SPIRA:
A Novel ACE2 Decoy Therapeutic for Covid-19

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Why is there a need a novel therapeutic for COVID-19?

1) There is a need for treatments for unvaccinated Individuals and Individuals Who Cannot Make An Immune Response

2) Virus Variants

3) Cost

1) The unvaccinated problem

For whatever reasons, some part of the population will always be unvaccinated. Thus there will always be a need for an anti-viral therapeutic.

2) Variants

It is very likely that viral variants will emerge that will escape the immune response generated by the contemporary vaccines. For example, individuals vaccinated with the Pfizer mRNA Vaccine are more susceptible to the Delta Variants than to the original Wuhan Virus. This is a very significant problem and a new therapeutic is needed that will combat all variants.

3) Cost

At present the existing therapeutic, the Regeneron Monoclonal Antibody Cocktail is provided free of charge. However, this is unlikely to continue in the future. Typical Monoclonal Antibody Therapies are very expensive (approx $200,000 per year). Thus any new therapeutics should be economical to make and develop.

So, to understand my novel approach, it is important to review the biology of Corona Virus Infection.

How SARS-COV-2 infects cells

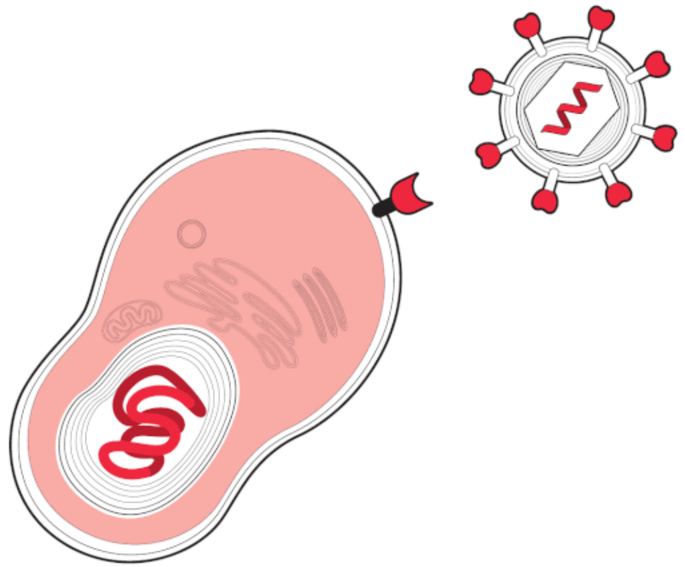
- SARS-COV-2 is a virus that belongs to the Coronavirus family and causes the respiratory illness, Covid-19.

- Spike is a SARS-COV-2 protein that binds to the ACE2 receptor, which is located on human lung, arterial, heart, kidney, and intestinal cells.

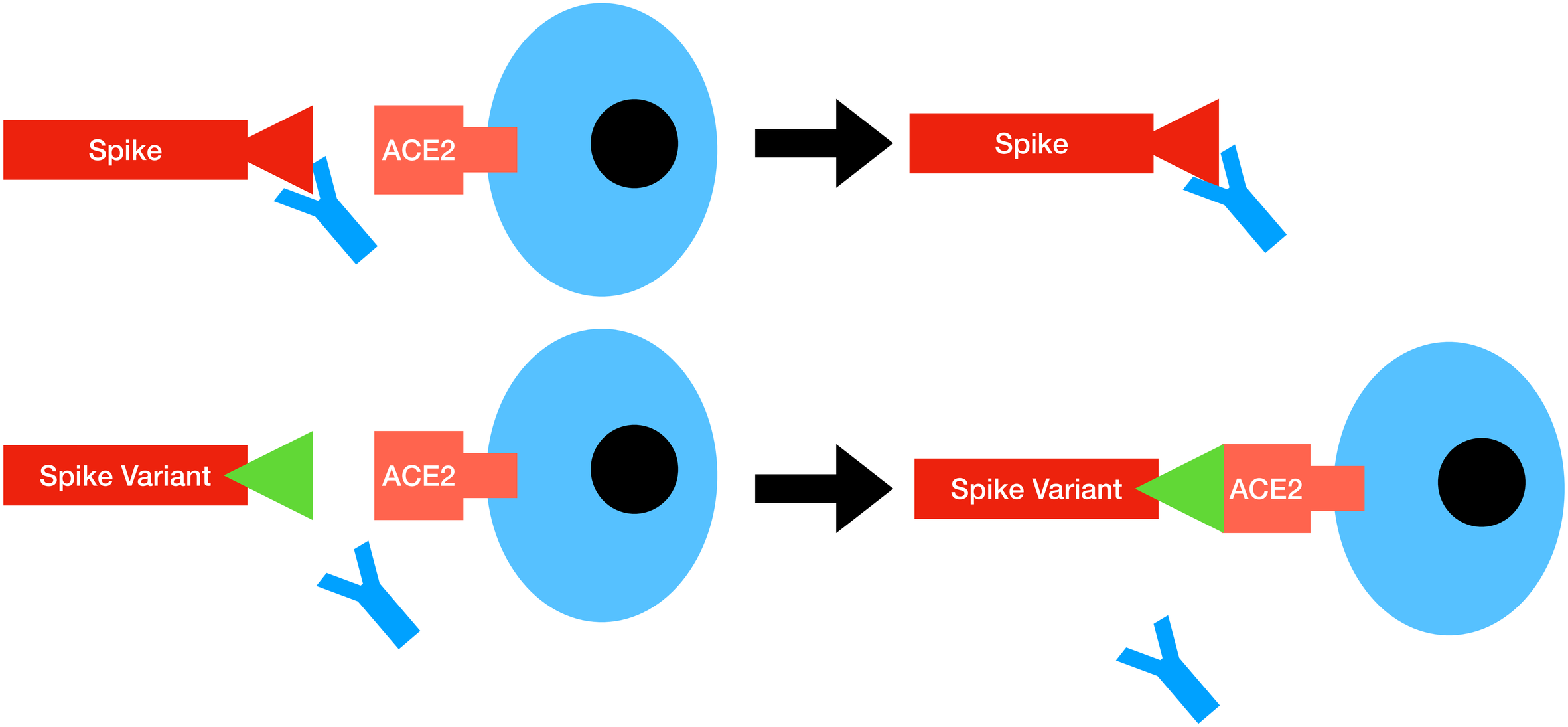
- When Receptor binding domain (RBD) of the spike protein binds to the ACE2 receptor, the ACE2 receptor changes the structure of the spike protein and this then enables the virus to enter the cell.

