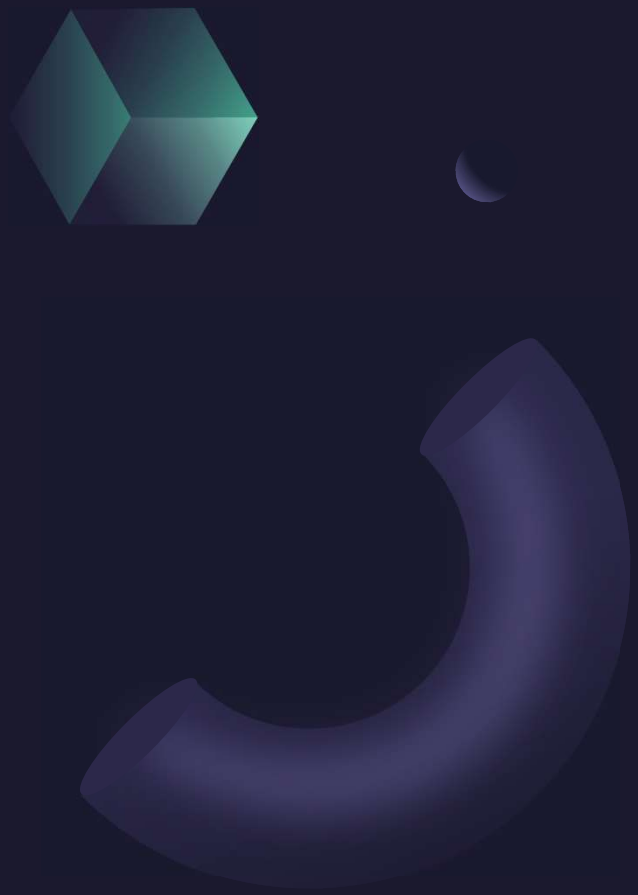


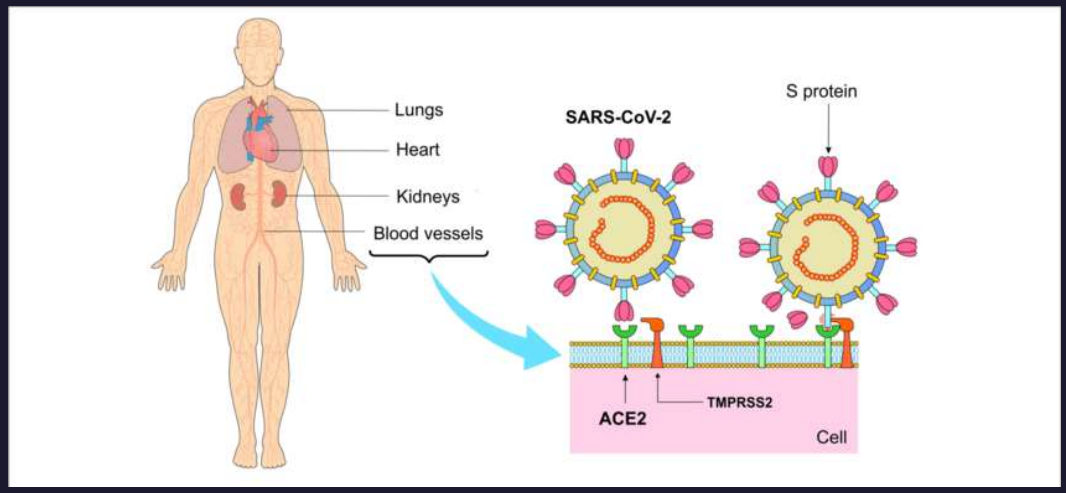
Coronavirus membrane fusion mechanism opportunistic model for SARS-CoV-2 fusion

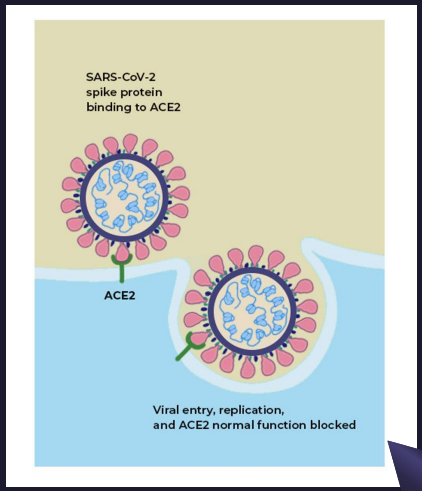
Overview

- Introduction
- Angiotensin-converting enzyme 2 (ACES-2)
- Function Of TMPRSS -2
- S protein structure
- Life Cycle of SARS-CoV-2
- Summarize

