A Recombinant SARS-CoV-2 Neutralizing Antibody Blocks the Spike RBD-ACE2 Interaction; A Biophysical Characterization Using SPR

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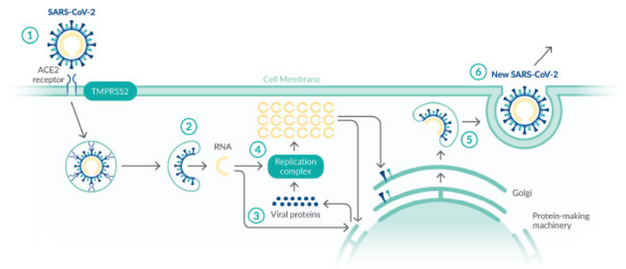
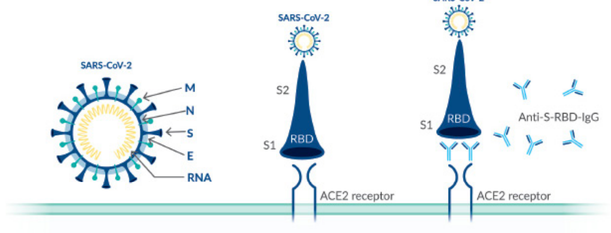
Investigating a Recombinant SARS-CoV-2 Neutralizing Antibody

- SARS-CoV-2 is an enveloped positive-stranded RNA virus, a member of the *Betacoronavirus* genus and the causative agent of COVID-19.
- As of September 2021, the number of COVID-19 cases worldwide had reached over 223 million. In addition, the number of deaths from COVID-19 was over 4.3 million.
- To aid in vaccine development, neutralizing antibodies against the SARS-CoV-2 spike glycoprotein receptor binding domain (RBD) have been explored.
- Spike glycoprotein is located on the outer envelope of the virion and composed of two subunits, S1 and S2.
- The S1 subunit contains the RBD and binds to ACE2.
- S1 and S2 cleavage by TMPRSS2 facilitates viral fusion with the host cell membrane followed by infection.

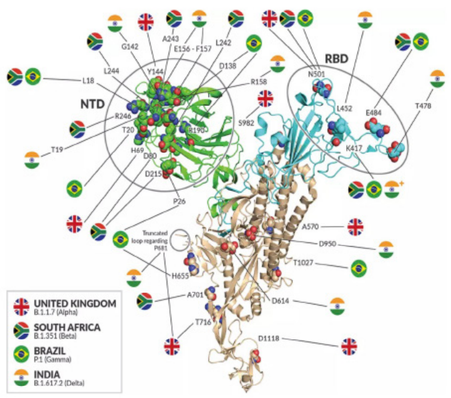


1 Spike protein on the virion binds to ACE2, a cell-surface protein. TMPRSS2, an enzyme, helps the virion enter. 2 The virion releases RNA. 3 Some RNA is translated into proteins by the cell's machinery. 4 Some of these proteins form a replication complex to make more RNA. 5 Proteins and RNA are assembled into a new virion in the Golgi and 6 released.

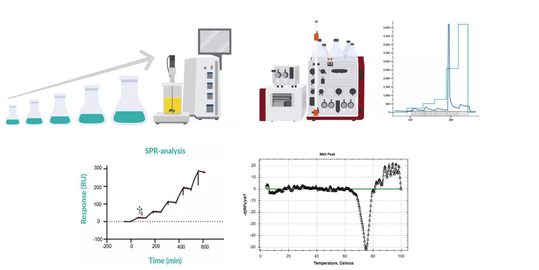
- Envelope protein (E), Membrane glycoprotein (M), Nucleocapsid protein (N), RiboNucleic Acid (RNA), and Spike protein (S) are structural components of SARS-CoV-2.
- Human neutralizing antibodies have been identified that bind to the S1 RBD, preventing further viral entry and infection through disruption of the S1 RBD-ACE2 interaction.



- This virus is rapidly mutating and now has several variants labeled as 'of concern' according to the WHO and CDC.
- In this study, variant effects on ACE2 binding were analyzed.
- N501Y, K417N, and E484K mutation effects on a recombinant SARS-CoV-2 nAbs were studied.



