Rational Antibody Design

INTRODUCTION

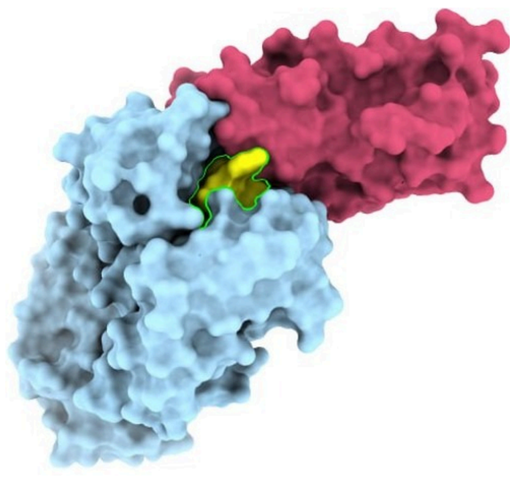
The objective of our Lab project is to determine the most efficient variant of the antibody in binding to the spike protein of the SARS-CoV-2 virus. As the virus continues to rapidly mutate, previously developed antibodies have been proven less effective in neutralizing the spike protein on the virus surface. This has prompted the scientific community to engineer antibodies that exhibit a higher affinity when interacting with the virus spike protein. Our methods include saturation mutagenesis, cloning, plasmid extraction, human cell transfection, and ELISA.
In parallel, we have performed the *in silico* study of the antibody binding to Wuhan Receptor Binding Domain (RBD). Within this framework, we have worked with AlphaFold and protein docking and we measured the effect of amino acids changes to the binding sites.

PROTEIN DOCKING

XR10 AA sequence → Introducing mutations in CDR3-H region → 3D structure prediction with AlphaFold2 → Docking to Wuhan RBD

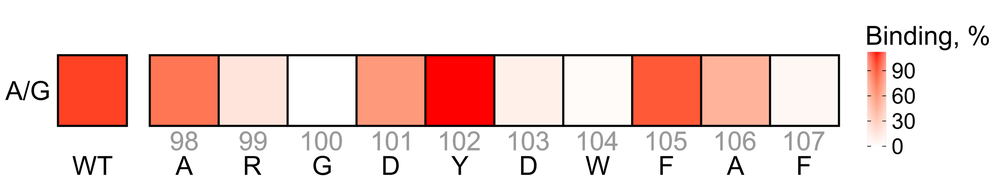
Pipeline of the protein docking project

SARS-CoV-2 **spike** protein (Wuhan variant), **RBD**



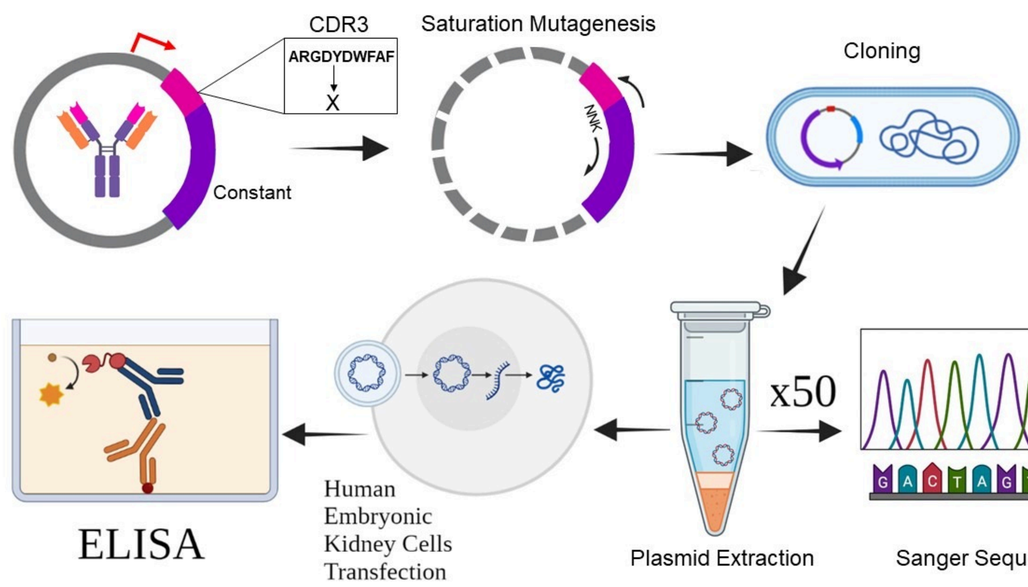
XR10 antibody bound to the **RBD**, **CDR3**

As a part of the docking team, our main objective was to profile the binding of XR10 antibody and its mutated variants to SARS-CoV-2 spike protein. We performed *in silico* alanine scanning for CDR3 of XR10 heavy chain using AlphaFold2 to predict the three-dimensional structure and HDOCK to assess the protein interaction. We estimated the influence of amino acid exchanges on the affinity and visualised the antibody-antigen interaction with ChimeraX.

