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SARS-Coronavirus-2 Gene Mutagenesis

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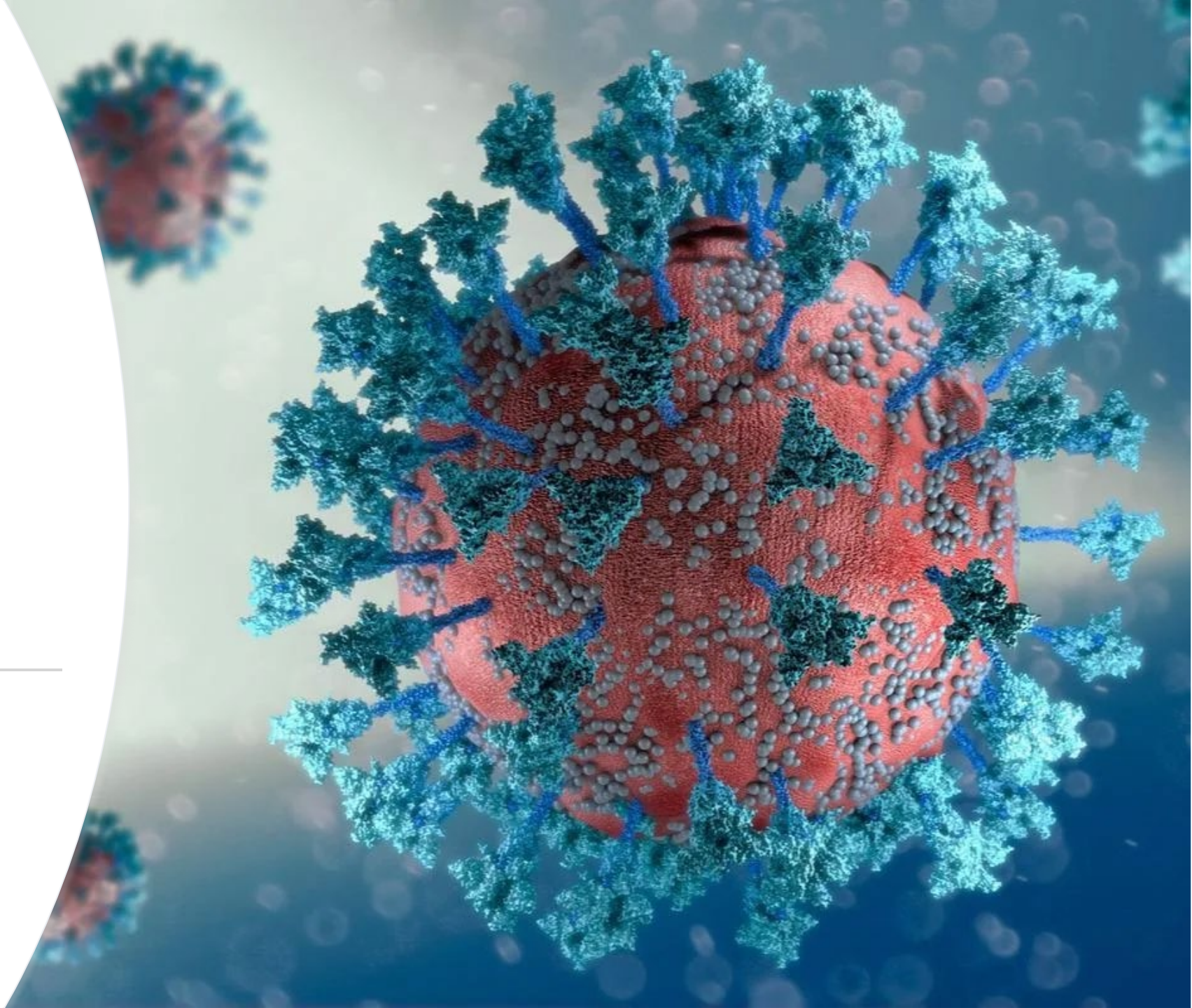
Salina Acharya

Testing the Functions of SARS-Coronavirus-2 Spike Gene Mutations

Anna Anderson & Salina Acharya



Project Goal

Short-term goal:

Generate specific mutants of the Spike (S) protein of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, by replacing amino acids in the SARS-CoV-2 Alpha variant S gene with the corresponding mutations in the SARS-CoV-2 Delta Variant S gene.

Long-term goal:

To test if a library of SARS-CoV-2 Spike Mutations (L452R, T478K, D614G, P681R, D950N) associated with changes in transmissibility and virulence affect viral entry kinetics and immune cell activation.

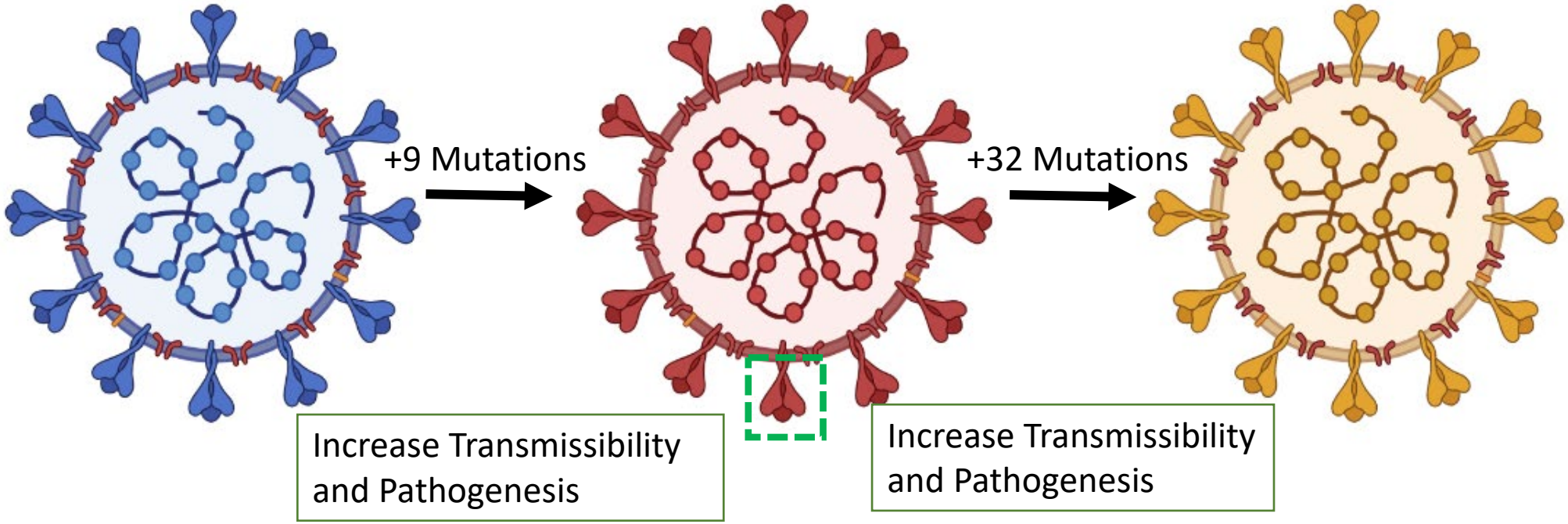
Why focus on mutations in the Spike Protein?

