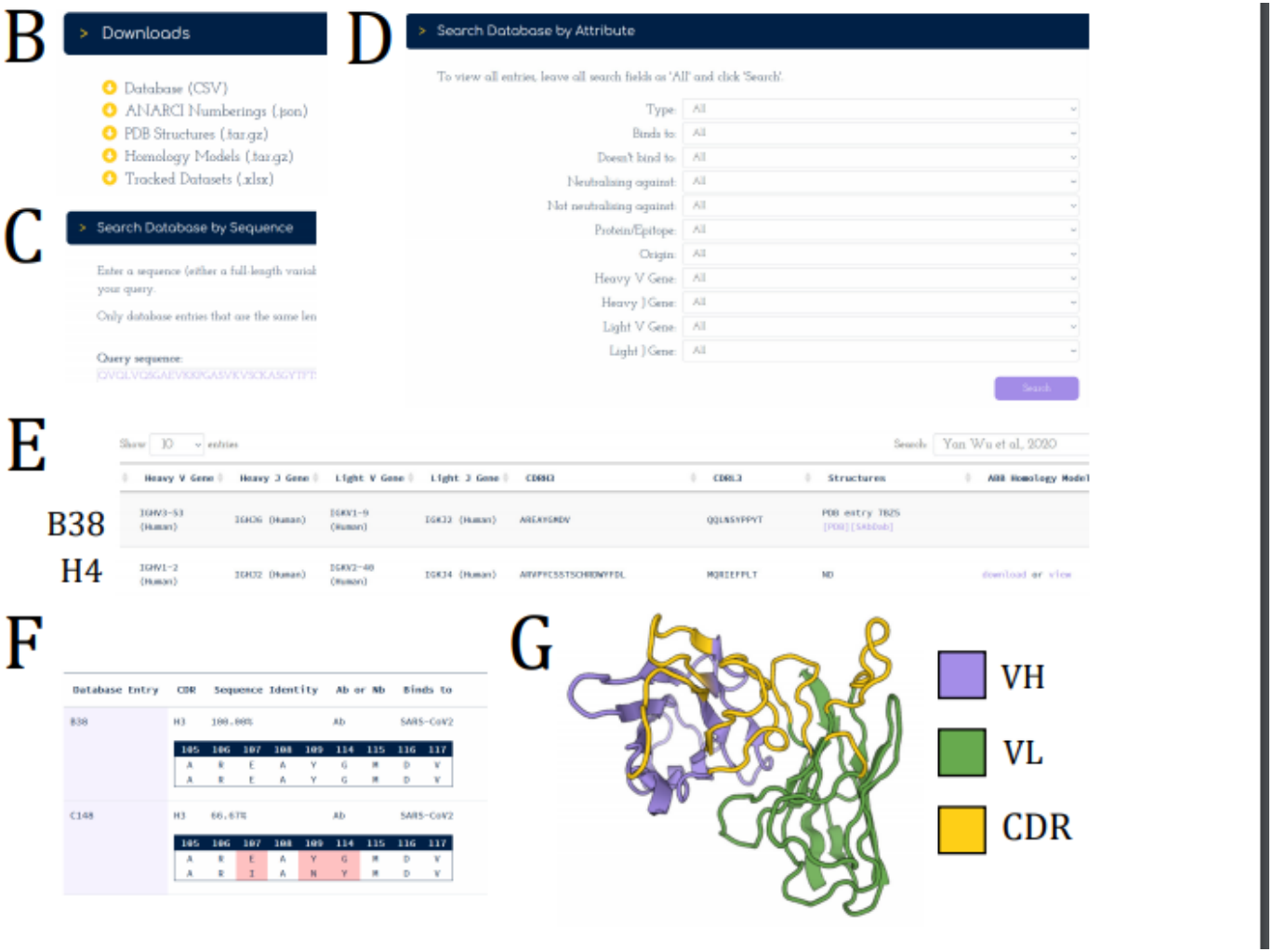
Interaction with antibodies

- Neutralizing antibodies binds with RBD domain – blocking ACE2 interaction



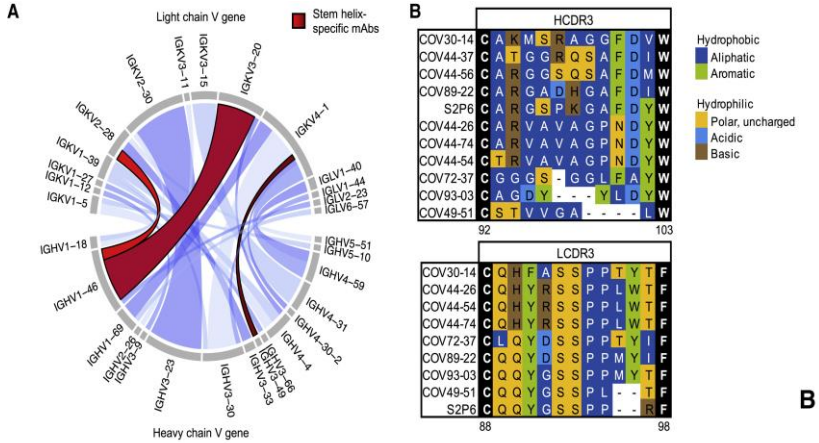
Protilátky

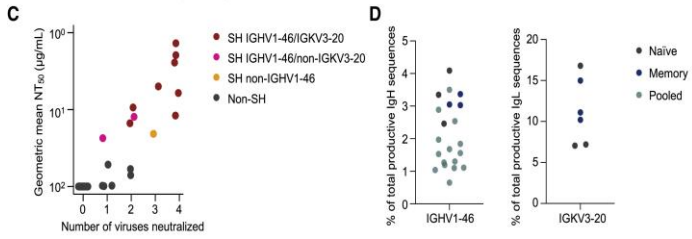
CoV-AbDab
The Coronavirus Antibody Database

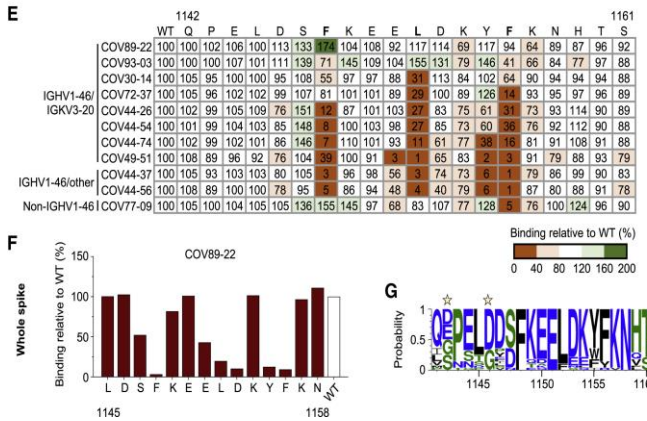


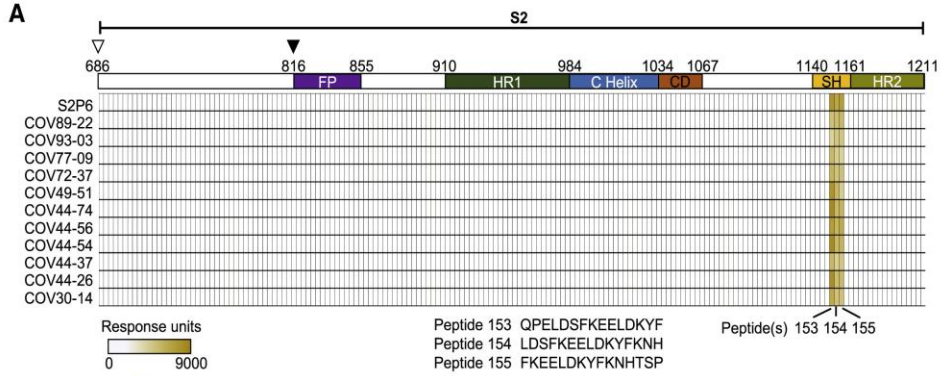
http://opig.stats.ox.ac.uk/webapps/covabdab/

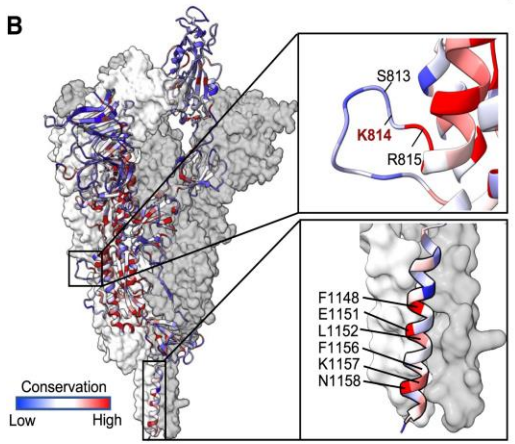
Rare, convergent antibodies targeting the stem helix broadly neutralize diverse betacoronaviruses

