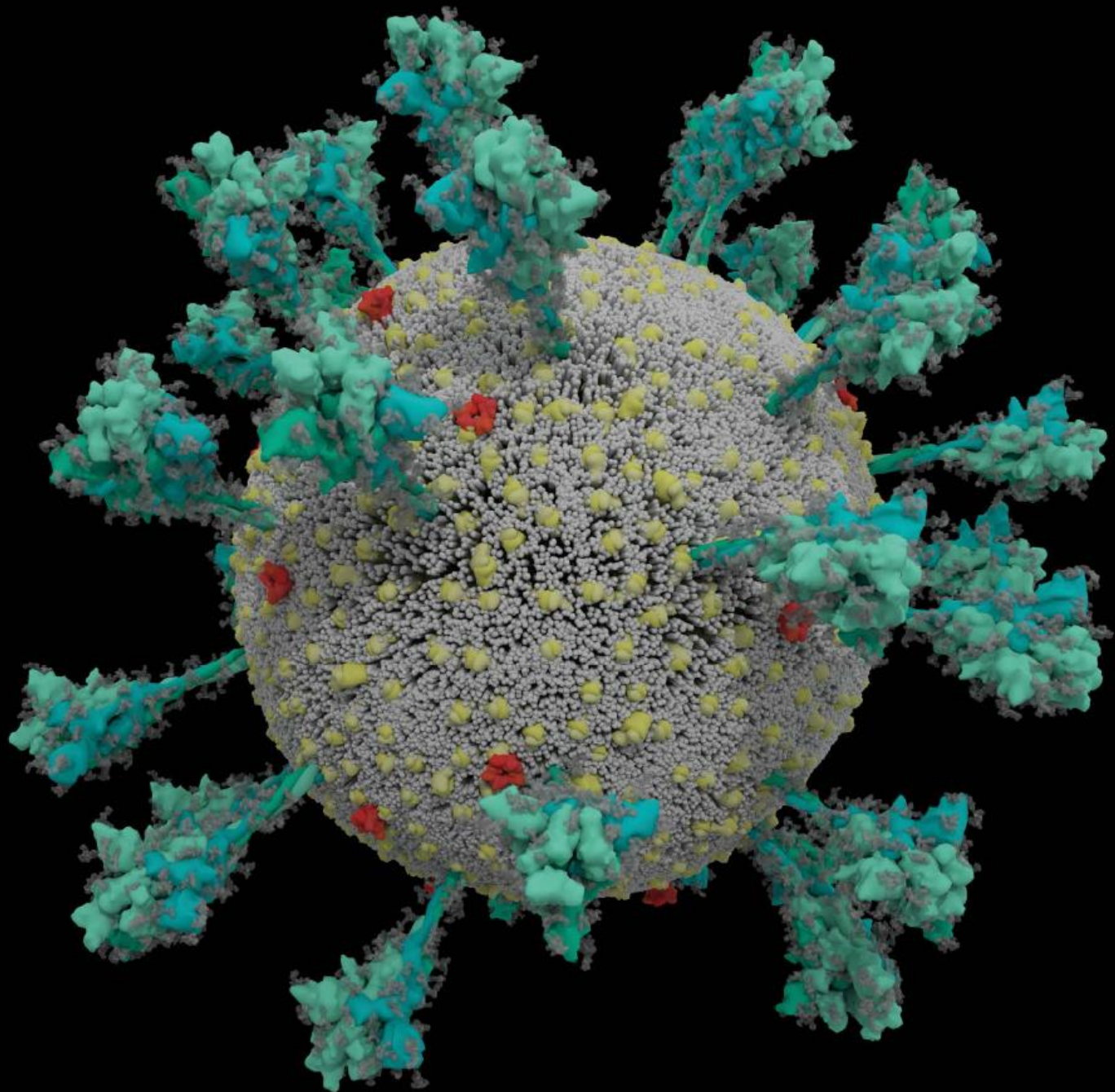


Molecular aspects of COVID-19

H. Rabbani



<https://www.nature.com/articles/d41586-021-02275-2>

LIFE CYCLE OF THE PANDEMIC CORONAVIRUS

A simplified account of how SARS-CoV-2 enters and exits cells.

Stage 1: Viral entry

The virus's spike protein binds to a receptor on the host cell called ACE2. Then, the host molecule TMPRSS2 cleaves the spike protein, exposing parts that fuse the viral membrane with that of the host.

Nucleocapsid
Spike protein
RNA
M protein

TMPRSS2

ACE2

Stage 2: Inside the cell

Viral RNA is translated into non-structural proteins (NSPs) that quickly suppress the translation of host messenger RNAs in favour of those belonging to the virus.

TMPRSS2 cuts the spike protein

Endoplasmic reticulum (ER)

NSPs

Viral proteins (NSPs)

Ribosome

ER remodelling

DMVs

Viral RNA

The spikes unravel and pull the membrane of the virus and host cell together

Stage 3: Remodelling the cell

The virus transforms the cell's ER — an internal membrane network — into bubble-like structures called double-membrane vesicles (DMVs). These might provide a safe haven for more viral RNA to be replicated and translated.

