ImmunoScience Academy
Partnering for Education & Optimizing Treatment in ImmunoScience

COVID-19 through the eyes of an immunologist

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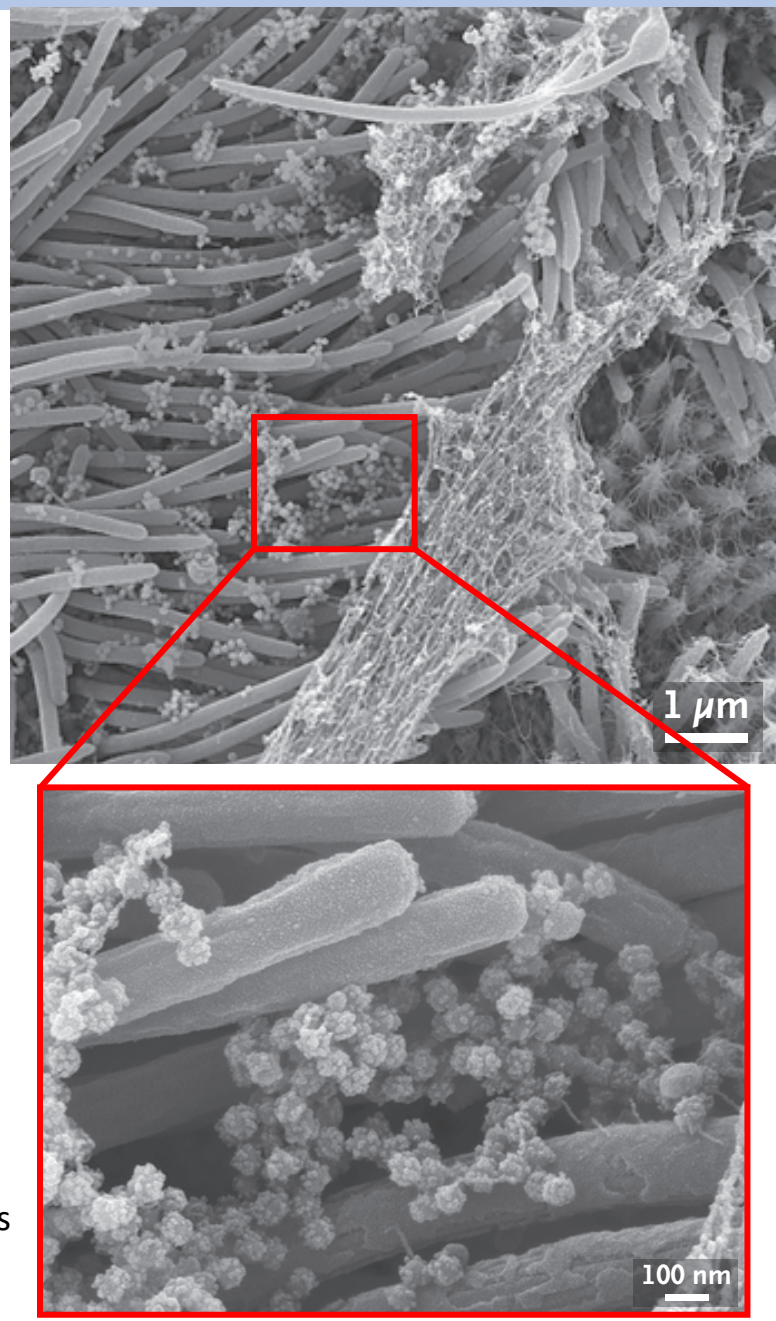
- **The virus: SARS-CoV-2**
  - very high infectivity
  - rare severe/letal cases
- The Spike protein
- Receptor and host factors
- The disease: COVID-19
- Innate and adaptive immune responses
- Vaccines



Viral particles produced by cultured human lung epithelial cells infected *in vitro* with SARS-CoV-2

Ehre 2020 NEJM 383:969

SARS-CoV-2

order *Nidovirales* → enveloped; positive-strand RNA (+ssRNA) (capped and polyadenylated); largest genomes among RNA viruses: 26-32 kb (Influenza: -ssRNA 13kb)

family *Coronaviridae*

… …

subfamily *Letivirinae*

Orthocoronavirinae

genus

- *α-coronavirus*, *β-coronavirus* } can infect mammals, including **man**
- *γ-coronavirus*, *δ-coronavirus* } infect only birds

α-CoVs	HCov-229E	endemic, non pathogenic	
	HCoV-NL63		
β-CoVs	HCov-OC43		
	HCoV-HKU1		
Receptors			
ACE2	SARS-CoV-1 (2003)	pathogenic	
DPP4	MERS-CoV (2012)		
ACE2	**SARS-CoV-2** (2019)		

Origin of SARS-CoV-2: bats (closest relative: Bat-CoV-RATG13 96.2% genome identity)

⇩

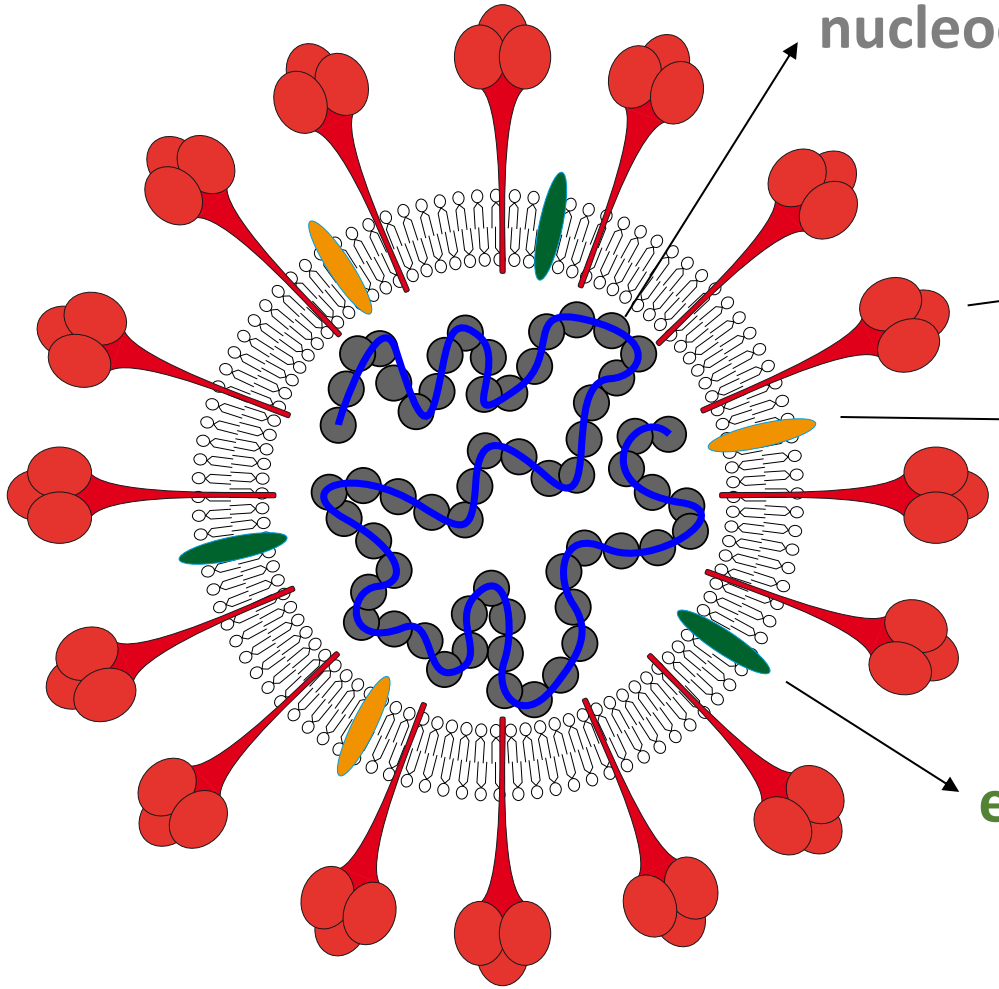
?

⇩

man

Tang D, et al. PLoS Pathog 2020;16:e1008536.

SARS-CoV-2 structural proteins



100 nm

nucleocapsid protein (419 aa)

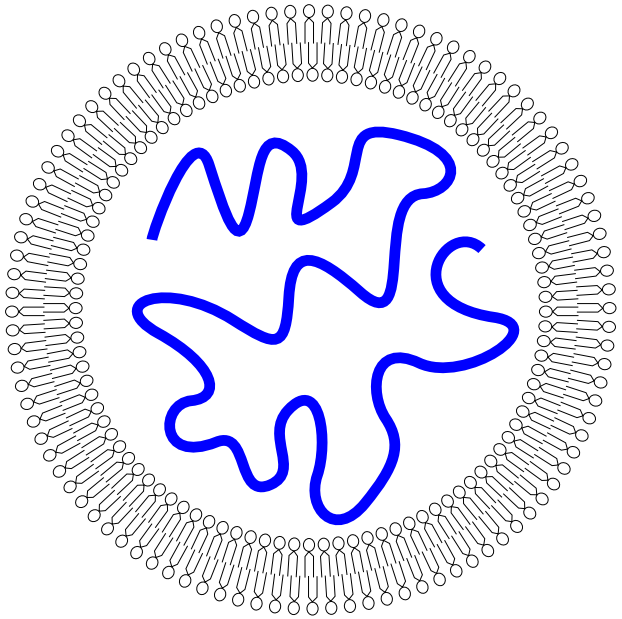
spike protein (1273aa)

responsible for the crown-like shape of CoV viral particles → 'coronaviruses'