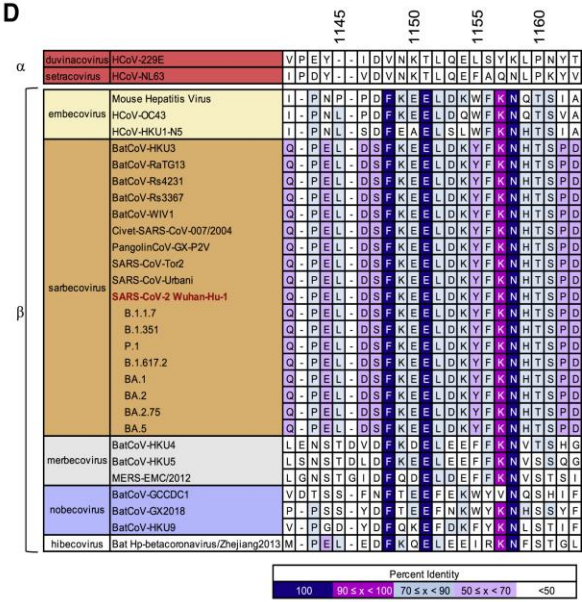


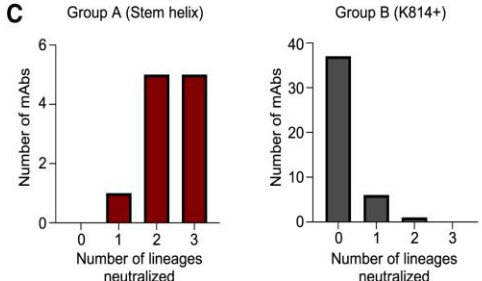




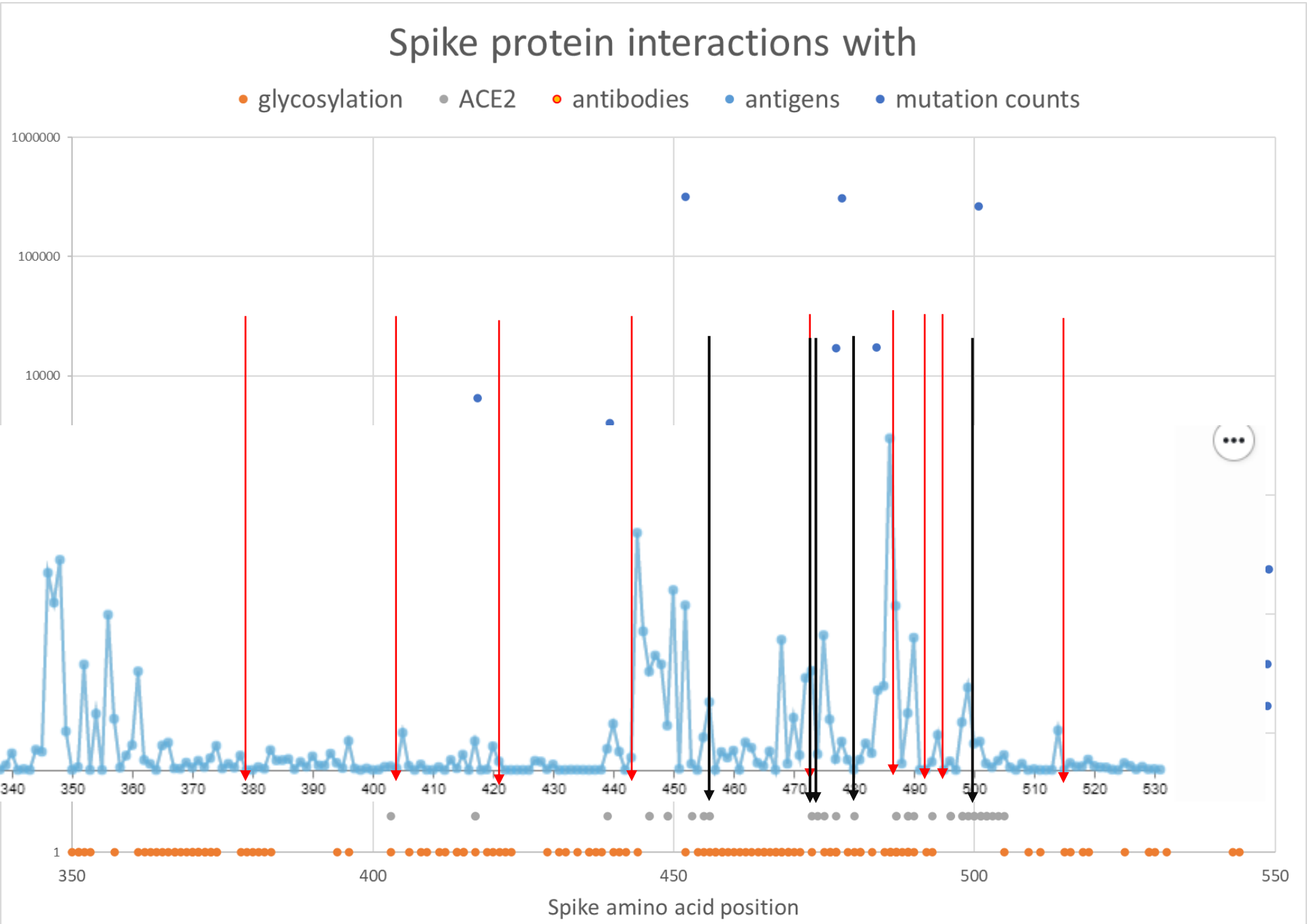




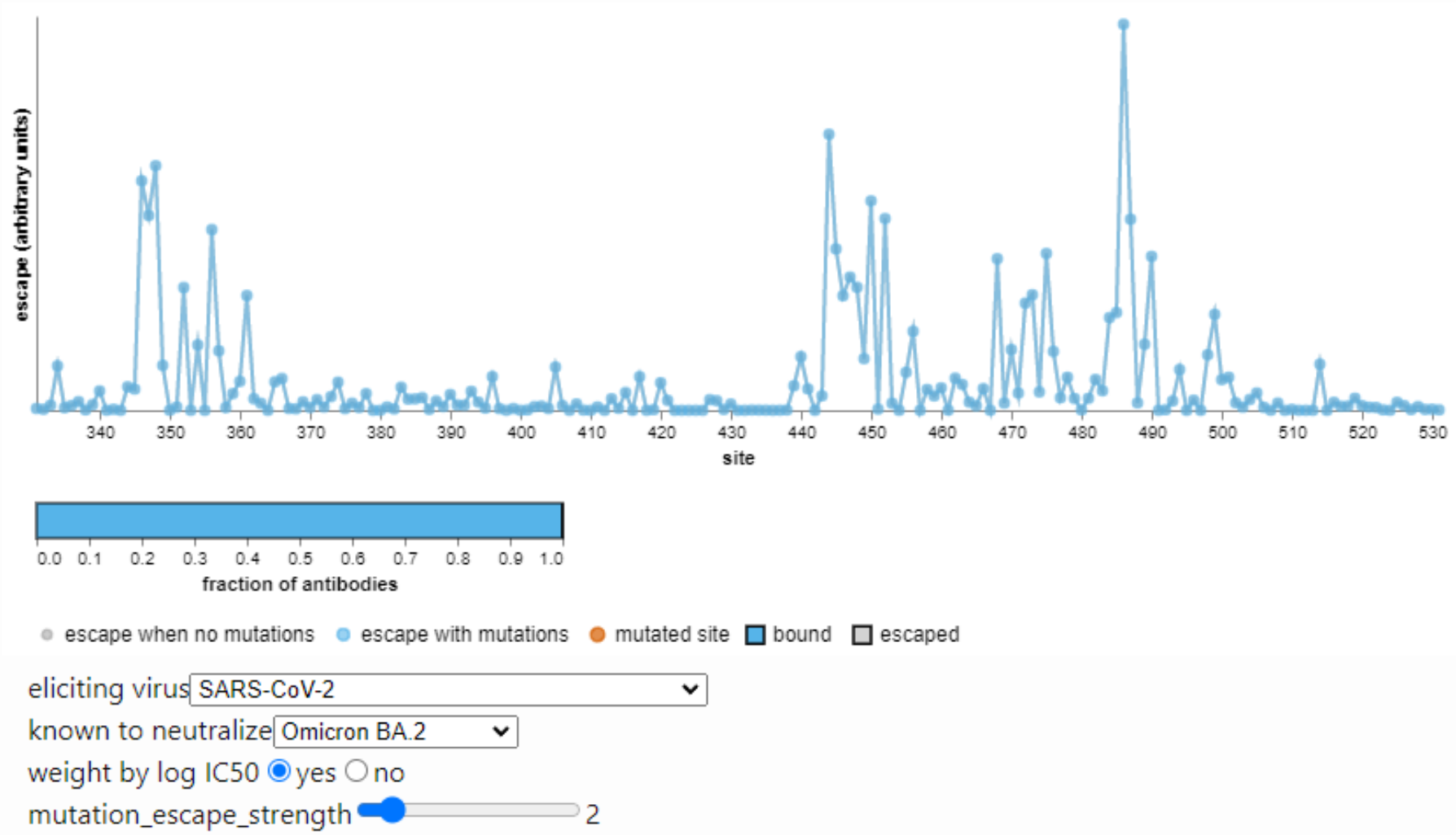




Dacon et al. Rare, convergent antibodies targeting the stem helix broadly neutralize diverse betacoronaviruses.
Cell Host & Microbe DOI: 10.1016/j.chom.2022.10.010


Spike protein interactions with
glycosylation
ACE2
antibodies
antigens
mutation counts
1000000
100000
10000
1
340
350
360
370
380
390
400
410
420
430
440
450
460
470
480
490
500
510
520
530
350
400
450
500
550
Spike amino acid position

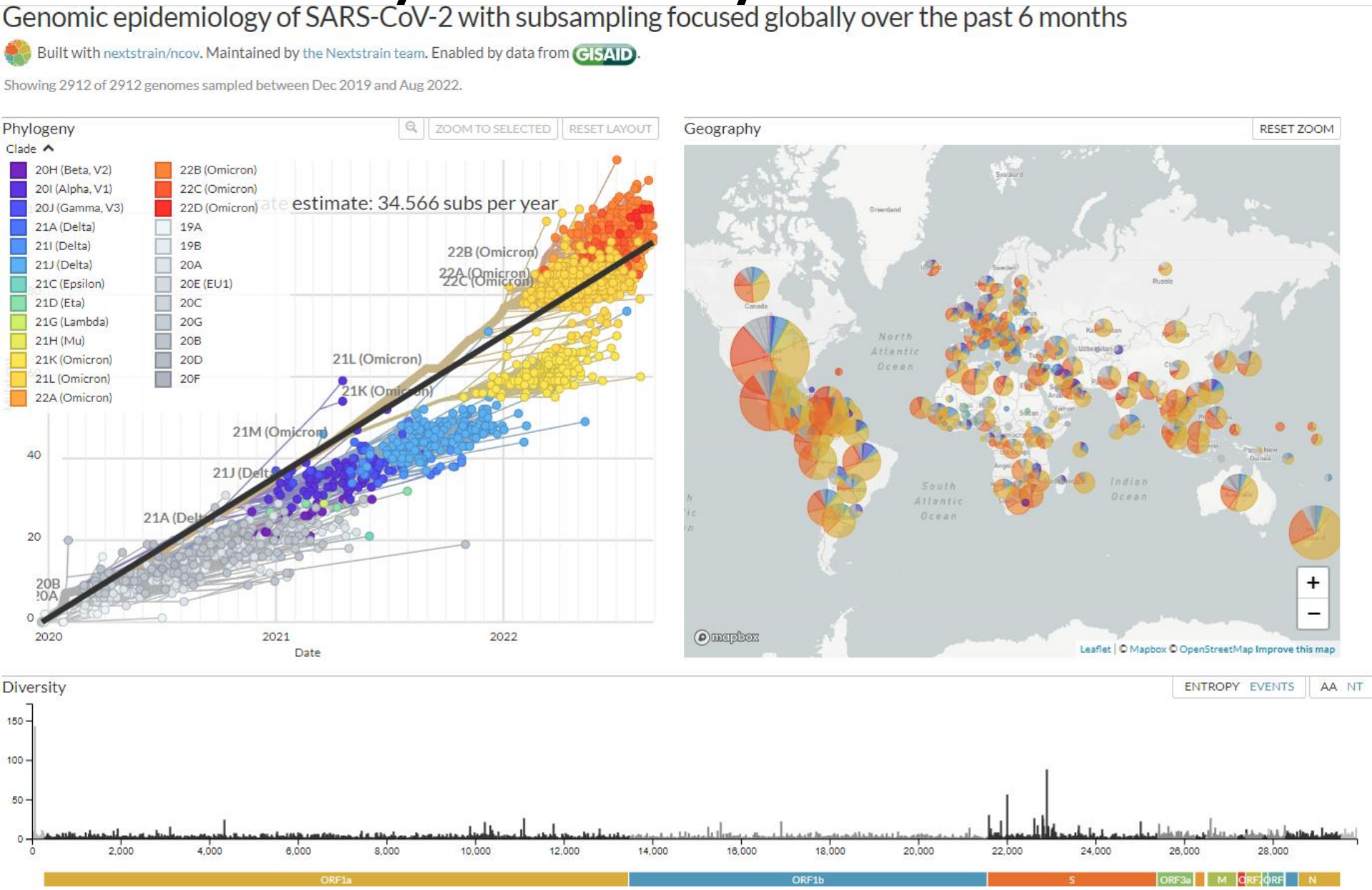
Escape calculator for SARS-CoV-2 RBD



https://jbloomlab.github.io/SARS2_RBD_Ab_escape_maps/escape-calc/
https://academic.oup.com/ve/article/8/1/veac021/6549895