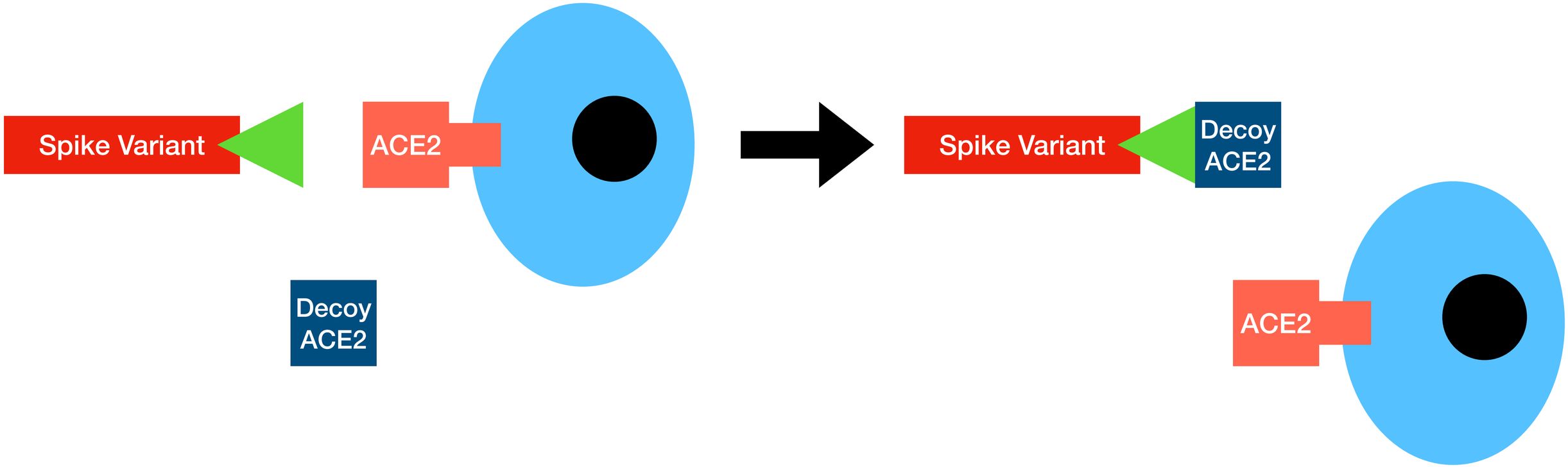
- Once inside the cell, the mRNA-virus sends its own RNA through our ribosomes, and duplicates. When the virus duplicates, the cell will die and the viruses will move into different cells.

(The Wall Street Journal, 2020)



Current therapeutics inhibit the binding of RBD(spike) to ACE2, however, they may not work on spike variants.





An ACE2 decoy will work on all variants!

Creating an ACE2 Decoy has been attempted before, however some critiques that I had were:

1) Decoy Still Had Enzymatic Function (It still processes Ang II to Ang 1-7, and thus would alter blood pressure)

