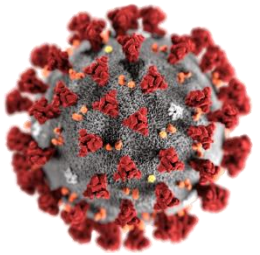
EACH | campus capital USP LESTE
Escola de Artes, Ciências e Humanidades
Universidade de São Paulo



Programa de Pós-Graduação em Bioquímica e Biologia Molecular

BBM5002 - Bioquímica e Biologia Molecular

SARS-CoV-2 e COVID-19
EaD

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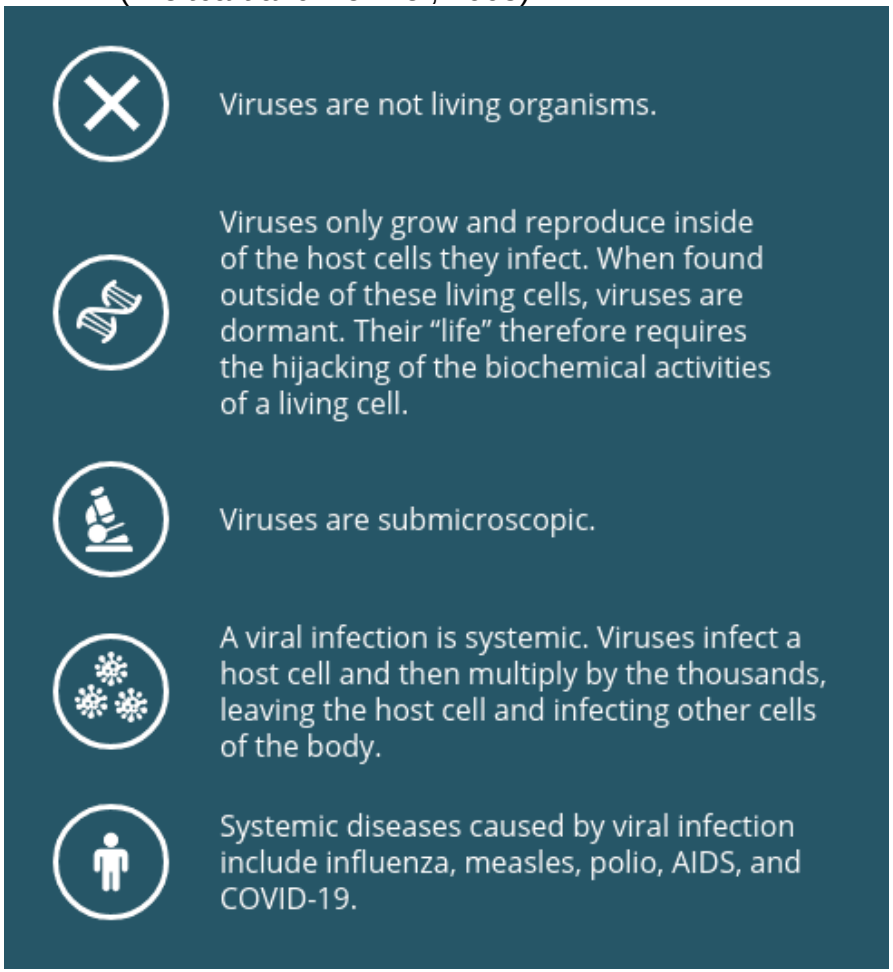
Laboratório de Bioquímica e Biotecnologia, EACH/USP

2021

Vírus

Estimado de aproximadamente 10^{31} vírus no planeta

(Breitbart and Rohwer, 2005)



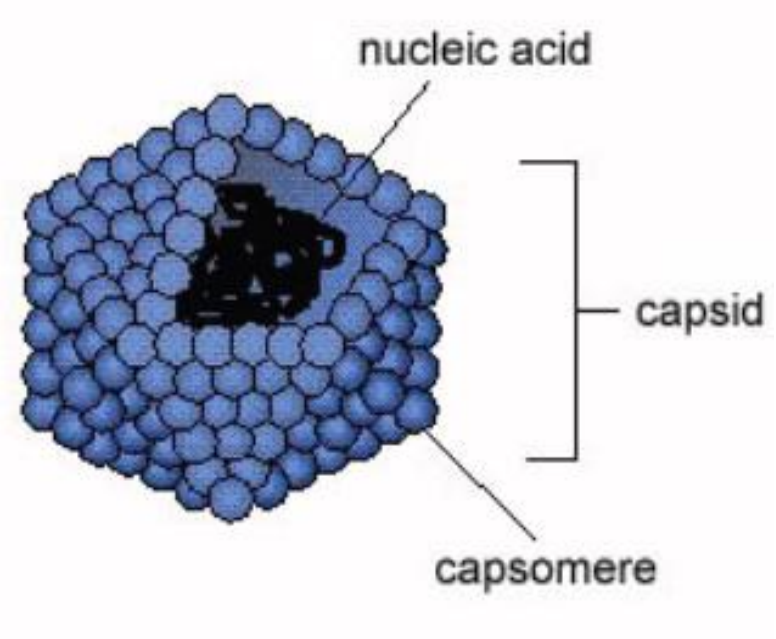
doi: 10.1093/nar/gkq901

Source: ***ViralZone:www.expasy.org/viralzone, SIB Swiss Institute of Bioinformatics***)

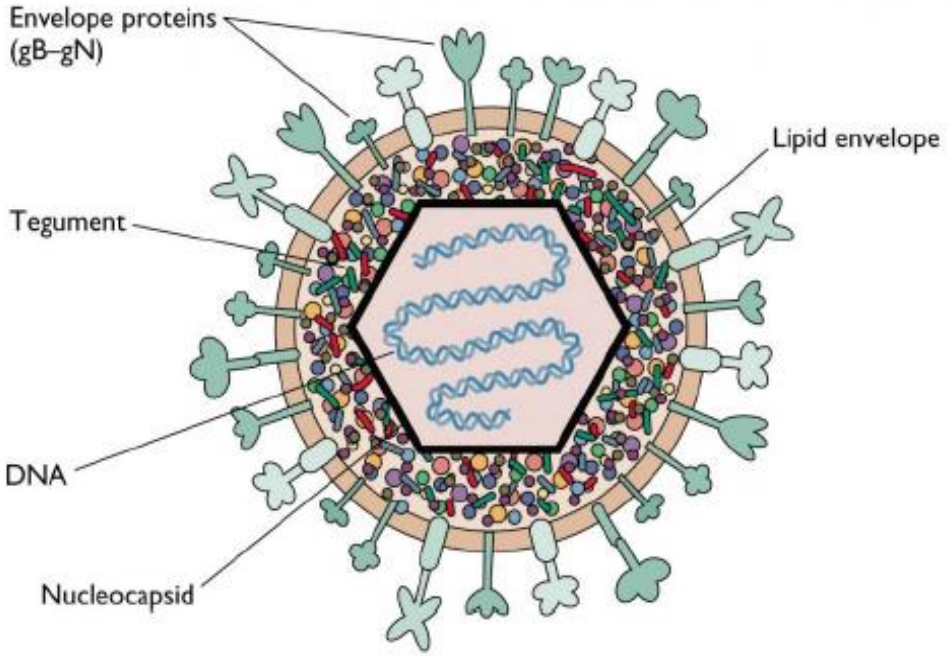
Estrutura dos vírus

Proteção do genoma pelo capsídeo viral.

Os capsídeos são constituídos por proteínas virais com propriedades de auto reconhecimento, o que propicia a formação de complexos chamados protômeros.



Não Envelopados
(Adenovírus)



Envelopados (Herpesvírus)

No caso dos vírus envelopados o capsídeo passa a ser chamado de nucleocapsídeo.

VÍRUS DNA

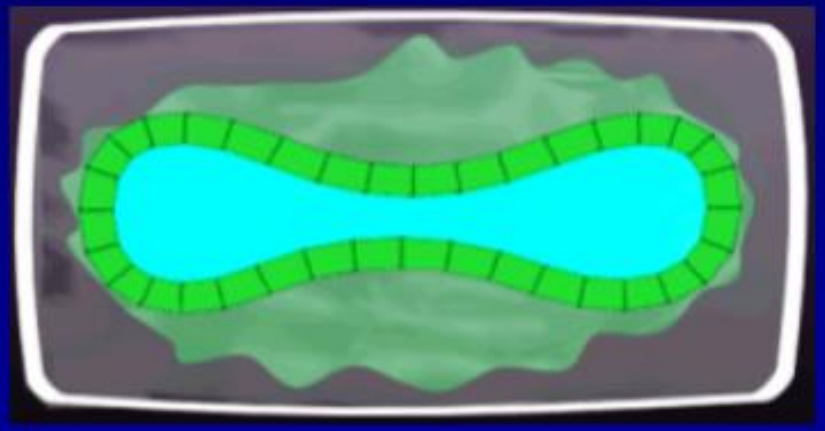
PARVOVIRIDAE

ADENOVIRIDAE

PAPOVAVIRIDAE

HERPESVIRIDAE

HEPADNAVIRIDAE



POXVIRIDAE

VÍRUS RNA

REOVIRIDAE

BUNYAVIRIDAE

TOGAVIRIDAE
FLAVIVIRIDAE

ARENAVIRIDAE

RHABDOVIRIDAE

PICORNAVIRIDAE

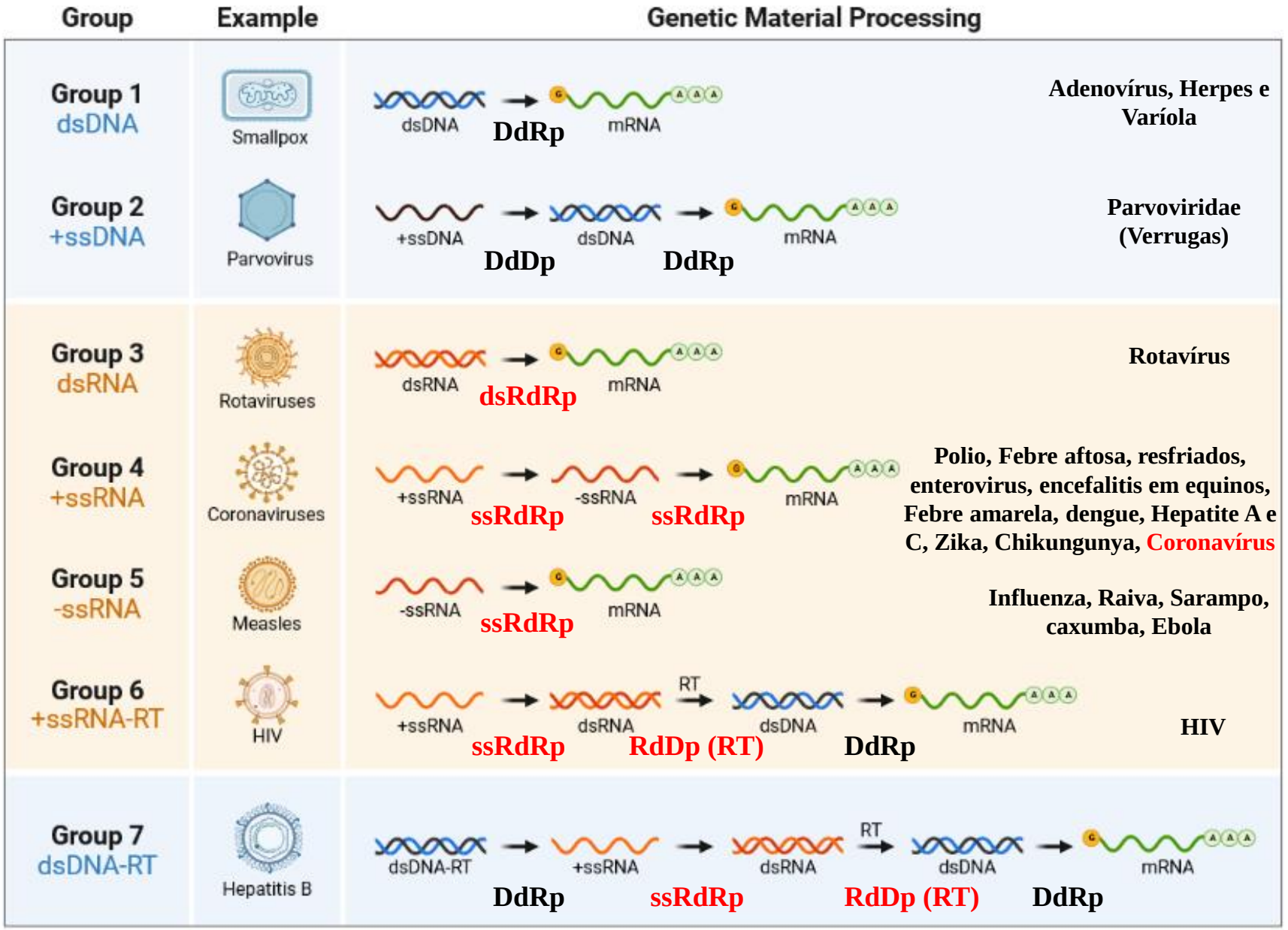
PARAMYXOVIRIDAE

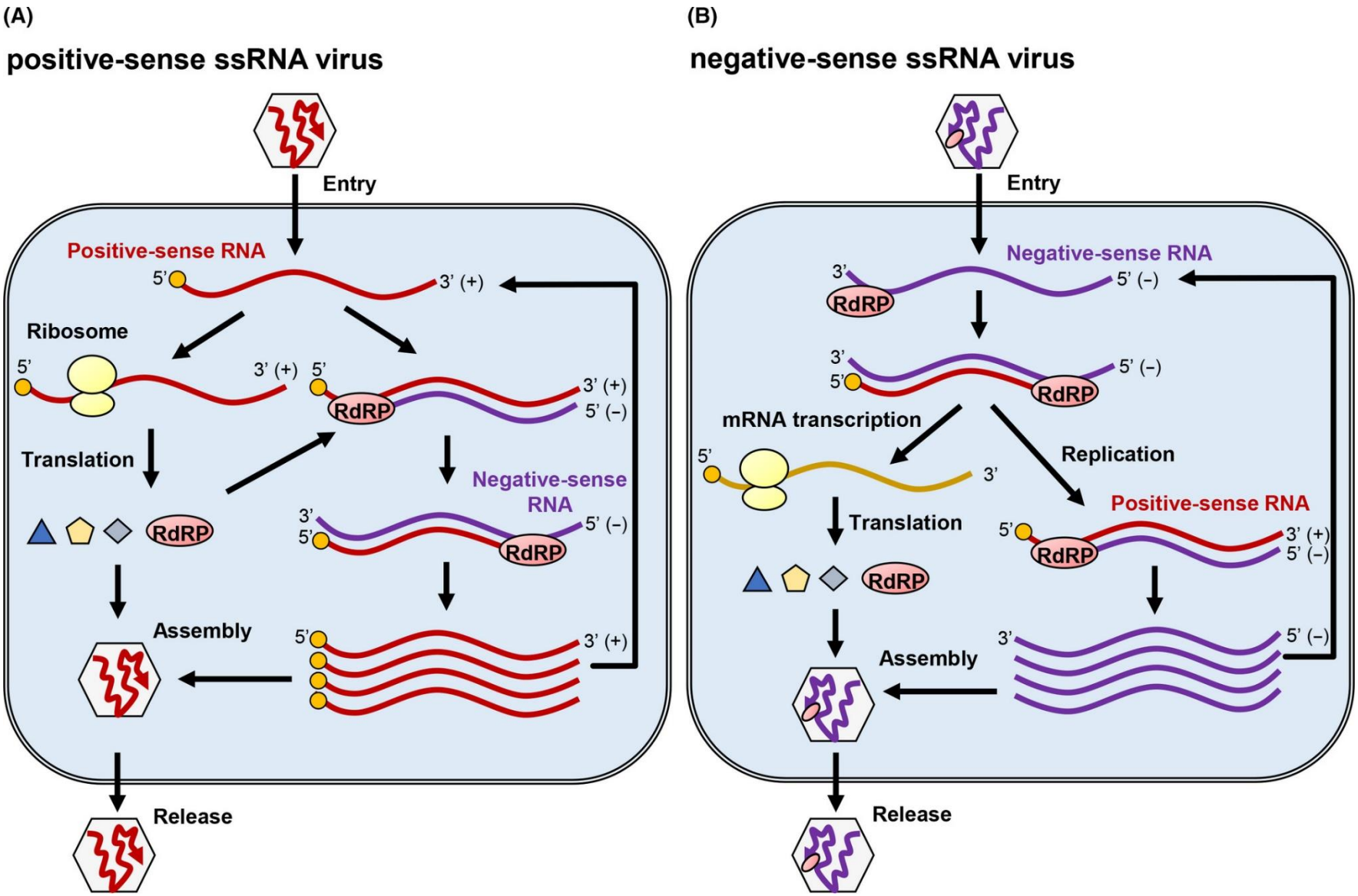
CORONAVIRIDAE

ORTHOMYXOVIRIDAE

RETROVIRIDAE

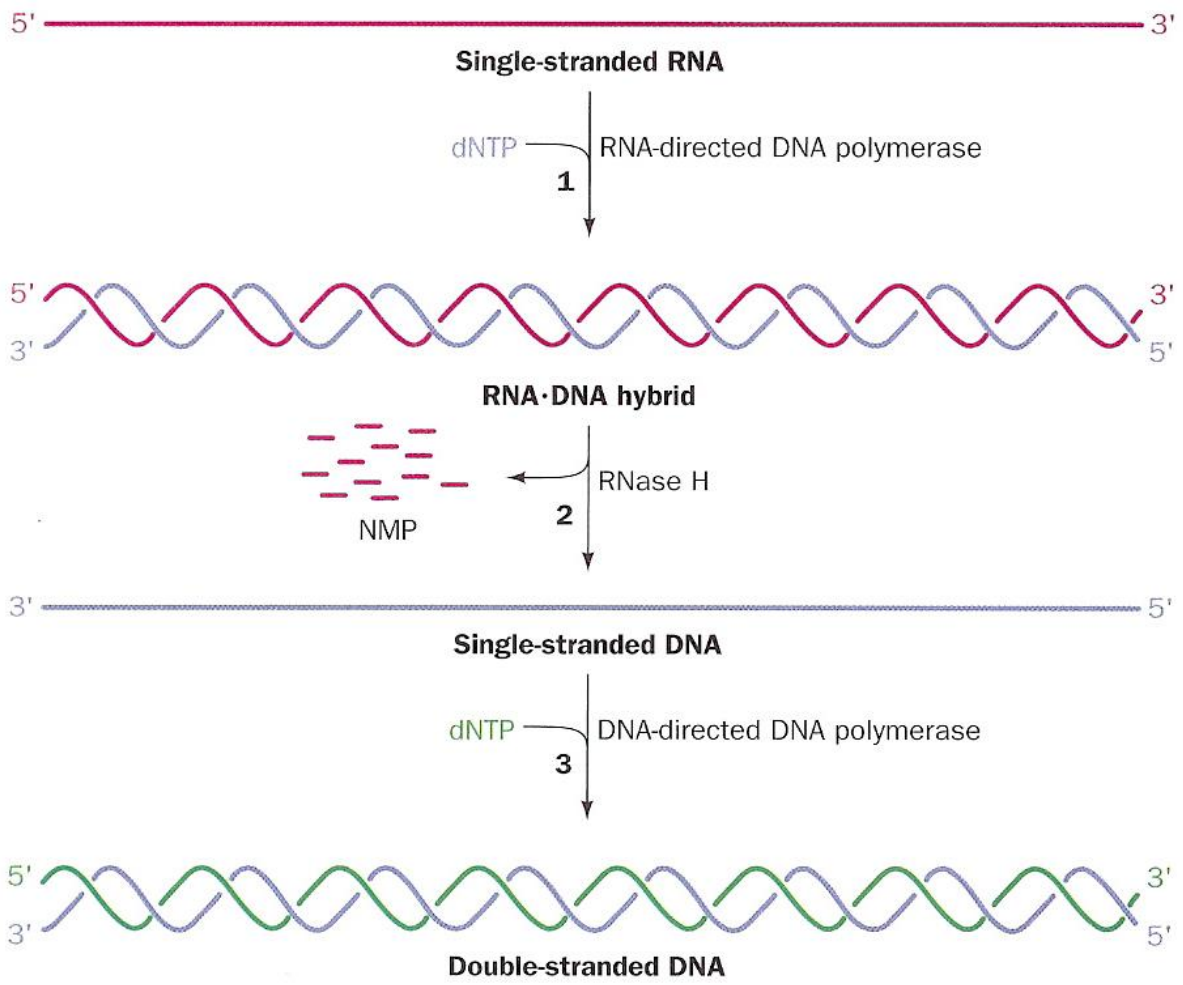
The Baltimore Classification e Transcrição Viral



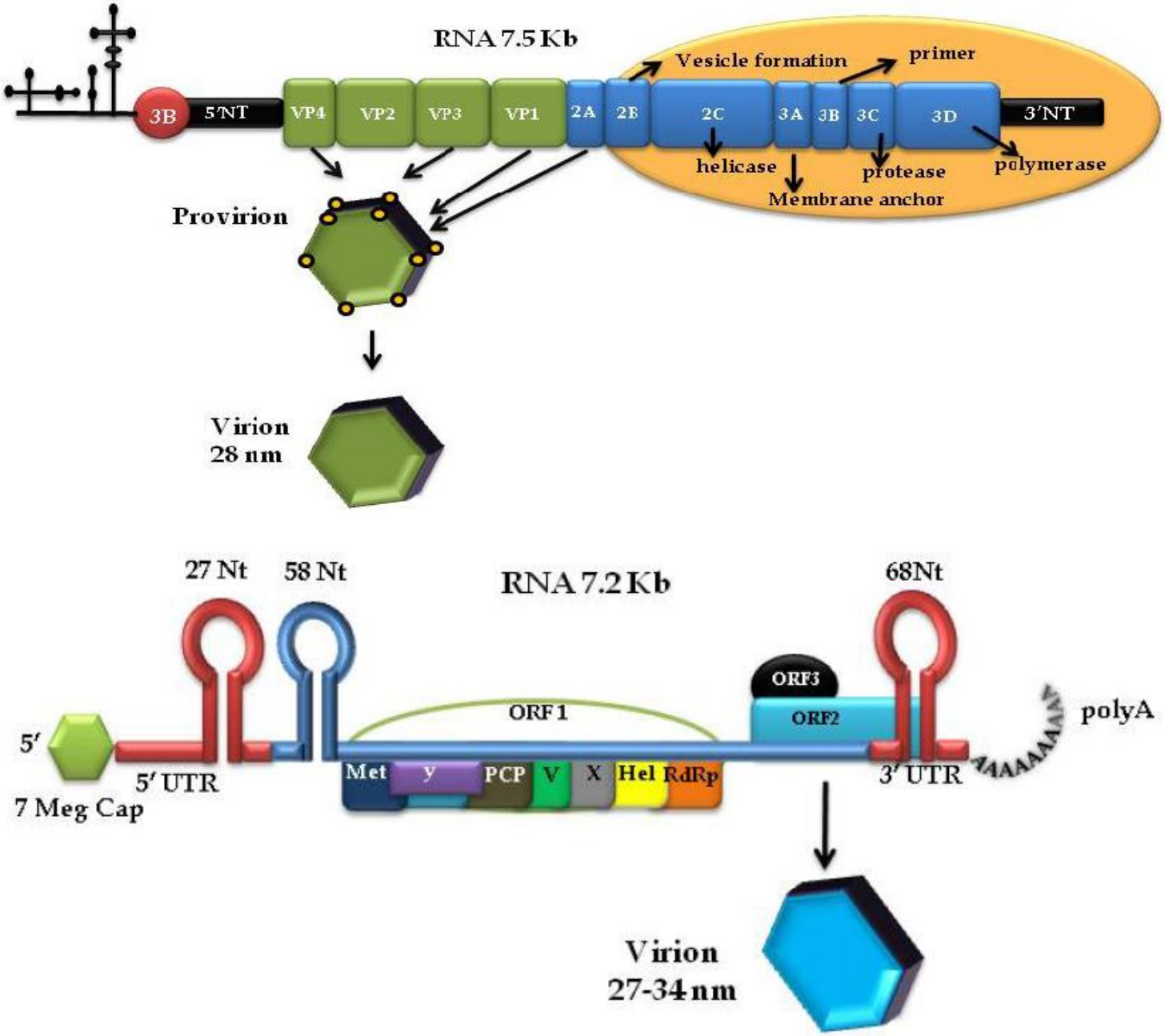


Cancer Science, Volume: 111, Issue: 11, Pages: 3976-3984, First published: 17 August 2020, DOI: (10.1111/cas.14618)

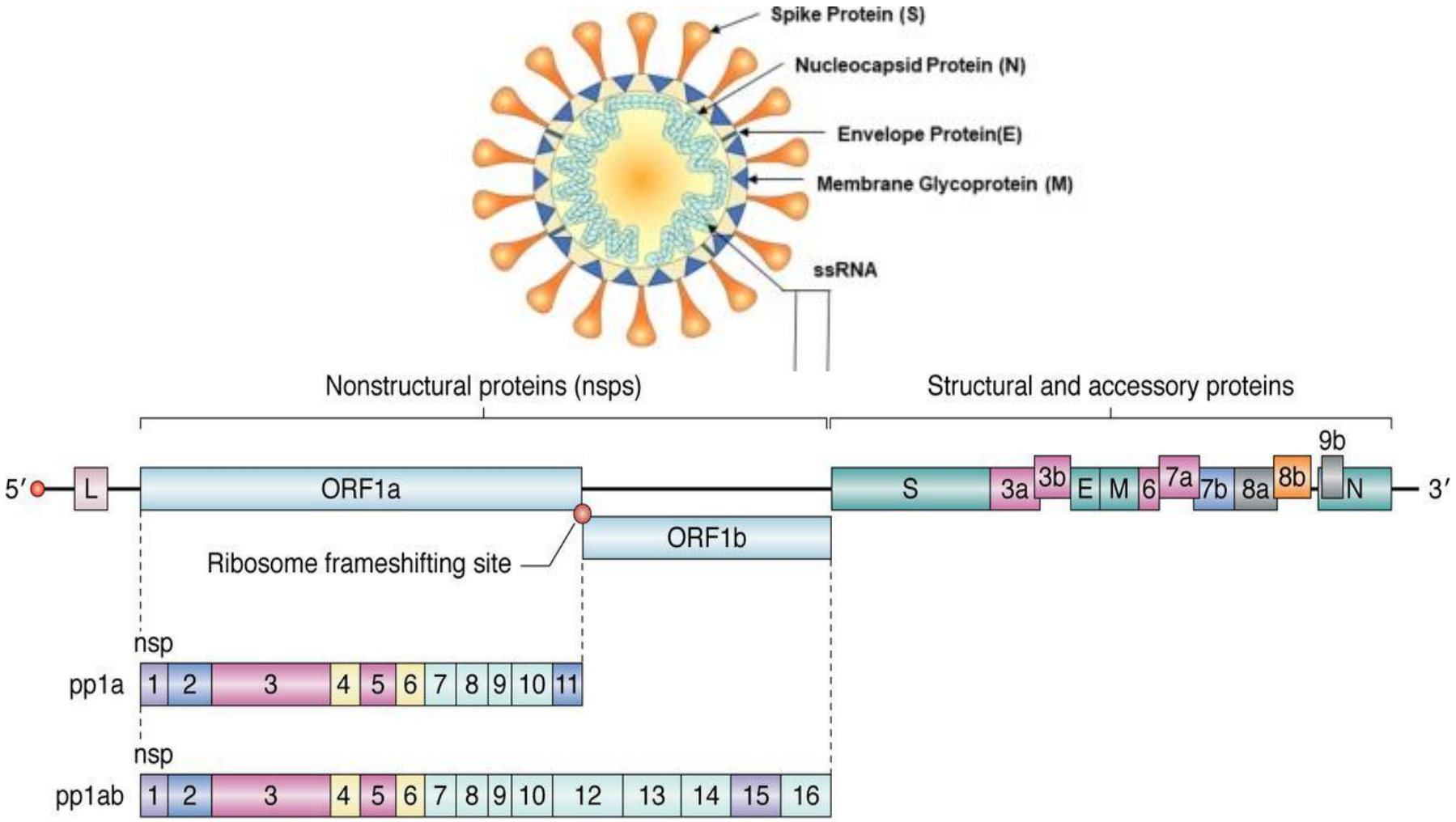
Transcriptasa reversa



Viral Genome Organization

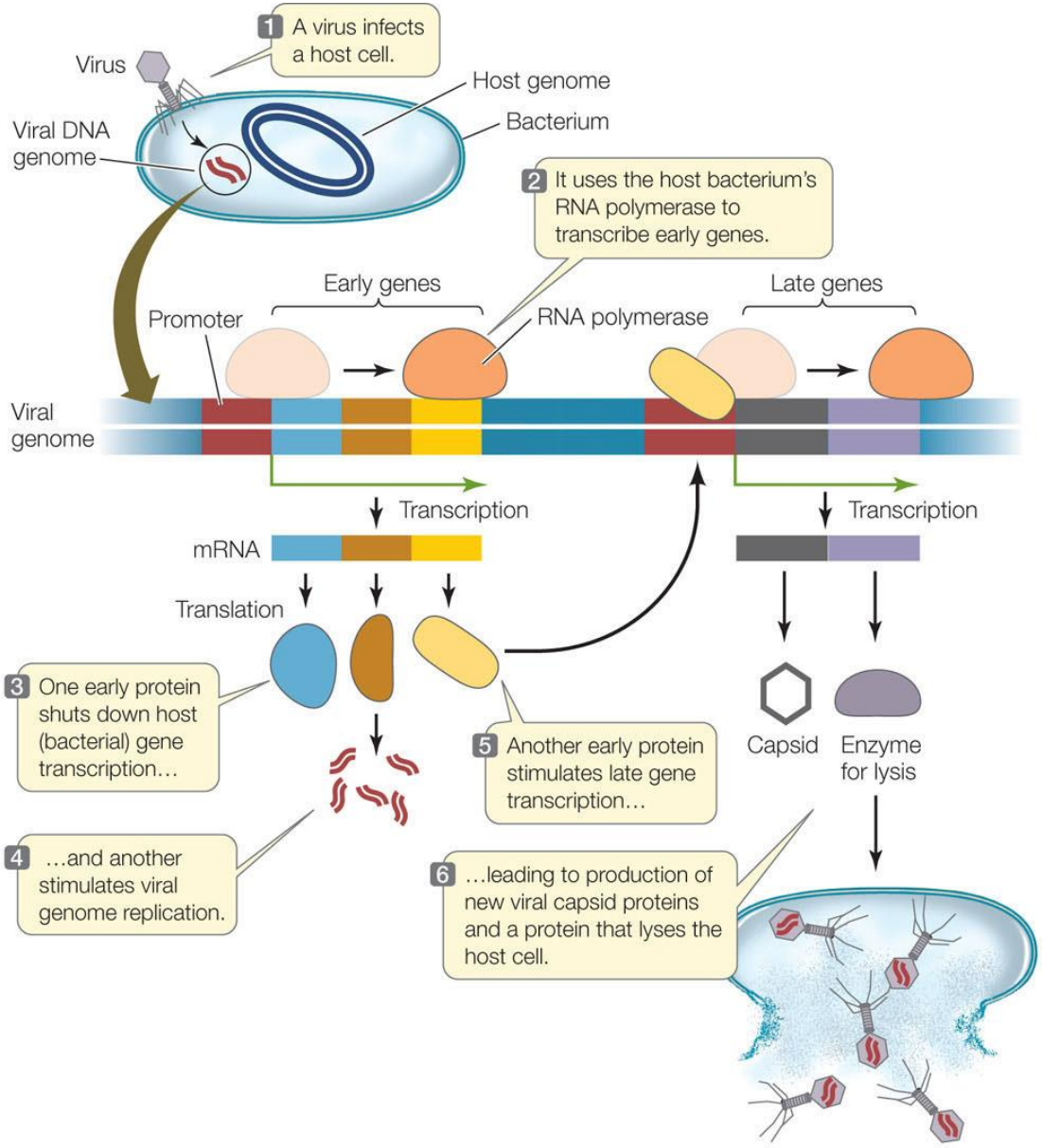


SARS-CoV-2 genome Structure



doi.org/10.1074/jbc.REV120.013930 - 2020

- For RNA viruses, most viral mRNAs are synthesized by Viral RDRP
- For DNA viruses, **viral mRNAs are generally synthesized by cellular RNA polymerases**.
  - Some exceptions: e.g. Poxviruses have their own DNA-dependent RNA polymerase.
- Transcription and expression of viral genes occurs in a strictly defined, reproducible sequence.
- Generally:
  - Genes for viral enzymes and regulatory proteins are transcribed early in infection.
  - Genes for structural proteins are transcribed later.



A Gene Regulation Strategy for Viral Replication In a host cell infected with a virus, the viral genome uses its early genes to shut down host transcription while it replicates itself. Once the viral genome is replicated, its late genes produce capsid proteins that package the new genomes, and other proteins that lyse the host cell.

Linha do Tempo – COVID-19

Data	Evento Chave
17/11/2019	Um caso confirmado do novo coronavírus surgiu em 17/11/2019, de acordo com relatórios de fontes oficiais do governo chinês , mas não foi reconhecido na época.
01/12/2019	O primeiro paciente conhecido começou a apresentar sintomas.
12/12/2019	A emissora estatal chinesa CCTV informou em uma transmissão de 12 de janeiro de 2020 que "um novo surto viral foi detectado pela primeira vez na cidade de Wuhan, na China.
21/12/2019	Epidemiologistas do Centro Chinês de Controle e Prevenção de Doenças (CCDC) publicam um artigo em 20/01/2020 afirmando que o primeiro grupo de pacientes com "pneumonia de causa desconhecida" foi identificado em 21/12/2019
31/12/2019	Autoridades de saúde da China informam a Organização Mundial da Saúde (OMS), a existencia de “27 casos de pneumonia de origem desconhecida, 7 de gravidade”, identificados em Wuhan, China.
03/01/2020	O primeiro genoma completo do vírus foi sequenciado (GenBank: MN908947) apartir de amostras de líquido de lavagem broncoalveolar de um paciente de Wuhan, o **novo vírus do gênero β coronavírus** foi designado como 2019-nCoV e a doença denominada pneumonia por infeção de novo coronavírus. Nas semanas seguintes, a infecção se espalhou pela China e outros países ao redor do mundo.
30/01/2020	A OMS declarou o surto uma Emergência de Saúde Pública de Interesse Internacional.
12/02/2020	A OMS nomeou a doença causada pelo novo coronavírus como Doença de Coronavírus 2019 (**COVID-19**) e o coronavírus 2 da síndrome respiratória aguda grave (**SARS-CoV-2**), como o agente etiológico
26/02/2020	Foi confirmado o primeiro caso de COVID-19 no Brasil (na cidade de São Paulo).
26/02/2020	Empresas de Biotecnologia (ModernaTX, Inc (https://www.modernatx.com/, USA) e Clover Biopharmaceuticals (http://www.cloverbiopharma.com, China)), anunciam o desenvolvimento da vacina contra o SARS-CoV-2.
11/03/2020	A epidemia se espalhou por diversos paises e a OMS declarou a COVID-19 uma **Pandemia**.
01/06/2021	**Globally, there have been 170.426.245 confirmed cases of COVID-19, including 3.548.628 deaths, reported to WHO. O ministério de Saúde do Brasil reporta 16.545.554 casos confirmados de COVID-19 e 462.791 óbitos.**

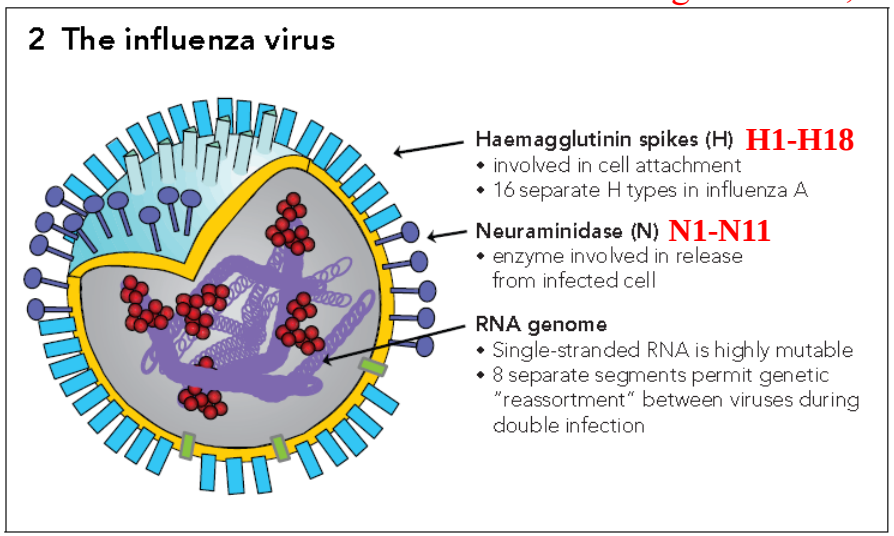
Pandemia

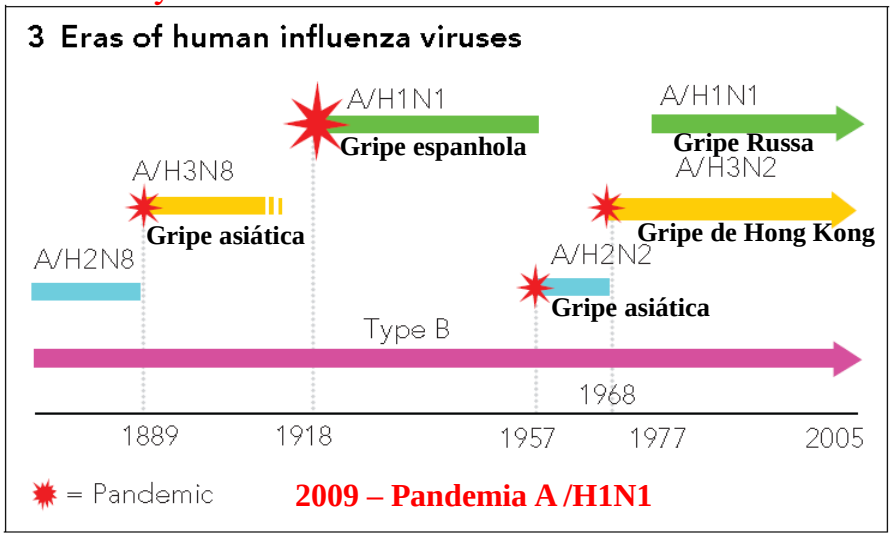
De acordo com a OMS, uma pandemia pode começar quando se reúnem três condições:

- O aparecimento de uma **nova doença** na população;
- O agente **infecta humanos**, causando uma doença séria;
- O agente **espalha-se fácil** e sustentavelmente entre humanos.

PREPARING FOR AN INFLUENZA PANDEMIC

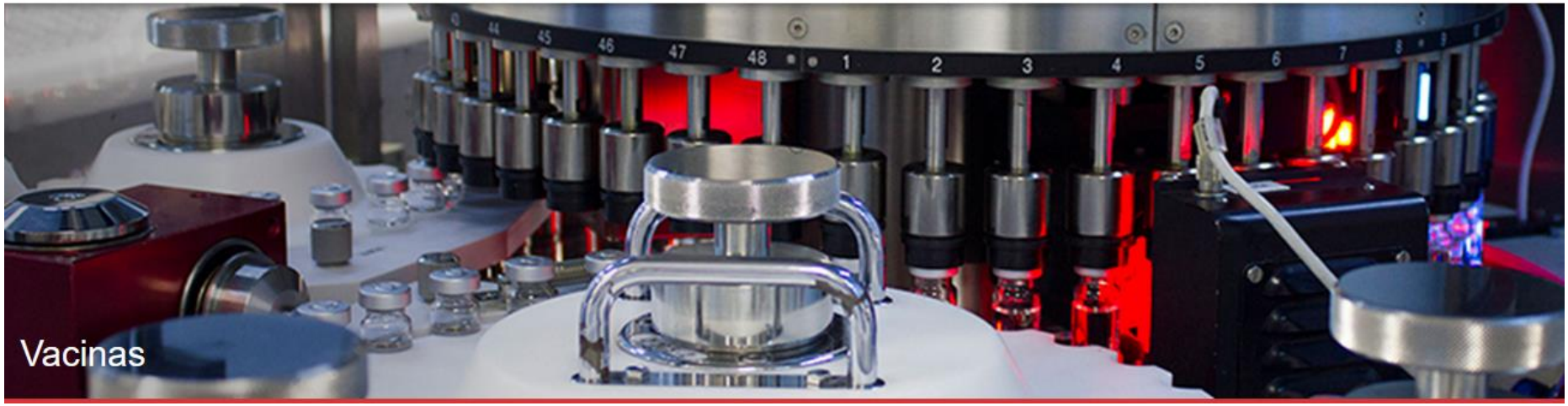
Pandemic influenza occurs at irregular and unpredictable intervals, and is the result of a major antigenic change known as “antigenic shift”, which occurs only in influenza A.





Myxovirus influenzae,também denominado vírus influenza. Vírus envelopados de RNA de fita simples segmentadas e subdividem-se nos tipos A, B e C, sendo que apenas os do tipo A e B têm relevância clínica em humanos.

doi: 10.5694/j.1326-5377.2006.tb00705.x - 2006



Vacinas em desenvolvimento

Um longo caminho tem sido percorrido pelo Butantan no desenvolvimento de vacinas e adjuvantes, desde a pesquisa básica e a aplicação em etapas piloto, até o escalonamento em nível industrial, envolvendo equipes multidisciplinares em colaborações nacionais e internacionais.

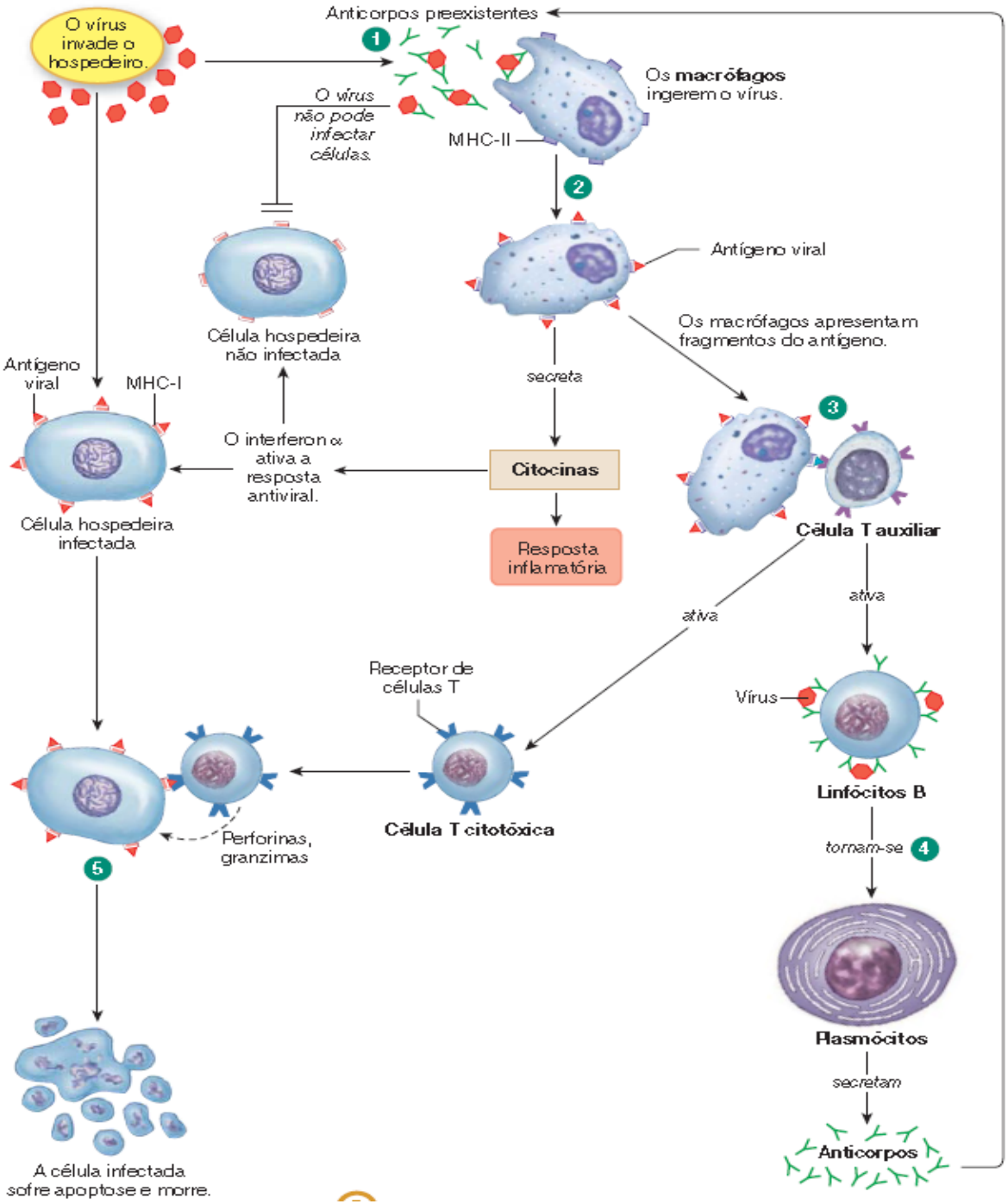
A aplicação de ferramentas biotecnológicas tem contribuído para avanços no desenvolvimento de novos antígenos e adjuvantes, o que aumenta a segurança sem comprometer a eficácia dos novos produtos.