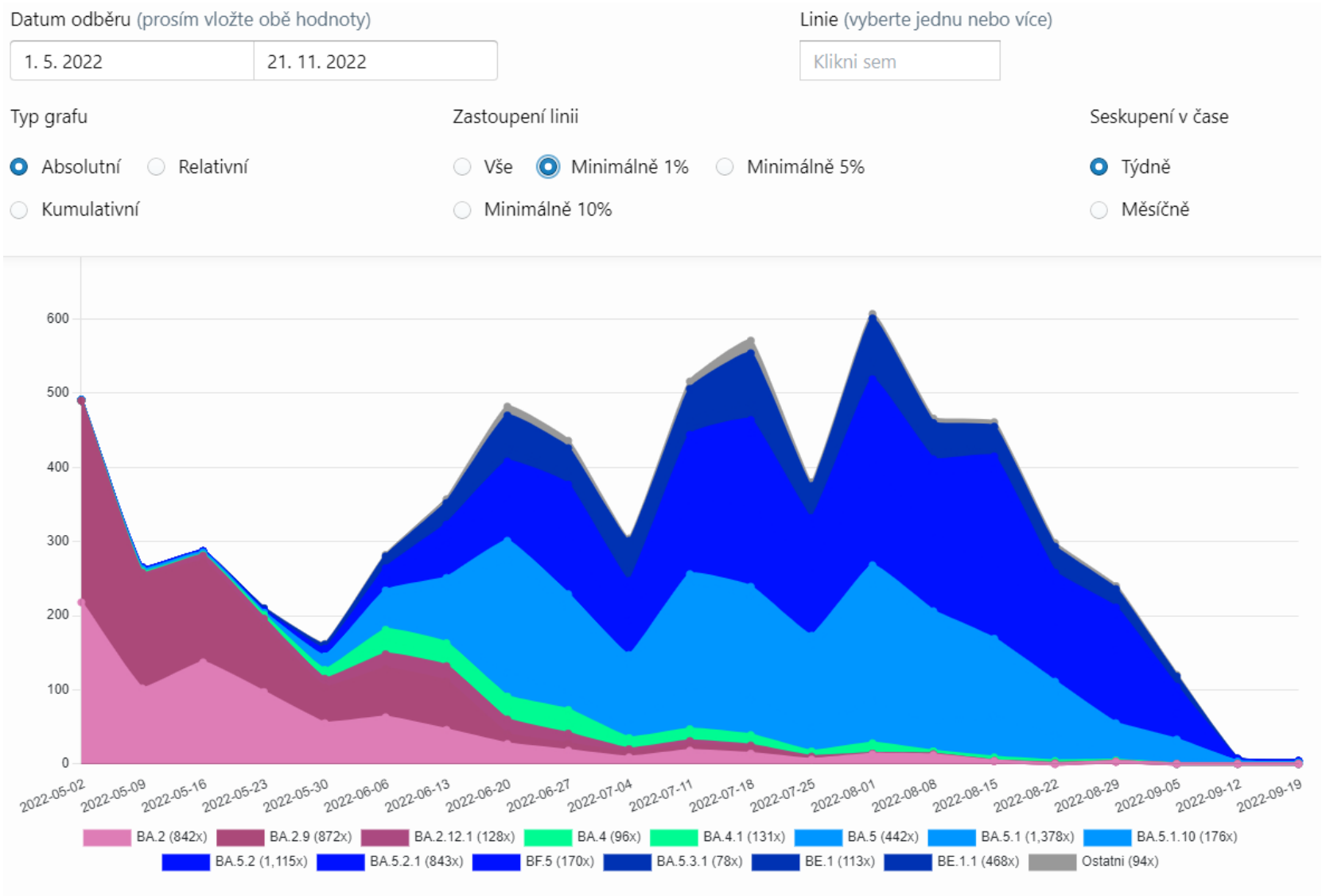
Genomic surveillance

Varianty - Genomy SARS-Cov-2



https://nextstrain.org/ncov/global?m=div

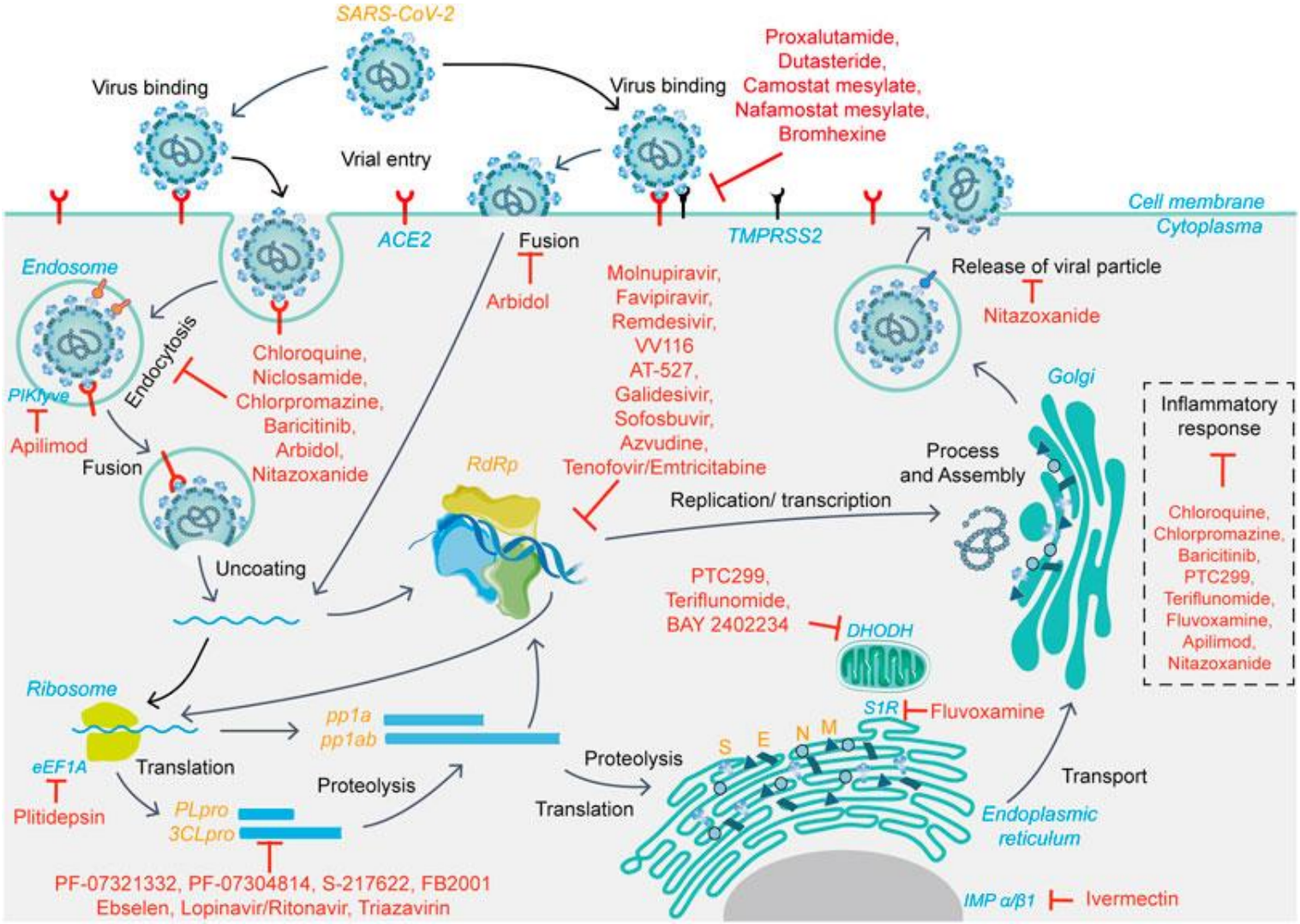
Varianty v ČR



https://virus.img.cas.cz/lineages

Therapeutics

Clinical trials



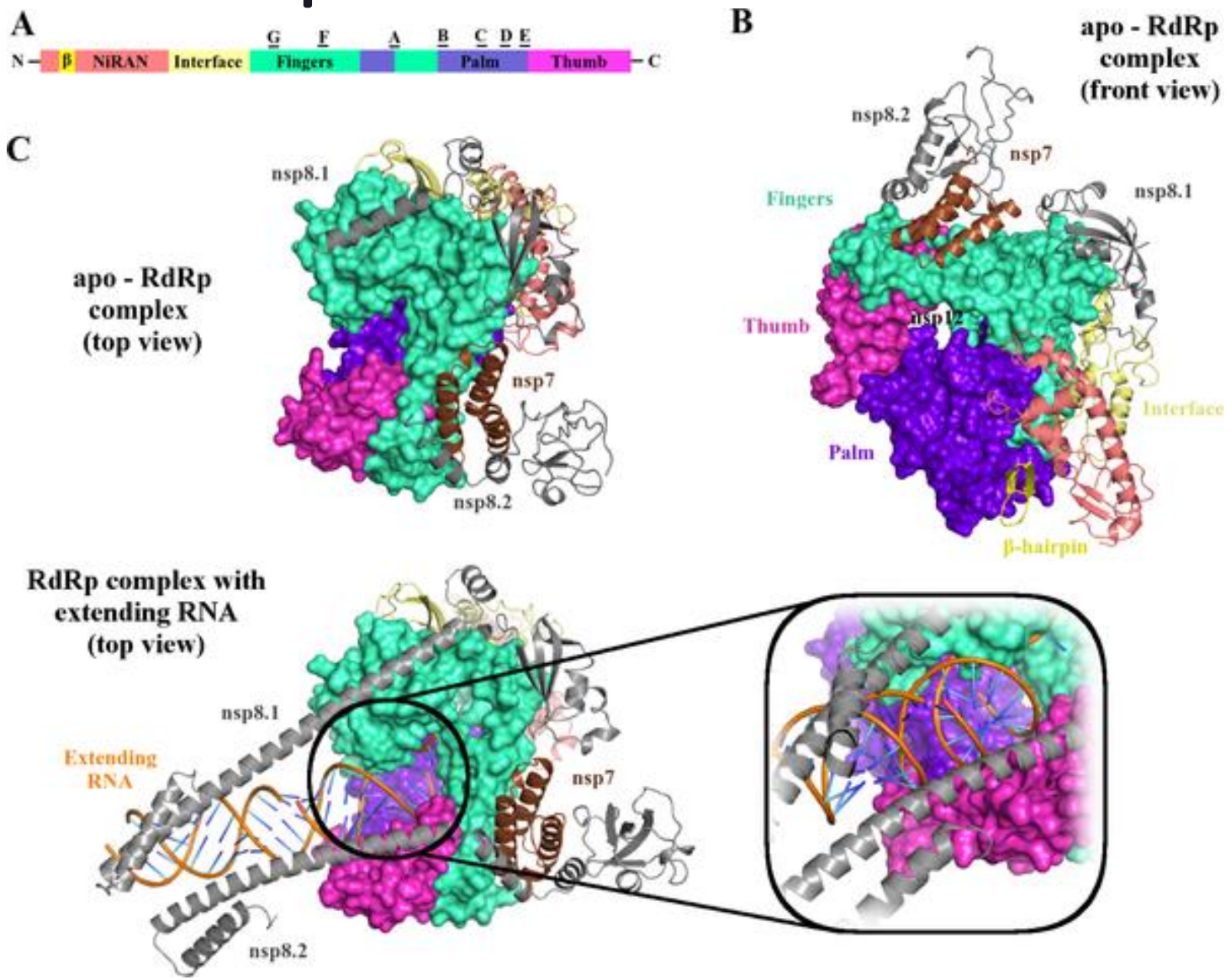
Zhao et al. Mechanism of Action of Small-Molecule Agents in Ongoing Clinical Trials for SARS-CoV-2: A Review
Front. Pharmacol., 25 February 2022 https://doi.org/10.3389/fphar.2022.840639

Drugs	No. of clinical trials registered[a]	Phase	Molecular target	Development strategy	Approval status (for COVID-19)
Remdesivir	77	4	RdRp	Repurposing	Approval by FDA
Favipiravir	46	4	RdRp	Repurposing	EUA in several countries
Molnupiravir	5	3	RdRp	Novel	Approval by MHRA; EUA by FDA
AT-527	3	3	RdRp	Novel	Non-approved
Galidesivir	1	1	RdRp	Repurposing	Non-approved
Sofosbuvir	8	4	RdRp	Repurposing	Non-approved
Azvudine	3	3	RdRp	Repurposing	Non-approved
Tenofovir/emtricitabine	5	3	RdRp	Repurposing	Non-approved
PF-07321332	8	3	3CLpro	Novel	EUA by FDA
PF-07304814	3	1	3CLpro	Novel	Non-approved
s-217622	—	2/3	3CLpro	Novel	Non-approved
FB2001	1	2/3	3CLpro	Novel	Non-approved
Ebselen	2	2	3CLpro	Repurposing	Non-approved
Lopinavir/ritonavir	24	4	3CLpro	Repurposing	Non-approved
Triazavirin	2	4	RNA synthesis/3CLpro	Repurposing	Non-approved
Chloroquine/hydroxychloroquine	46/276	4	Endosomal entry	Repurposing	EUA by FDA at earlier outbreak (chloroquine)
Umifenovir/arbidol	3	4	Endosomal entry	Repurposing	Non-approved
Niclosamide	11	3	Endosomal entry	Repurposing	Non-approved
Chlorpromazine	2	3	Endosomal entry	Repurposing	Non-approved
Baricitinib	20	4	Endosomal entry	Repurposing	EUA by FDA
Proxalutamide	5	3	Androgen receptor antagonist	Repurposing	Non-approved
Dutasteride	1	2	5-alpha-reductase inhibitor	Repurposing	Non-approved
Camostat mesylate	5	3	TMPRSS2 inhibitor	Repurposing	Non-approved
Nafamostat mesylate	2	2	TMPRSS2 inhibitor	Repurposing	Non-approved
PTC299	1	2	DHODH inhibitor	Repurposing	Non-approved
Teriflunomide	3	3	DHODH inhibitor	Repurposing	Non-approved
Nitazoxanide	23	4	Endosomal entry/Inflammatory response regulation	Repurposing	Non-approved
Fluvoxamine	1	3	Sigma-1 receptors agonist	Repurposing	Non-approved
Plitidepsin	3	3	eEF1A inhibitor	Repurposing	Non-approved
Ivermectin	69	4	IMPA/β1 inhibitor	Repurposing	Non-approved
Apilimod	1	2	PIKFYVE inhibitor	Repurposing	Non-approved

[a]*Registered on ClinicalTrials.gov.*

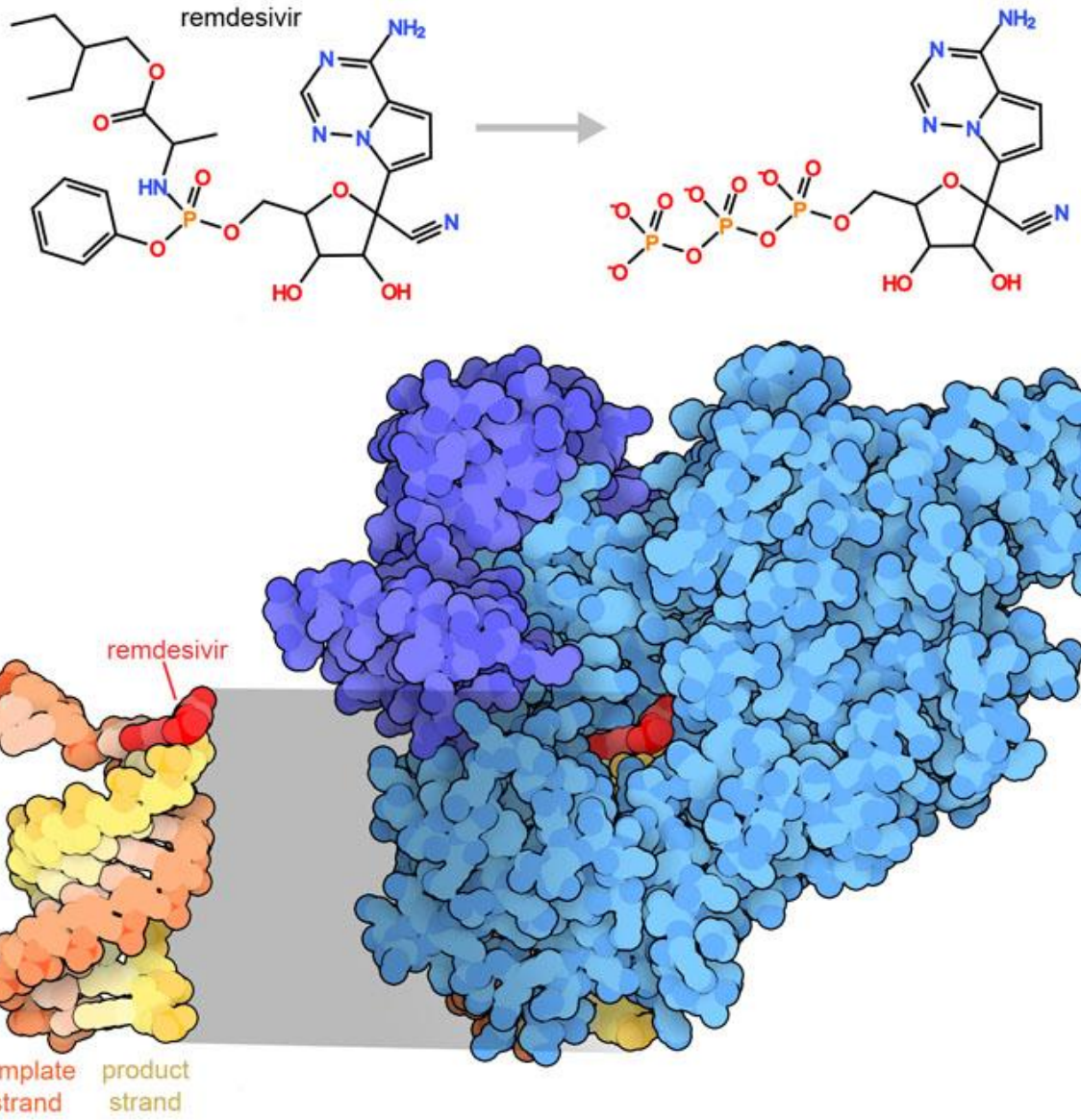
Zhao et al. Mechanism of Action of Small-Molecule Agents in Ongoing Clinical Trials for SARS-CoV-2: A Review
Front. Pharmacol., 25 February 2022 https://doi.org/10.3389/fphar.2022.840639

Cryo-EM RdRp of SARS-CoV-2.



