

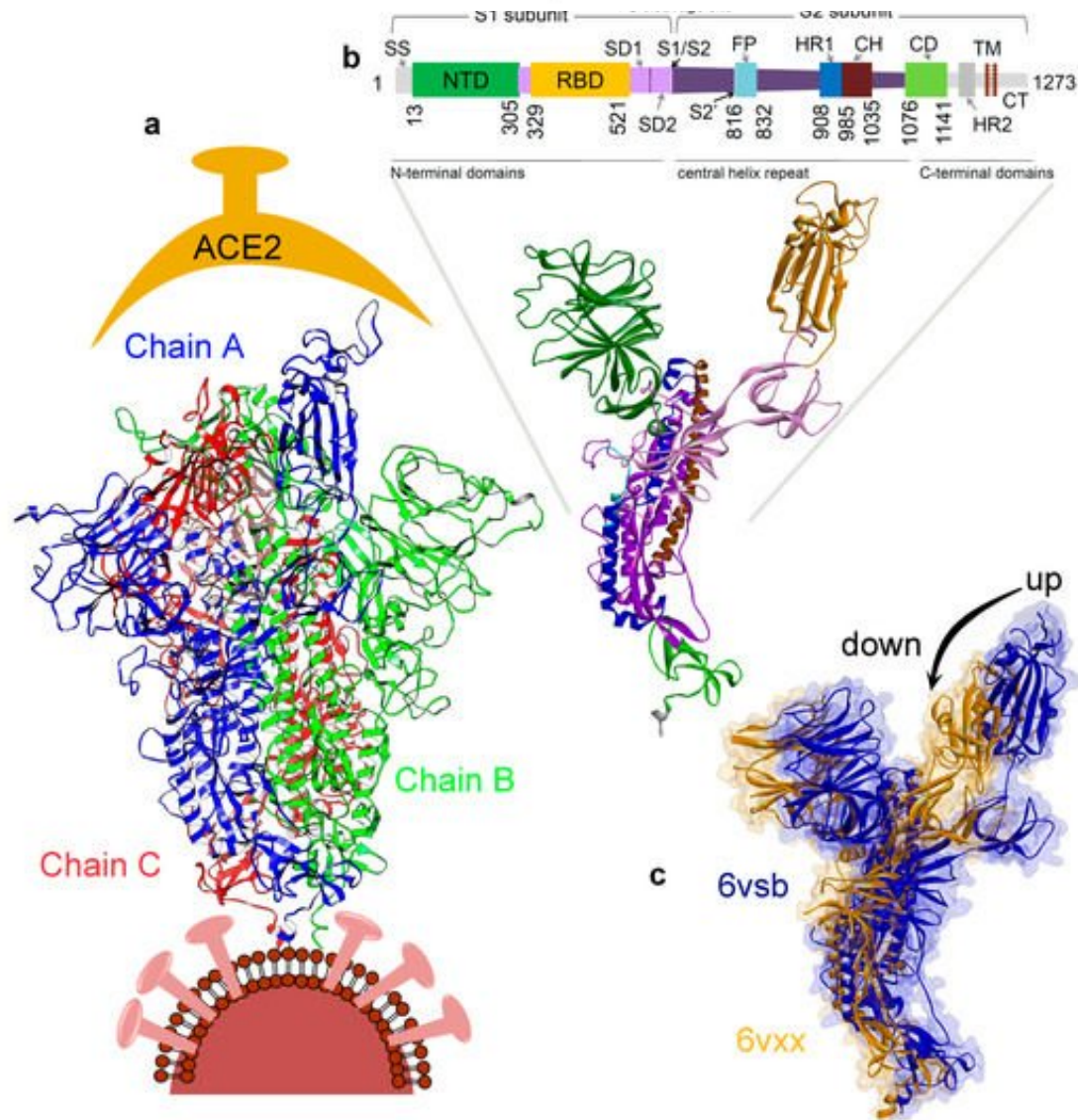
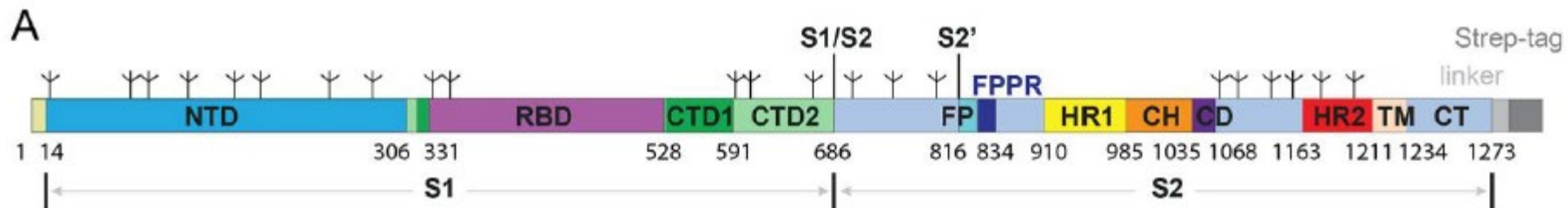
COMPUTATIONAL STUDIES OF THE SARS-COV-2 SPIKE PROTEINS: MOLECULAR MECHANISMS OF ANTIBODY-INDUCED NEUTRALIZATION AND ALLOSTERIC DRUG DISCOVERY

Gennady Verkhivker

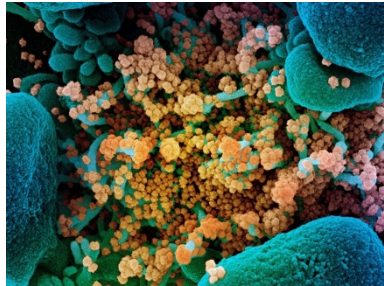
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Chapman University School of Pharmacy, Irvine CA

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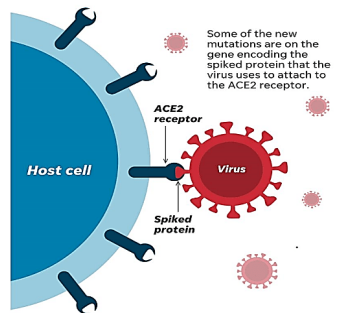
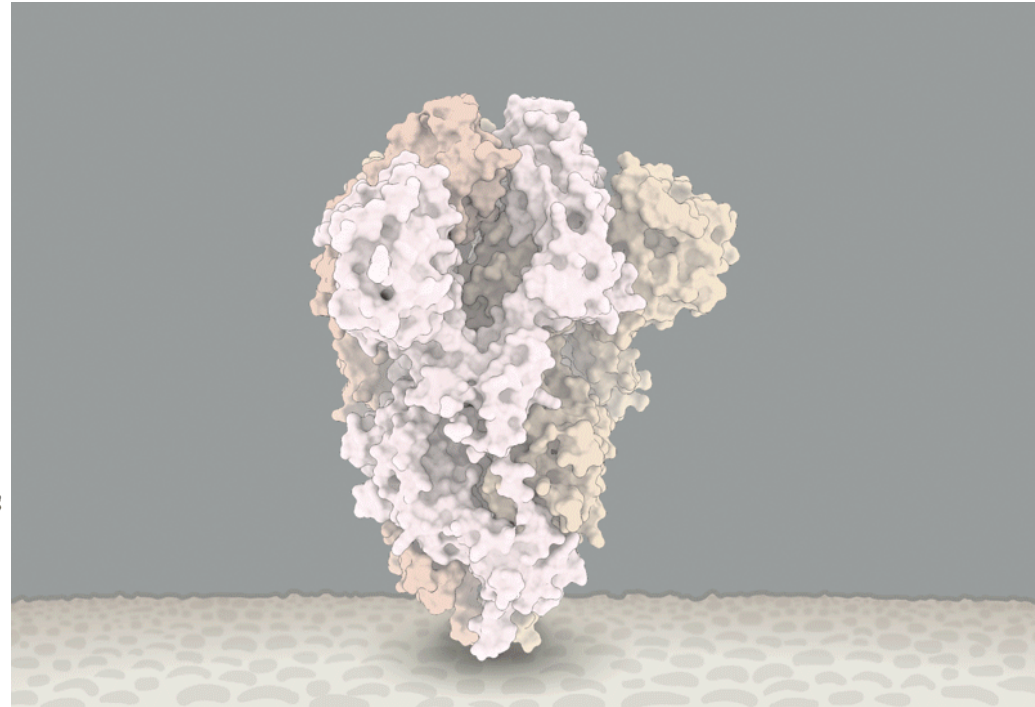
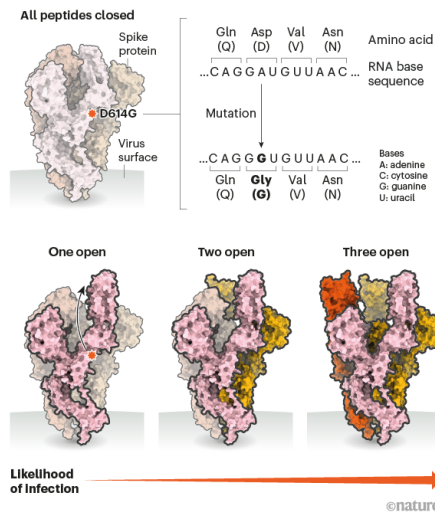


SARS-CoV-2 Spike Proteins are Versatile and Adaptable Molecular Machines that can accommodate many mutational variants