Vacina da Dengue 1, 2, 3, 4 (atenuada)

Vacina Influenza H7N9

Uma estratégia da Organização Mundial da Saúde (OMS) definiu o desenvolvimento e a produção de uma vacina contra a gripe causada pelo vírus Influenza aviário A (H7N9). O Butantan foi escolhido para ser um dos laboratórios mundiais a produzir e fornecer essa vacina, na iminência de uma pandemia pelo vírus H7N9. A vacina encontra-se em ensaio clínico fase II para a comprovação da segurança e eficácia.

Resposta imune a Vírus



1. Os anticorpos atuam como opsoninas, recobrindo as partículas virais e tornando-as alvos melhores para os macrófagos.

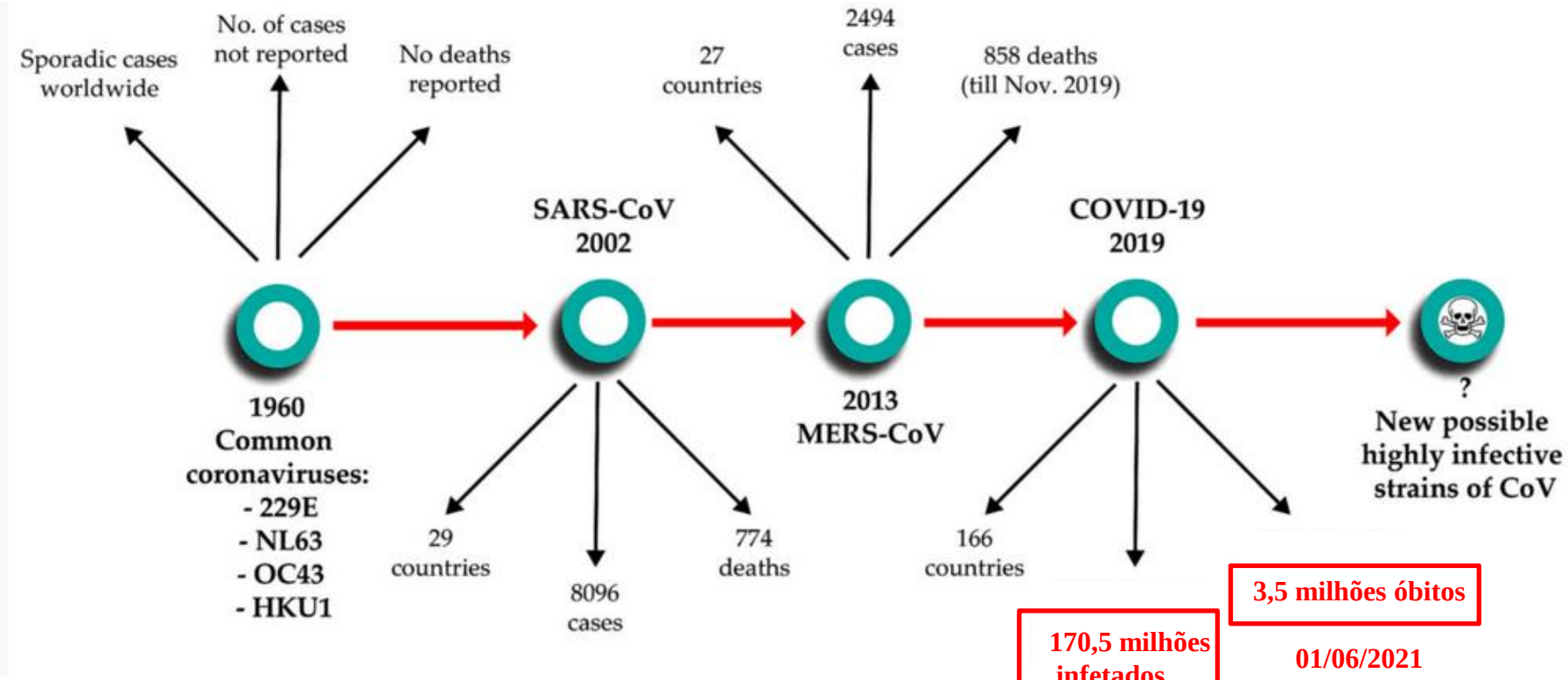
2. Os macrófagos ingerem vírus e inserem antígenos virais nas moléculas de MHC-II em suas membranas. Os macrófagos ativados também secretam citocinas.

3. As células T auxiliares ligam-se a antígenos virais nos macrófagos e tornam-se ativadas. Estas células T_H ativadas estimulam linfócitos B e células citotóxicas.

4. Os linfócitos B de memória ativados tornam-se plasmócitos, o que resulta em produção adicional de anticorpos.

5. As células T citotóxicas ativadas atacam e destroem células hospedeiras infectadas.

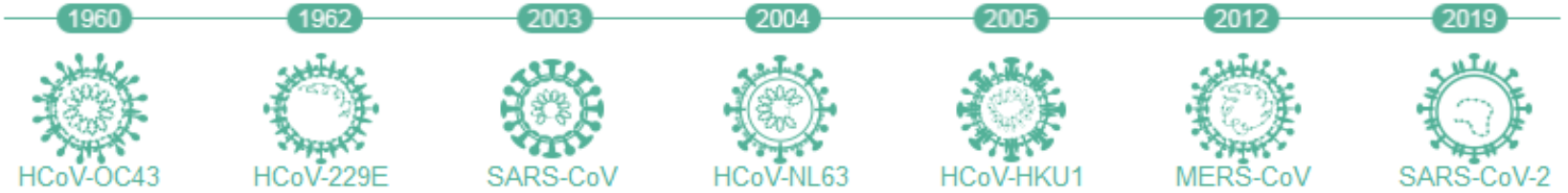
The History of Human Coronavirus



Zoonotic coronaviruses were discovered in the 1960s. Since then pathogenic human coronaviruses were identified beginning with the discovery of SARS-CoV in 2002. With the recent detection of SARS-CoV-2, there are now seven human coronaviruses. Those that cause mild diseases are the 229E, OC43, NL63 and HKU1, and the pathogenic species are SARS-CoV, MERS-CoV and SARS-CoV-2

Malays J Pathol. 2020 Apr;42(1):3-11.

Coronavírus

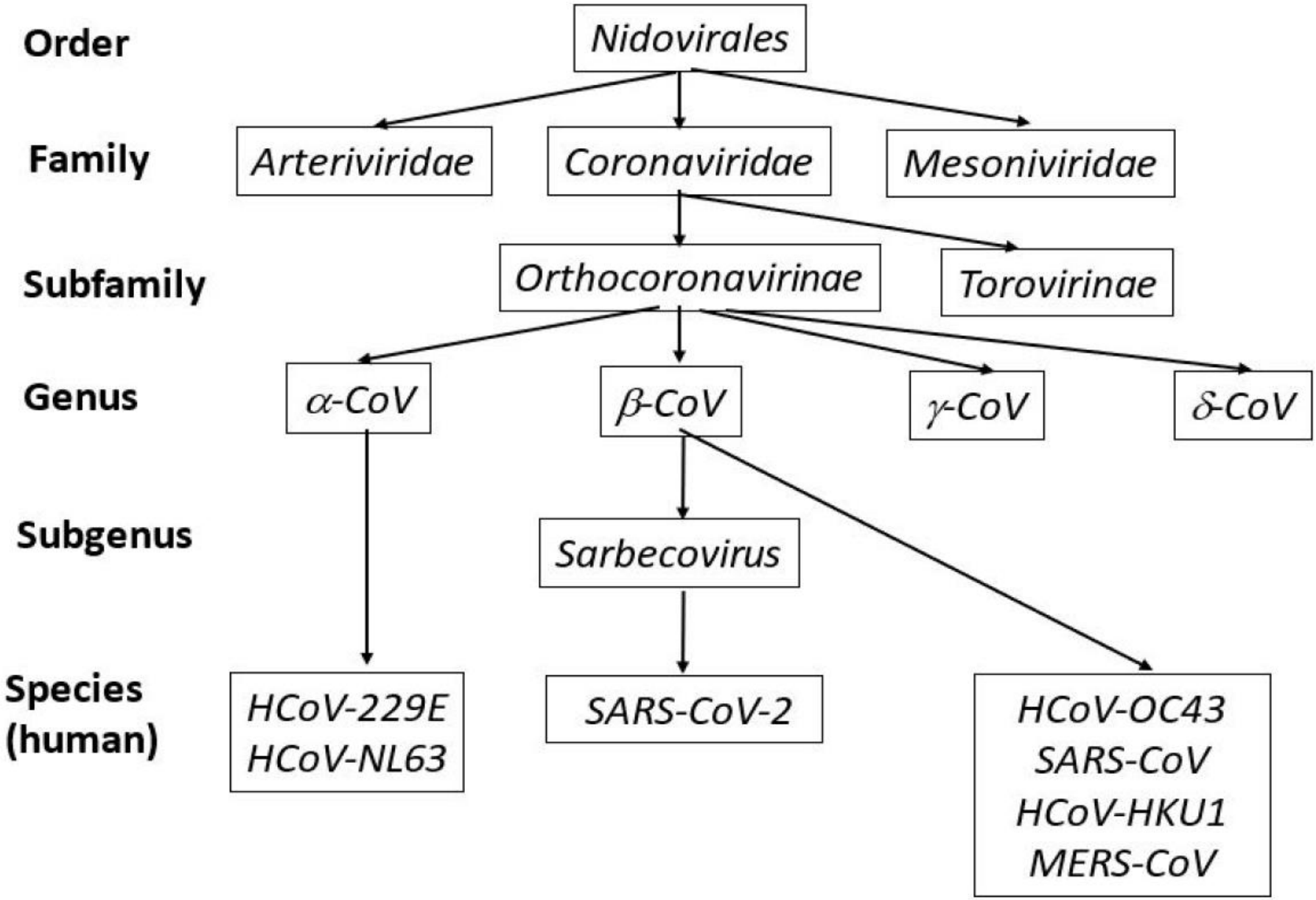


Epidemia **Epidemia** **Pandemia**

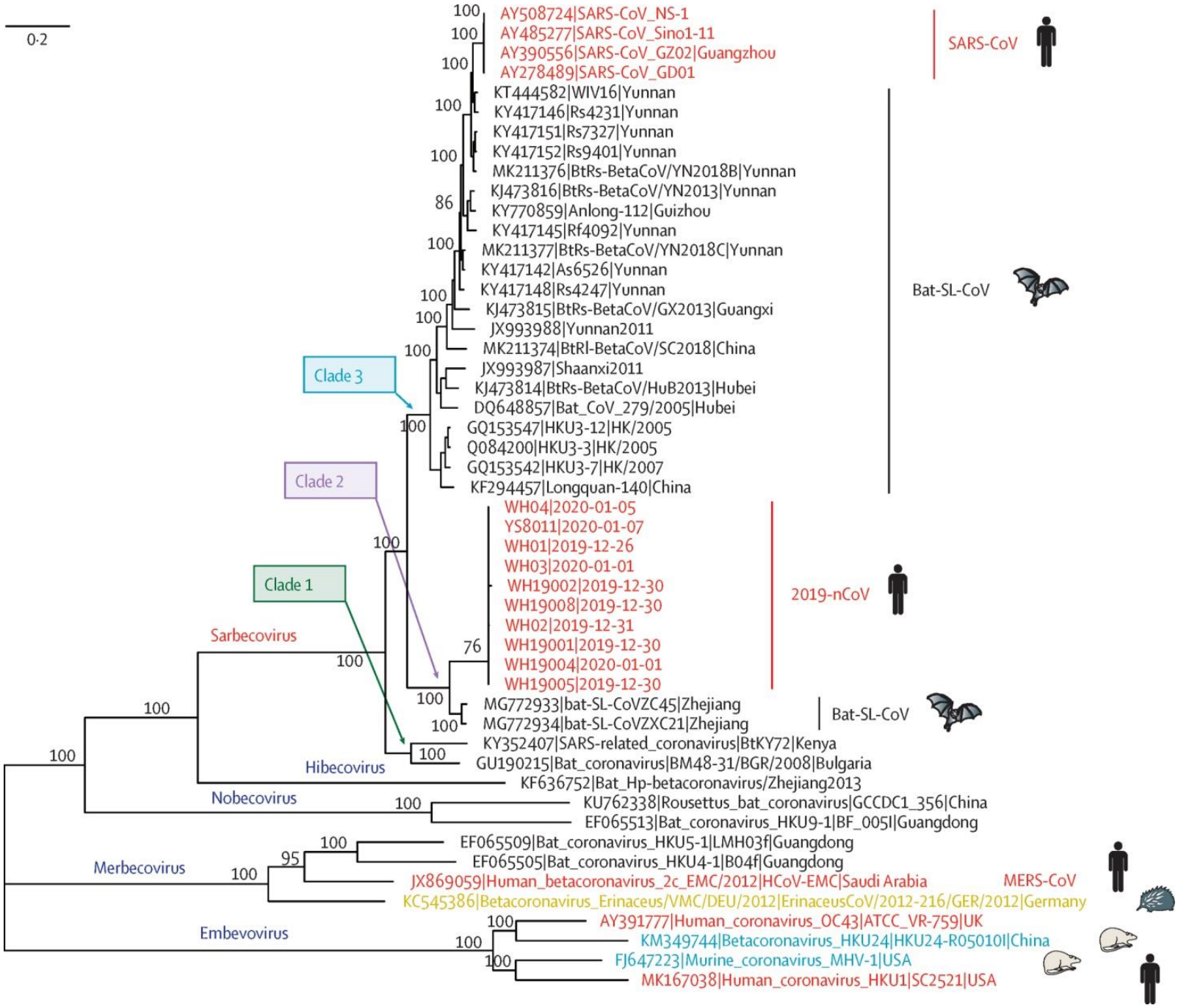
Types	Genera	Disease
SARS-CoV-2 (2019-nCoV)	Betacoronavirus	Coronavirus disease 2019 (COVID-19). As of 20th Mar, >240,000 infected, >9,800 death
SARS-CoV	Betacoronavirus	Severe acute respiratory syndrome(SARS), mortality rate 9%
MERS-CoV	Betacoronavirus	Middle East respiratory syndrome(MERS), mortality rate >30%
HCoV-HKU1	Betacoronavirus	Upper and lower respiratory tract disease
HCoV-NL63	Alphacoronavirus	Common cold
HCoV-OC43	Betacoronavirus	Common cold
HCoV-229E	Alphacoronavirus	Common cold

https://www.sinobiological.com/research/virus/coronavirus-overview

Classificação Taxonômica

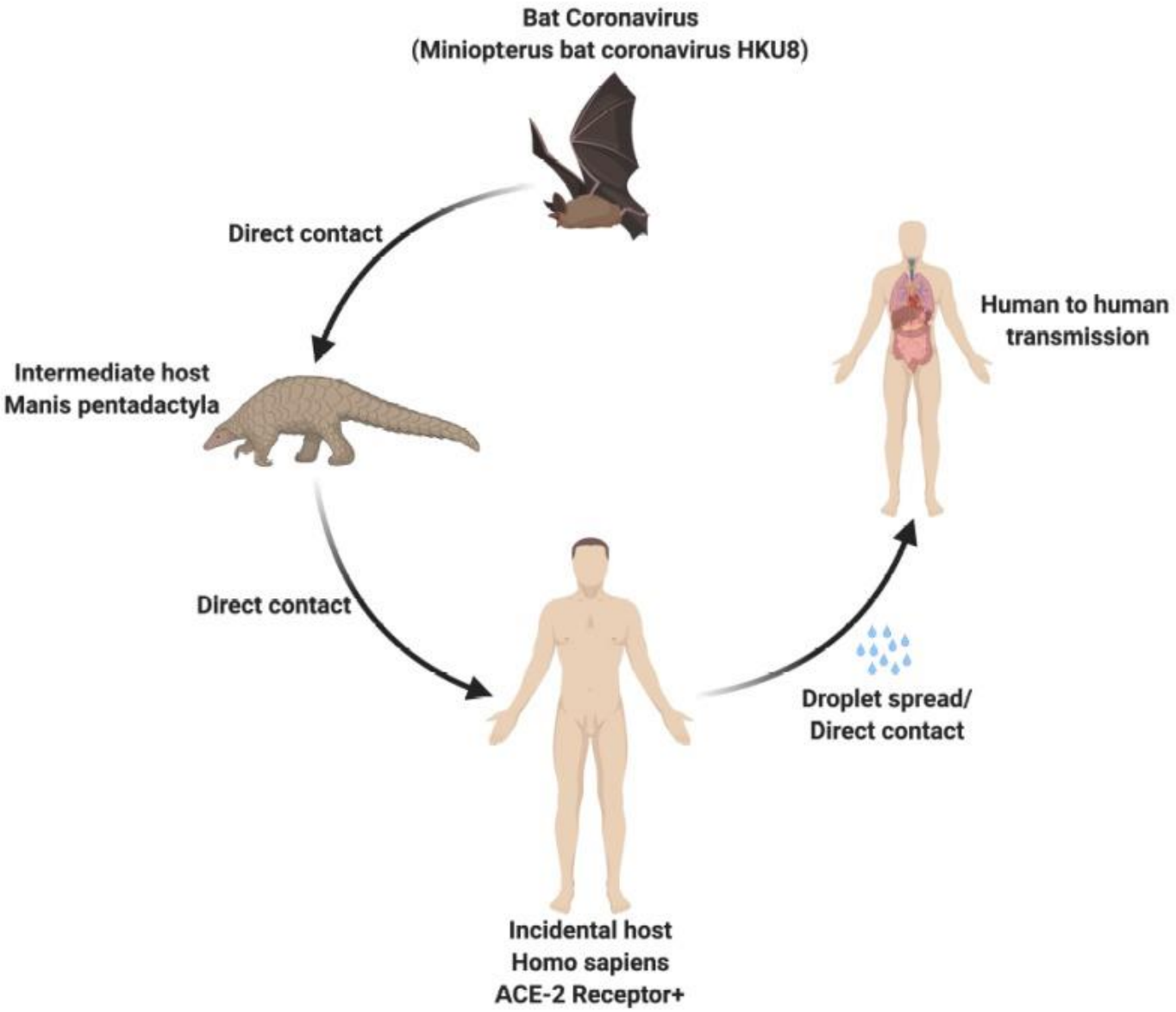


Malays J Pathol. 2020 Apr;42(1):3-11.

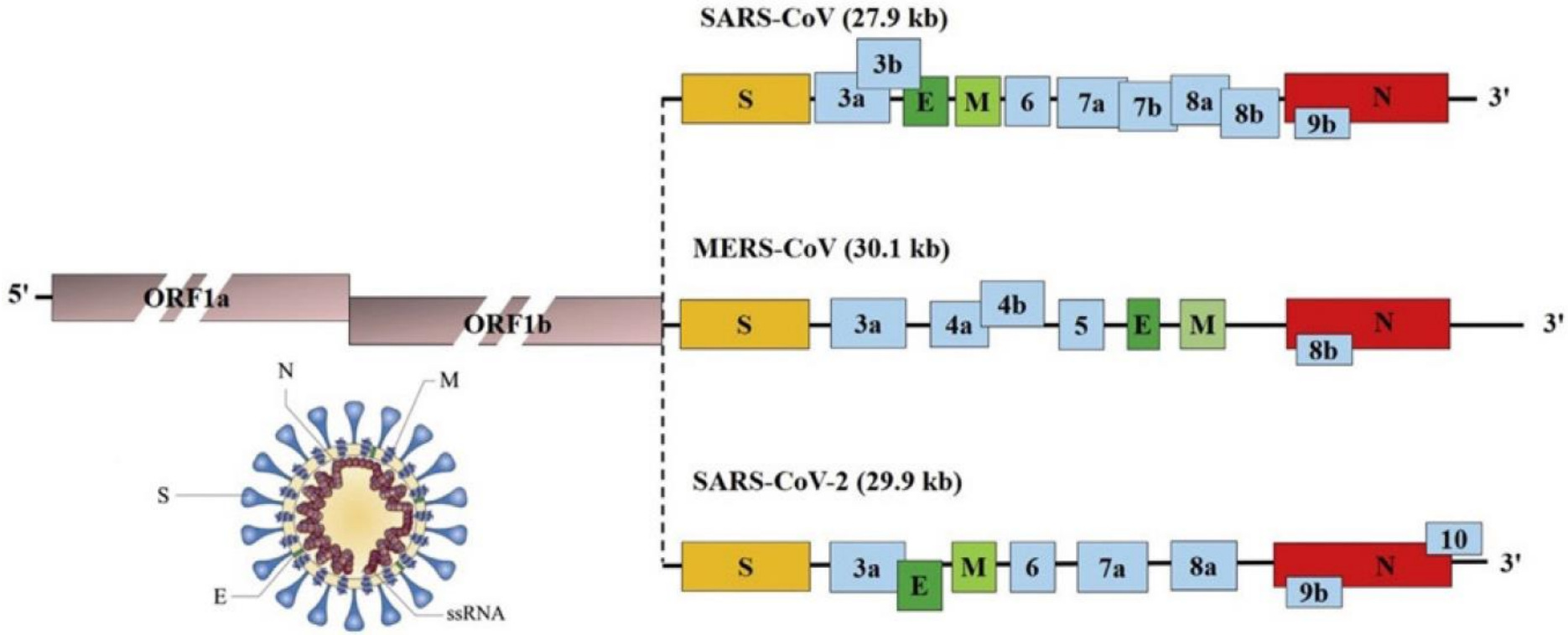


doi.org/10.1016/S0140-6736(20)30251-8

Transmission Cycle of SARS CoV 2



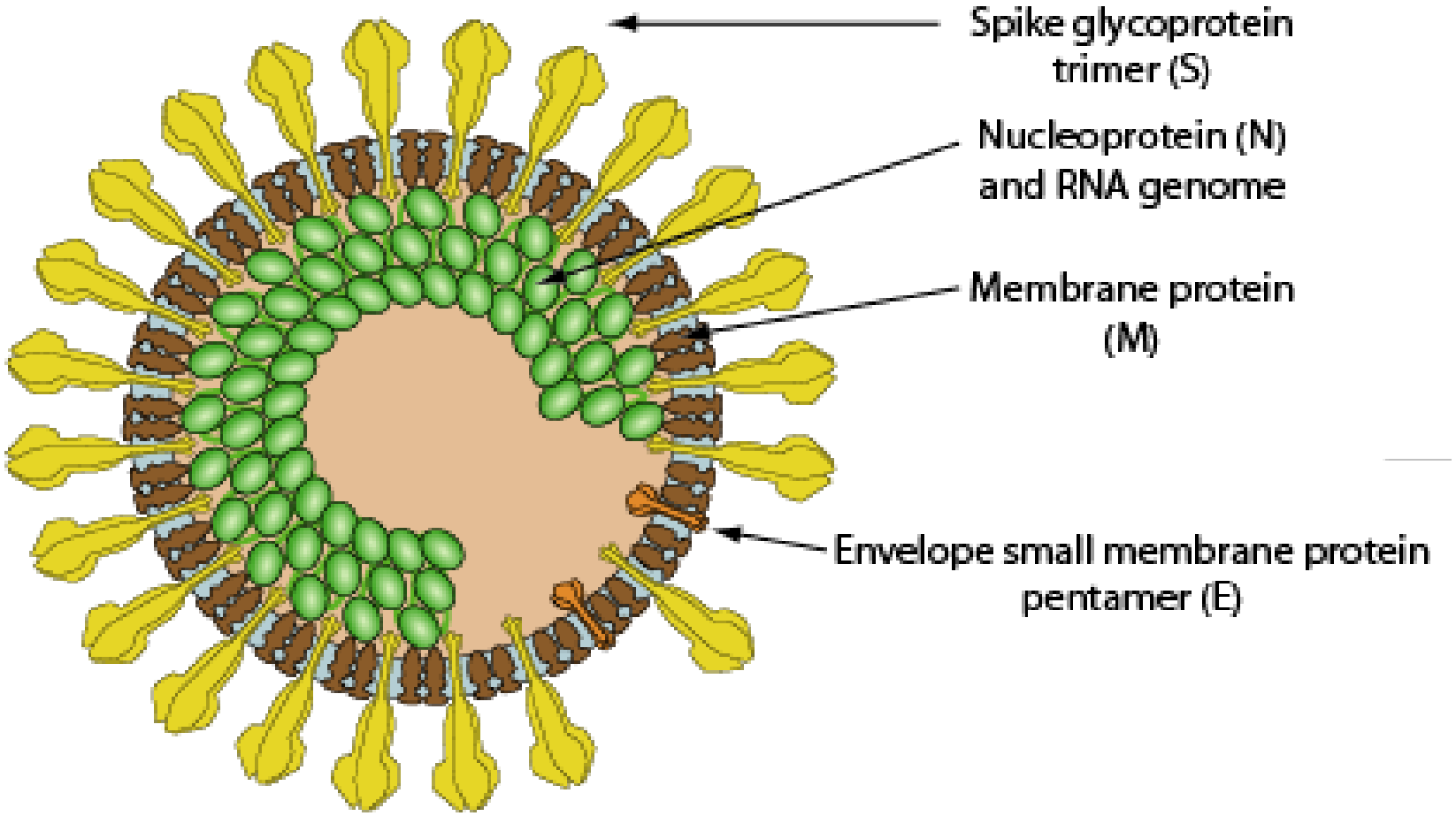
Estrutura Genômica de Coronavírus



Four major viral structural proteins, namely spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins 3–5, that follow the characteristic gene order [5′-replicase (*rep* gene), spike (S), envelope (E), membrane (M), nucleocapsid (N)-3′] with short untranslated regions at both termini.

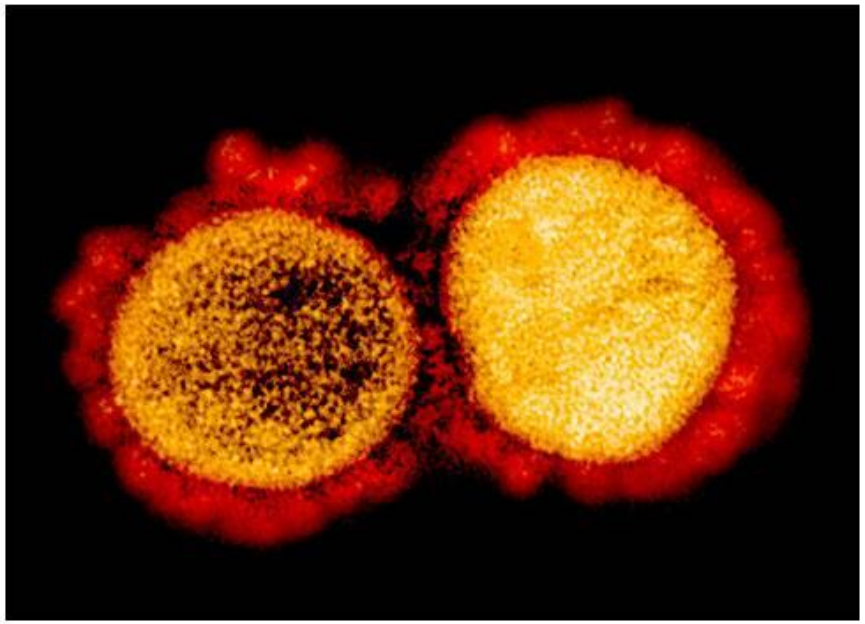
Malays J Pathol. 2020 Apr;42(1):3-11.

Um novo coronavírus (SARS-CoV-2) é uma nova cepa que não foi previamente identificada em humanos e causa a doença que foi denominada "doença de coronavírus 2019" (abreviada como "COVID-19")



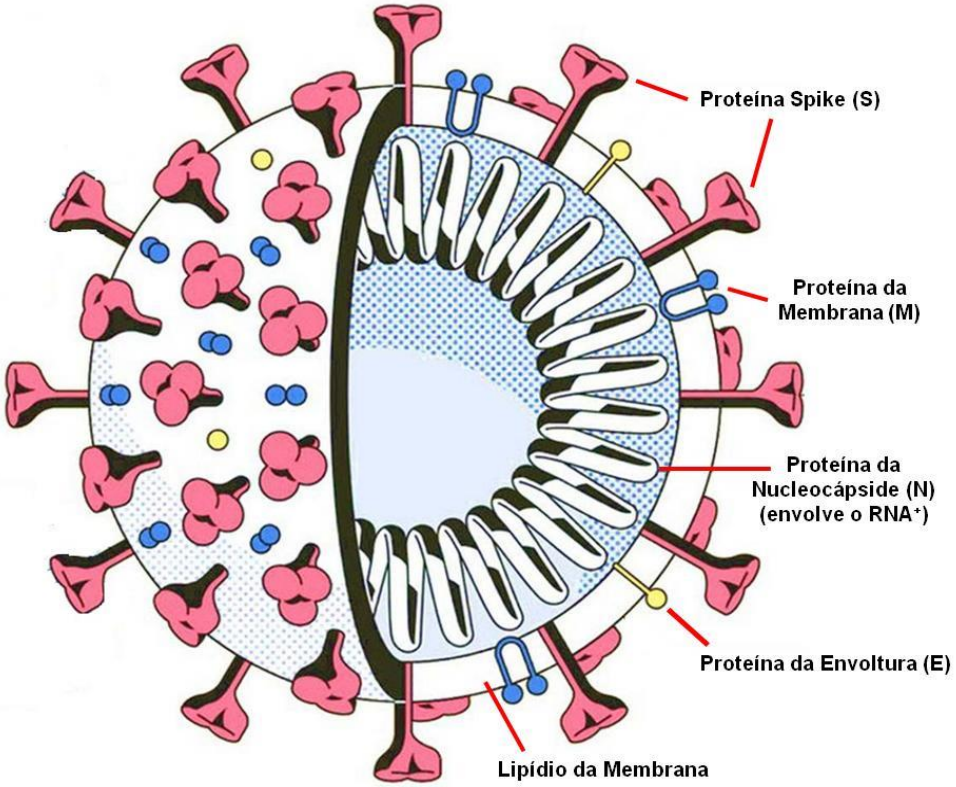
In the last two decades, coronavirus has caused two large-scale pandemics, SARS in 2002 and the Middle East respiratory syndrome (MERS) in 2012. In December 2019, a novel coronavirus (SARS-CoV-2) induced an outbreak of pneumonia in Wuhan, China, restated the risk of coronaviruses posed to public health. The infection routes and pathogenesis of SARS-CoV-2 are not fully understood, and the study of SARS-CoV-2 host cell receptor ACE2 could be valuable for the prevention and treatment of the COVID-19.

SARS-CoV-2



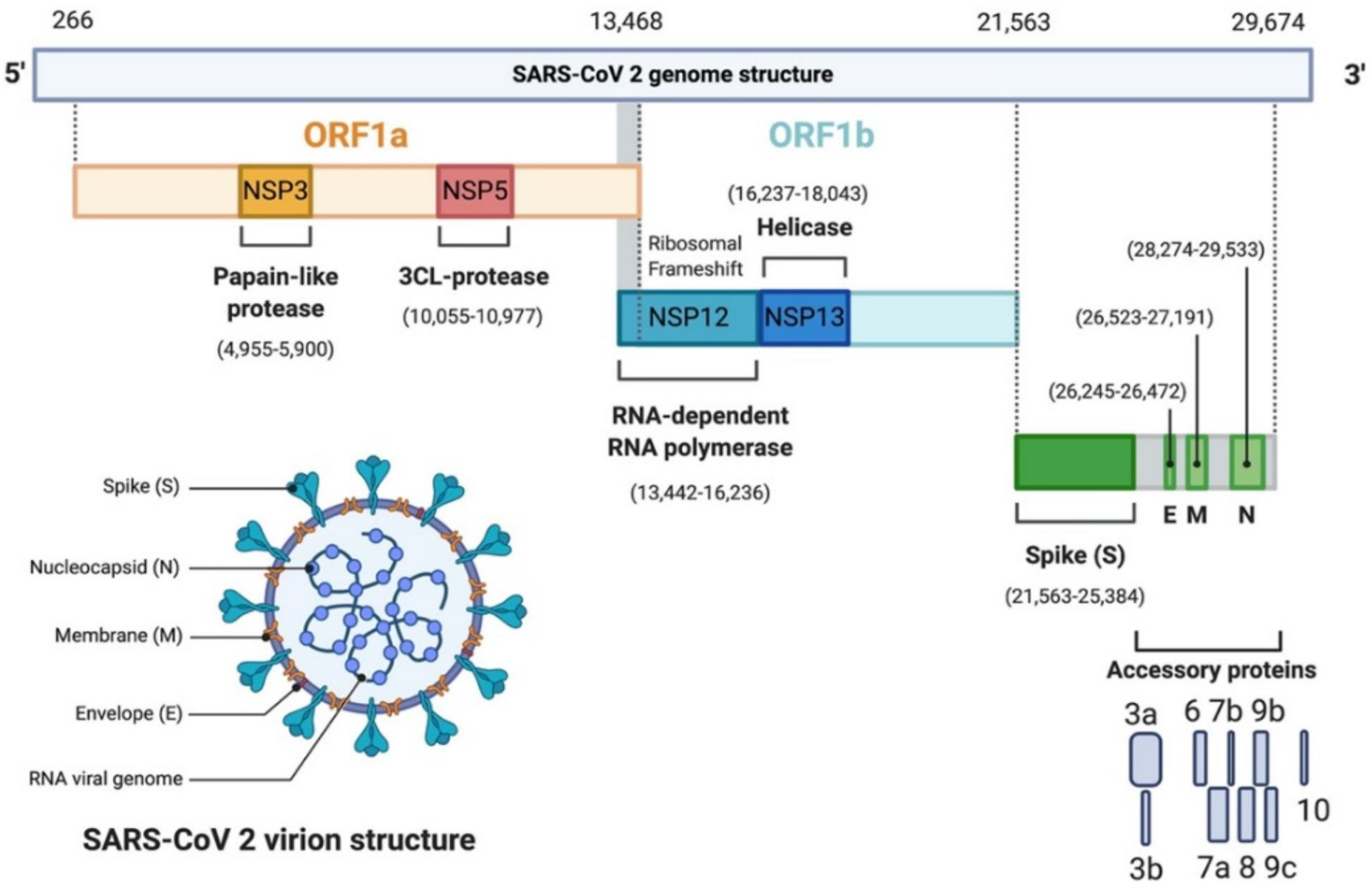
Novel Coronavirus SARS-CoV-2

Transmission electron micrograph of SARS-CoV-2 virus particles, isolated from a patient. Image captured and color-enhanced at the NIAID Integrated Research Facility (IRF) in Fort Detrick, Maryland. Credit: NIAID



É um novo vírus do gênero β coronavírus, família de vírus RNA (~30 kb) de cadeia simples e positivo, com envoltório, de morfologia esférica ou pleomórfica com um diâmetro de 80-120 nm, que podem infectar, principalmente o trato respiratório e intestinal, de diferentes espécies, causando uma ampla gama de sintomas em animais e pessoas

doi.org/10.1146/annurev-micro-020518-115759
doi.org/10.1186/s12985-019-1182-0



Pathogens **2020**, *9*(5), 331; ttps://doi.org/10.3390/pathogens9050331

Origem de SARS-CoV-2

"O salto de Espécie"

Fig. 2

Fig. 3

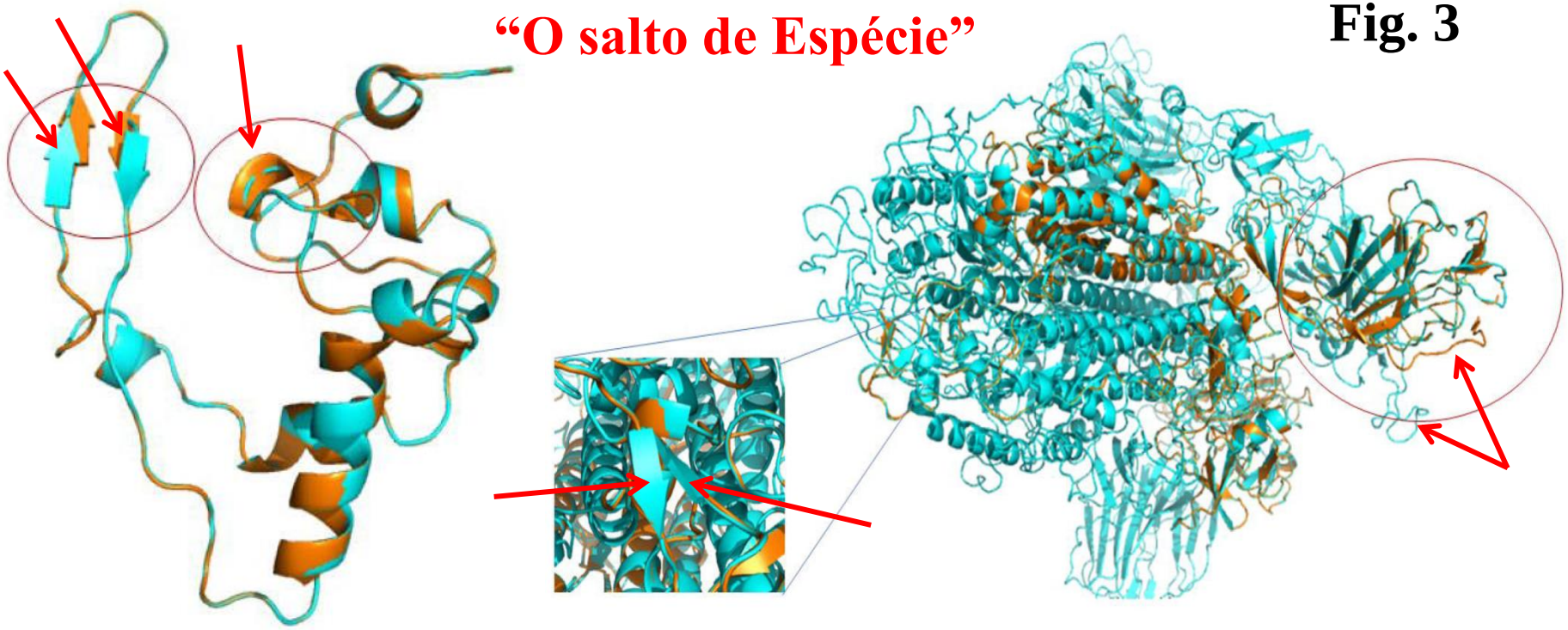


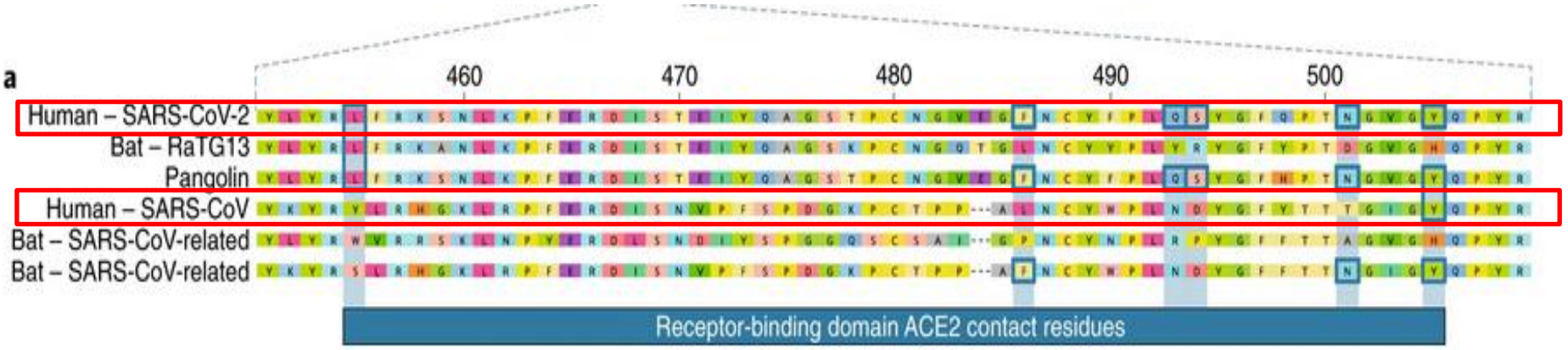
Figure 2. Cartoon model of the structural superposition between the homology model of the 2019-nCoV in blue and the Nucleocapsid protein of SARS Coronavirus (PDB code 2jw8.1) in orange. the presence of an alpha-helix on the SARS-CoV and not present on the 2019 n-CoV structure and e positional difference of the beta-sheets.