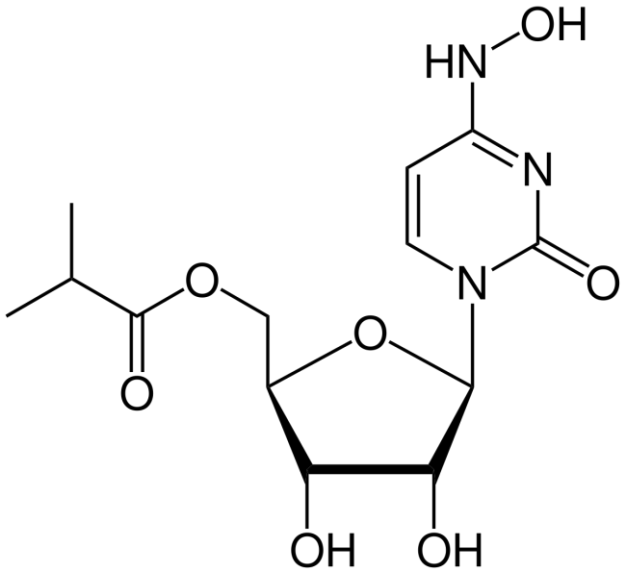
Mittal A, Manjunath K, Ranjan RK, Kaushik S, Kumar S, et al. (2020) COVID-19 pandemic: Insights into structure, function, and hACE2 receptor recognition by SARS-CoV-2. PLOS Pathogens 16(8): e1008762. https://doi.org/10.1371/journal.ppat.1008762
https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1008762

Remdesivir

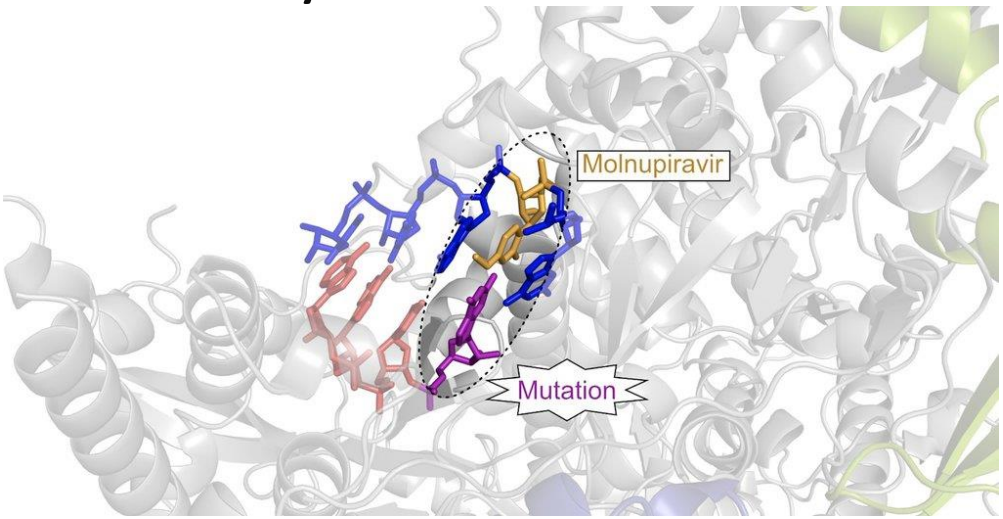


http://pdb101.rcsb.org/learn/resources-to-fight-the-covid-19-pandemic/the-search-for-drugs-to-fight-covid-19

Molnupiravir

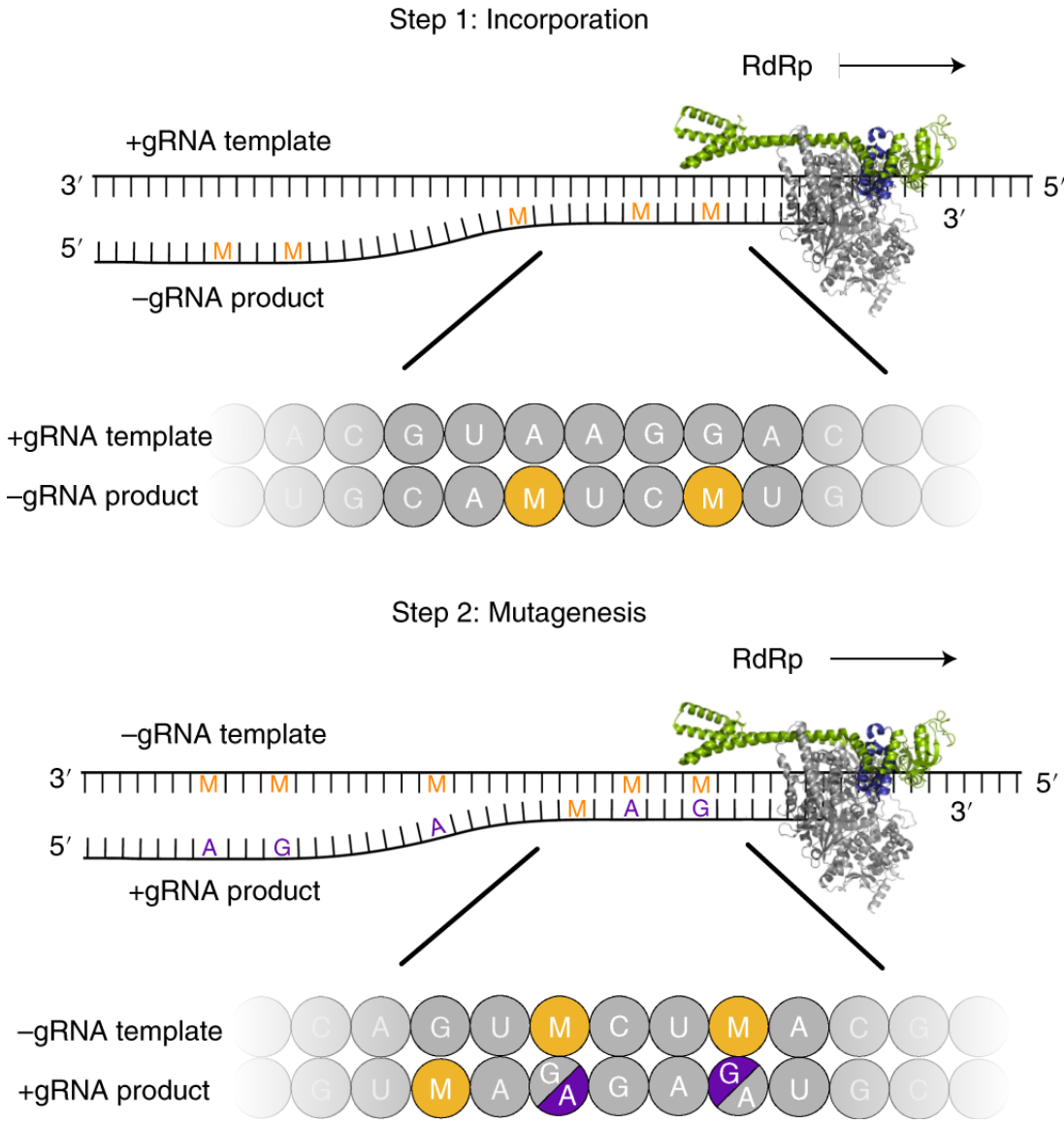


- Pilulka
- Blokuje replikaci SARS-CoV-2
- Žádné závažné vedlejší efekty na dobrovolnících
- prevence hospitalizace se závažnými formami a smrtí



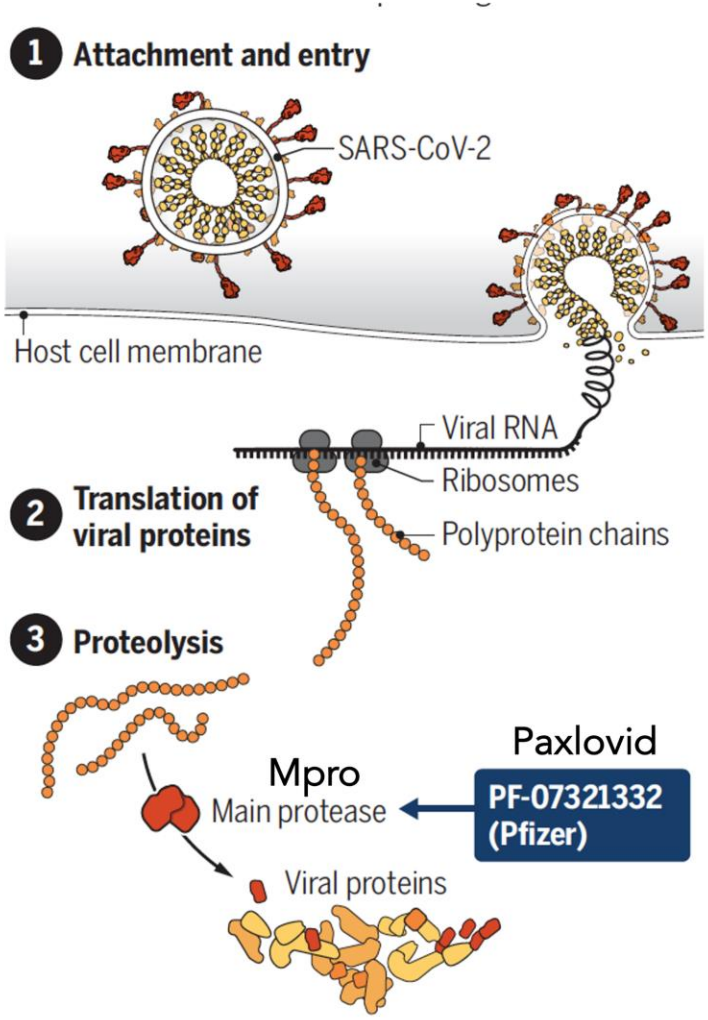
https://www.mpg.de/17362204/0812-bich-molnupiravir-152115-x

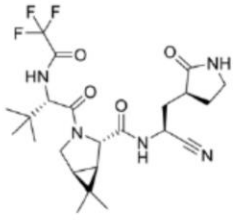
Molnupiravir MoA



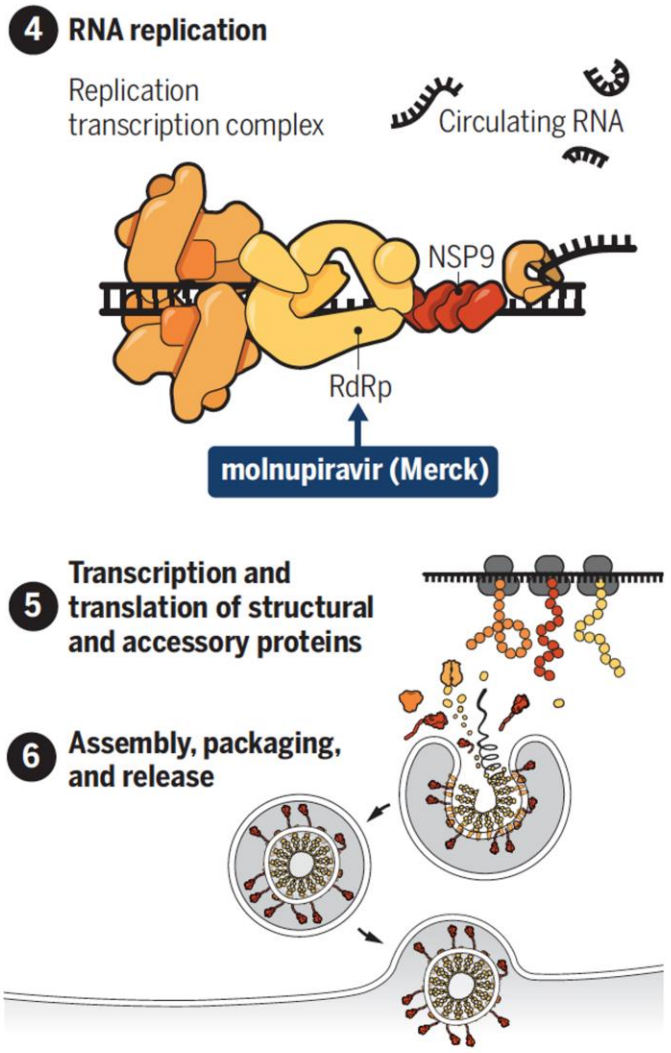
Kabinger, F., Stiller, C., Schmitzová, J. *et al.* Mechanism of molnupiravir-induced SARS-CoV-2 mutagenesis. *Nat Struct Mol Biol* **28**, 740–746 (2021). https://doi.org/10.1038/s41594-021-00651-0