Golgi apparatus

Furin cut

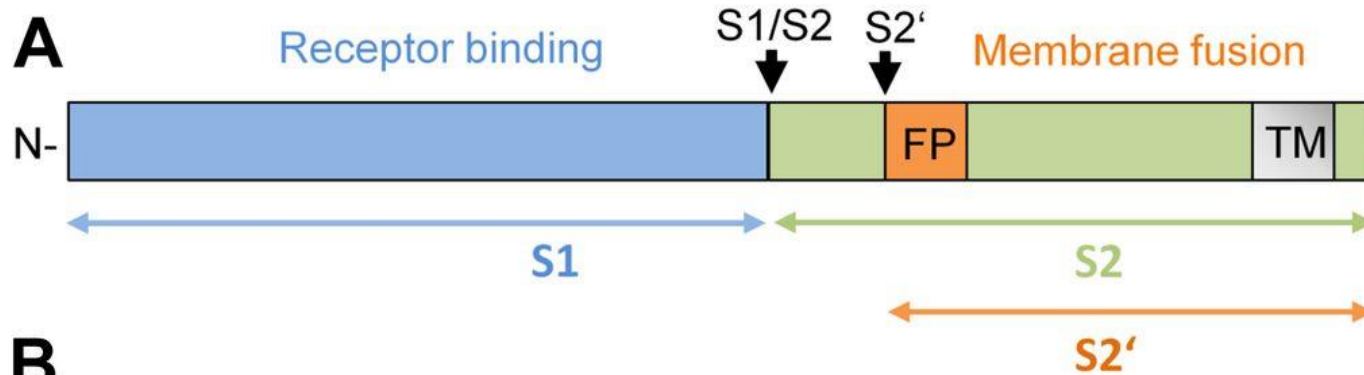
Stage 4: Exit

Once the newly made molecules assemble into a complete virus particle, this leaves the cell through an organelle called the Golgi apparatus, or perhaps through lysosomes, which are cellular rubbish bins.

Stage 5: The last slice

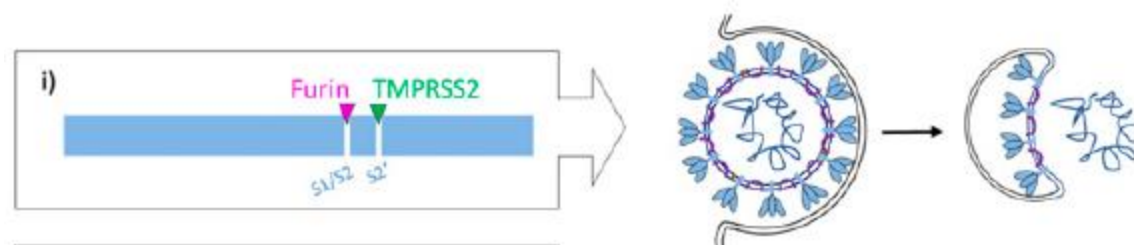
A host enzyme named furin makes a crucial cut at a site of five amino acids on the spike protein. This prepares the virus to strike another cell. Variants have a higher proportion of snipped spike proteins, helping them to infect cells more efficiently.