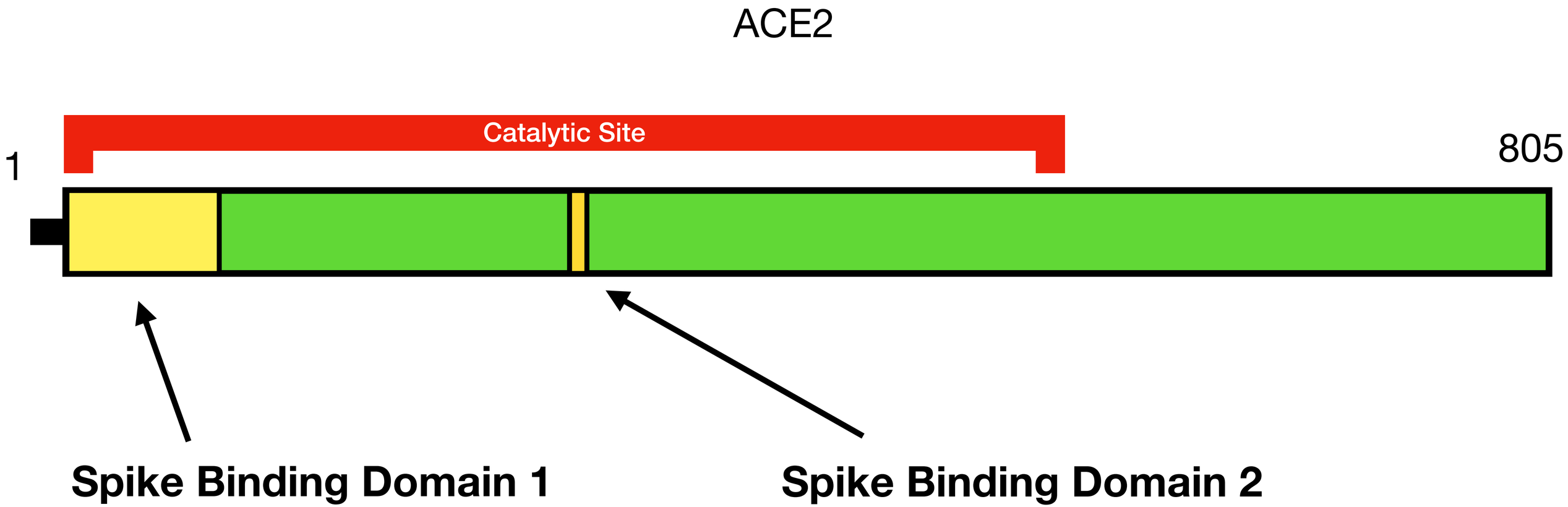
2) The Binding Affinity Could be Improved

3) Decoy Construct May Not Have Maintained Necessary Tertiary Structure

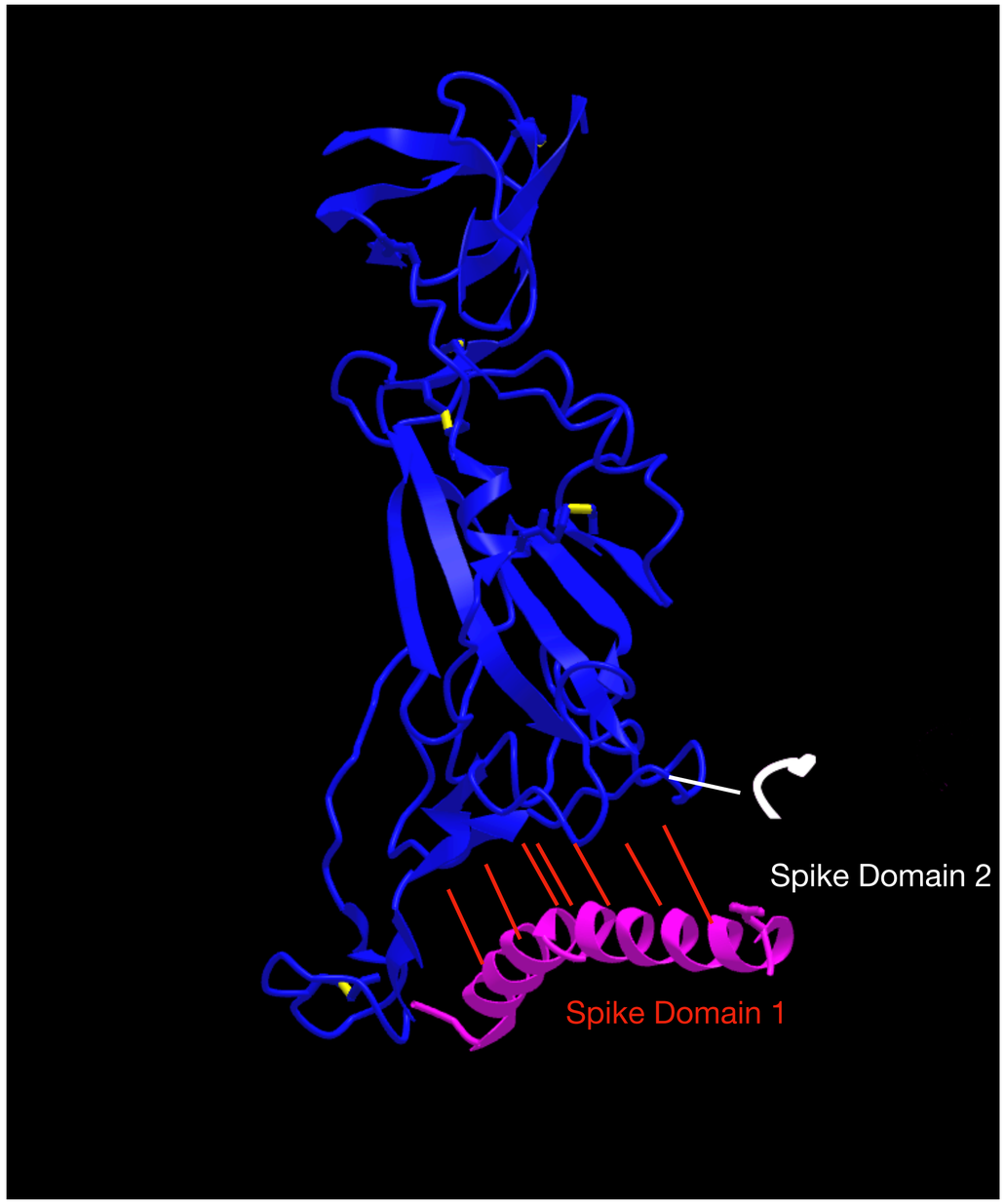
My Design Aims for SPIRA.1(the therapeutic!)