Paxlovid vz Molnupiravir



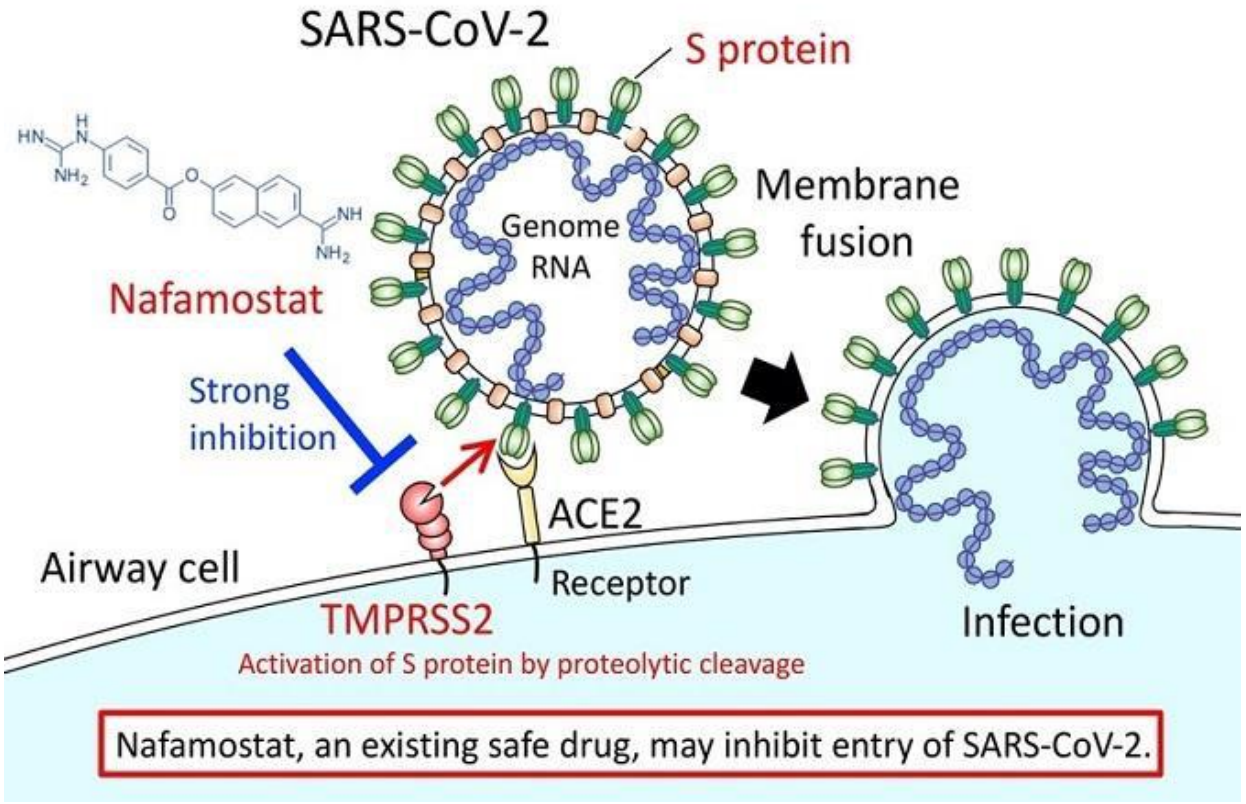


Chemical structure

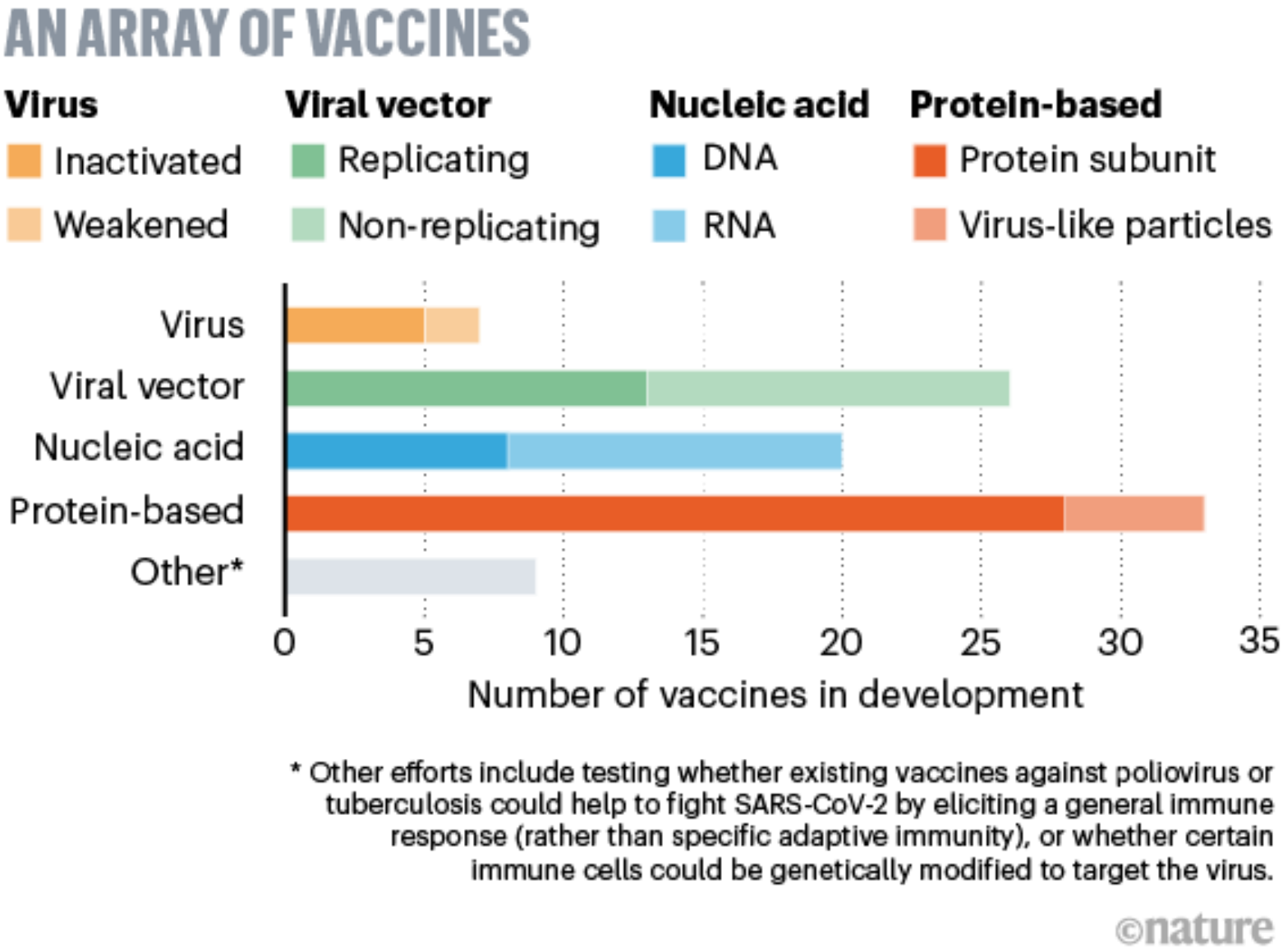


https://erictopol.substack.com/p/why-paxlovid-is-a-just-in-time-breakthrough

Protease Inhibitors – Spike and Maturation

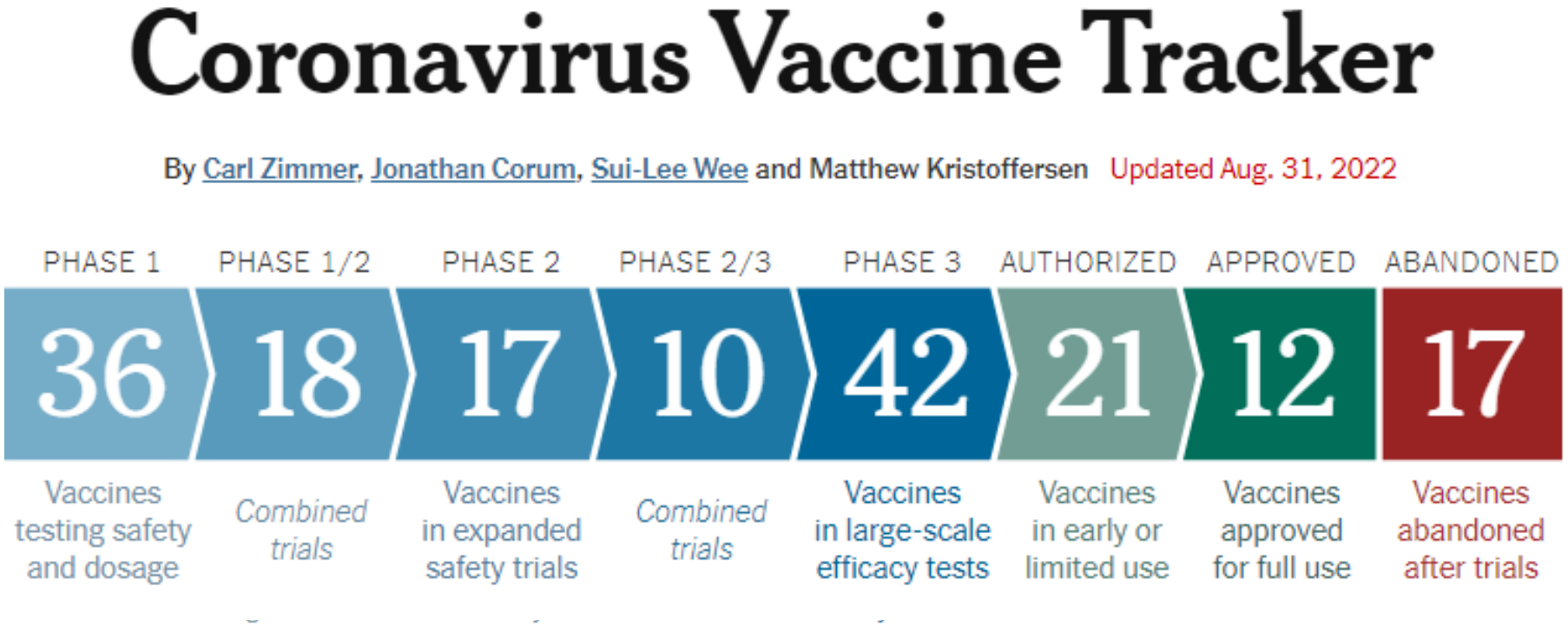


Vakcíny



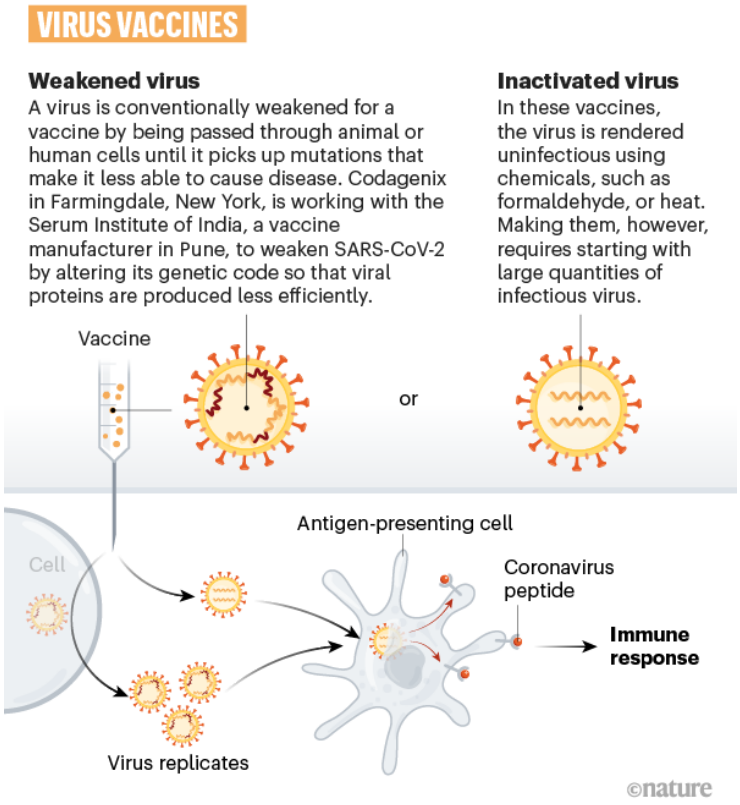
https://www.nature.com/articles/d41586-020-01221-y as of April 2020