Furin and TMPRSS2 cleavage sites

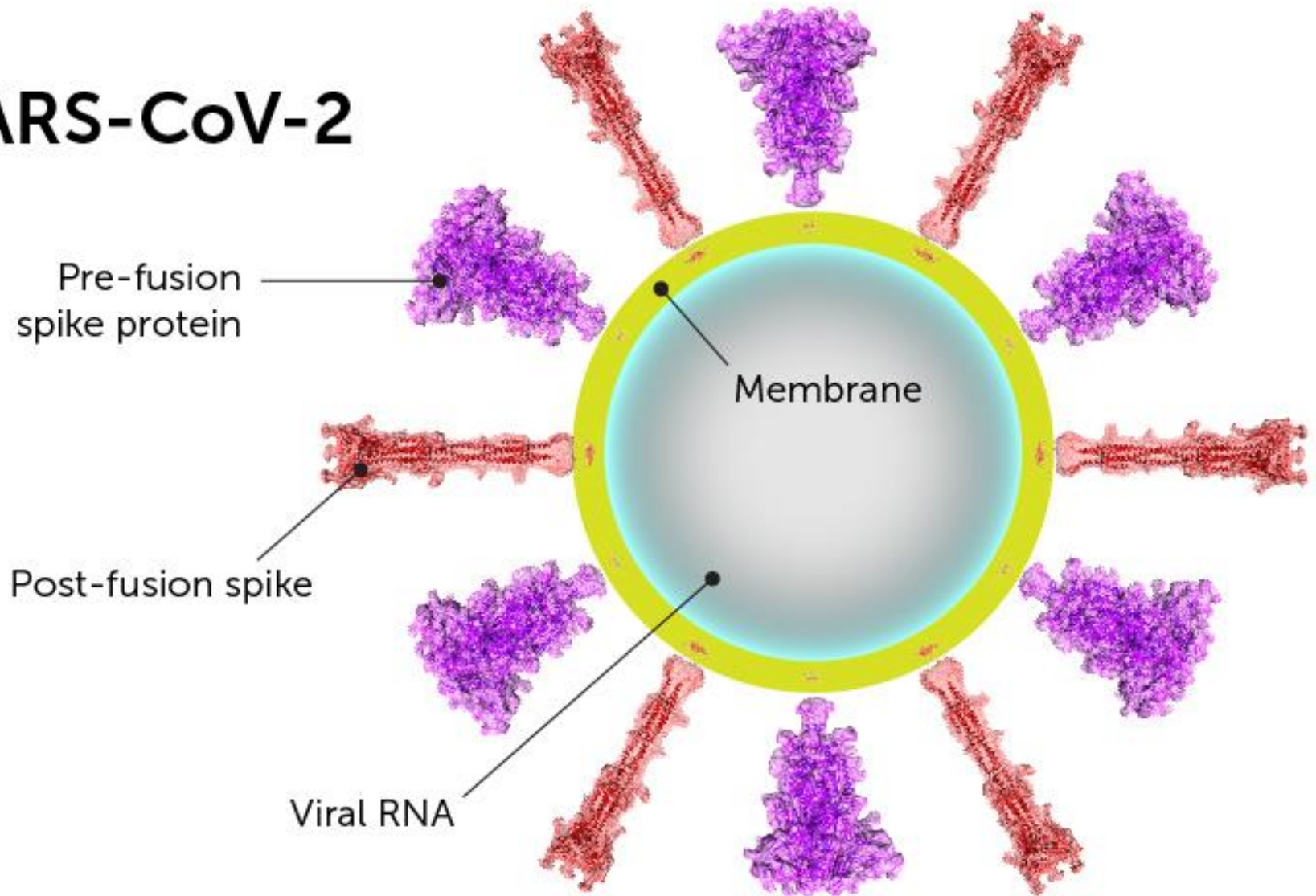


B

Virus	S1/S2 site	S2' site	GenBank sequence
SARS-CoV-2	TNSP RRAR ↓SVA	PSKPSK R ↓SFIEDL	YP_009724390
SARS-CoV	S----LL R ↓STS	PLKPTK R ↓SFIEDL	AAP13441.1
MERS-CoV	TLTP RSVR ↓SVP	TGS RSAR ↓SAIEDL	QFQ59587.1
HCoV-OC43	SKT RRSRR ↓AIT	CSKASS R ↓SAIEDL	AMK59677.1
HCoV-229E	SIIAVQ PR ↓NVS	GSRVAG R ↓SAIEDI	APT69890.1
HCoV-NL63	SLIPVR PR ↓NSS	SSRIAG R ↓SALEDL	AFV53148.1
HCoV-HKU1	SSS RRKRR ↓SIS	CGS-SS R ↓SFFEDL	AAT98580.1
IBV Beaudette	NGT RRFRR ↓SIT	PSS RRKR ↓SLIEDL	CAC39300.1



SARS-CoV-2



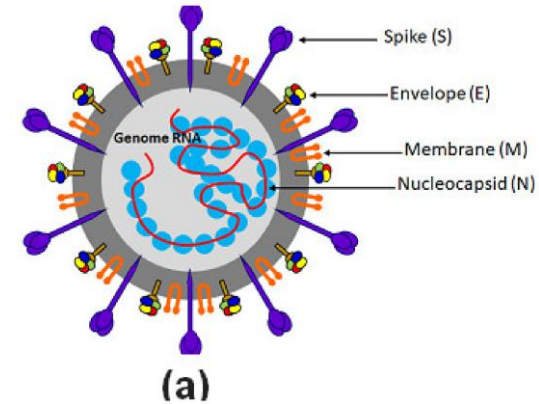
COVID-19 structural proteins

- Consists of 16 major proteins:

- Replicase polyprotein 1ab
- Replicase polyprotein 1a
- Spike glycoprotein
- Protein 3a
- Envelope small membrane protein
- Membrane protein
- Non-structural protein 6
- Protein 7a
- Protein non-structural 7b
- Non-structural protein 8
- Nucleoprotein
- ORF10 protein
- Protein 9b
- Uncharacterized protein 14
- Finally 26 known proteins

Number of COVID-19 structural proteins

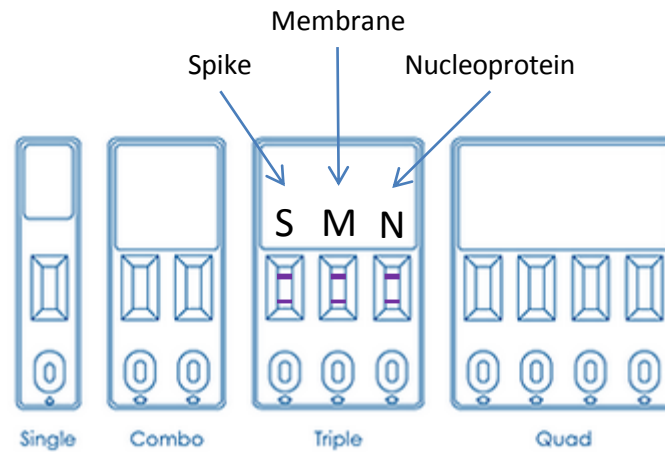
- 20x **E** pentamers (**100** proteins, 75 A.A)
 - 1000x **M** dimers (**2000** proteins, 222 A.A)
 - 30x **S** trimers (**90** proteins, 1273 A.A)
 - 1000x **N** monomers (**1000** proteins, 419 A.A)
- Reference: DOI: <https://doi.org/10.7554/eLife.57309>
- <https://www.nature.com/articles/d41586-021-02039-y>