Evolution of the SARS-CoV-2

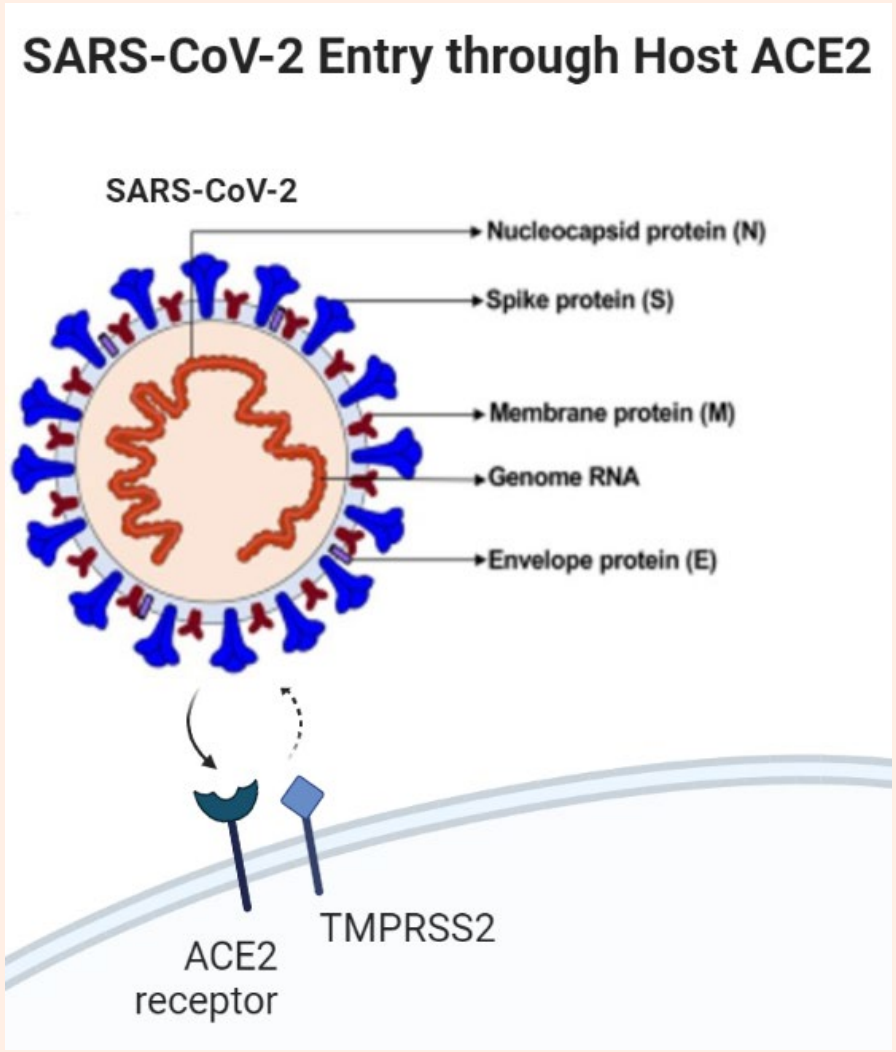
SARS-CoV-2 Alpha

SARS-CoV-2 Delta

SARS-CoV-2 Omicron



SARS CoV-2 Spike binds to host receptor to infect lung cell

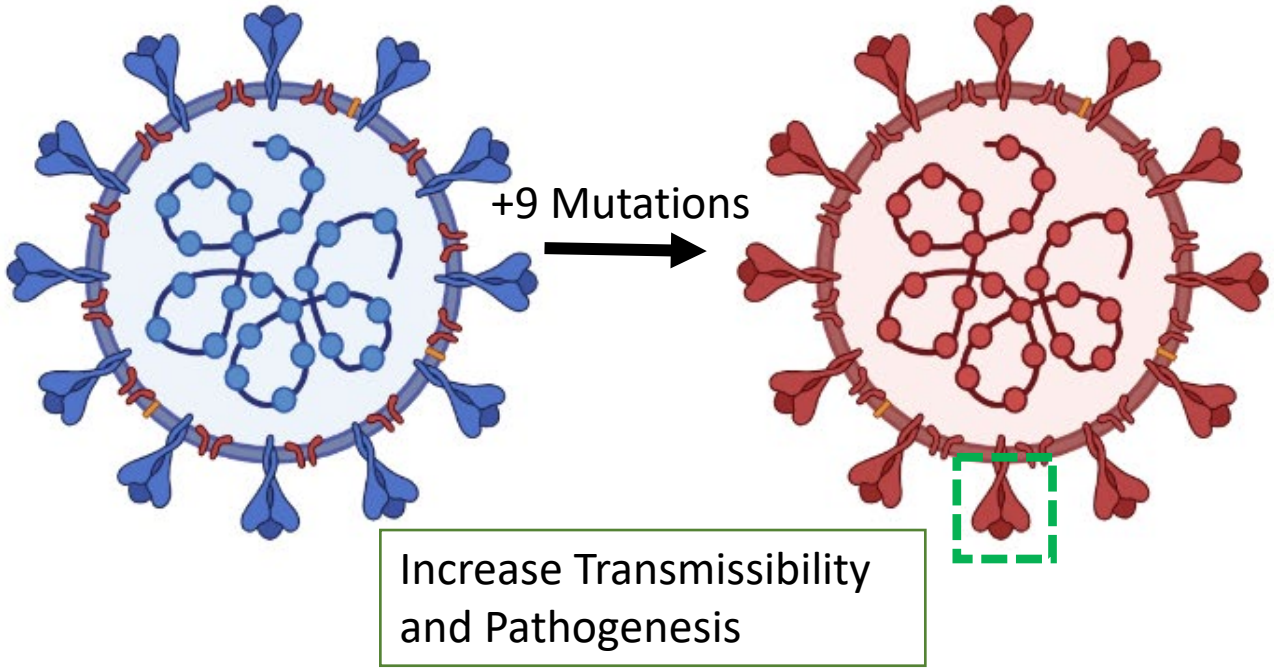


- Binds to ACE2 Receptor
- Mediates entry of the virus into the lung cell cytoplasm.
- Mutations in the S protein affect transmission from human to human, and virulence. (Disease severity)

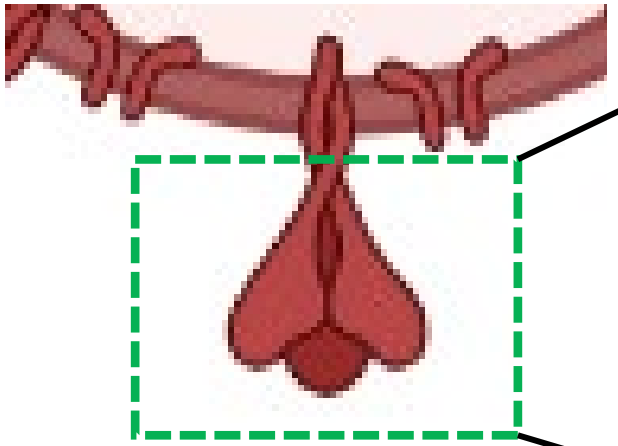
How did the Delta variant increase transmission and virulence?