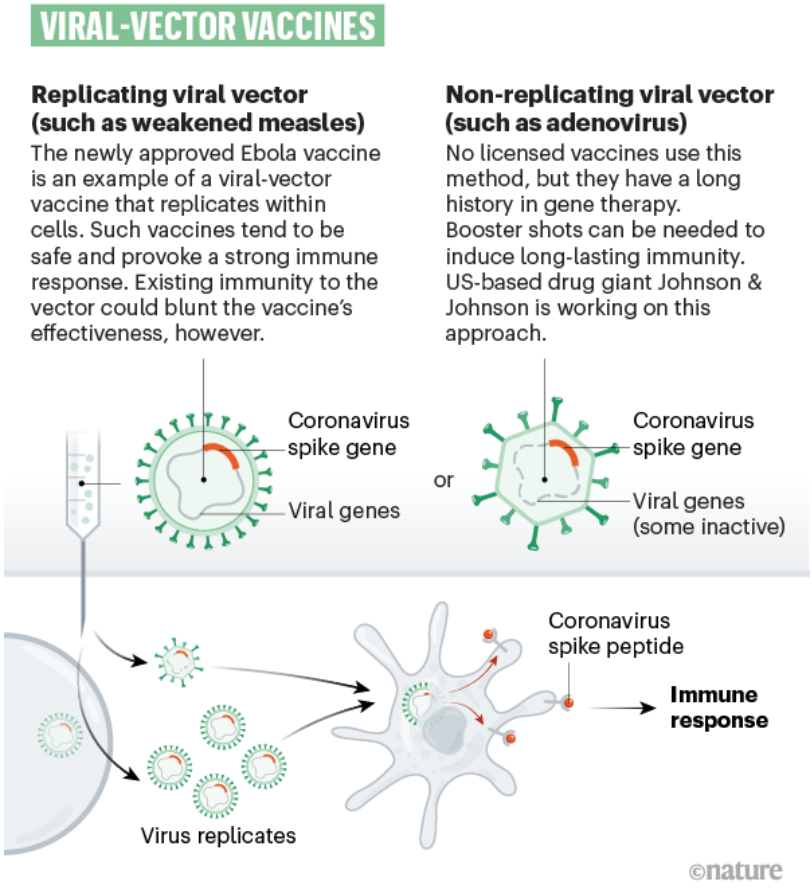
Vakcíny ve vývoji



https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html

Vakcíny I

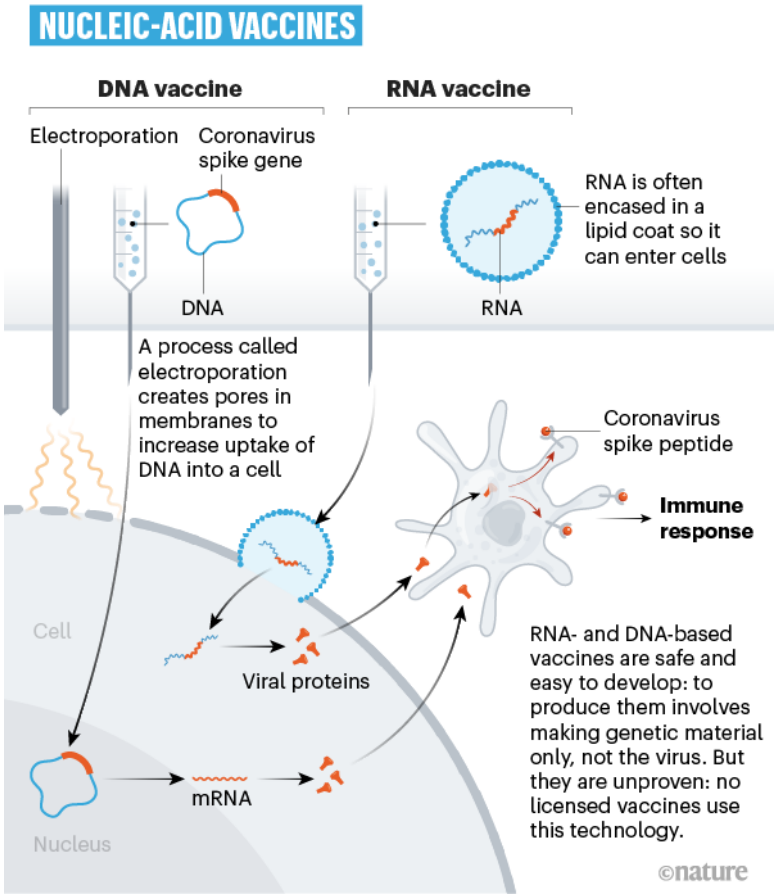


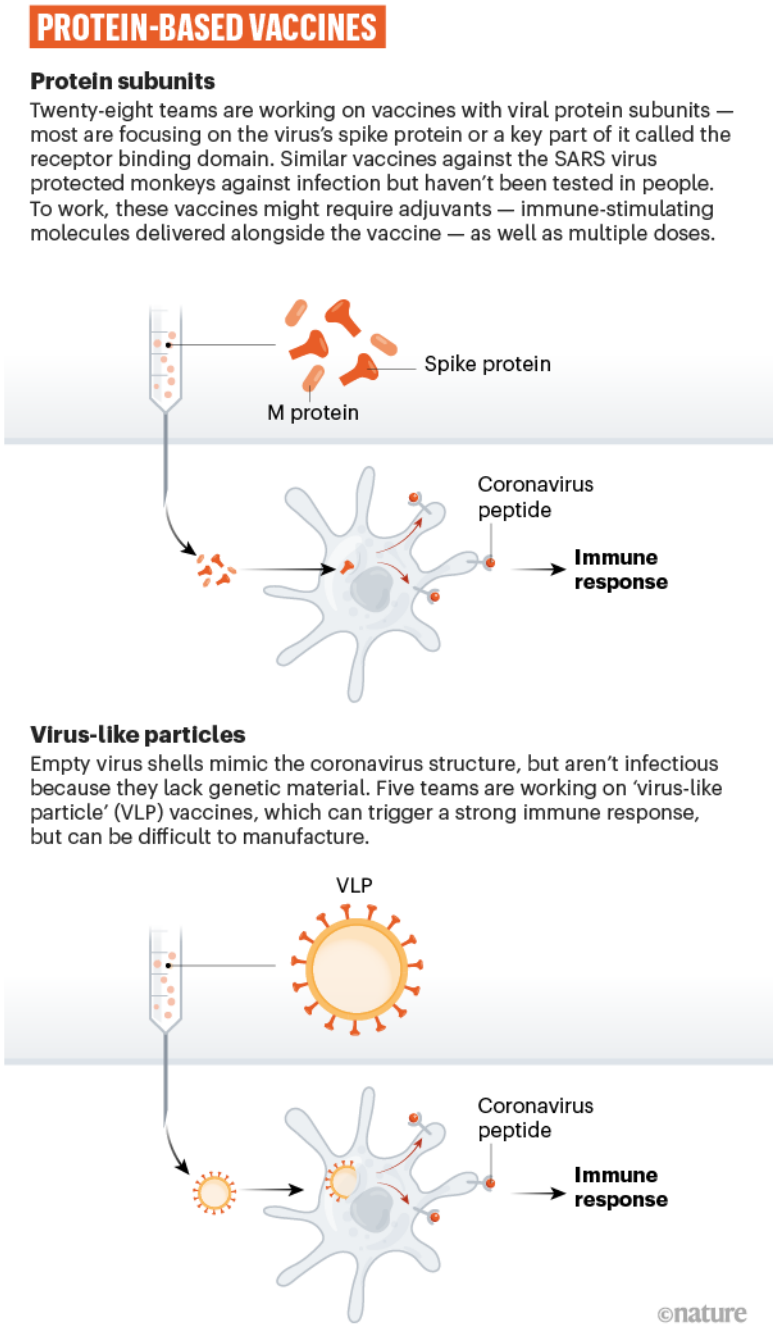


Sputnik V - two adenovirus vectors with Spike protein- rAd26-S + rAd5-S

https://www.nature.com/articles/d41586-020-01221-y

Vakcíny II





Pfizer-BioNtech – mRNA for Spike protein

Moderna - mRNA-1273 for Spike protein

https://www.nature.com/articles/d41586-020-01221-y

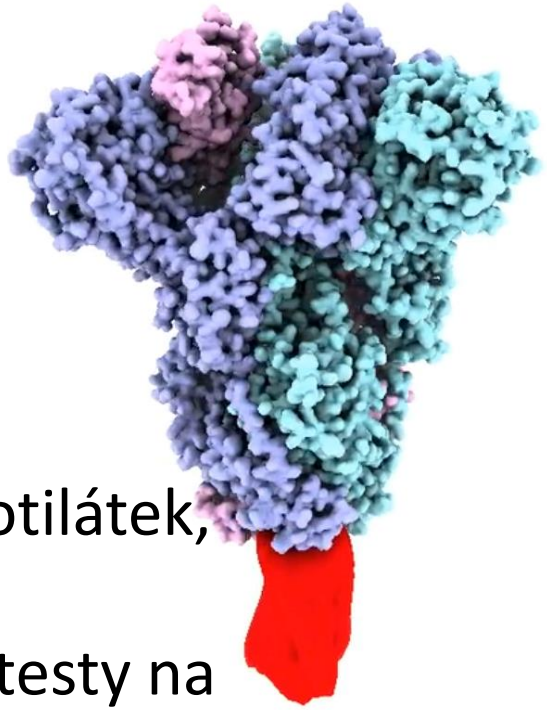
Leading vaccines

Developer	How It Works	Phase	Status
Pfizer-BioNTech	mRNA	3	Approved in U.S., other countries. Emergency use in many countries.
Sinopharm	Inactivated	3	Approved in China, Bahrain. Emergency use in many countries.
Oxford-AstraZeneca	ChAdOx1	2 3	Approved in Brazil, India. Emergency use in many countries.
Sinovac	Inactivated	3	Approved in China. Emergency use in many countries.
Moderna	mRNA	3	Approved in U.S., Canada, Switzerland. Emergency use in many countries.
Novavax	Protein	3	Approved in Canada, South Korea. Emergency use in several countries.
Bharat Biotech	Inactivated	3	Approved in India. Emergency use in other countries.
Johnson & Johnson	Ad26	3	Approved in Canada. Limited in U.S. Emergency use in many countries.
Baylor-Biological E	Protein	3	Emergency use in India, Botswana.
Gamaleya	Ad26, Ad5	3	Approved in Russia. Emergency use in many countries.

Zamítnuté vakcíny – zvláštní zmínka

ABANDONED

THE UNIVERSITY OF QUEENSLAND AUSTRALIA CSL



- Opuštěna 10.12.2020
- Na křečcích fungovala skvěle
- Fáze I – červenec 2020 – skvělé, hodně protilátek, žádné závažné vedlejší účinky
- Ale pak – dobrovolníci začali mít pozitivní testy na HIV, aniž by HIV virus měli
- Důvod: Aby udrželi S protein ve správném tvaru – drželi ho na místě pomocí „molecular clamp“ – ke kterému použili segment HIV proteinu – a ten chytaly protilátkové testy na HIV

