Protein Display to Discover and Engineer Viral Neutralizing Antibodies

Maxwell A. Stefan, Ph.D.

Postdoctoral Appointee: Antibody Engineering

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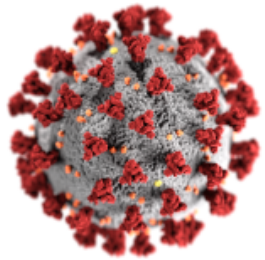
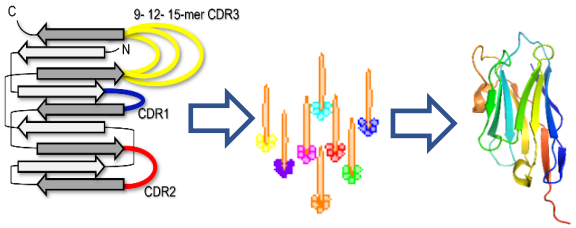
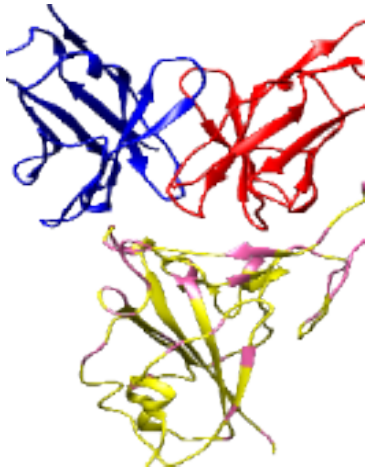
Research Projects at Sandia National Laboratories

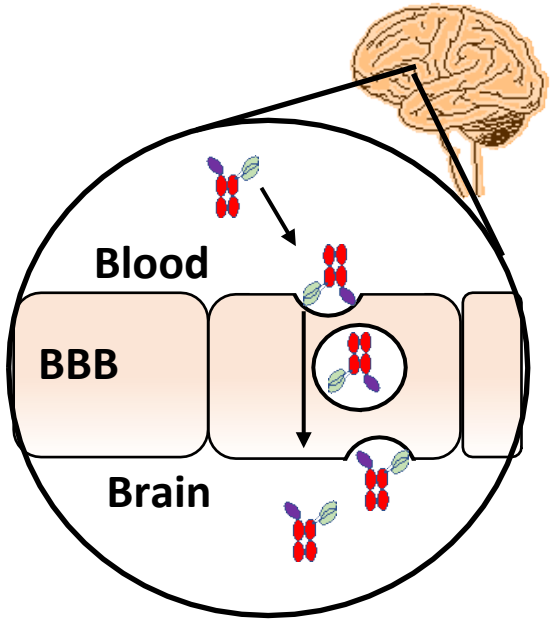
Antibody Engineering/ Assay Devt

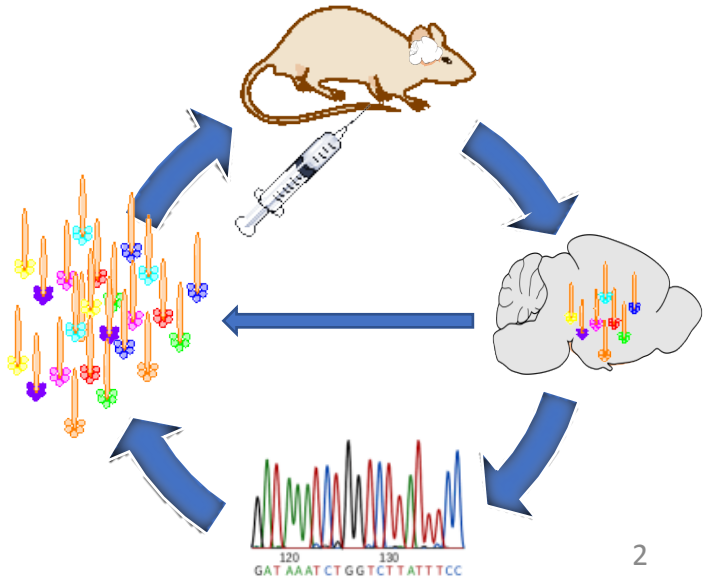
- Discovery of viral neutralizing antibodies for coronaviruses
- Computational and Phage Display to Adapt Existing mAbs to Related Viruses
- Blood-Brain Barrier (BBB) transmigrating bispecific antibodies for encephalitic alphaviruses
- In vivo biopanning to identify novel receptor-mediated transcytosis (RMT) targets