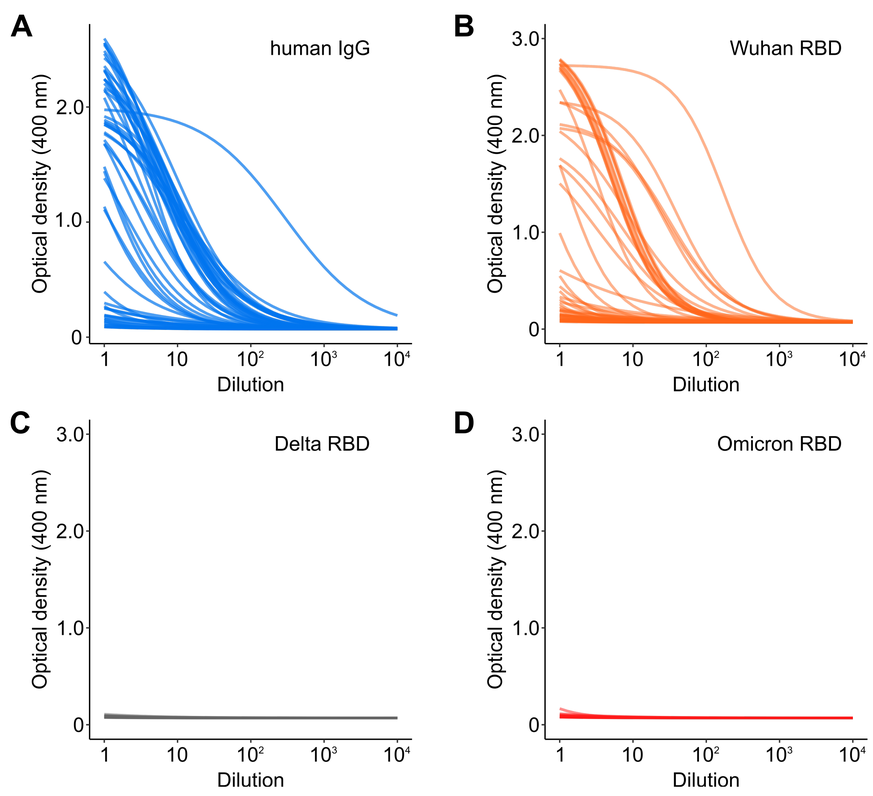


Effect of the amino acid exchanges on binding of the XR10 antibody to Wuhan RBD estimated *in silico*

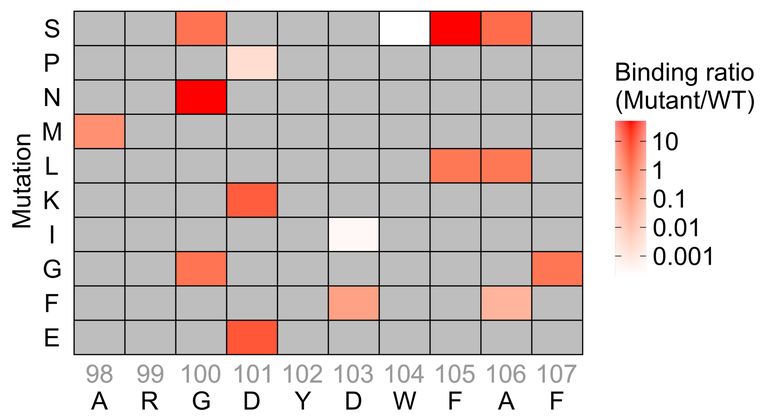
METHODS



RESULTS



Antibody concentration was assessed by anti-human IgG ELISA (A). Antibody mutants binding to the Wuhan (B), Delta (C), and Omicron BA.1 (D) SARS-CoV-2 RBD.



XR10 mutants binding relative to the wild-type (WT) sequence estimated by ELISA.

CONCLUSIONS

1. We were able to determine the CDR3 amino acid exchanges improving the XR10 binding to Wuhan RBD (G100N and F105S) along with the mutations drastically decreasing the affinity (D103I and W104S).
2. None of the mutants tested was able to bind Delta or Omicron BA.1 RBD.
3. *In silico* 3D structure prediction and docking allowed us to pinpoint the crucial interaction residues in CDR3-H region (G100, W104)

Resources and additional info