Molecular Diagnosis

- At RNA level: RT-PCR (200 copies/ml)= about 70%
- At protein level: look for antigen
- Anti-Membrane (2000 proteins) antibody
- Anti-Nucleoprotein (1000 proteins) antibody
- Double

lateral flow Immunoassay (LFA)



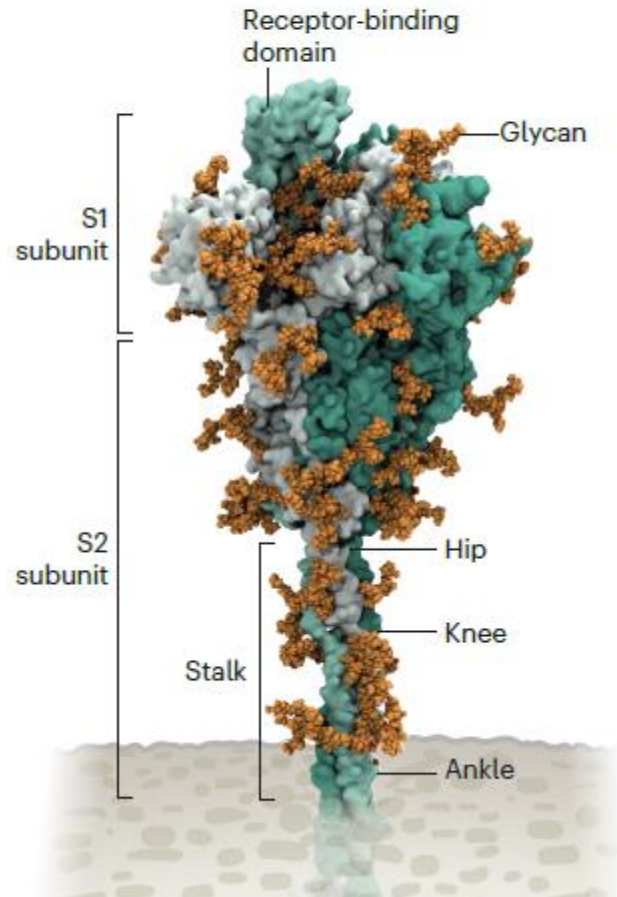
Lateral Flow Immunoassay (LFA) or Biosensors for antigen detection

- More reliable and sensitivity
- Easy to produce
- Much faster results
- No need to special device and could be done at any geographical conditions

Targeting

A HIDDEN SPIKE

The spike protein of SARS-CoV-2 is coated in sugar molecules, or glycans, which disguise it from the immune system. It can hinge at three points on the stalk, giving it flexibility.



Furin cuts

- In SARS-CoV, less than 10% of spike proteins are primed to infect a human cell
- In SARS-CoV-2, Alpha variant 50%
- In the highly transmissible Delta variant >75% of spikes are primed to infect a human cell.

Alpha variant (spike)

Alpha variant 50%

MFVFLVLLPLVSSQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLP
FNDGVYFASTEKSNIIRGWI FGTTLD SKTQSL LIVNNATNVVIKVCEFQFCNDPFLGVYHKNKSWMESEFRVYSSANNCTFEYV
SQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTL LALHRSYLT PGDSSSG
WTAGAAAYYVGYLQPRTFLLKYNENG TITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFN
ATRFASVYAWNRRKRI SN CVADYSVLNSASFSTFKCYGVSP TKLNDLCFTNVYADSFVIRGDEV RQIAPGQTGKIADYNYKL PDDE
TGCVIAWNSNNLDSKVGGNYNYLYRLEFRKSNLKPFERDISTEIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTYGVGYQPYRVVLS
FELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIDDTTDAVRDPQTLEILDITPCSFGGVSVITP
GTNTSNQVAVLYQG V NCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGA EHVNNSYECDIPIGAGICASYQTQTNSHRRAR
SVASQSIIAYTMSLGAENSVAYSNN SIAIPINFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIA
VEQDKNTQE VFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNG
LTVLPPLLTDemiaQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSL SSTAS
ALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILARLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKM
SECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICH DGKAHFPREGV FVSNGTHWFVTQRNFYEPQII
TTHNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQ
ELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT

Beta variant (spike)

Beta variant 50%

MFVFLVLLPLVSSQCVN**F**TTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVS GTNGTKRF**A**NPVLP
FNDGVYFASTEKSNIIRGWIFGTTLD SKTQSL LIVNNATNVVIKVCE FQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYV
SQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVR**G**LPQGFSALEPLVDLPIGINITRFQT**LLA**LHRSYLTPGDSSS
GWTAGAAAYYVG YLQPRTFLLKYNENG TITDAVDCALDPLSETKCTLKSF TVEKGIYQTSNF**RVQPTESIVRFPNITNLCPFGEVF**
NATRFASVYAWN RKRISNCVADYSVLNSASFSTFKCYGVSPTKLN DLCFTNVYADSFVIRGDEV RQIAPGQTGN**IADYNYKL PDD**
FTGCVIAWNSNNLDSKVGGNYNYLYRLF**RKSNLKPFERDISTE I**YQAGS**TPCNGV**KGFNCYFPLQSYGFQPT**Y**GVGYQPYRVVVL****
SFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVIT
PGTNTSNQVAVLYQ**G**VNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGA EHVNNSYECDIPIGAGICASYQTQTNS**PRRAR**
SVASQSI IAYTMSLGV**ENSVAYSNN SIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIA**
VEQDKNTQE VFQAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVT LADAGFIKQYGDCLGDIAARDLICAQKFNG
LTVLPPLLTD EMI AQYTSALLAGTITSGWTFGAG AALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFN SAIGKIQDSLSTAS
ALGKLQDVVNQNAQALNTLVKQLSSNF GAISSVLNDILSR LDKVEAEVQIDRLITGRLQSLQTYVTQQ LIRAAEIRASANLAATKM
SECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICH DGKAHFPREGVFVSNGTHWFVTQRNFYEPQII
TTDN T FVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPD VDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQ
ELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPV LKGVKLHYT