Figure 3. Cartoon model of the structural superposition between the homology model of the 2019-nCoV in blue and the spike glycoprotein of SARS Coronavirus (PDB code 6acc.1) in orange. the red circle highlights the presence of a variable region on the 2019 n-CoV at the beginning of the protein while the blue square highlights the presence of 2 beta-sheets on the 2019 n-CoV (401:KYR and 440:LND) that are not present on the SARS-CoV structure.

doi.org/10.1101/2020.01.24.915157

Origem de SARS-CoV-2

Genome analysis of SARS-CoV-2 sequences revealed that the complete genome sequence recognition rates of **SARS-CoV** and **bat SARS** coronavirus (SARSr-CoV-RaTG13) were **79.5%** and **96%** respectively



With coordinates based on

SARS-CoV-2: **Leu455, Phe486, Gln493, Ser494, Asn501 and Tyr505**

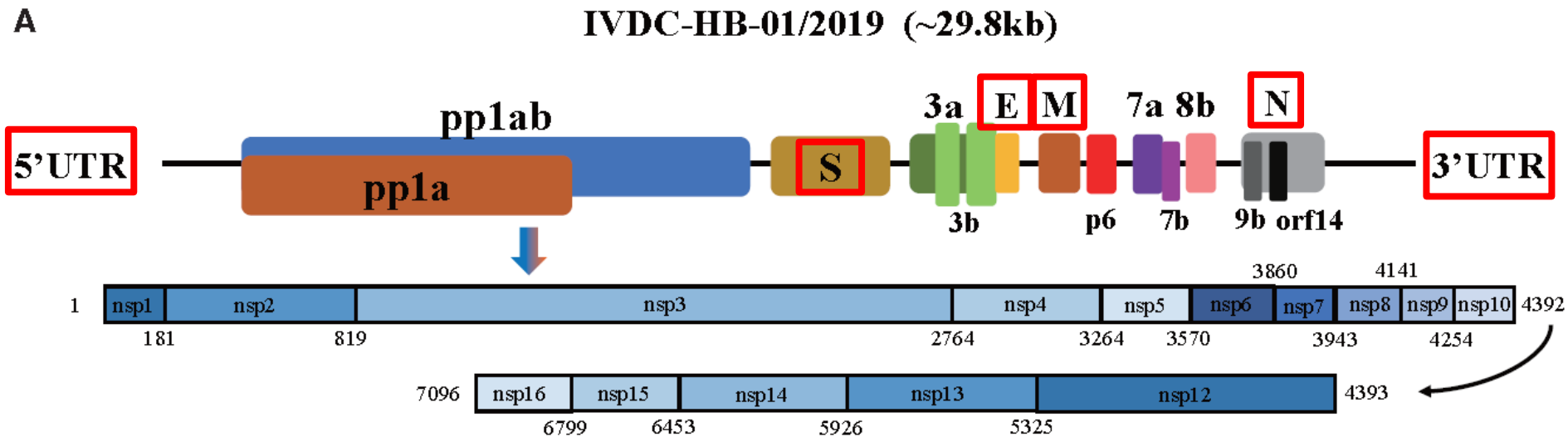
SARS-CoV: **Tyr442, Leu472, Asn479, Asp480, Thr487 and Tyr491**

Five of these six residues differ between SARS-CoV-2 and SARS-CoV.
On the basis of structural studies and biochemical experiments, SARS-CoV-2 seems to have an RBD that binds with high affinity to ACE2 from humans, ferrets, cats and other species with high receptor homology

Analyses clearly show that SARS-CoV-2 is not a laboratory construct or a purposefully manipulated virus.

doi.org/10.1038/s41591-020-0820-9

SARS-CoV-2 Genoma



O genoma **de ~30 kb**, codifica para **~14 ORFs**, com a seguinte ordem:

5´ - UTR 265 nucleotídeos (nt)-ORF1ab (codifica 16 proteínas não estruturais, nsp) - proteína Spike (**S**) - ORF3a-ORF3b-proteína do envoltório (**E**); proteína da membrana (**M**)-ORF6-ORF7a--ORF7b- ORF8-proteína do nucleocapsídeo (**N**)---ORF9b-ORF9c-ORF10(14)-229 nt UTR **-3´**

Os genes S, E, M e N codificam proteínas estruturais, sendo a proteína S a responsável pela ligação ao receptor Enzima Conversora da Angiotensina 2
(ECA 2) em humanos.

doi.org/10.1016/j.chom.2020.02.001
doi.org/10.1101/2020.03.22.002386
doi.org/10.1038/s41586-020-2008-3

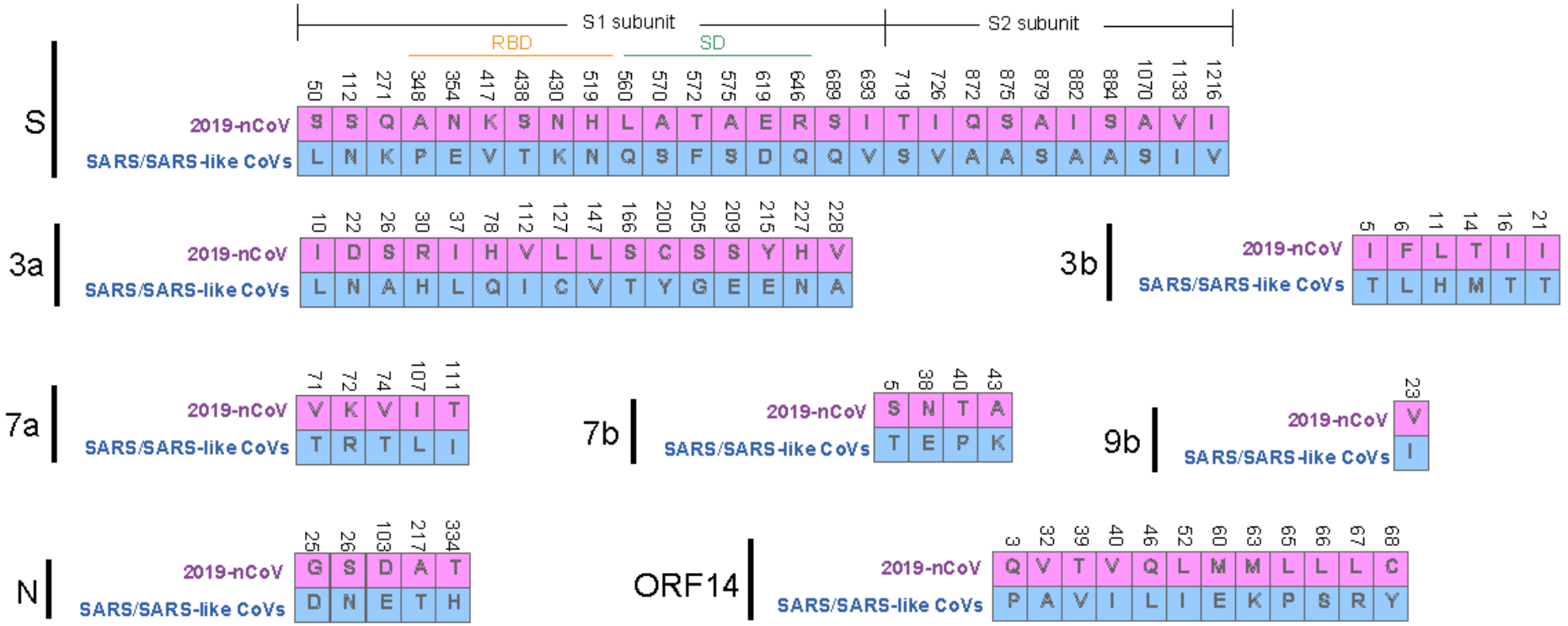
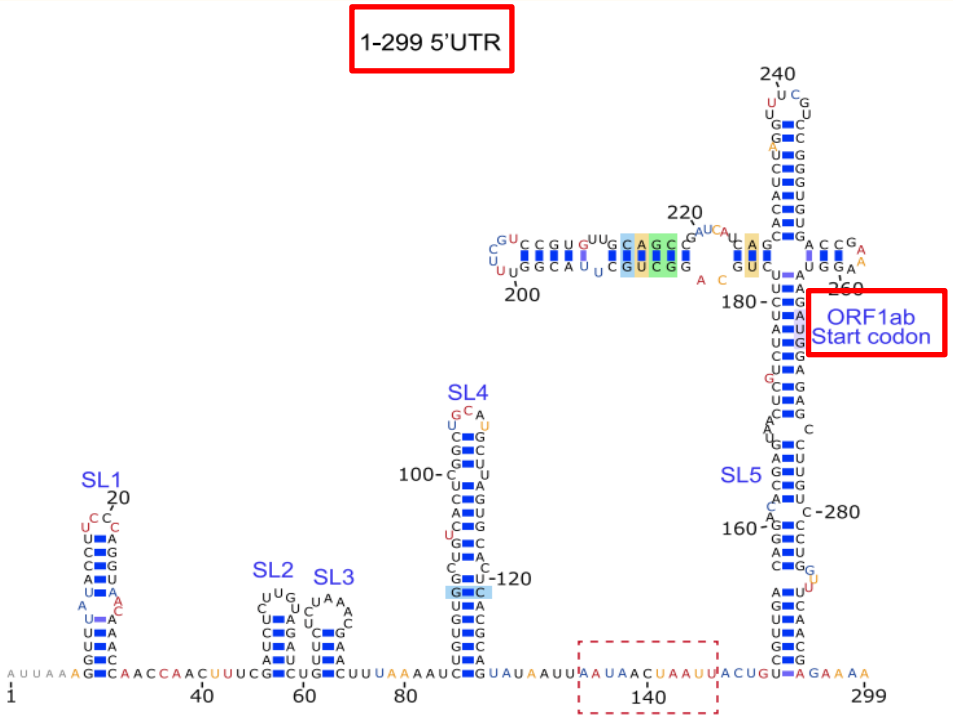


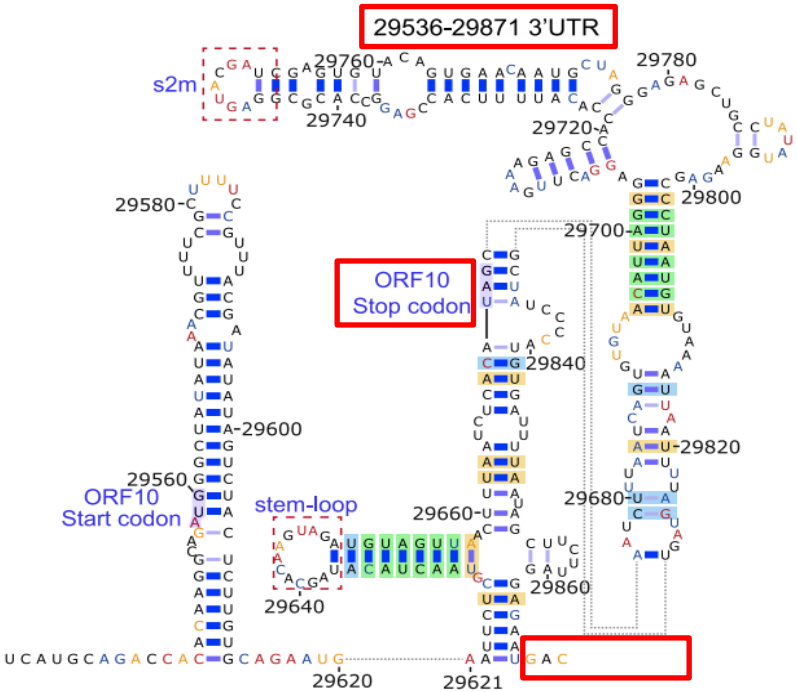
Figure 2. Amino Acid Substitutions of 2019-nCoV against SARS and SARS-like Viruses

All 27 proteins encoded by 2019-nCoV have been aligned against SARS-CoVs and SARS-like bat CoVs using the FFT-NS-2 algorithm in MAFFT (version v7.407) (The number of aligned proteins were listed in Table S1E). An amino acid substitution was defined as an absolutely conserved site in the group of SARS and SARS-like CoVs but different from that of 2019-nCoV. In total, 380 amino acid substitutions have been identified between the amino acid sequences of 2019-nCoV (HB01) and the corresponding consensus sequences of SARS and SARS-like CoVs.

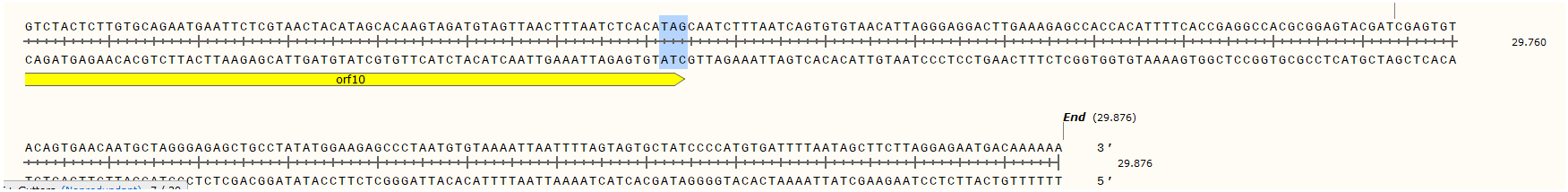
doi.org/10.1016/j.chom.2020.02.001

Structural overview of the SARS-CoV-2 RNA genome

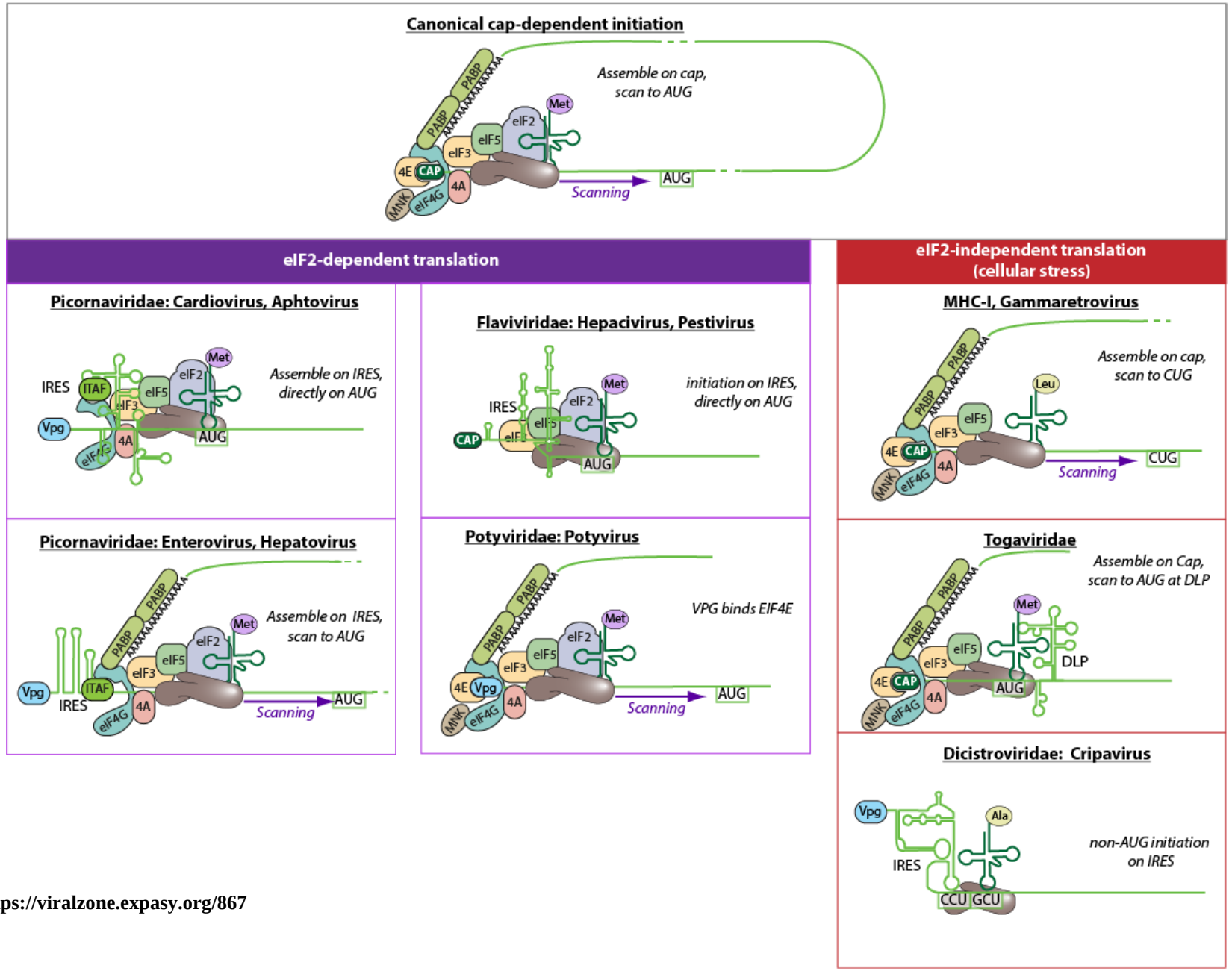




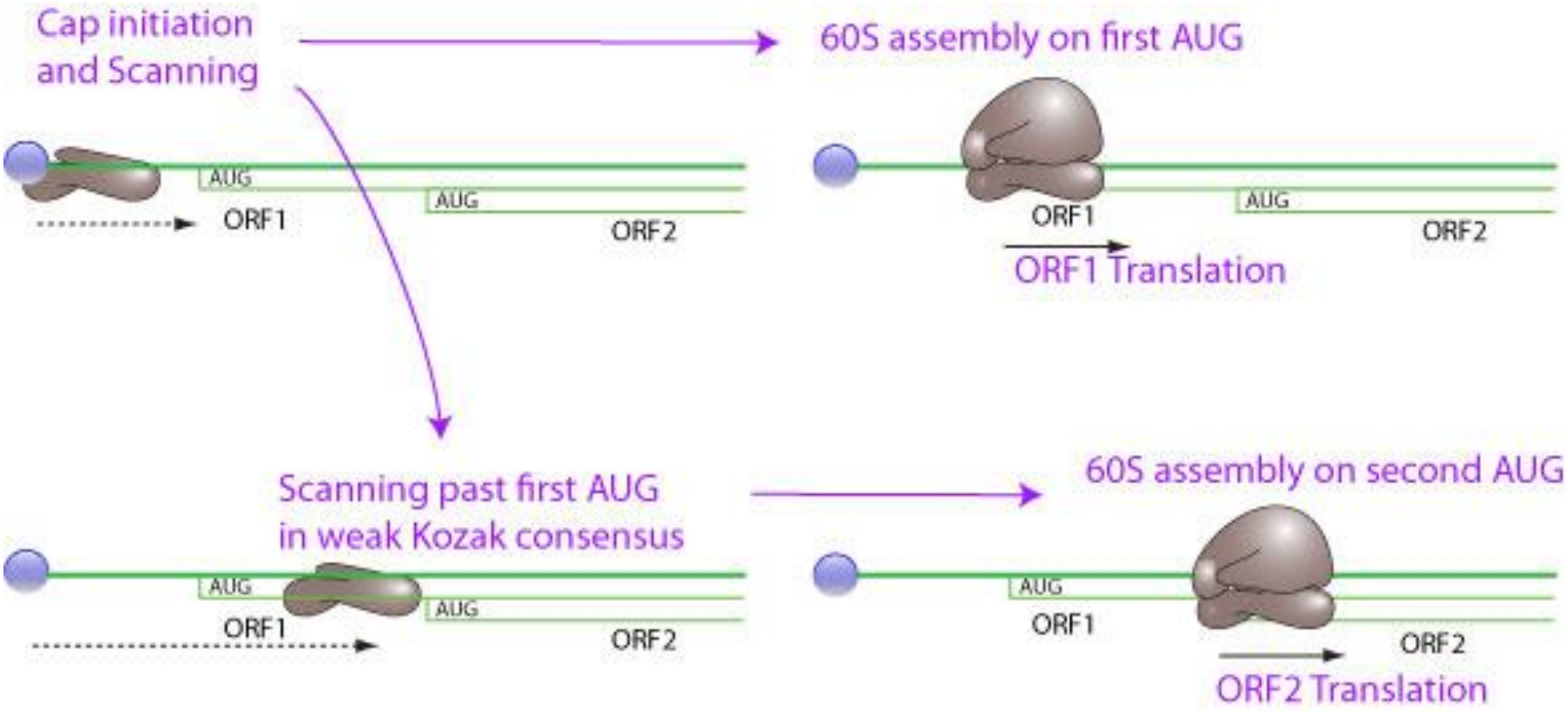
https://doi.org/10.1016/j.cell.2021.02.008



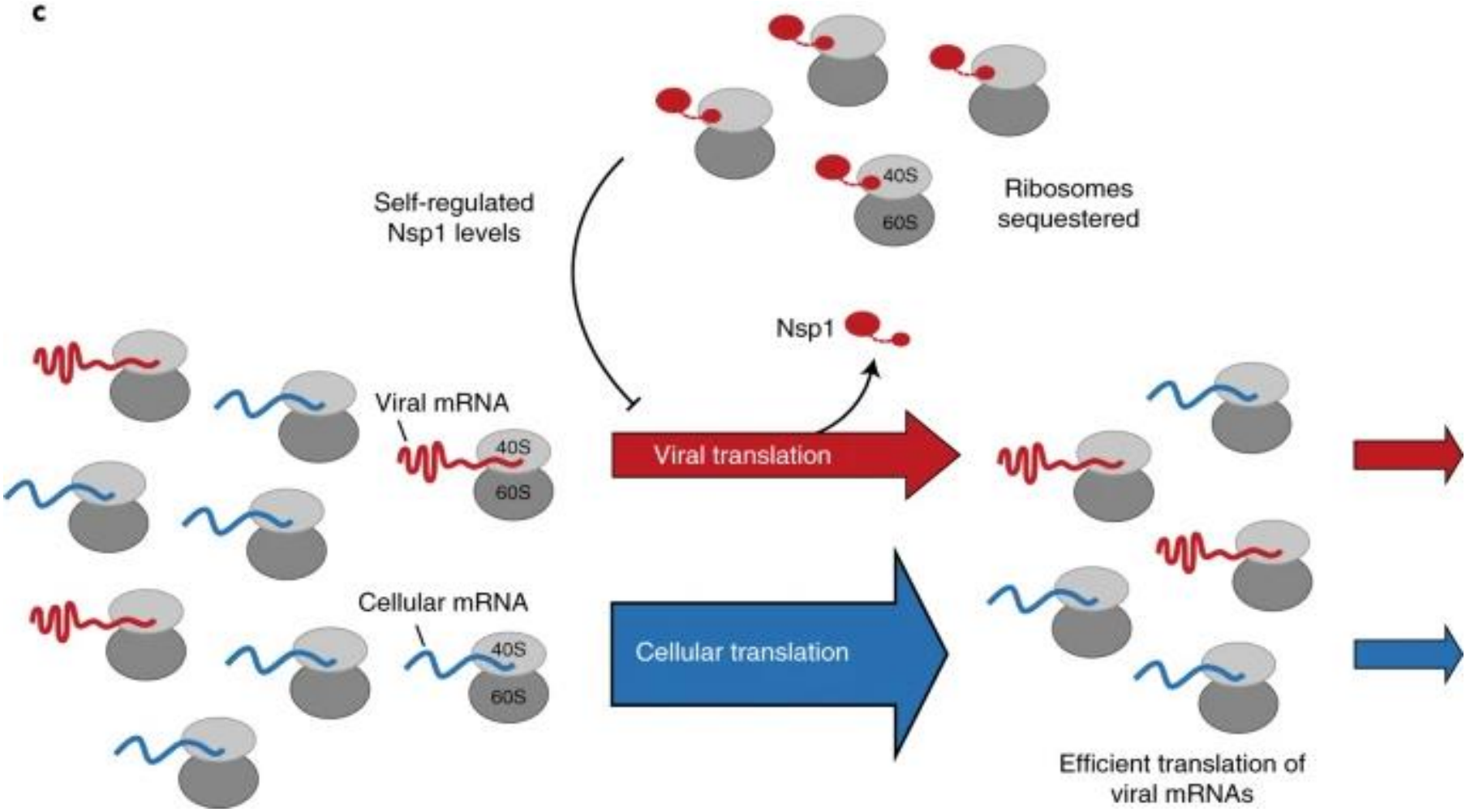
Viral initiation of translation



https://viralzone.expasy.org/867



Leaky scanning is a phenomenon in which a weak initiation codon triplet on mRNA is sometimes skipped by ribosome in translation initiation. The 40S ribosomal subunit continues scanning to further initiation codon. The weak initiation codon can be an ACG, or an ATG in a weak Kozak consensus context.
This way a mRNA can encode for several different proteins if the AUG are not in frame, or for proteins with different N-terminus if the AUG are in the same frame.



Model for translation inhibition by Nsp1. Following viral infection and translation of viral genomic mRNA (red), Nsp1 acts as a translation inhibitor, reducing the pool of ribosomes that can engage in translation. Under such ribosome-limiting conditions, translation of viral mRNAs will be favored over translation from less efficient cellular 5′ UTRs (blue).
Nat Struct Mol Biol **27,** 959–966 (2020). https://doi.org/10.1038/s41594-020-0511-8

SARS-CoV-2 - Transdução

Protein	Mol. weight (kDa)	Seq. similarity with SARS-CoV	Description	
Nsp1	19.8	91.1%	Suppresses host antiviral response	
Nsp2	70.5	82.9%		
Nsp3	217.3	86.5%	Nsp3-Nsp4-Nsp6 complex involved in viral replication	
Nsp4	56.2	90.8%	Nsp3-Nsp4-Nsp6 complex involved in viral replication	
Nsp5	33.8	98.7%	Main protease (3C-like)	Alvo para Inibidor
Nsp6	33.0	94.8%	Nsp3-Nsp4-Nsp6 complex involved in viral replication	
Nsp7	9.2	100.0%	Nsp7-Nsp8 complex is part of RNA polymerase	
Nsp8	21.9	99.0%	Nsp7-Nsp8 complex is part of RNA polymerase	
Nsp9	12.4	98.2%	ssRNA binding	
Nsp10	14.8	99.3%	Essential for Nsp16 methyltransferase activity	
Nsp11	1.3	92.3%	Short peptide	
Nsp12	106.7	98.3%	RNA polymerase	Alvo para Inibidor
Nsp13	66.9	100.0%	Helicase/triphosphatase	
Nsp14	59.8	98.7%	3′–5′ exonuclease	
Nsp15	38.8	95.7%	Uridine-specific endoribonuclease	
Nsp16	33.3	98.0%	RNA-cap methyltransferase	
S	141.2	87.0%	Spike protein, mediates binding to ACE2	Alvo para Inibidor Alvo para Vacina
Orf3a	31.1	85.1%	Activates the NLRP3 inflammasome	
Orf3b	6.5	9.5%		
E	8.4	96.1%	Envelope protein, involved in virus morphogenesis and assembly	
M	25.1	96.4%	Membrane glycoprotein, predominant component of the envelope	
Orf6	7.3	85.7%	Type I IFN antagonist	
Orf7a	13.7	90.2%	Virus-induced apoptosis	
Orf7b	5.2	84.1%		
Orf8	13.8	45.3%		
N	45.6	94.3%	Nucleocapsid phosphoprotein, binds to RNA genome	Alvo para Inibidor Alvo para Vacina
Orf9b	10.8	84.7%	Type I IFN antagonist	
Orf9c	8.0	78.1%		
Orf10	4.4	-		

doi: https://doi.org/10.1101/2020.03.22.002386. - 2020

Estrutura do Complexo de Replicação

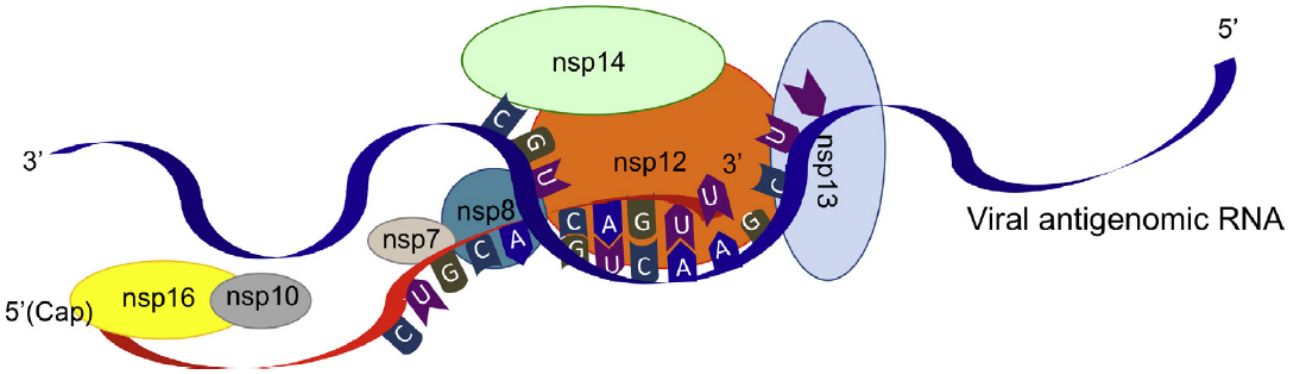
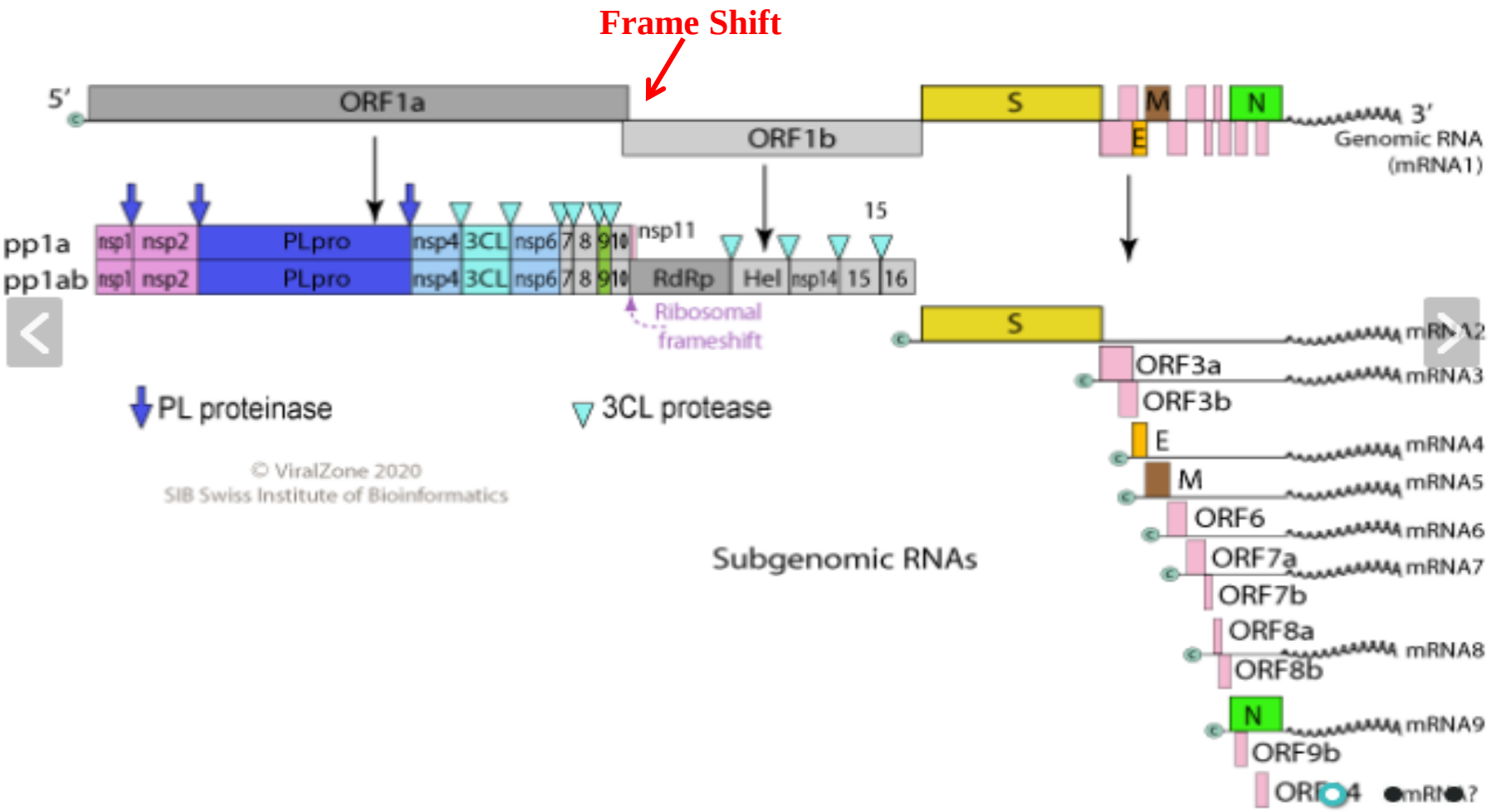


Fig. 5. Model of how coronavirus enzymes associate in the replicative complex. A model of the current state of knowledge on coronavirus enzymes and their co-factors is depicted. At the head of the complex nsp13 is in charge of the first capping step and disrupts RNA secondary structures during replication. Nsp12 may be in charge of the GMP transfer to RNA, while the third step of capping is performed by nsp14, bound to nsp12, and also in charge of RNA proofreading during nsp7/nsp8/nsp12-mediated RNA synthesis. Nsp16, in complex with nsp10, performs 2′-*O*-methylation. Since nsp16 has not been shown to tightly interact with other ORF1b-encoded nsps, nsp10/nsp16 complex may act right after replication has started.

The replication-transcription complex (RTC) directs and coordinates processes in order to replicate and transcribe the coronavirus genome, a single-stranded, positive-sense RNA of outstanding length (27–32 kilobases). SARS-CoV replicative enzymes encoded in the **ORF1b**, i.e., the main RNA-dependent RNA polymerase (nsp12), the helicase/triphosphatase (nsp13), two unusual ribonucleases (nsp14, nsp15) and RNA-cap methyltransferases (nsp14, nsp16), enzymes co-operate with other viral co-factors (nsp7, nsp8, and nsp10) to regulate their activity.

http://dx.doi.org/10.1016/j.antiviral.2013.11.006

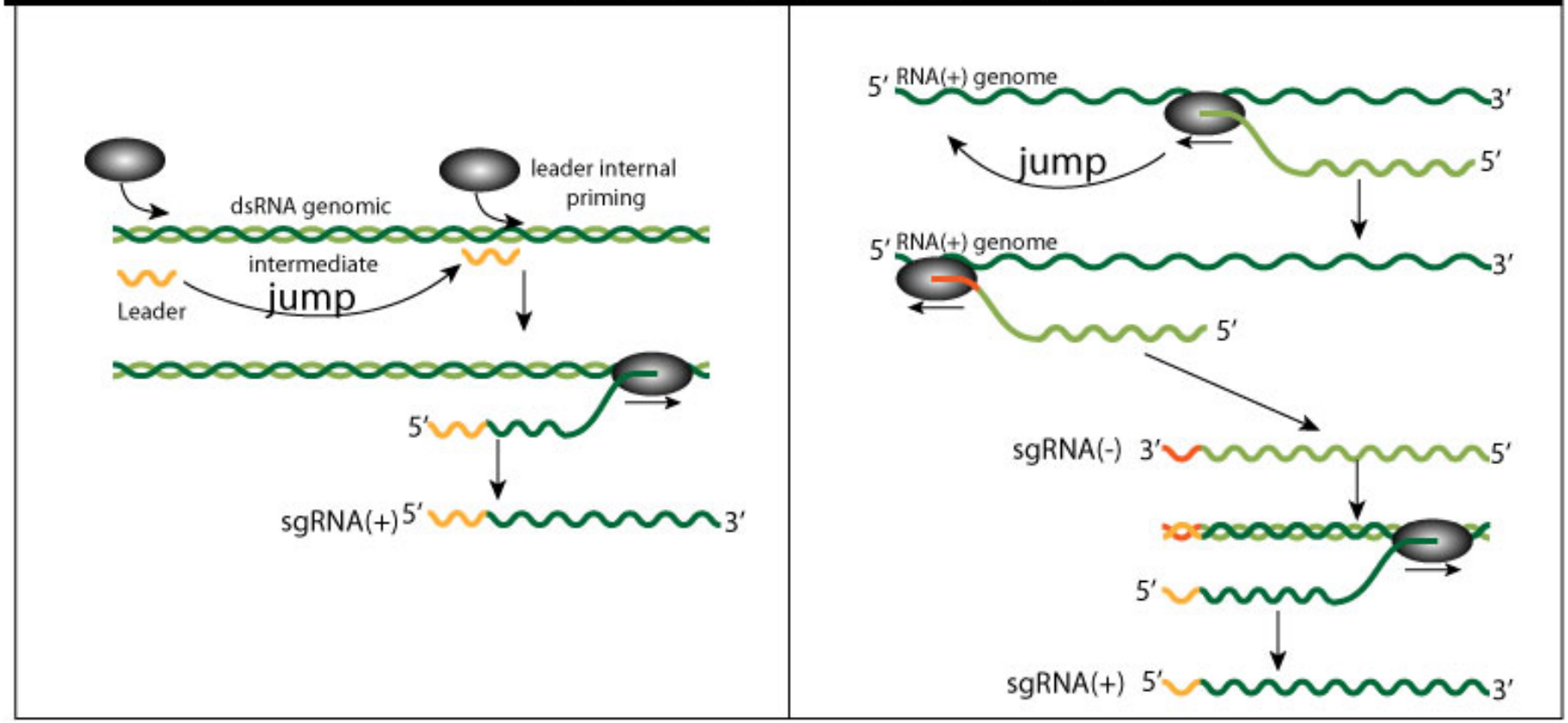


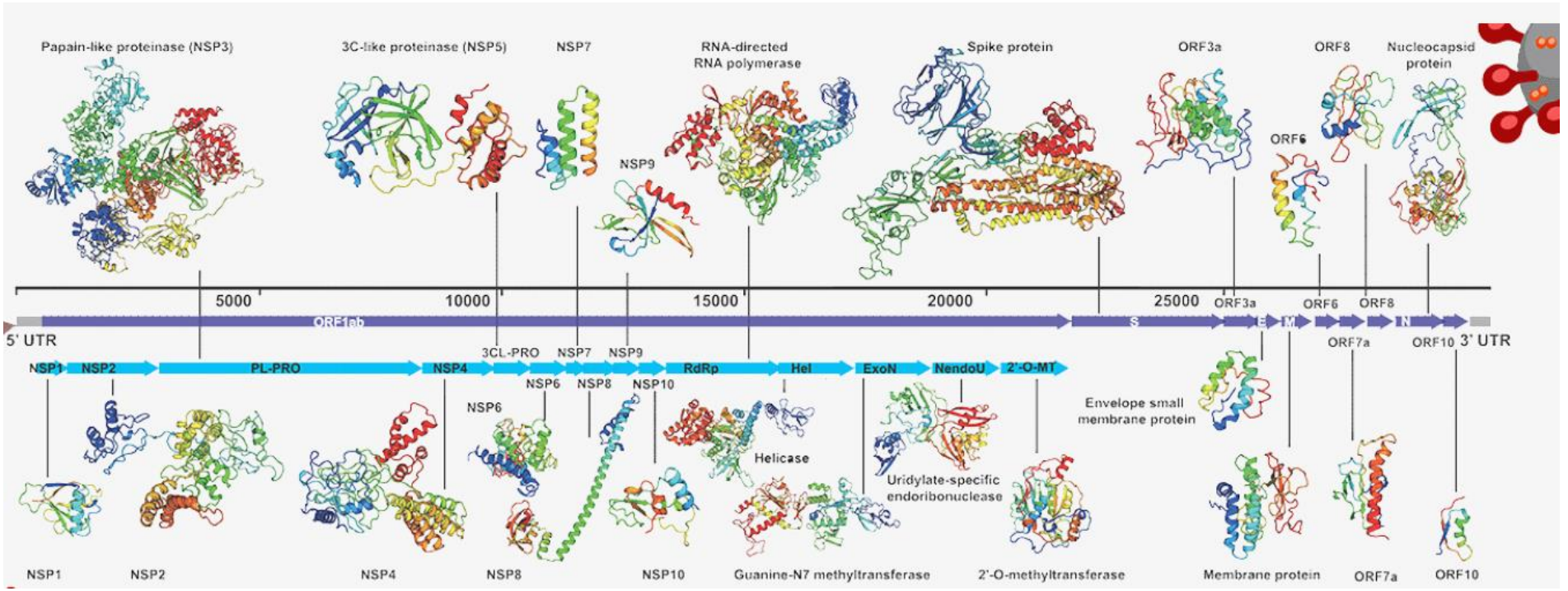
https://viralzone.expasy.org/764?outline=all_by_species

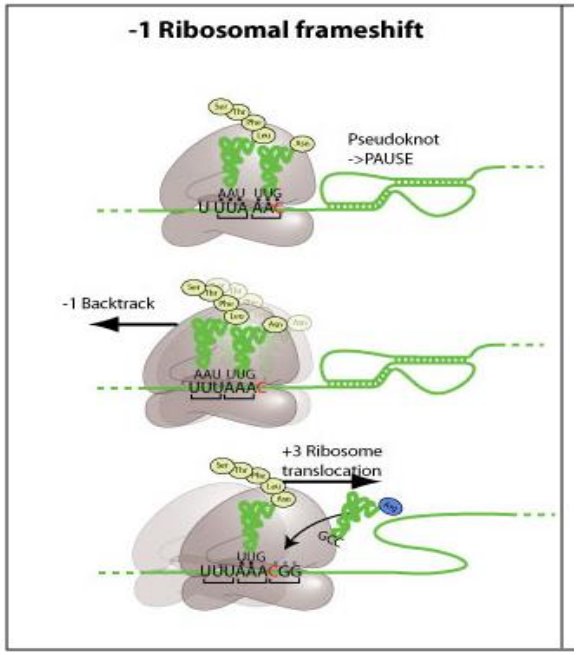
Discontinuous transcription

Discontinuous transcription can happen in the two models above. It's a feature well described in *Nidovirales*, for which the PT model is favored. Somehow, a leader RNA from the 5' genomic RNA is added to all subgenommic RNA. In internal initiation model it means that leaders RNA are transcribed and serve to prime subgenomic RNAs. In the premature termination model the polymerase would "jump"

DIscontinuous transcription sg RNA synthesis







-1 Ribosomal frameshift

SARS coronavirus -1 frameshift

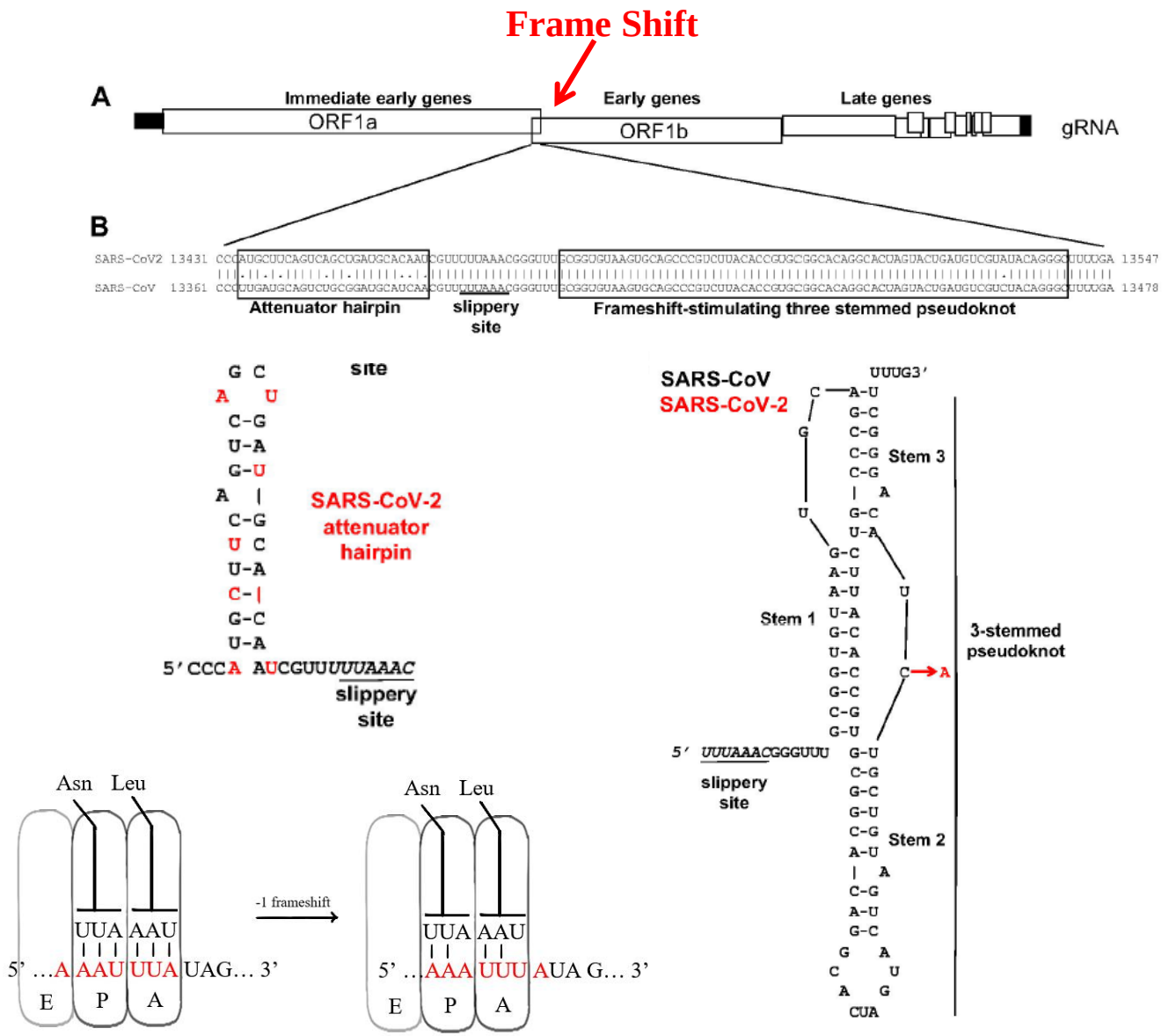
Ser	Thr	Phe	Leu	Asn	Gly	Phe	Ala	ORF1
UCA	ACG	UUU	UUA	AAC	GGG	UUU	GCG	

Arg	Val	Cys	ORF2
UCA	ACG	UUU	UUA	AA	C	GG	GUU	UGC	G

-1 frameshift

https://zhanglab.ccmb.med.umich.edu/COVID-19/

https://viralzone.expasy.org/764?outline=all_by_species



O mecanismo geralmente aceito de –1 Frame Shift é que a estrutura secundária do mRNA induz os ribossomos a fazer uma pausa com os sitios A e P (ribossomais) posicionados no local escorregadio (**Slippery site**). A pausa permite reparar o tRNA no códon de quadro -1 puxando uma base na direção 5 'ao longo do mRNA.

https://doi.org/10.1101/2020.03.13.991083.