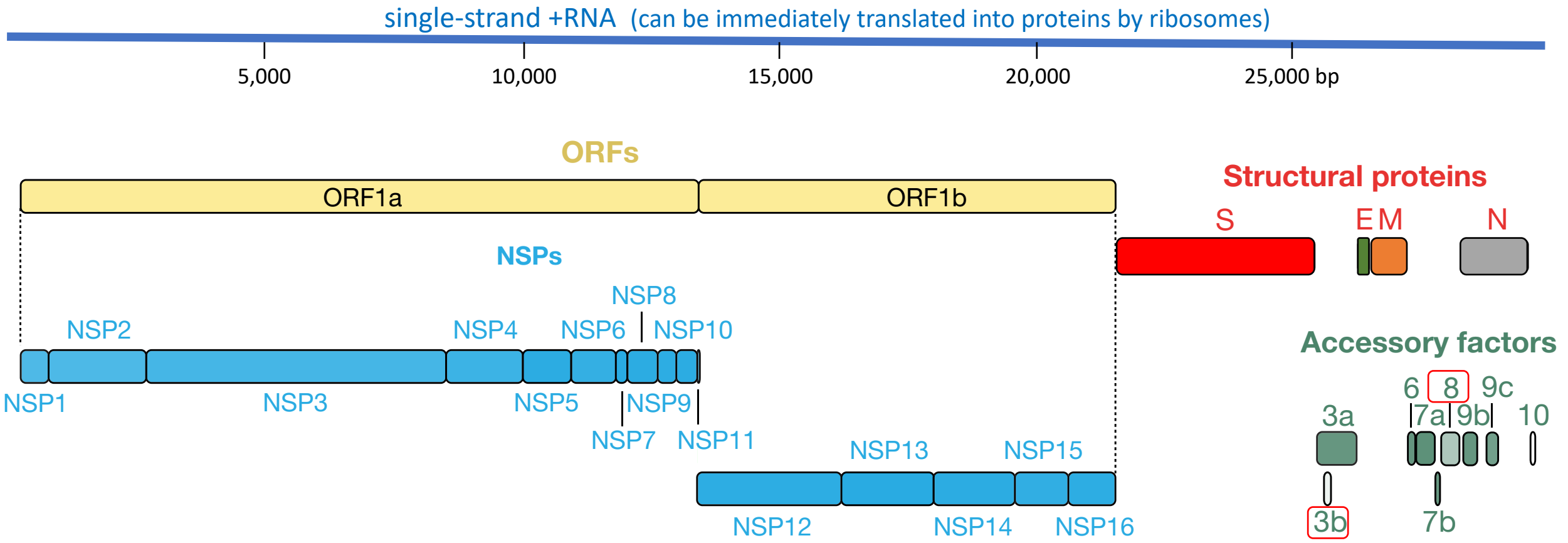
membrane protein (222 aa)

envelope protein (75 aa)

SARS-CoV-2 genome



SARS-CoV-2 genome



two new proteins !

- structural proteins
- proteins required for viral replication and production (in ORF1a and 1b)
- 'accessory factors', functions not completely known, participation in pathogenesis?

Gordon D, et al. Nature 2020;583:459–68.

- The virus: SARS-CoV-2
- **The Spike protein**
- Receptor and host factors
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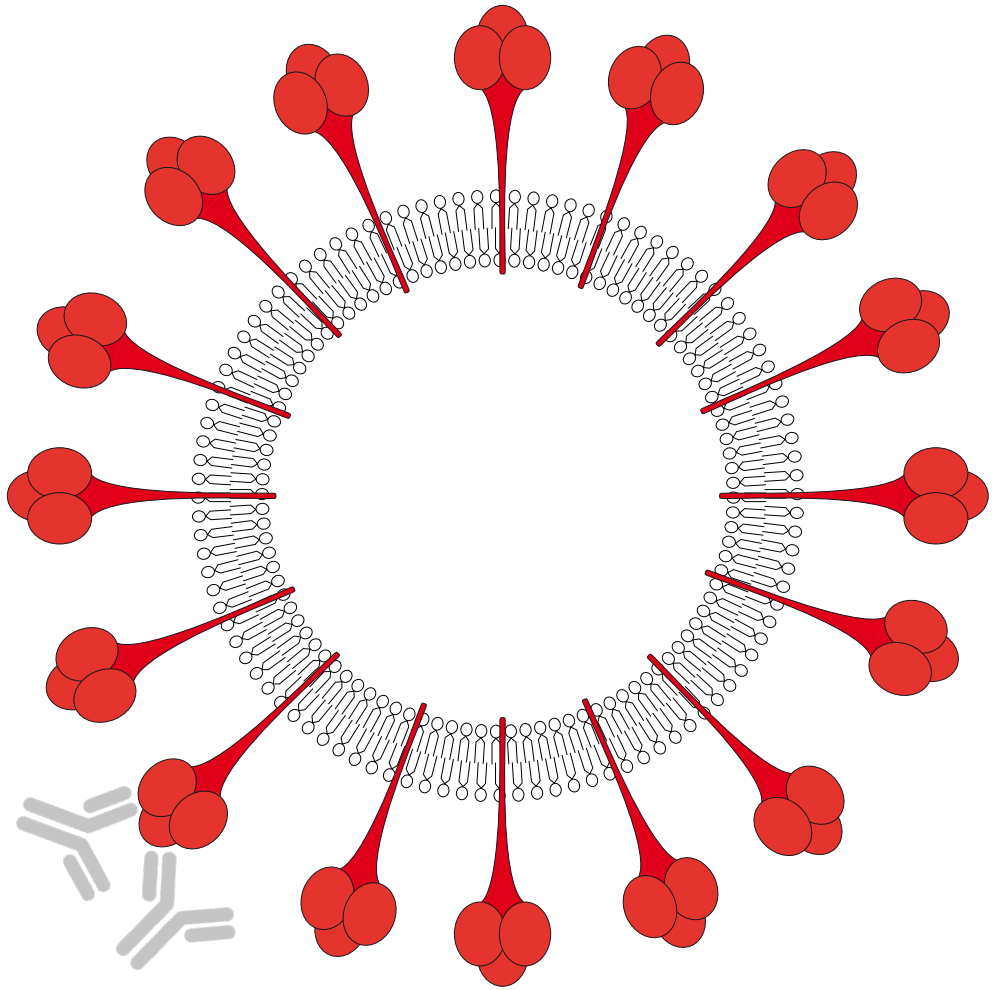
- Vaccines

SARS-CoV-2 spike protein

- ±40 per virion
- 20 nm
- trimer
- heavily glycosylated
- flexible



(drawn to scale)

Turonova B, et al. Science 2020;370:203-208.

SARS-CoV-2 spike protein

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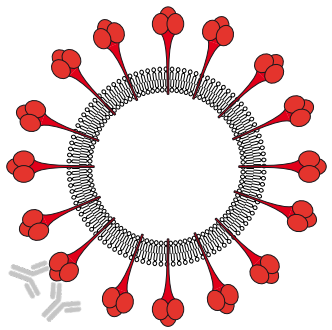
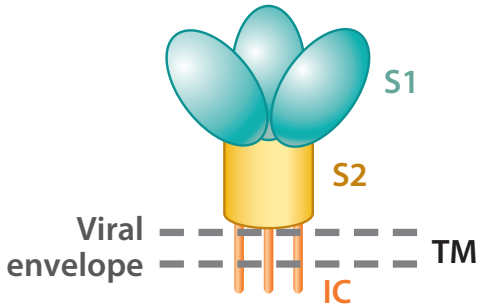
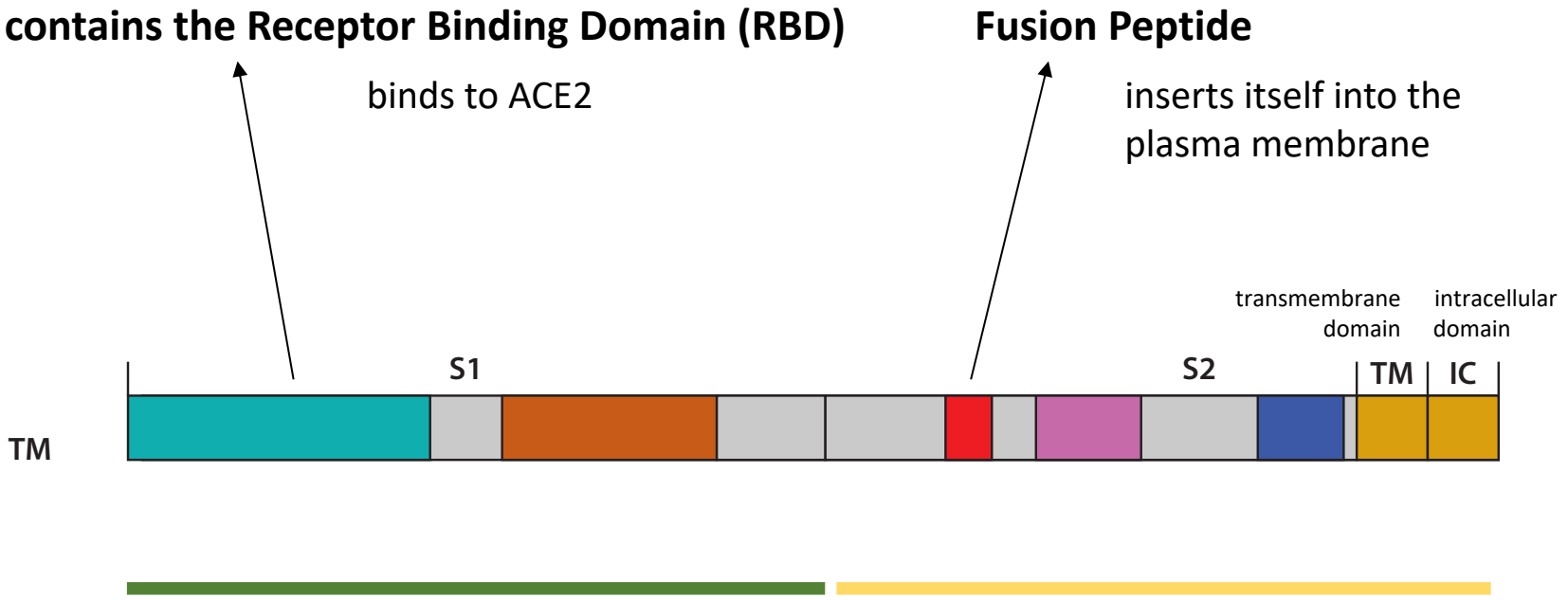