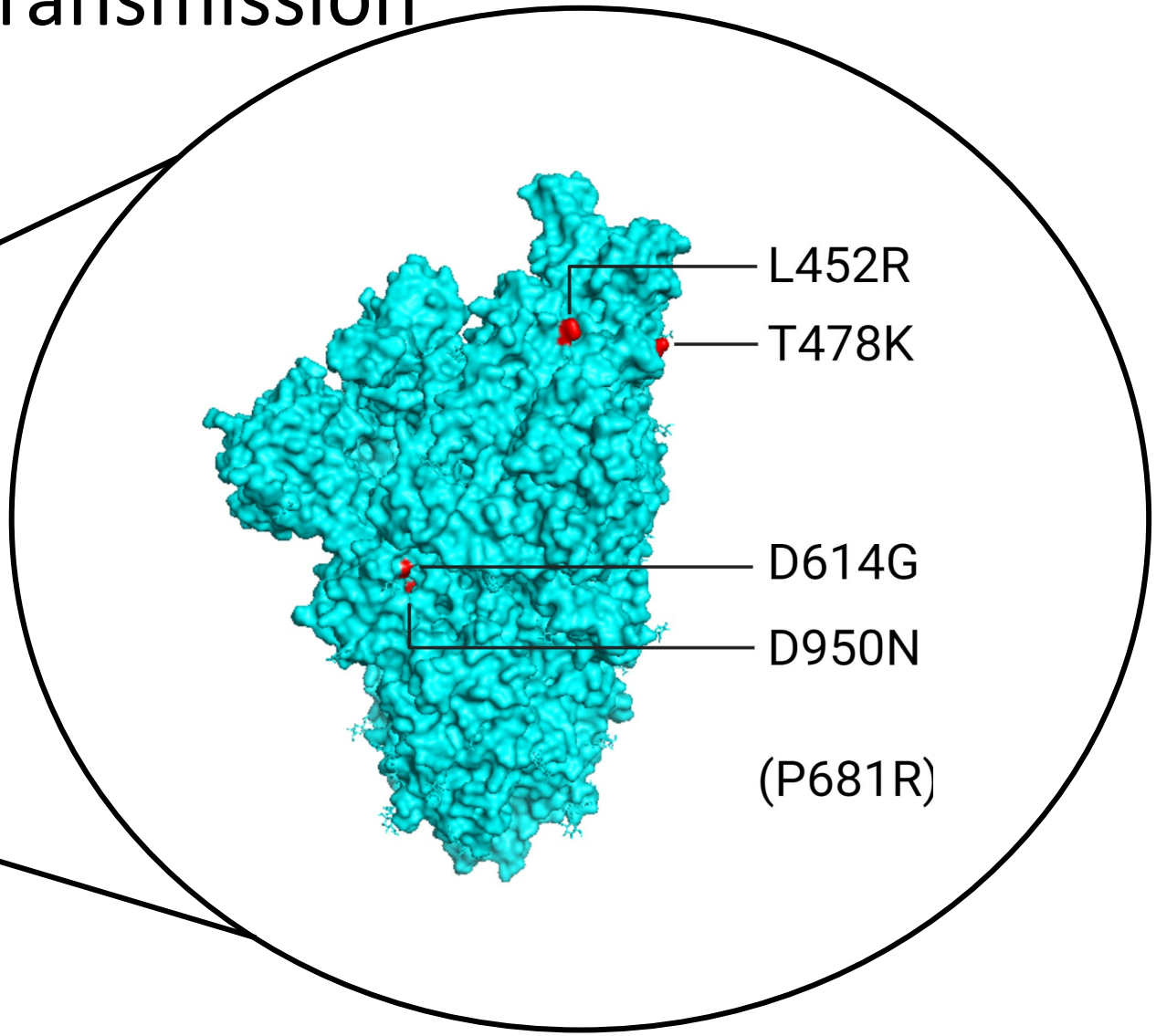
Evolution of the SARS-CoV-2

SARS-CoV-2 Alpha SARS-CoV-2 Delta

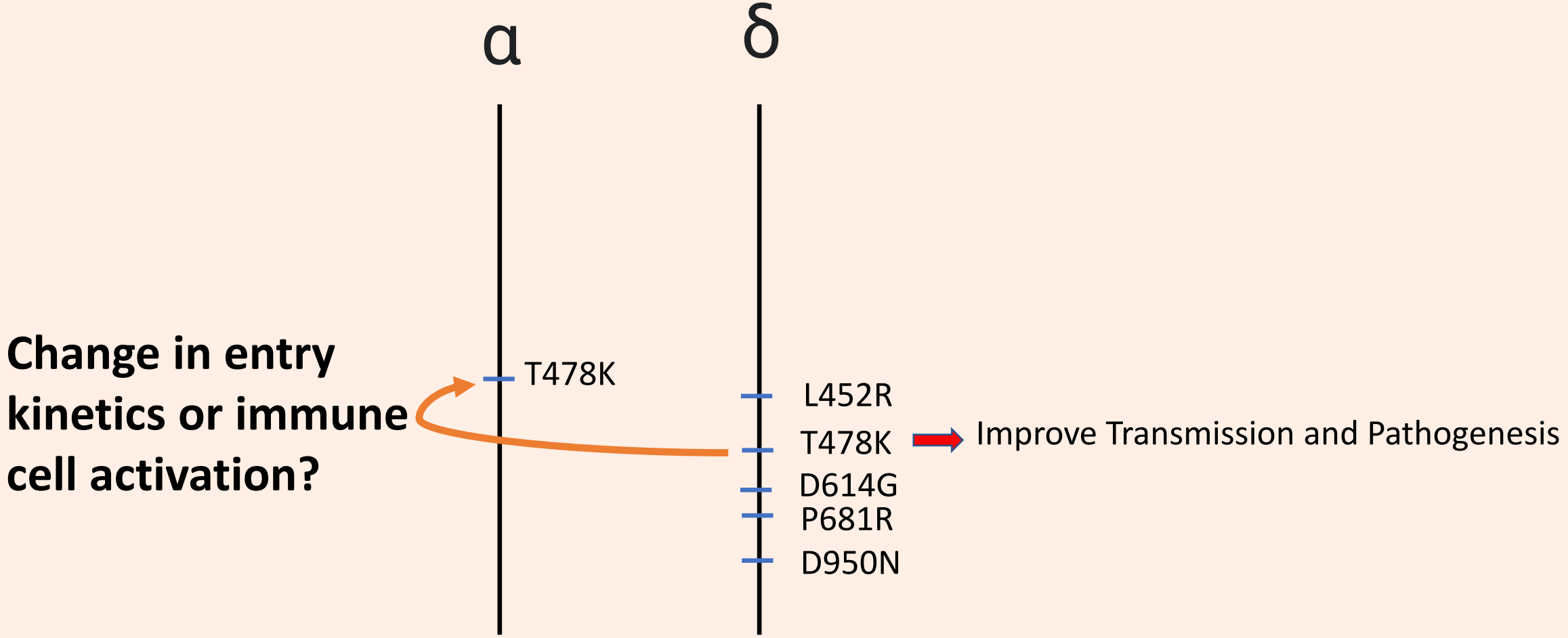


Delta Variant Spike Protein Mutations associated with changes in transmission and virulence.

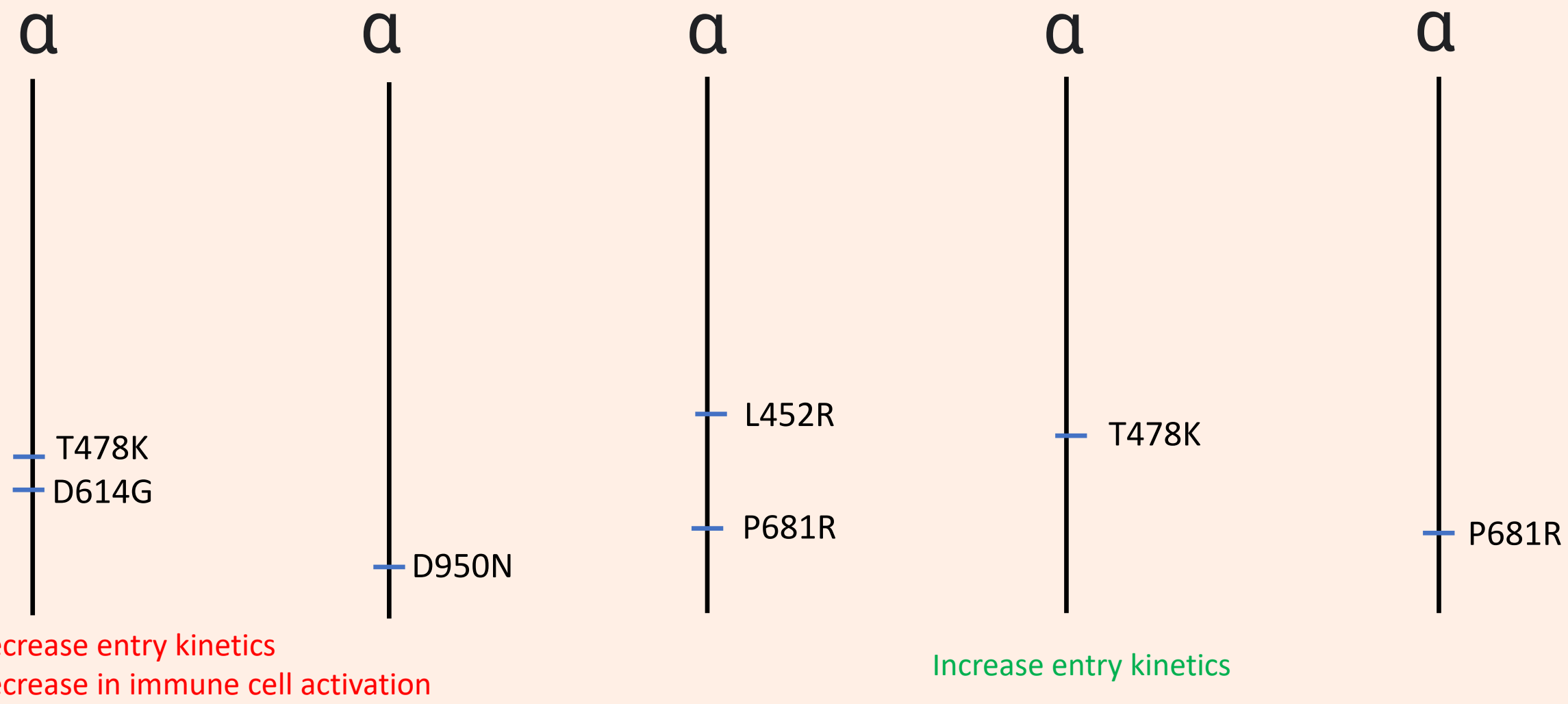




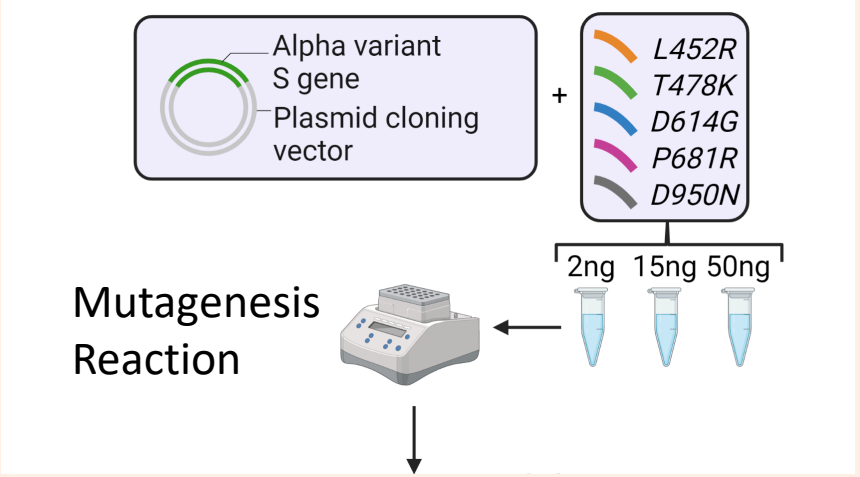
Strategy for generating S gene mutants to be tested for gain or loss of function



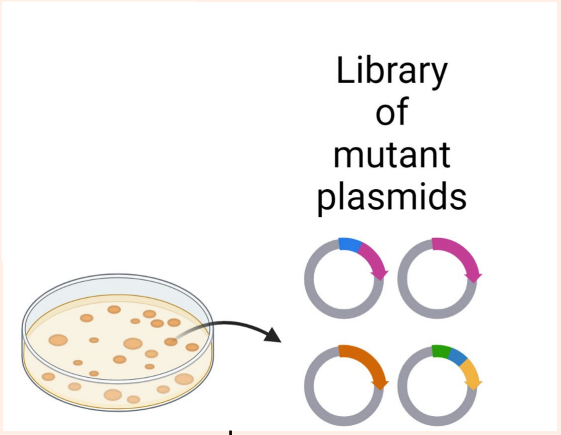
Testing viral mutants for gain or loss of functions



Preview of Experiment



Transformation



Prep DNA

Send for Sequencing

The Experiment: Mutagenesis

- Using the QuikChange Multi site-directed mutagenesis kit
- 3 mutagenesis reactions using **3 different primer concentrations** (2ng, 10ng, 50ng) of 5 primers
  - 1: L452R
  - 2: T478K
  - 3: D614G
  - 4: P681R
  - 5: D950N

Performing Mutagenesis: Primers, DNA template, and reaction buffers react in Thermocycler to generate random mutations into Alpha virus S gene. Dpn 1 is added to dissolve original template leaving only mutant S genes.