- To design an ACE2 Decoy that still binds to Spike but does not have enzymatic activity
- To design an ACE2 decoy that has significantly increased affinity for the Spike Protein
- To Design an ACE2 Decoy that may preserve the key tertiary structure (this is not in the talk today, but I will talk about it later)

To design an ACE2 Decoy that binds to Spike but does not have enzymatic activity



Will the Spike Binding Domain 1 work by itself?



Evidence that spike domain will work by itself

Spike Domain 1 is an Alpha Helix and has 12 contacts where as Spike Binding Domain 2 is a loop structure that only has 1 contact. So, I predicted that Spike Binding Domain 1 would work by itself.

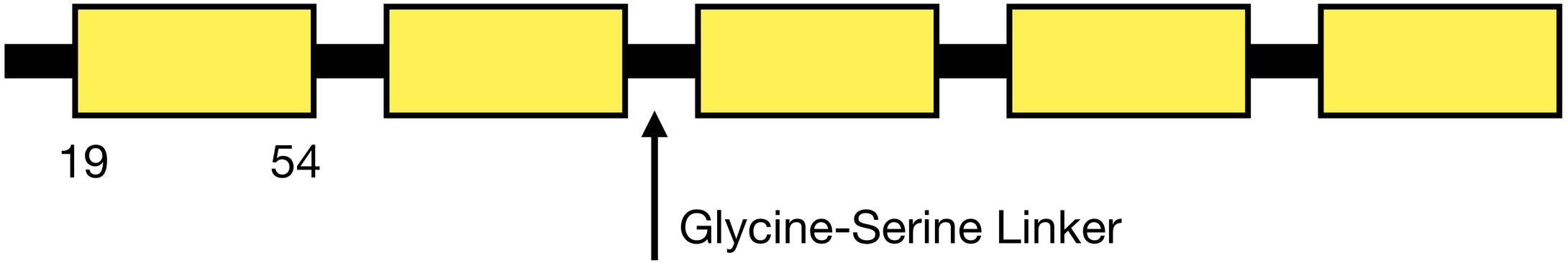
What piece of Spike Binding Domain 1 should I use in my decoy construct?

On the bases of this structure, I decided to go with Amino Acids 19-54 and this my SPIRA Core unit.

19-54

To design an ACE2 decoy that has significantly increased affinity for the Spike Protein