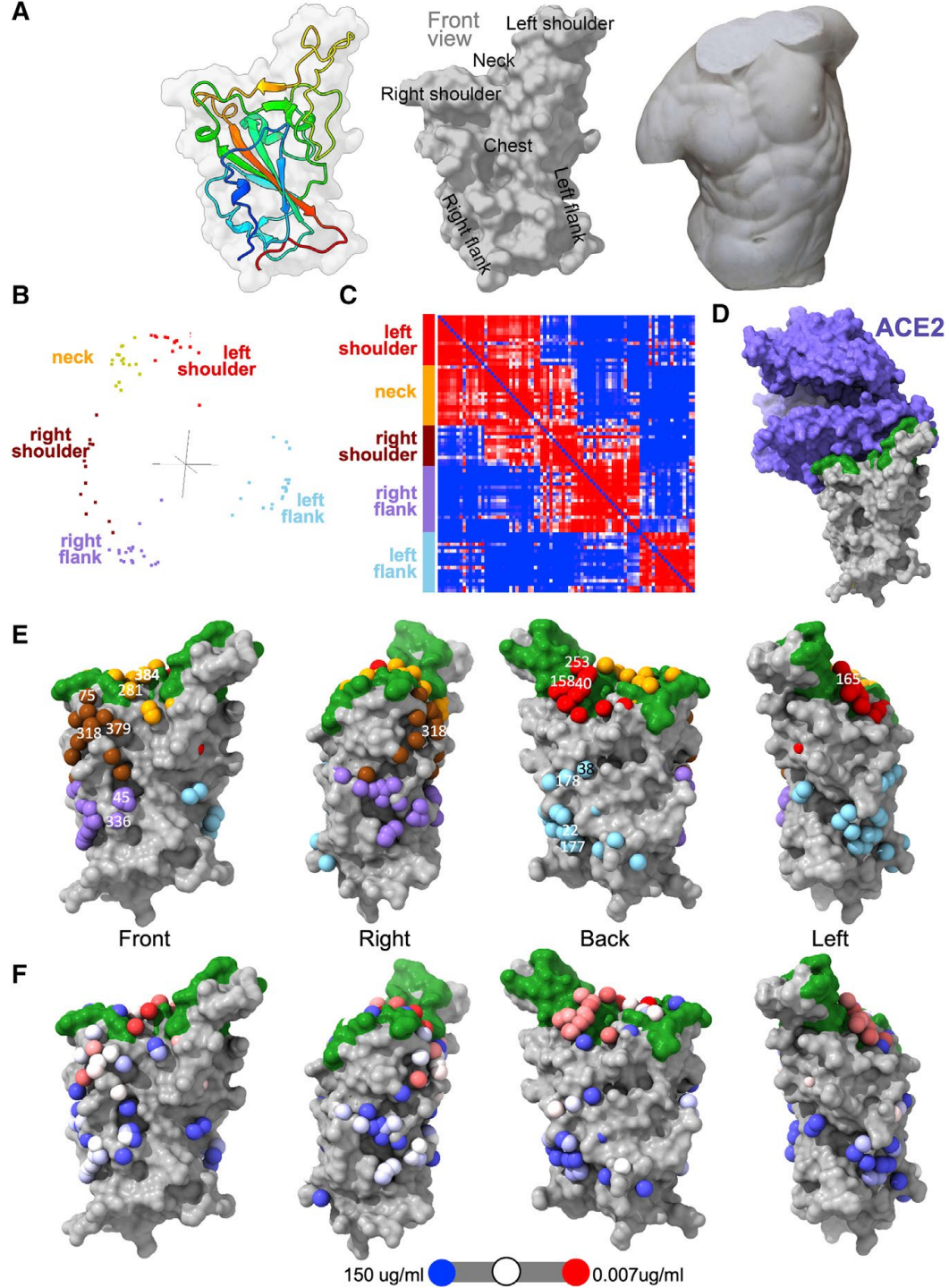
THE MUTATION THAT LOOSENS THE SPIKE PROTEIN

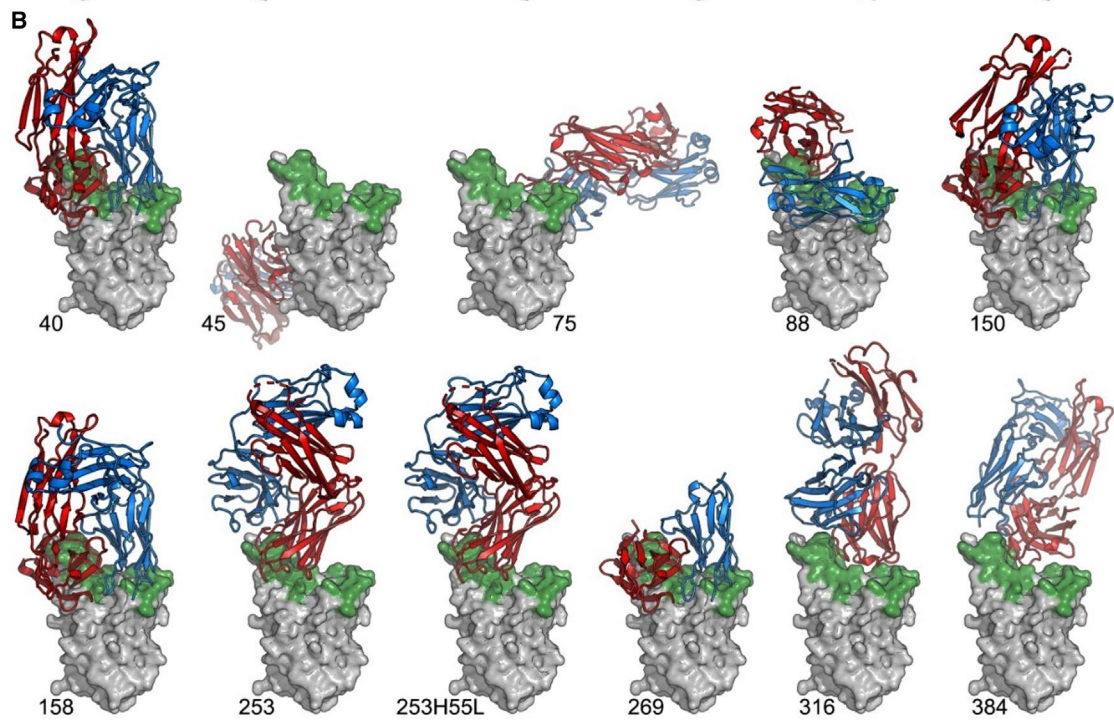
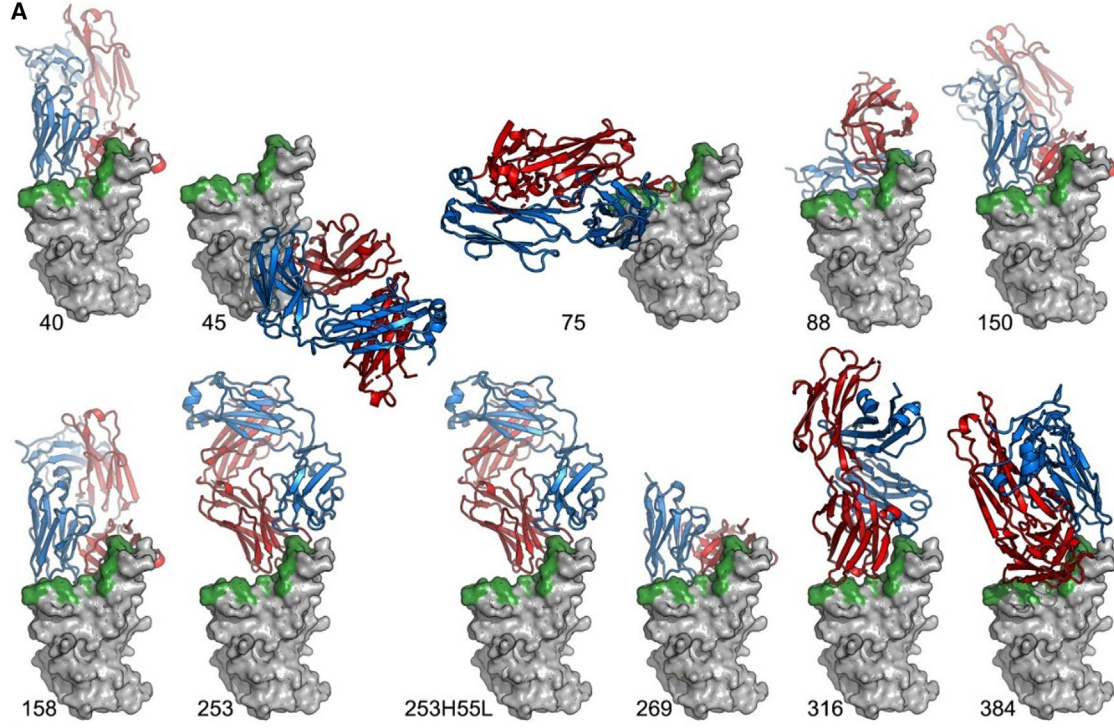
Spike proteins on SARS-CoV-2 bind to receptors on human cells, helping the virus to enter. A spike protein is made up of three smaller peptides in 'open' or 'closed' orientations; when more are open, it's easier for the protein to bind. The D614G mutation — the result of a single-letter change to the viral RNA code — seems to relax connections between peptides. This makes open conformations more likely and might increase the chance of infection.



Bioinformatics
 Coevolutionary analysis
 Molecular simulations
 Deep mutational scanning

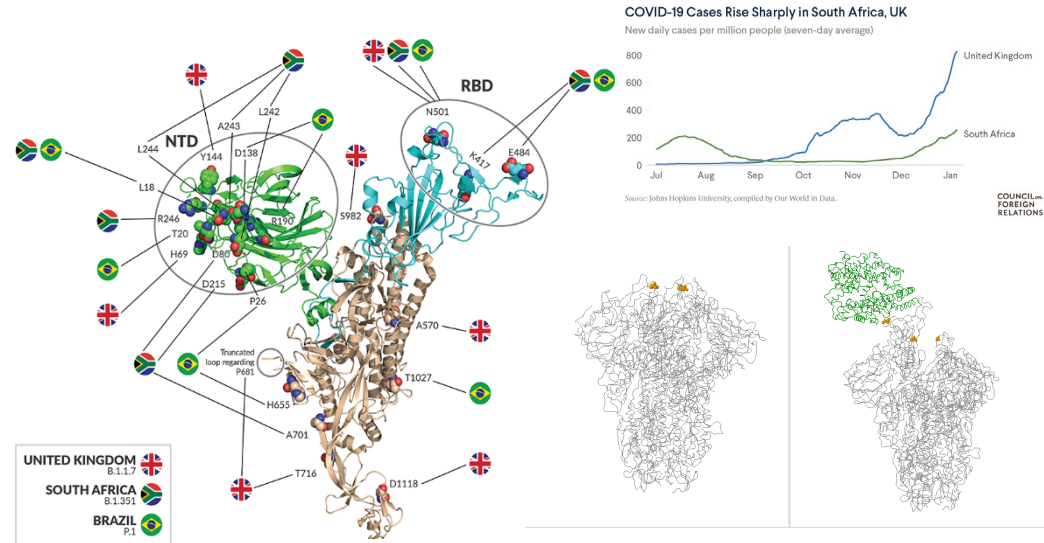
Perturbation-response scanning
 Network Biology
 Machine Learning





Novel variants may reduce the capacity of the antibodies to neutralize the virus in individuals who have previously contracted, or had a vaccination against, COVID-19.