Gamma variant (spike)

Gamma variant 50%

MFVFLVLLPLVSSQCVN**F**T**N**RTQLP**S**AYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLP
FNDGVYFASTEKSNIIRGWIFGTTLDSTQSLIVNNATNVVIKVECFQFCN**Y**PFLGVYYHKNNKSWMESEFRVYSSANNCTFEYV
SQPFLMDLEGKQGNFKNL**S**EFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLALHRSYLTTPGDSSSG
WTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSN**F**RVQPTESIVRFPNITNLCPFGEVFN
ATRFASVYAWNRKRISNCVADYSVLNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQT**G**TIADYNYKLPDDE
TGCVIAWNSNNLDSK**VGGNYNYLYRLE**RKSNLKPFERDISTE**IYQAGS**TPCNGV**KGFNCYFPLQSYGFQPT****Y**GVGYQPYRVVLS
FELLHAPATVCGPKKSTNLVKNKCVNFNENGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTL**EILDITPCSF**GGVSVITP
GTNTSNQVAVLYQ**G**VNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAE**Y**VNNSYECDIPIGAGICASYQTQTN**PRRAR**
SVASQSIIAYTMSLGAENSVAYSNN**SI**AIPNFTISVTTEILPVSM**TKTSVDCTMYICGDSTEC**SNLL**LQYGSFCTQLNR**ALT**GIA**
VEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVT**LADAGFIKQYGDCLGDIAARDL**ICAQKFNG
LTVLPPLLTD**EMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQ**MAYRFNGIGVTQNVLYENQK**LIANQFN**SAIGKIQDSLSSTAS
ALGKLQDVVNQNAQALNTLVKQLSSNFGA**ISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAA****I**KM
SECVLGQSKRVDFCGKGYHLM**SFPQSAPHGVVFLHV**TYVPAQ**EKNFTTAPAICH**DGKAHFPREGV**FVSNGTHWFVTQRNFYEPQII**
TTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELD**KYFKNHTSPD**VDLGD**ISGINAS****F**VNIQKEIDRLNEVAKNLNESLIDLQ
ELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCC**SCLKGCCSCGSCCKF**DEDDSEPV**LKGVKLHYT**

Delta variant (spike)

Delta variant >75%

MFVFLVLLPLVSSQCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPF
NDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVECFQFCNDPFLGVYYHKNNKSWMESEFGVYSSANNCTFEYV
SQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSG
WTAGAAAYYVGYLQPRTFLLKYNGGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFN
ATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDF
TGCVIAWNSNNLDSKVGGGNYNYRYRLFRKSNLKPFERDISTEIYQAGSKPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLS
FELLHAPATVCGPKKSTNLVKNKCVNFNENGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTL*ILDITPCSF*GGVSVITP
GTNTSNQVAVLYQGVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAHEVNNSYECDIPIGAGICASYQTQTNSRRRAR
SVASQSIIAYTMSLGAENSVAYSNNSI*AIPTNFTISVTTEILPVSM*TKTSVDCTMYICGDSTEC*SNLLLQYGSFCTQLNRALTGIA*
VEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNG
LTVLPPLLTDemiaQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTAS
ALGKLQNVVNQNAQALNTLVKQLSSNFGAIISSVLNDILSRDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKM
SECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFHTTAPAICHDKAHFPREGVSVSNGTHWFVTQRNFYEPQII
TTDNTFVSGNCDVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQ
ELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT

Studies have found that Pfizer and Johnson & Johnson vaccines are less effective against the delta variant, compared to the alpha strain, per [Popular Science](#).

Lambda variant (spike)

Lambda variant ?%

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTN**VI**KRFDNPVLPF
NDGVYFASTEKSNIIRGWIFGTTLDSTQSLNATNVVIKVECFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVS
QPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLALH**RSYLT**PGN**SSS**
GWTAGAAAYYVGYLQPRFTLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVF
NATRFASVYAWNRRKISNCVADYSVLNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDD
FTGCVIAWNSNNLDSK**VGGNYNYQYRLF**ERKSNLKPFERDISTE**IYQAGS****T**PCNGV**EGFNCYS**PLQSYGFQPTNGVG**YQPYRVVVL**
SFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVIT
PGTNTSNQVAVLYQ**G**VNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAELVNNSECDIPIGAGICASYQTQNTS**PRRAR**
SVASQSI IAYTMSLGAENSVAYSNNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIA
VEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNG
LNVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTAS
ALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKM
SECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFHTTAPAICHGKAHFPRGVFVSNGTHWFTQRFYEPQII
TTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQ
ELG**KYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCC**MTSCCCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT

Lambda Variant Shows Vaccine Resistance

The loss of B cell epitope in Lambda variant



Emerging
N-glycosylation
site

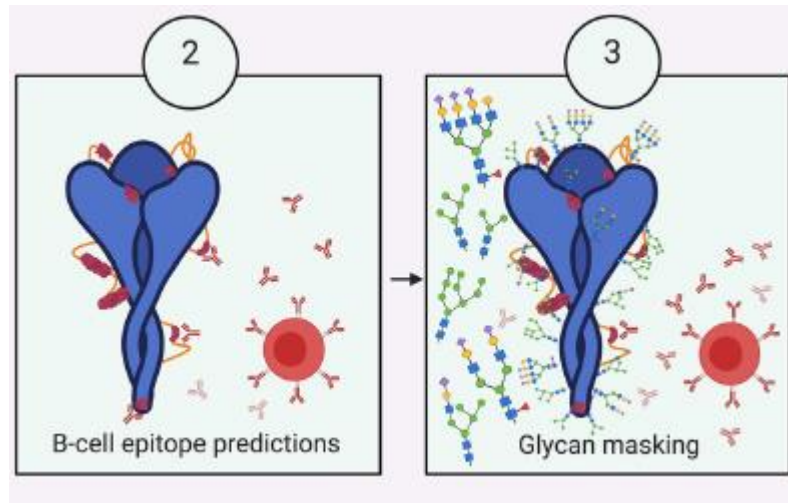
N-glycosylation

Spike is a glycoprotein

- 22x N- and O-glycosylation sites
- This glycosylation alters B cell epitope recognition

RBD-ACE2

- interaction between RBD and ACE2 involves residues 445-456, 473-477, and 484-505

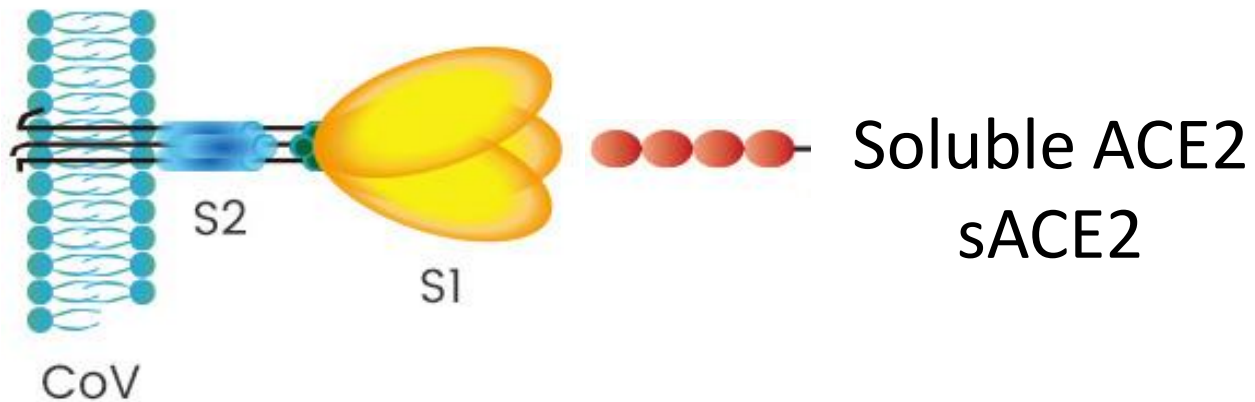
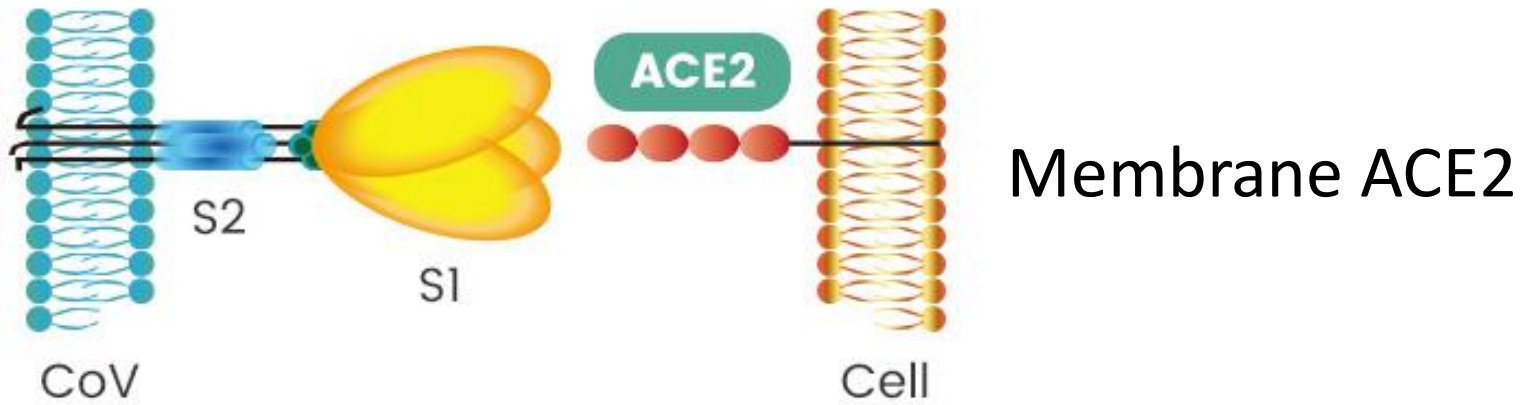