- We made recombinant human antibody, expressed in CHO cells using engineered constructs based on sequences of antibodies from COVID-19 patients (and one of the most frequently elicited antibody families (IGHV3-53, CC12.3, and CC12.1).
- The binding of this recombinant antibody to the S1 RBD and its ability to block the S1 RBD-ACE2 interaction were characterized using SPR.



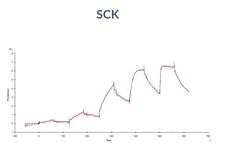
SARS-CoV-2 Neutralizing Recombinant Antibody Binding Analysis Studied by SPR

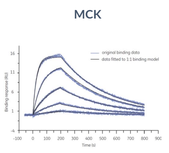
Goals:

- Evaluate the direct binding kinetics (on- and off-rate, K_D) to optimize target engagement (on-rate) and residence time on the target (off-rate) directly
- Compare the affinity of SARS-CoV-2 nAb (Fab) towards S1 RBD WT, Alpha, Beta variants, and blocking against ACE2
- Establish a competition assay between SARS-CoV-2 nAb (Fab) and ACE2 against S1 RBD and determine whether the nAb has reduced neutralizing capabilities towards the variants *versus* the WT using SPR

Protocols:

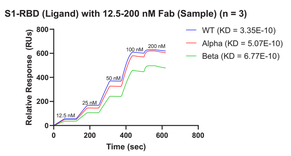
- Optimize SPR protocol for S1 RBD WT, Alpha and Beta with SARS-CoV-2 nAb (Fab), and ACE2
- Run SCK assay for ACE2/S1 RBD (rFc-tagged)
- Run SCK assay for SARS-CoV-2 nAb (Fab)/S1 RBD (rFc-tagged)
- Run SCK competition assay for SARS-CoV-2 nAb (Fab) and ACE2/S1 RBD (rFc-tagged)
- Run MCK competition assay for SARS-CoV-2 nAb (Fab)/S1 RBD (rFc-tagged) and ACE2



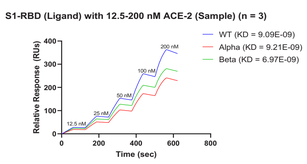


SCK Binding and Competition Assays for SARS-CoV-2 nAb (Fab) and ACE2 with S1 RBD

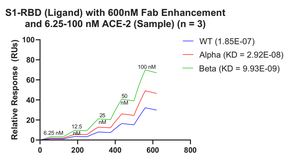
- Based on the SPR results of SCK runs, ACE2 had similar affinity towards S1-RBD WT, Alpha, and Beta variants and slightly higher affinity towards Beta variant (6.9 nM *vs.* 9 nM).
- The SARS-CoV-2 nAb (Fab) had very high affinity (K_D below 1 nM) towards S1-RBD WT, Alpha, and Beta variant and slightly higher affinity towards the WT (0.3 nM *vs.* 0.55 nM).
- For the competition assay, nAb, provided better blocking against ACE2 for WT and Alpha and Beta variants, respectively (185 nM *vs.* 29 nM and 9 nM for WT, Alpha, and Beta variants respectively).



	ka (1/Ms)	kd (1/s)	KD (M)
WT	8.65E+05	2.86E-04	3.35E-10
Alpha	9.16E+05	4.65E-04	5.07E-10
Beta	1.40E+06	8.38E-04	6.77E-10



	ka (1/Ms)	kd (1/s)	KD (M)
WT	8.98E+04	8.17E-04	9.09E-09
Alpha	9.22E+04	8.48E-04	9.21E-09
Beta	1.04E+05	7.12E-04	6.97E-09



	ka (1/Ms)	kd (1/s)	KD (M)
WT	1.75E+04	1.72E-03	1.85E-07
Alpha	3.40E+04	9.74E-04	2.92E-08
Beta	7.40E+04	7.33E-04	9.93E-09

MCK Binding and Competition Assays for SARS-CoV-2 nAb (Fab) and ACE2 with S1 RBD

- MCK competition assays were run to look at SARS-CoV-2 nAb's ability to neutralize S1 RBD WT and variants when ACE2 is added at K_D, 4x K_D, and ¼ K_D *(10 nM ACE2 data shown below).*
- Overall data correlated well with SCK, and nAb provided better blocking against ACE2 binding.
- The nAb (Fab) provided better blocking against ACE2 binding with WT and the Alpha variant (IC_{50s} = 64 nM and 68 nM, respectively) compared to the Beta variant (IC_{50s} = 120 nM).
- The same results were obtained using *in vitro* and cell-based assays.