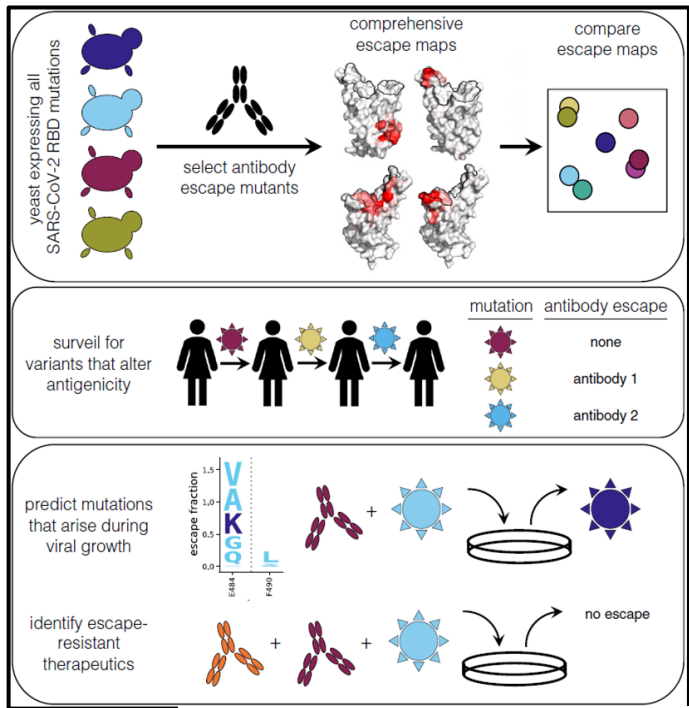
Name(s)	Distribution	Notable mutations	Potential effects on transmissibility, virulence, and immune escape
B.1.1.7, 20I/501Y.V.1, VOC20201.2/01	First identified in late December in the UK, it has spread to 62 countries in Europe, Asia, the US , and elsewhere.	17 recent mutations, including N501Y , P681H, HV 69–70 deletion, and four more on the spike protein; the ORF8 Q27stop mutation outside the spike protein	<ul style="list-style-type: none"> • Thought to have greater than 40 percent increased transmissibility • Increased virulence suggested but remains unresolved • Little concern around current vaccine efficacy
B.1.351, 20C/501Y.V.2	Identified in late December in South Africa and now spotted in Africa, Europe, Asia, and Australia	21 mutations , including N501Y, E484K, and K417N on the spike protein, and ORF1b deletion outside the spike protein	<ul style="list-style-type: none"> • Suggested to have heightened transmissibility • No evidence of increased virulence • In vitro studies suggest a potential for immune escape following natural infections and a small effect on the potency of vaccine-induced antibodies • Effect on transmissibility and/or virulence unknown • Anecdotes of reinfections reported, but potential for immune evasion remains unresolved
P.1, 20J/501Y.V.3	Discovered in travelers from Brazil during screening at a Japanese airport in January; now known to widely circulate in Brazil's Amazonas state and also observed in the Faroe Islands, South Korea, and the US	17 amino acid changes, including N501Y, E484K, and K417N on the spike protein; ORF1b deletion outside the spike protein	<ul style="list-style-type: none"> • Effect on transmissibility and/or virulence unknown • Anecdotes of reinfections reported, but potential for immune evasion remains unresolved



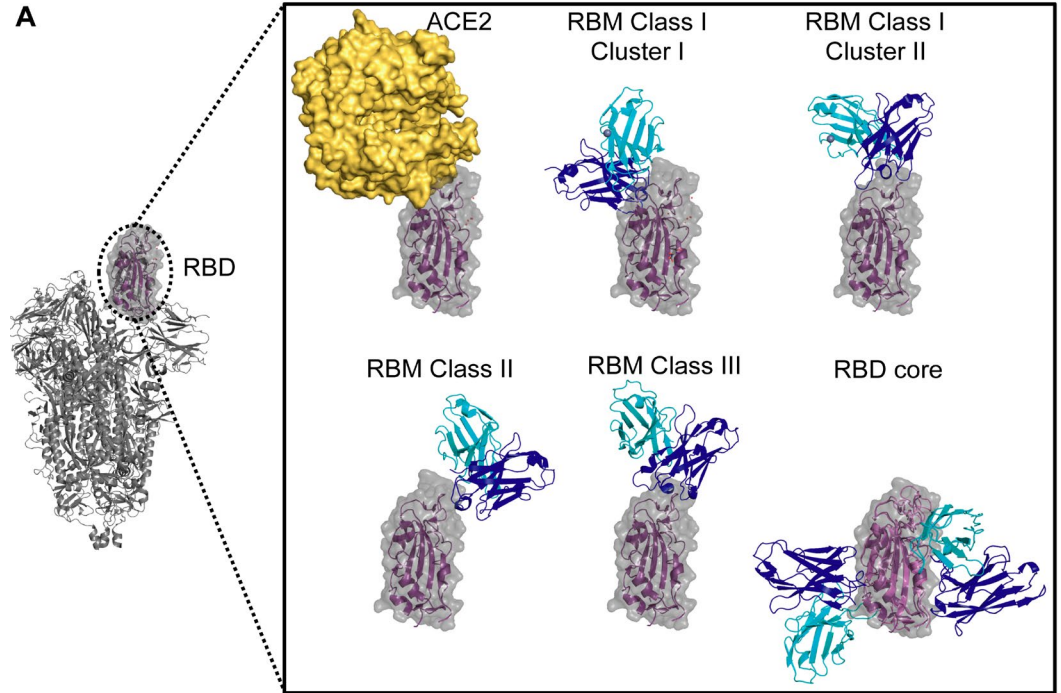
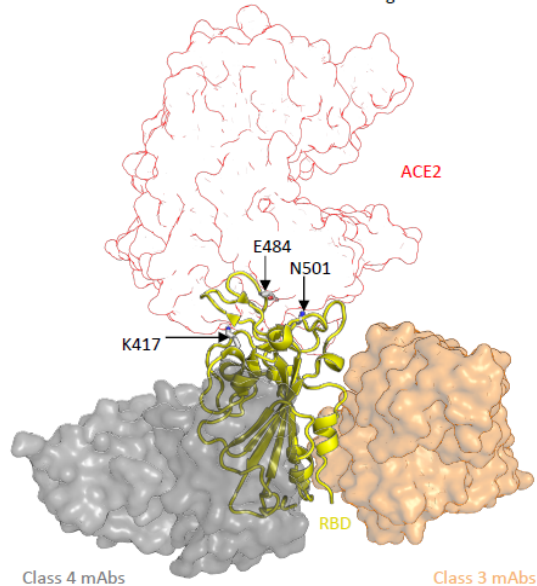
The virus is mutating in positions to avoid antibodies

Antibodies from hospitalized patients with more severe disease were more effective against the virus compared to those who had only mild symptoms

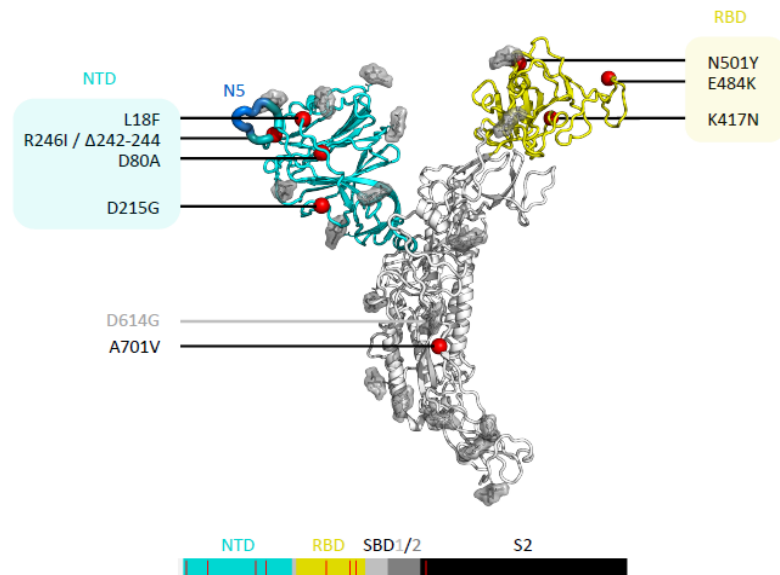
Neutralizing antibodies and Deep mutational mapping of viral variants that escape antibodies