- npj Vaccines (2020) 5:81 ;
<https://doi.org/10.1038/s41541-020-00237-9>

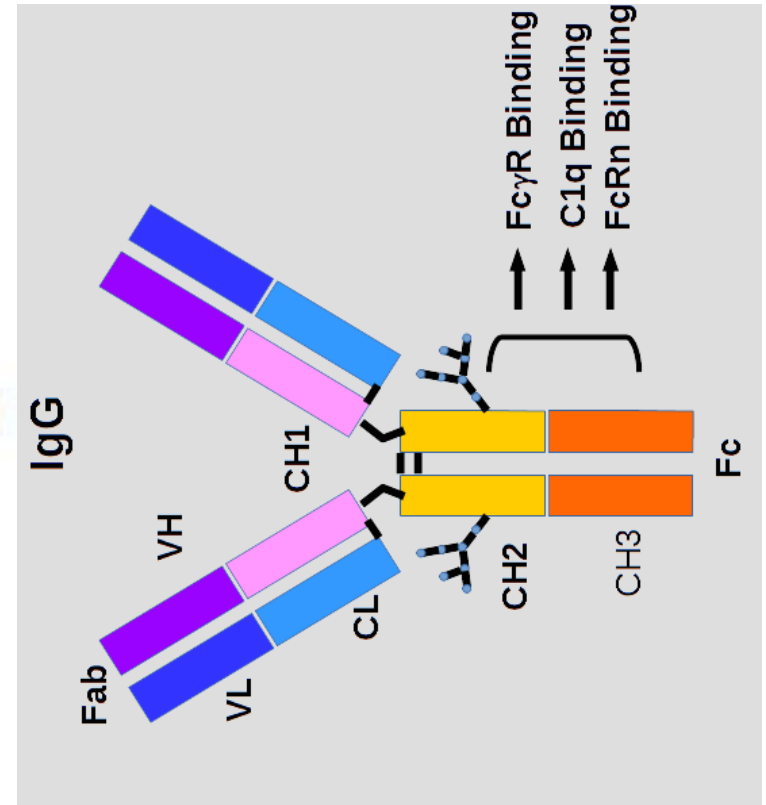
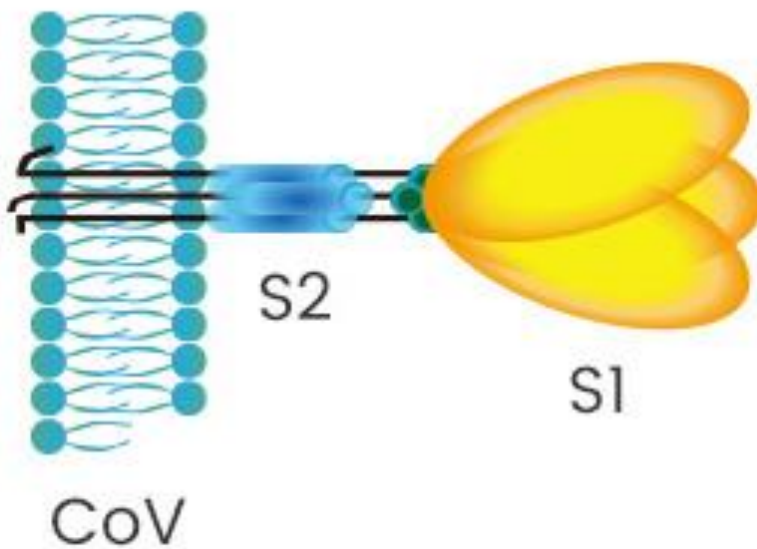
Therapy

- Different types of vaccines:
- Whole inactivated COVID-19
- Recombinant spike protein
- mRNA coding spike
- dsDNA in adenovirus encoding spike protein