A. Repeating Core SPIRA Unit to increase affinity for binding, 5x total units

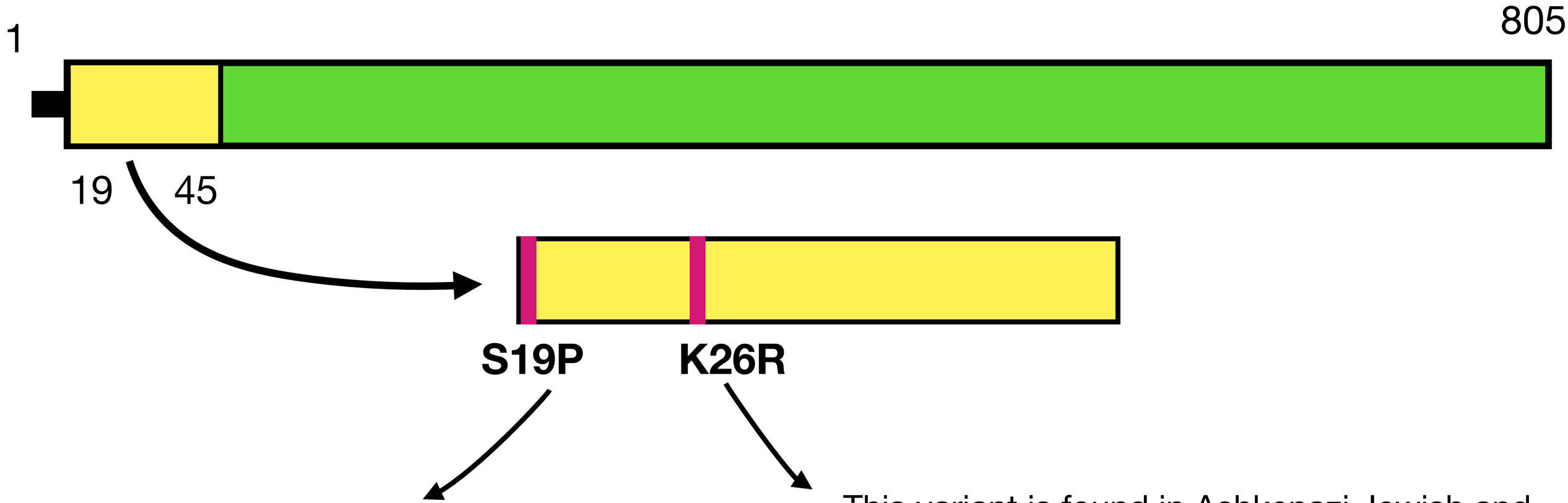


Another way to increase affinity

There are natural sequence variants In human ACE2 that cause better affinity to Spike.

Can I use them to generate an ACE2 Decoy with increased affinity?

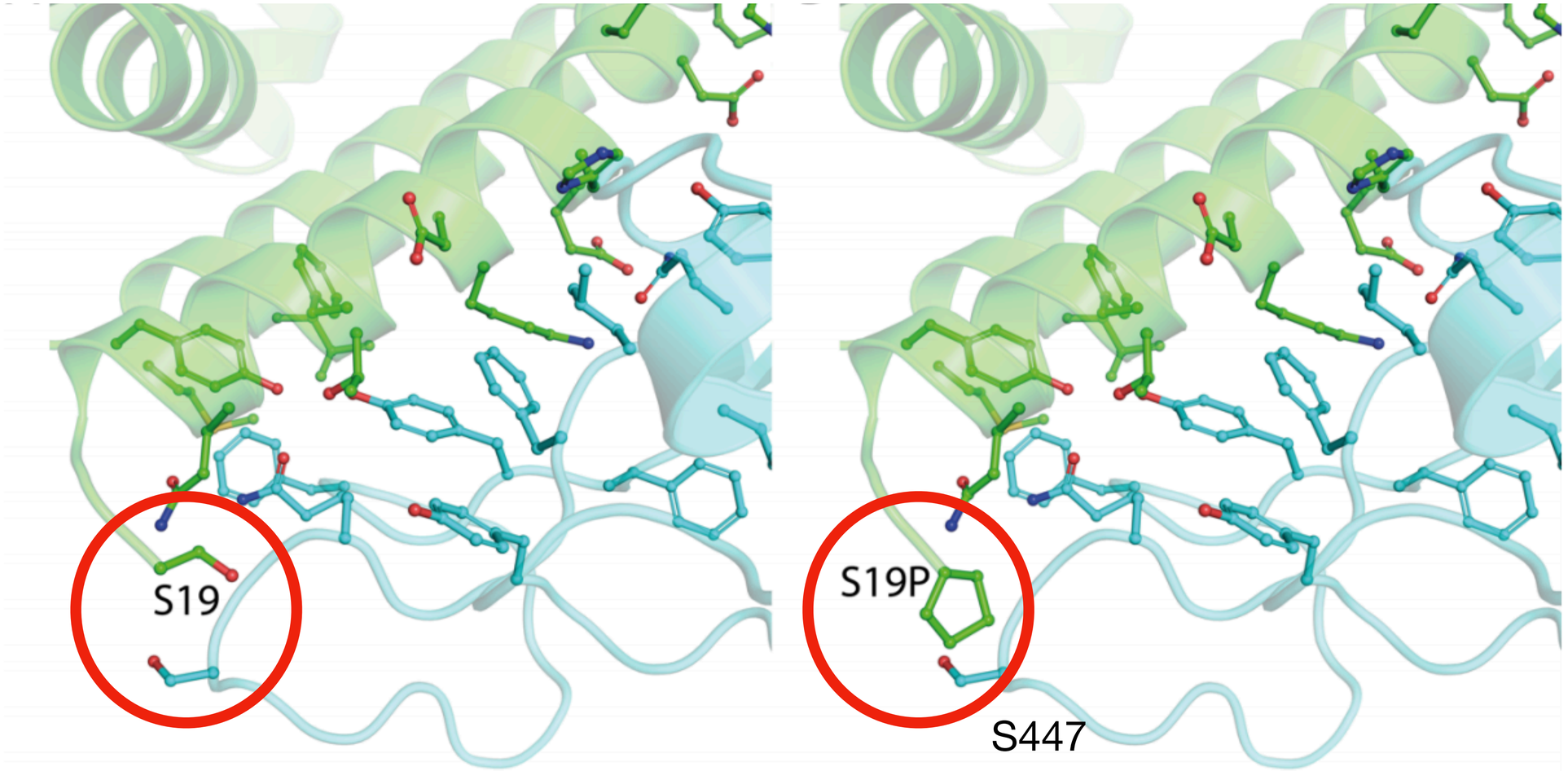
Natural mutations in ACE2 that increase binding to Spike and thus increase susceptibility to COVID-19



This variant is found in African Americans. The change of Serine to Proline makes the helix more stable and results in an increase in affinity.