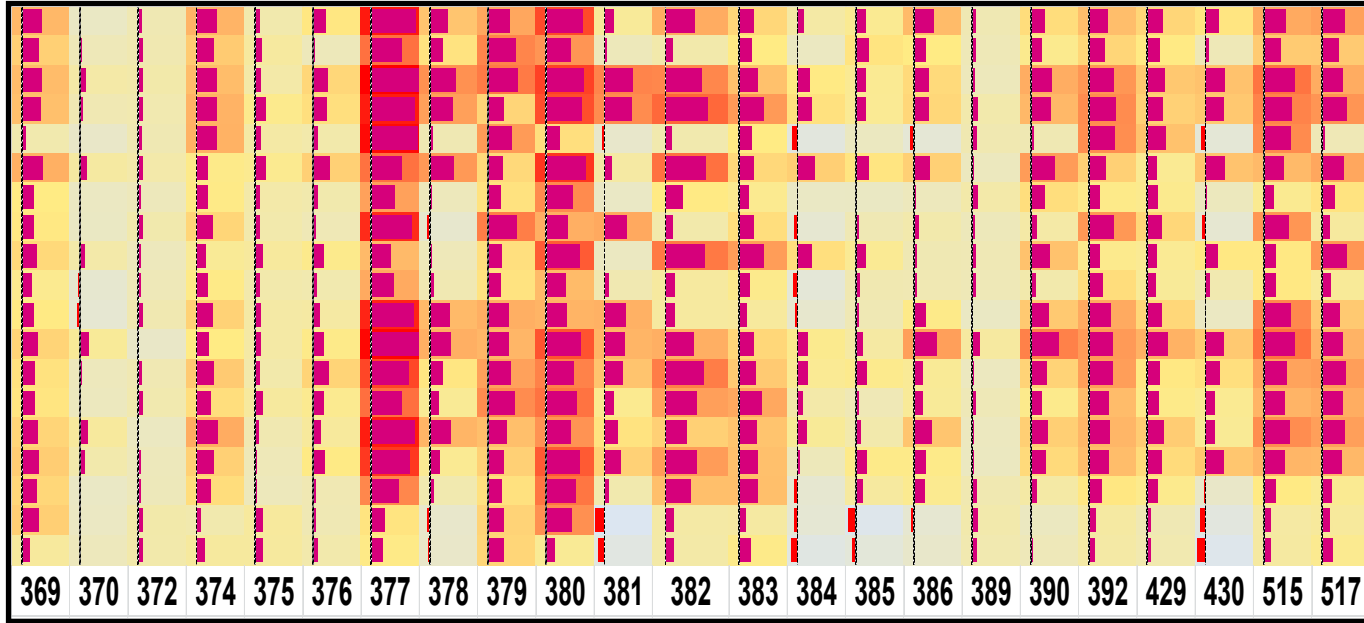
SARS-CoV-2 RBD bound to ACE2 and neutralizing antibodies



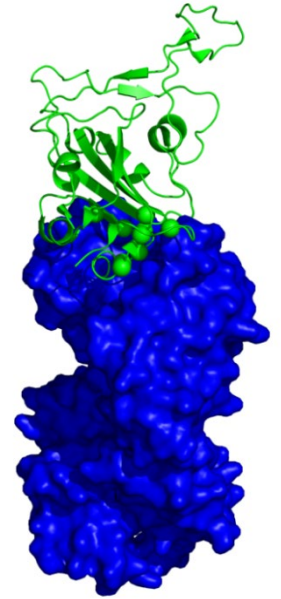
SARS-CoV-2 501Y.V2 Spike



In silico Deep mutational scanning

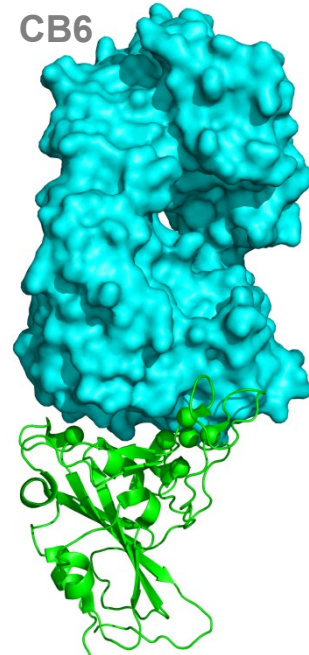


SARS-CoV-2 RBD

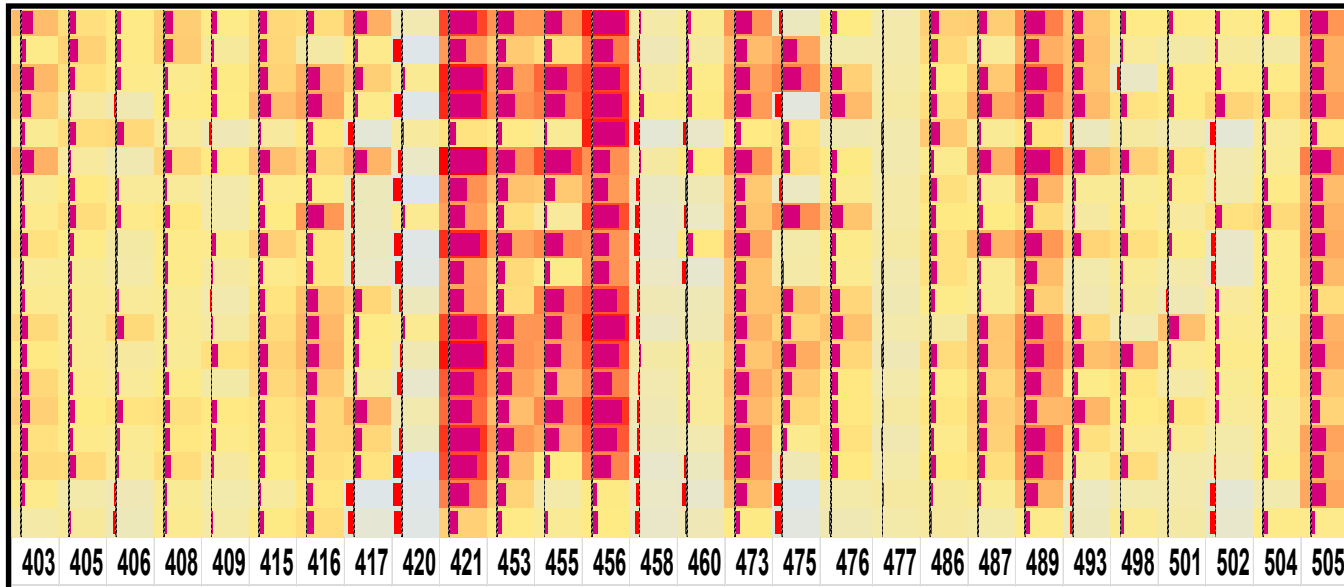


CR3022

CB6



SARS-CoV-2 RBD



SARS-CoV-2 RBD-ACE2



Integrated Biophysical Modeling

Molecular simulations

Mutational sensitivity analysis

Perturbation response scanning

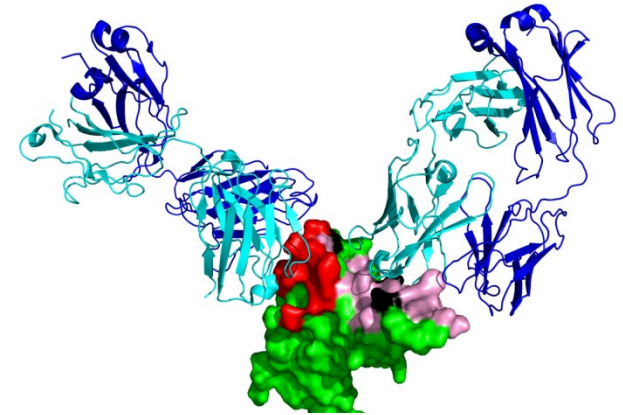
Hierarchical network modeling

Allosteric regulatory
Model of Spike Protein
Binding

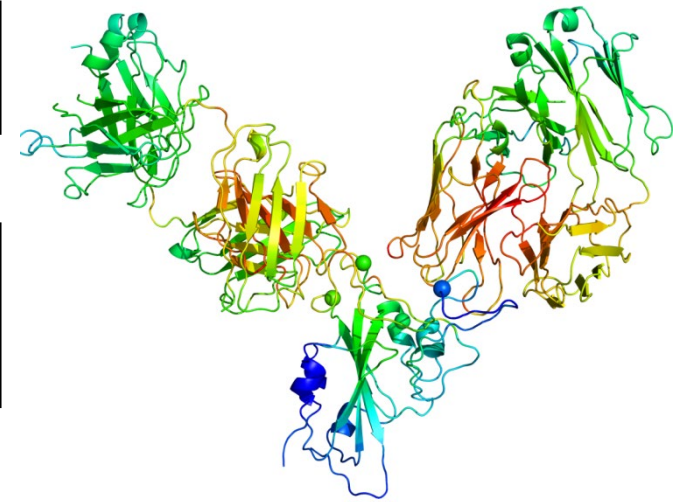
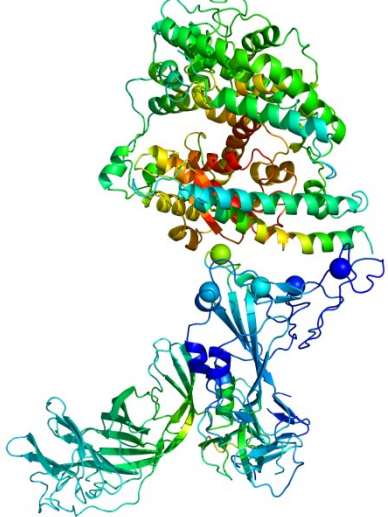
SARS-CoV-2 RBD-REGN/COV2