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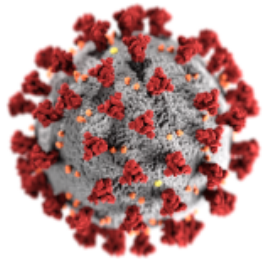
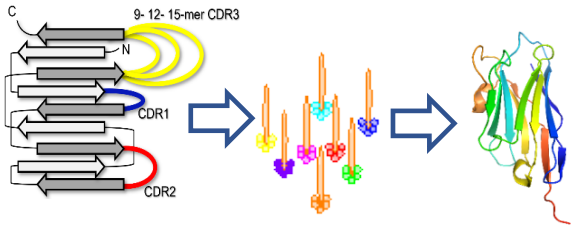
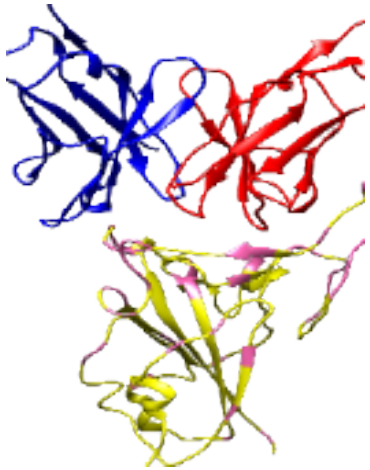
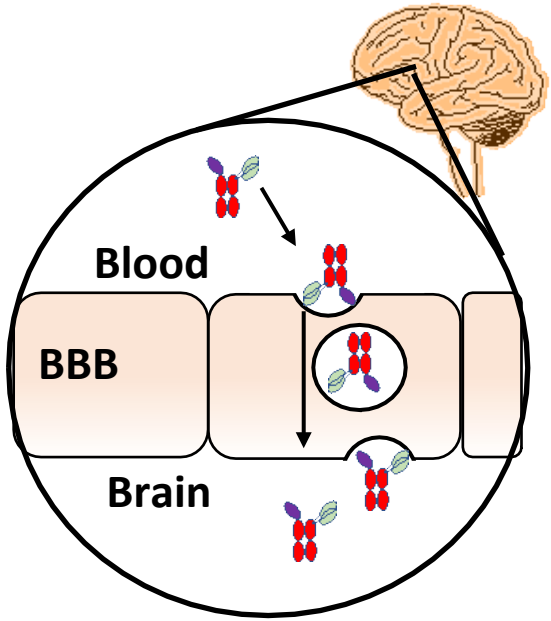
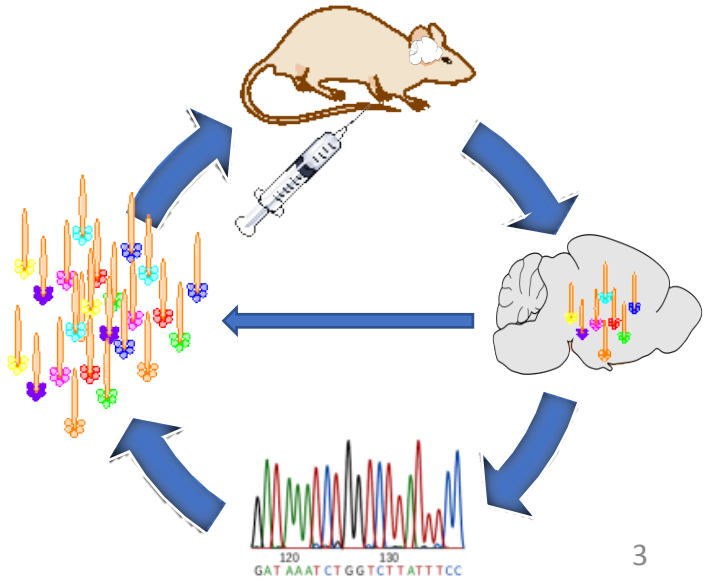