ACES-2 Angiotensin-converting enzyme 2

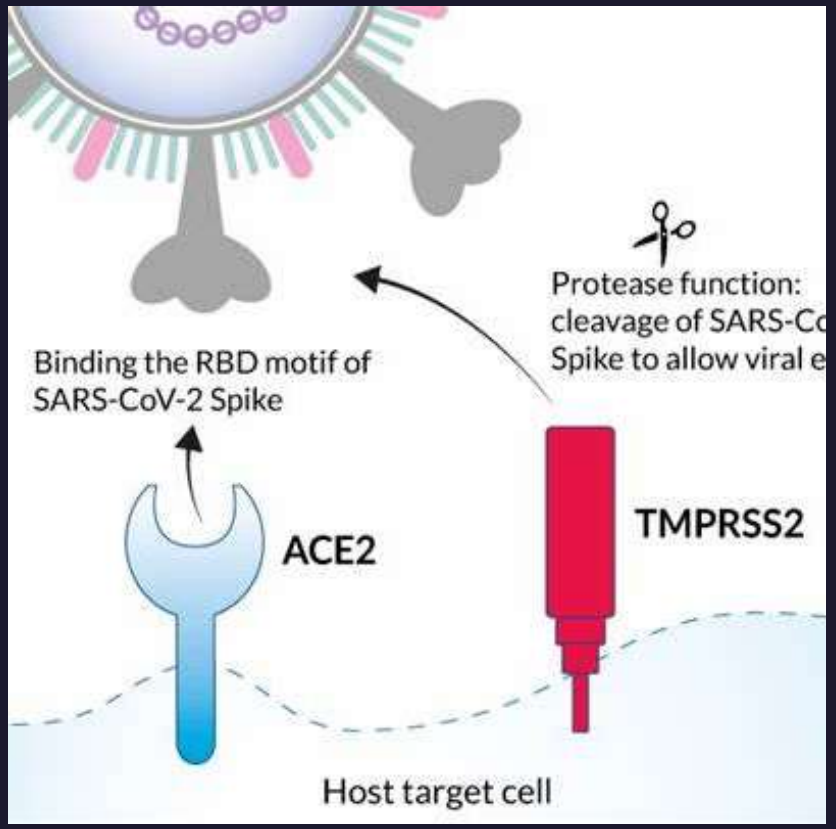
- ACE2 is a protein on the surface of many cell types. It is an enzyme that generates small proteins – by cutting up the larger protein angiotensinogen – that then go on to regulate functions in the cell.
- Using the spike-like protein on its surface, the SARS-CoV-2 virus binds to ACE2 – like a key being inserted into a lock – prior to entry and infection of cells. Hence, ACE2 acts as a cellular doorway – a receptor – for the virus that causes COVID-19.





Function Of TMPRSS -2

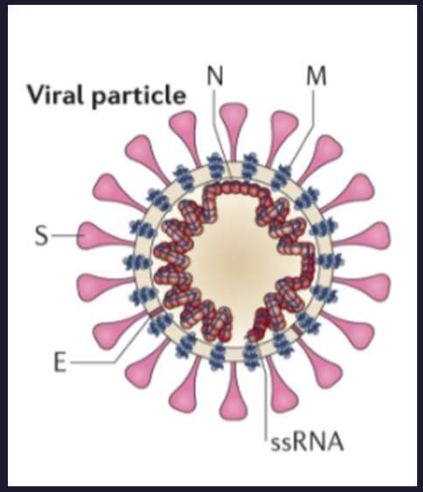
- Both ACE2 and TMPRSS2 are expressed in nasal, bronchial, and gastrointestinal epithelium. TMPRSS2 activates; or primes, the spike protein domain (a key glycoprotein found on coronaviruses) which leads to the virus fusing to the respiratory epithelia on the cell surface through binding to ACE2. As TMPRSS2 is a serine protease, it primes the spike-domain (S) of SARS-CoV-2 by cleaving as the S1/S2 sites.
- TMPRSS2 is expressed less in Type II alveolar cells and alveolar macrophages than in bronchial epithelial cells; additionally, no TMPRSS2 protein was found in Type I alveolar cells of the respiratory surface in this study
- Type II alveolar epithelial cells are a heterogeneous population that have critical secretory and regenerative roles in the alveolus to maintain lung.