TABLE 1: Functions of coronavirus non-structural proteins (nsps)

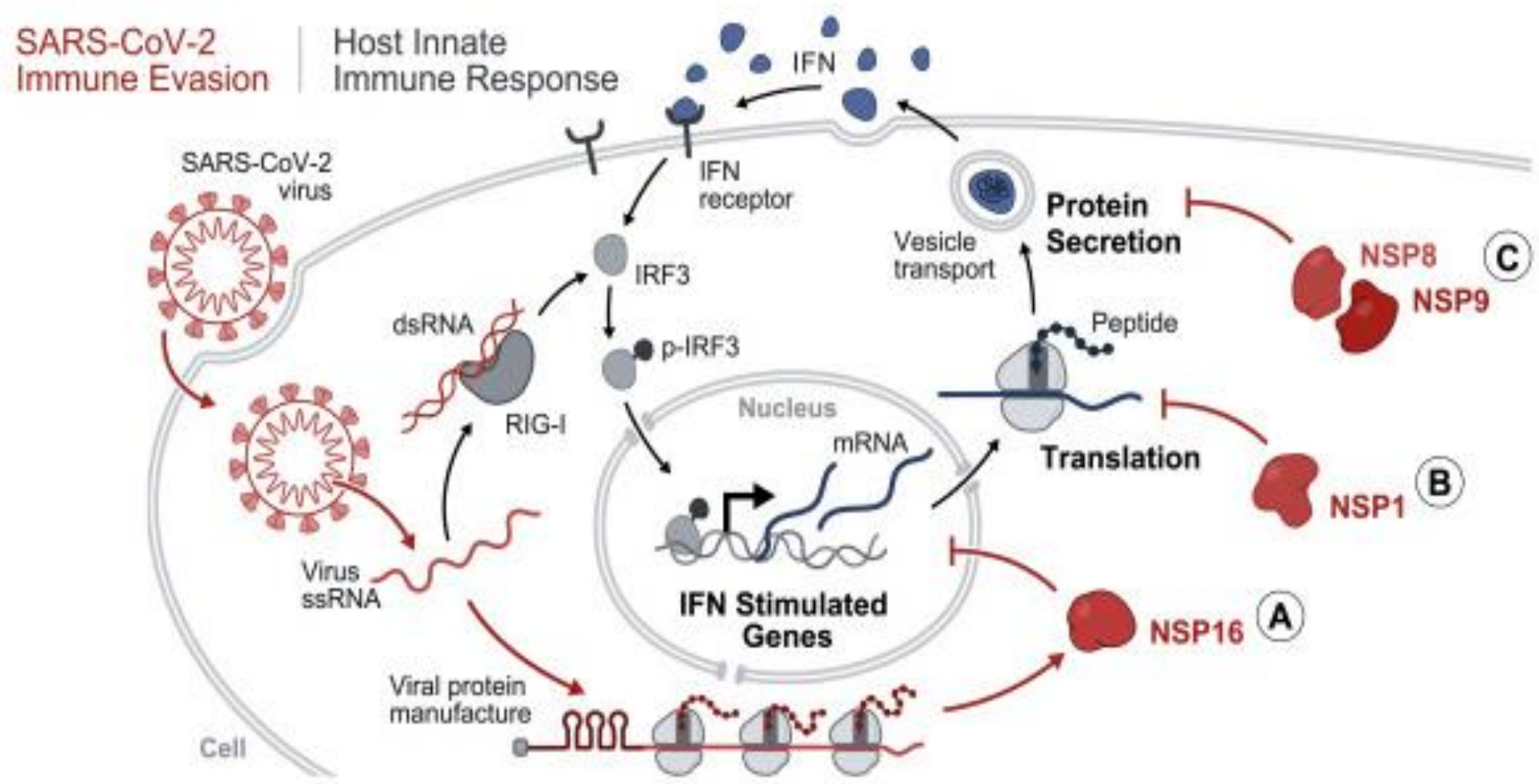
Protein	Functions
nsps1	Promotes cellular mRNA degradation and blocks host cell translation, results in blocking innate immune response
nsps2	No known function, binds to prohibitin proteins
nsps3	Large, multi-domain transmembrane protein, activities include: Ubl1 and Ac domains, interact with N protein ADRP activity, promotes cytokine expression PLPro/Deubiquitinase domain, cleaves viral polyprotein & blocks host innate immune response Ubl2, NAB, G2M, SUD, Y domains, unknown functions
nsps4	Potential transmembrane scaffold protein, important for proper structure of DMVs
nsps5	Mpro, cleaves viral polyprotein
nsps6	Potential transmembrane scaffold protein
nsps7	Forms hexadecameric complex with nsp8, may act as processivity clamp for RNA polymerase
nsps8	Forms hexadecameric complex with nsp7, may act as processivity clamp for RNA polymerase; may act as primase
nsps9	RNA binding protein
nsps10	Cofactor for nsp16 and nsp14, forms heterodimer with both and stimulates ExoN and 2-O-MT activity
nsps12	RdRp
nsps13	RNA helicase, 5′ triphosphatase
nsps14	N7 MTase) and 3′-5′ exoribonuclease, ExoN; N7 MTase adds 5′ cap to viral RNAs, ExoN activity is important for proofreading of viral genome
nsps15	Viral endoribonuclease, NendoU
nsps16	2′-O-MT; shields viral RNA from MDA5 recognition

Note: Ubl, ubiquitin-like; Ac, acidic; ADRP, ADP-ribose-1 '-phosphate; PLPro, papain-like protease; NAB, nucleic acid binding; SUD, SARS-unique domain; DMVs, double-membrane vesicles; Mpro, main protease; RdRp, RNA-dependent RNA polymerase; MTase, methyltransferase; Viral exoribonuclease, ExoN; Viral endoribonuclease, NendoU; 2 '-O-MT, 2 '-O-Methyltransferase; MDA5, Melanoma differentiation associated protein 5.

Coronavirus proteins affecting innate immune responses.

Protein	CoV	Immune function
Nsp1	SARS-CoV	Antagonizes type I IFN production and signaling by inducing host mRNAs shut off, promoting the degradation of host mRNAs and preventing phosphorylation of STAT1 Upregulates CCL5, CXCL10, and CCL3 in human lung epithelial cells via the activation of NF-κB
Nsp3	SARS-CoV	Prevents IFN production by blocking IRF3 phosphorylation, most probably by interacting with STING
	MHV	Antagonizes type I IFN
	MERS-CoV	Antagonizes type I IFN
Nsp7	SARS-CoV	Antagonizes type I IFN
Nsp15	SARS-CoV	Antagonizes type I IFN
S	SARS-CoV	Induces the expression of IL6, IL8, CXCL10 and TNF through NF-κB activation in macrophages
M	SARS-CoV	Blocks IFN-β production by impairing the formation of TRAF3–TANK–TBK1/IKKε complex
N	SARS-CoV	Antagonizes type I IFN production by blocking IRF-3 phosphorylation Activates NF-κB and upregulates the expression of IL-6 Activates AP-1 Induces the expression of IL8 via AP-1 activation
3a	SARS-CoV	Downregulates the expression of the type I IFN receptor (IFNAR), leading to a blockade on type I IFN signaling Increases NF-κB and JNK activity and upregulates TNF, IL8 and CCL5 production
3b	SARS-CoV	Antagonizes type I IFN production by blocking IRF-3 phosphorylation. Inhibits IFN signaling Induces transcriptional activity of AP-1, through activation of JNK and ERK pathways, leading to CCL2 upregulation
6	SARS-CoV	Antagonizes type I IFN production by blocking IRF-3 phosphorylation Inhibits IFN signaling by blocking the nuclear translocation of the transcription factor STAT1
7a	SARS-CoV	Activates NF-κB and upregulates the expression of the proinflammatory mediators IL8 and CCL5
Nsp3	NL63	Antagonizes type I IFN
Nsp1	MHV	Antagonizes type I IFN
N	MHV	Acts as an interferon antagonist and prevents RNA degradation by inhibiting RNaseL activity
2	MHV	Antagonizes type I IFN signaling and prevents activation of the cellular endoribonuclease RNase L
5a	MHV	Antagonizes type I IFN
4a	MERS-CoV	Block interferon induction at the level of MDA5 activation presumably by direct interaction with double-stranded RNA
4b	MERS-CoV	Antagonizes type I IFN
7	TGEV	Reduces the expression of genes involved in the immune response, the interferon response, and inflammation
7a	FIPV	Antagonizes type I IFN

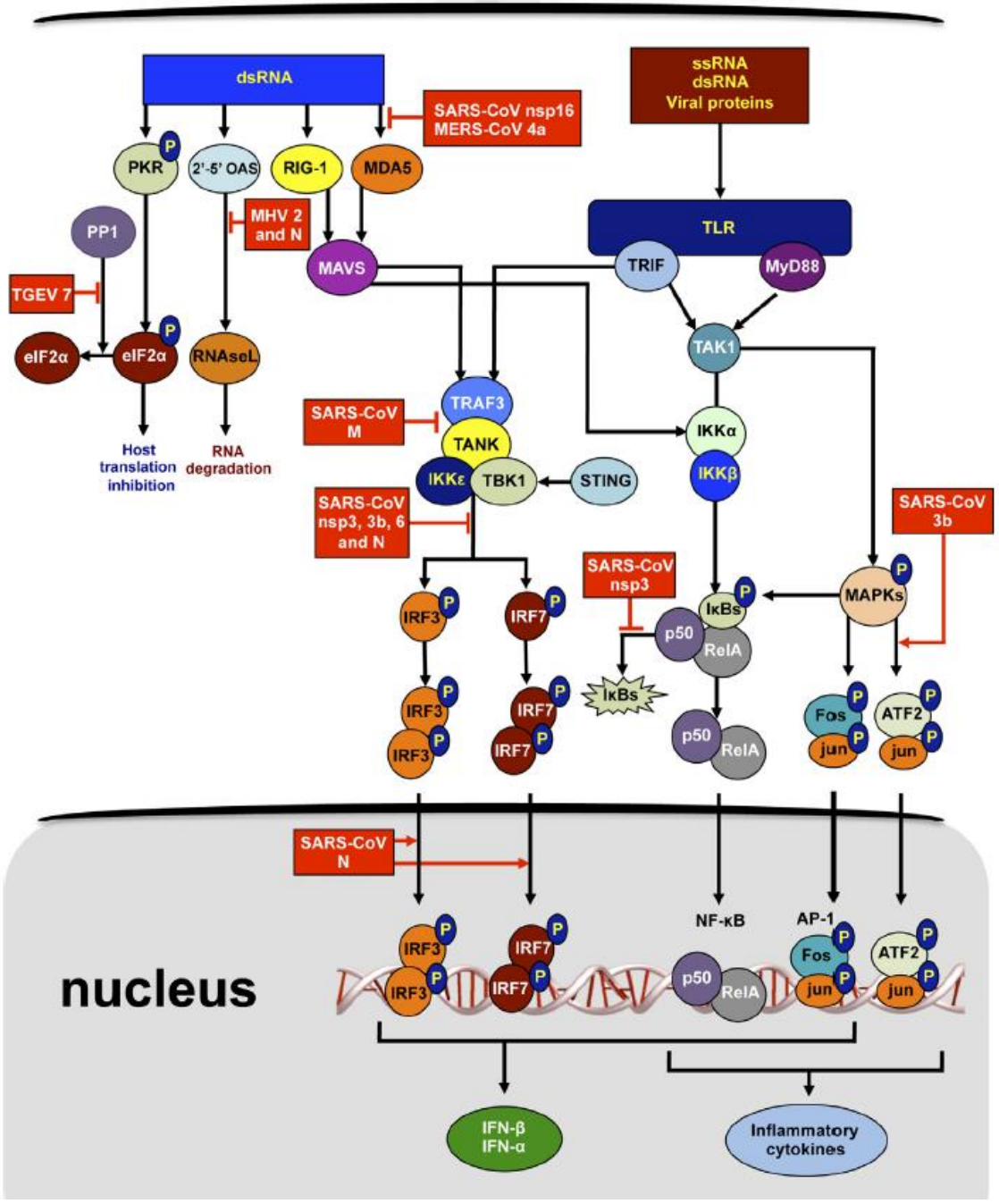
Supressão da resposta Imune

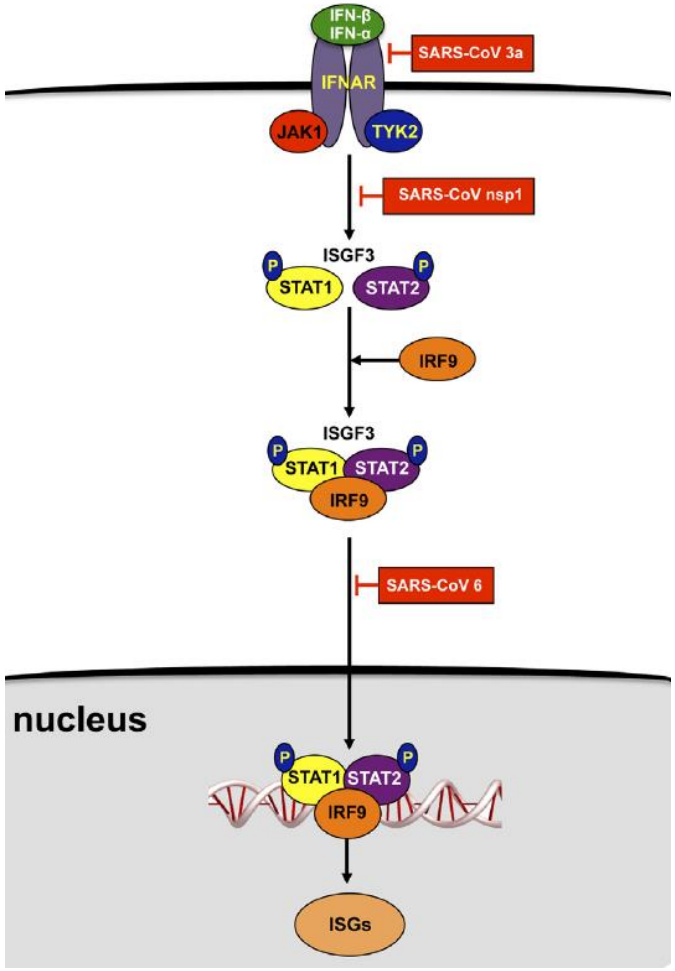


A model of how SARS-CoV-2 suppresses host immune responses through multi-pronged inhibition of core cellular functions. Cellular mechanisms are shown in gray and viral mechanisms in red.

Cell Volume 183, Issue 5, 25 November 2020, Pages 1325-1339.e21 https://doi.org/10.1016/j.cell.2020.10.004

Effect of coronavirus proteins on cellular signaling pathways associated with the innate immune response





Interferon-stimulated genes

M.L. DeDiego et al. / Virus Research 194 (2014) 124–137

Mapa de Interação Proteínas SARS-CoV-2 - Humano

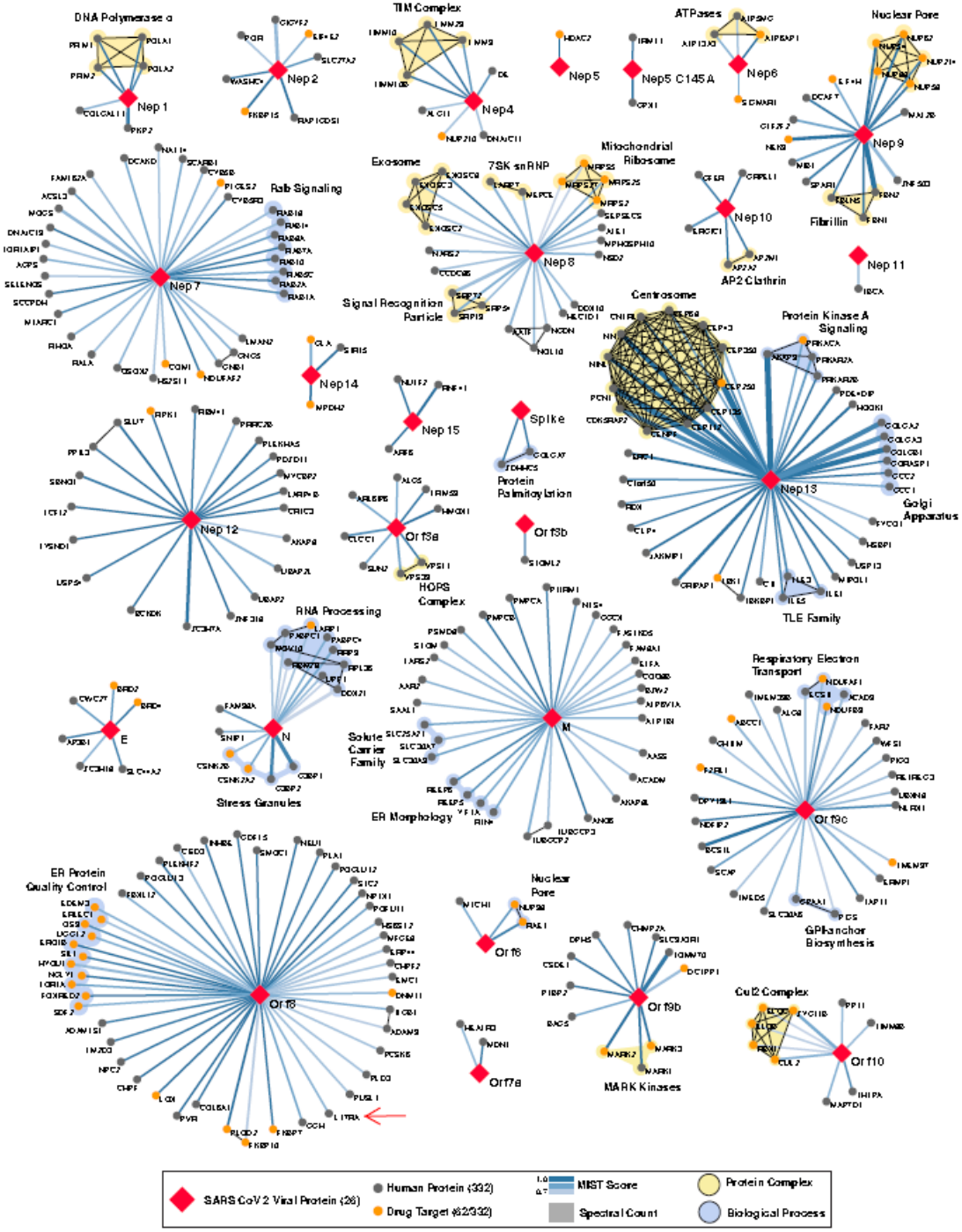
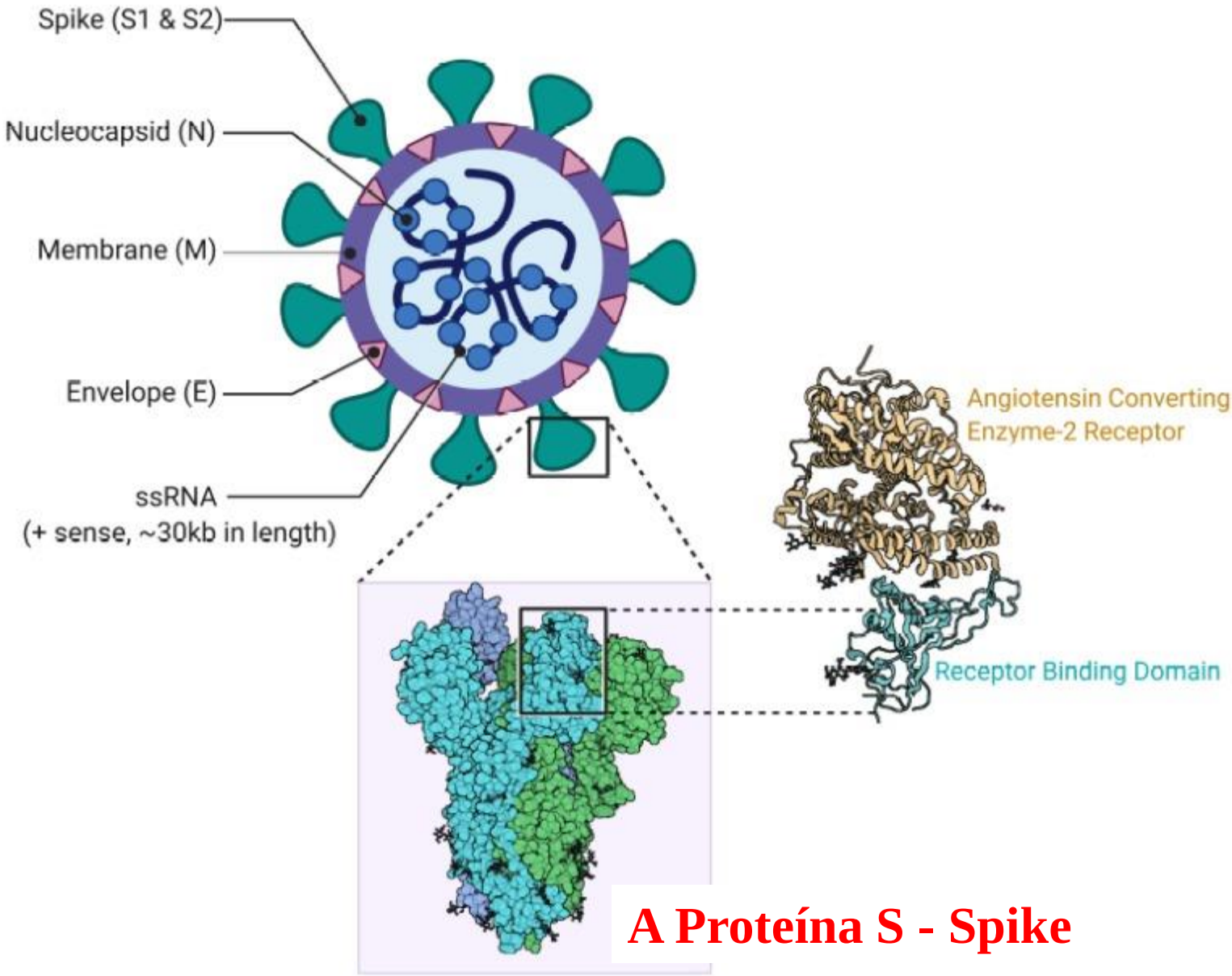


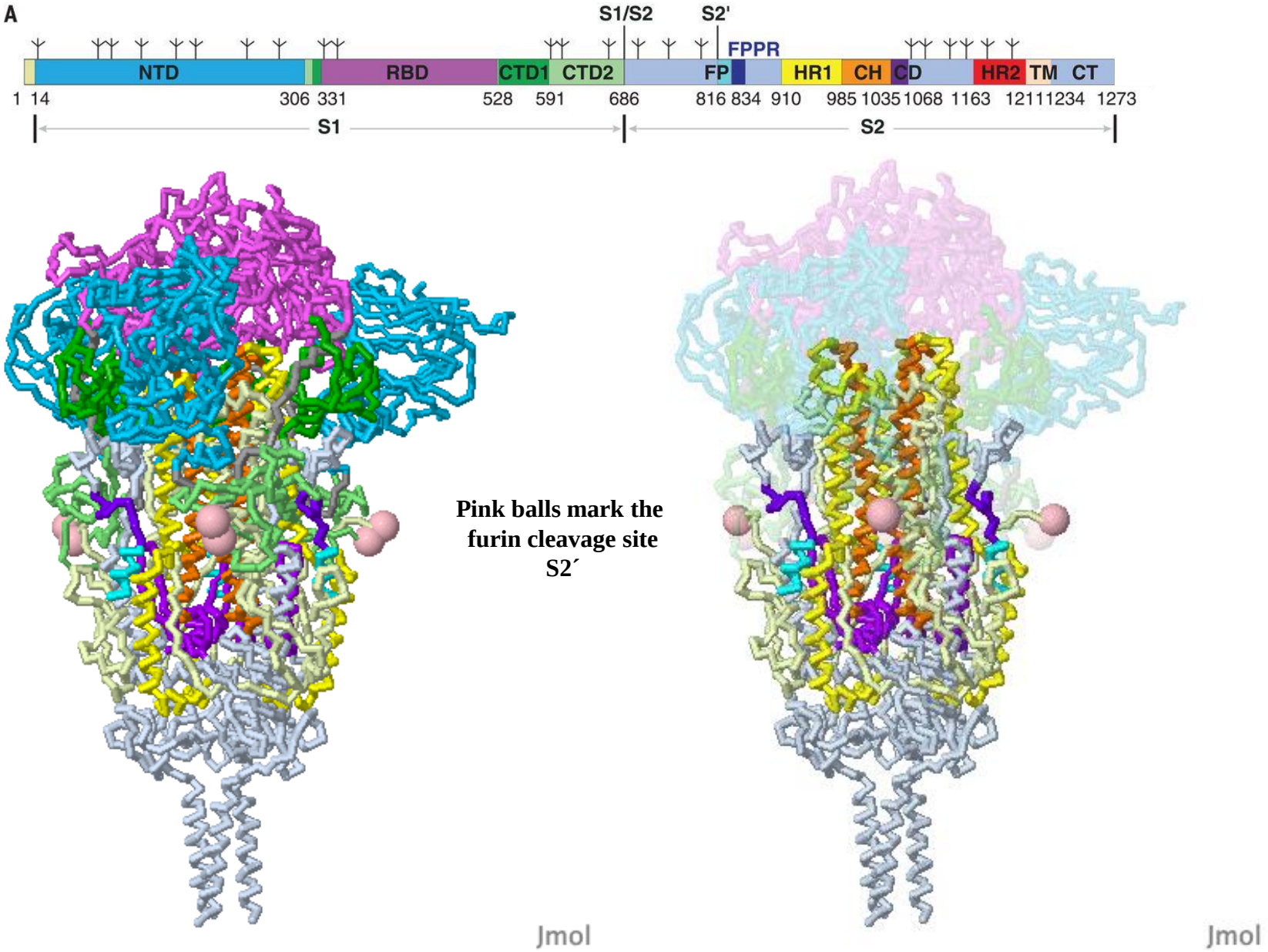
Figure 3: SARS-CoV-2 Protein-Protein Interaction Network. In total, 332 high confidence interactions are represented between **26 SARS-CoV-2** proteins and their human interactors. Red diamonds represent a SARSCoV-2 viral protein, interacting human host proteins are represented with circles, with drug targets in orange. Edge color is proportional to MiST score and edge thickness proportional to spectral counts. Physical interactions among host proteins are noted as thin black lines, protein complexes are highlighted in yellow, and proteins sharing the same biological process are highlighted in blue.

doi.org/10.1101/2020.03.22.002386.

SARS-CoV 2 Structure

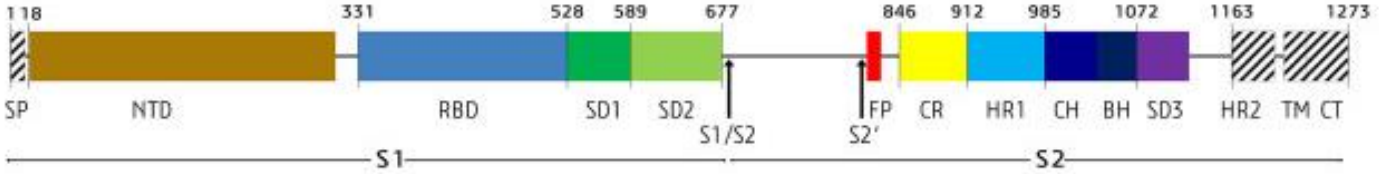


A Proteína S - Spike



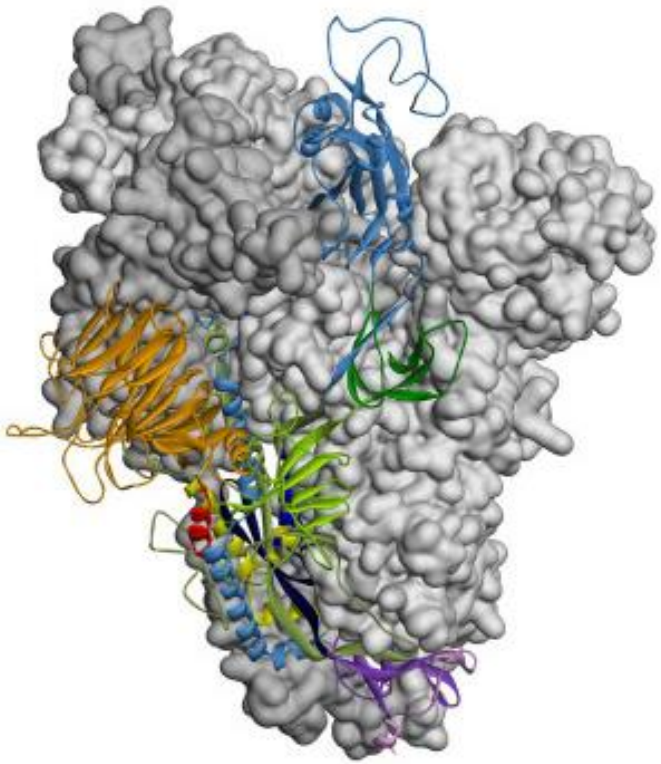
https://proteopedia.org/wiki/index.php/SARS-CoV-2_spike_protein_fusion_transformation#Membrane_Fusion_Schematic

Cai Y, Zhang J, Xiao T, Peng H, Sterling SM, Walsh RM Jr, Rawson S, Rits-Volloch S, Chen B. Distinct conformational states of SARS-CoV-2 spike protein. Science. 2020 Jul 21. pii: science.abd4251. doi: 10.1126/science.abd4251. PMID:32694201 doi:http://dx.doi.org/10.1126/science.abd4251

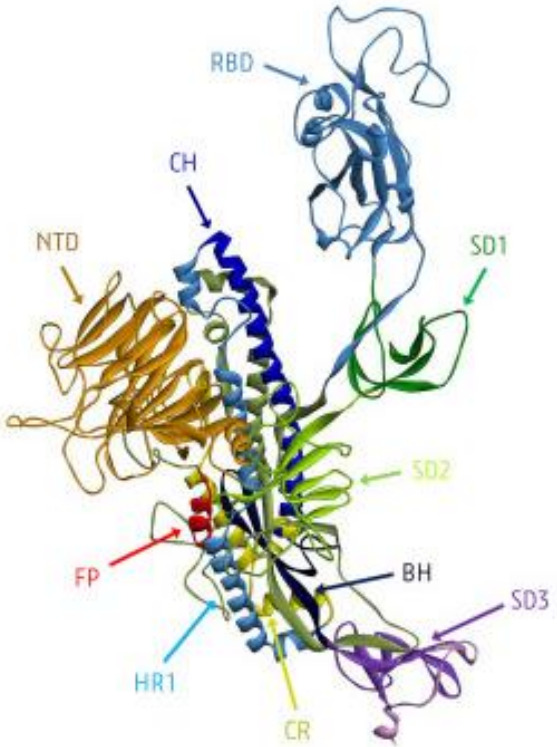


SARS-CoV-2 Spike Protein

c

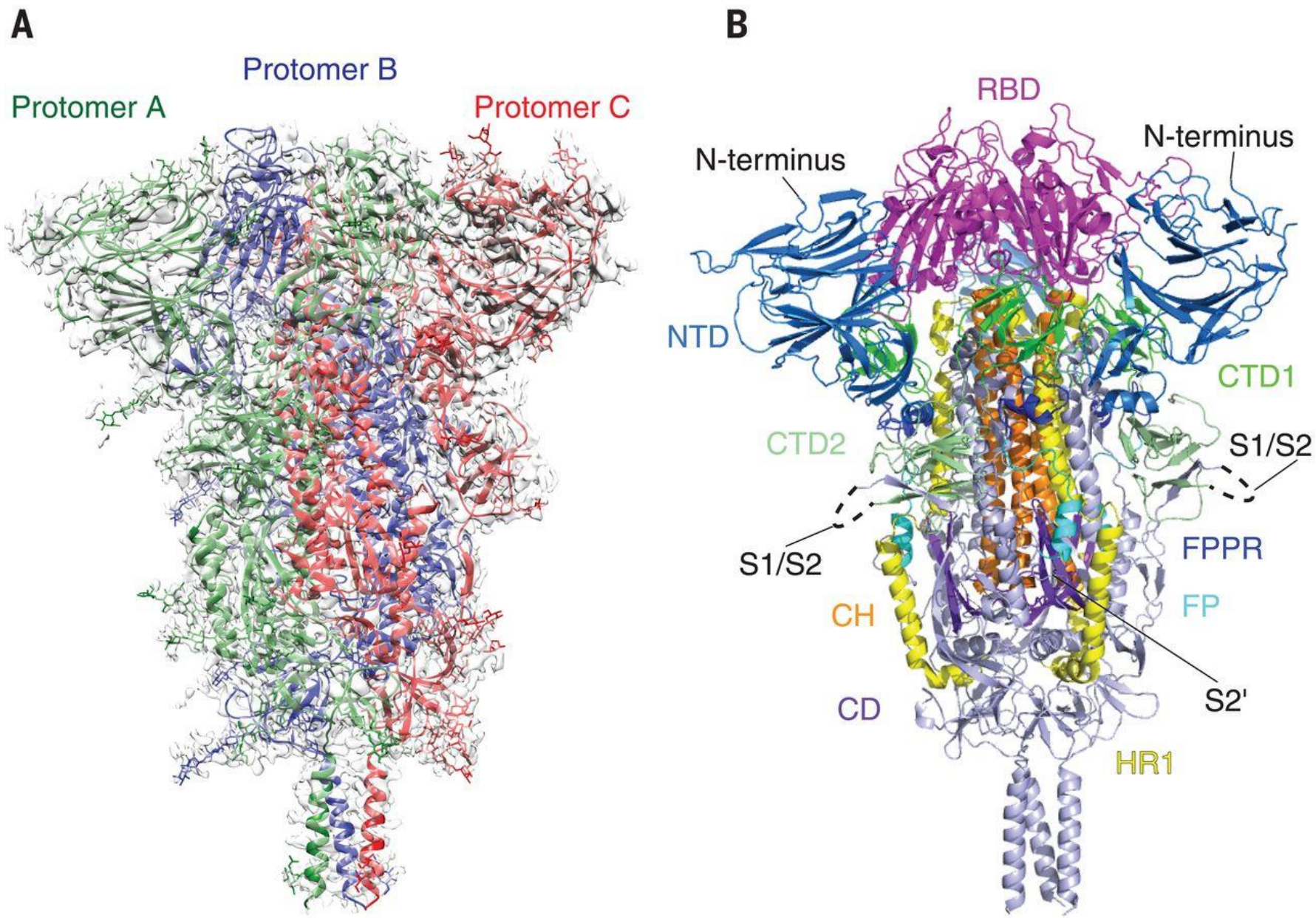


Homology Model of
SARS-CoV-2 Spike Protein Trimer

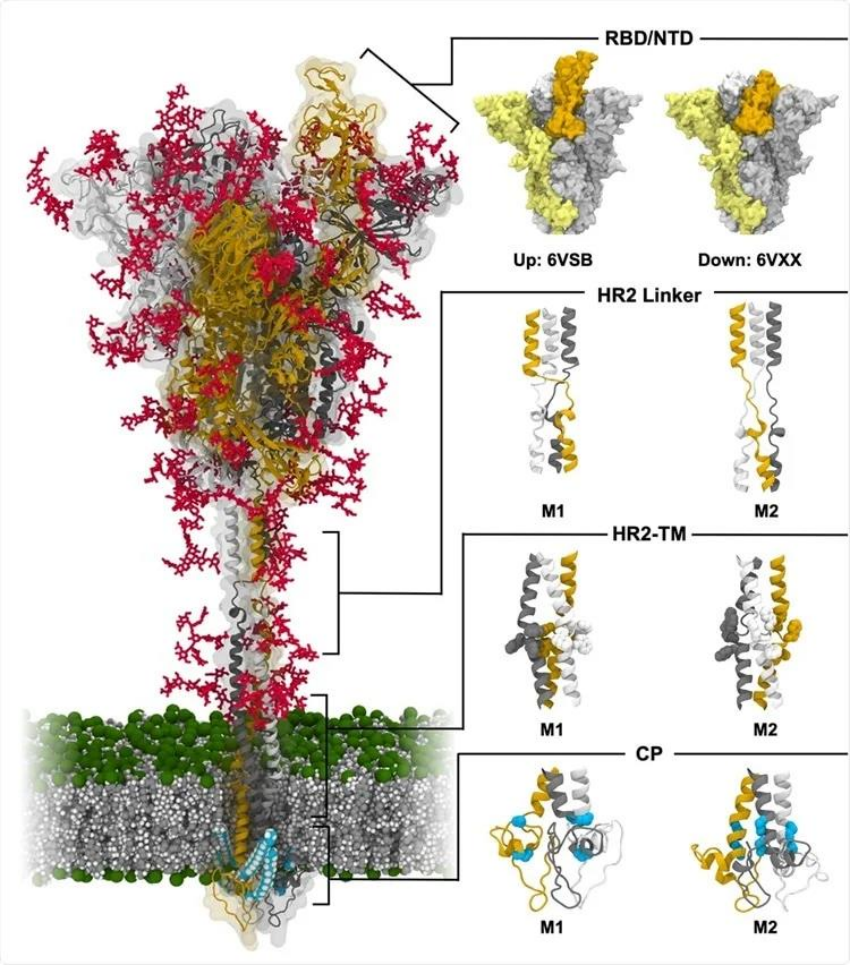


Ribbon diagram of
SARS-CoV-2 Spike Protein Monomer

Infect Genet Evol. 2020. doi: 10.1016/j.meegid.2020.104389



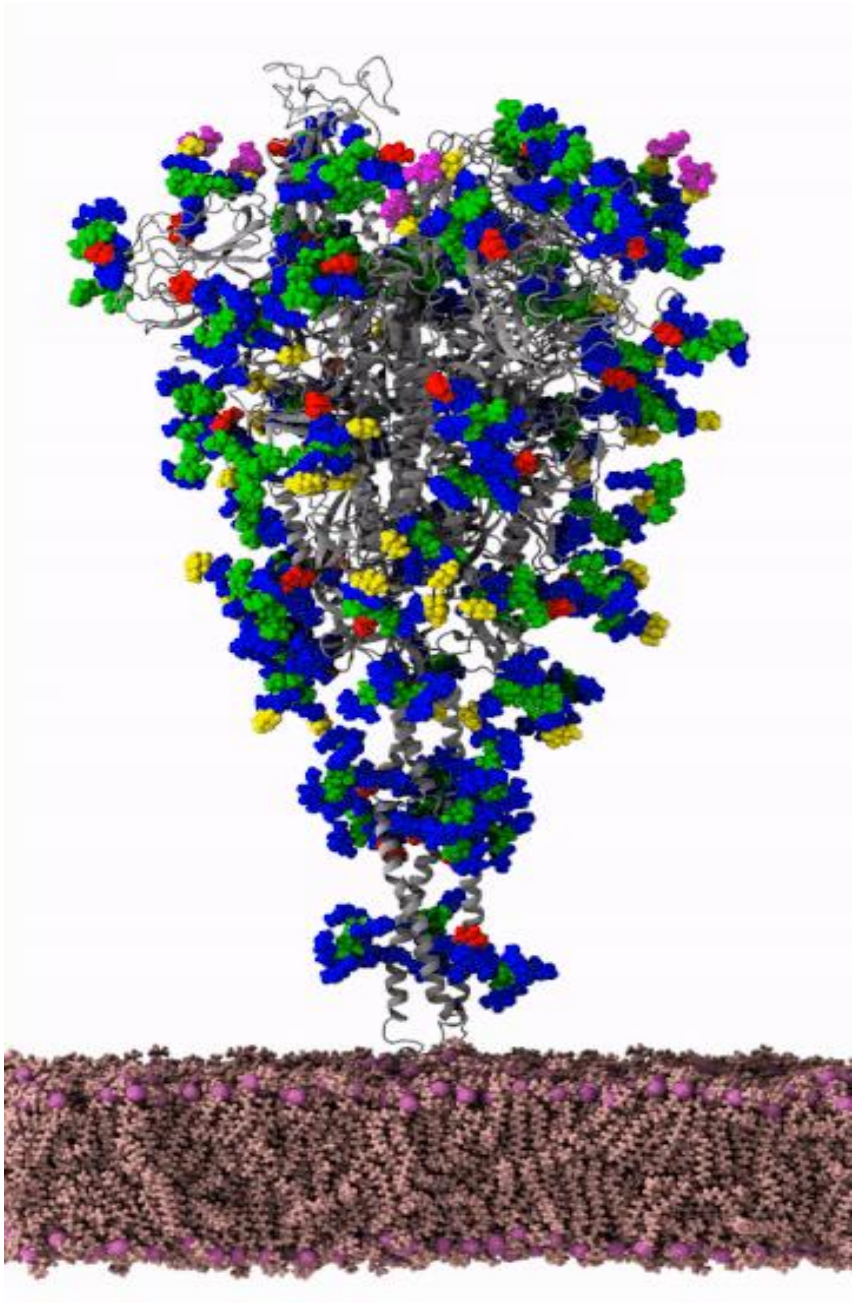
Yongfei Cai et al. Science 2020;369:1586-1592



Estrutura modelo de proteína completo completo-glycosylated de SARS-CoV-2 S em uma membrana viral. Uma estrutura modelo da proteína de SARS-CoV-2 S é mostrada no painel esquerdo. Dois modelos para o linker RBD/NTD, HR2, o HR2-TM, e o PC são ampliados no painel direito. As três correntes individuais da proteína de S estão coloridas em amarelo, em cinzento, e em branco, respectivamente, quando os glycans forem representados como varas vermelhas. Os locais do palmitoylation da proteína de S são destacados em ciano. O fosfato, o carbono, e os átomos de hidrogênio da membrana viral são coloridos em verde, em cinzento, e em branco, respectivamente. Para maior clareza, as moléculas de água e os íons são omitidos. Todas as ilustrações foram criadas usando Dynamics30 molecular (VMD) visual.

https://www.news-medical.net/news/20201019/23765/Portuguese.aspx

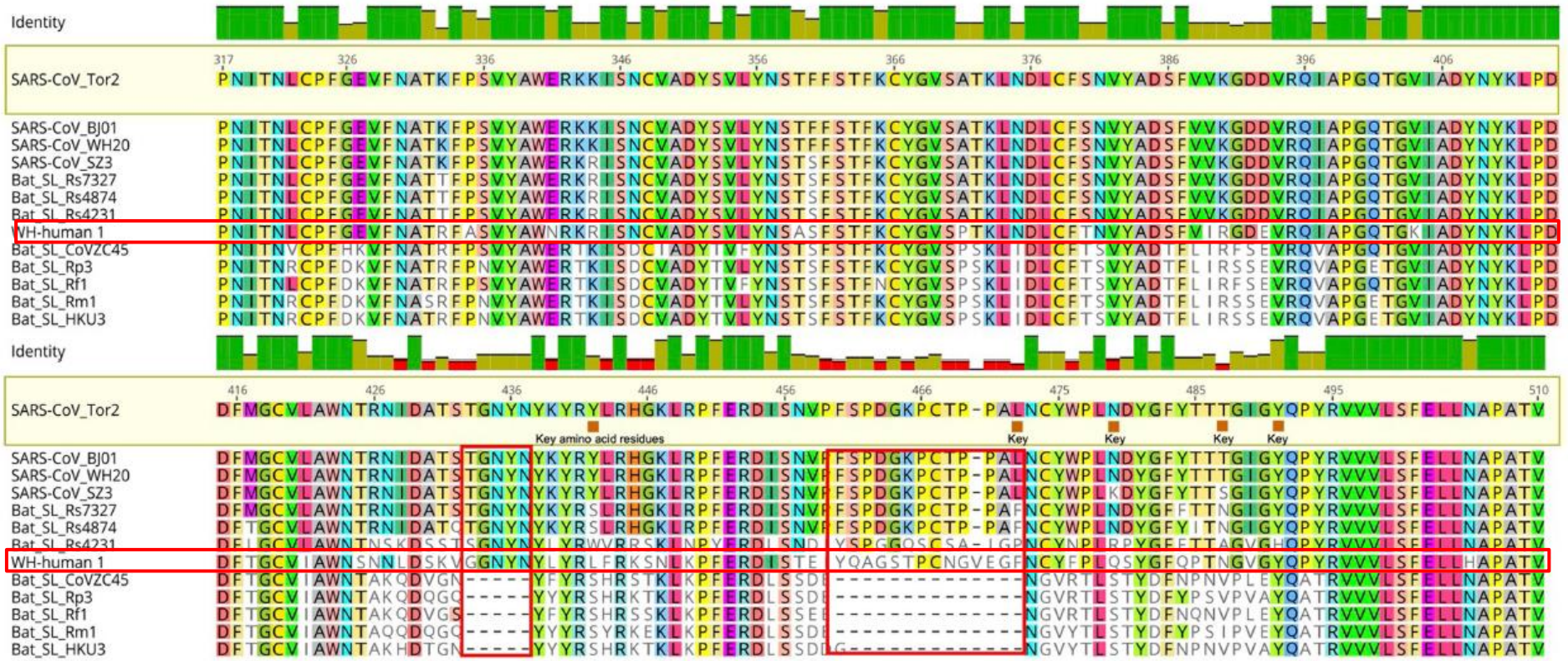
Animação mostrando a proteína spike SARS-CoV-2 (cinza) com glicanos espalhados em sua superfície. A estrutura balança, o que pode afetar a forma como os anticorpos ou outras moléculas se ligam a ela.



https://www.the-scientist.com/news-opinion/scientists-scan-for-weaknesses-in-the-sars-cov-2-spike-protein-67404

doi.org/10.1016/S0140-6736(20)30251-8

Analysis of RBD of the spike protein of WHCV coronavirus.