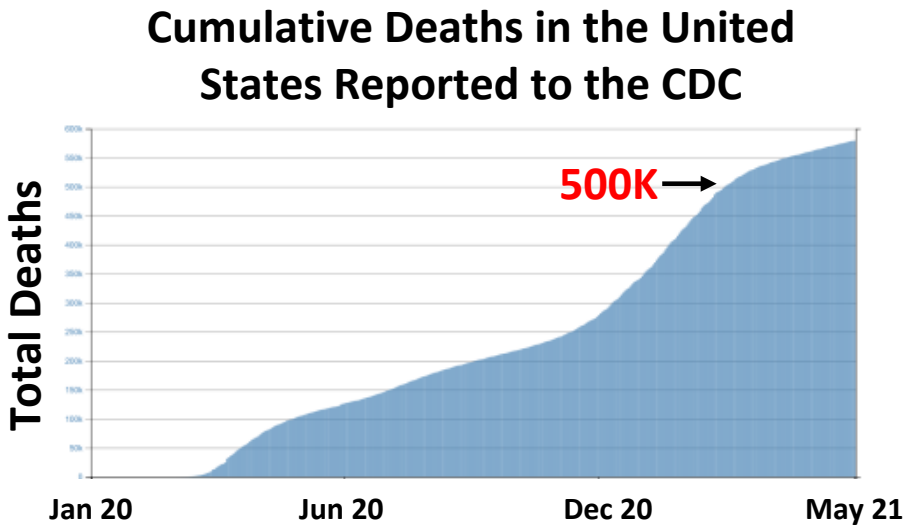


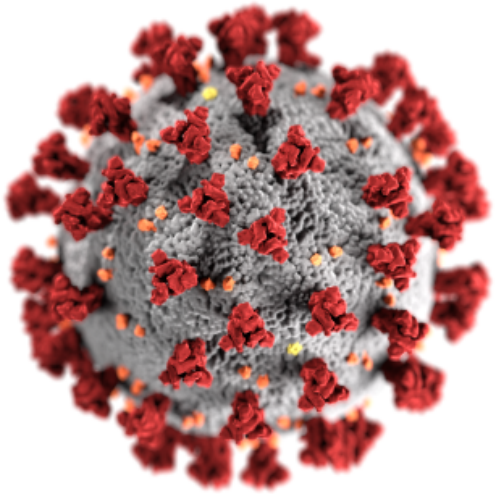
COVID-19 Pandemic and SARS-CoV-2

- Coronavirus Disease-19 Metrics
  - Respiratory/Inflammatory disease
  - >159,000,000 confirmed infections
  - >3,300,000 deaths globally

- Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2)
  - (+)-sense RNA virus – prone to mutation and neutralization escape
  - Cell entry mediated by Angiotensin-converting enzyme 2 (ACE2)

- Massive Global Response
  - Public health measures (masks, social distancing, required shutdowns, etc.)
  - Therapeutic intervention (Neutralizing antibodies, mRNA vaccines, antivirals)





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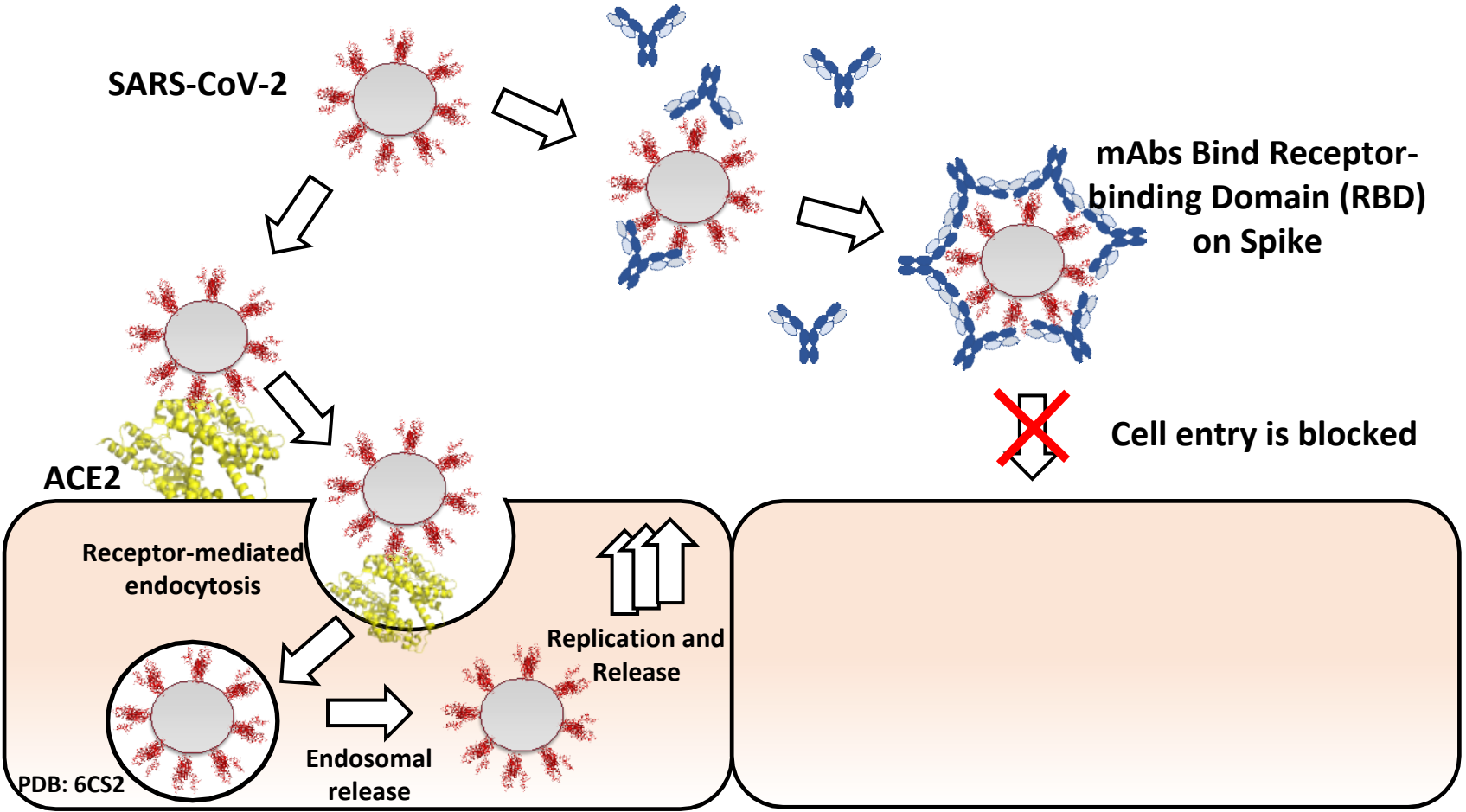
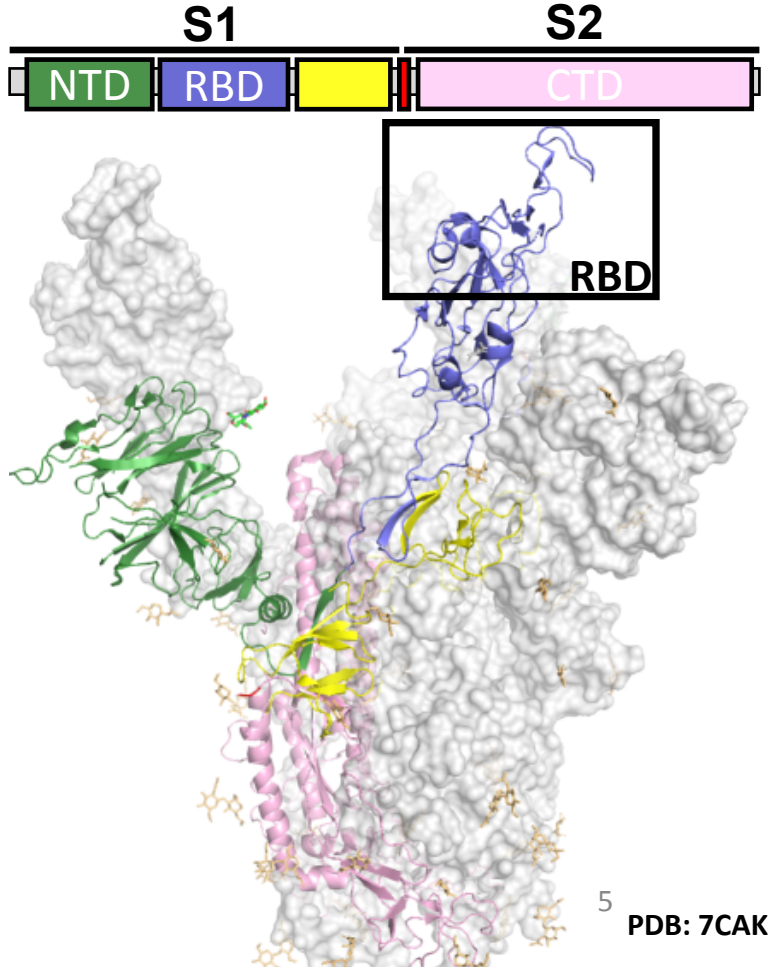
Viral Neutralizing Antibodies