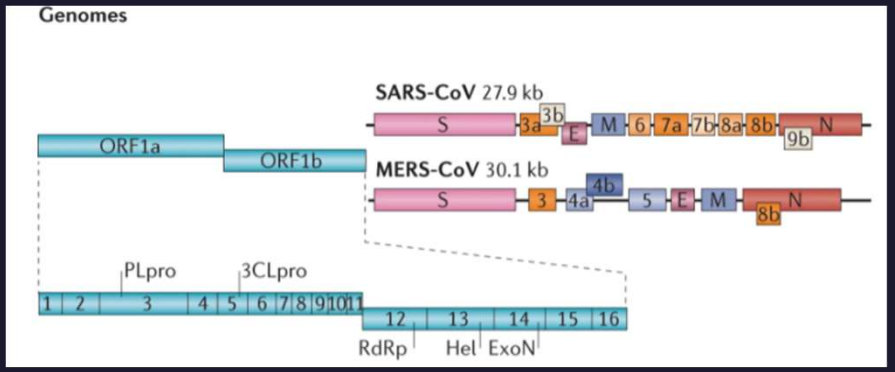


Transmembrane serine protease 2

S protein structure

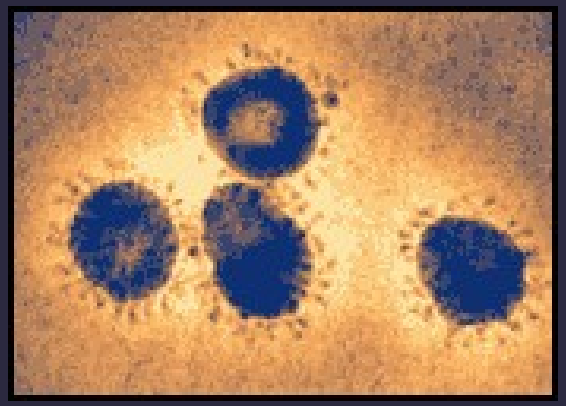
- **ORFs (open reading frames) are** two large polyproteins, pp1a and pp1b with name ORF1a and ORF1b respectively.
- The CoV single stranded genome encodes for 16 non-structural proteins, including the papain-like protease (PLpro), 3C-like protease (3CLpro), RNA-dependent RNA polymerase (RdRp), helicase (Hel), and exonuclease (ExoN)
- SARS-CoV and MERS-CoV form spherical particles that consist of four structural proteins.
- The envelope **glycoprotein spike (S)** forms a layer of glycoproteins
- **envelope (E) and membrane (M)**. Inside the viral envelope resides the helical nucleocapsid (N)

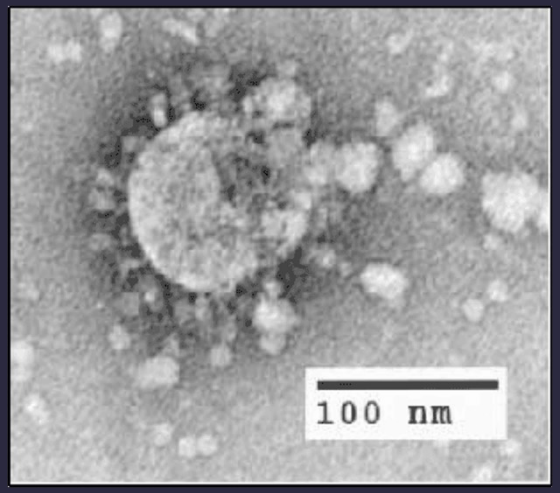


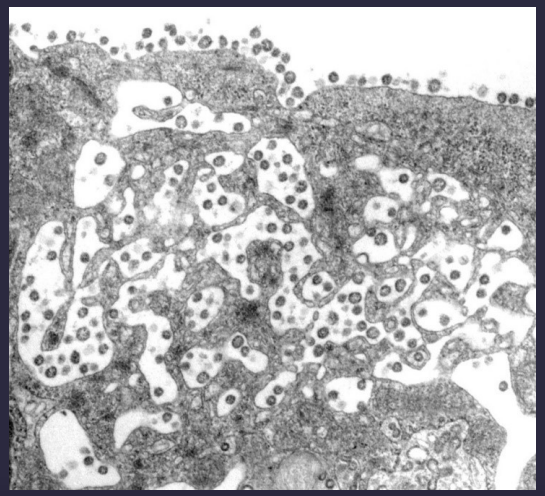


Severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV)