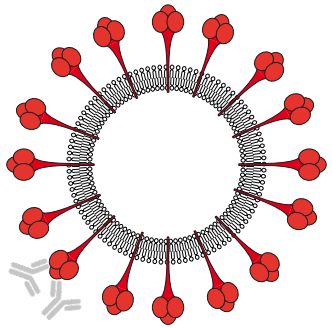


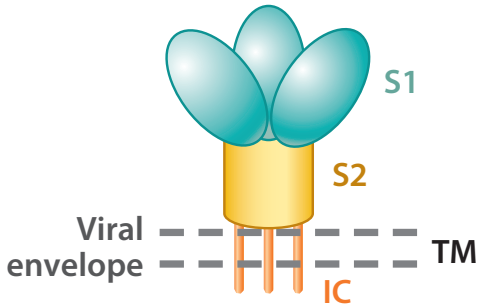


Li F. Annu Rev Virol 2016;3:237-61.

Monomer of SARS-CoV-2 spike protein

- ±40 per virion
- 20 nm
- trimer
- heavily glycosylated
- flexible





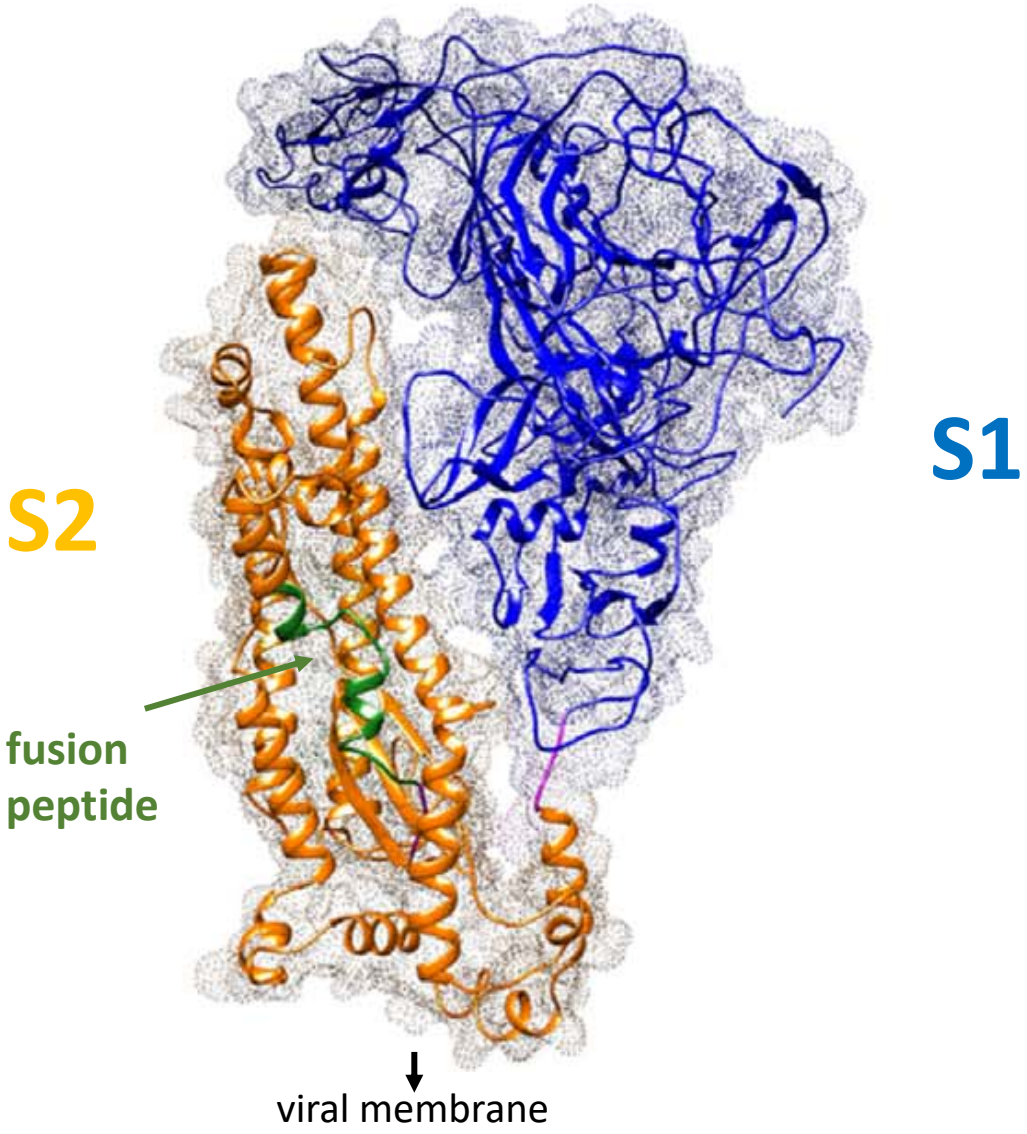
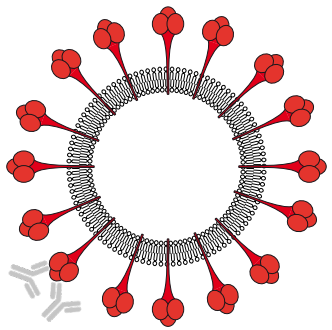
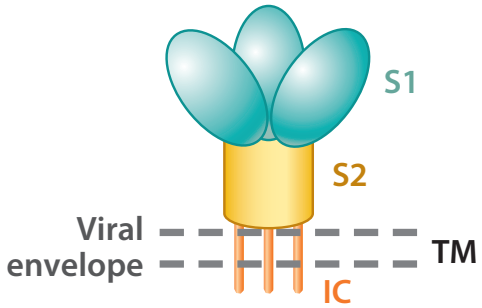


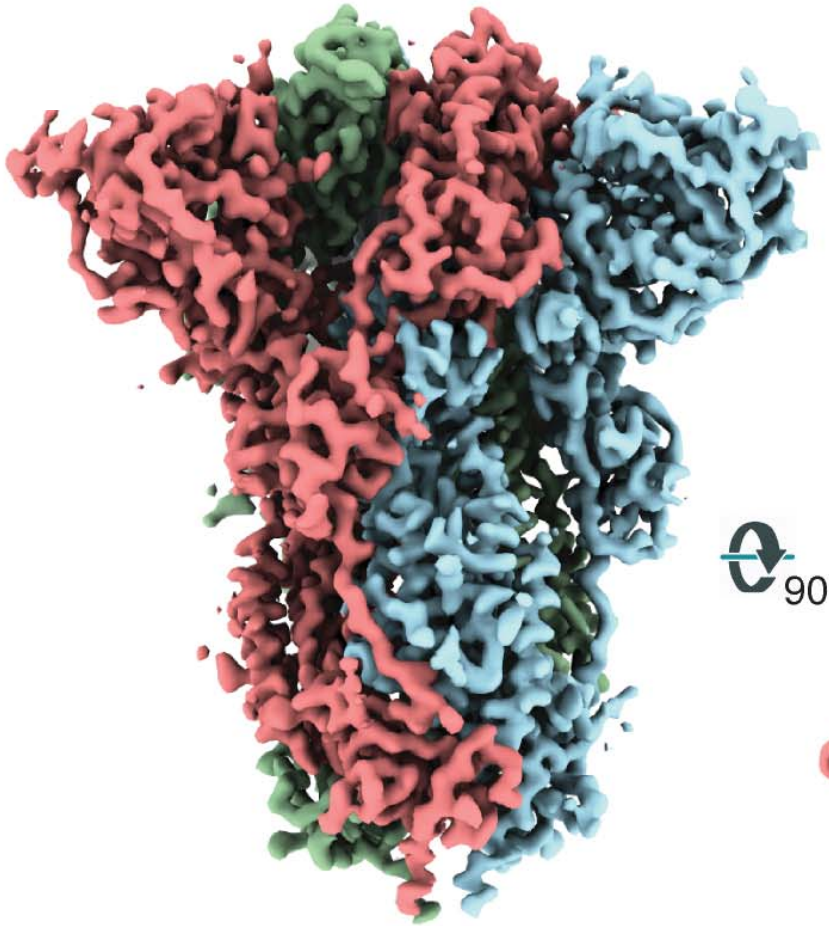
Figure adapted from Millet J and Wittaker G. Virology 2018;517:3-8.