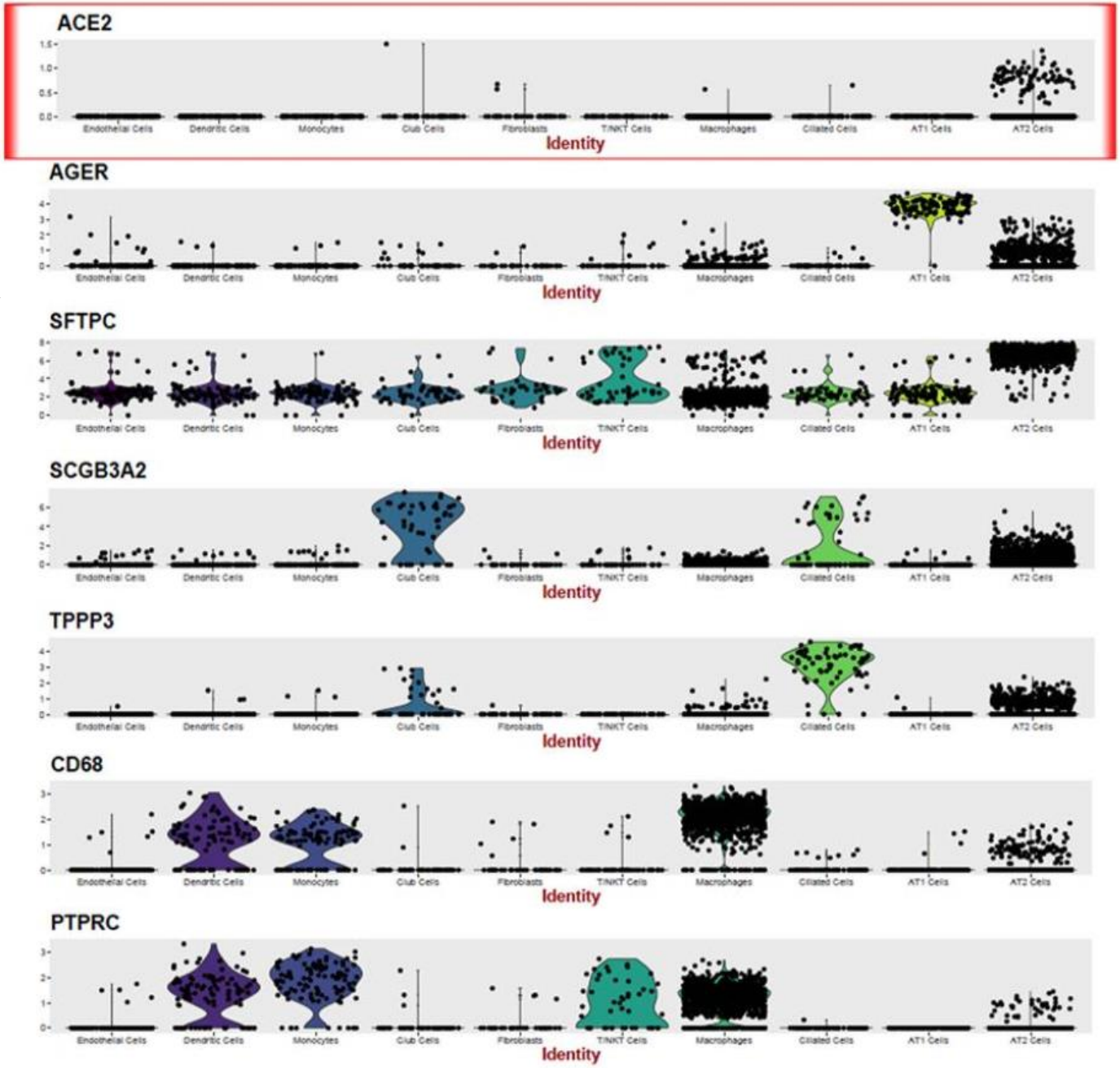


doi.org/10.1038/s41586-020-2008-3

Our analysis suggested that the expression of ACE2 is concentrated in a special population of AT2 which expresses many other genes favoring the viral process. The abundant expression of ACE2 in a population of AT2 explained the severe alveolar damage after infection.

Alveolar type 2 progenitor cells (AT2) seem closest to clinical translation, specifying the evidence that AT2 may satisfactorily control the immune response to decrease lung injury by stabilizing host immune-competence and a classic and crucial resource for lung regeneration and repair.

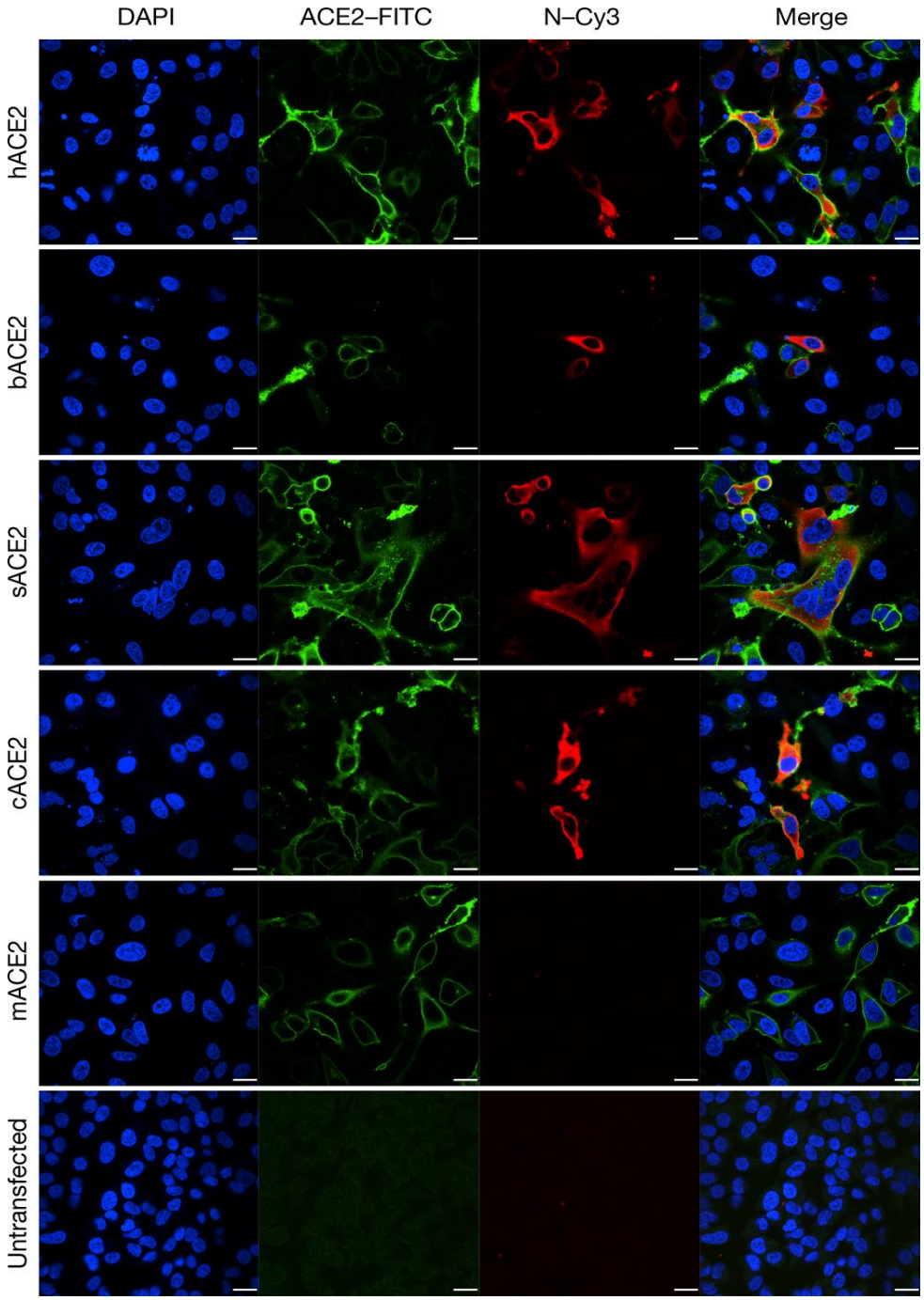
AT2 cells are important for the production of surfactant. The AT2 cell secretes, synthesizes, and reutilizes the protein and lipid constituents of pulmonary surfactant. **(doi: 10.1038/s41420-019-0147-9)**



https://doi.org/10.1101/2020.01.26.919985

Analysis of the receptor use of SARS-CoV-2

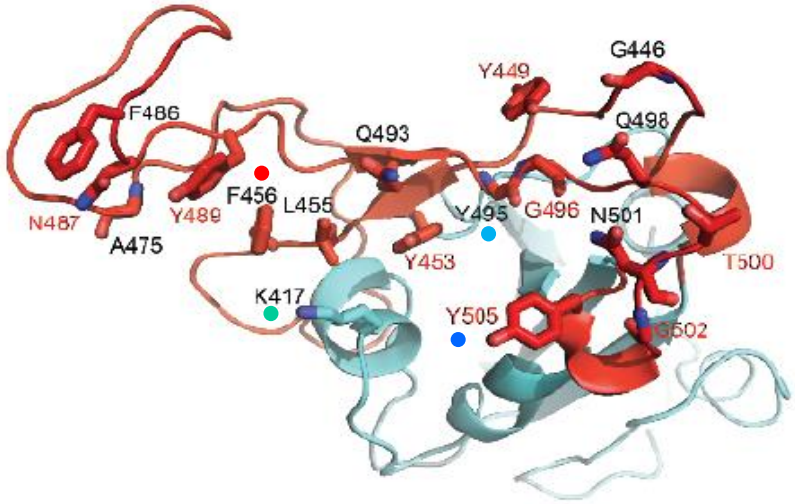
Os sintomas clínicos típicos desses pacientes são febre, tosse seca, dificuldades respiratórias (dispneia), dor de cabeça e pneumonia. O início da doença pode resultar em insuficiência respiratória progressiva devido a dano alveolar (como observado pelas imagens transversais de tomografia computadorizada do tórax) e até morte. Determinou-se que a doença era causada por pneumonia induzida por vírus de acordo com sintomas clínicos e outros critérios, incluindo aumento da temperatura corporal, diminuição do número de linfócitos e glóbulos brancos (embora os níveis destes últimos às vezes sejam normais), novos infiltrados pulmonares na radiografia de tórax e nenhuma melhora óbvia após o tratamento com antibióticos por três dias



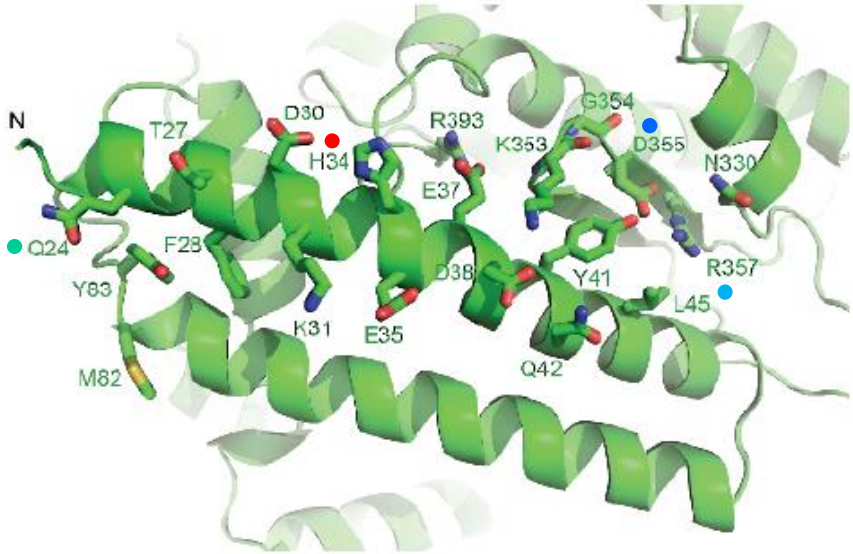
hACE2, human ACE2;
bACE2, ACE2 of *Rhinolophus sinicus* (bat);
cACE2, civet ACE2;
sACE2, swine ACE2 (pig);
mACE2, mouse ACE2.
Green, ACE2; red, viral protein (N); blue, DAPI (nuclei).

doi.org/10.1038/s41586-020-2012-7

Interação Proteína S e ECA2



2019-nCoV RBD



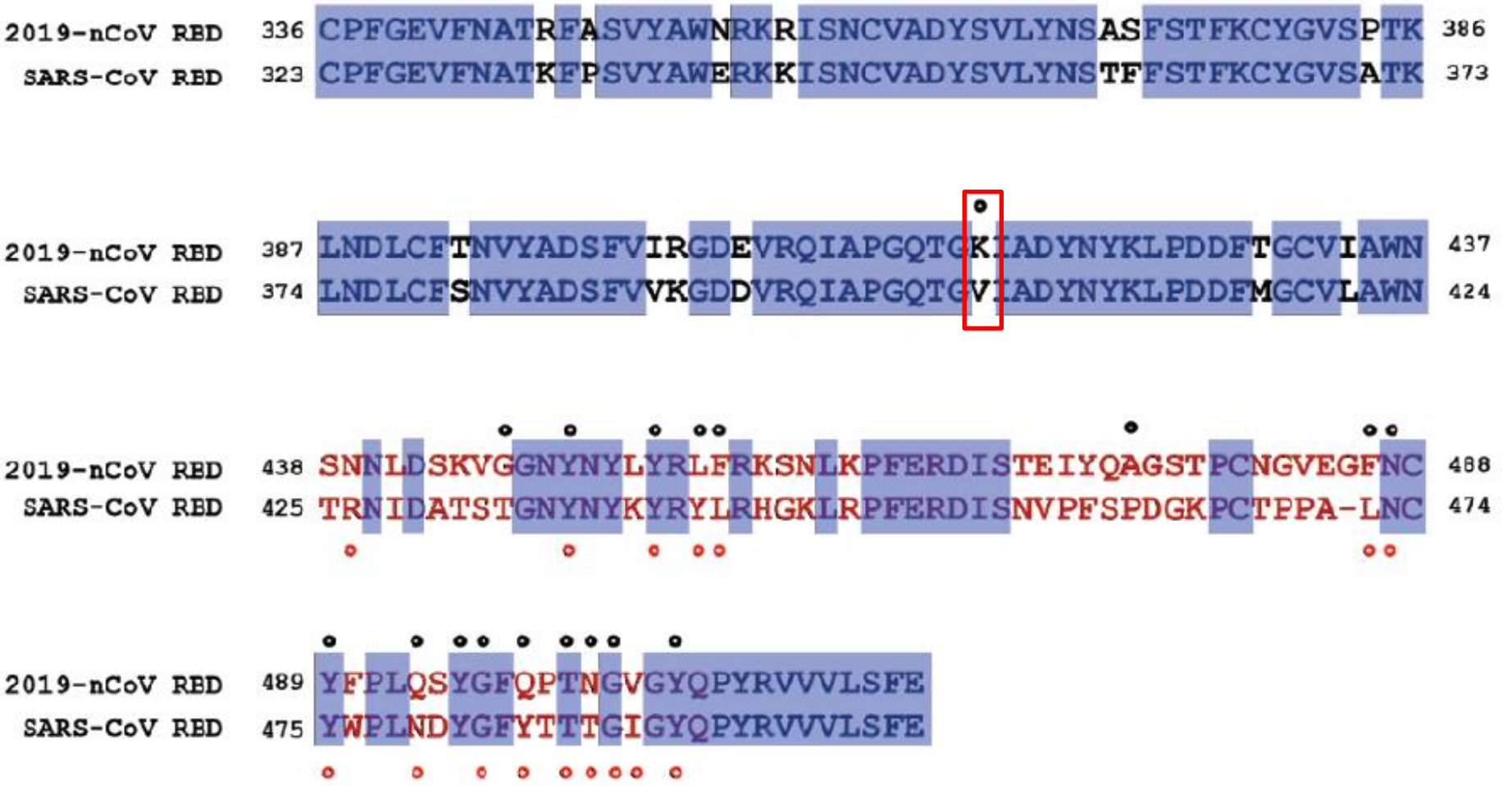
human ACE2

2019-nCoV RBD-ACE2 interface	
ACE2	2019-nCoV RBD
24Q	417K
27T	446G
28F	449Y
30D	453Y
31K	455L
34H	456F
35E	475A
37E	486F
38D	487N
41Y	489Y
42Q	493Q
45L	495Y
82M	496G
83Y	498Q
330N	500T
353K	501N
354G	502G
355D	505Y
357R	
393R	

With a distance cutoff of 4 Aº, a total of 18 residues of the RBD contact 20 residues of the ACE2. PDB: 2AJF.

doi: https://doi.org/10.1101/2020.02.19.956235 - 2020.

Interação Proteína S e ECA2

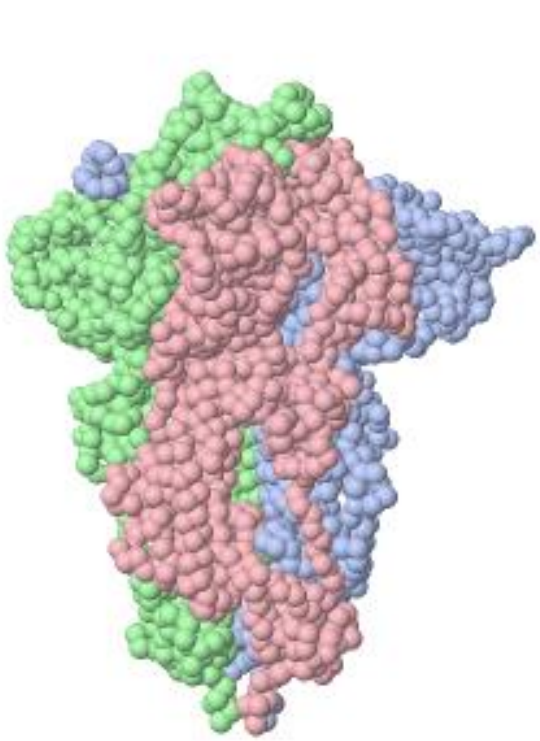


Sequence alignment of 2019-nCoV RBD and SARS-CoV RBD. Contacting residues in the 2019-nCoV RBD are indicated by black dots; contacting residues in the SARS CoV RBD are indicated by red dots. Outside RBM, there is a unique ACE2-interacting residues Lys417 in the 2019-nCoV, forming a salt bridge with ACE2 Asp30 . This position is replaced by a valine in the SARS-CoV RBD that fails to participate in ACE2 binding

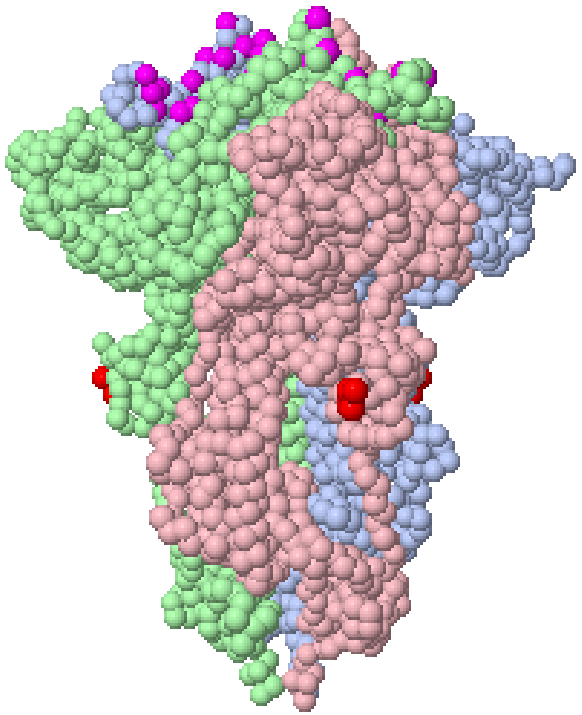
doi: https://doi.org/10.1101/2020.02.19.956235 - 2020.

Two Steps: Priming and Activation

Spike protein binds to antiotensin converting enzyme 2 (ACE2) on host cells to initiate entry and infection. Spike protein is initially in a closed conformation in which the receptor binding motif is less accessible. Proteases such as furin, amply expressed in the respiratory tract[2], clip the spike protein, priming or pre-acivating it to undergo a conformational change that extends the receptor binding motif on one chain (here light green), making it more accessible[1]. Binding to ACE2 and a second proteolytic cut, typically by a host cell membrane-bound protease such as transmembrane serine protease 2 (TMPRSS2) activates spike protein for membrane fusion, which inserts the virus genome into the host cell.
Scenes showing how the closed form of the coronavirus spike protein changes its conformation when it is primed by the human protease furin.
Spike protein conformational change when primed by furin
This morph (restore initial scene) shows the transition of SARS-CoV-2 protein S ("spike protein") from closed (6ZGI) to open (6ZGG) conformation induced by proteolytic cleavage by furin[3]. The transition probably goes only towards the open (extended) conformation, but this animation goes both ways to help visualize the conformational change. (The model is simplified, showing only enlarged alpha carbon atoms, with glycosylation hidden. The model lacks 62 "stem" residues at the C-terminal [narrow] end, and also lacks 65 C-terminal transmembrane and cytoplasmic residues.)

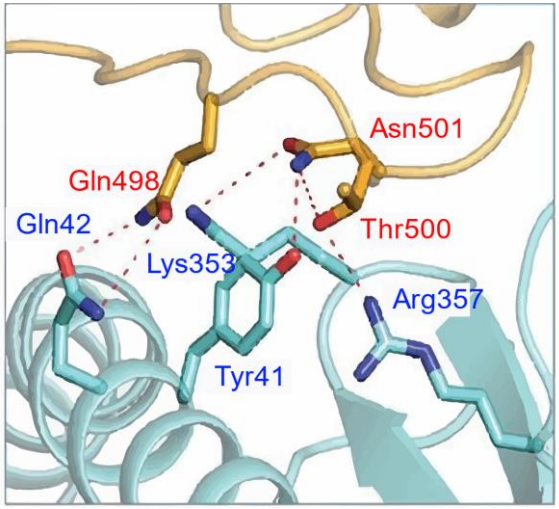


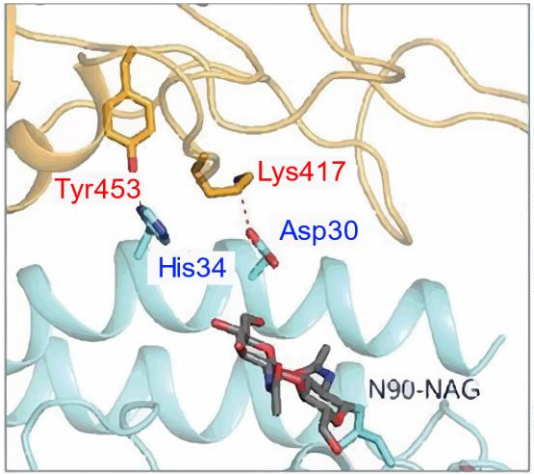
ACE2 binding site;
Furin cleavage site.

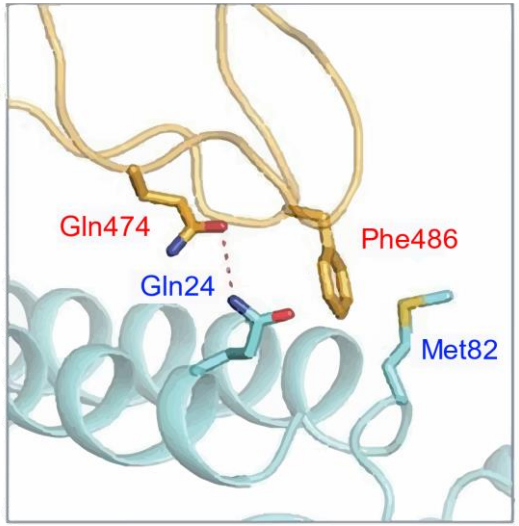


https://proteopedia.org/wiki/index.php/SARS-CoV-2_spike_protein_priming_by_furin

Interfase entre RBD-SARS-CoV-2 e o receptor ECA2





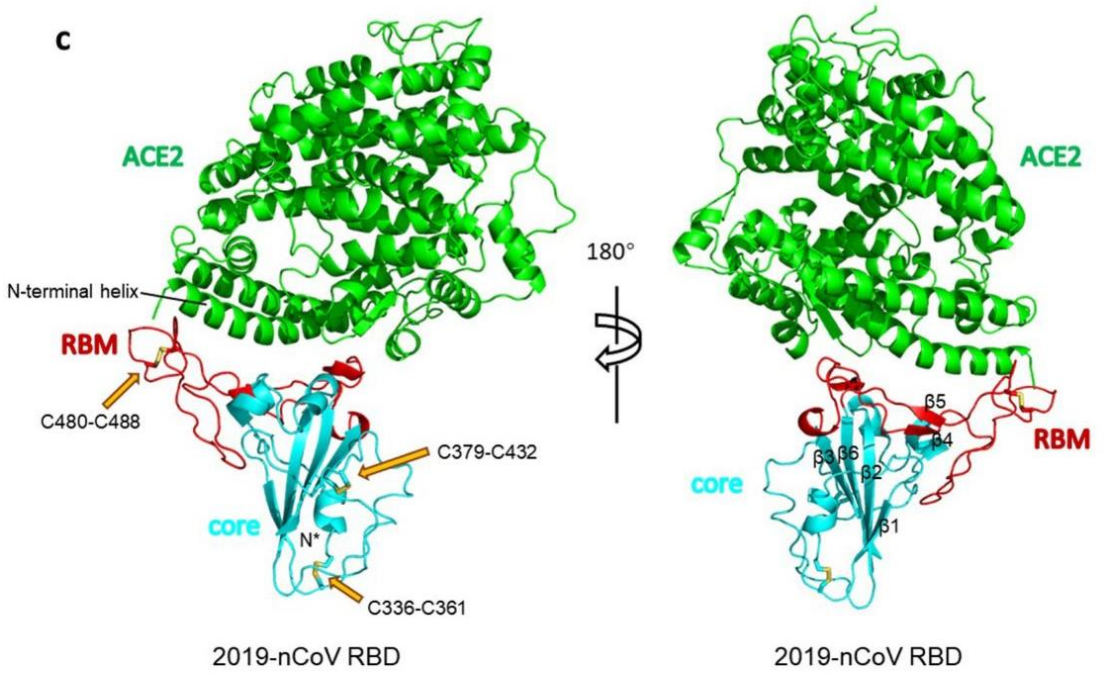


B to D) Detailed analysis of the interface between SARS-CoV-2-RBD and ACE2. Polar interactions are indicated by red dashed lines. NAG, N-acetylglucosamine. The contact can be divided into three clusters. The two ends of the bridge interact with the N and C termini of the α1 helix as well as small areas on the α2 helix and loop 3-4. The middle segment of α1 reinforces the interaction by engaging two polar residues (Fig. 4A). At the N terminus of α1, Gln498, Thr500, and Asn501 of the RBD form a network of H-bonds with Tyr41, Gln42, Lys353, and Arg357 from ACE2 (Fig. 4B). In the middle of the bridge, Lys417 and Tyr453 of the RBD interact with Asp30 and His34 of ACE2, respectively (Fig. 4C). At the C terminus of α1, Gln474 of the RBD is H-bonded to Gln24 of ACE2, whereas Phe486 of the RBD interacts with Met82 of ACE2 through van der Waals forces (Fig. 4D).

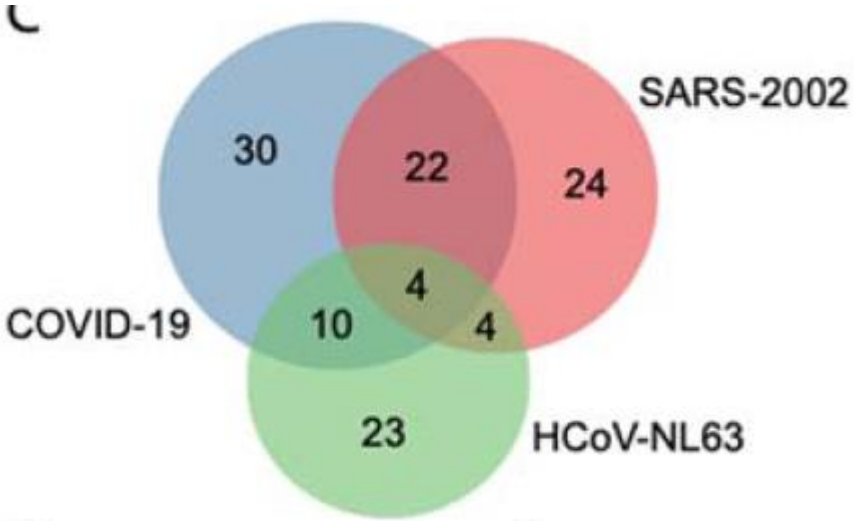
Proteína S e ECA2

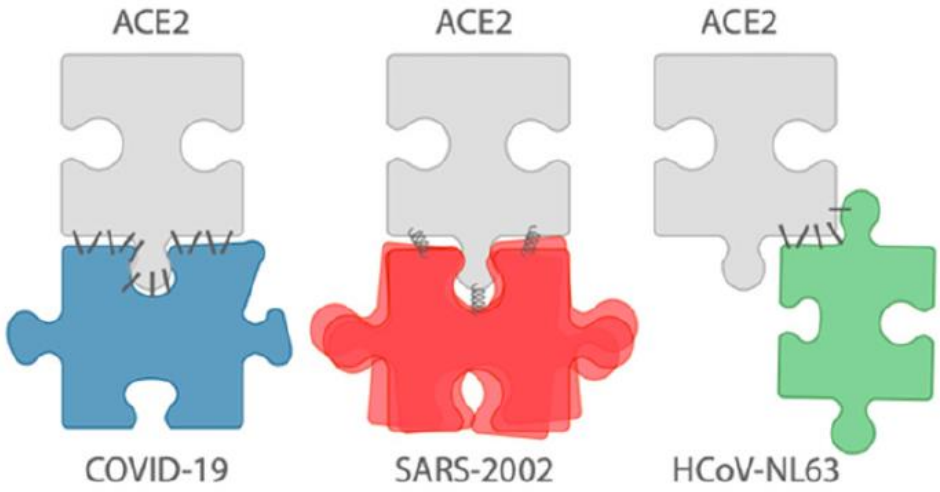
1273 aa e 141.2 kD.
SARS-CoV-2 utilizes an extensively glycosylated spike (S) protein that protrudes from the viral surface to bind to angiotensin-converting enzyme 2 (ACE2), the host cell receptor, to mediate cell entry3. The S protein is a trimeric class I fusion protein that is composed of two functional subunits responsible for receptor binding (S1 subunit) and membrane fusion (S2 subunit), with each trimer displaying 66 N-linked glycosylation sites

doi.org/10.1101/2020.03.26.010322



doi.org/10.1101/2020.02.19.956235





Interação de resíduos ECA2

doi:10.3390/v12050497

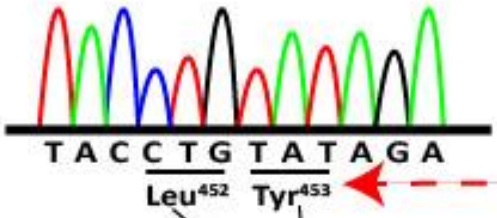
Estratégia de ligação

Variantes SARS-CoV-2

DNA SARS-CoV-2 Parental

L= Leucina (Leu)

Y= Tirosina (Tyr)



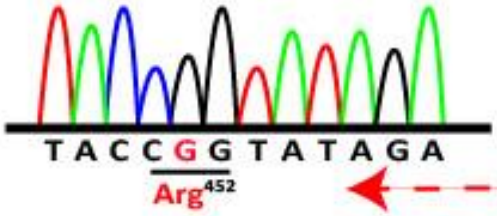
Observe os aminácidos Leu e Tyr presentes nos primeiros isolados do coronavírus SARS-CoV-2

Estrutura da Proteína Spike (S) que liga ao receptor presente na célula humana

RBM= Motivo de ligação ao receptor

DNA SARS-CoV-2 Mutação L452R

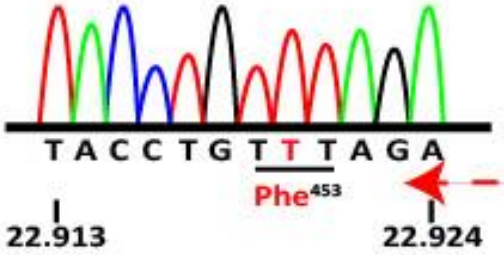
R= Arginina (Arg)



Observe a troca do aminácido Leu por Arg na variante do coronavírus SARS-CoV-2

SARS-CoV-2 Mutação Y453F

F= Fenilalanina (Phe)



Observe a troca do aminácido Tyr por Phe na variante do coronavírus SARS-CoV-2

Posição dos nucleotídeos

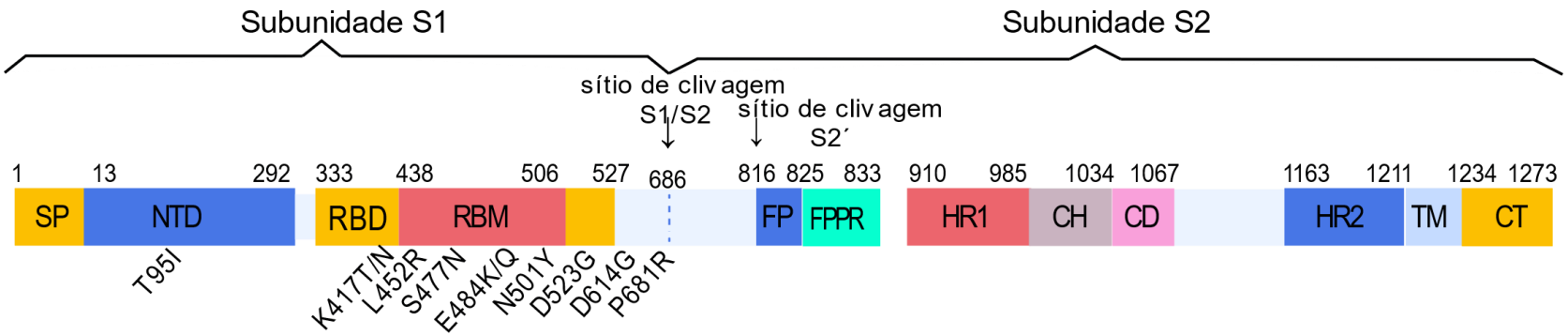


Tabela 1. Variantes de Atenção (VOC), identificadas*.

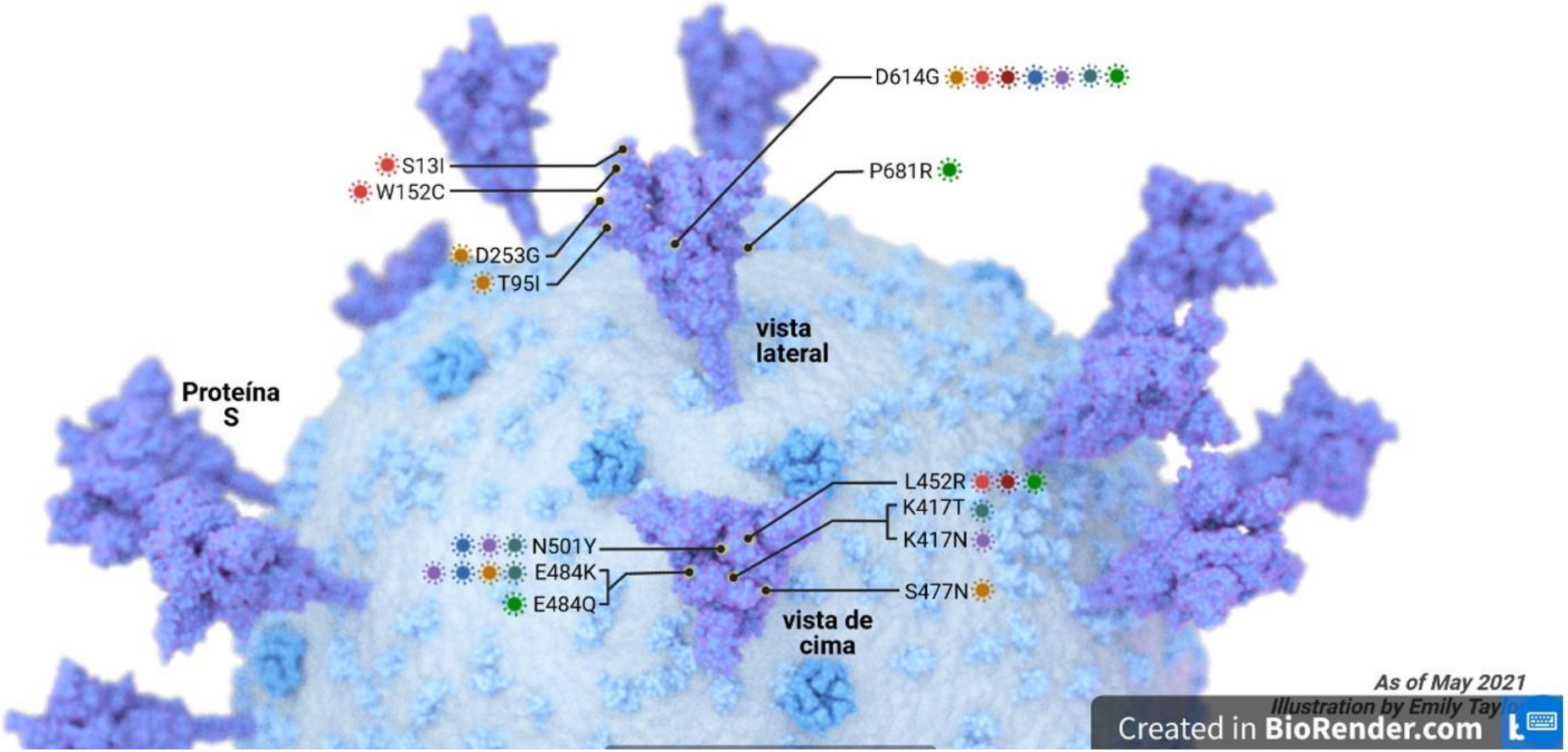
Variante	Local de Identificação	Mutação identificada
B.1.1.7	Reino Unido	E484K, N501Y, D614G
B.1.351	África do Sul	K417N, N501Y, D614G
P.1	Brasil/Japão	K417T, E484K, N501Y, D614G
B.1.526	Estados Unidos (NY)	T95I, S477N, E484K, D523G, D614G
B.1.427	Estados Unidos (CAL)	L452R, D614G
B.1.429	Estados Unidos (CAL)	S13I, W152C, L452R, D614G
B.1.617	Índia	L452R. E484Q, D614G, P681R

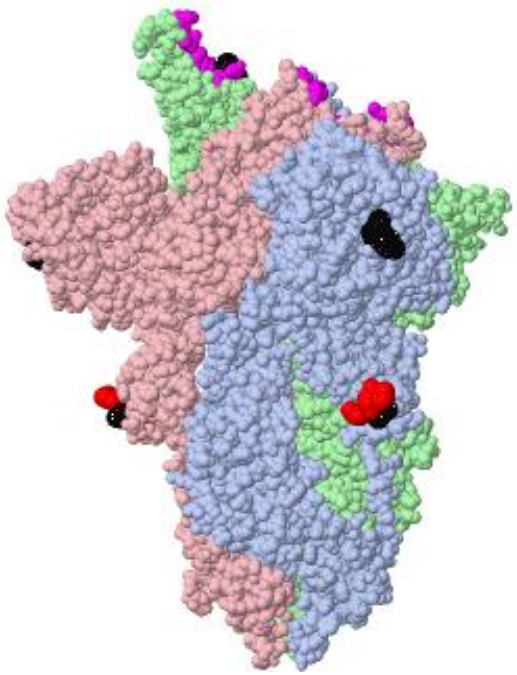
*Fonte: (3). E= Ácido glutâmico; K= Lisina; N= Asparagina; Y= Tirosi
D= Ácido aspártico; G= Glicina; T= Treonina; I= Isoleucina; S= Serina
L= Leucina; R= Arginina; W= Triptofano; C= Cisteína; Q= Glutamina;
P= Prolina.