- Neutralizing antibodies (mAbs) are naturally produced by the adaptive immune response
- Can be produced as recombinant monoclonal mAb and administered as a therapeutic





High-Diversity V_HH Library Design

Nanobodies (V_HH)

- Highly soluble and stable – developability
- Modular – easily incorporated into multispecific antibody formats (bispecific, trispecific, etc.)
- Smaller – impacts immunogenicity, antigen recognition

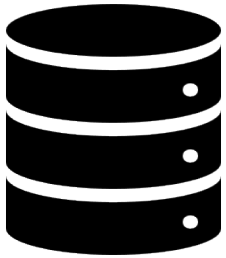
Natural Sources

Synthetic Sources

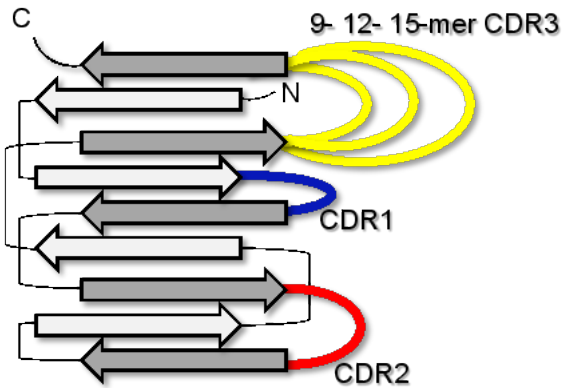
EVQLQASGGGFVQPG...
EVQLQASGGGFVQPG...
EVQLQASGGGFVQPG...
EVQLQASGGGFVQPG...
EVQLQASGGGFVQPG...