Trimer of SARS-CoV-2 spike protein

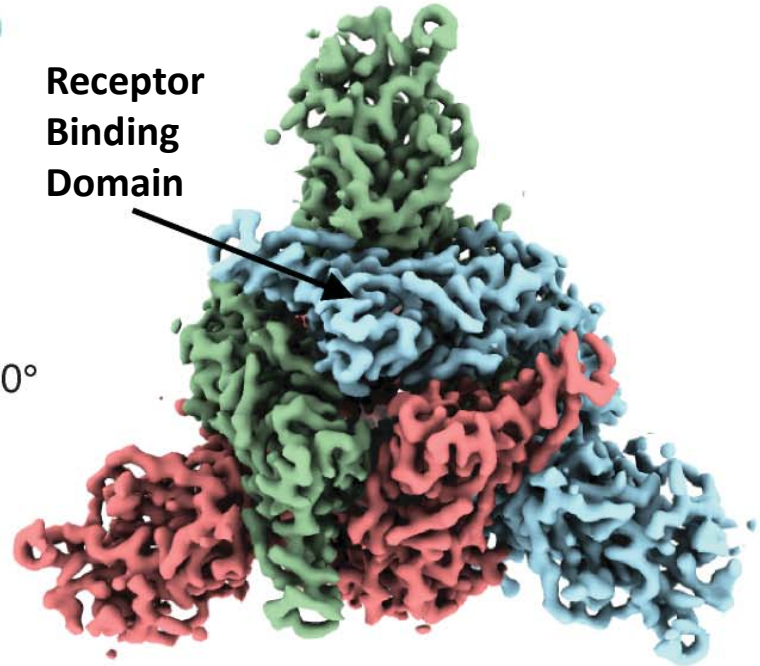
- ±40 per virion
- 20 nm
- trimer
- heavily glycosylated
- flexible







90°



Bangaru S, et al. Science 2020;370:1089-94.

SARS-CoV-2 spike protein: polysaccharides in color

- ±40 per virion
- 20 nm
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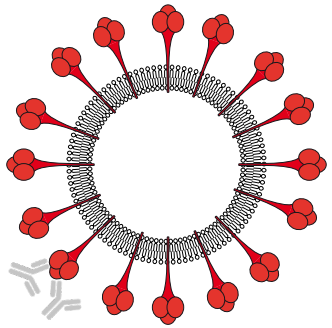
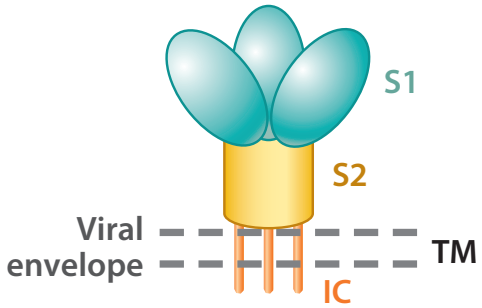
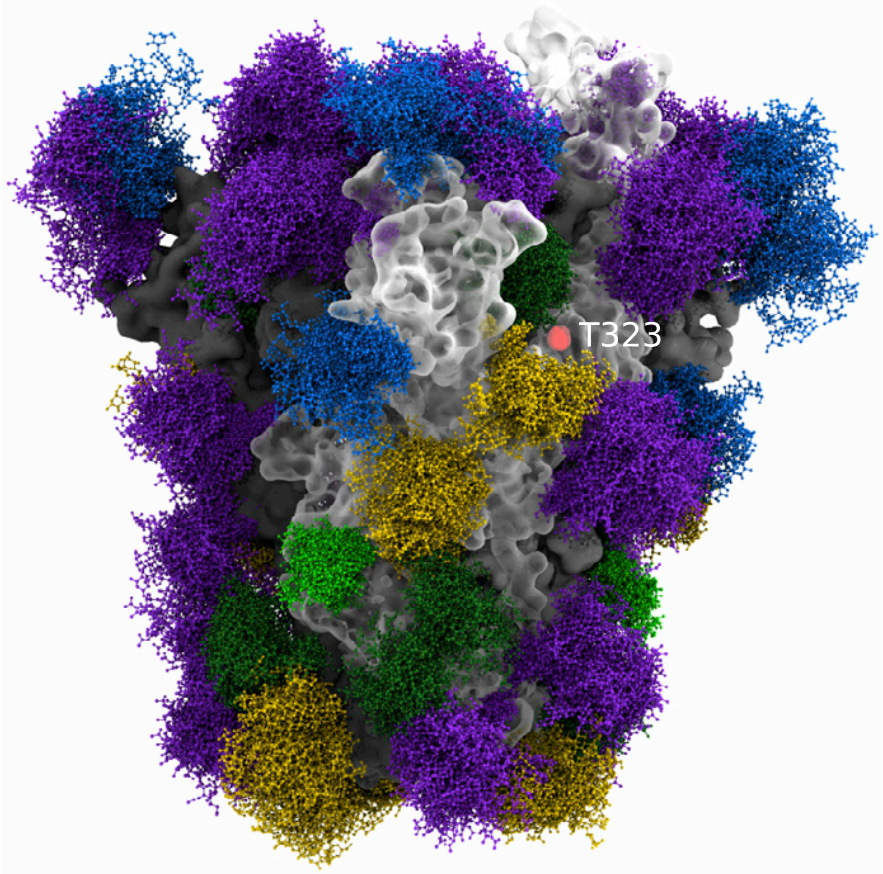






(T323: unique site of O-glycosylation)

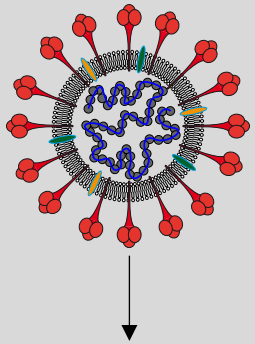
Multiple sites of N-glycosylation

→ many proteic epitopes are masked
= virus partial protection against neutralizing antibodies

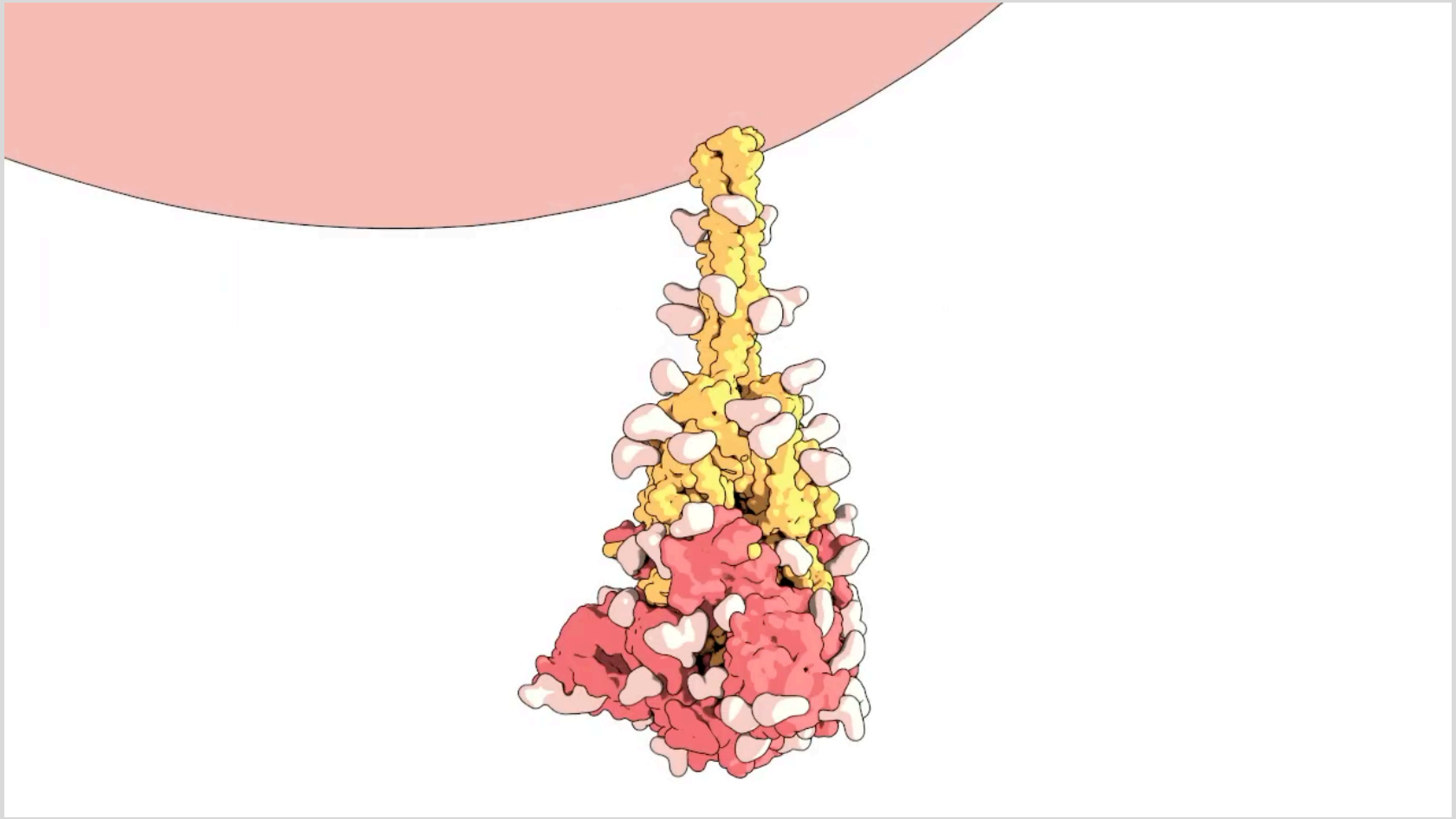
→ protective effect of blood group O

Zhao P, et al. Cell Host Microbe 2020;28:586-601.