SARS-CoV Images



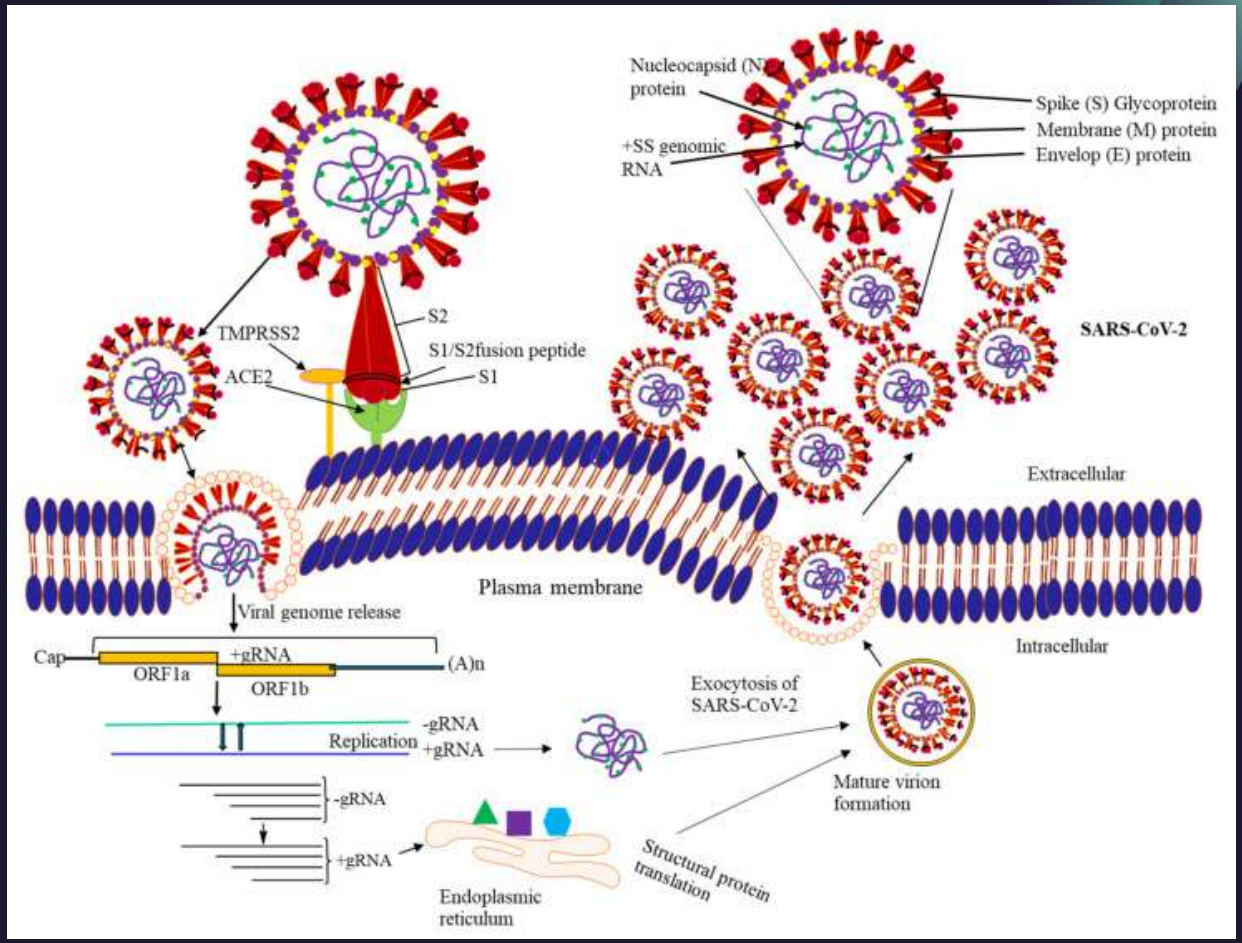


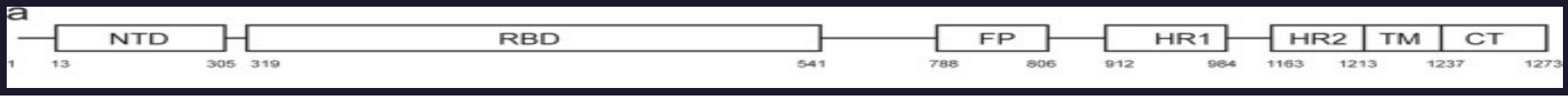