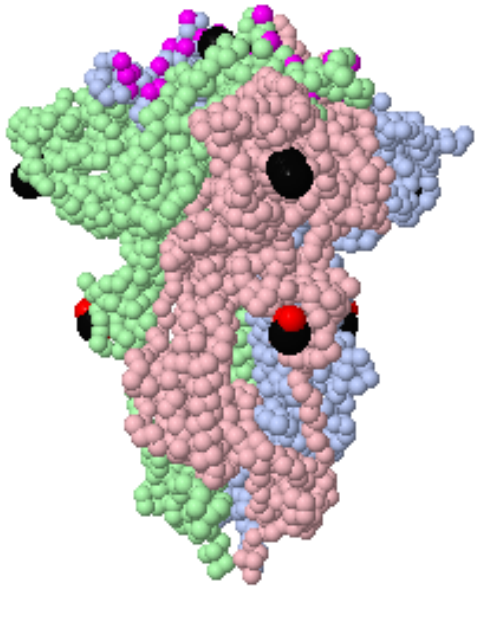
Referências:
1. Tortora, Gerard J. Microbiologia. 10. ed. Porto Alegre: Artmed, 2012.; 2. https://www.who.int/csr/don/31-december-2020-sars-cov-
-variants/en/; 3. https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html
4. doi: 10.1038/s41401-020-0485-4. ;5. doi: 10.1126/science.abb2507.

Variantes SARS-CoV-2 de Atenção/Preocupante (Variant of Concern, VOC).

Alpha	Beta	Gamma	Iota	Epsilon	Alpha	Kappa
B.1.1.7	B.1.351	P.1	B.1.526	B.1.427	B.1.429	B.1.617
Local e Data Inicial de Identificação: Reino Unido 09/2020	Local e Data Inicial de Identificação: África do Sul 09/2020	Local e Data Inicial de Identificação: Japão/Brasil 12/2020	Local e Data Inicial de Identificação: Estados Unidos 11/2020 (NY)	Local e Data Inicial de Identificação: Estados Unidos 12/2020 (CAL)	Local e Data Inicial de Identificação: Estados Unidos 11/2020 (CAL)	Local e Data Inicial de Identificação: Índia 02/2021







SARS-CoV-2 protein S is a homotrimer composed of three sequence-identical protein chains with a mature length 1,261 amino acids each (total 3,783 amino acids). The host protease furin cuts these chains at the sequence PRRAR[3][4][1] (sequence range 681-685), cutting the chain into fragments S1 and S2. The site cut by furin is part of a 12-amino acid surface loop that could not be resolved in the model, but we can mark the adjacent residues in red.

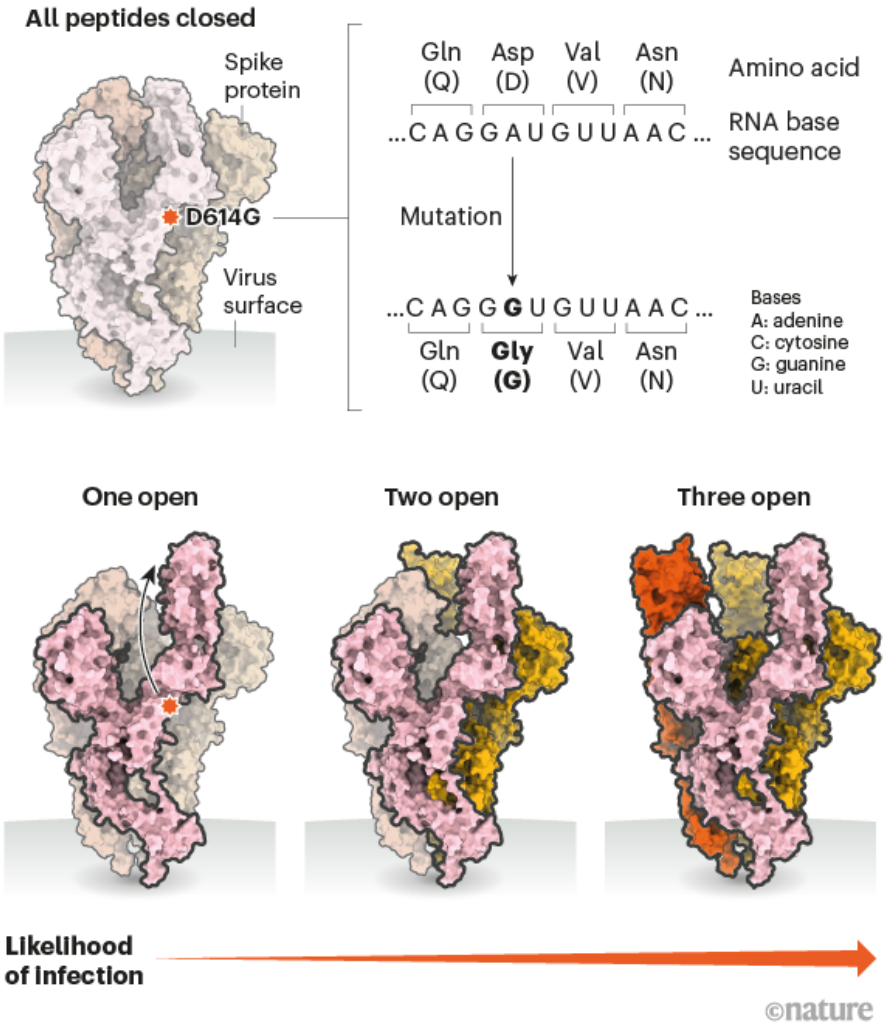
This shows the priming conformational change, whereas the interactive scenes above show the static open conformation. The scenes above show all atoms, while the movie shows only alpha carbons. As above for lineage B.1.1.7: each chain of the homo-trimer is a different color; **Four mutations (black)**; **ACE2 binding site**; **furin cleavage site**.

Primed (open) spike protein (6zgg). **Mutations of concern** in lineage B.1.1.7. ACE2 binding amino acids. Furin cleavage site for priming.

https://proteopedia.org/wiki/index.php/SARS-CoV-2_spike_protein_mutations

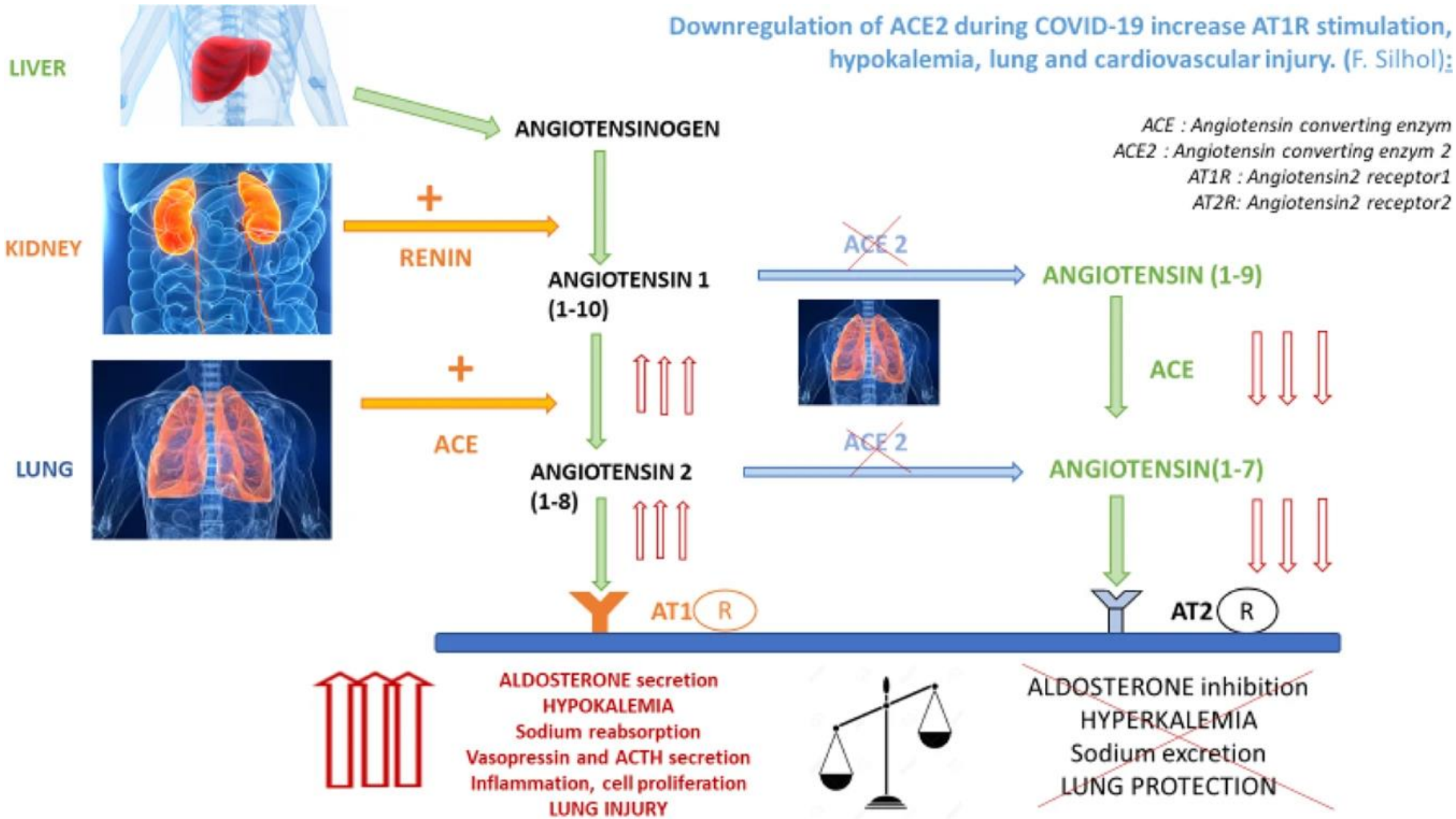
THE MUTATION THAT LOOSENS THE SPIKE PROTEIN

Spike proteins on SARS-CoV-2 bind to receptors on human cells, helping the virus to enter. A spike protein is made up of three smaller peptides in 'open' or 'closed' orientations; when more are open, it's easier for the protein to bind. The D614G mutation — the result of a single-letter change to the viral RNA code — seems to relax connections between peptides. This makes open conformations more likely and might increase the chance of infection.

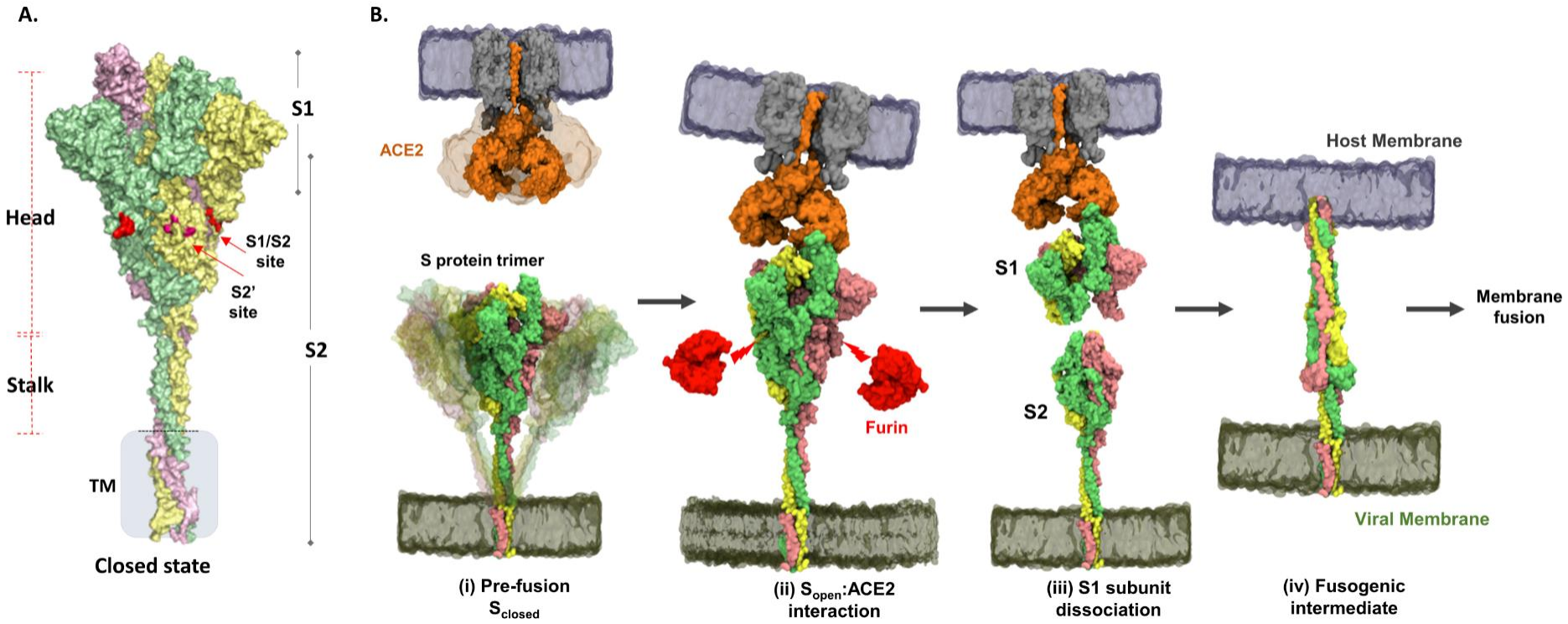


Nature 585, 174-177 (2020) doi: https://doi.org/10.1038/d41586-020-02544-6

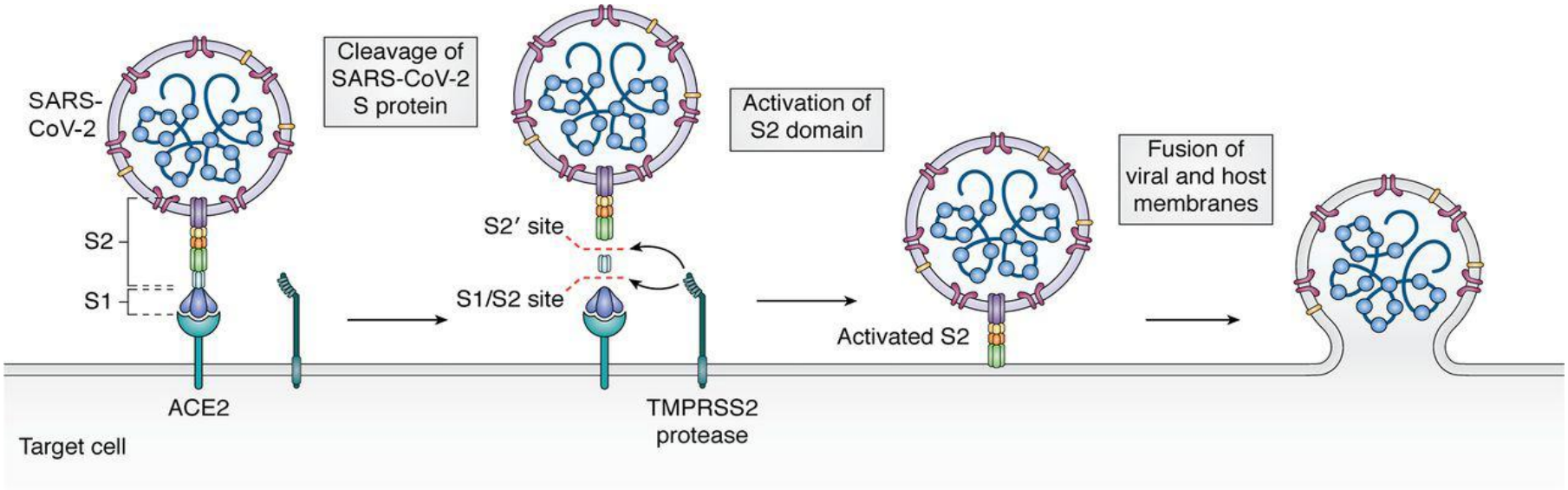
Downregulation of ACE2 induces overstimulation of the renin–angiotensin system in COVID-19



Hypertens Res 43, 854–856 (2020). https://doi.org/10.1038/s41440-020-0476-3



Cite as: eLife 2021;10:e63646 doi: 10.7554/eLife.63646



Mechanism of SARS-CoV-2 viral entry. The SARS-CoV-2 S protein engages with the host ACE2 receptor and is subsequently cleaved at S1/S2 and S2′ sites by TMPRSS2 protease. This leads to activation of the S2 domain and drives fusion of the viral and host membranes.

doi.org/10.1074/jbc.REV120.013930 - 2020

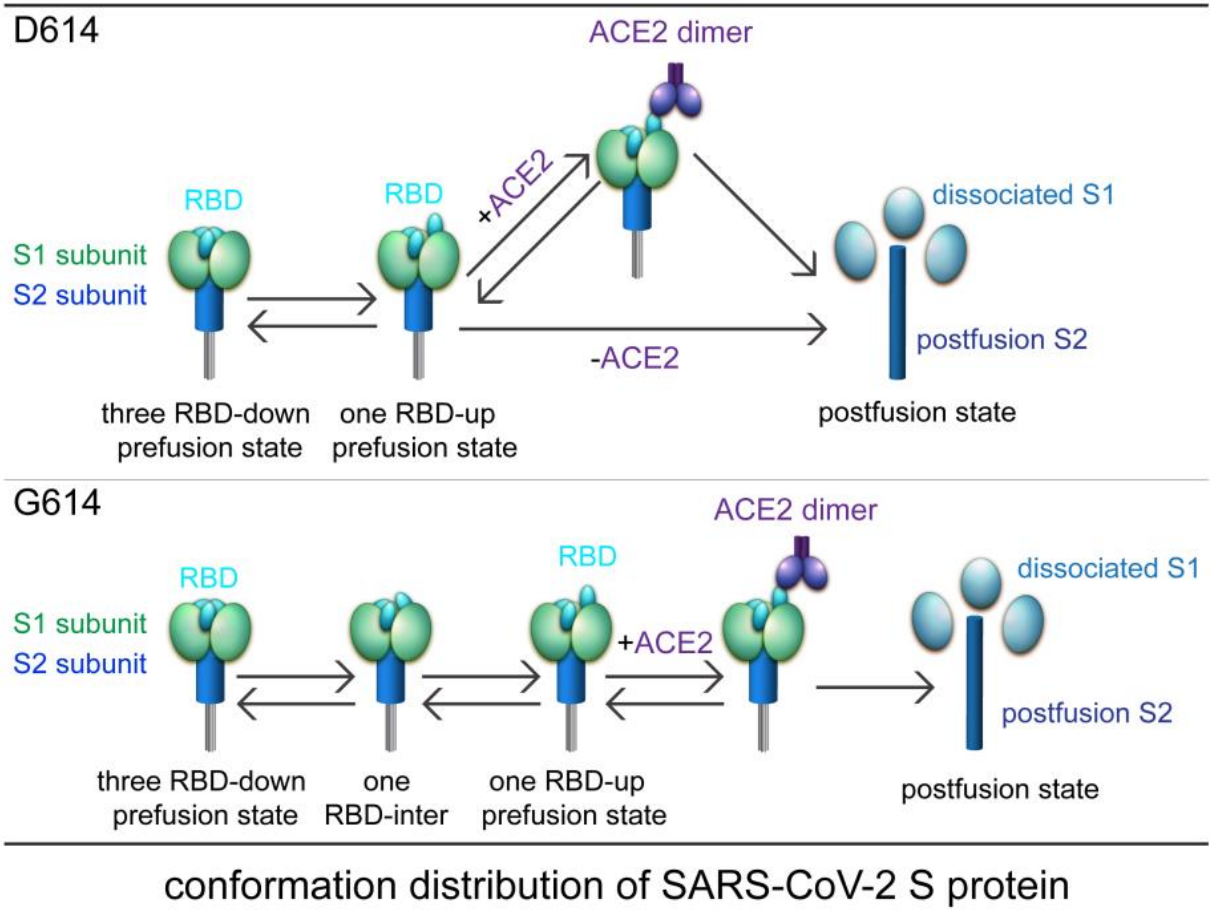
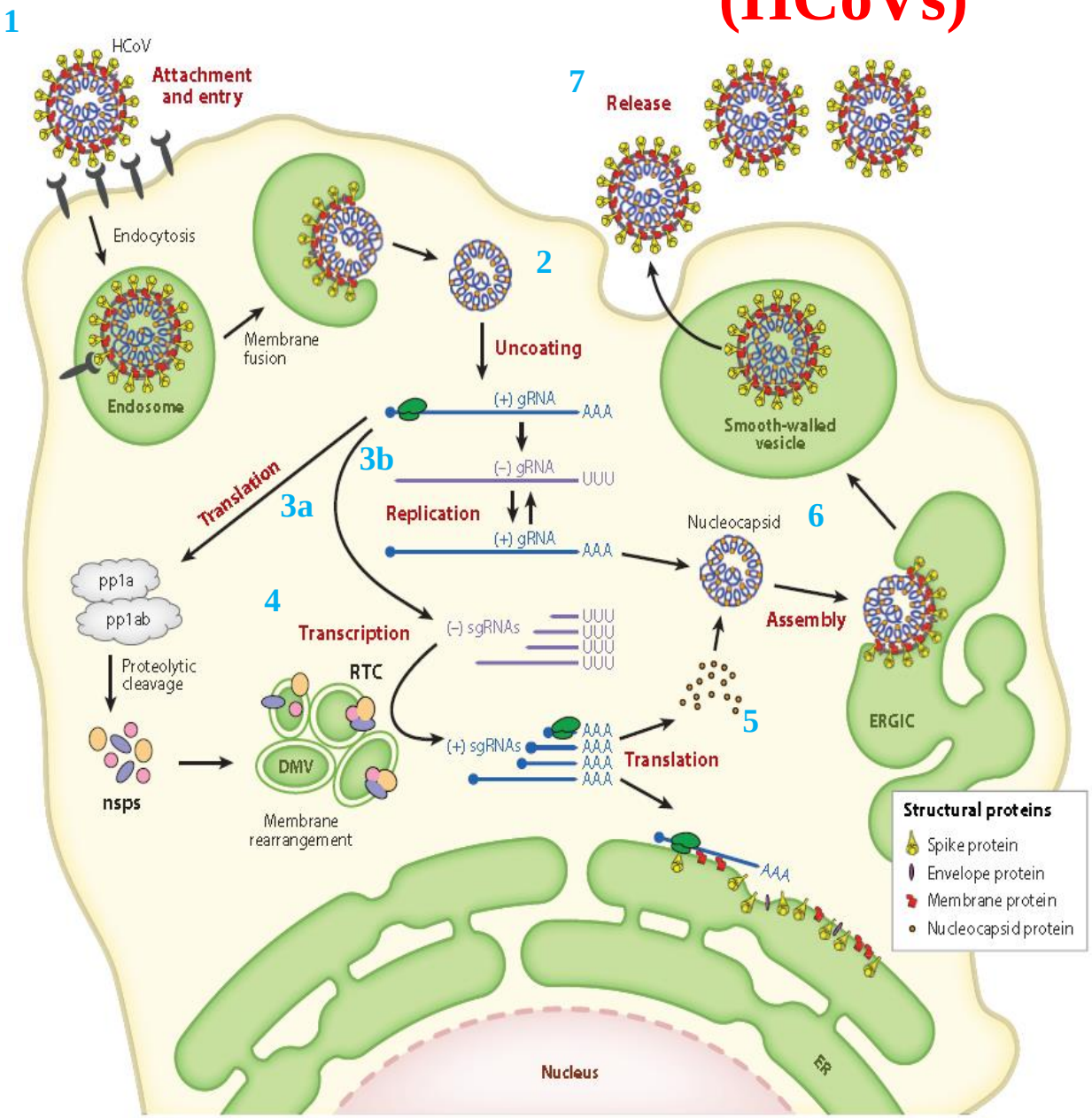


Figure S15. Proposed conformation distribution of the SARS-CoV-2 S trimer. The D614 S trimer samples the closed, one RBD-up, ACE2-bound and postfusion conformations, while the G614 S trimer samples the closed, RBD-intermediate, one RBD-up, ACE2-bound and postfusion conformations.

Structural and biochemical studies on a full-length G614 spike trimer showed that there are interactions not present in D614 that prevent premature loss of the S1 subunit that binds angiotensin-converting enzyme 2. This stabilization effectively increases the number of spikes that can facilitate infection.

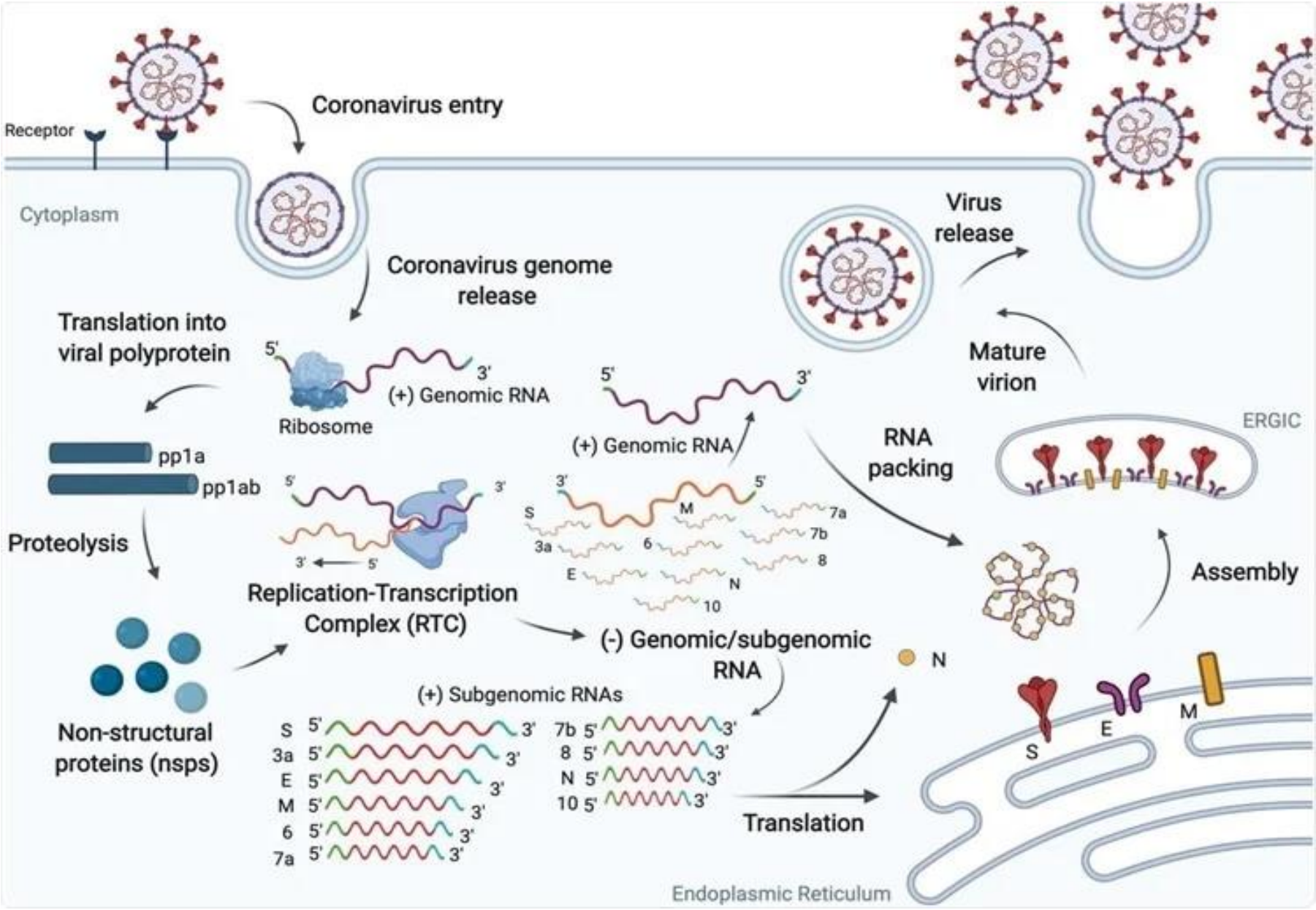
Science. 2021 Apr 30;372(6541):525-530. doi: 10.1126/science.abf2303

Ciclo geral de replicação de coronavírus humanos (HCoVs)



1. Adesão e ingresso do virus à célula hospedeiro;
2. Remoção da envoltura e liberação do gRNA;
3a. Síntese de proteínas virais;
3b. Síntese de novas moléculas de gRNA;
4. Síntese de pequenas e novas moléculas de gRNA;
5. Síntese de proteínas virais;
6. Montagem de novas partículas virais;
7. Liberação ao meio extracelular

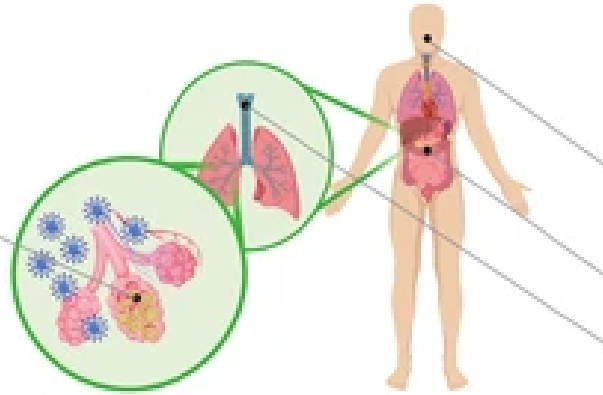
doi.org/10.1146/annurev-micro-020518-115759


Receptor
Coronavirus entry
Cytoplasm
Coronavirus genome release
Translation into viral polyprotein
5'
3'
(+) Genomic RNA
Ribosome
pp1a
pp1ab
Proteolysis
Non-structural proteins (nsps)
Replication-Transcription Complex (RTC)
(+) Genomic RNA
S
M
7a
3a
6
7b
E
8
N
10
(-) Genomic/subgenomic RNA
(+) Subgenomic RNAs
S 5' 3'
3a 5' 3'
E 5' 3'
M 5' 3'
6 5' 3'
7a 5' 3'
7b 5' 3'
8 5' 3'
N 5' 3'
10 5' 3'
Translation
Endoplasmic Reticulum
N
S
E
M
Assembly
RNA packing
ERGIC
Mature virion
Virus release

Host Cells

(tentative list; number of cells per person)

Type I & II pneumocytes (~10^{11} cells)
Alveolar macrophage (~10^{10} cells)
Mucous cell in nasal cavity (~10^{9} cells)
Host cell volume: ~10^{3} μm^3 = 10^{3} fL



virions not to scale

Concentration

(maximal observed values following diagnosis)

Nasopharynx: 10^{6}-10^{9} RNAs/swab
Throat: 10^{4}-10^{8} RNAs/swab
Stool: 10^{4}-10^{8} RNAs/g
Sputum: 10^{6}-10^{11} RNAs/mL

RNA counts can markedly overestimate infectious virions

Antibody Response - Seroconversion

Antibodies appear in blood after: ≈10-20 days
Maintenance of antibody response:
≈2-3 years (measured for SARS-CoV-1)

Virus Environmental Stability

(relevance to personal safety unclear)

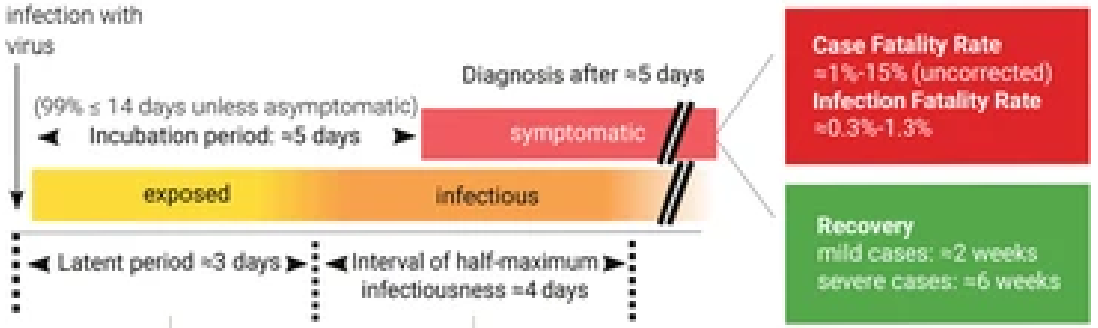
Half-life
(time to decrease 2-fold, not strictly constant)

Aerosols: ≈1 hr
Surfaces: ≈1-10 hr
e.g. plastic, glass, paper and metals

Based on quantifying infectious virions.
Numbers will vary between conditions and surface types.
Viral RNA observed on surfaces even after a few weeks.

"Characteristic" Infection Progression in a Single Patient

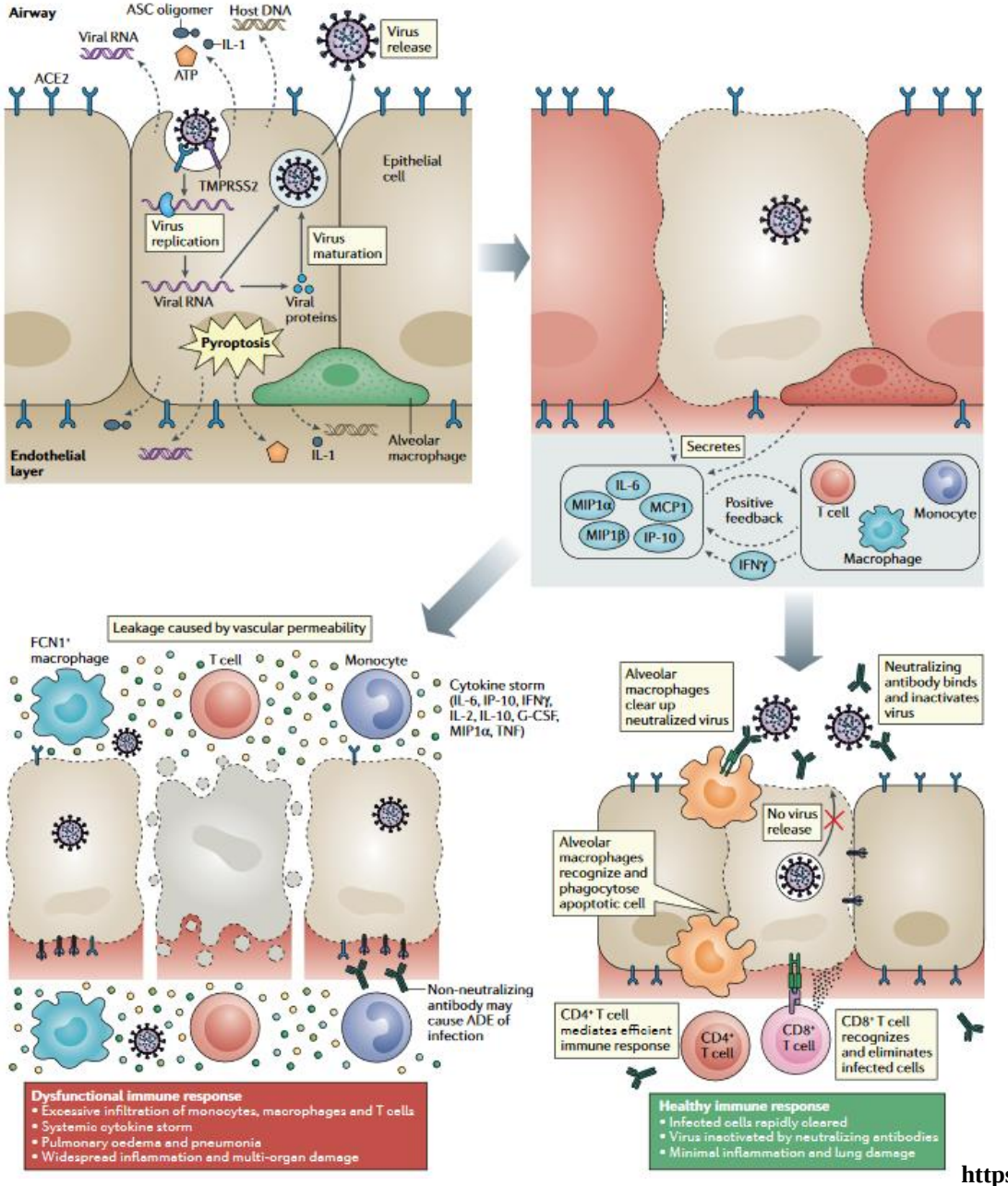
Basic reproductive number R_0: typically 2-4
Varies further across space and time
(number of new cases directly generated from a single case)



Inter-individual variability is substantial and not well characterized. The estimates are parameter fits for population median in China and do not describe this variability.

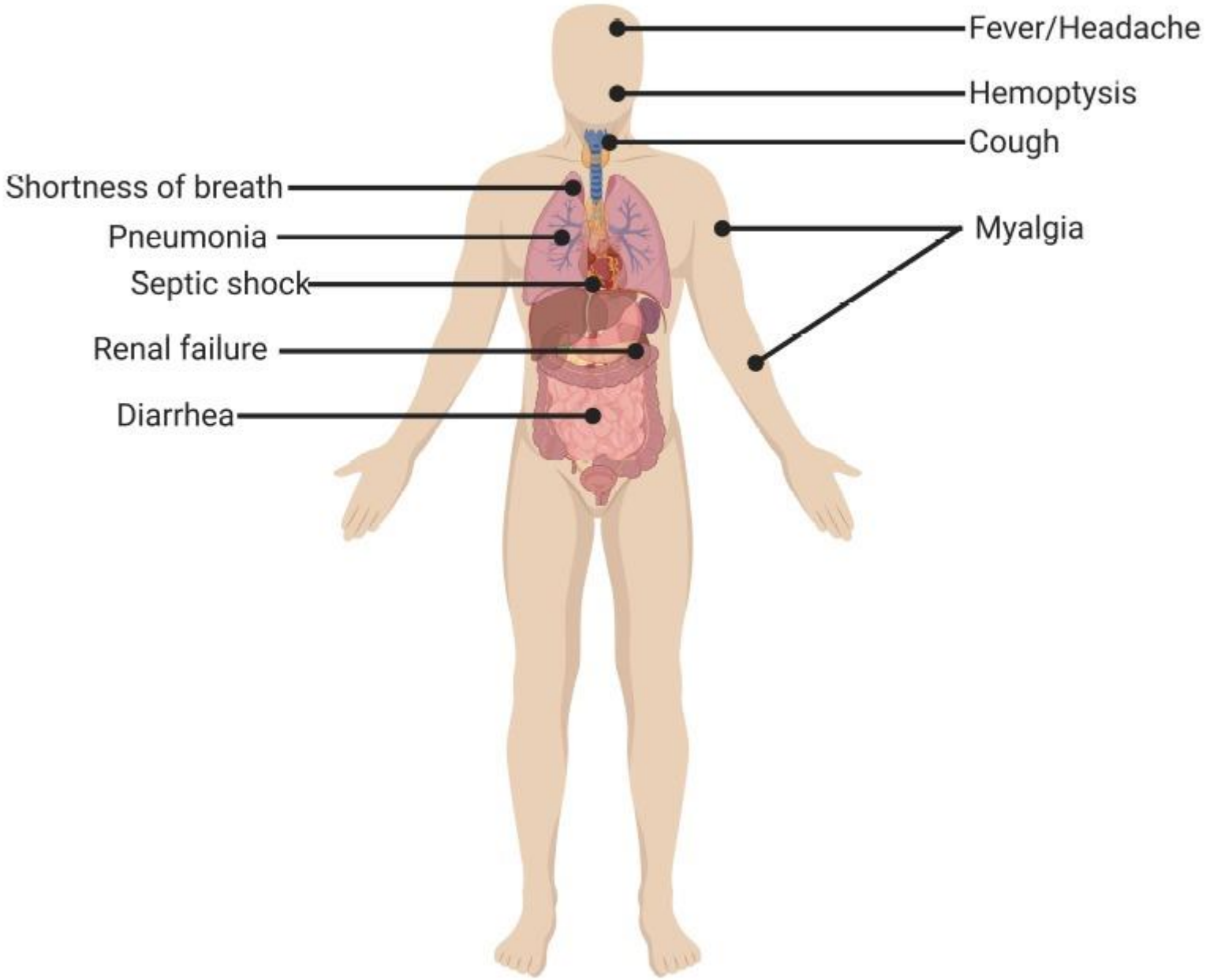
Note the difference in notation between the symbol ≈, which indicates "approximately" and connotes accuracy to within a factor 2, and the symbol ~, which indicates "order of magnitude" or accuracy to within a factor of 10.

Events during SARS-CoV-2 infection



https://doi.org/10.1038/s41577-020-0311-8

Clinical presentation of patients with CoVID-19

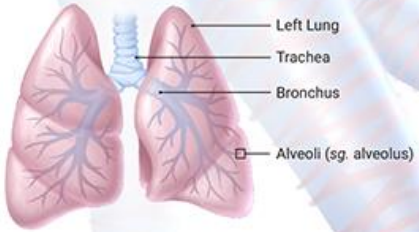


COVID-19

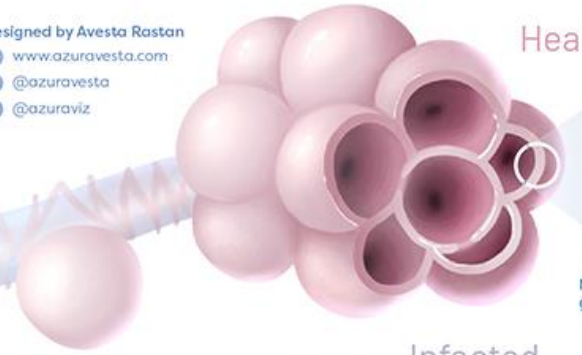
HOW DOES IT AFFECT YOU?

Designed by Avesta Rastan
www.azuravesta.com
@azuravesta
@azuraviz

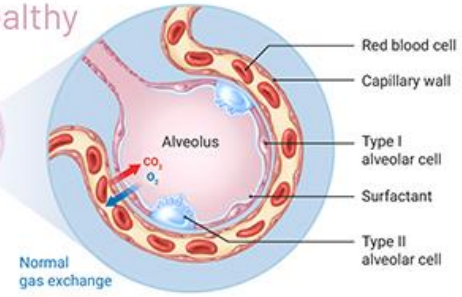
Coronavirus Disease 2019 (COVID-19) is a pandemic caused by Severe Acute Respiratory Syndrome Coronavirus 2, also called SARS-CoV-2. Despite the widespread awareness regarding COVID-19, many are still unaware about how it affects the human body.



SARS-CoV-2 starts its journey in the nose, mouth, or eyes and travels down to the alveoli in the lungs. Alveoli are tiny sacs of air where gas exchange occurs.



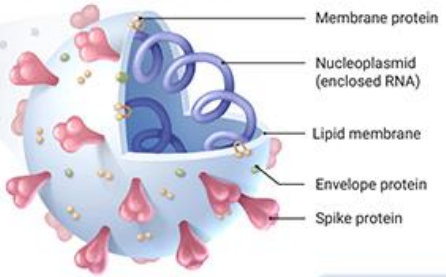
Healthy



Gas Exchange

Each sac of air, or alveolus, is wrapped with capillaries where red blood cells release **carbon dioxide** (CO_2) and pick up **oxygen** (O_2). Two alveolar cells facilitate gas exchange; **Type I** cells are thin enough that the oxygen passes right through, and **Type II** cells secrete **surfactant** – a substance that lines the alveolus and prevents it from collapsing.