Model of membrane fusion by ß-coronavirus spike protein



cell



Virion entry into cell:
± 10 min

Eclipse period: ± 10 hrs

Burst size: ± 1,000 virions

This process is THE main target for antibody-mediated virus neutralization

Khao J. and McGill G. Digizyme Inc.

- The virus: SARS-CoV-2

- The Spike protein

- **Receptor and host factors**

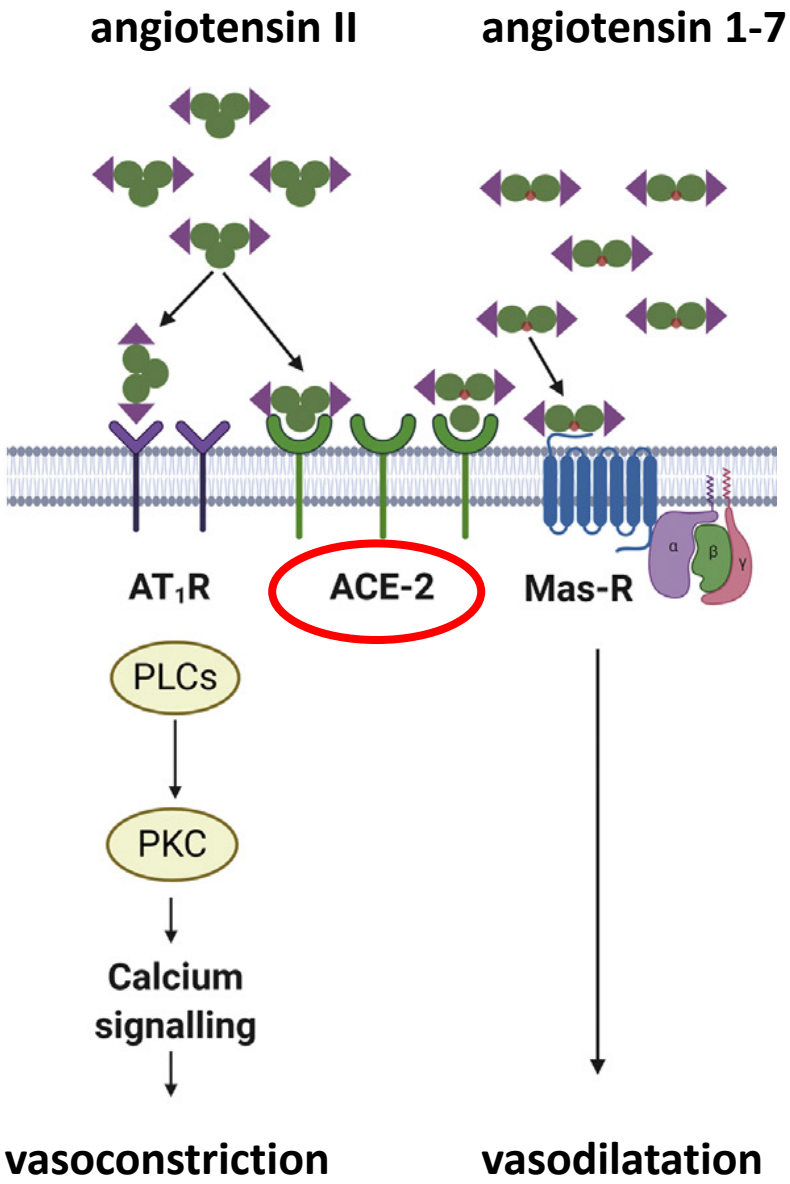
- The disease: COVID-19

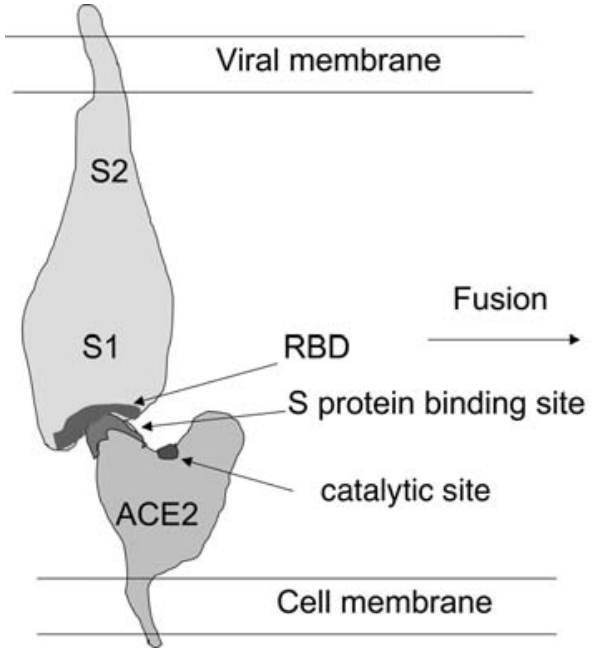
- Innate and adaptive immune responses

- Vaccines

The receptor: angiotensin converting enzyme 2 (ACE2)

surface carboxypeptidase





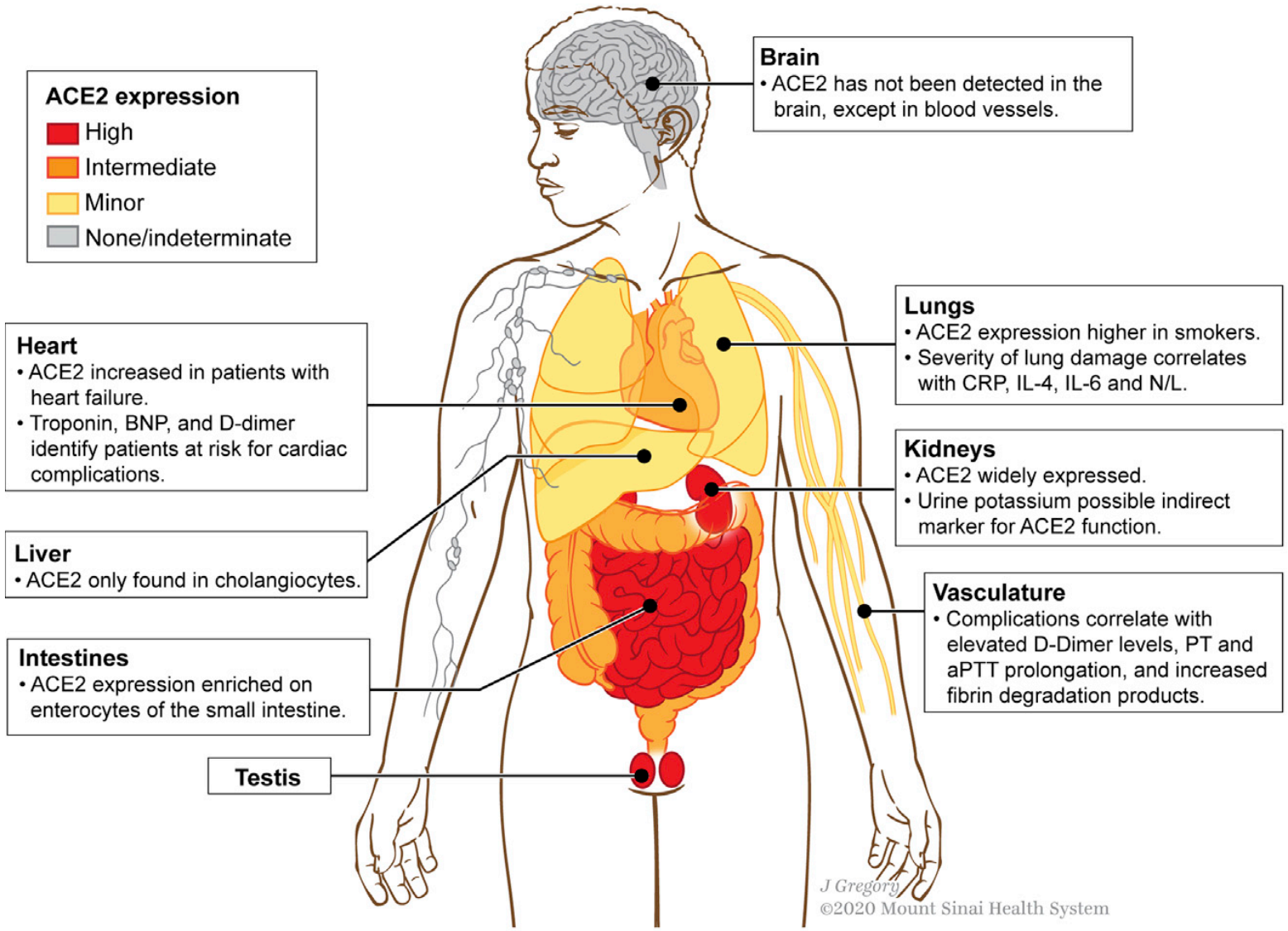
Model of SARS-CoV-**1** binding to ACE2

2004 BBRC 314:235

(MERS-CoV receptor: ≠ACE2 but DPP4)

Adapted from Groß S et al. J Mol Cell Cardiol 2020;144:47-53.