This variant is found in Ashkenazi Jewish and European populations. The change from Lycine to Arginine increases affinity because it can make a stronger connection to spike (more positive and larger amino acid).

The S19P variant make ACE2 interacts better with spike



S19P

(MacGowan, 2021)

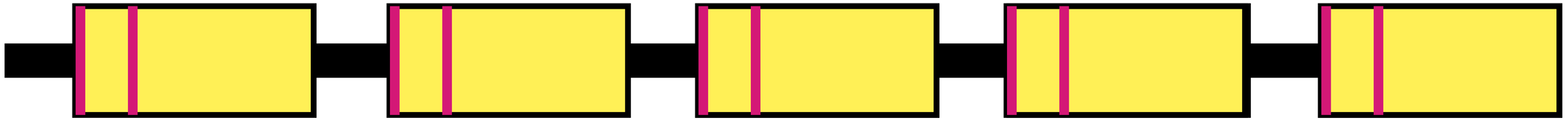
I decided to use both enhancing variants:

S19P and K26R

Summary

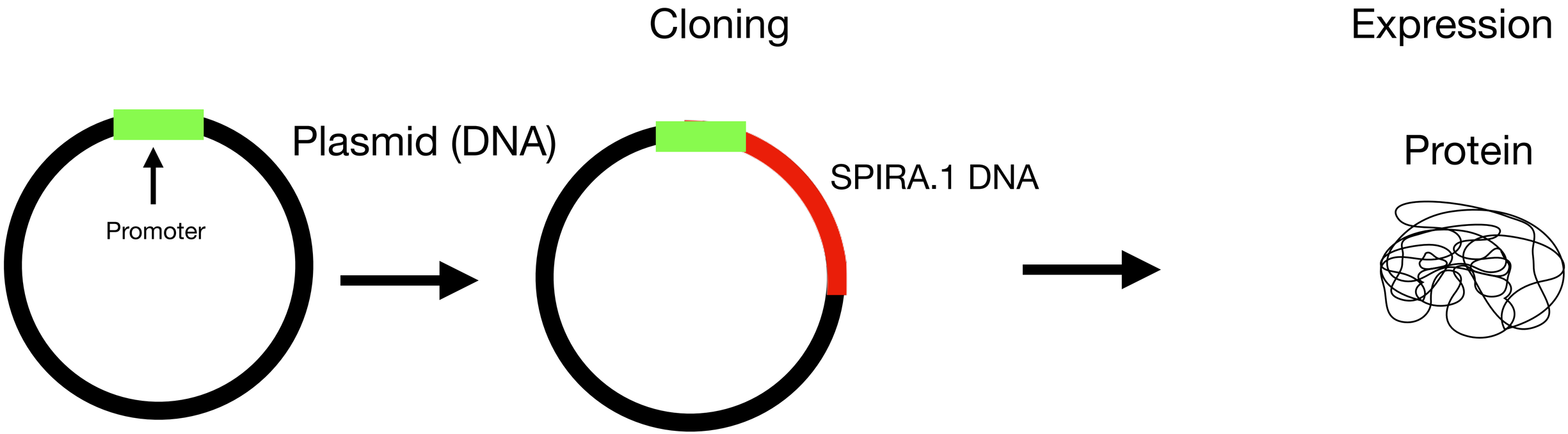
- I have designed a Decoy ACE2 Therapeutic that lacks ACE2 Enzymatic Activity
- Using repetitive elements and naturally occurring Variants, I’ve designed a therapeutic that will likely have a higher Affinity