Life Cycle of SARS-CoV-2

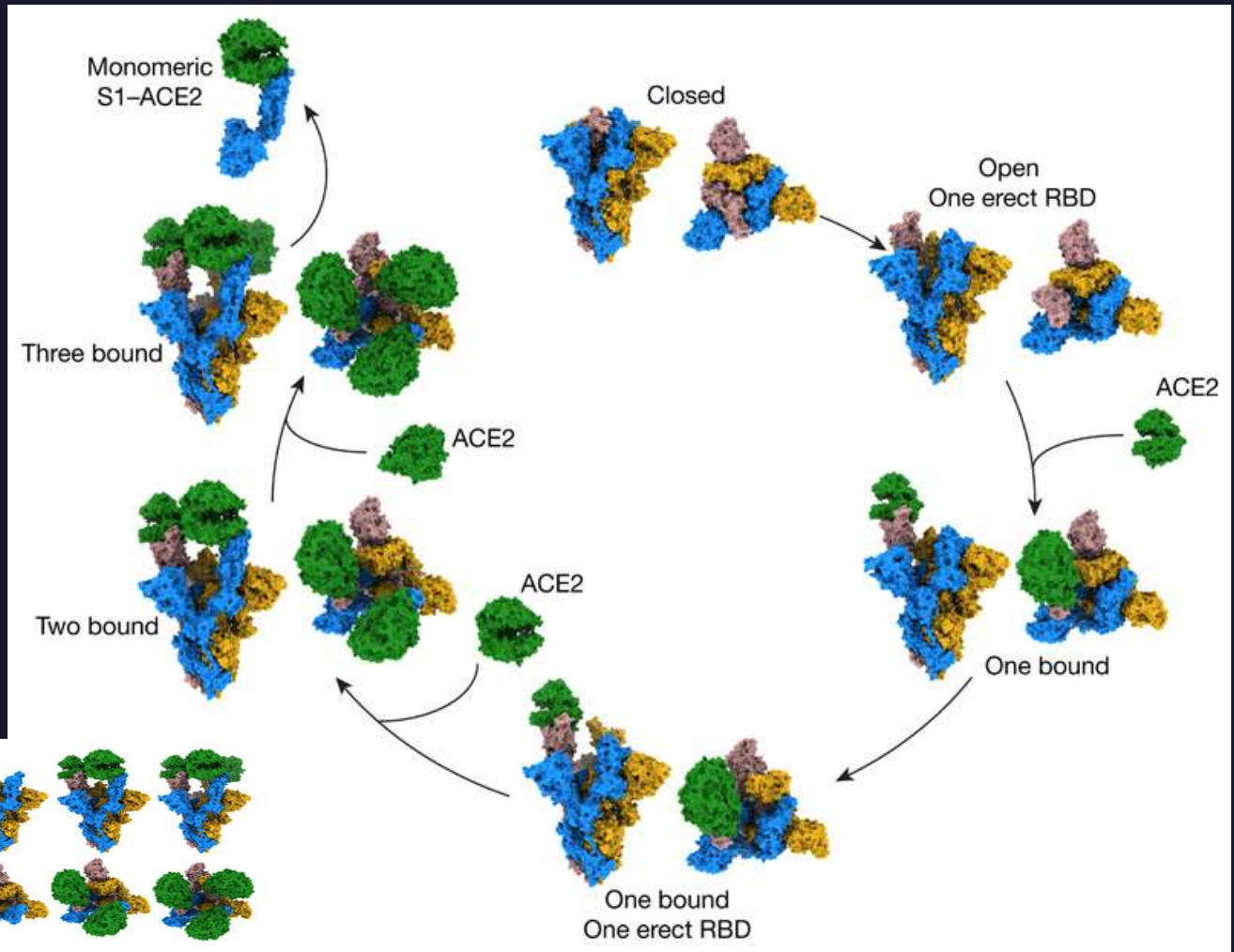
- Life cycle of SARS-CoV-2 consists of following 5 steps