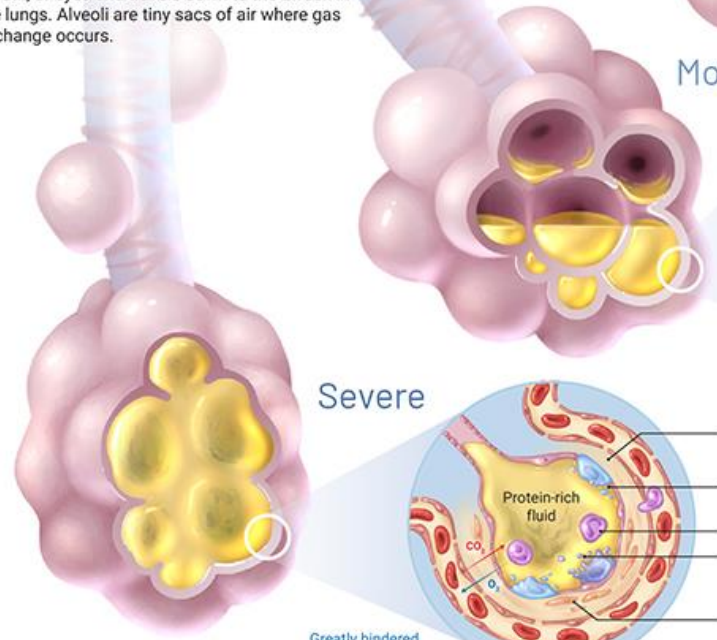
Infected



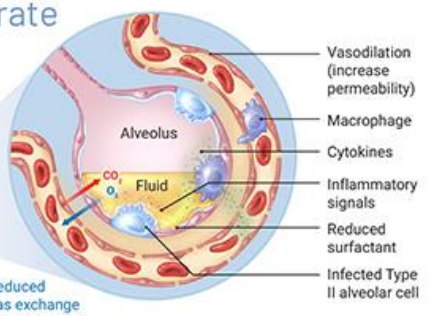
SARS-CoV-2 Structure

Viral Infection

The spike proteins covering the coronavirus bind ACE2 receptors primarily on type II alveolar cells, allowing the virus to inject its RNA. The RNA "hijacks" the cell, telling it to assemble many more copies of the virus and release them into the alveolus. The host cell is destroyed in this process and the new coronaviruses infect neighbouring cells.



Moderate



Severe

Impaired Gas Exchange

When the immune system attacks the area of infection it also kills healthy alveolar cells. This results in three things that hinder gas exchange:

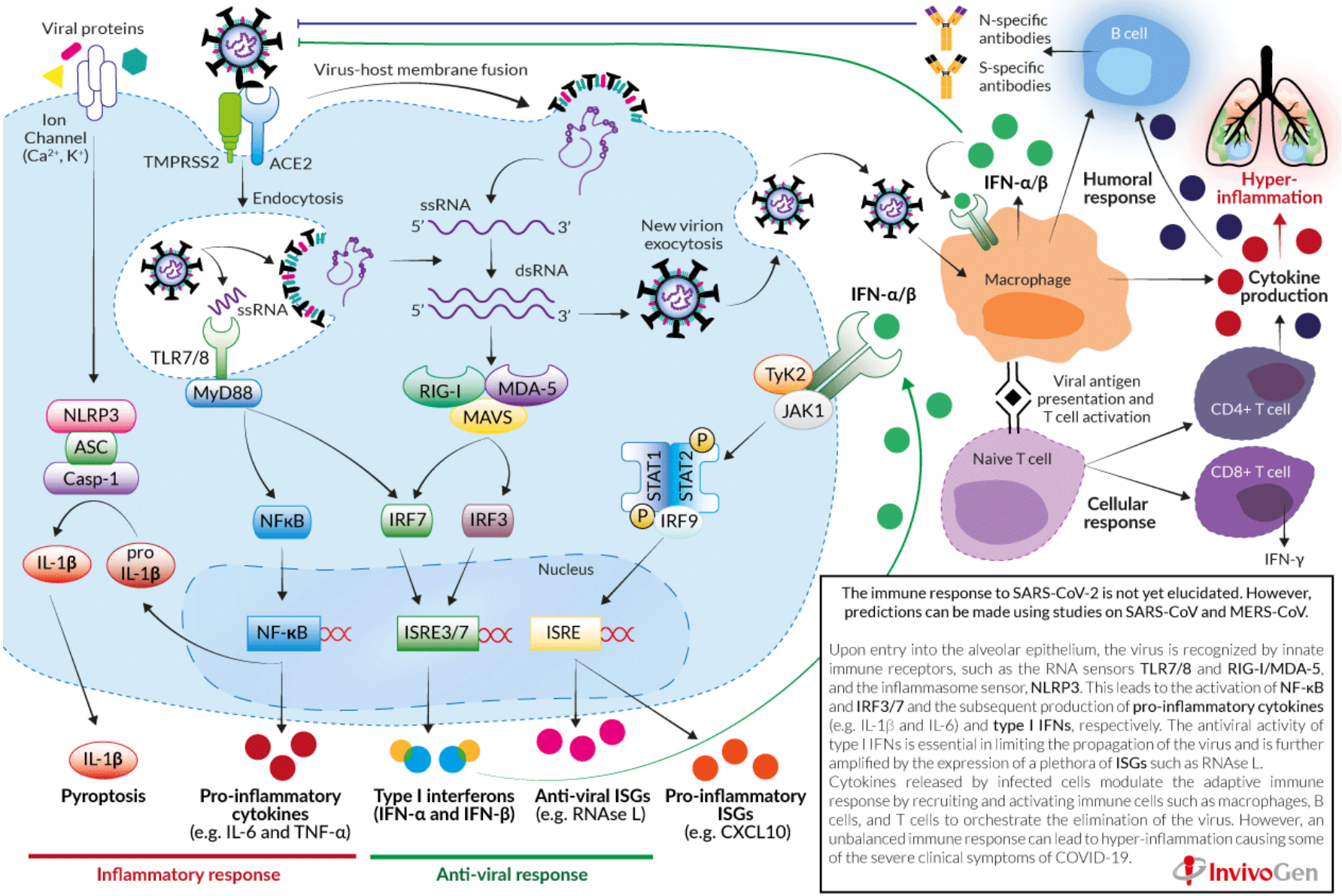
1) Alveolar collapse due to loss of surfactant from Type II cells
2) Less oxygen enters the blood-stream due to lack of Type I cells
3) More fluid enters the alveolus

Immune Response

1. After infection, Type II cells release **inflammatory signals** that recruit **macrophages** (immune cells).
2. Macrophages release **cytokines** that cause vasodilation, which allows more immune cells to come to the site of injury and exit the capillary.
3. Fluid accumulates inside the alveolus.
4. The fluid dilutes the surfactant which triggers the onset of alveolar collapse, decreasing gas exchange and increasing the work of breathing.
5. **Neutrophils** are recruited to the site of infection and release Reactive Oxygen Species (ROS) to destroy infected cells.
6. Type I and II cells are destroyed, leading to the collapse of the alveolus and causing **Acute Respiratory Distress Syndrome** (ARDS).
7. If inflammation becomes severe, the protein-rich fluid can enter the bloodstream and travel elsewhere in the body, causing **Systemic Inflammatory Response Syndrome** (SIRS).
8. SIRS may lead to **septic shock** and **multi-organ failure**, which can have fatal consequences.

https://www.visualcapitalist.com/visualizing-what-covid-19-does-to-your-body/

Predicted host immune responses to SARS-CoV-2



The immune response to SARS-CoV-2 is not yet elucidated. However, predictions can be made using studies on SARS-CoV and MERS-CoV.

Upon entry into the alveolar epithelium, the virus is recognized by innate immune receptors, such as the RNA sensors **TLR7/8** and **RIG-I/MDA-5**, and the inflammasome sensor, **NLRP3**. This leads to the activation of **NF-κB** and **IRF3/7** and the subsequent production of **pro-inflammatory cytokines** (e.g. IL-1β and IL-6) and **type I IFNs**, respectively. The antiviral activity of type I IFNs is essential in limiting the propagation of the virus and is further amplified by the expression of a plethora of **ISGs** such as RNAse L.

Cytokines released by infected cells modulate the adaptive immune response by recruiting and activating immune cells such as macrophages, B cells, and T cells to orchestrate the elimination of the virus. However, an unbalanced immune response can lead to hyper-inflammation causing some of the severe clinical symptoms of COVID-19.

InvivoGen

Tempestade de Citocinas

A fisiopatologia da SARS induzida SARS-CoV-2 induz a superprodução de citocinas pró-inflamatórias (fator de necrose tumoral [TNF] , IL-6 e IL-1β) denominada como **Tempestade de Citocinas**, levando a um risco aumentado de hiperpermeabilidade vascular, falência de órgãos e, eventualmente, morte quando as altas concentrações de citocinas não diminuem ao longo do tempo

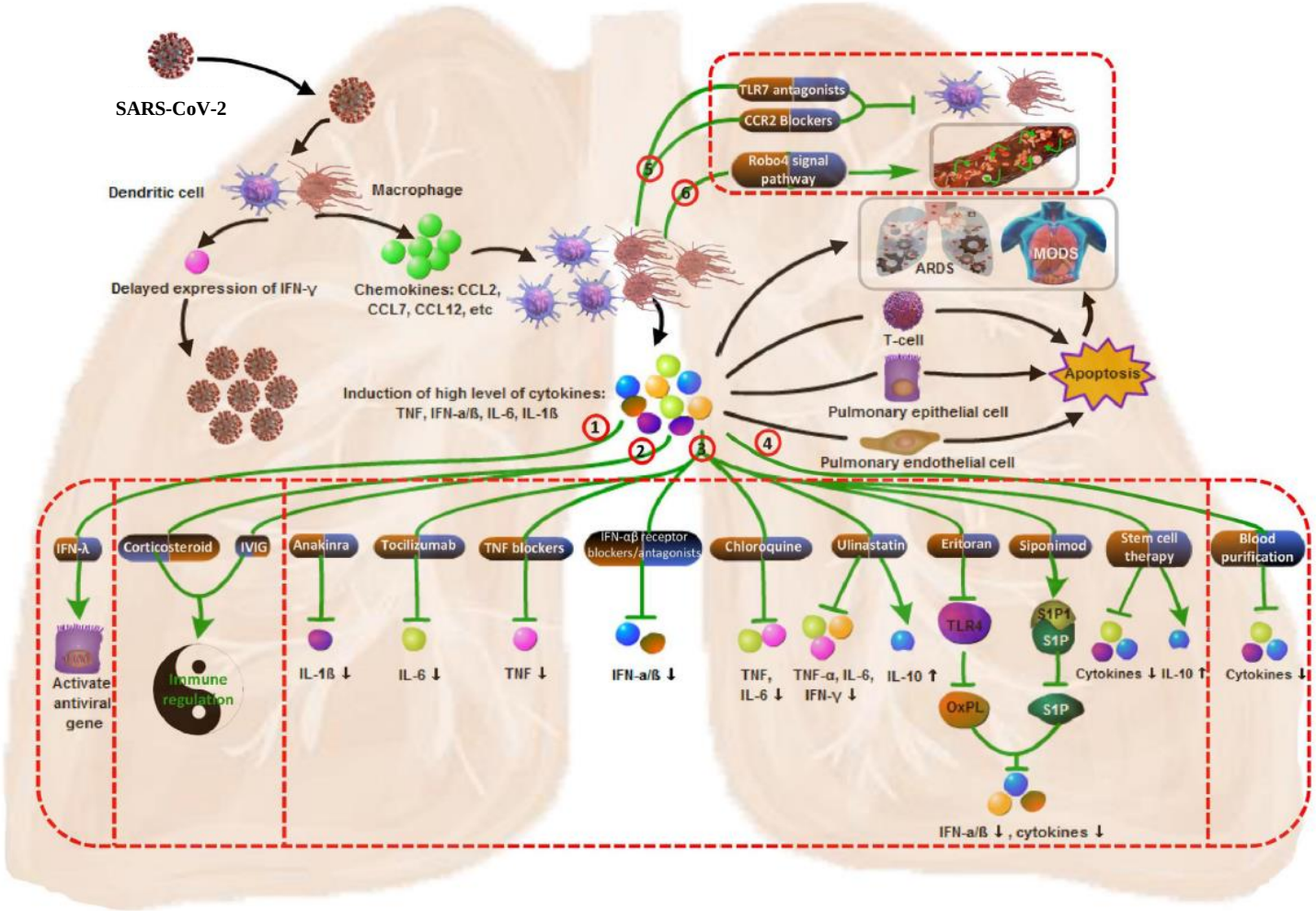
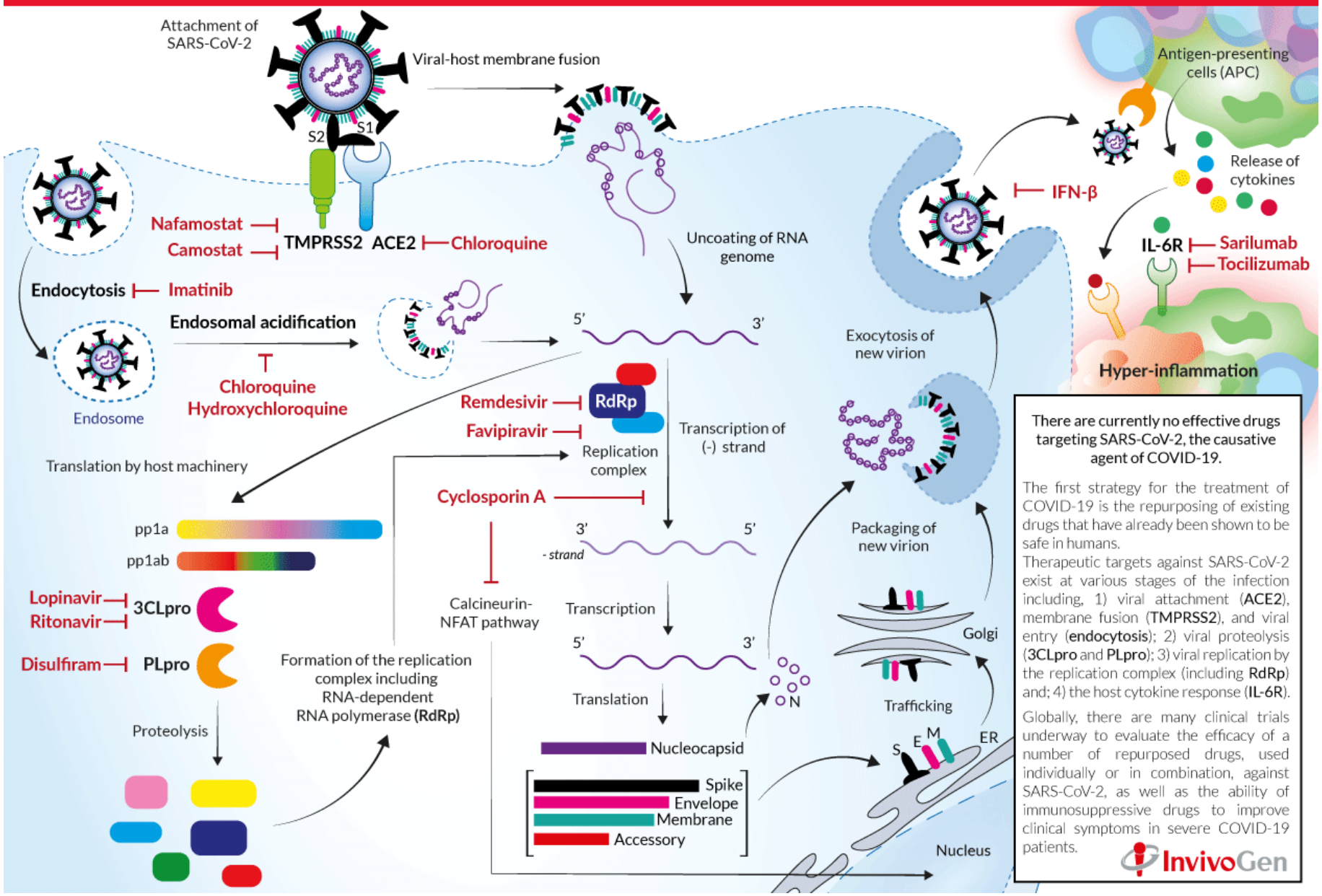


Fig. 1. Mechanism of cytokine storm in COVID-19 and potential therapy.
① Supplement with IFN-λ to activate the innate immunity; ② Using immunomodulator to restore immune balance; ③ Inhibiting the production of cytokines; ④ Scavenging cytokines; ⑤ Inhibiting mononuclear macrophage recruitment and function; ⑥ Strengthening the vascular barrier by activating of the endothelial Slit-Robo4 signal pathway.

doi: 10.1016/j.jinf.2020.03.037

Repurposing approved drugs for targeting SARS-CoV-2



There are currently no effective drugs targeting SARS-CoV-2, the causative agent of COVID-19.

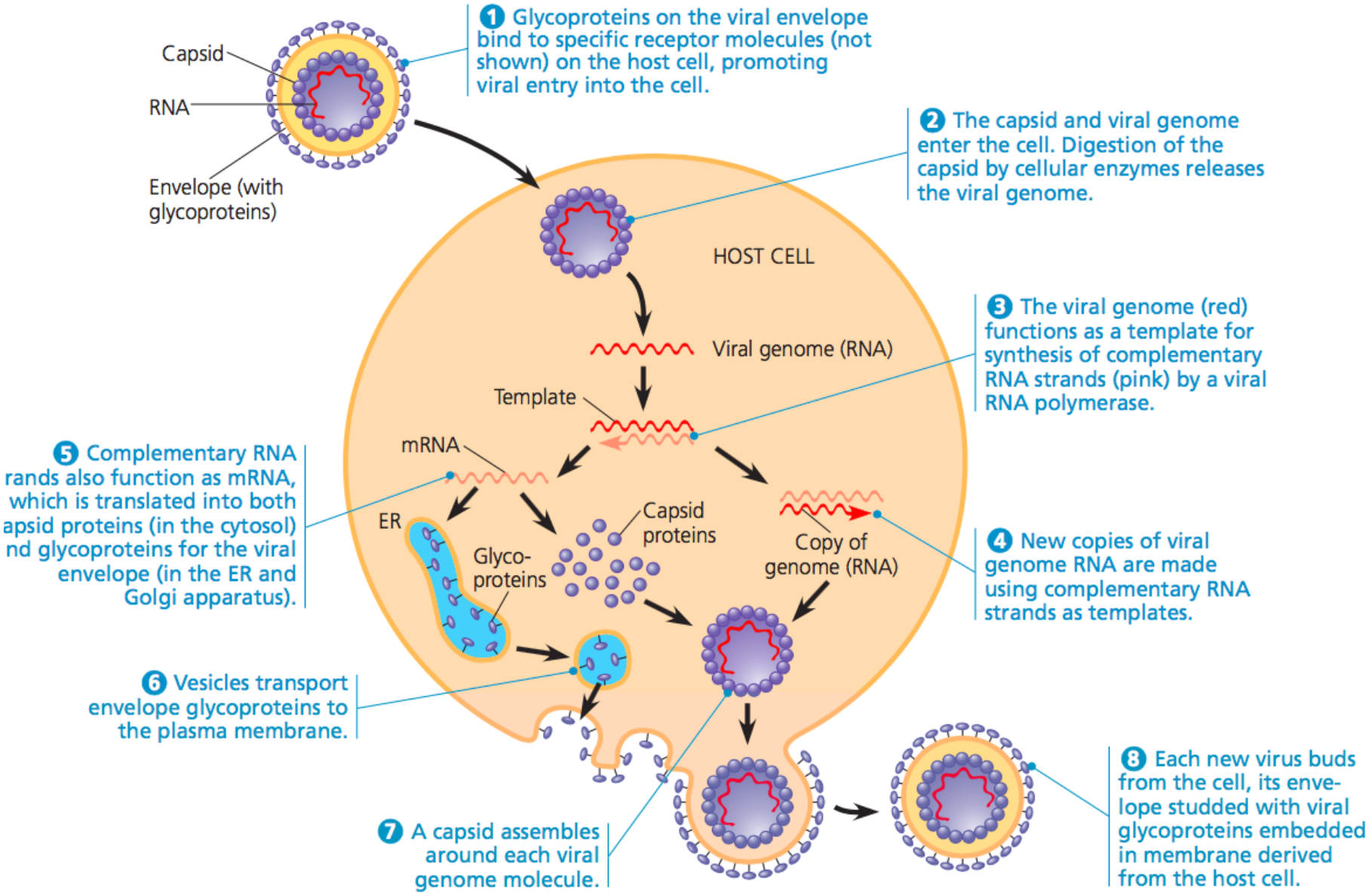
The first strategy for the treatment of COVID-19 is the repurposing of existing drugs that have already been shown to be safe in humans.

Therapeutic targets against SARS-CoV-2 exist at various stages of the infection including, 1) viral attachment (**ACE2**), membrane fusion (**TMPRSS2**), and viral entry (**endocytosis**); 2) viral proteolysis (**3CLpro** and **PLpro**); 3) viral replication by the replication complex (including **RdRp**) and; 4) the host cytokine response (**IL-6R**).

Globally, there are many clinical trials underway to evaluate the efficacy of a number of repurposed drugs, used individually or in combination, against SARS-CoV-2, as well as the ability of immunosuppressive drugs to improve clinical symptoms in severe COVID-19 patients.

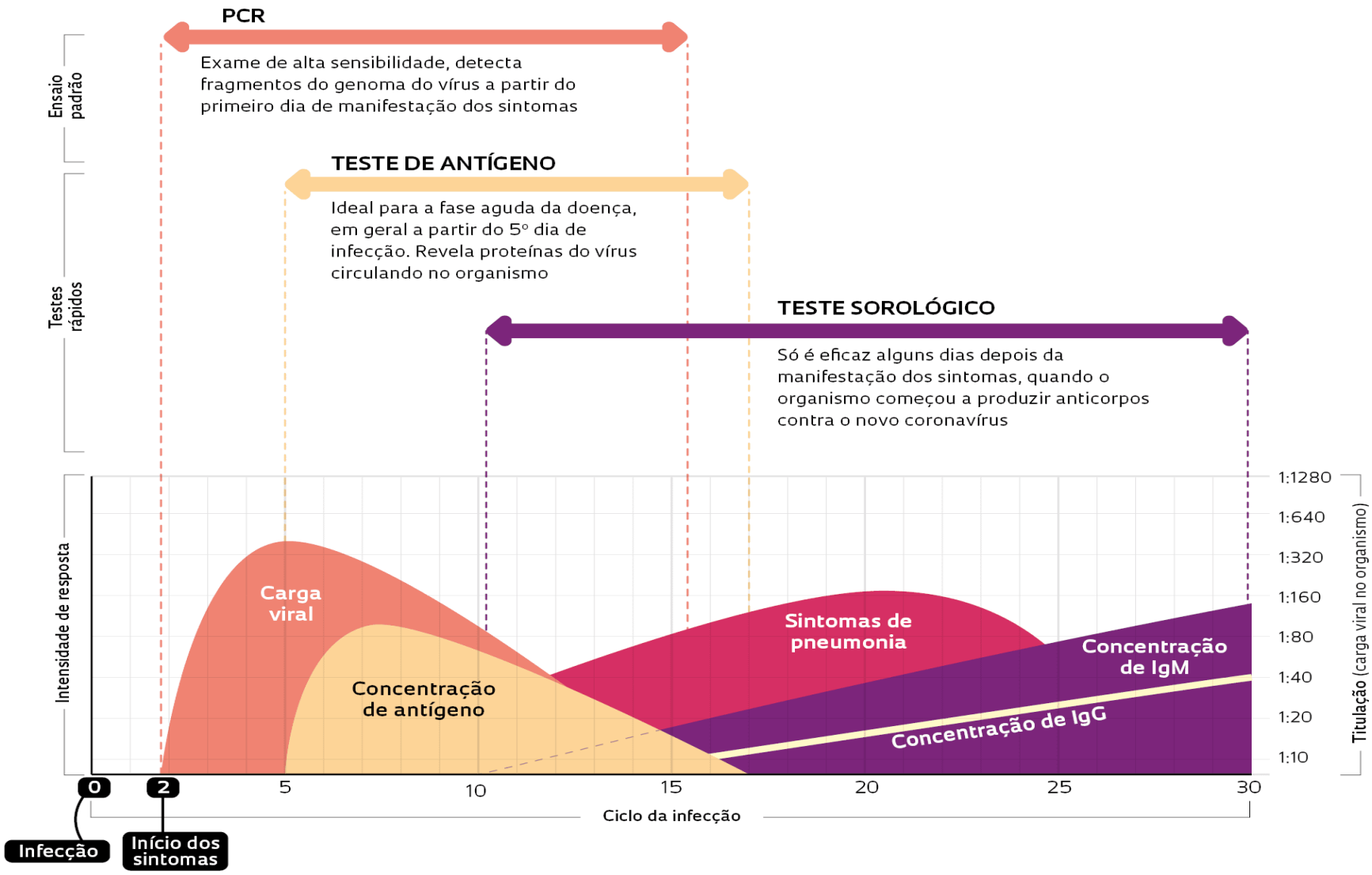
InvivoGen

Liberação/Brotamento



https://biology.stackexchange.com/

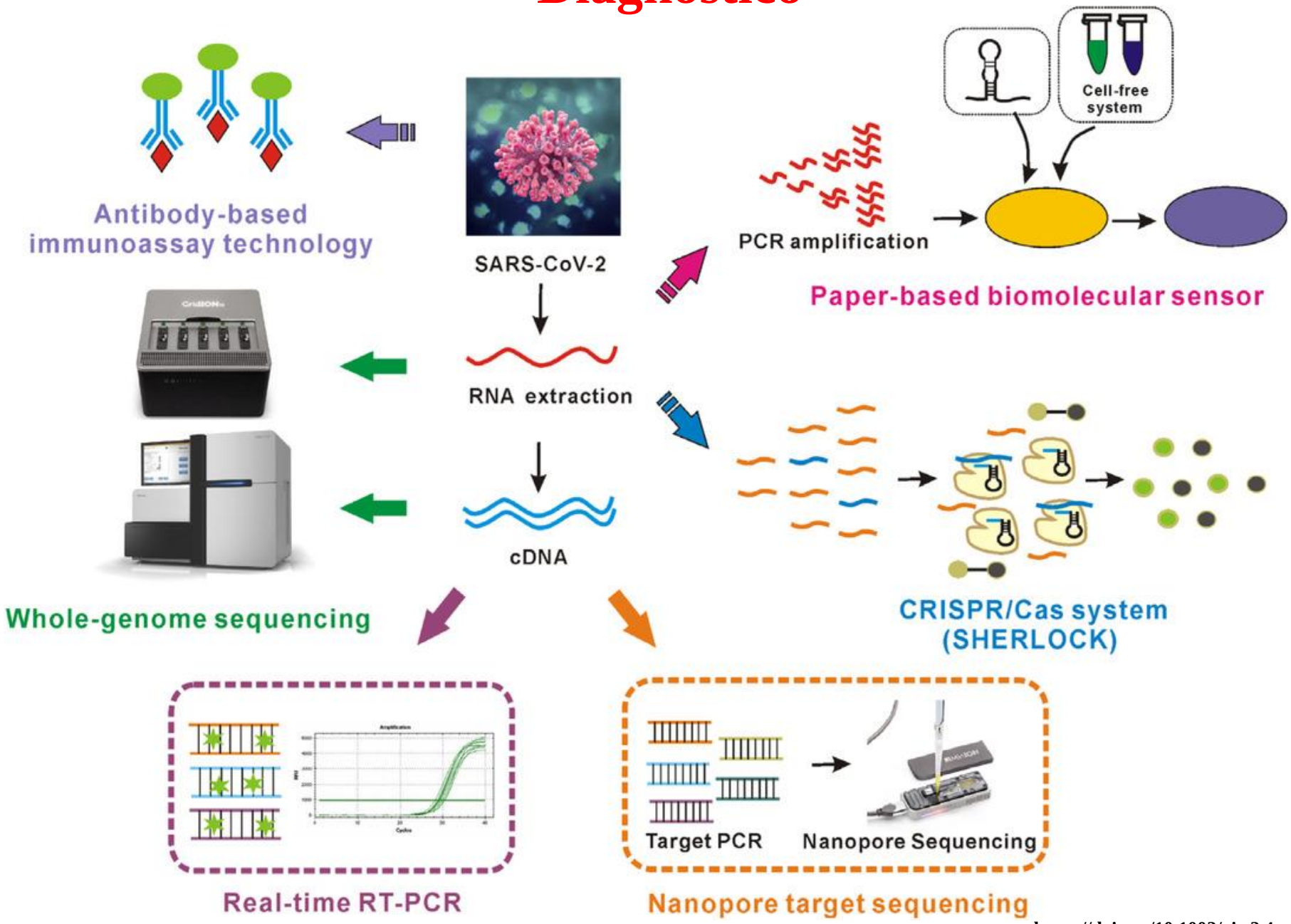
Diagnóstico



FONTE ECO DIAGNÓSTICA, UTILIZANDO COMO REFERÊNCIA O ARTIGO CELLULAR IMMUNE RESPONSES TO SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS (SARS-COV) INFECTION IN SENESCENT BALB/C MICE: CD4+ T CELLS ARE IMPORTANT IN CONTROL OF SARS-COV INFECTION

https://revistapesquisa.fapesp.br/2020/04/09/

Diagnóstico



https://doi.org/10.1002/viw2.4

Diagnóstico

	ENSAIO RT-PCR	EXAME SOROLÓGICO	TESTE DE ANTÍGENO
O que é	Sigla para transcrição reversa seguida de reação em cadeia de polimerase. Esse teste molecular é o exame padrão para detecção da doença no país. Revela a presença de fragmento do genoma do vírus na amostra coletada	Teste rápido que verifica a resposta imunológica do organismo ao vírus, detectando a presença dos anticorpos IgM e IgG	Exame rápido que demonstra a presença de proteínas próprias do vírus na amostra
Amostra	Secreções do fundo do nariz (nasofaringe) e da garganta (orofaringe)	Sangue, soro ou plasma sanguíneo	Secreções do fundo do nariz (nasofaringe) e da garganta (orofaringe)
Maior eficácia	A partir do primeiro dia de manifestação dos sintomas	A partir de alguns dias de manifestação dos sintomas, período em que o organismo já está produzindo anticorpos	Na fase aguda da doença, quando os sintomas começam a se manifestar
Onde é feito	Em laboratório, com uso de equipamento específico e mão de obra capacitada	Em hospitais, postos de saúde e unidades volantes, com aparelho portátil fácil de manusear	Em hospitais, postos de saúde e unidades volantes, com aparelho portátil fácil de manusear
Resultado	Cerca de 24 horas	Em até 30 minutos	Em até 30 minutos

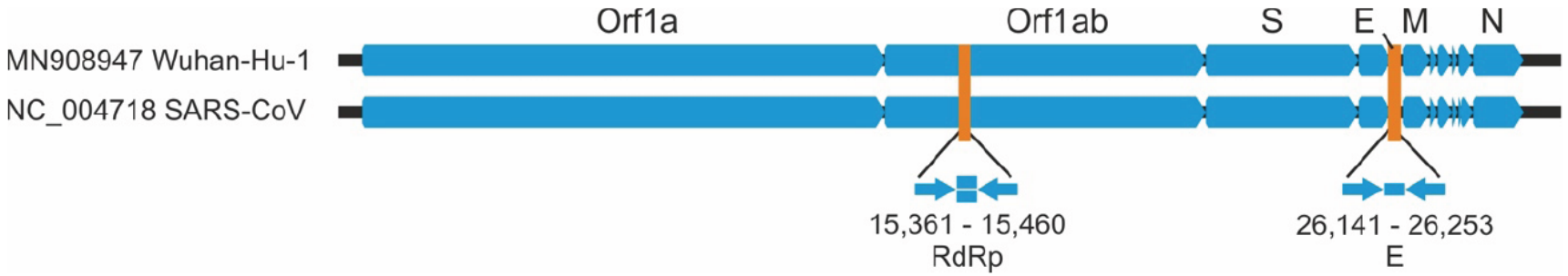
FONTES PESQUISADORES E EMPRESAS CONSULTADOS PELA REPORTAGEM

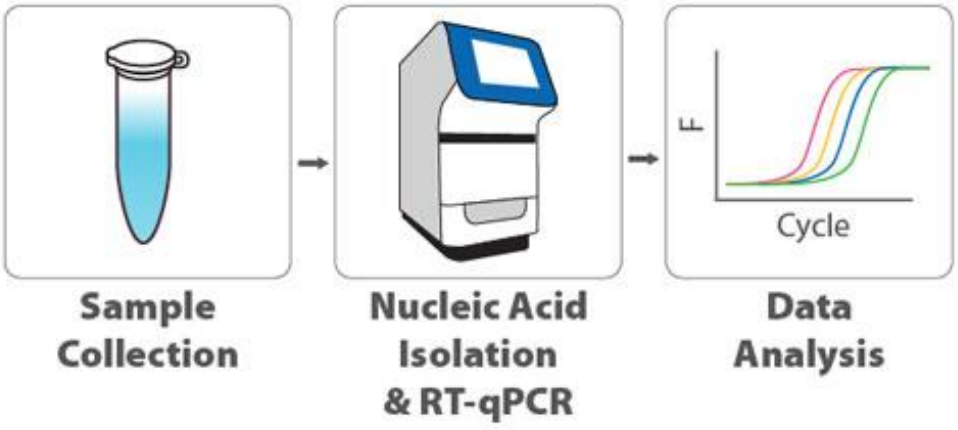
https://revistapesquisa.fapesp.br/2020/04/09/

Diagnóstico: RT-PCR

Protocolos já estabelecidos e reconhecidos (Protocolo de Berlim e Pasteur)
- **Extração do RNA** do material proveniente do Swab pelo método do Brazol
- RNA submetido a análise por PCR quantitativo (**qPCR ou PCR em Tempo Real**), usando um conjunto de três primers, em reações individuais, em duplicatas:

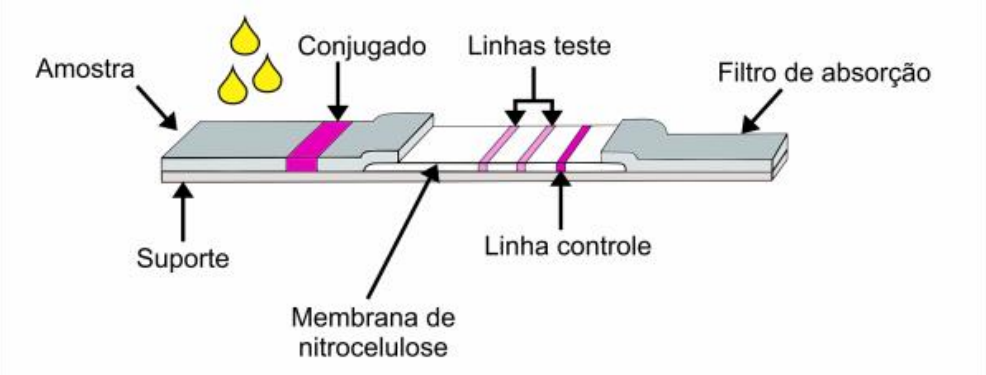
1) Primer para avaliação da presença de RNA humano (para a **RNAseP**), o qual indica a presença de células humanas nas amostras e o sucesso tanto da coleta quanto da **extração do RNA**. Deve ser positivo para todas as amostras.
2) Primer para o gene **E** do vírus. Deve ser **positivo** para amostras contendo RNA viral e para o controle positivo (RNA sintético do vírus).
3) Primer para o gene **RdRp** do vírus. Deve ser positivo para amostras contendo RNA viral e para o controle positivo, correspondendo a um teste **confirmatório** que pode discriminar entre outros coronavírus.

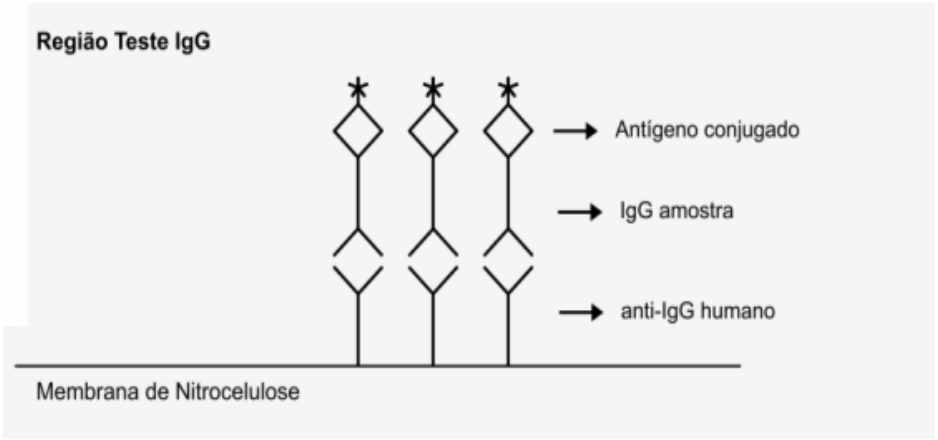


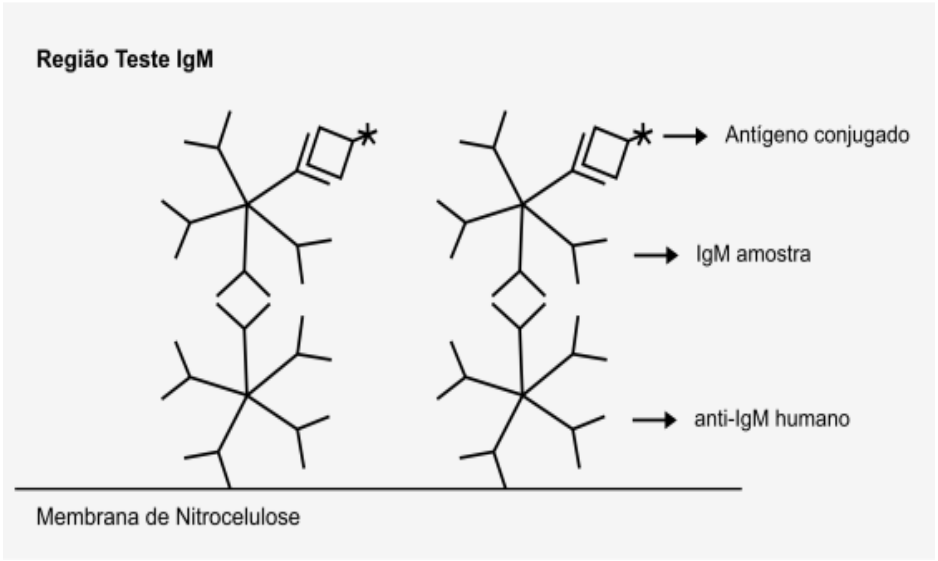


doi: 10.2807/1560-7917.ES.2020.25.3.2000045

Teste rápido: Sorologia



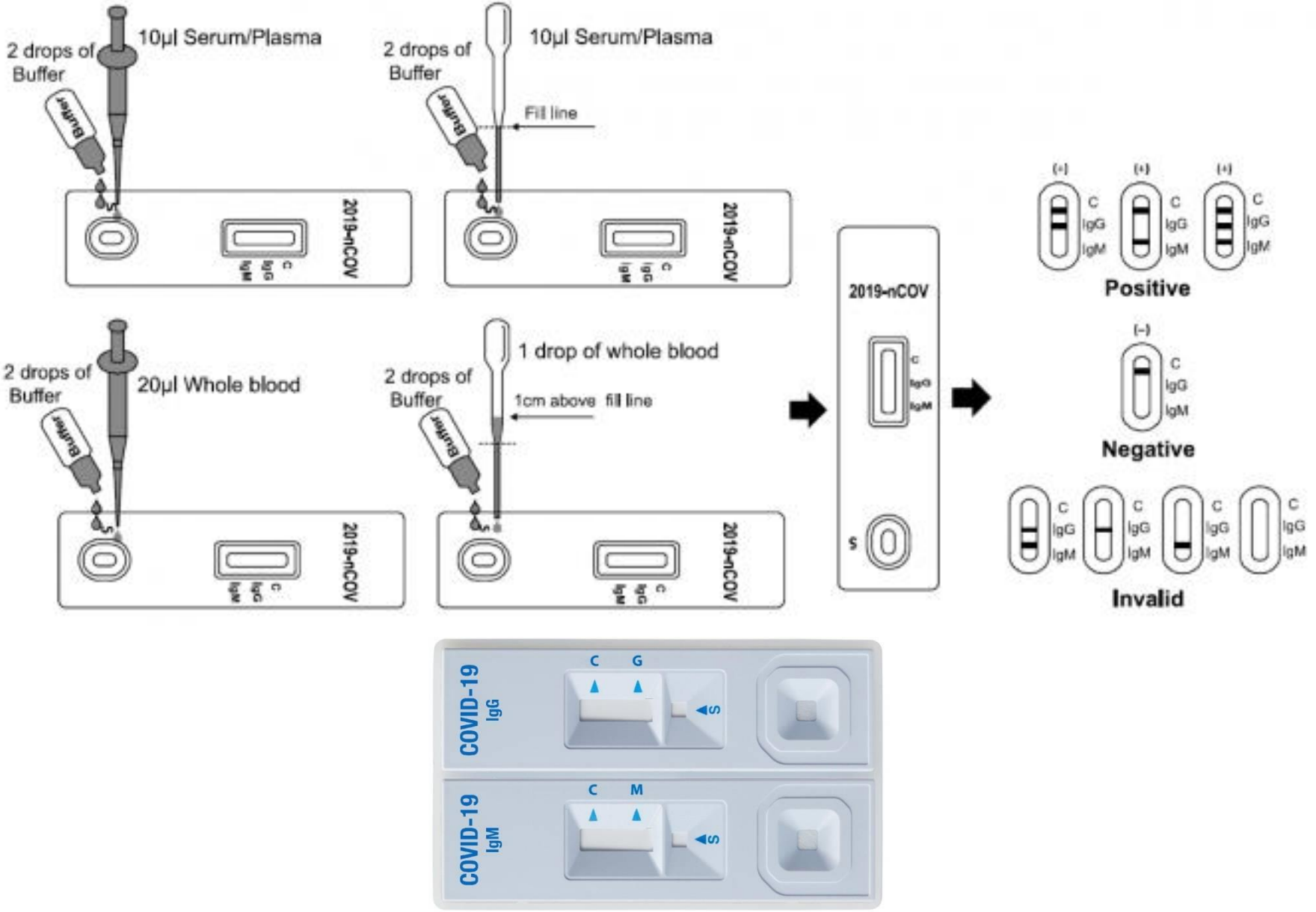




O princípio do teste consiste em anticorpos anti-IgG e anti-IgM humano imobilizados na membrana de nitrocelulose, nas regiões teste IgG e IgM, respectivamente.
O conjugado contém partículas de ouro coloidal ligadas aos antígenos recombinantes do COVID-19. Durante o teste, os anticorpos específicos anti-COVID-19 presentes na amostra interagem com o conjugado e migram cromatograficamente através da membrana.
Quando encontram as regiões teste, são imobilizados e formam uma linha colorida. A presença desta linha indica um resultado positivo e a sua ausência indica um resultado negativo. Independente do resultado, o ensaio é considerado válido desde que a linha controle apareça.

https://labtest.com.br/labtest-lanca-teste-rapido-para-covid-19/

Teste rápido: Sorologia



https://labtest.com.br/labtest-lanca-teste-rapido-para-covid-19/

Diagnóstico

Os novos testes aprovados pela Anvisa

A maioria deles é do tipo sorológico, que detecta anticorpos no sangue

SOROLÓGICOS

- One step Covid-2019 test
- Coronavírus rapid test
- Medteste coronavírus 2019-nCoV IgG/IgM
- Teste rápido em cassete (Covid-19) IgG/IgM
- Covid-19 IgG/IgM eco teste
- Coronavírus IgG/IgM (Covid-19)
- Anti Covid-19 IgG/IgM rapid test
- Lumiratek Covid-19 (IgG/IgM)
- Maglumi IgM de 2019-nCoV (CLIA)
- Maglumi IgG de 2019-nCoV (CLIA)
- DPP Covid-19 IgM/IgG System
- Smart test Covid-19 Vyttra

RT-PCR

- Família kit de detecção por PCR em tempo real viasure Sars-CoV2
- Família Cobas Sars-CoV2
- Família kit Xgen Master Covid-19

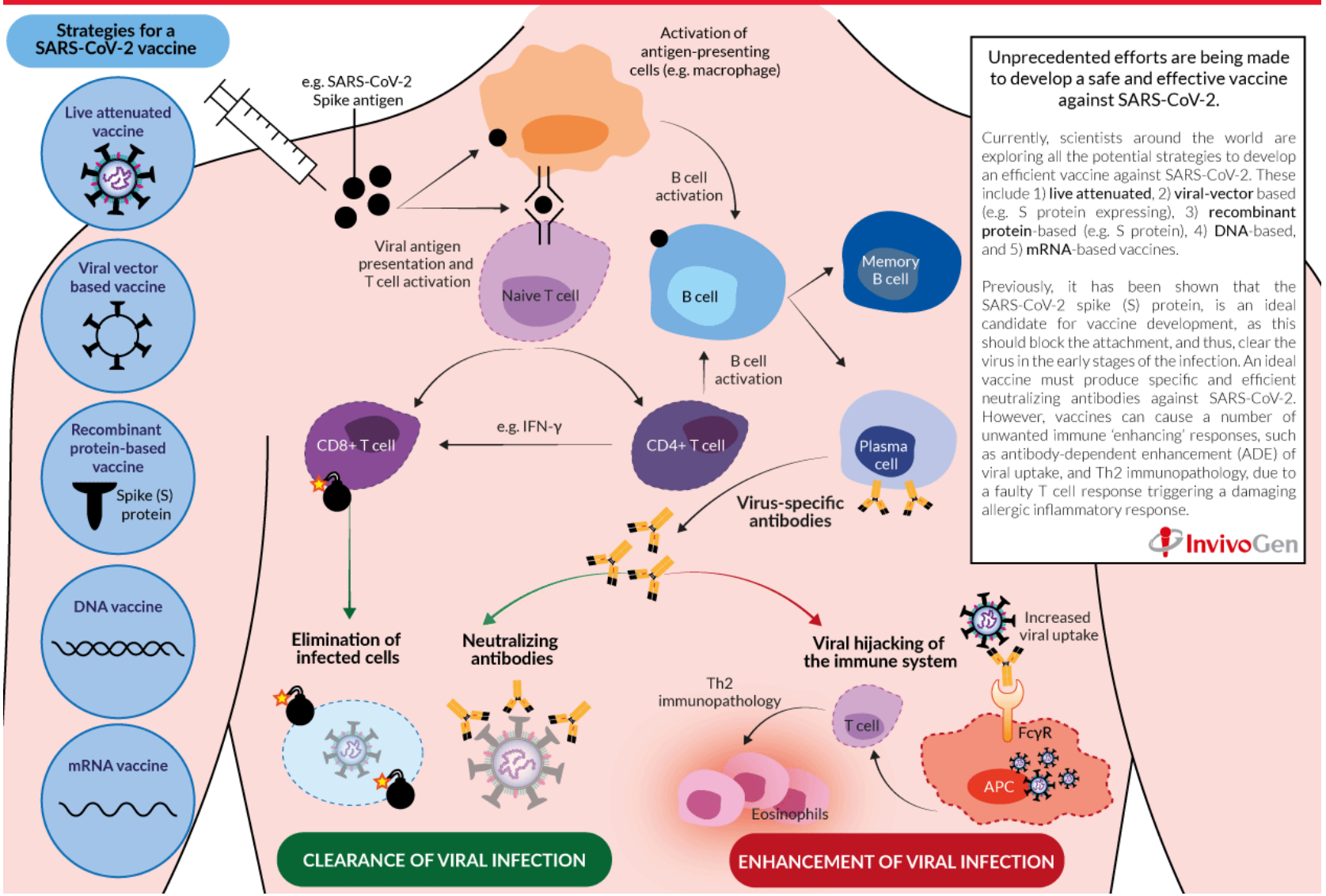
TESTE DE ANTÍGENO

- Eco F Covid-19 Ag
- Covid-19 Ag eco teste

OBS. ATÉ 1/4/2020
FONTE AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA (ANVISA)

https://revistapesquisa.fapesp.br/2020/04/09/a-importancia-de-testar-em-larga-escala/

Developing a successful vaccine against SARS-CoV-2



Unprecedented efforts are being made to develop a safe and effective vaccine against SARS-CoV-2.