The cloning and production of SPIRA.1



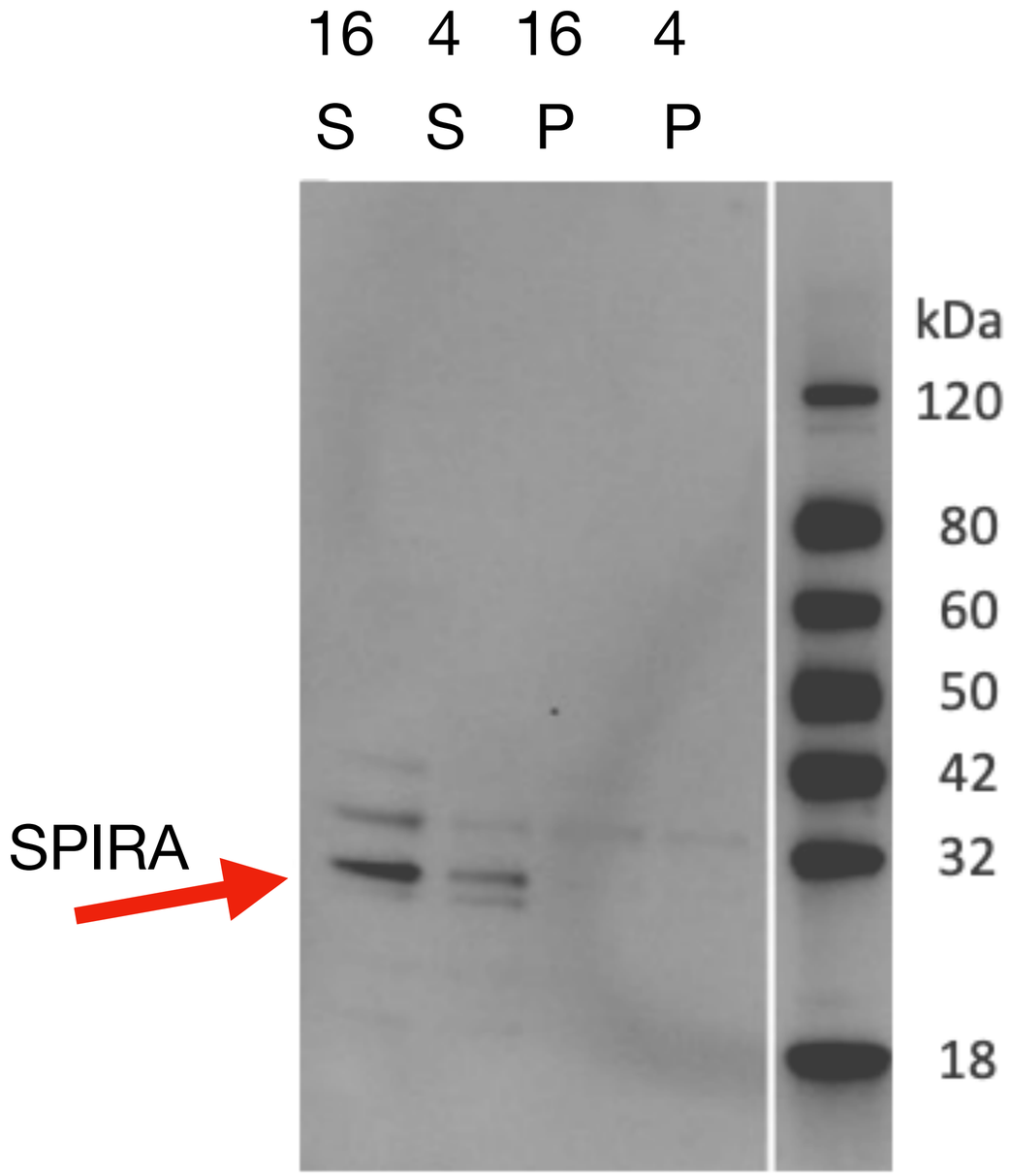
This is my final design for SPIRA.1 ACE2 Decoy. The next step is to synthesis the DNA and clone the construct into an expression vector.

Cloning and expression of SPIRA.1



This work was contracted to Genscript

My SPIRA Protein is cloned and expressed in bacterial Cells!

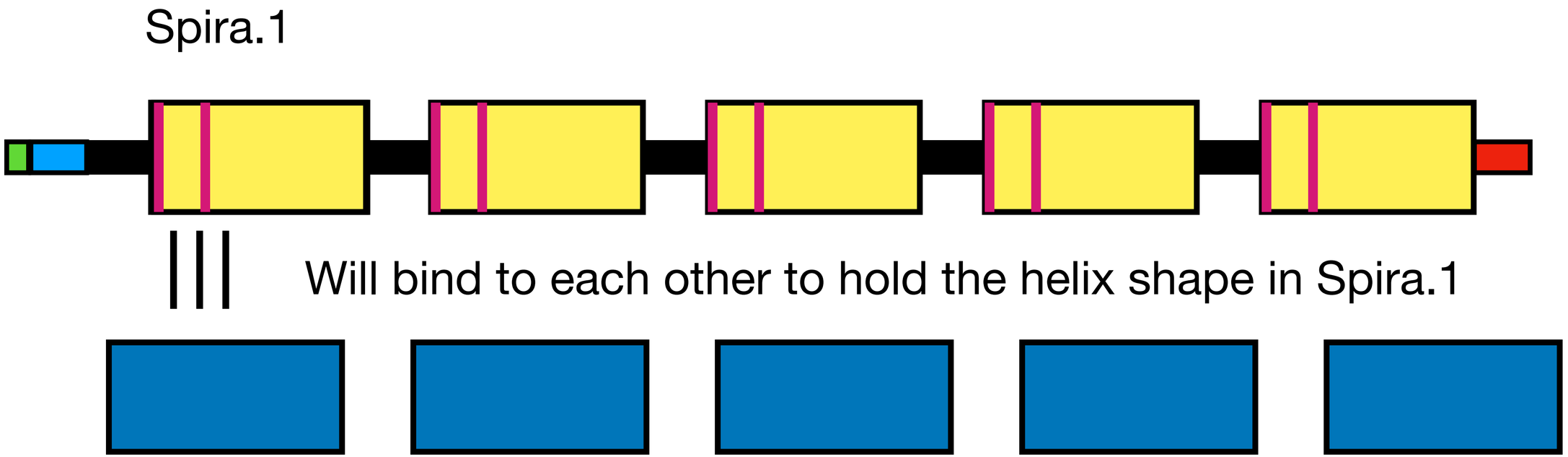


Next Step, Make a large amount of the protein and test that it binds to spike.