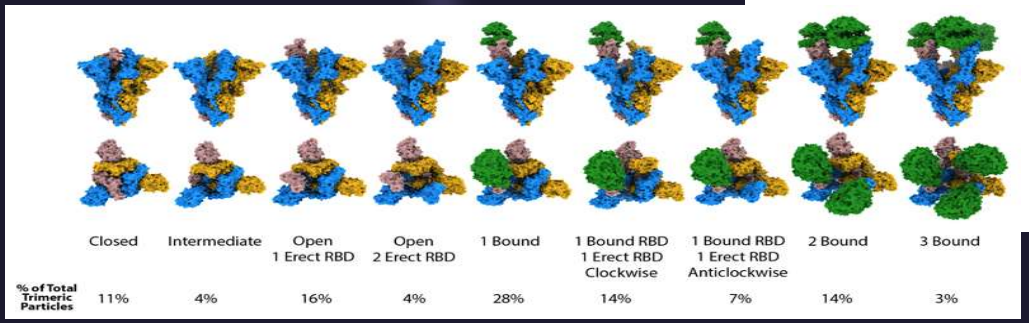
- 1. Attachment to Host Cell Surface
- 2.Viral Penetration and Uncoating
- 3. Replication-Transcription Complex (RTC) Formation
- 4. Synthesis of Viral RNA
- 5. Molecular Assembly and Release of SARS-CoV-2





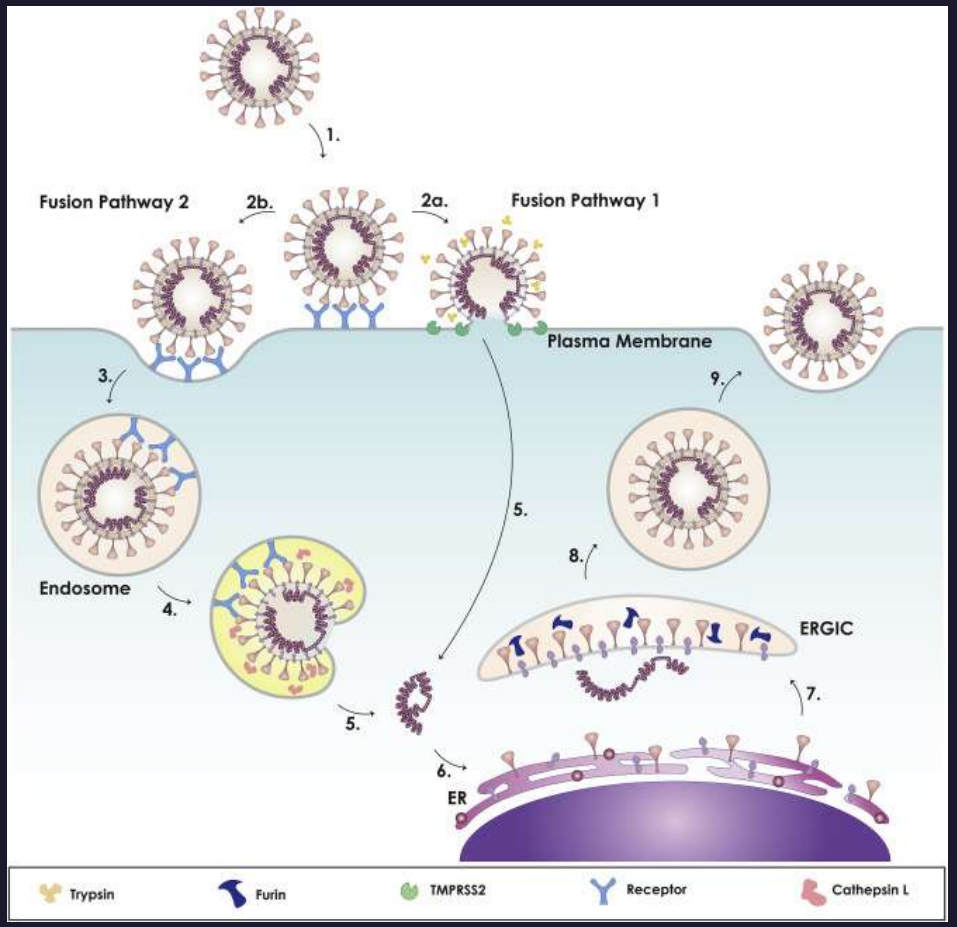
Sequential steps in ACE2 binding of the SARS-CoV-2 spike protein