Functional V_HH antibodies

sdAb-DB Database



Sandia High-Diversity V_HH Library ~3.2 x 10^{10}



DNA Produced by Twist Biosciences

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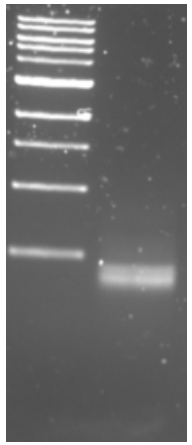
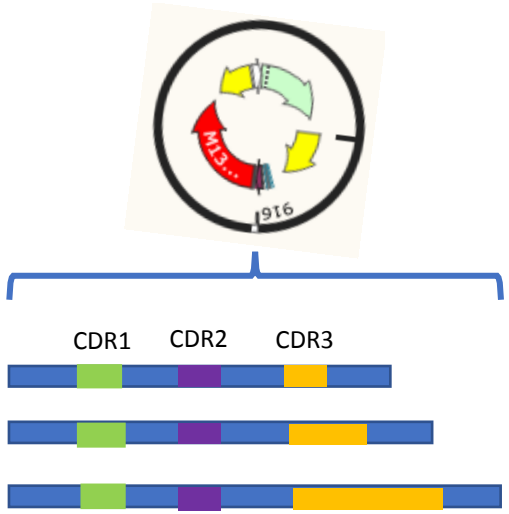
Library Construction and Transformation

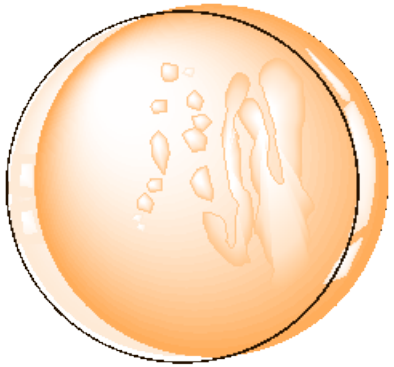
PCR Amplification on Twist Library (5 cycles)



Ligation (Vector: Insert = 1:2)