The Experiment: Transformation

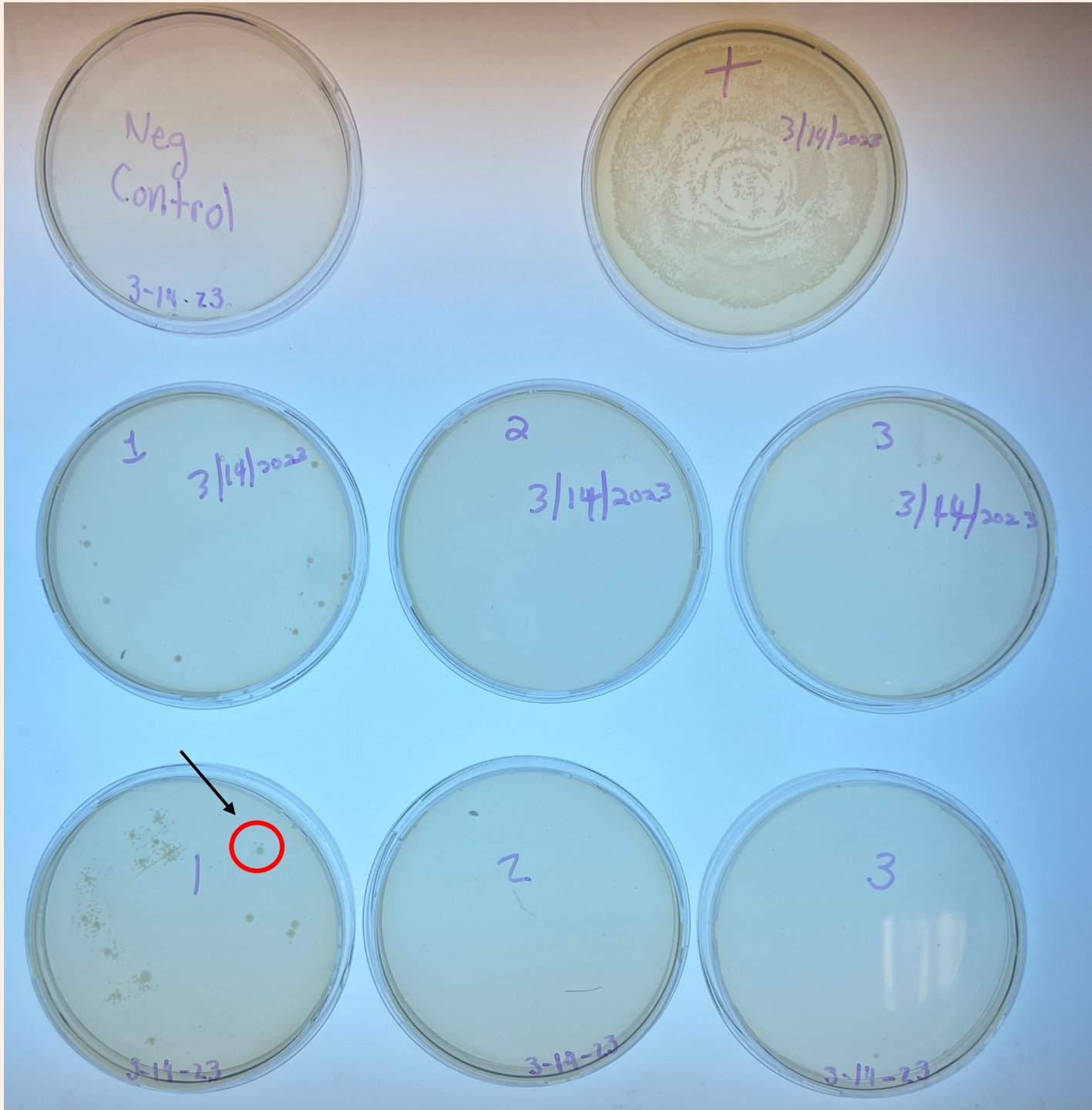
- Purpose: To harvest and separate different mutant S gene containing plasmids from mutagenesis tubes into bacteria so that each colony will contain one S gene.

- Transformation Protocol

  - To place plasmids into competent bacteria, heat shock to take up DNA, plate bacteria on agar plate which will select Bacteria that contain plasmids. Plasmids have mutant S genes in them.

Result of Transformation Colonies of Bacteria containing S genes

Plate	Mutagenesis primer Dilutions	Numbers of Colonies
1	2ng	+++
2	10ng	-
3	50ng	+



Testing SARS-CoV S protein mutations in entry and immune cell activation assays

- The Entry Assay tests mutant virus kinetics of infection
  - Testing an aspect of transmissibility

- Stimulation assays test the mutant virus' ability to stimulate immune cells
  - Testing an aspect of virulence

Results and Conclusion

- Performed Mutagenesis
- Transformed Bacteria with 3 mutagenesis reaction plasmids
- Harvested 24 colonies of bacteria containing S genes
- Performed mini-preps to isolate the plasmid DNA containing S genes.
- Sent DNA for sequencing analysis to check S gene mutation success.
  - If we have successfully mutated the Alpha spike protein, we will perform assays that can assess the mutations' effect on entry kinetics and immune cell activation.

Acknowledgements

Anna Anderson
Salina Acharya
Colton Kline
Professor Osvaldo Martinez
The WSU Biology Department
Early-Year Research & Creative Mentoring Program
Ramaley Research Celebration Event Organizers

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SARS-CoV-2 delta

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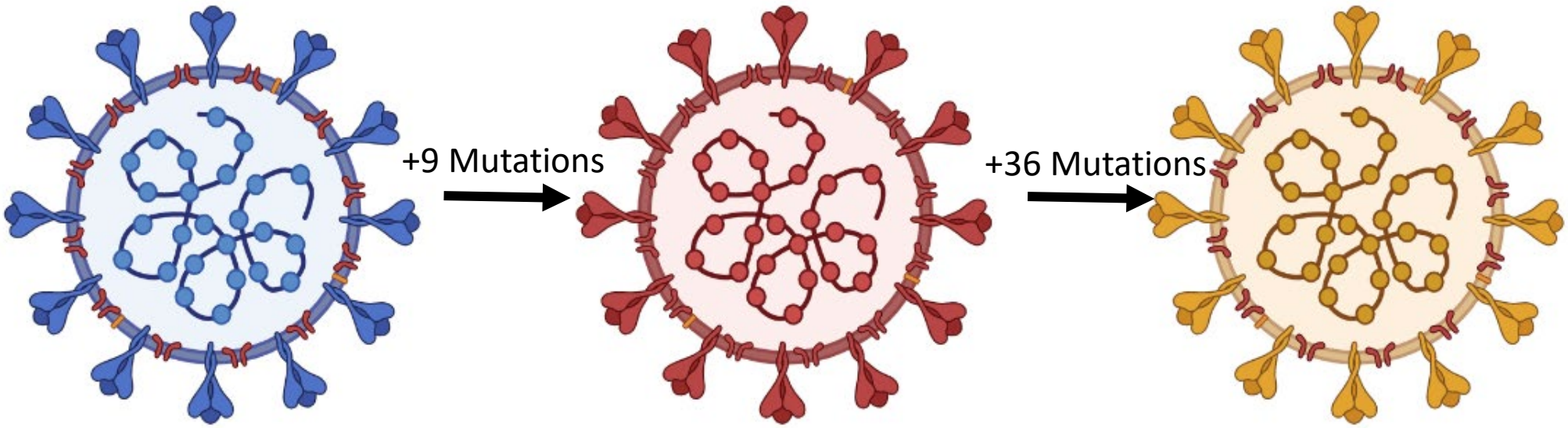