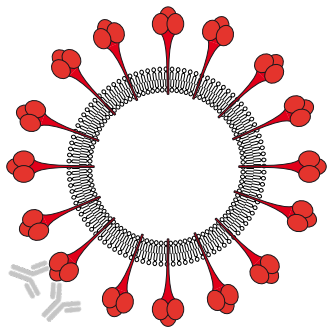
ACE2 expression profile is key to COVID-19 symptoms

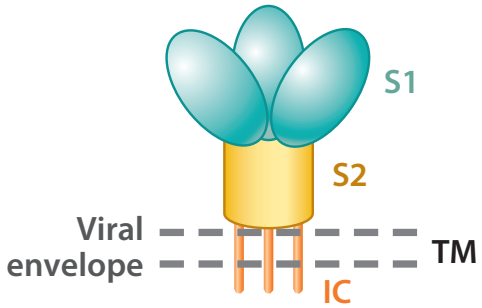


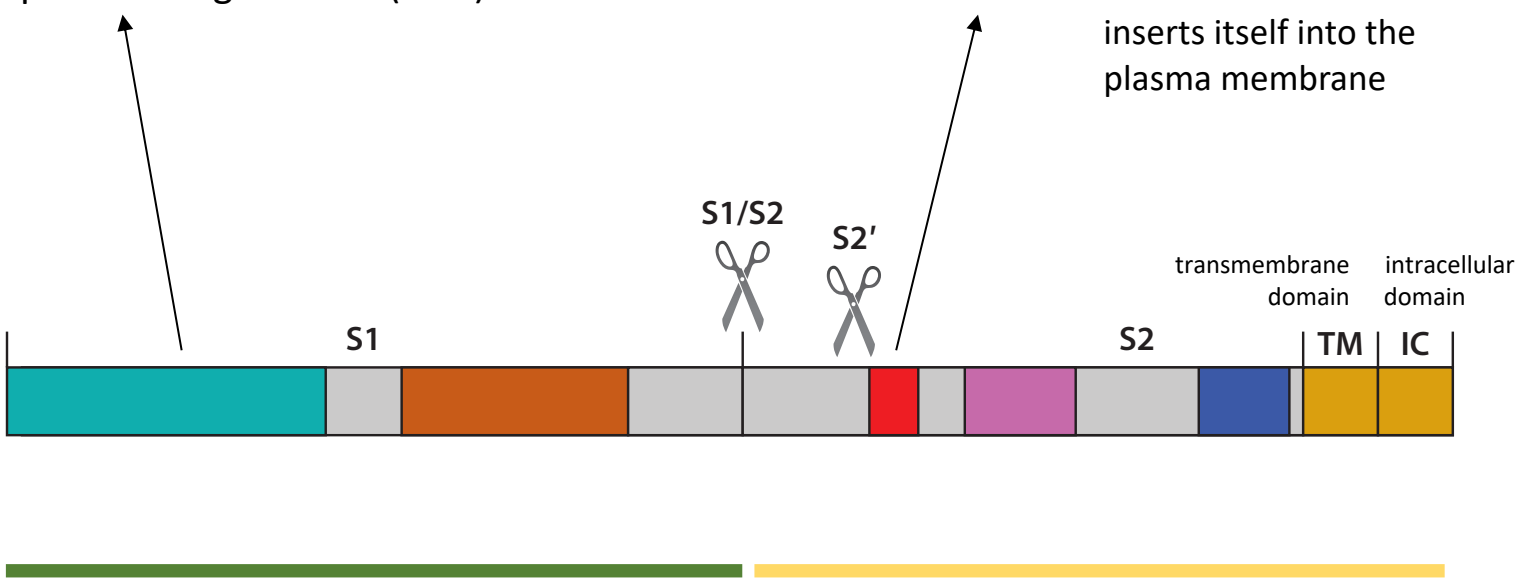
Vabret et al. 2016 Immunity 52:910

SARS-CoV-2 spike protein: processing required

- ±40 per virion
- 20 nm
- trimer
- heavily glycosylated
- flexible







Li F, et al. Annu Rev Virol 2016;3:237-61.

SARS-CoV-2 spike protein: processing required for virus entry

- ß-coronavirus S protein requires 2 proteolytic cleavages:

  - between domains S1 and S2

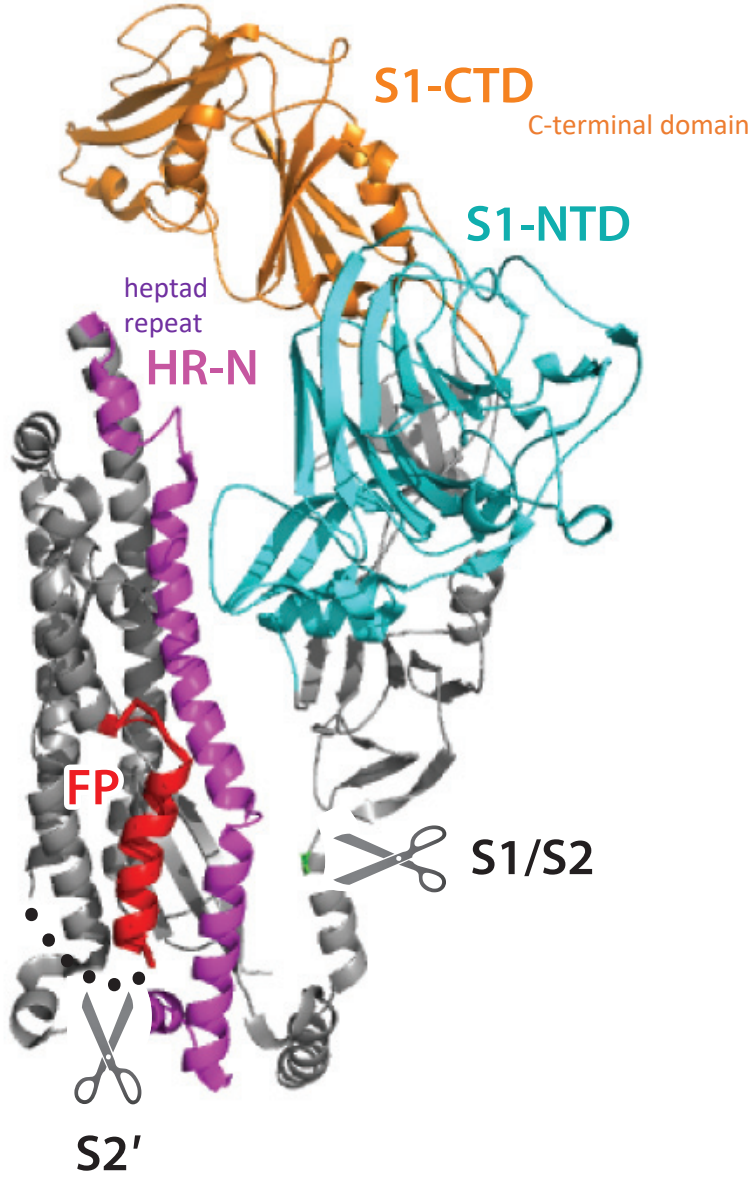
    to favor changes of S1 conformations:

    closed conformation: RBD buried
    open conformation: RBD accessible to ACE2

    to allow for the detachment of S1, unmasking S2

  - upstream of the fusion peptide, to allow its insertion in the plasma membrane

- efficacy and extent of these activation steps regulate cellular tropism and viral pathogenesis



Li F, et al. Annu Rev Virol 2016;3:237-61; Coutard B, et al. Antiviral Res 2020;176:104742.

Host factor: TMPRSS2

- transmembrane serine protease 2

- cleaves at S1/S2 and at S2'

- surface co-expression of ACE2 and TMPRSS2 on the same cells (in cis) appears optimal for SARS-CoV-2 infection of human cell lines *in vitro*

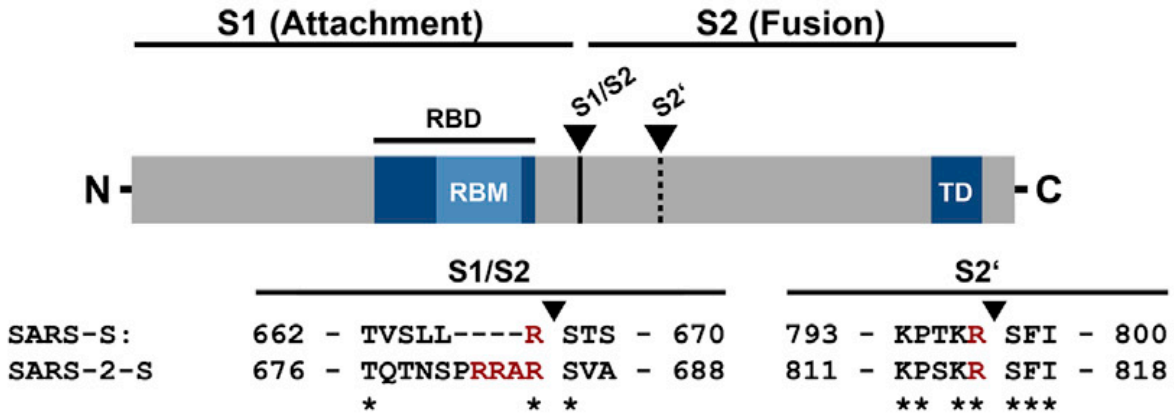
in vivo, coexpression by nasal epithelial cells, bronchial transient secretory cells

(Sungnak et al. 2020 Nat Med 26:681) *(Lukassen et al. 2020 EMBO J e105114)*

- *ACE2* and *TMPRSS2* genes are induced in various proinflammatory conditions (obesity, diabetes, ...)