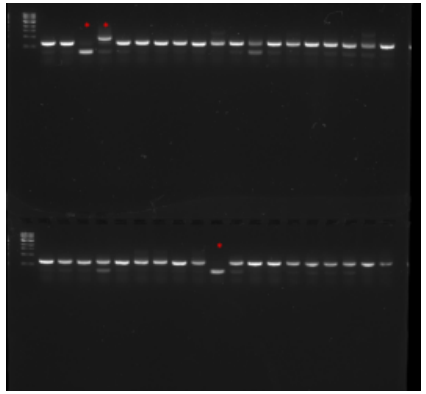


Transformation: Electroporation and Plates



3.38 x 10^{10} colonies recovered

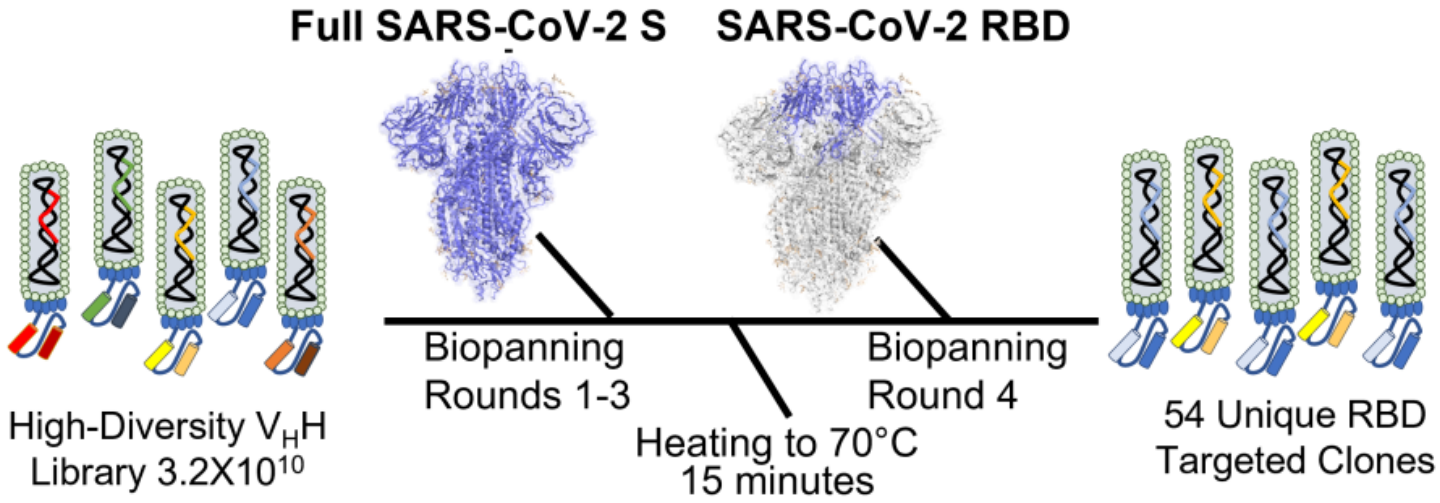
Colony PCR and NGS

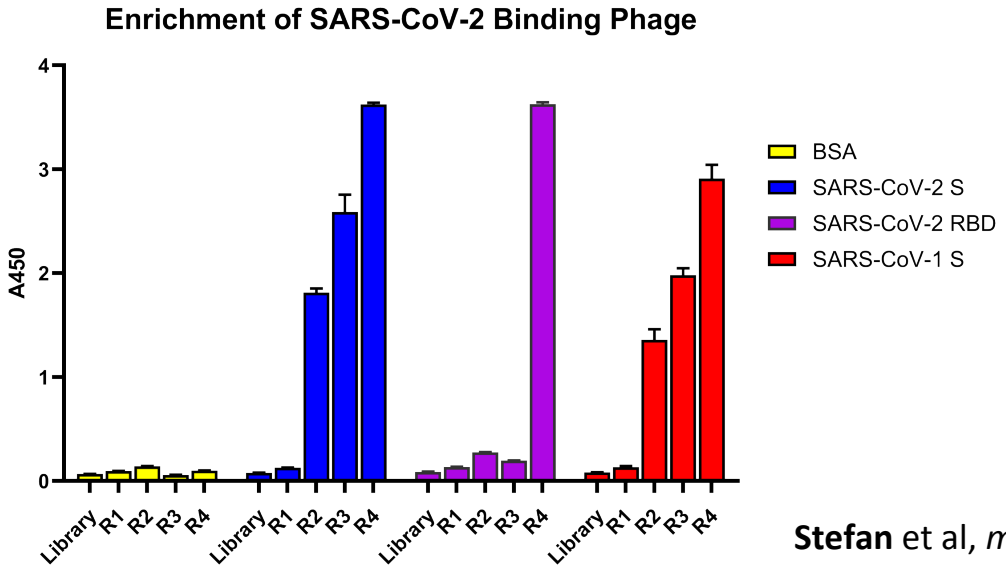


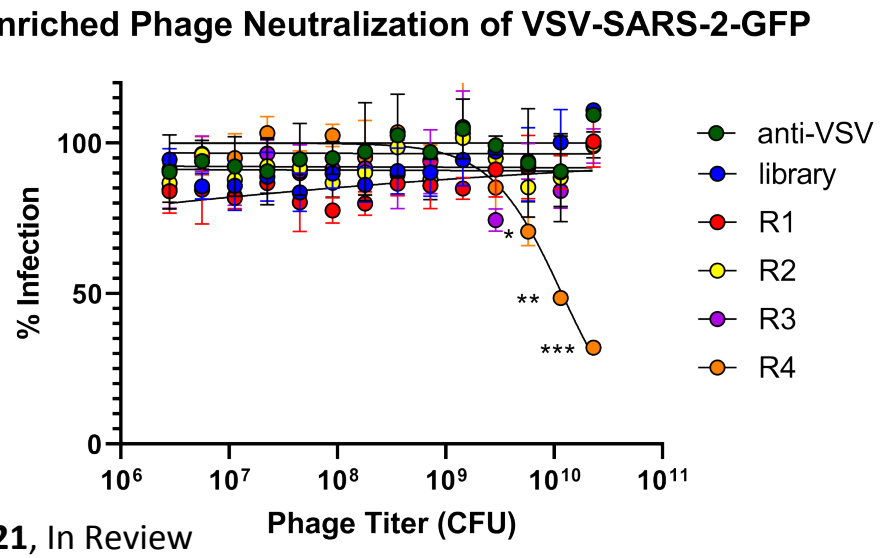
395 / 408 (95.9%)

3.2 x 10^{10}

Biopanning Campaign for Neutralizing mAbs







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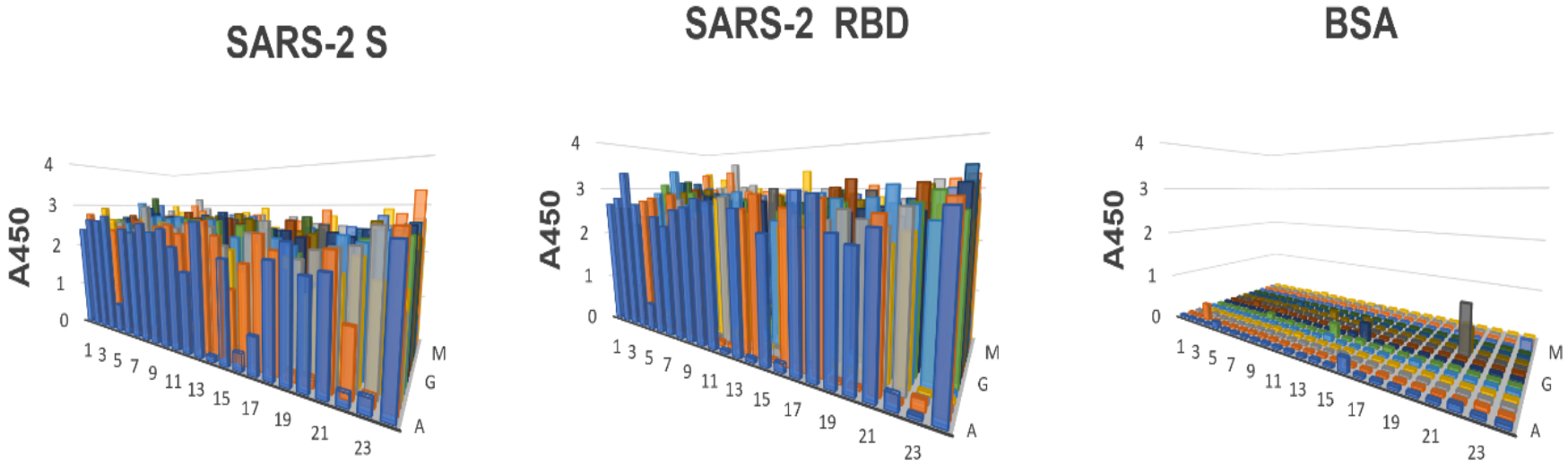
Phage Quantification and Clonal Selection