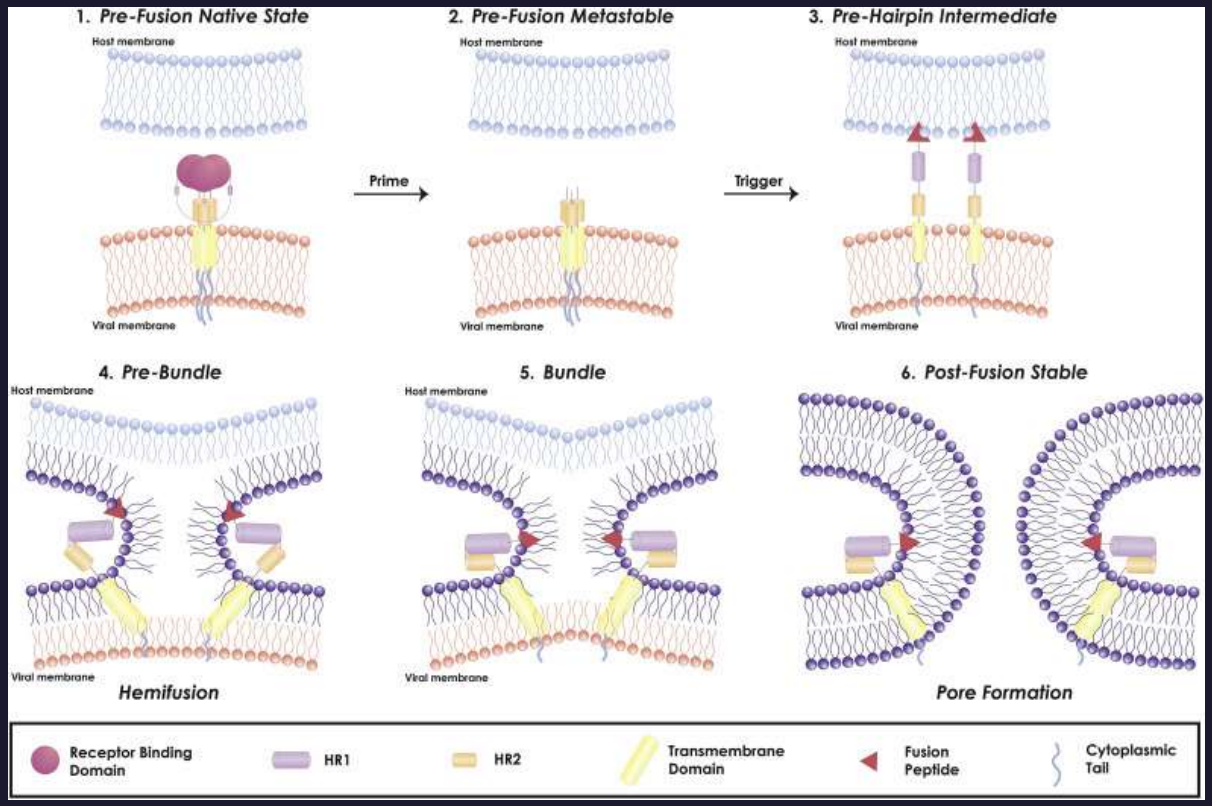
Summarize

- Model of coronavirus dual entry pathway. This model depicts the two methods of viral entry: early pathway and late pathway. As the virus binds to its receptor (*1*), it can achieve entry via two routes: plasma membrane or endosome. For SARS-CoV: The presence of exogeneous and membrane bound proteases, such as trypsin and TMPRSS2, triggers the early fusion pathway (*2a*). Otherwise, it will be endocytosed (*2b*, *3*). For MERS-CoV: If furin cleaved the S protein at S1/S2 during biosynthesis, exogeneous and membrane bound proteases, such as trypsin and TMPRSS2, will trigger early entry (*2a*). Otherwise, it will be cleaved at the S1/S2 site (*2b*) causing the virus to be endocytosed (*3*). For both: Within the endosome, the low pH activates cathepsin L (*4*), cleaving S2′ site, triggering the fusion pathway and releasing the CoV genome. Upon viral entry, copies of the genome are made in the cytoplasm (*5*), where components of the spike protein are synthesized in the rough endoplasmic reticulum (ER) (*6*). The structural proteins are assembled in the ER-Golgi intermediate compartment (ERGIC), where the spike protein can be pre-cleaved by furin, depending on cell type (*7*), followed by release of the virus from the