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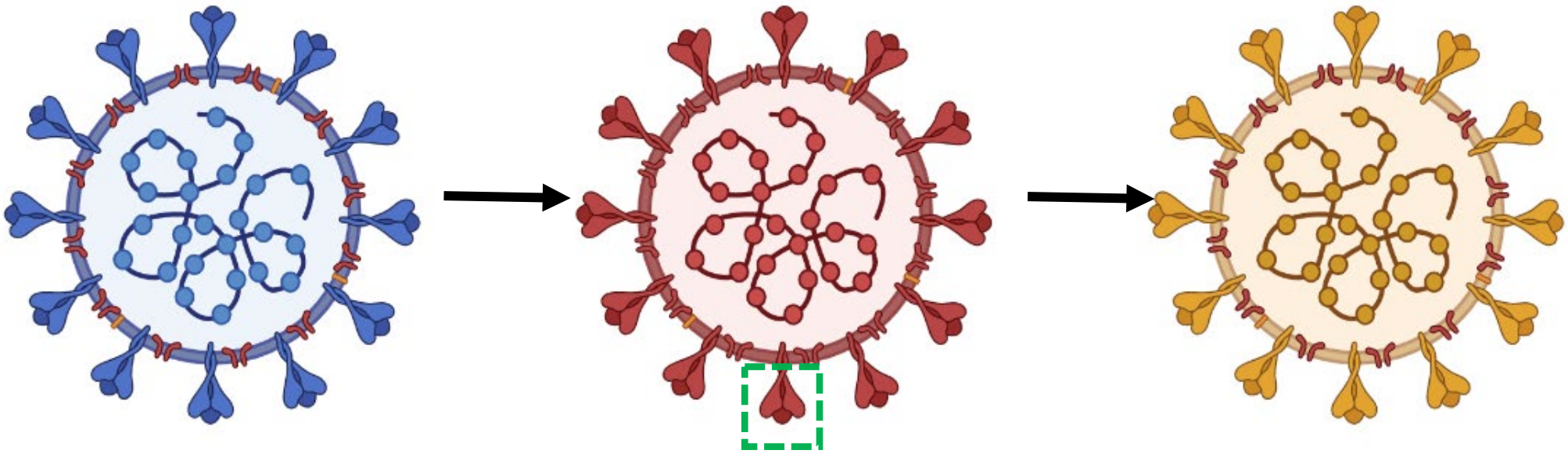
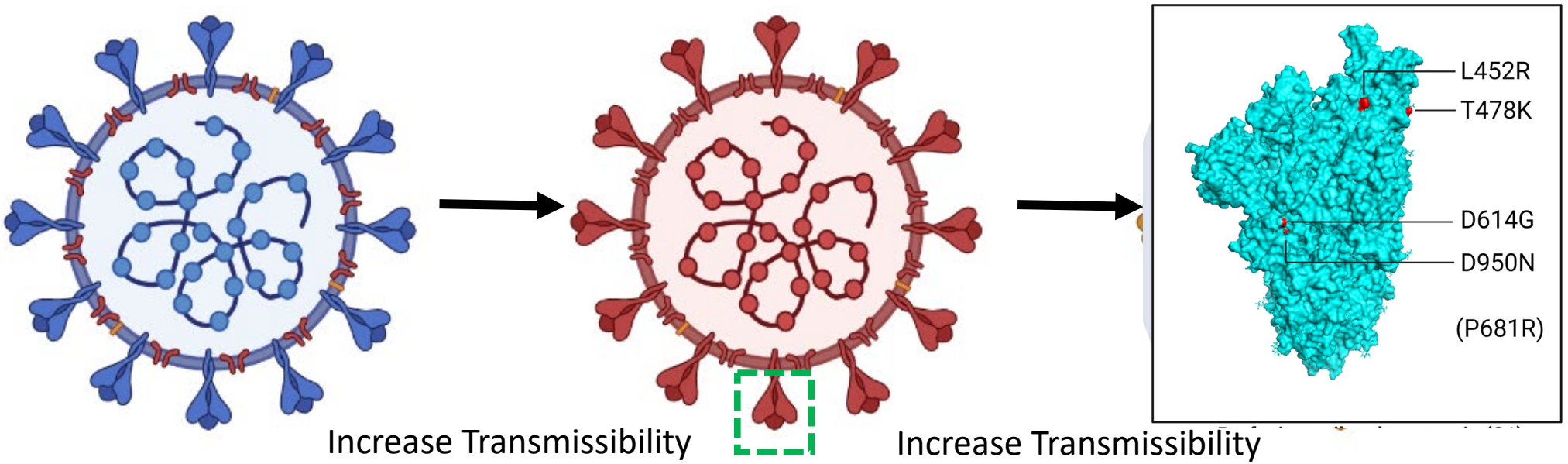


Focus on Spike mutations that have been linked to changing transmissibility and pathogenesis

SARS-CoV-2 alpha

SARS-CoV-2 delta



Increase Transmissibility and Pathogenesis

Increase Transmissibility and Pathogenesis

Introducing the Virus

What is a virus?

- A virus is an infectious microbe consisting of a segment of nucleic acid (either DNA or RNA) surrounded by a protein coat. (NIH)

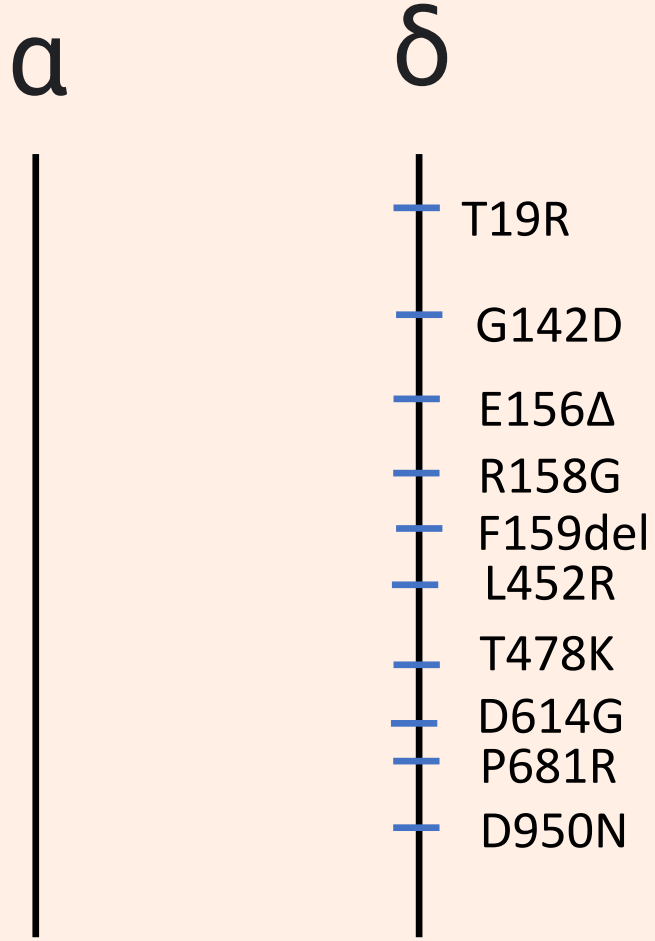
What is covid?

- Coronavirus disease (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus. (WHO)

Why look at this virus?

Mutations are happening in real time.