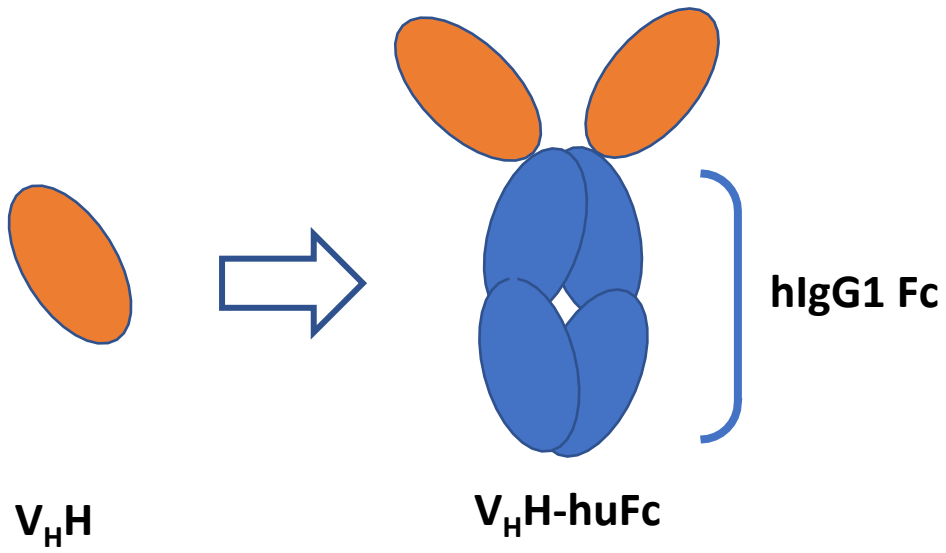
Biopanning Round	Antigen	Input Phage (CFU)	Output Phage (CFU)	Phage Recovered (%)
R1	SARS-2 S	1.6×10^{13}	3.1×10^{8}	0.002
R2	SARS-2 S	1.0×10^{12}	2.6×10^{7}	0.003
R3	SARS-2 S	1.0×10^{12}	3.5×10^{6}	0.004
R4	SARS-2 RBD	1.0×10^{11}	4.6×10^{5}	0.0005