REGN10987

REGN10933

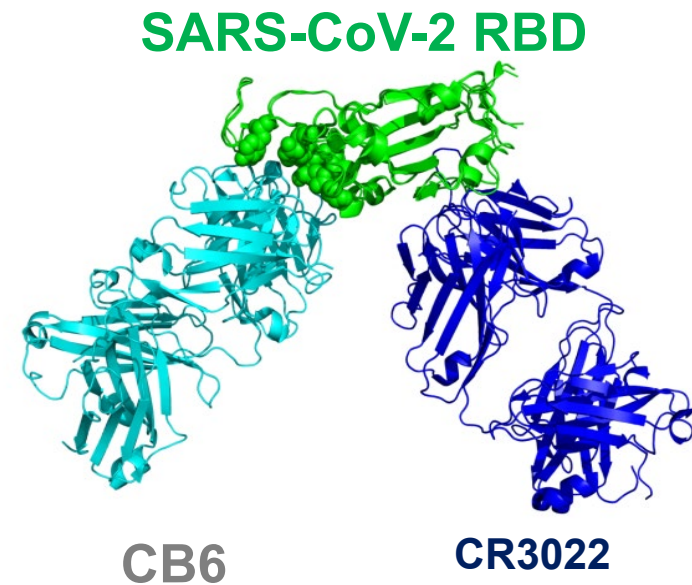
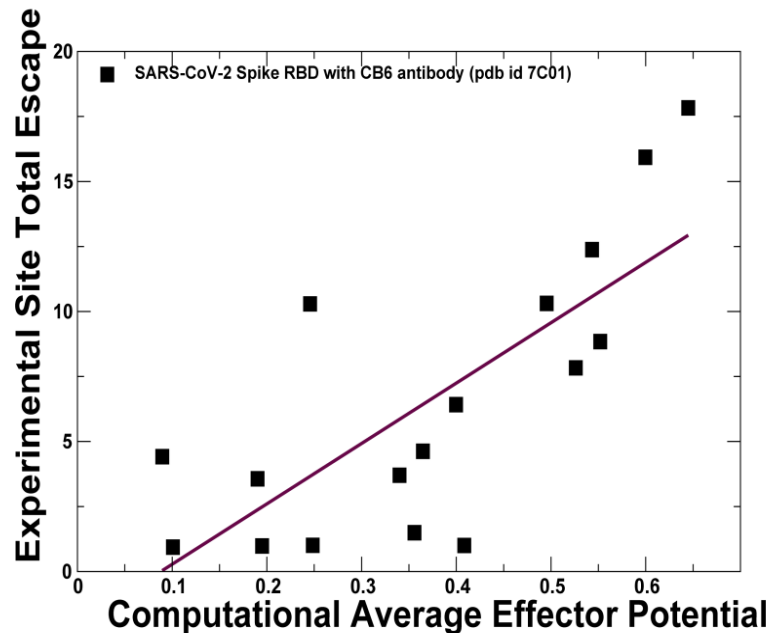
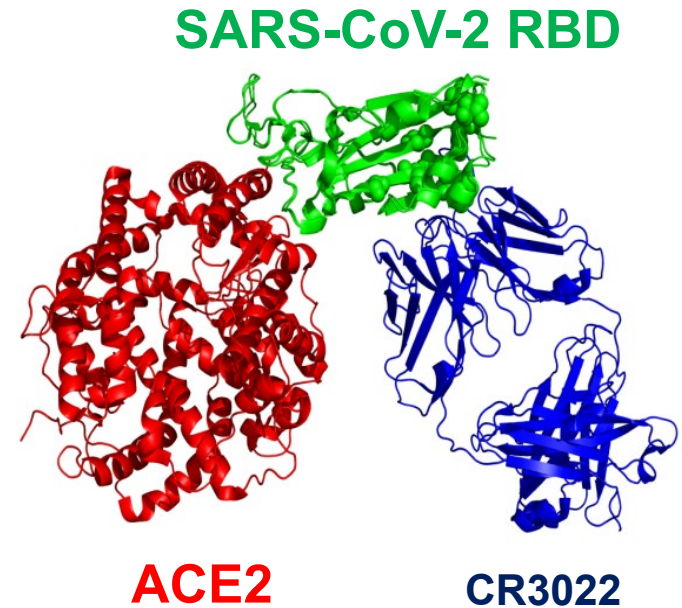
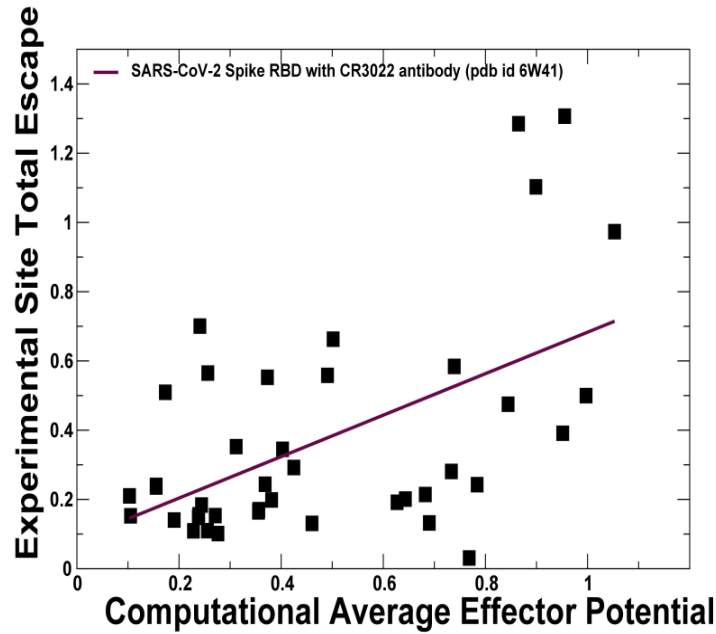


S1/RBD-ACE2

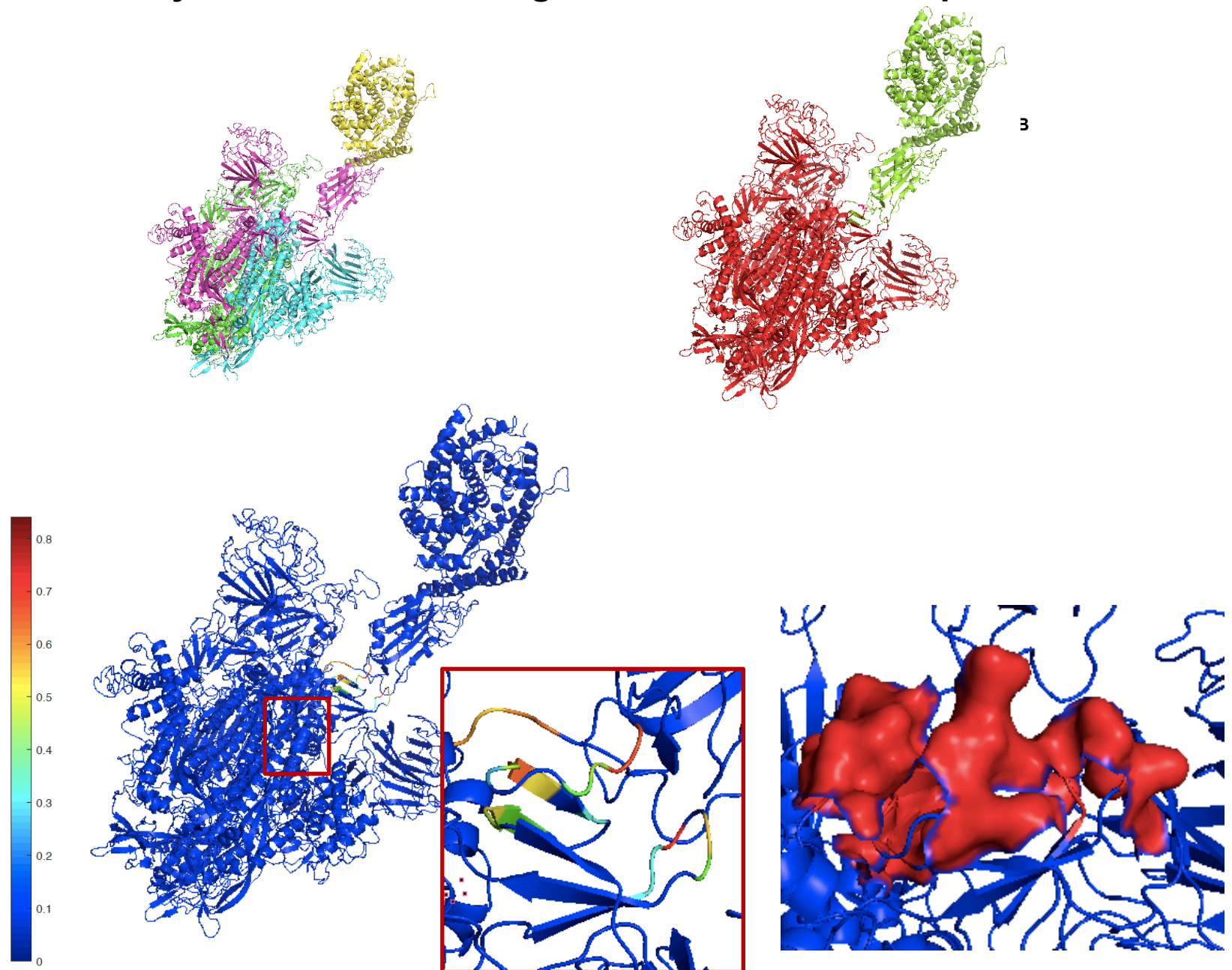


Structurally Adaptable Allosteric Communication Hotspots Define Spike Sites Targeted by Global Circulating Mutations and Antibody-Escaping Mutations