Gkogkou et al. 2020 Redox Biol 36:101615

A unique feature of SARS-CoV-2, absent in other members of its clade



- insertion of 12 nucleotides upstream of S1/S2 in SARS-CoV-2

- absent in SARS-CoV-1, MERS-CoV and Bat-CoV-RATG13

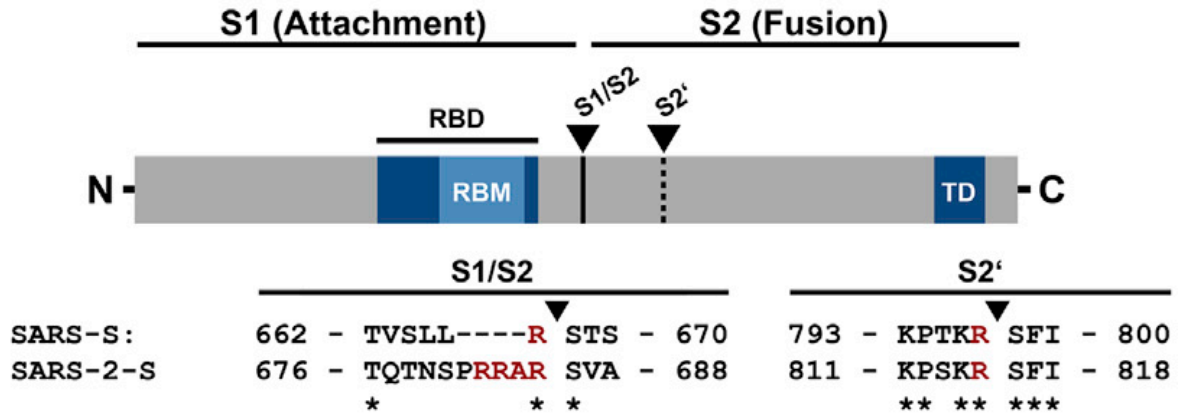
- creates a **furin**-like cleavage site
  (furin: endoprotease in the secretory pathway compartments)

- furin is highly expressed in lungs

- participates in SARS-CoV-2 pathogenesis ? (very high contagiousness and rapid spread)

Coutard B, et al. Antiviral Res 2020;176:104742; Johnson B, et al. bioRxiv 2020;2020.08.26.268854.

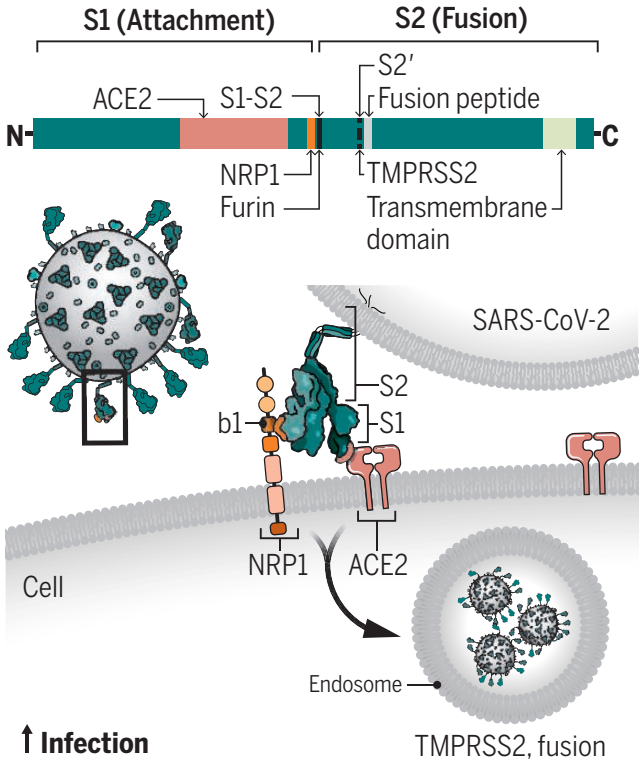
Host factor: neuropilin 1



- neuropilins (NRP1 and NRP2):
  - surface receptor for semaphorins and VEGF
  - highly expressed in almost all pulmonary and olfactory cells
  - highest levels of expression in endothelial cells

- neuropilin 1 binds furin-cleaved sustrates (the polybasic RRAR carboxyterminal sequence on S1)

- blocking this interaction reduces SARS-CoV-2 infectivity *in vitro*



Daly J, et al. Science 2020;370:861-65; Canti-Castelvetri L, et al. Science 2020;370:856-60.

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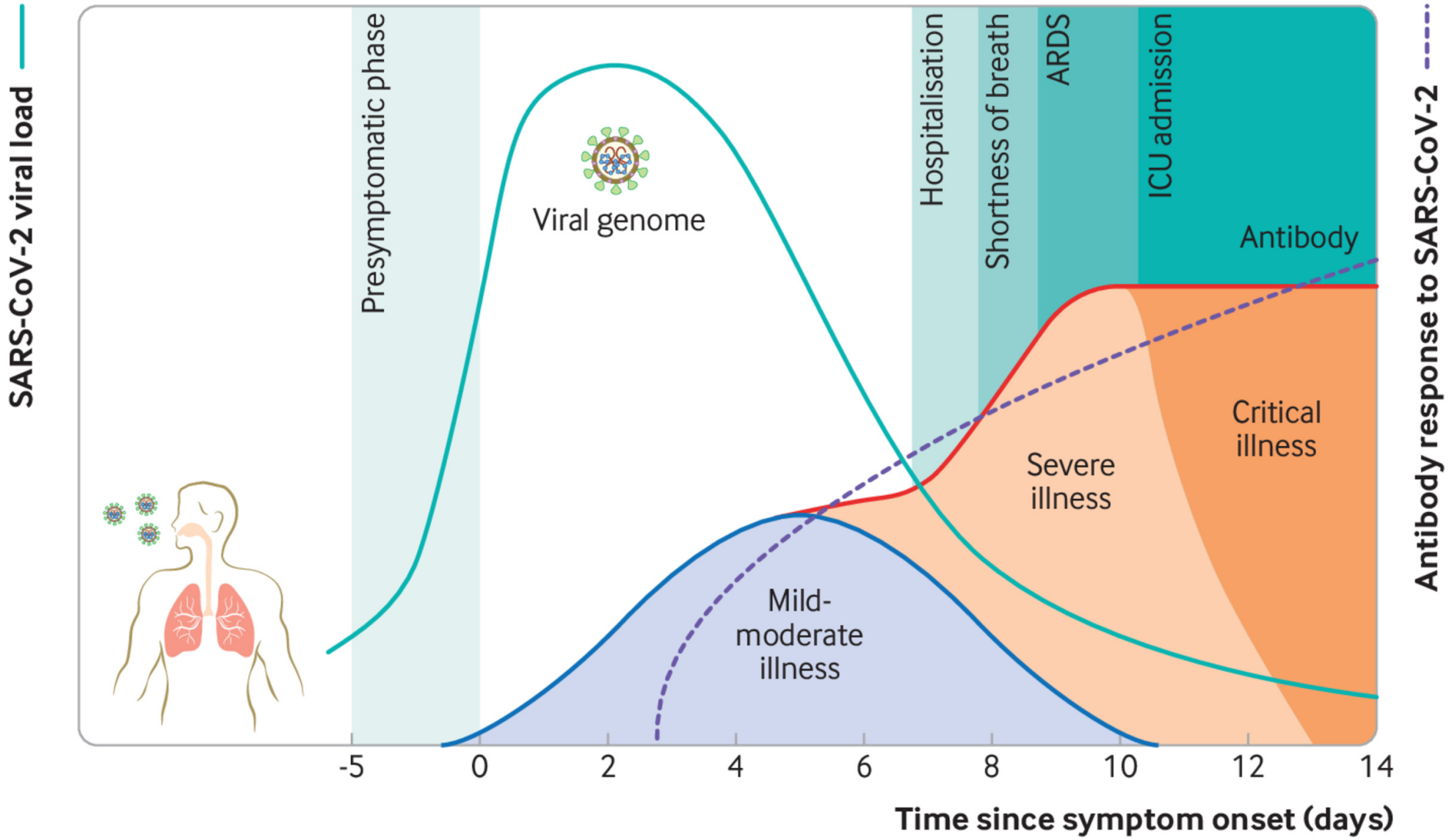
- Innate and adaptive immune responses

- Vaccines

COVID-19



Cevik et al. 2020 BMJ 371:m3862

- The virus: SARS-CoV-2
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- The disease: COVID-19
- **Innate and adaptive immune responses**
- Vaccines

Innate immune response to viruses

- **detection** by cells of pathogen-associated molecular patterns (PAMP)

  - main sensor for SARS-CoV-2: TLR3

  - detected PAMP: virus-specific aberrant RNA structures (dsRNA, ...)

- **transcriptional response**: two antiviral programs

  COVID-19

  - type-I (IFN-α, IFN-β) and type-III interferons (IFN-λ) → cellular antiviral defenses — too little, too slow

  - chemokines → recruitment and coordination of subsets of leukocytes — too much

Blanco-Melo D, et al. Cell 2020;181:1036-1045.