Strategy for generating S gene mutants



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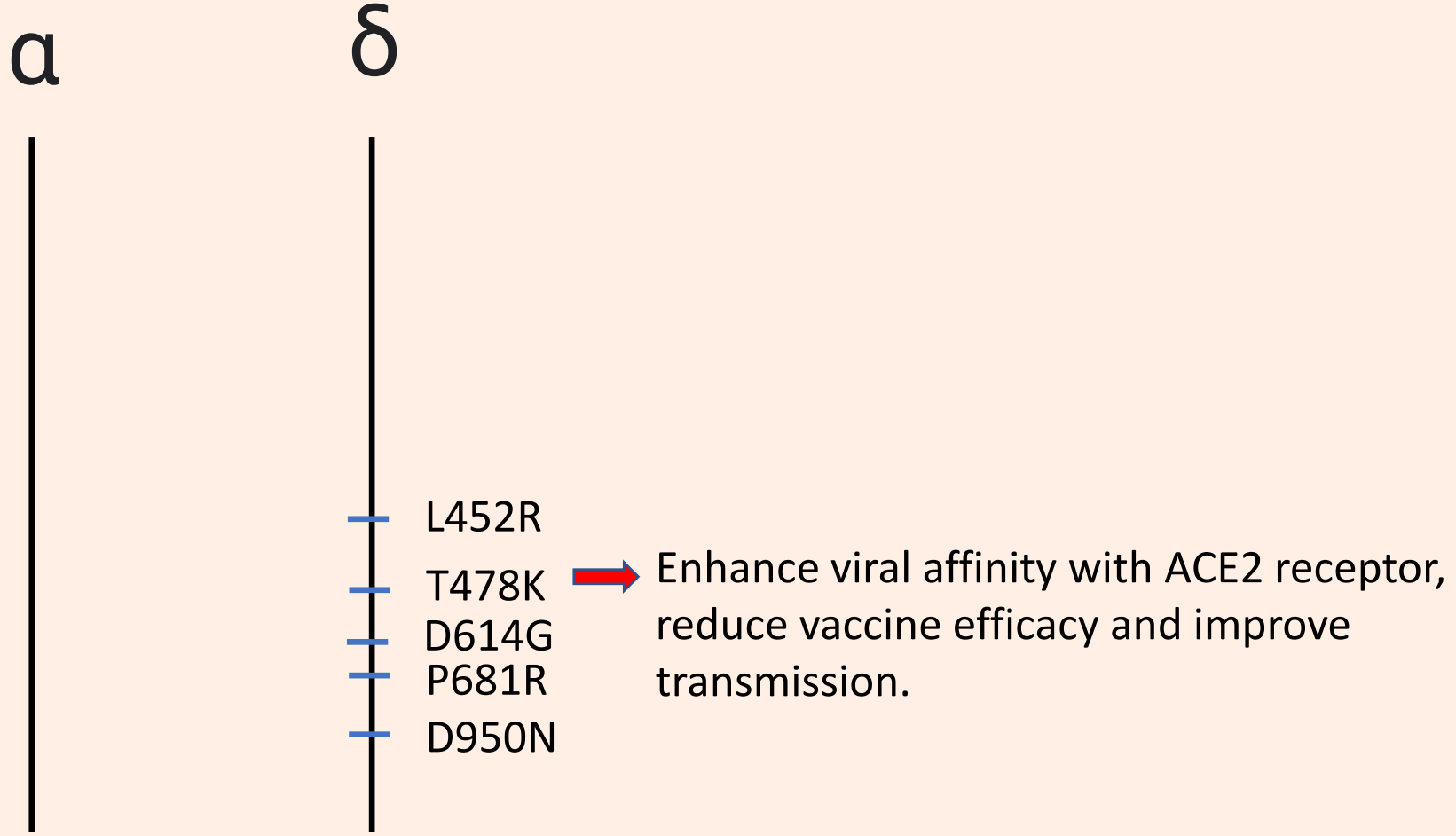
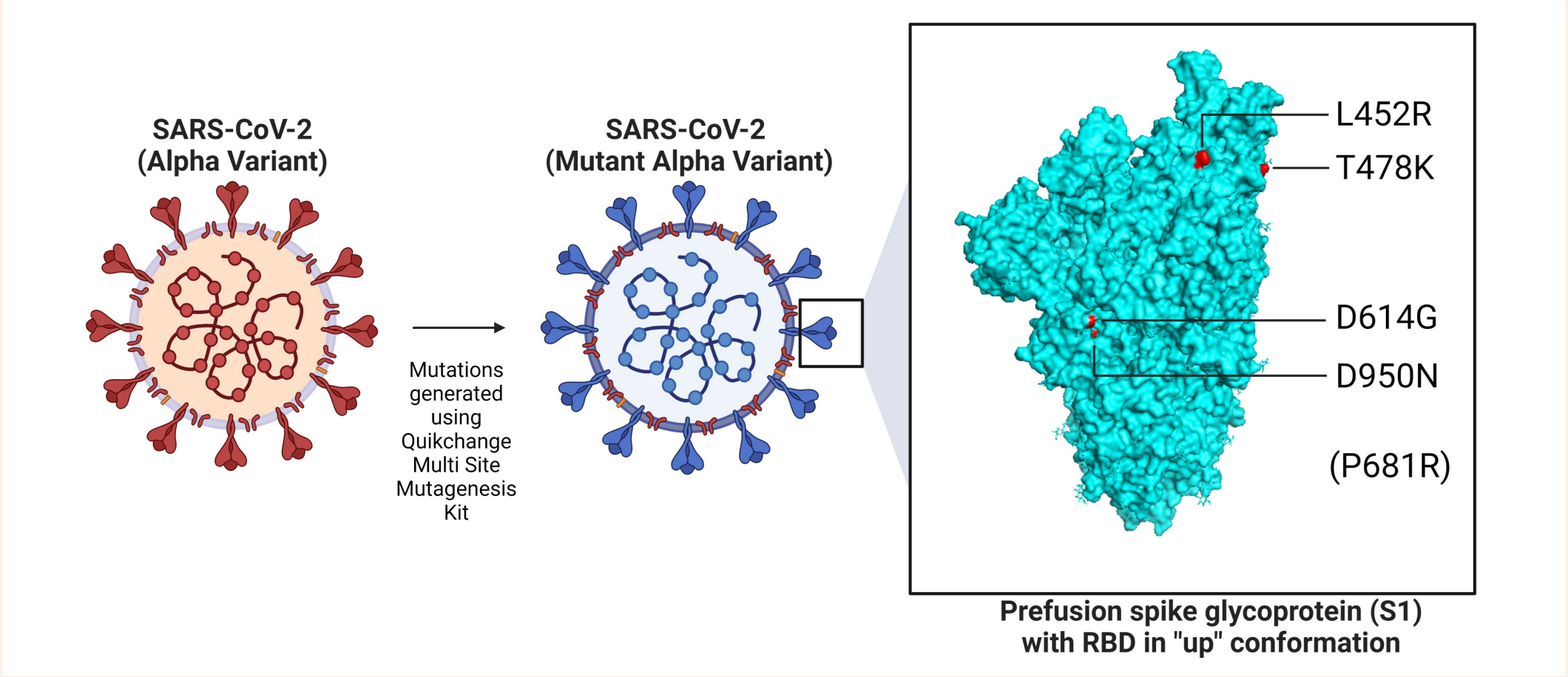
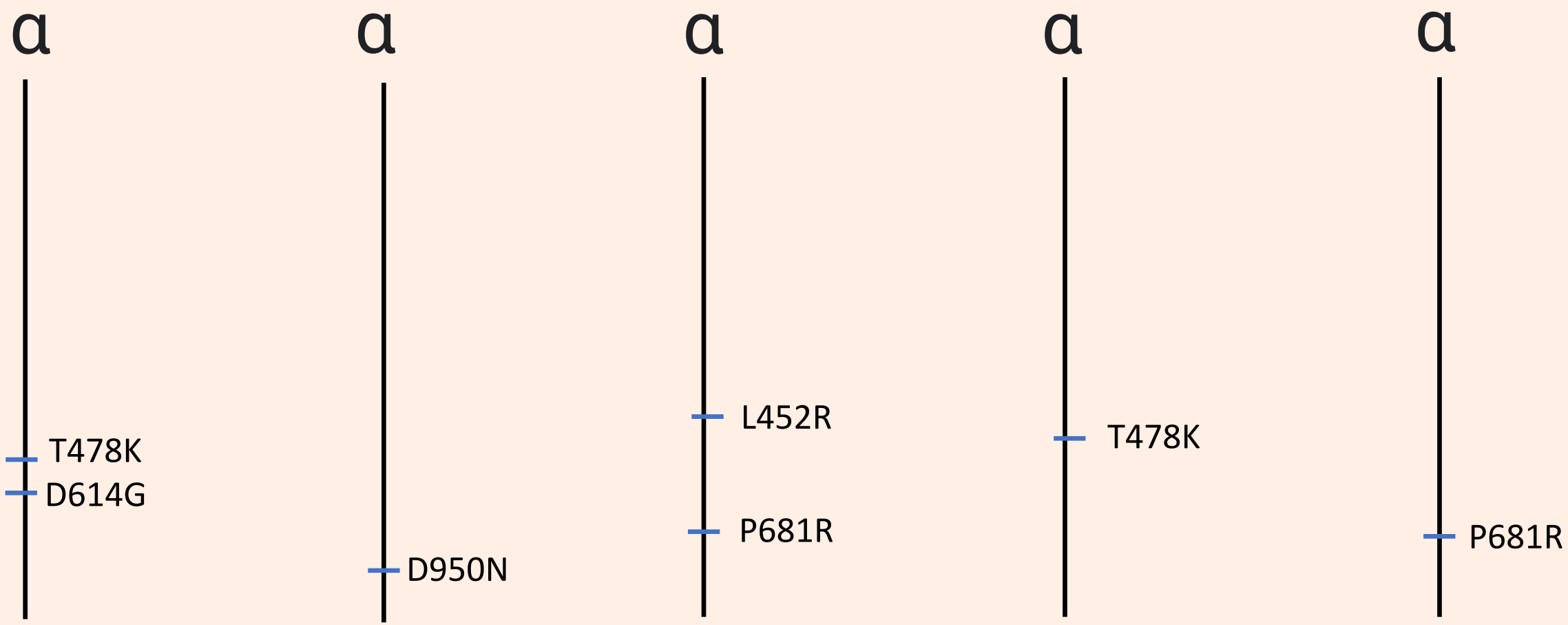


Generate a mutant alpha variant SARS-CoV-2 to test in entry and immune cell activation assays