Perturbation response scanning

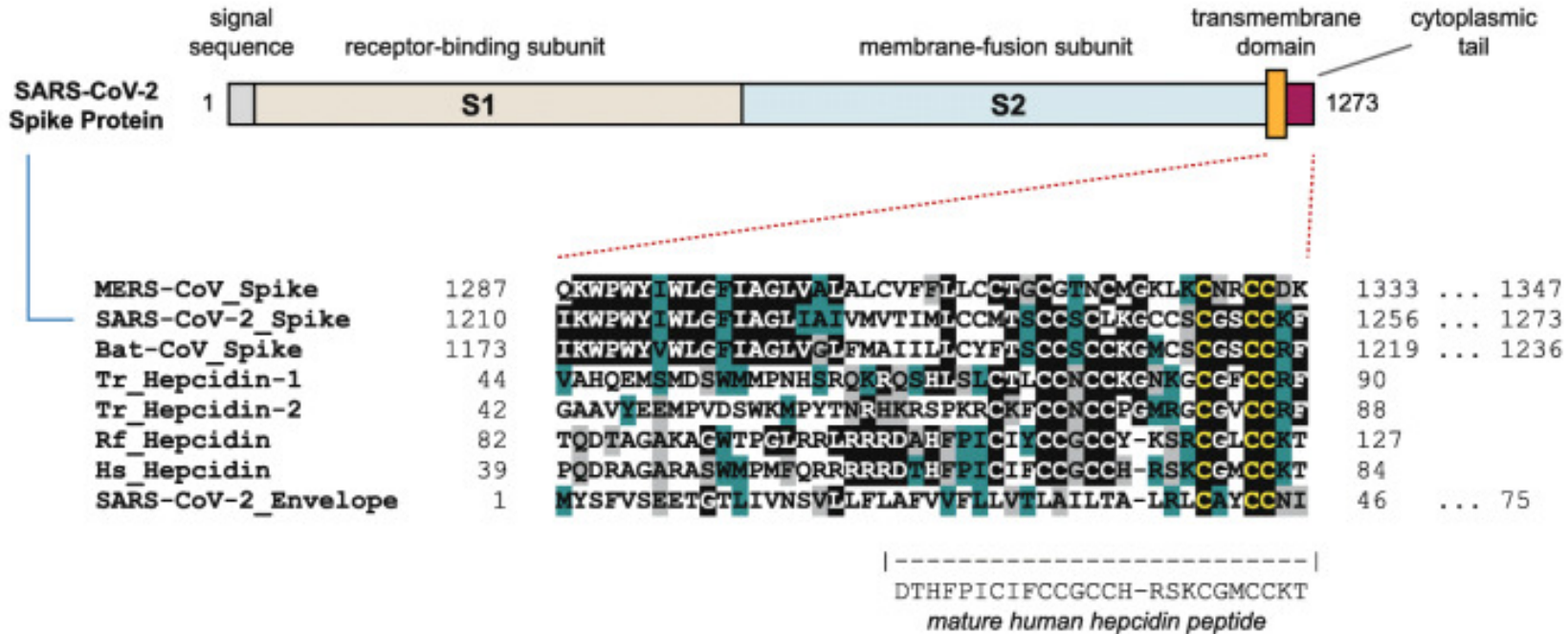


Discovery of Allosteric Binding Site on SARS-CoV-2 Spike

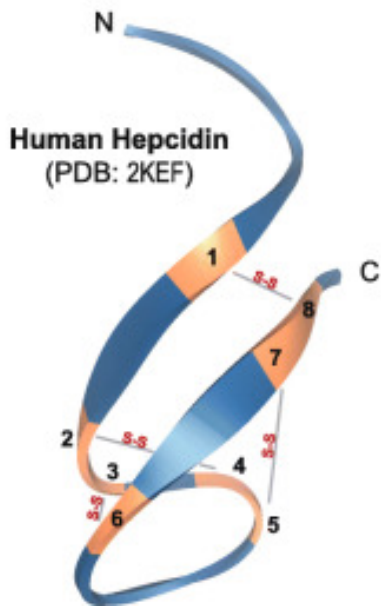


In Quest for Allosteric Modulators of the SARS-CoV-2 Spike

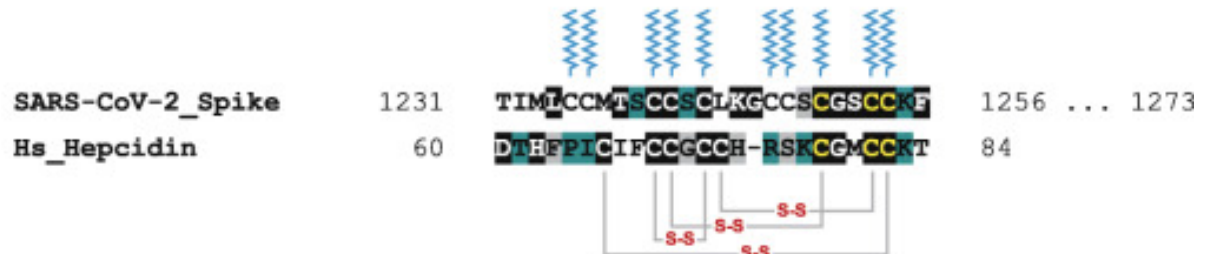
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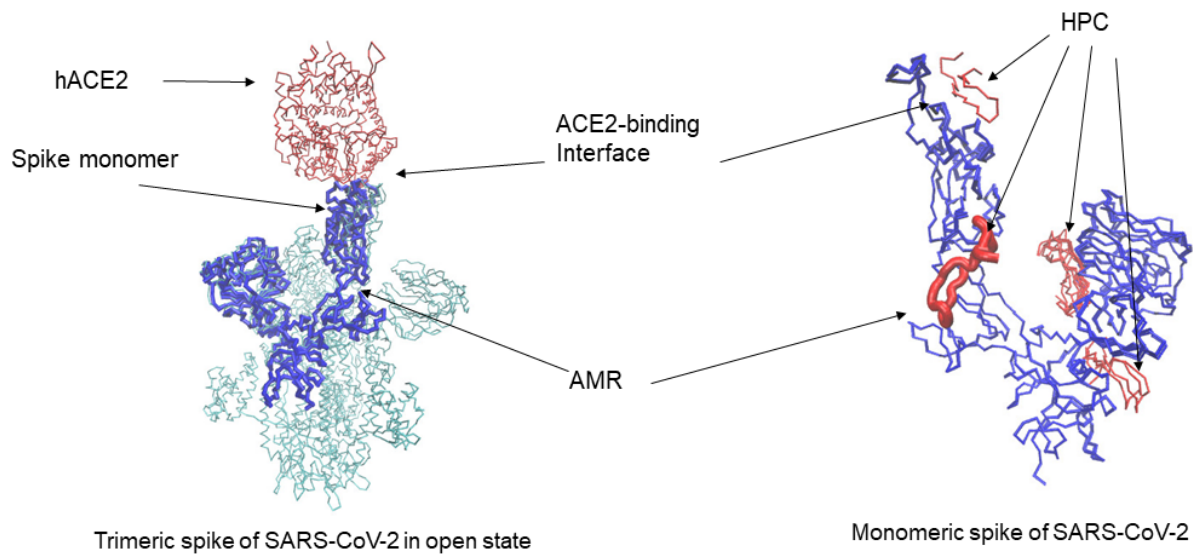
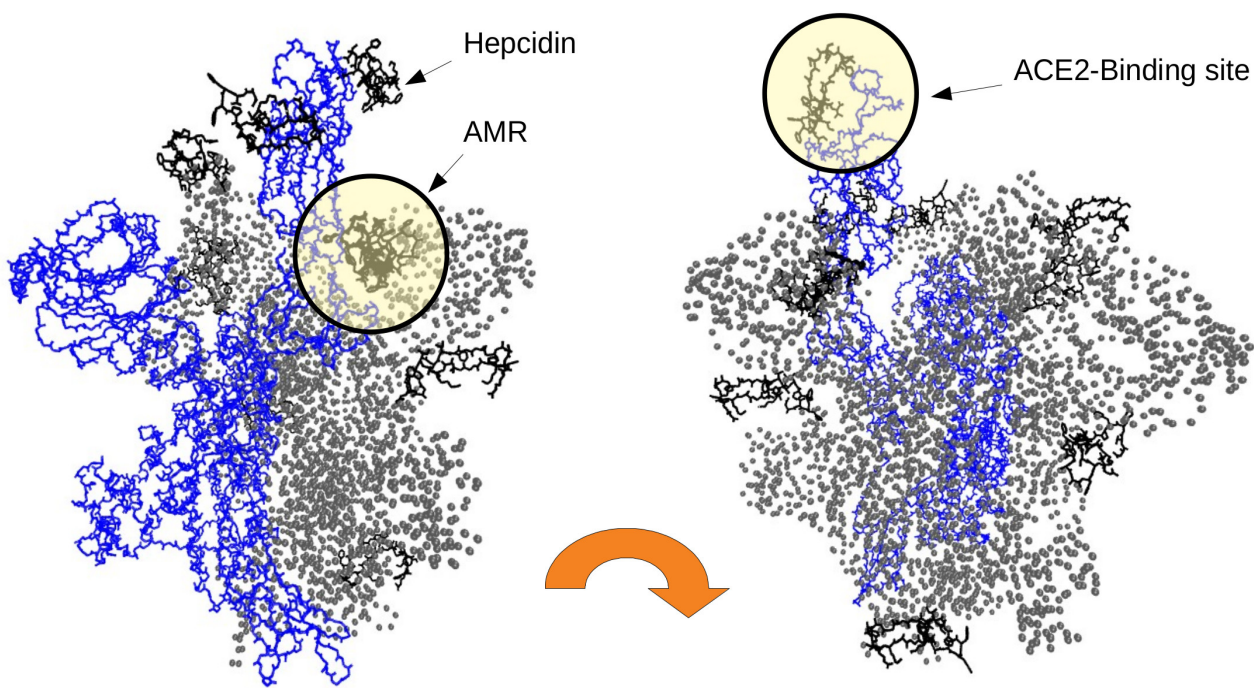
B



C

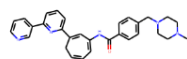


Hepcidin is the key regulator of iron metabolism in humans through its inhibition of the iron-exporting protein ferroportin

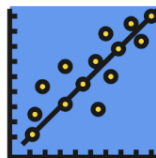
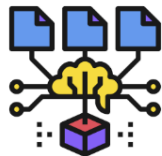


Machine Learning Strategies

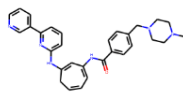
Perturbation of Known Drug Substances



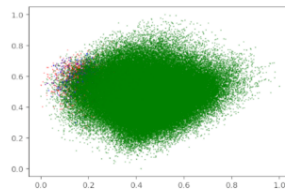
Known drug substances are obtained from the ZINC database



The molecules are encoded into latent variables, randomly perturbed, and decoded back into newly altered molecules



De Novo Generation via Bayesian Optimization



Instantiate a bayesian optimizer with 1000 random locations in the latent space

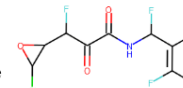


Calculate the scoring function at all points, and use the newly found information to warm up the gaussian process



Perform search on the latent space using the information learned from the exploration step to estimate high likelihood locations to sample from

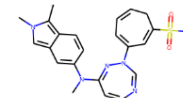
Alteration of Small Molecules via GAN



Obtain a random small molecule from the GDB-17 database

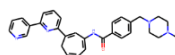


Pass it through the generator neural network, which makes an alteration to the molecule



Alterations performed by the GAN will result in molecules that are more similar to SRC kinase inhibitors

Perturbation Strategy



[0.1, 0.35, 0.6, ... , 0.8, 0.65]

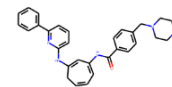
Known drug substances are downloaded from the ZINC database