Viral resistance to innate immunity

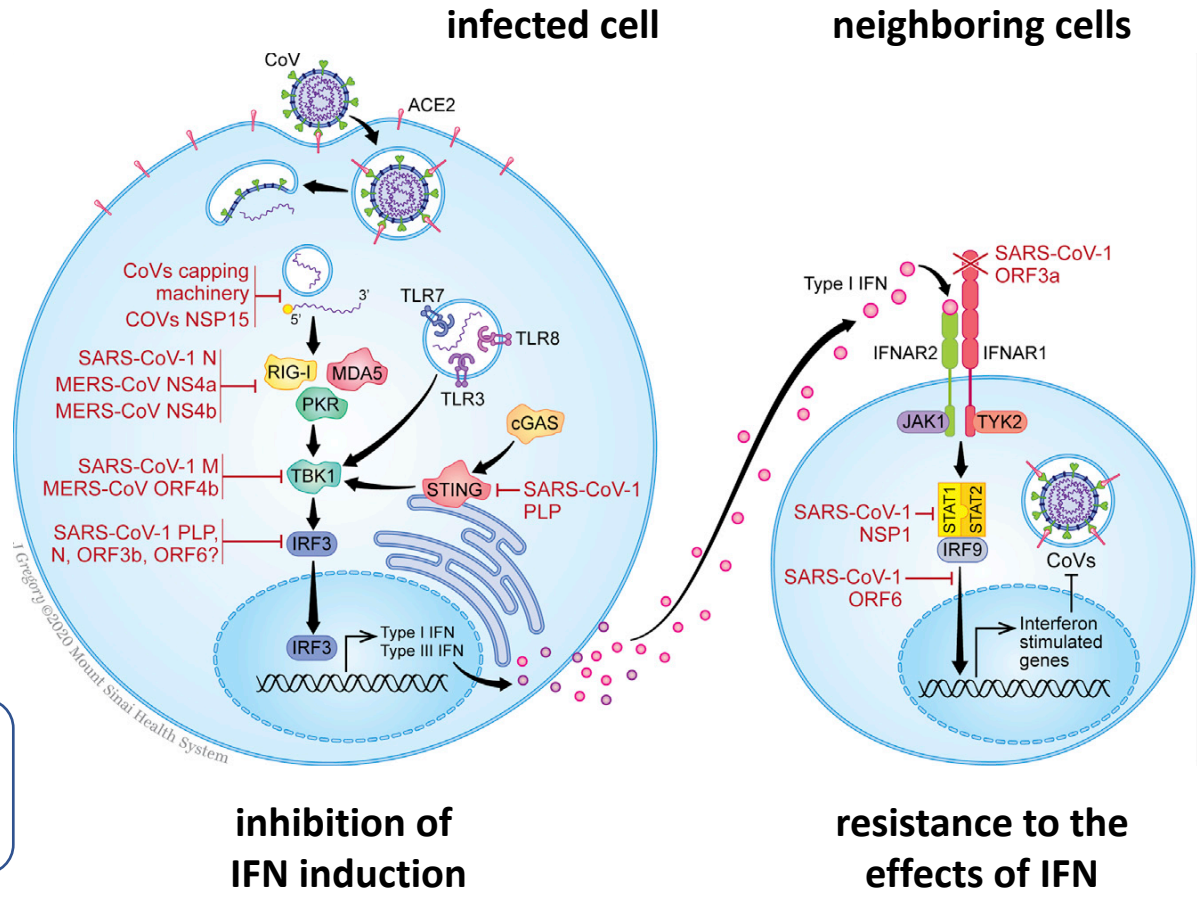
- all pathogens, viruses and others, have acquired mechanisms to partially resist immunity (innate and adaptive)

- several known mechanisms of IFN-resistance in coronaviruses

- **SARS-CoV-2** is probably even more resistant than SARS-CoV-1 and MERS-CoV

mechanism(s) ?

Using *in vitro* infection of human cell lines, most authors report higher infection rates and lower IFN-I production with SARS-CoV-2 than with SARS-CoV-1 or MERS-CoV.



Vabret N, et al. Immunity 2020;52:910-41.

Inflammatory cells

- local (lungs) production of inflammatory cytokines (IL-1ß, IL-6, TNF, ...) and chemokines

  levels of gene and protein expression are correlated with disease severity

  especially in severe cases: associated with impaired type I IFN responses (comparisons with RSV of influenza A)

  'cytokine storm' and viral production remain present in the inflamed lungs of deceased patients

- followed by the accumulation of inflammatory cells, including neutrophils, in the lungs

  immature myeloid cells found in blood (reactive myelopoiesis)

- the higher level of basal inflammation associated with age ('inflammaging') or obesity (inflammasome activation) might be risk factors

Altman DM, et al. Sci Immunol 2020;5:eabd6160; Hadjadj J, et al. Science 2020;369:718.

Adaptive immunity to viruses

COVID-19

- activation of B cells → production of **IgM** →
  - production of **IgA** and **IgG**
  - higher affinity of antibodies (somatic mutations in *IgV* regions)
  - memory B cells

present

- activation of CD4 T cells → 'help' to B cells

present

- activation of CD8 T cells → cytolysis of SARS-CoV-2-infected cells

present

- stronger and broader responses in severe cases, assumed to have had higher viral burden
- long term protective effects of these responses ?
- T lymphodepletion at late sages of disease (observed also in sepsis from other causes)

Altman DM, et al. Sci Immunol 2020;5:eabd6160

Adaptive immunity to SARS-CoV-2

- antibodies against a dozen of viral proteins, not only S
- a fraction of these antibodies are neutralizing, mostly anti-S

↓

prevent infection of cells

- activation of B cells → production of **IgM** →
  - production of **IgA** and **IgG**
  - higher affinity of antibodies (somatic mutations in *IgV* regions)
  - memory B cells

- activation of CD4 T cells → 'help' to B cells

- activation of CD8 T cells → cytolysis of SARS-CoV-2-infected cells

Altman DM, et al. Sci Immunol 2020;5:eabd6160

Is there something 'immunologically' remarkable with SARS-CoV-2 ?

- For the immunologist, SARS-CoV-2 is a perfectly **normal virus**

  It induces innate and adaptive immunity with classical effector mechanisms to which it partially resists.

- Today, the peculiarity of SARS-CoV-2 appears to be a proportionally **low IFN-I response**.

  This low IFN response is responsible for more viruses during a longer time, and these viruses continue to trigger the inflammatory response (chemokines and inflammatory cytokines).

  Together, these mediators continue to recruit innate immune cells (of myeloid origin) at sites of virus production, notably the lungs.

N.B. Inborn errors of IFN-I induction or signalling were found in adult patients (n=23/659) with life-threatening COVID-19 pneumonia, but these genotypes were silent until infection with SARS-CoV-2. These pathways are however known to be associated with influenza respiratory distress. Thus we appear to depend more on IFN-I to control SARS-CoV-2 than other viruses.

Zhang Q, et al. Science 2020;370:eabd4570.

- The virus: SARS-CoV-2

- The Spike protein

- Receptor and host factors

- The disease: COVID-19

- Innate and adaptive immune responses

- Vaccines (therapeutic antibodies are not discussed here)

SARS-CoV-2 vaccines

Objective:

Stimulate the production of **neutralizing** anti-SARS-CoV-2 antibodies (the other option is to transfer such antibodies)

All currently proposed vaccines aim at inducing anti-Spike protein antibodies

Method:

Inoculate the antigen(s) in such a way that they are **immunogenic** (stimulate an *in vivo* adaptive immune response)

Vaccination modality is a key factor for immunogenicity:

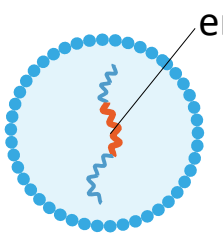
- either co-inject antigen and immunological adjuvants (increase antigen half-life and induce local inflammation)
- or inject DNA or RNA encoding the antigen, which is then produced by the vaccinee's own cells

DNA or RNA has to be 'packaged' (lipid nanoparticles, recombinant viruses)

Roles of packaging: protect against DNAse/RNAse and promote entry into cells

SARS-CoV-2 vaccines for Belgium (dec 2020)

mRNA in lipid nanoparticles

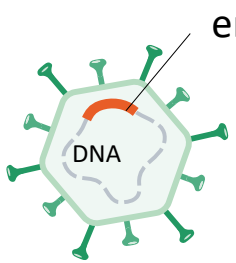


Pfizer-BioNTech, Moderna, Curevac

5 M 2 M 2.9 M doses pre-ordered

replication-incompetent chimpanzee adenovirus

(after the first injection, production of neutralizing antibodies against the virus)



AstraZeneca

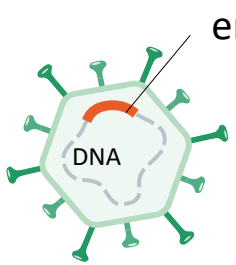
7.7 M

Thus far, no major differences between the immunological or clinical efficacies of these vaccines.

replication-incompetent human recombinant adenovirus 26

(low seroprevalence to Ad26, contrary to Ad5)

(russian vaccine: prime Ad26 boost Ad35)



Janssen

5 M

Krammer F. Nature 2020;586:516-27.

Expected immune responses with (SARS-CoV-2) vaccines

	B cells	CD4 T cells	CD8 T cells	
mRNA ?	+	+	+	Pfizer-BioNTech Moderna Curevac
replication-incompetent adenovirus	+	+	+	AstraZeneca Janssen Gamaleya (Spoutnik V)
protein + adj	+	+	-	GSK-Sanofi Novavax
inactivated virion + adj	+	+	-	Sinovac Biotech
live attenuated virus	+	+	+	Pasteur-Merck Measles-spike prot Pasteur-TheraVectys Lentivirus-spike prot

not for immunocompromised individuals

Krammer F. Nature 2020;586:516-27.