https://www.nature.com/articles/d42473-020-00504-2?source=globalbiodefense

Summary

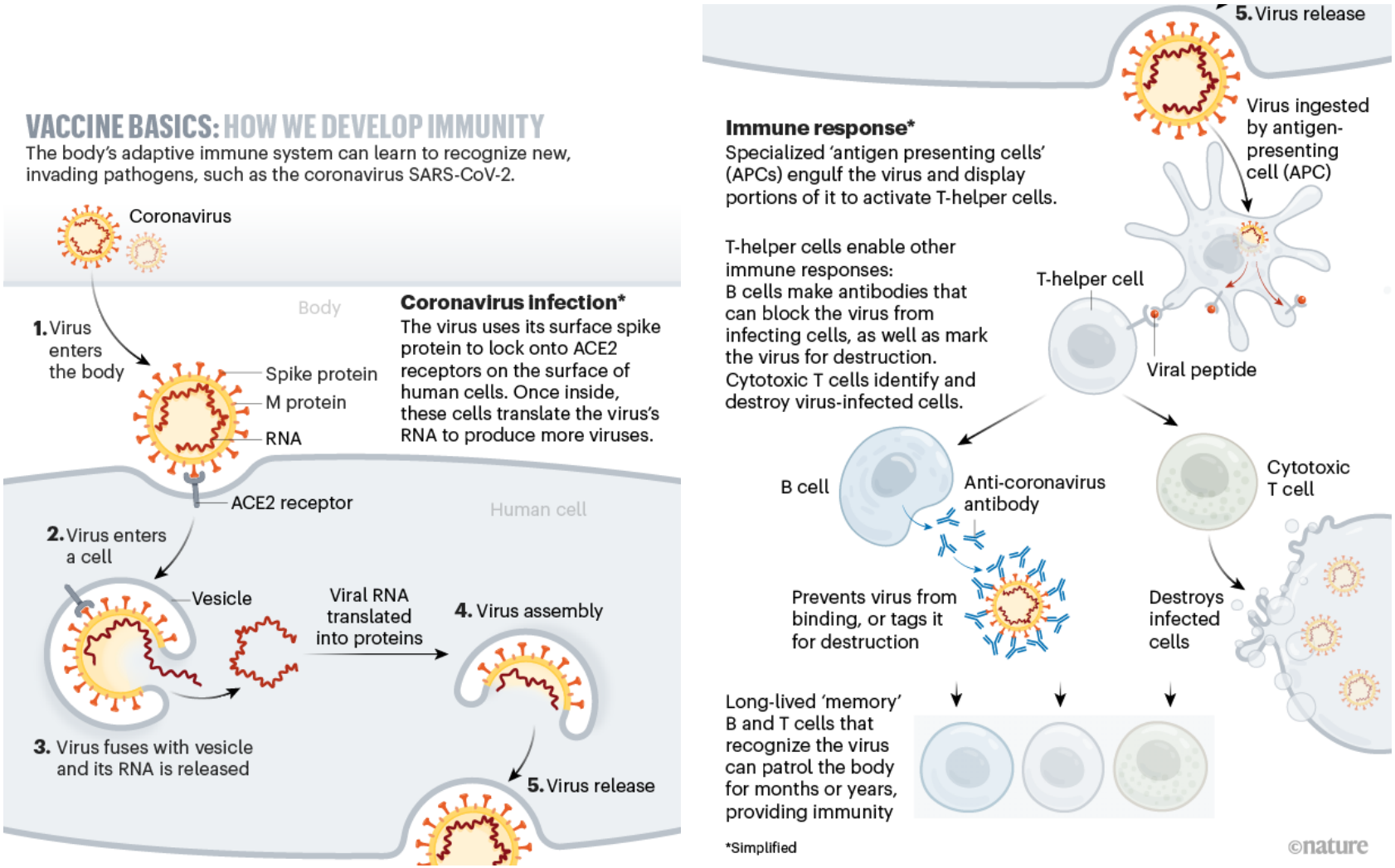
Závěry

- Máme už docela dobrou představu ohledně struktury a funkce drtivé většiny proteinů SARS-CoV-2
- Vede to k návrhu léčiv a vakcín
- Sledováním mutací můžeme odhalit, které protilátky už na nové varianty nebudou plně fungovat
- Bohužel ani po nákaze tolika lidí stále má virus i v rámci RBD domény kde mutovat…

Zdroje

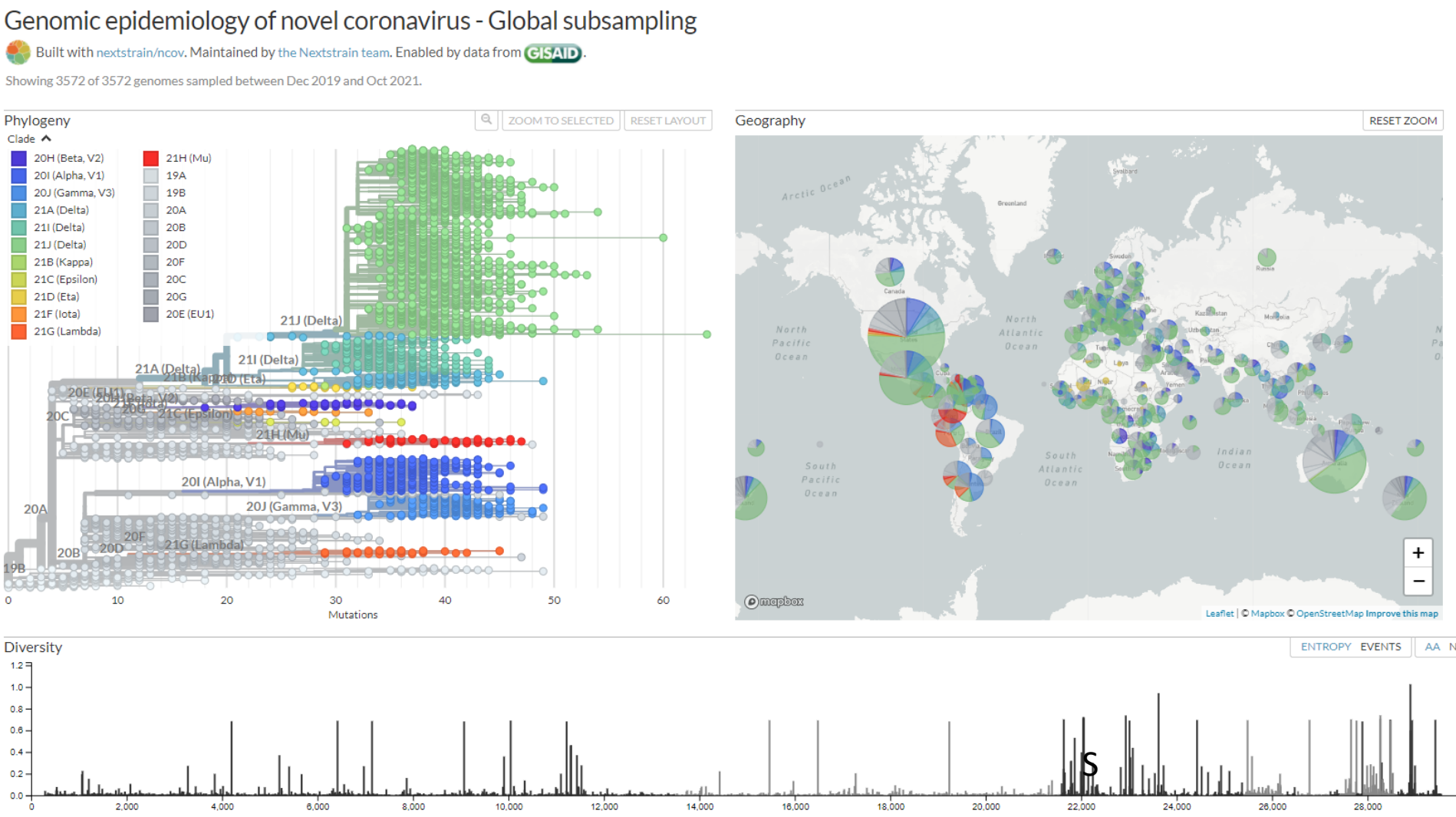
- virus.img.cas.cz
- covid19dataportal.org
- pdb101.rcsb.org/teach/covid-19
- pdb101.rcsb.org/sci-art/goodsell-gallery
- swissmodel.expasy.org/repository/species/2697049
- ebi.ac.uk/pdbe/covid-19
- http://home.sandiego.edu/~josephprovost/Biochem_Covid_Teaching.html

Imunita



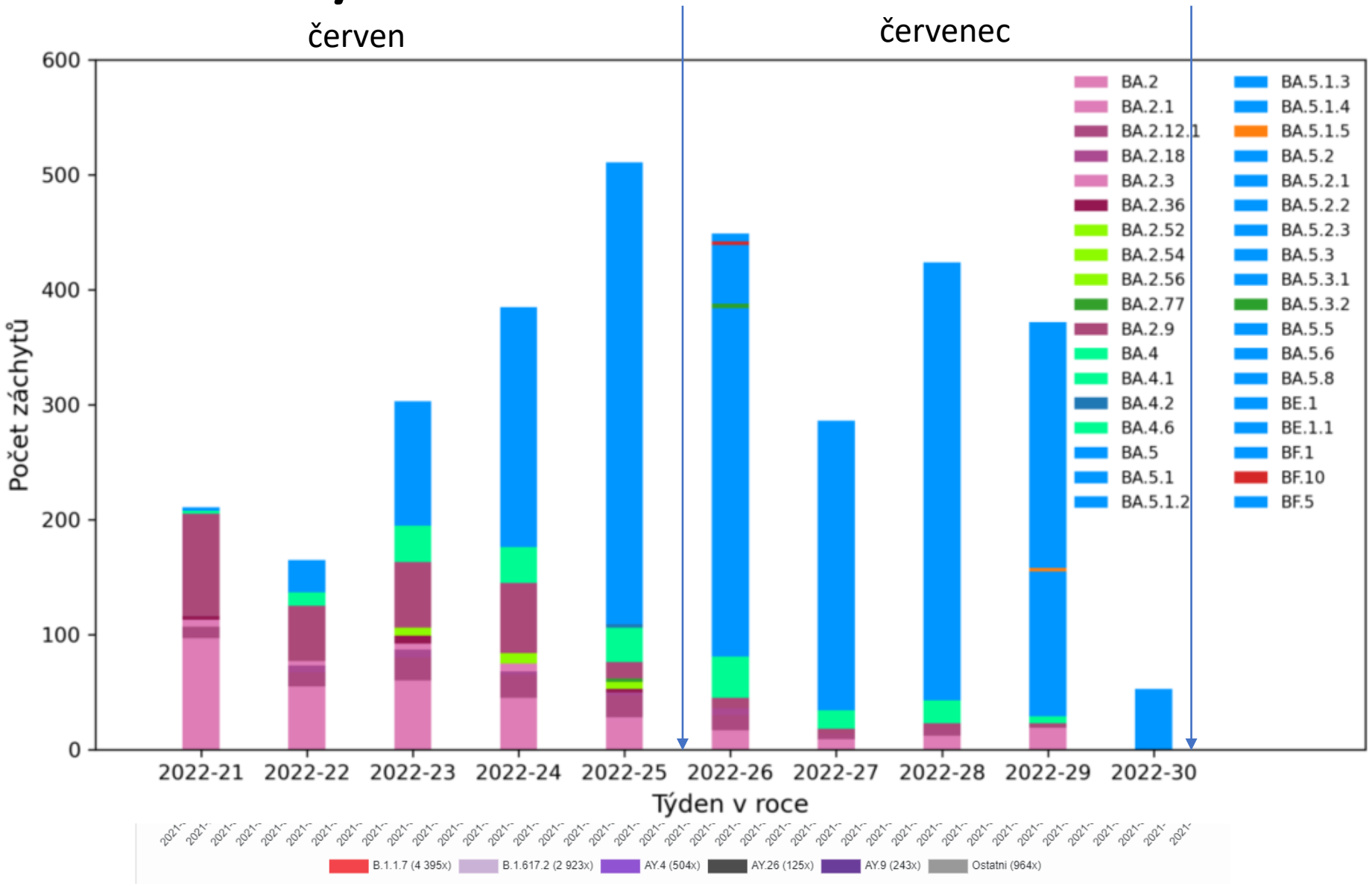
https://www.nature.com/articles/d41586-020-01221-y

Genomy SARS-Cov-2



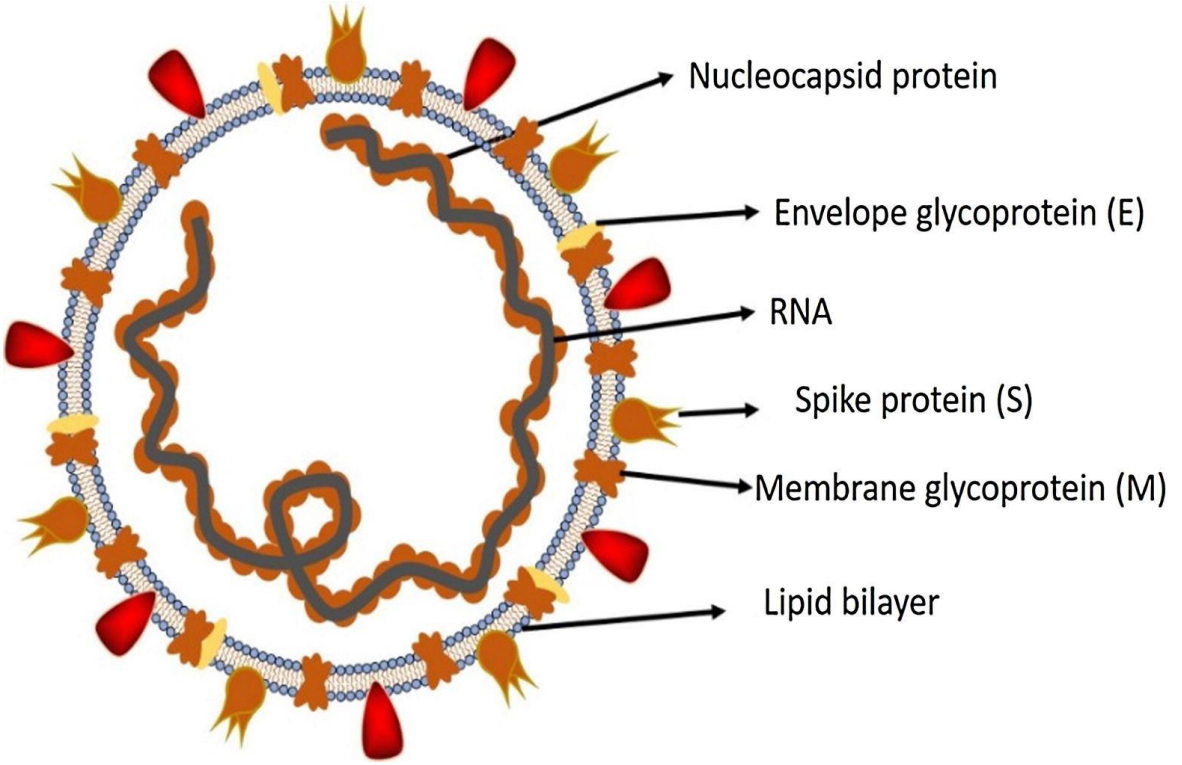
https://nextstrain.org/ncov/global?m=div