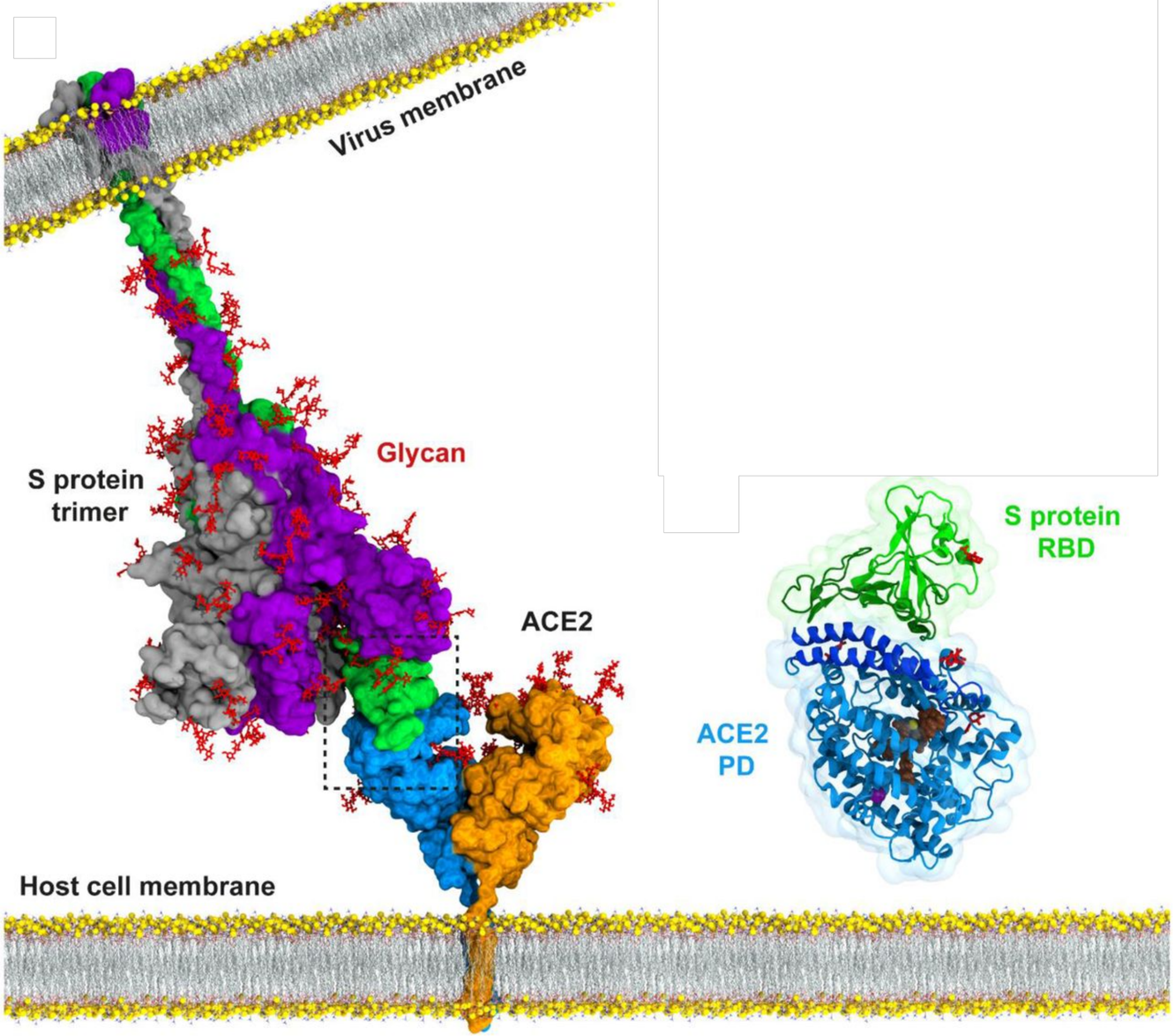
Thanks for listening!

3:To design an ACE2 Decoy that may stabilize the key tertiary structure

Will a complex of SPIRA.1 and Spike stabilize tertiary structure and generate a better therapeutic?



Using the techniques I've already discussed the plan is to make an RBD Recombinate construct and generate a decoy complex.



(BioRxiv, E.Taka)

SARS-COV-2 may have multiple Variants but they all bind to ACE2, so, the key to blocking SARS-COV-2 from entering cells is making a protein that outcompetes ACE2 in binding with spike.

Summary

- I have designed a Decoy ACE2 Therapeutic that lacks ACE2 Enzymatic Activity
- Using repetitive elements and naturally occurring Variants, I've designed a therapeutic that will likely have a higher Affinity
- I have formulated a novel approach to maintaining the tertiary structure of the ACE2 Decoy.