These molecules are fed through the variational autoencoder and their latent representation is obtained



[0.125, 0.335, 0.599, ... , 0.811, 0.671]

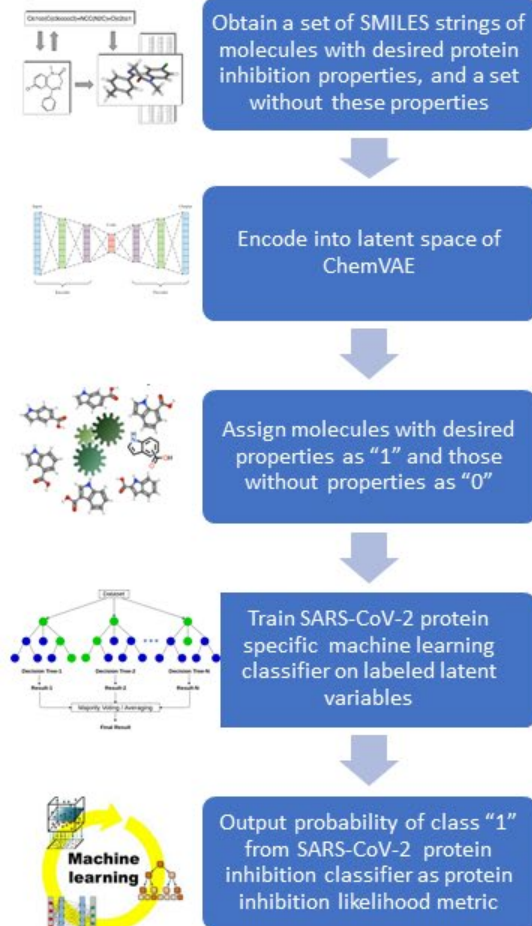
Noise is added to the vectors in various levels to search in close locations



The perturbed vector is fed back through the variational autoencoder, and its SMILES string is obtained

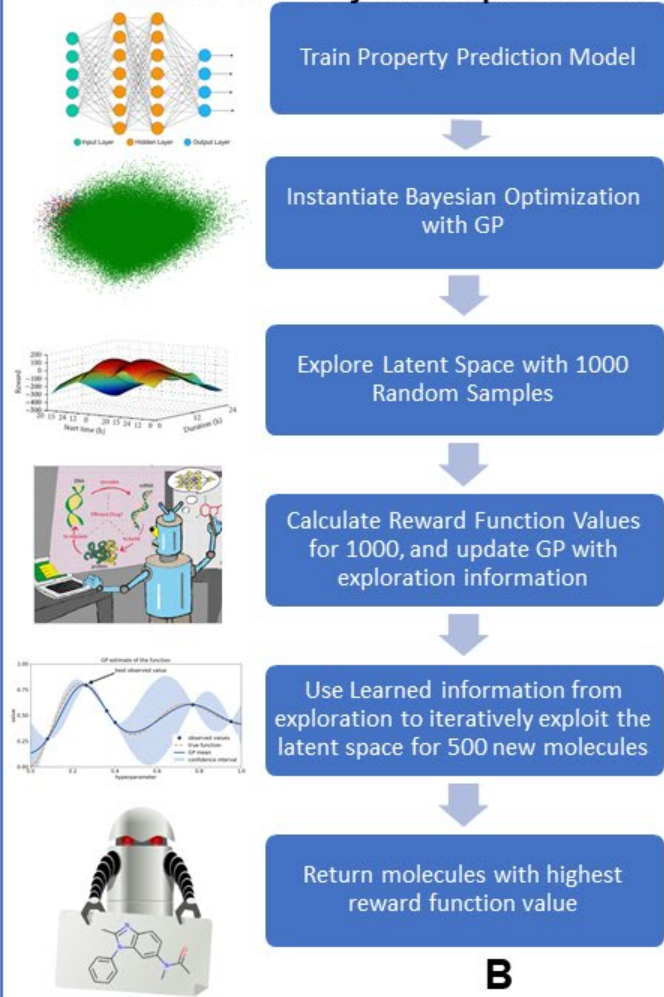
Experiment-Guided Machine Learning Strategies

SARS-CoV-2 Protein Inhibition Likelihood Classifier



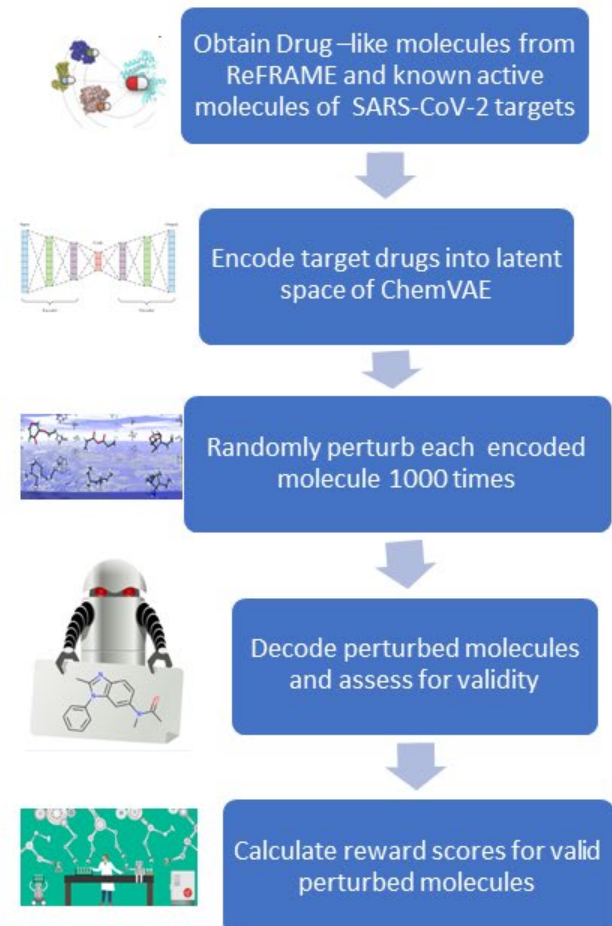
A

De Novo Generative Learning of Allosteric Probes with Bayesian Optimization



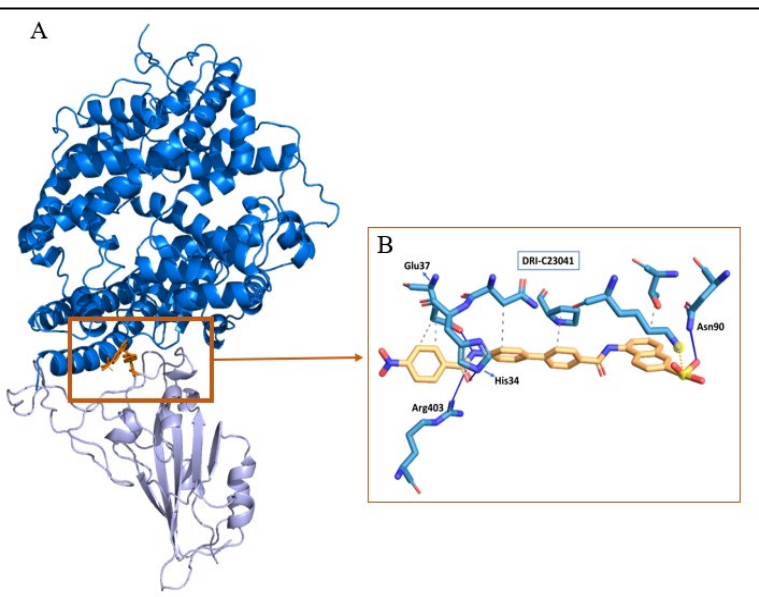
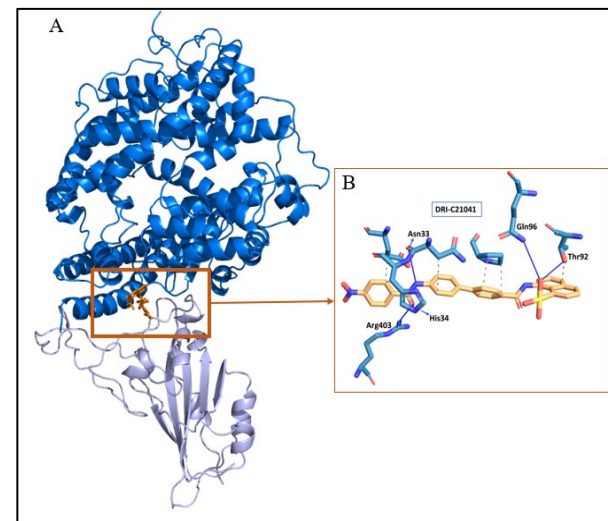
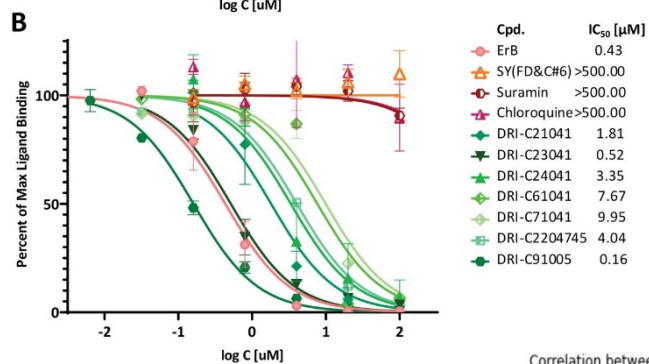
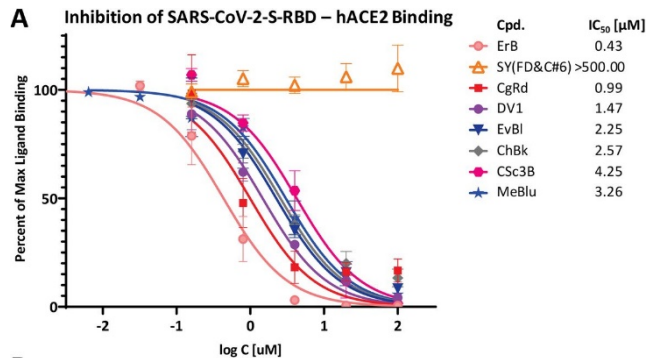
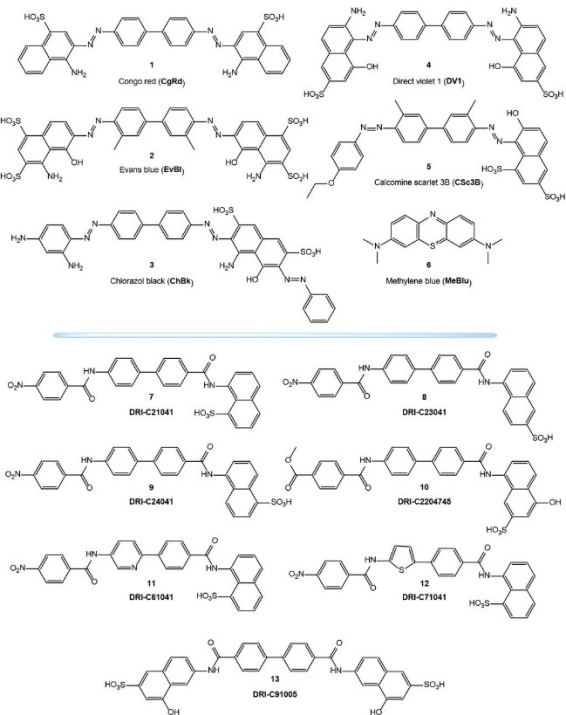
B