Strategy for generating S gene mutant library



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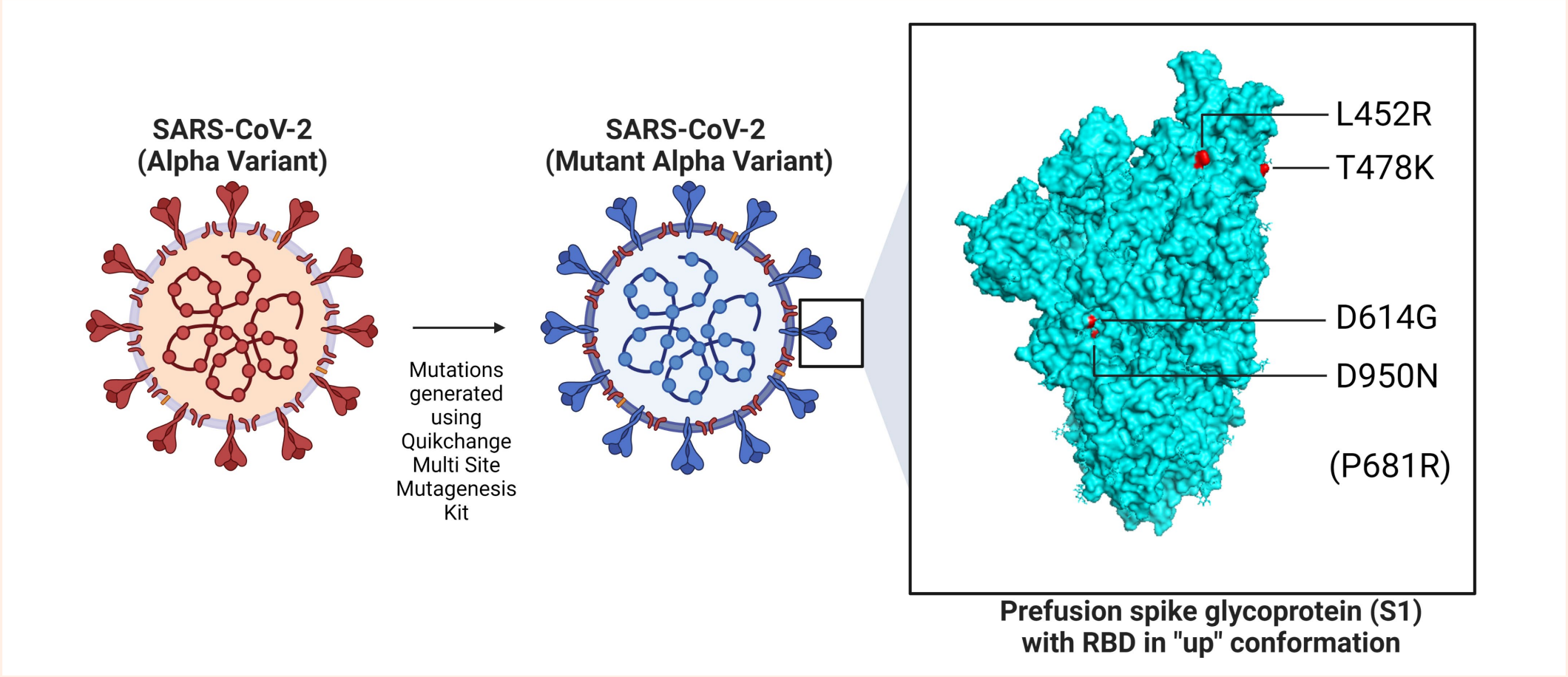
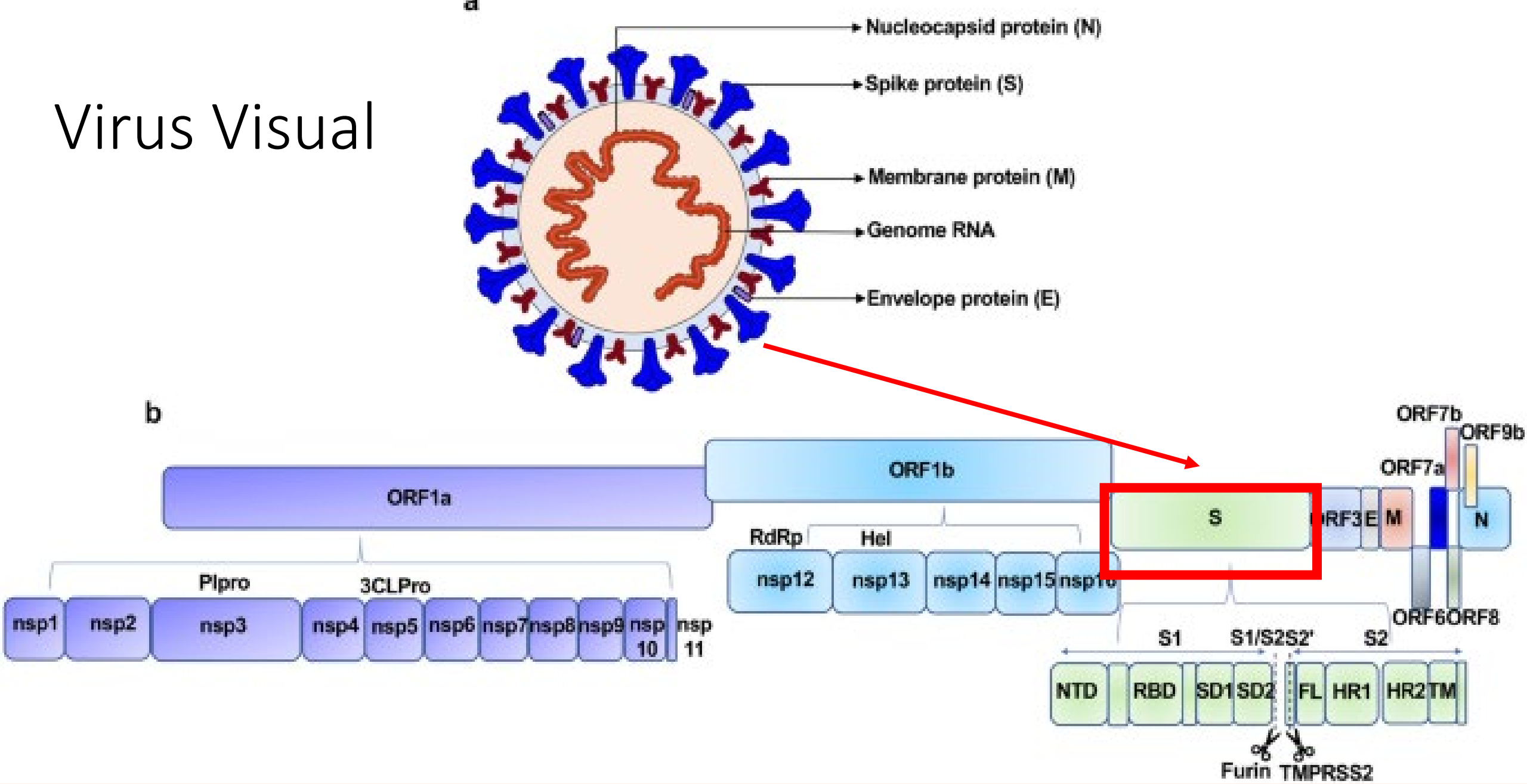


Our Mutations

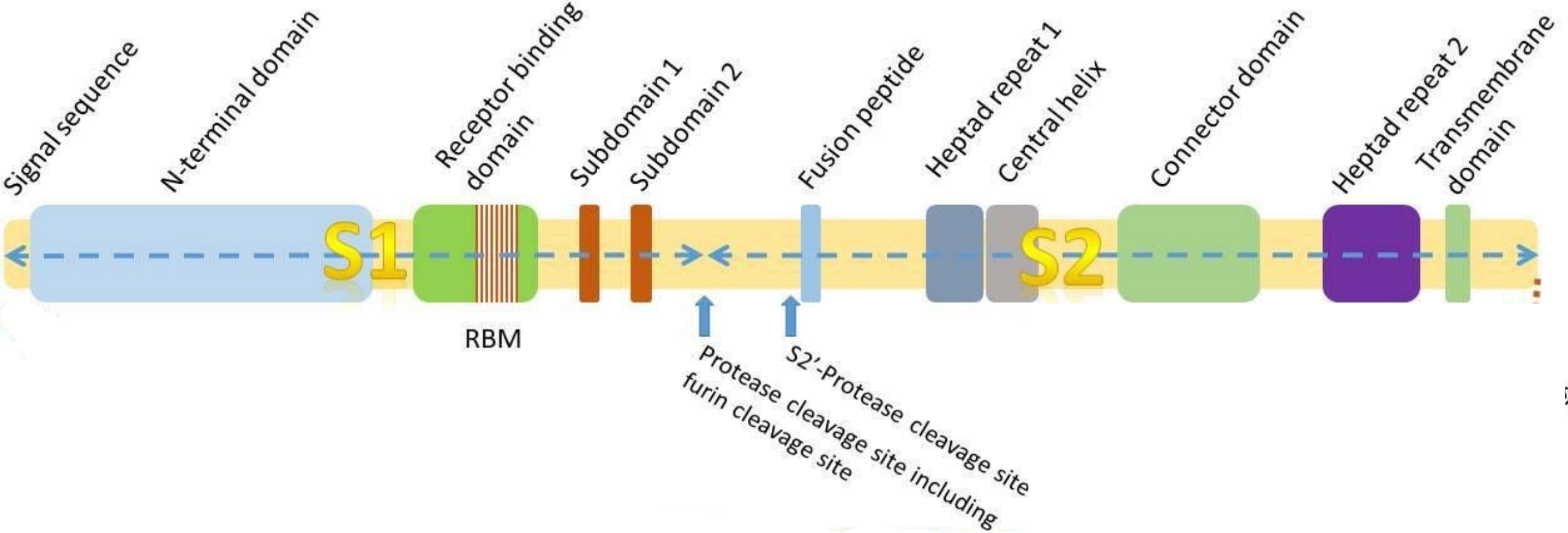
L452R	Involved in the escape from anti-RBD antibodies ; increases protein stability, viral infectivity, and potentially promotes viral replication. indicated decreased binding to select monoclonal antibodies (mAbs) and may affect their neutralization potential. Evades cellular immunity and increases infectivity
T478K	Found in epitope region of neutralizing antibodies categorized as class 1. may possibly result in increased ACE2 binding. The effect of the mutations L452R and T478K on ACE2 binding was also observed as enhanced stabilization of the RBD–ACE2 complex.
D614G	Escape from anti-RBD antibodies; no reduction in affinity of RBD for ACE2. Enhanced replication in upper respiratory tract through increased virion infectivity.
P681R	may enhance the fusogenic activity of the spike protein. could increase the rate of S1-S2 cleavage, resulting in better transmissibility.
D950N	D950N mutation mapped to the trimer interface, suggesting that this mutation may contribute to the regulation of spike protein dynamics.