cell (*8*, *9*). For SARS-CoV-2: Studies currently show that SARS-CoV-2 can utilize membrane bound TMPRSS2 or endosomal cathepsin L for entry and that the S protein is processed during biosynthesis. Other factors that can influence the viral entry pathway are calcium and cholesterol (*not shown*).

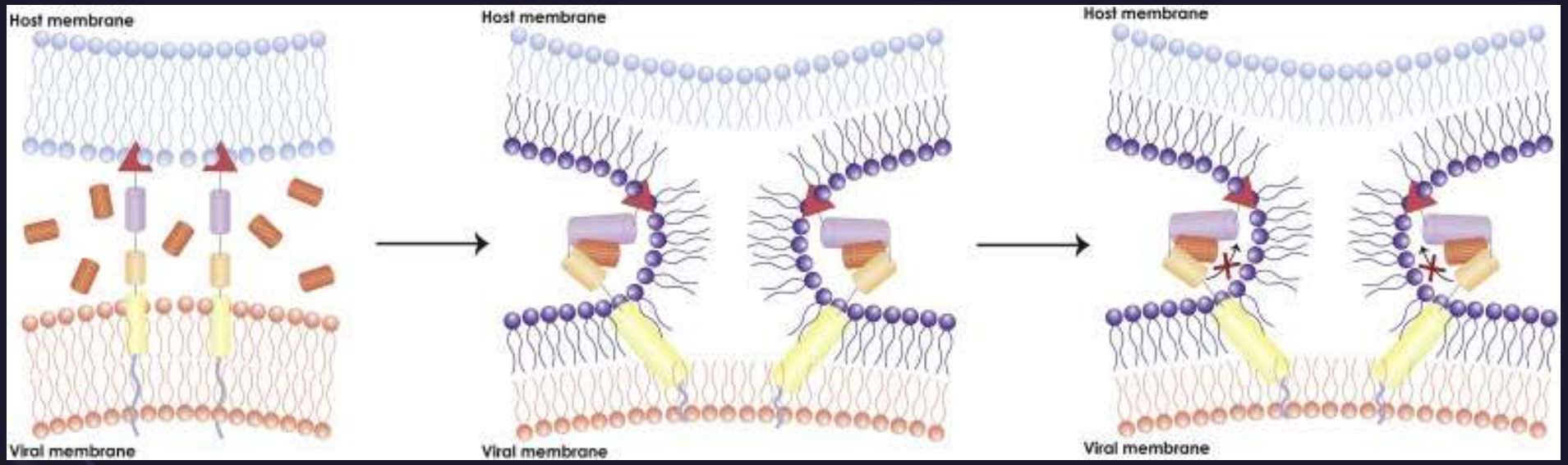


Coronavirus viral fusion pathway model based on class I fusion protein understanding

In this metastable state, the fusion protein must overcome a kinetic barrier to transition to the next state. The energy to overcome this barrier can be provided by a trigger that will interact with the fusion protein, resulting in a series of conformational changes that will enable the fusion protein to insert its FP into the host membrane, forming a pre-hairpin intermediate state. The triggering event(s) are usually environmental cues that inform the virus about its microenvironment. As an example, the influenza virus fusion protein is triggered by low pH; as the virus is trafficked through the endosome, the increasingly acidic conditions eventually destabilize its fusion protein, so that the fusion peptide is able to insert into the endosomal membrane and commence the fusion process.



- Model of major antiviral inhibitor pathway



Thank You