Problem



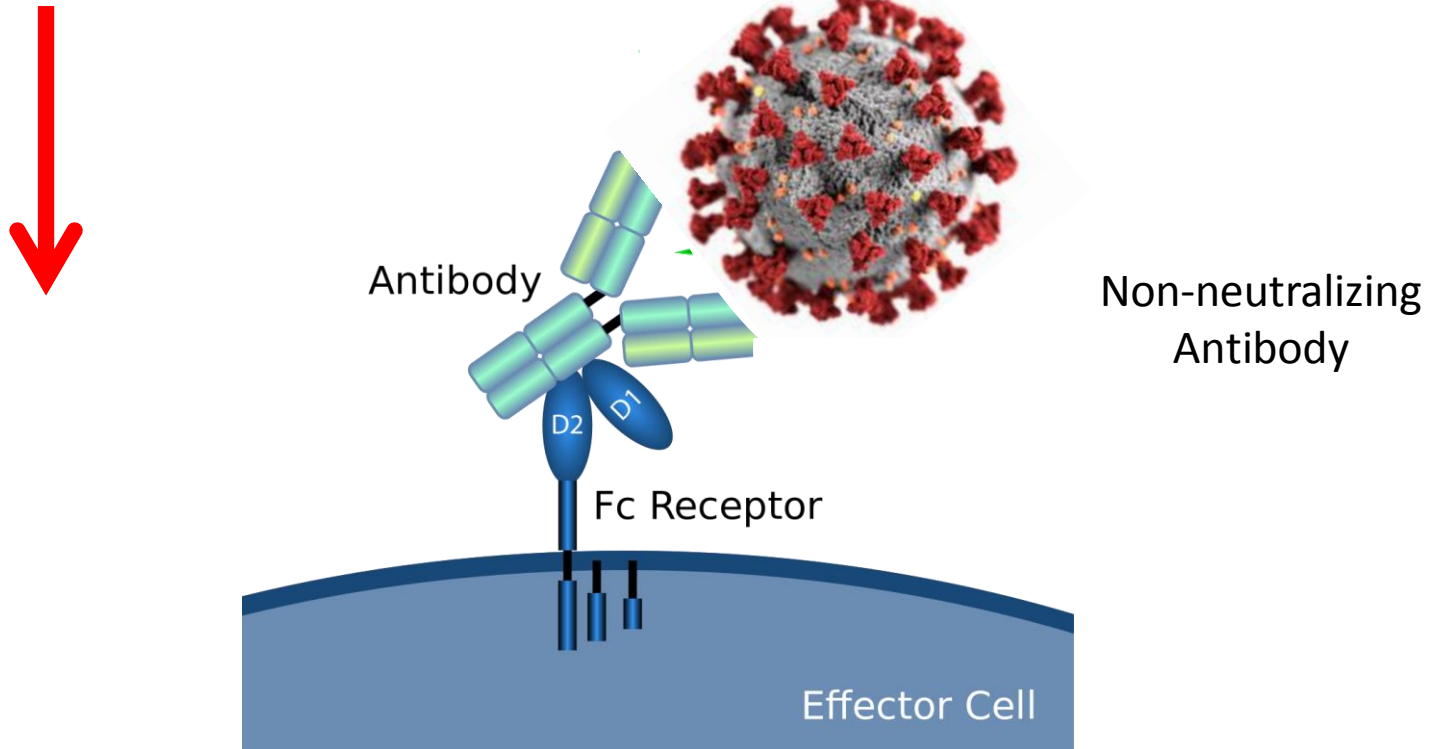
Problem



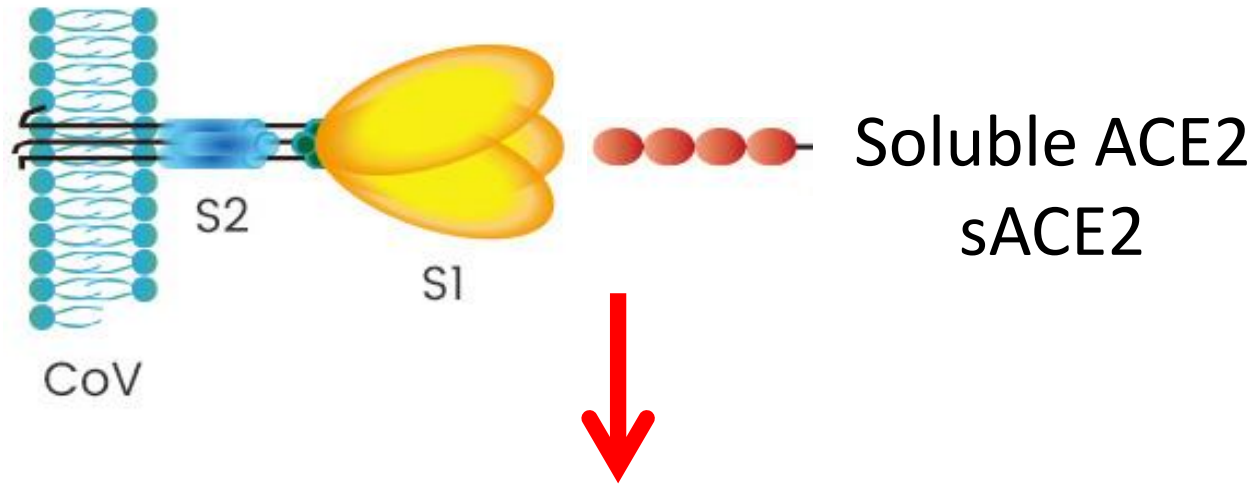
Antibody-Dependent Enhancement (ADE)

Fc γ RIIa, Fc γ RIIIa (CD16a)

- CD4+ effector T cells



Problem



Anti-ACE2 antibody (Autoimmunity)

Antibody response

- Total amino acids of the virus is about 7000 A.A.
- Only 25% of the proteins are B cell epitopes
- The best region to develop neutralizing antibody is spike protein (1273 A.A.)
- RBD is about 60 A.A. (25% B cell epitopes)
- Only 0.7-1% of the pooled antibody would be neutralizing antibody

The affinity of neutralizing antibody must be higher than ACE2 affinity in binding to the virus

Antibody > ACE2

Vaccinated people

- Still prone to infection but with lower viral load

Strategy

- A cocktail of monoclonal antibodies (**Passive immunotherapy**)
- Peptide-based vaccination (**Active vaccination**)

Summary and conclusion

- The neutralizing antibody may not necessarily target the RBD
- Peptide-based vaccines are more safe, provided increasing the antigenicity