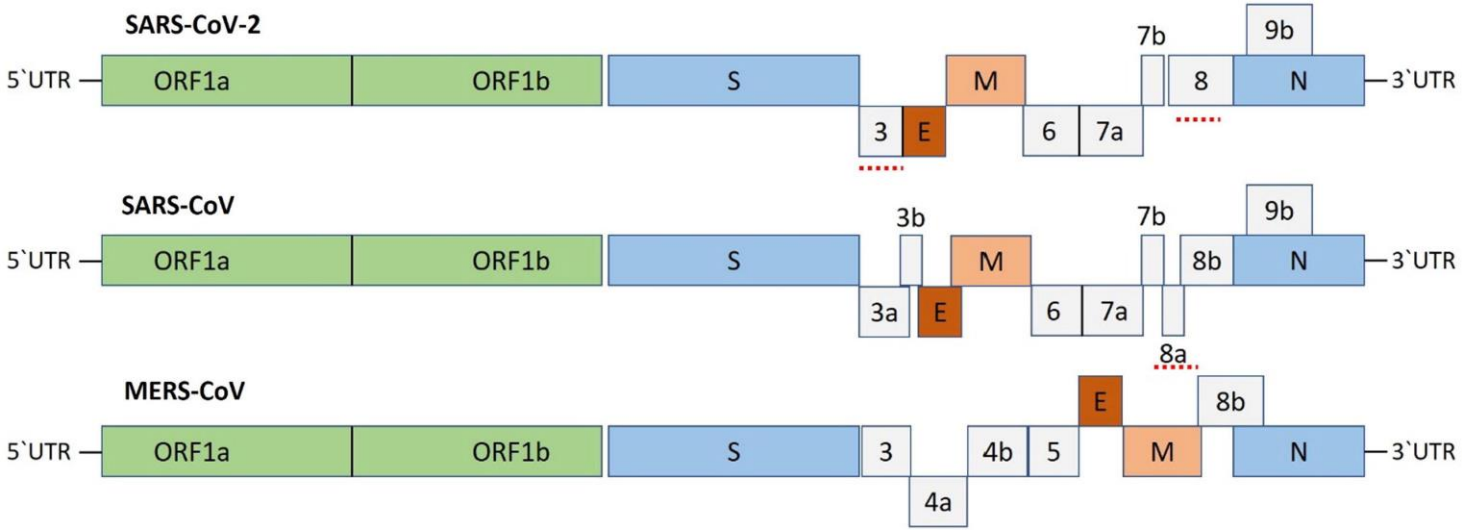
Varianty v ČR



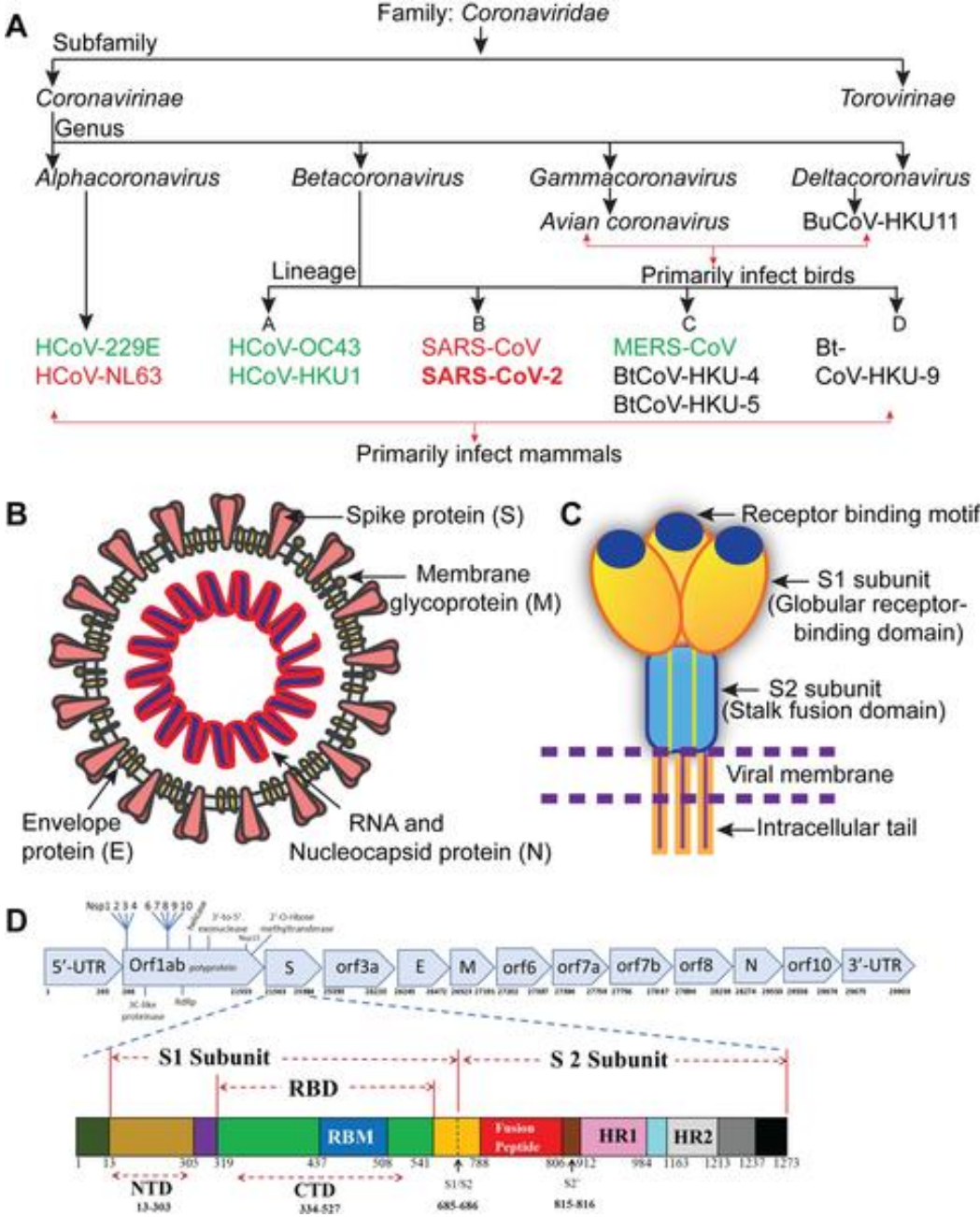
https://virus.img.cas.cz/lineages

SARS, MERS, COVID-19 "parts list"



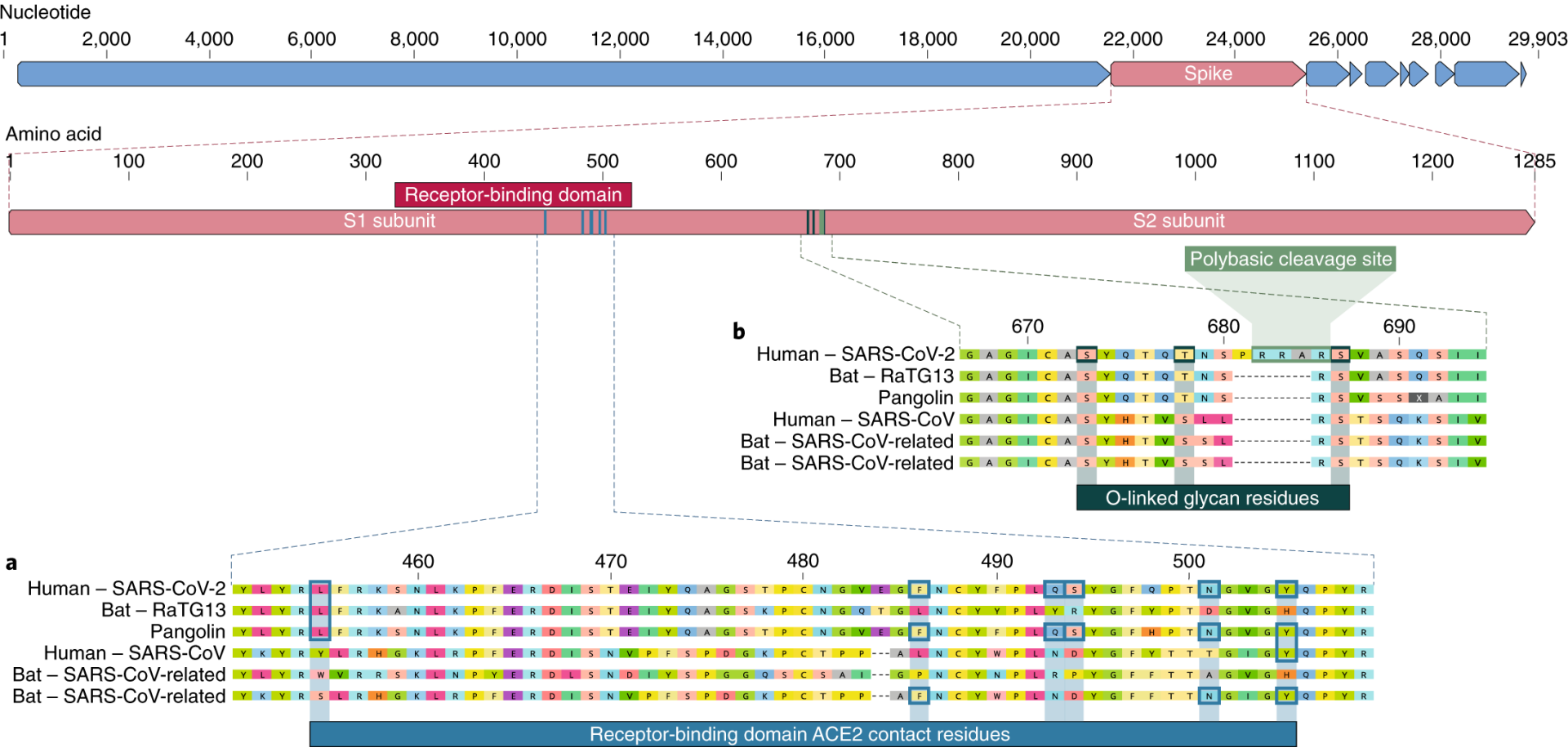


Klasifikace a struktury koronavirů



Mittal A, Manjunath K, Ranjan RK, Kaushik S, Kumar S, et al. (2020) COVID-19 pandemic: Insights into structure, function, and hACE2 receptor recognition by SARS-CoV-2. PLOS Pathogens 16(8): e1008762. https://doi.org/10.1371/journal.ppat.1008762
https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1008762

Spike Protein

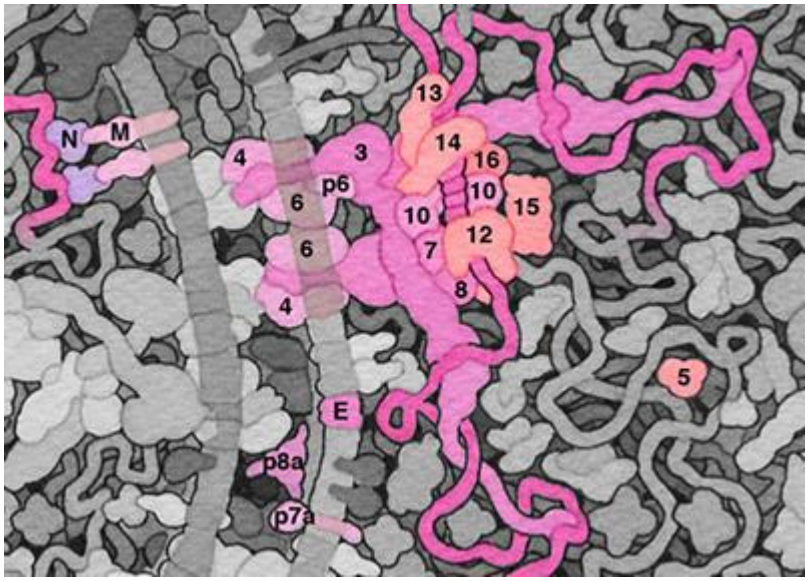


Detail komplexu proteinů viru

Nsp3 -- a multidomain protein that includes RNA-binding domains, a membrane-anchoring domain, and the papain-like protease
Nsp4 and 6 -- membrane-spanning proteins that remodel the ER membrane
Nsp5 -- main protease that cuts the viral polyproteins into functional pieces
Nsp7, 8 and 10 -- proteins involved in organizing the replicase complex
Nsp12 -- RNA-directed RNA polymerase creates new viral RNA strands
Nsp13 -- helicase separates strands in an RNA double helix
Nsp14 -- guanine N7-methytransferase includes an exoribonuclease involved in proofreading
Nsp15 -- uridylate-specific endoribonuclease breaks RNA, the function of which is still under study
Nsp16 -- 2'-O-methyltransferase is involved in formation of the RNA cap

Three structural proteins are also shown:
Nucleocapsid (N) condenses the viral genomic RNA
Membrane (M) protein works with N to package the RNA into the virion
Envelope (E) is involved in the process of budding

sizes and shapes of each protein are based on current structural information, but the arrangement within the complex is largely speculative:



Several accessory proteins (p6, p7a, and p8a) are also shown. These are dispensable for replication of the virus, but are involved in the virulence of infection.

http://pdb101.rcsb.org/sci-art/goodsell-gallery/coronavirus-life-cycle