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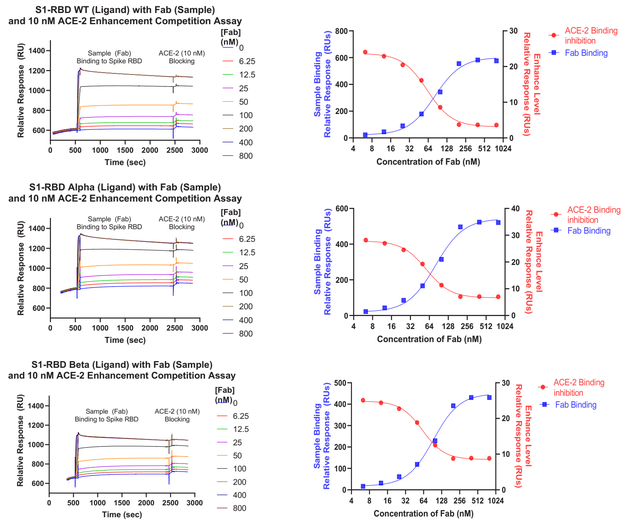
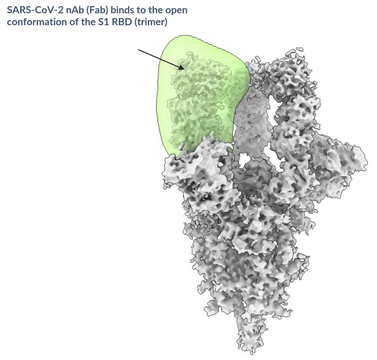


Cryo-EM Single Particle Analysis of SARS-CoV-2 6P Spike-Fab (nAb) Complex

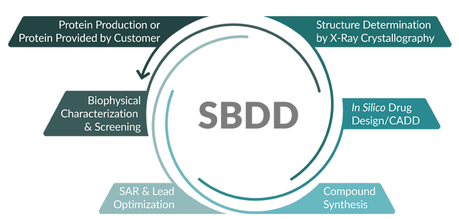
- In collaboration with Thermo Fisher Scientific, we were successful in obtaining the cryo-EM structure of the SARS-CoV-2 Spike bound nAb (Fab) complex at 2.9Å.
  - SARS-COV-2 6P Spike-Fab (nAb) Complex in the presence of fluorinated octyl maltoside (FOM)
  - Data acquisition on Krios Using EPU and Selectris-Falcon 4 by Ieva Drulyte, Thermo Fisher Scientific
- Based on the cryo-EM structure, Fab binds to the trimer Spike glycoprotein in open conformation and dominates recognition of the ACE2–binding site in S1 RBD.



Cryo-EM study was performed by Ieva Drulyte at Thermo Fisher Scientific.

Conclusion

- In this study, we investigated SARS-CoV-2 neutralizing recombinant antibody blocking the Spike RBD–ACE2 interaction using the biophysical technique, SPR.
- We made recombinant antibodies, hACE2, and S1 RBD WT and variants in-house using our established mammalian expression system and further characterized their binding *via* Surface Plasmon Resonance (SPR) using our Biacore instrument.
- We established an SPR platform to evaluate the binding kinetics and affinity measurements for ACE2 and nAb towards S1 RBD.
- Essentially, this engineered recombinant antibody can block the interaction of ACE2 with S1 RBD WT and Alpha variant better than that with the Beta variant.
- Knowledge of these structural data will help further design more potent and selective recombinant antibodies towards the new variants of the virus.



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