Currently, scientists around the world are exploring all the potential strategies to develop an efficient vaccine against SARS-CoV-2. These include 1) **live attenuated**, 2) **viral-vector** based (e.g. S protein expressing), 3) **recombinant protein**-based (e.g. S protein), 4) **DNA**-based, and 5) **mRNA**-based vaccines.

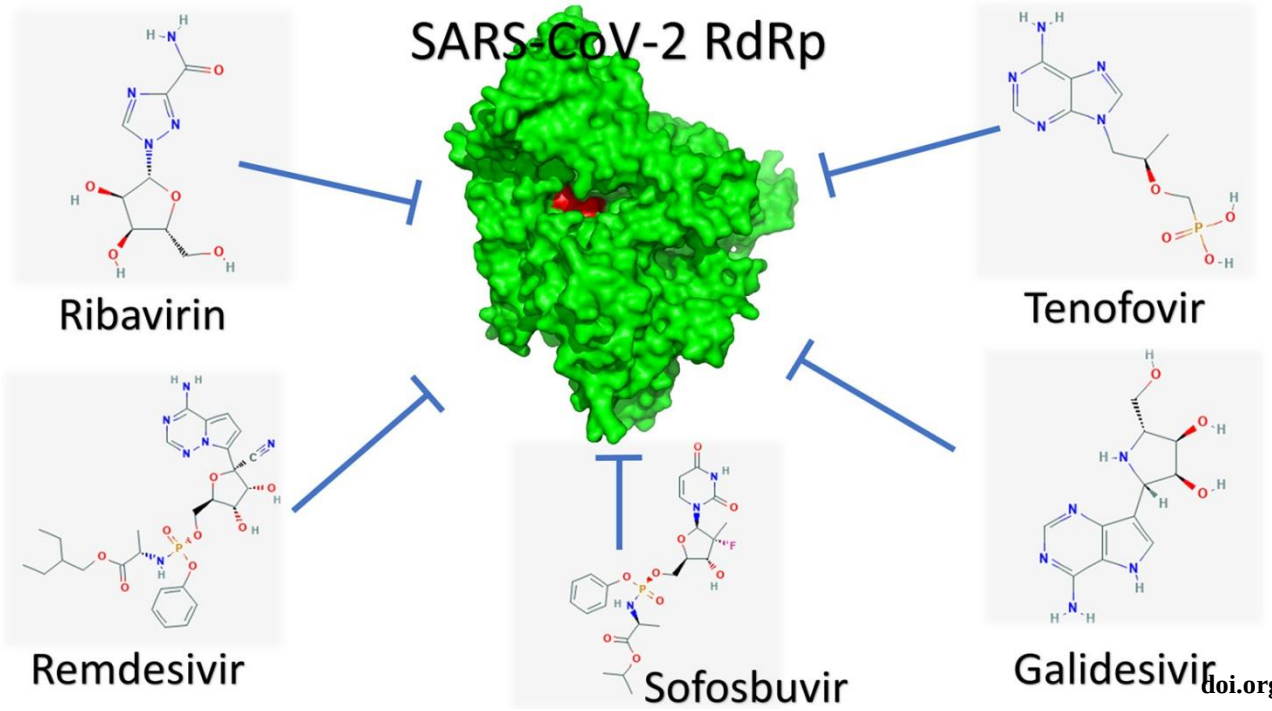
Previously, it has been shown that the SARS-CoV-2 spike (S) protein, is an ideal candidate for vaccine development, as this should block the attachment, and thus, clear the virus in the early stages of the infection. An ideal vaccine must produce specific and efficient neutralizing antibodies against SARS-CoV-2. However, vaccines can cause a number of unwanted immune 'enhancing' responses, such as antibody-dependent enhancement (ADE) of viral uptake, and Th2 immunopathology, due to a faulty T cell response triggering a damaging allergic inflammatory response.

InvivoGen

https://www.invivogen.com/spotlight-covid-19-vaccine-development

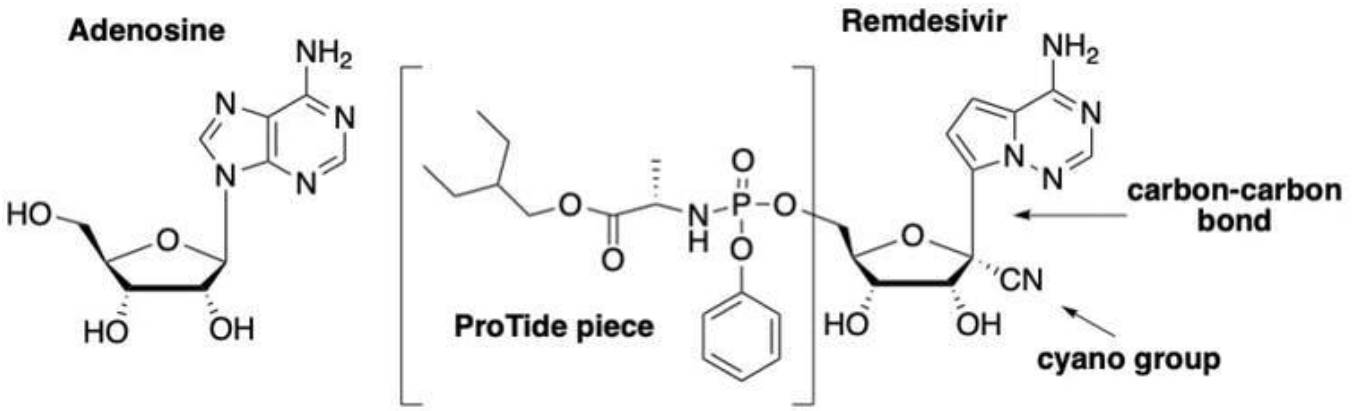
nsp12 RNA-dependent RNA polymerase (RdRp)

The SARS-CoV nsp12 is 932 a.a. in length and 106,7 kD; Complex of nsp8 and the nsp7-nsp8 complex within the viral RNA synthesis complex.

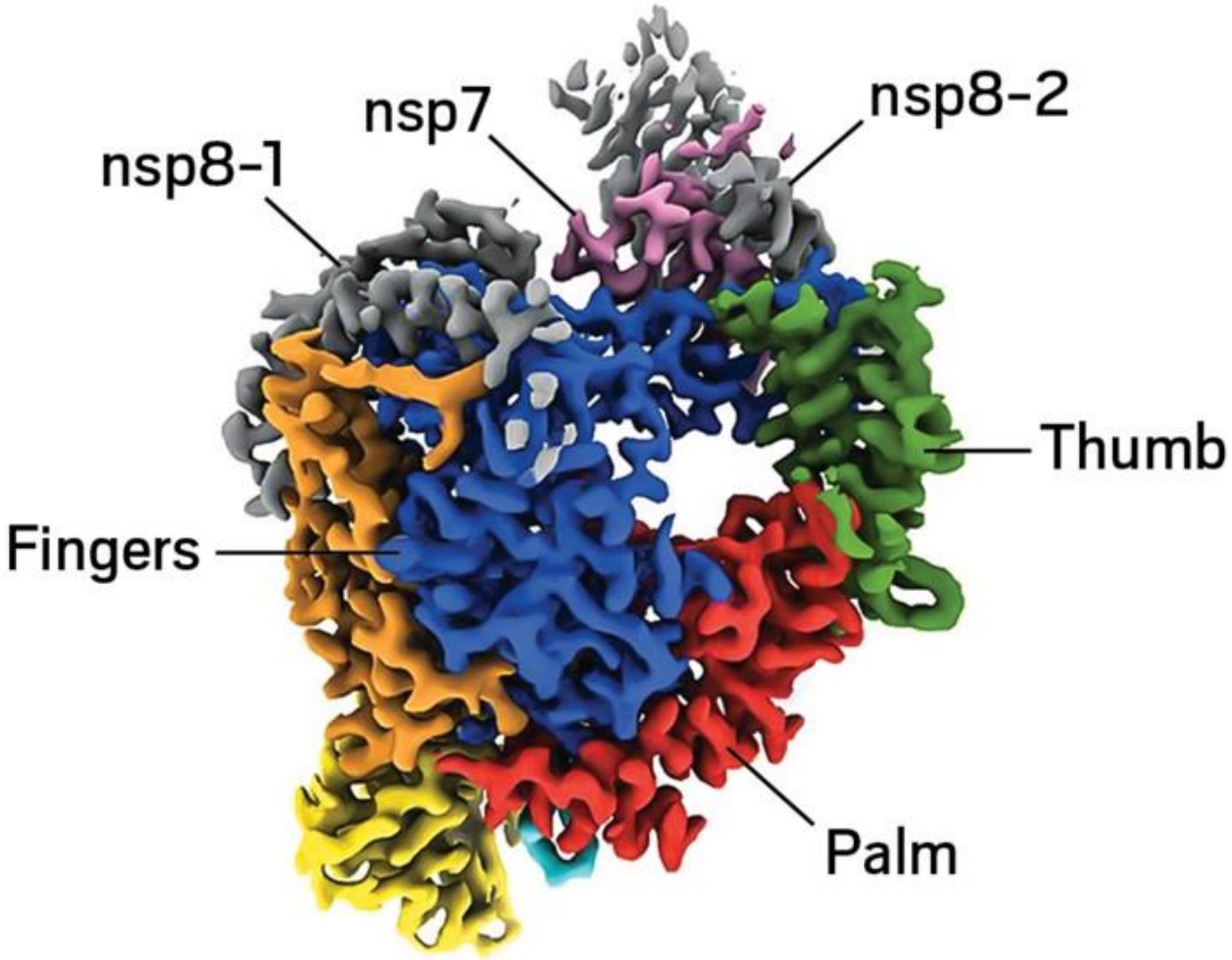


doi.org/10.1016/j.lfs.2020.117592

O **remdesivir** é incorporado na cadeia crescente de RNA, a presença do grupo CN faz com que o formato do açúcar fique deformado, o que, por sua vez, distorce o formato da cadeia de RNA, de forma que apenas mais três nucleotídeos possam ser adicionados. Isso termina a síntese da cadeia de RNA e a replicação do vírus.



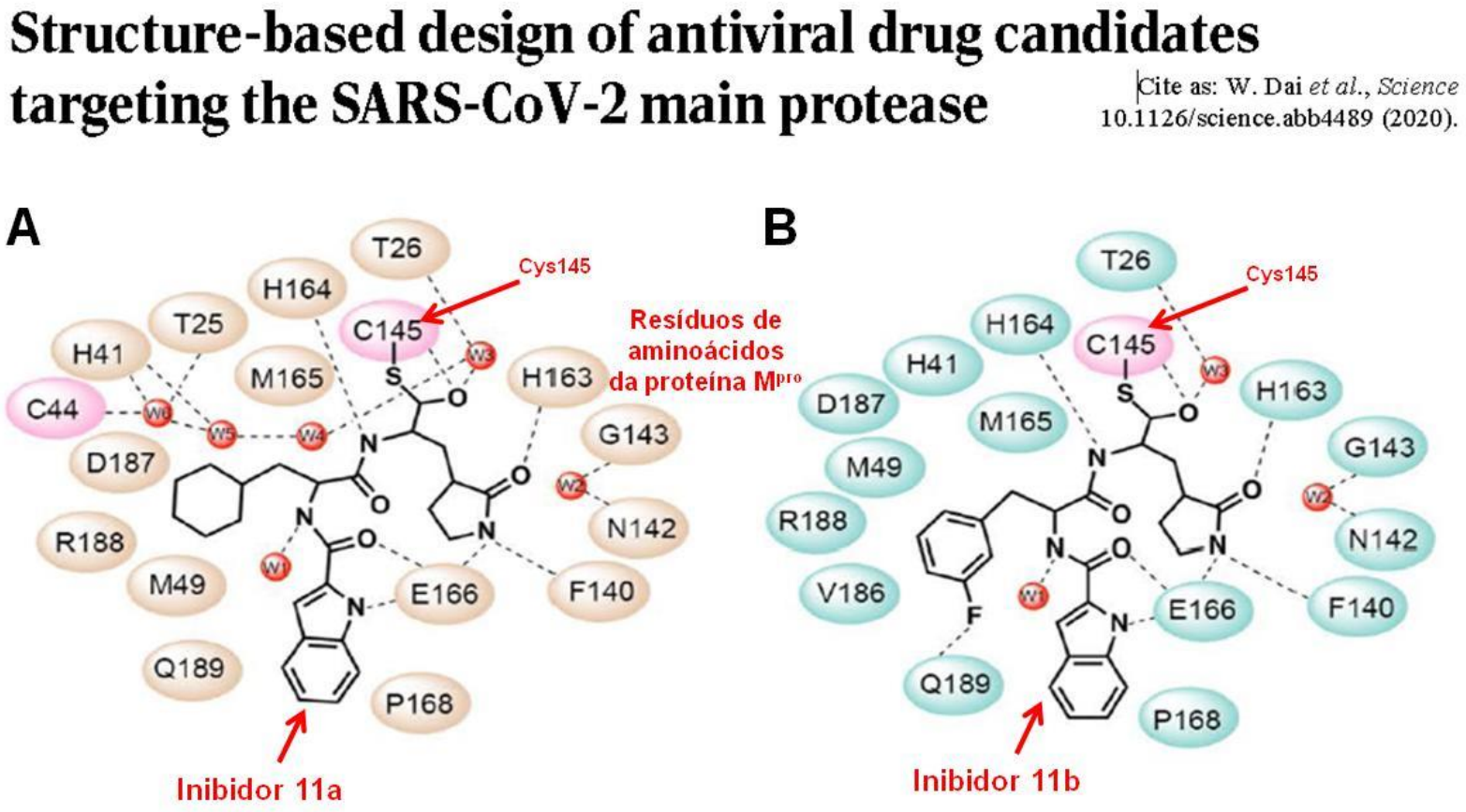
doi.org/10.1038/s41467-019-10280-3



The RNA-dependent RNA polymerase of the novel coronavirus is a complex that includes the small proteins nsp7 and nsp8. (*Science* 2020 10.1126/science.abb7498).

Nsp5 – Protease (3C-like main protease, M^{pro}, $3CL^{pro}$)

ORF1ab encodes replicase polyprotein 1 ab. After cleaved by two proteases (3CLpro and Plpro), replicase proteins showed multifunction involved in transcription and replication of viral RNAs. PLpro cleaves three sites at the N-terminus and 3CLpro cuts at the other 11 sites at the C-terminus, and forming 15 non-structural proteins. Among them, Nsp3 is proteolytic enzyme Plpro; Nsp5 is 3CLpro, Nsp12 is an RdRp, and Nsp13 is helicase. One crystal structure of 3CLpro has been deposited in PDB (pdb code: 6LU7).



Ligação dos Inibidores 11a (A) e 11b (B) na Protease M^{pro} de SARS-Cov-2. Mostra que o grupo aldeído de 11a e 11b ligam na Cys145 de M^{pro}, apresentando boa atividade inibitória e contra infecção por SARS-CoV-2 *in vitro*.

(Adaptado de: DOI: 10.1126/science.abb4489)

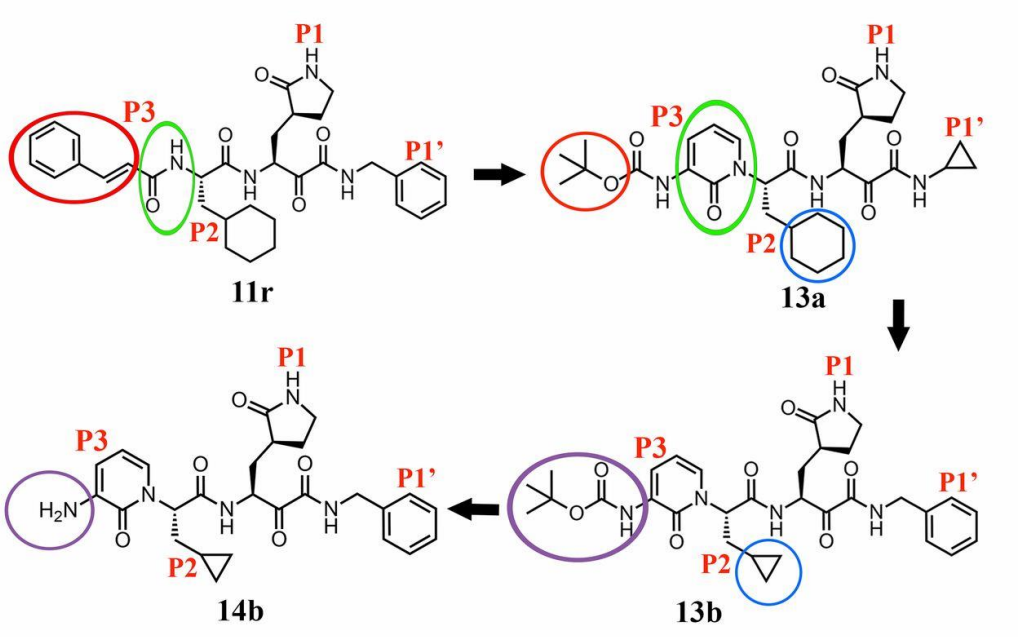
Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors

Linlin Zhang[1,2], Daizong Lin[1,3], Xinyuanyuan Sun[1,2], Ute Curth[4], Christian Drosten[5], Lucie Sauerhering[6,7], Stephan Becker[6,7],...
+ See all authors and affiliations

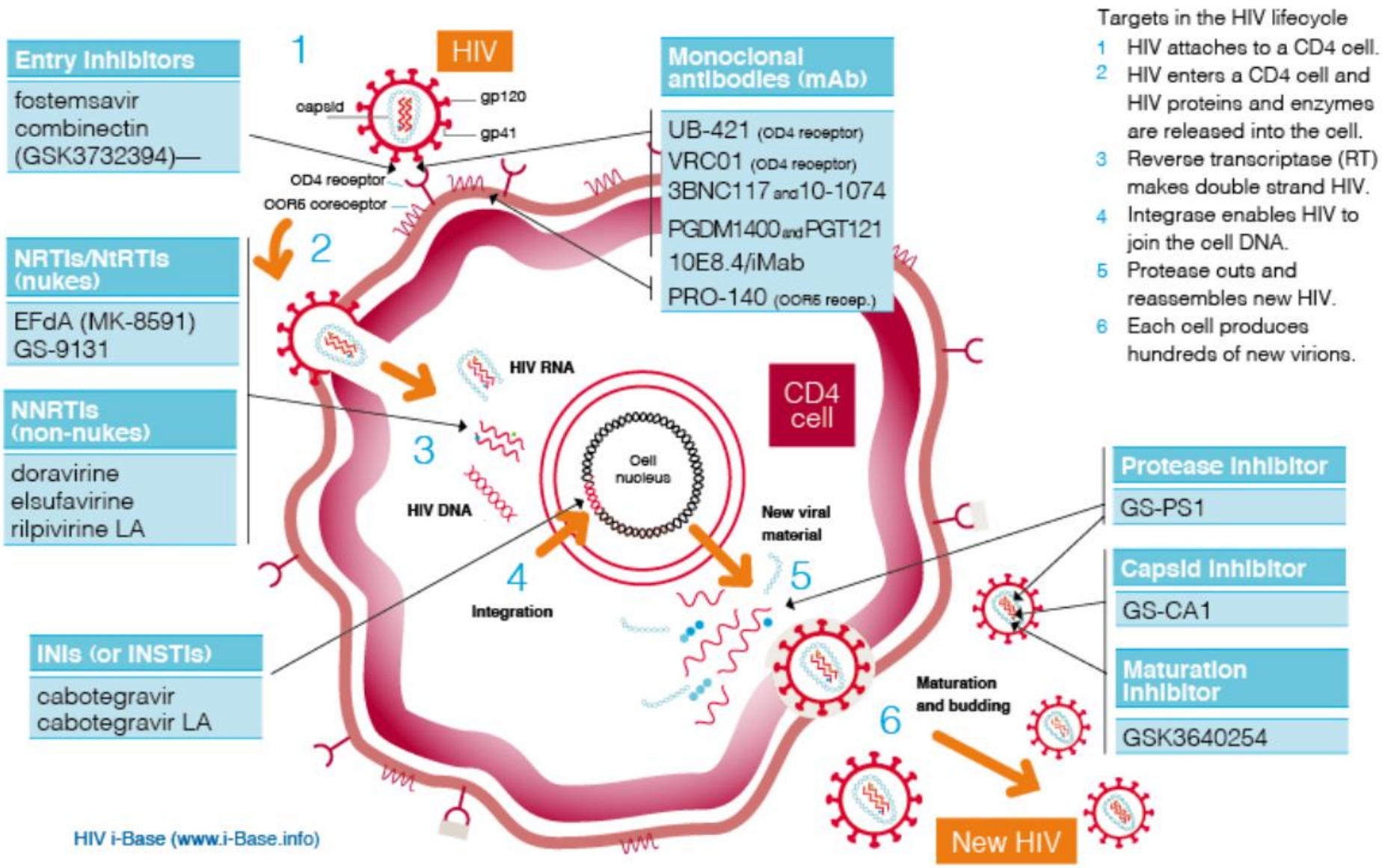
Science 20 Mar 2020:
eabb3405
DOI: 10.1126/science.abb3405

Abstract

An attractive drug target among coronaviruses is the main protease (M^{pro}, $3CL^{pro}$), due to its essential role in processing the polyproteins that are translated from the viral RNA. We report the X-ray structures of the unliganded SARS-CoV-2 M^{pro} and its complex with an α-ketoamide inhibitor. This was derived from a previously designed inhibitor but with the P3-P2 amide bond incorporated into a pyridone ring to enhance the half-life of the compound in plasma. Based on the structure, we developed the lead compound into a potent inhibitor of the SARS-CoV-2 M^{pro}. The pharmacokinetic characterization of the optimized inhibitor reveals a pronounced lung tropism and suitability for administration by the inhalative route.

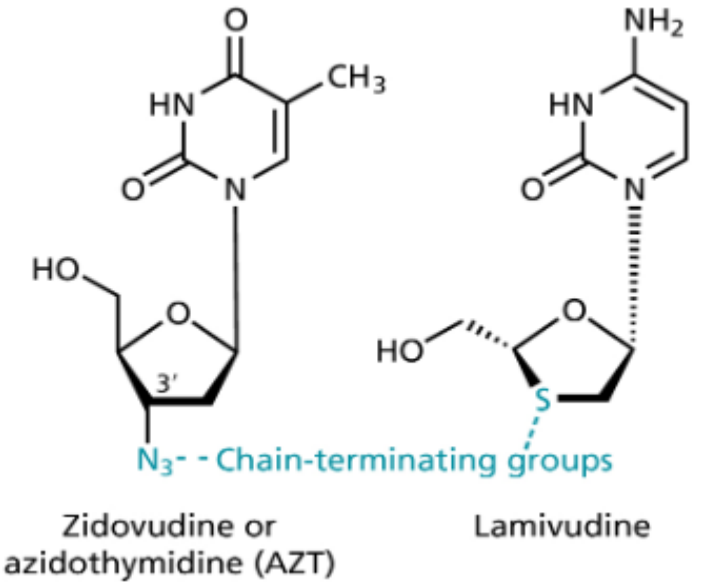


Antivirais



http://i-base.info/htb/wp-content/uploads/2018/07/PIPELINE-2018-full-version.pdf

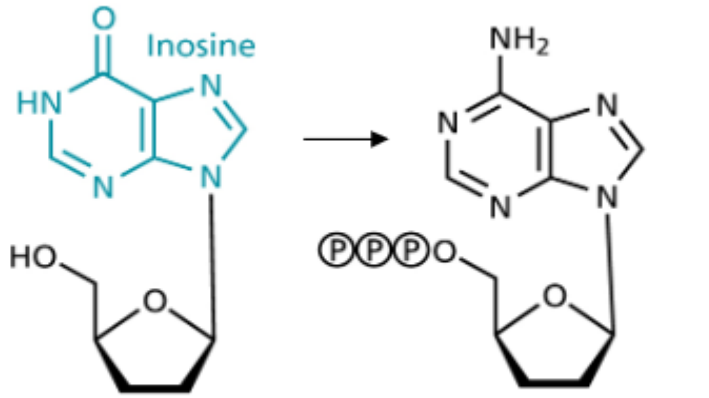
Nucleosídeos Inibidores de Transcriptase reversa (NRTIs)



Zidovudine or azidothymidine (AZT)

Lamivudine

→ Também utilizada para tratamento da hepatite B



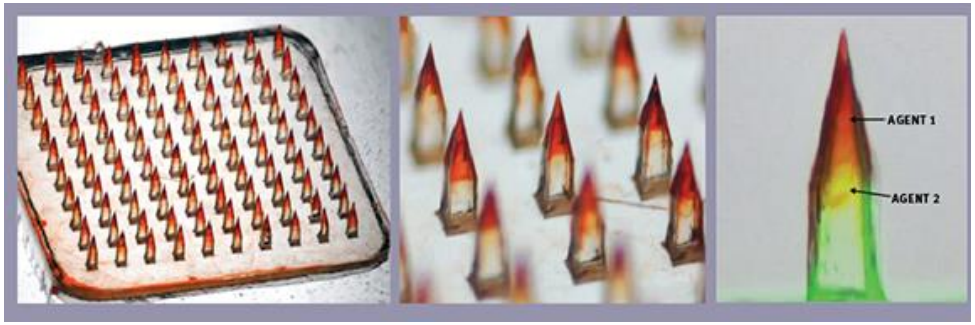
Didanosine

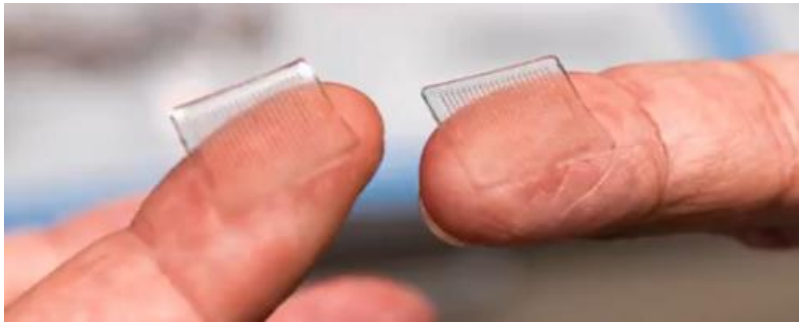
2′,3′-Dideoxyadenosine triphosphate

April 2020: SARS-CoV-2 Coronavirus Vaccine News

- April 2, 2020 - University of Pittsburgh School of Medicine scientists today announced a potential vaccine against SARS-CoV-2, the new coronavirus causing the COVID-19 pandemic. When tested in mice, the vaccine, delivered through a fingertip-sized patch, produces antibodies specific to SARS-CoV-2 at quantities thought to be sufficient for neutralizing the virus. The paper appeared today in EBioMedicine, which is published by The Lancet, and is the first study to be published after critique from fellow scientists at outside institutions that describes a candidate vaccine for COVID-19.

- April 2, 2020 – Applied DNA Sciences Inc. and Takis Biotech announced an expansion of their COVID-19 vaccine development program to include a 5th vaccine candidate. Production of all vaccine candidates is expected to be completed this month. All vaccine candidates have also been approved by Italy's Ministry of Health for preclinical animal testing that is scheduled to begin in late April 2020.





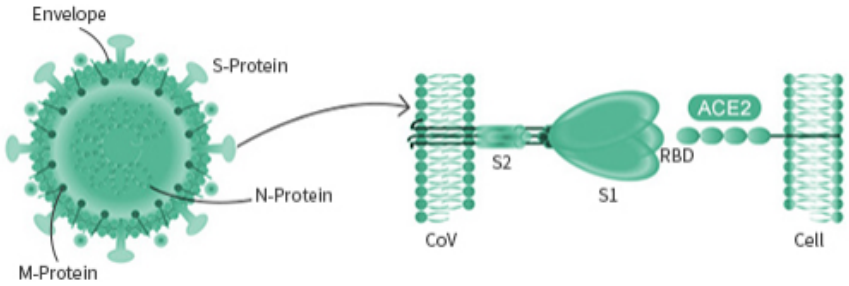
https://www.coronavirustoday.com/coronavirus-vaccine-candidates

Human Coronavirus Antigens

CoV Antigens	Description
Spike	Receptor binding and membrane fusion Target for antiviral treatment and vaccines
Nucleocapsid	Genome replication and cell signaling regulation A diagnostic marker
HE	Receptor interaction
PIpro	Viral polyprotein cleavage and host innate immune response blockage; Target for drugs development
3CLPro	Polypeptides cleavage and IFN signaling inhibition Target for drugs development
E	Assembly and release of the virus Vaccine candidates; Target for drugs development
M	Membrane and virion structure

Host Receptor of Human Coronavirus

HCoV Types	Host receptors
HCoV-229E	APN (aminopeptidase N, CD13)
HCoV-NL63	ACE2 (angiotensin-converting enzyme 2)
HCoV-HKU1	O-ac Sia
HCoV-OC43	O-ac Sia
MERS-CoV	DPP4 (dipeptidyl peptidase 4)
SARS-CoV	ACE2 (angiotensin-converting enzyme 2)
SARS-CoV-2	ACE2 (angiotensin-converting enzyme 2)



Prevenção

Como se proteger do novo coronavírus

Lave as mãos com frequência com água e sabão e use álcool gel 70%, principalmente se:

Tossir ou espirrar.

Após cuidar de pessoas.

Depois de ir ao banheiro.

Antes e depois de comer.

No convívio social:

Não compartilhe objetos de uso pessoal, como talheres, pratos, copos e escovas de dente.

Mantenha os ambientes bem arejados.

Evite contato próximo de pessoas com febre ou tosse.

Fuja de locais com aglomeração de pessoas.

Evite beijos, abraços e apertos de mão.

Se for tossir ou espirrar:

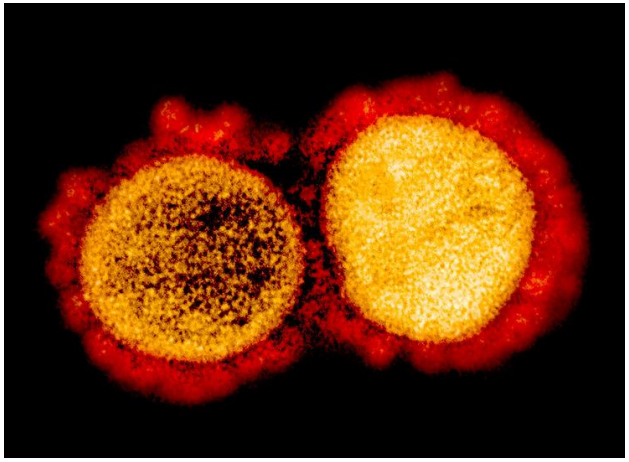
Cubra o nariz e a boca. Use os braços ou lenço descartável. Evite usar as mãos. Se usar, lembre-se de higienizá-las.

Se usar um lenço, jogue-o fora imediatamente.

Evite tocar os olhos, nariz e boca.

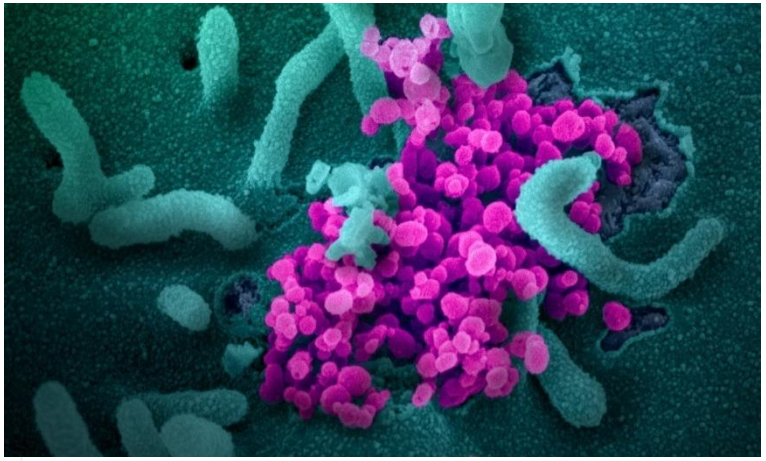
Obrigatório uso de Mascara

https://eephcfmusp.org.br/portal/wp-content/uploads/2020/05/e-book-covid-19.pdf



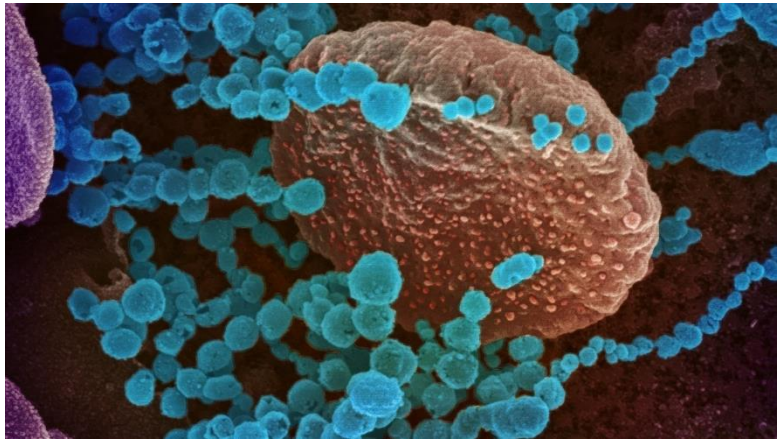
Novel Coronavirus SARS-CoV-2

Transmission electron micrograph of SARS-CoV-2 virus particles, isolated from a patient. Image captured and color-enhanced at the NIAID Integrated Research Facility (IRF) in Fort Detrick, Maryland. Credit: NIAID



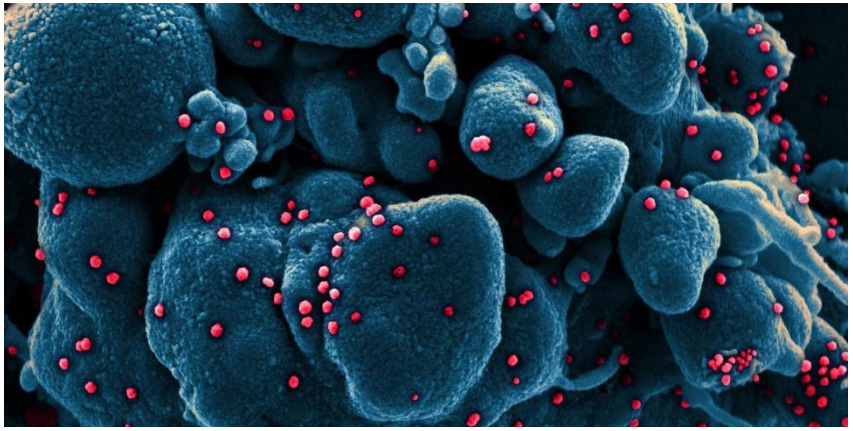
Novel Coronavirus SARS-CoV-2

This scanning electron microscope image shows SARS-CoV-2 (round magenta objects) emerging from the surface of cells cultured in the lab. SARS-CoV-2, also known as 2019-nCoV, is the virus that causes COVID-19. The virus shown was isolated from a patient in the U.S. Credit: NIAID-RML



Novel Coronavirus SARS-CoV-2

This scanning electron microscope image shows SARS-CoV-2 (round blue objects) emerging from the surface of cells cultured in the lab. SARS-CoV-2, also known as 2019-nCoV, is the virus that causes COVID-19. The virus shown was isolated from a patient in the U.S. Credit: NIAID-RML

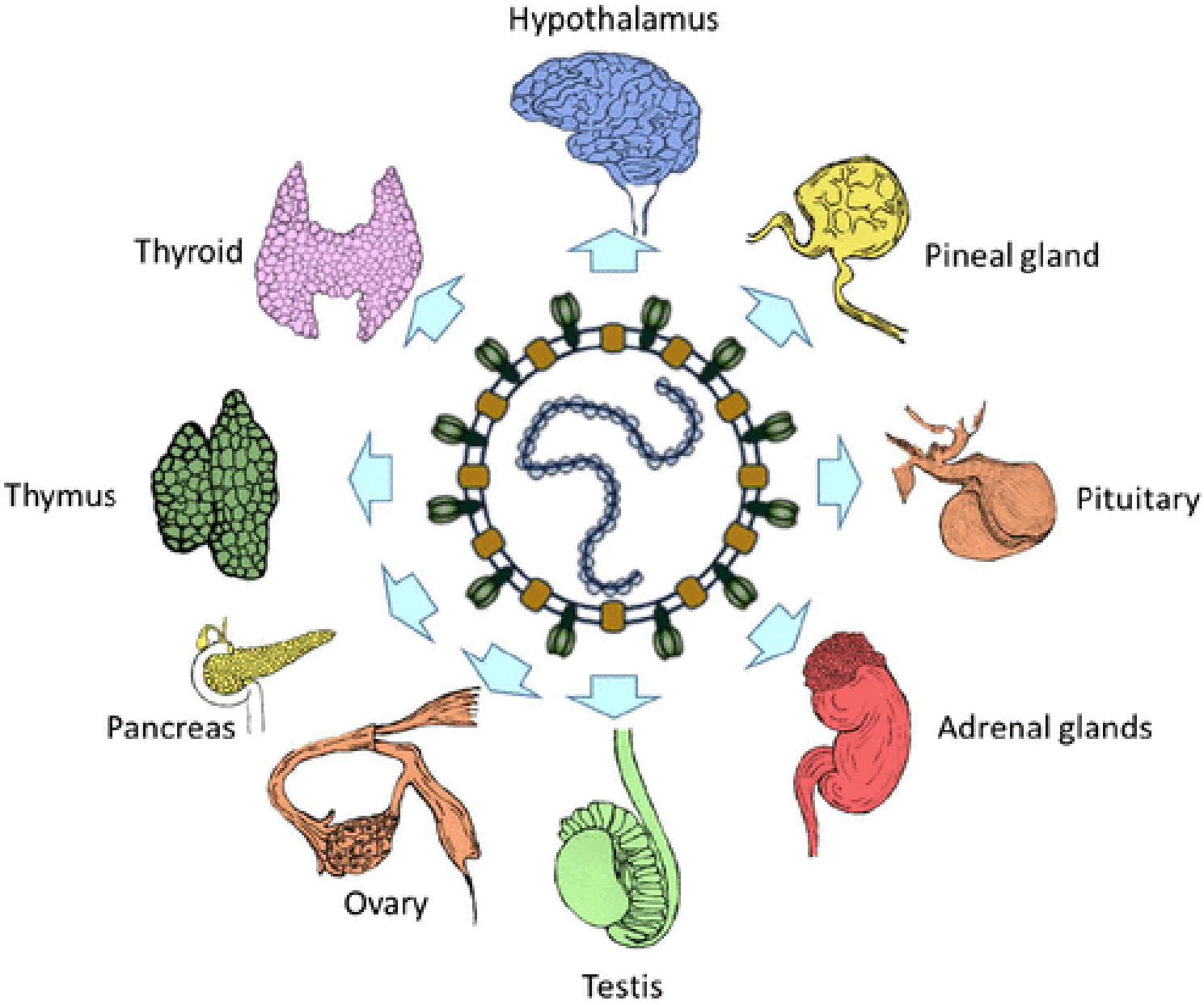


Novel Coronavirus SARS-CoV-2

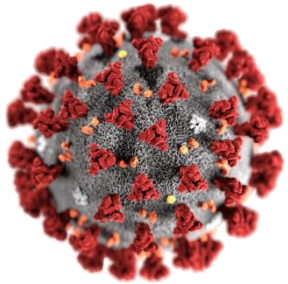
Colorized scanning electron micrograph of an apoptotic cell (blue) infected with SARS-COV-2 virus particles (red), isolated from a patient sample. Image captured at the NIAID Integrated Research Facility (IRF) in Fort Detrick, Maryland. Credit: NIAID

https://www.niaid.nih.gov/diseases-conditions/coronaviruses

SARS-CoV-2 e o Sistema endócrino



https://doi.org/10.1152/ajpendo.00480.2020 - 2021



Obrigado

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USP – 1º Semestre 2021