Generative Perturbation Learning for Drug Repurposing into Allosteric Probes

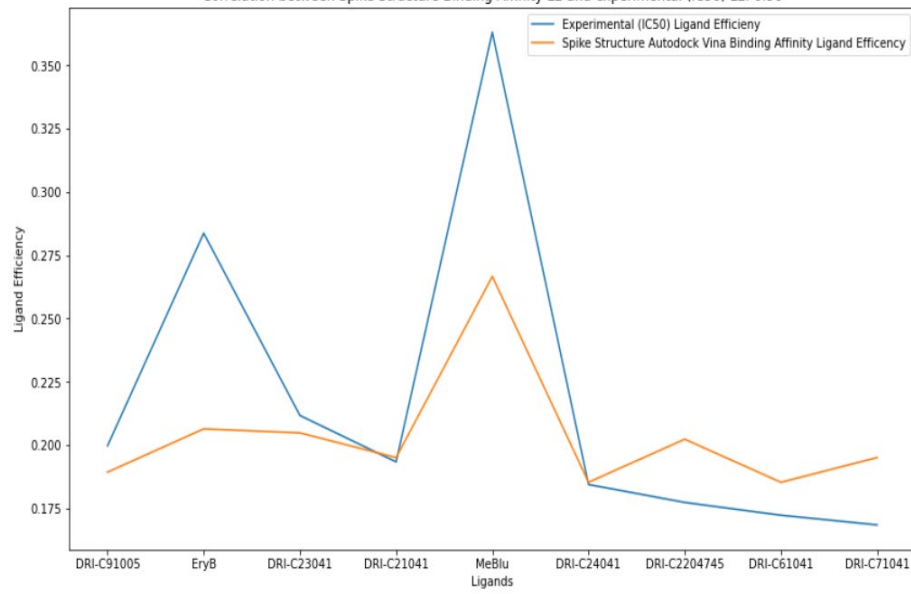


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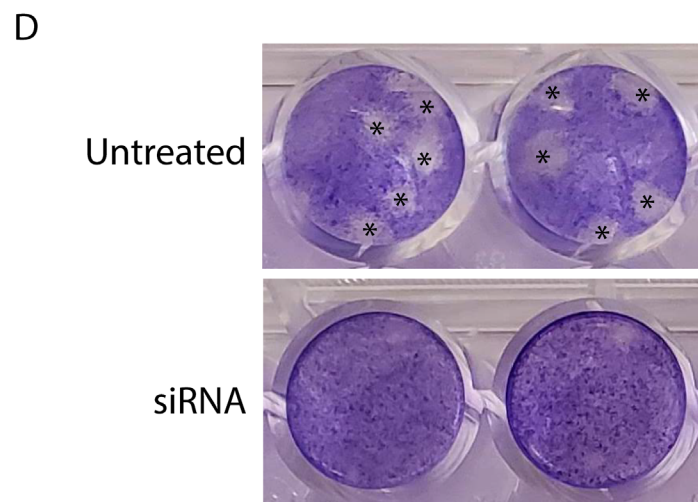
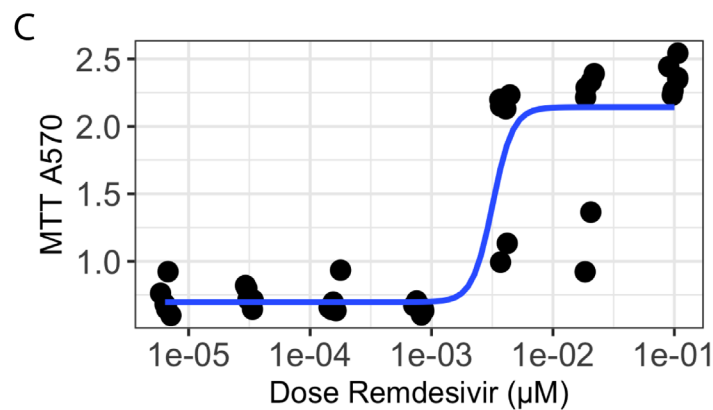
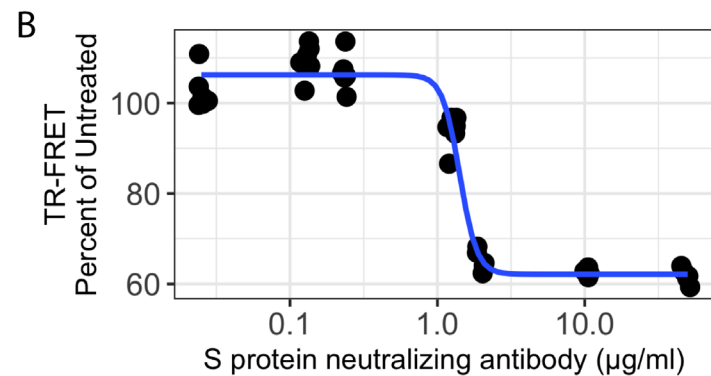
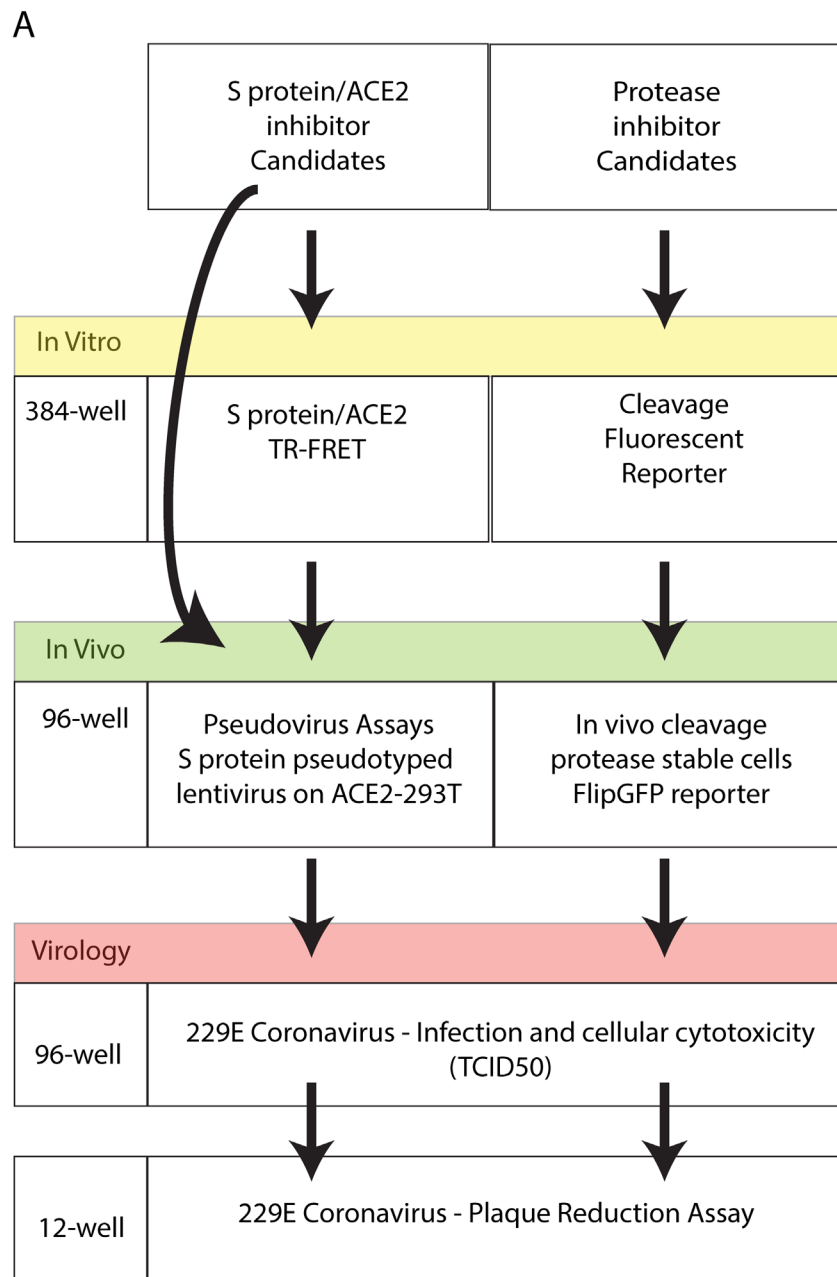
Small-Molecule *In Vitro* Inhibitors of the Coronavirus Spike – ACE2 Protein-Interaction as Blockers of Viral Attachment and Entry for SARS-CoV-2



Correlation between Spike Structure Binding Affinity LE and experimental (IC₅₀) LE: 0.90



Biochemical and pseudovirus entry assays for screening and validation of allosteric modulators of the SARS-CoV-2 protein targets.



Acknowledgments



<http://compbiosciences.chapman.edu>