Virus Visual



Pillay, Tahir S. “Gene of the Month: The 2019-nCoV/SARS-CoV-2 Novel Coronavirus Spike Protein.” *Journal of Clinical Pathology*, vol. 73, no. 7, 2020, pp. 366–69, https://doi.org/10.1136/jclinpath-2020-206658.

Spike (S) Protein Visual Expanded



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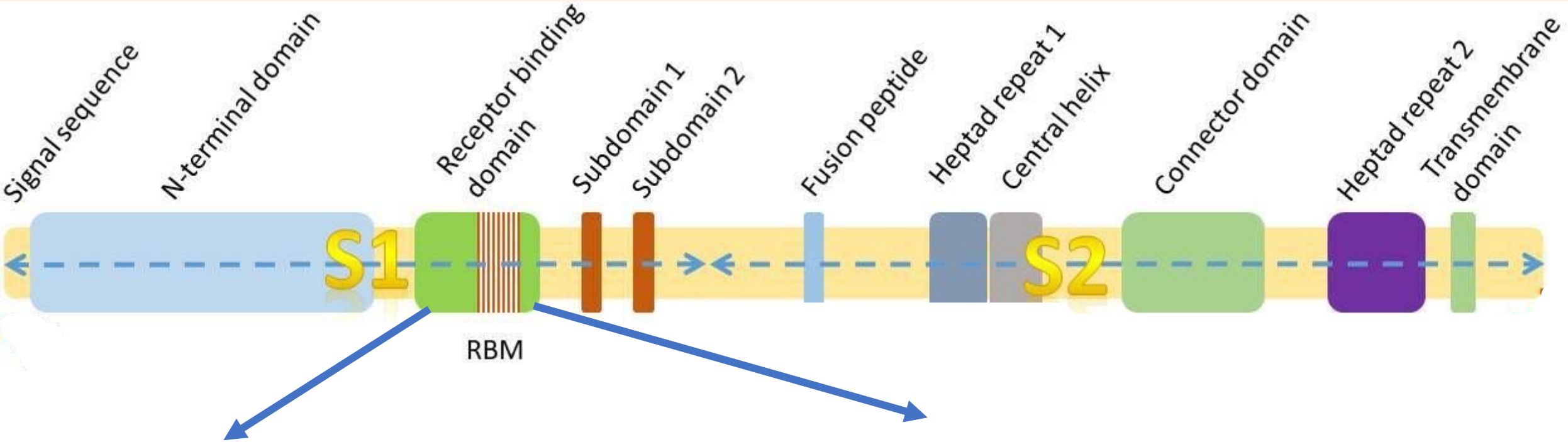


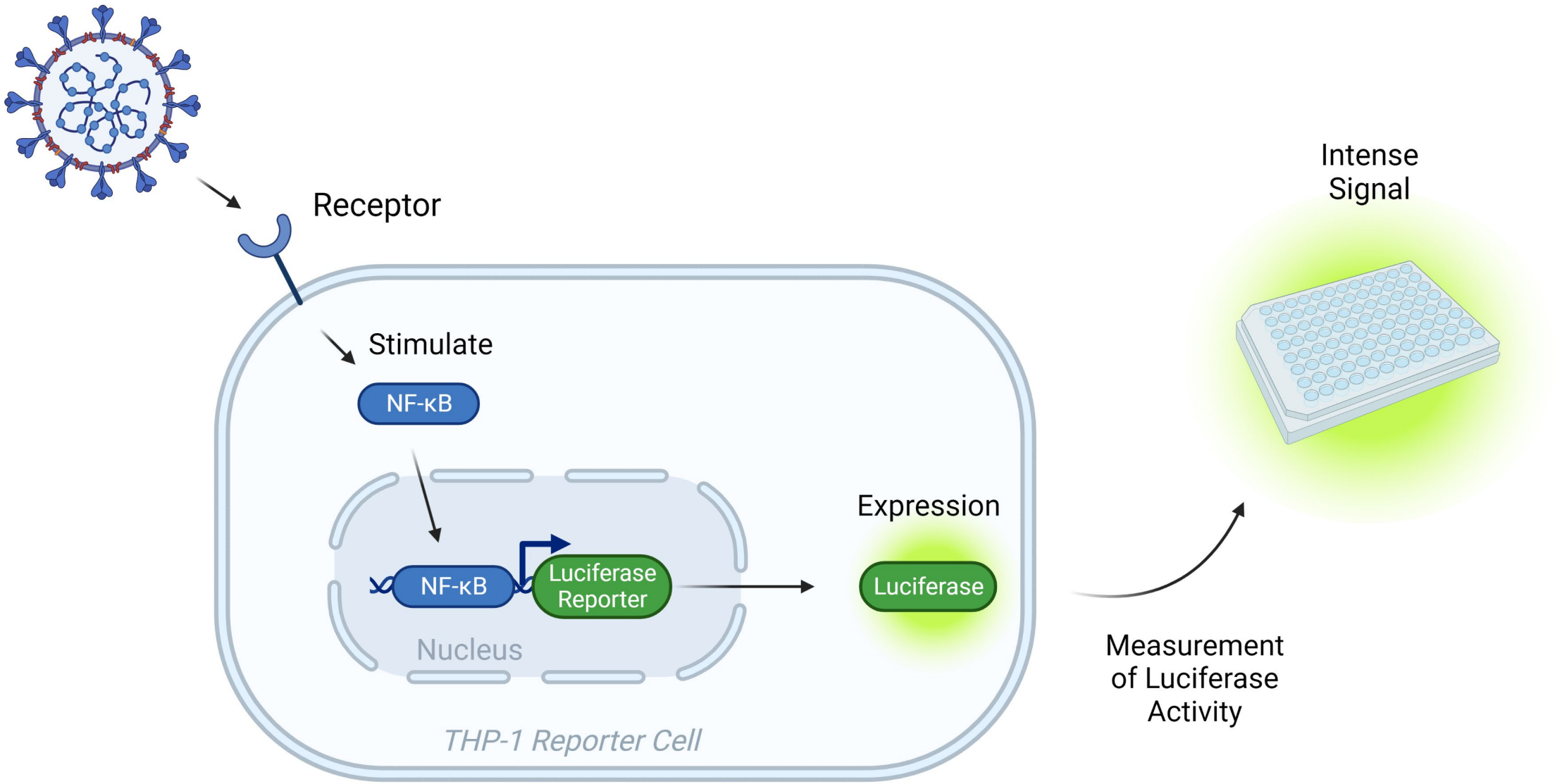
T478K Mutation: Changes threonine for a lysine.

- Found in epitope region of neutralizing antibodies categorized as class 1.
- May possibly result in increased ACE2 binding.

L452R Mutation: Changes leucine for an arginine.

- Increases protein stability, viral infectivity, and potentially promotes viral replication.
- Indicated decreased binding to select monoclonal antibodies (mAbs) and may affect their neutralization potential.

The effect of the mutations L452R and T478K on ACE2 binding was also observed as enhanced stabilization of the RBD–ACE2 complex.


Receptor
Stimulate
NF-κB
NF-κB
Luciferase Reporter
Nucleus
THP-1 Reporter Cell
Expression
Luciferase
Intense Signal
Measurement of Luciferase Activity