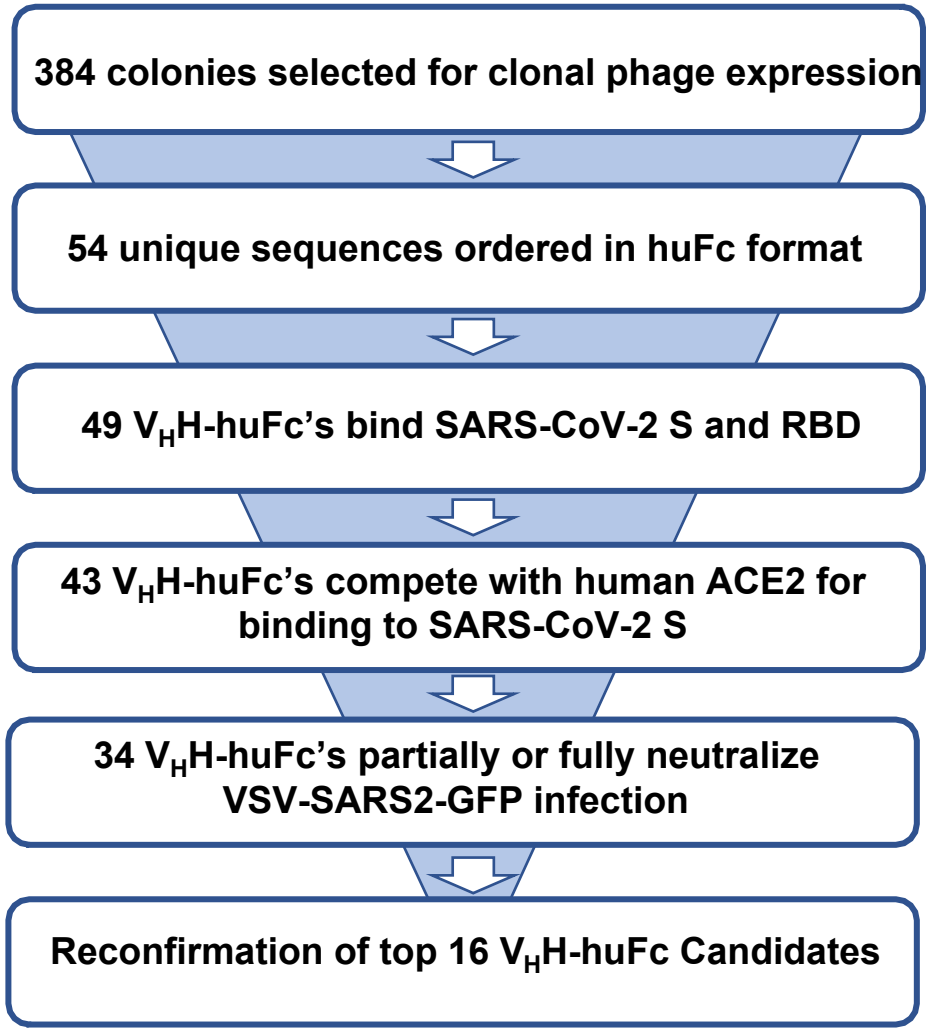


Candidate V_HH Selection

Candidates were produced with huFc

- Improved serum half-live
- Effector function (ADCC, CDC, ADCP)





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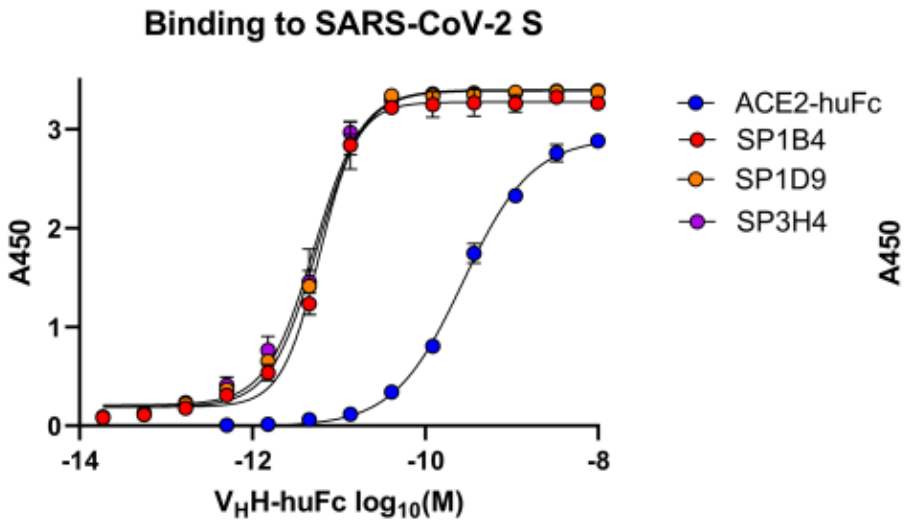
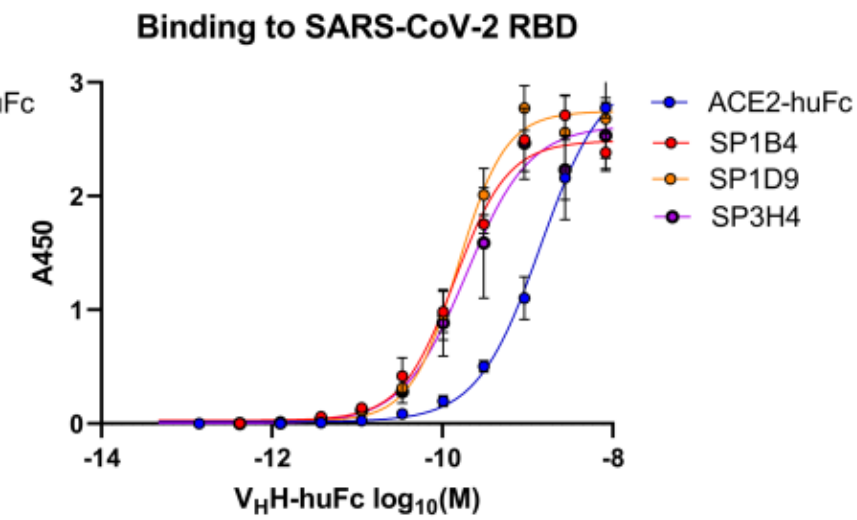
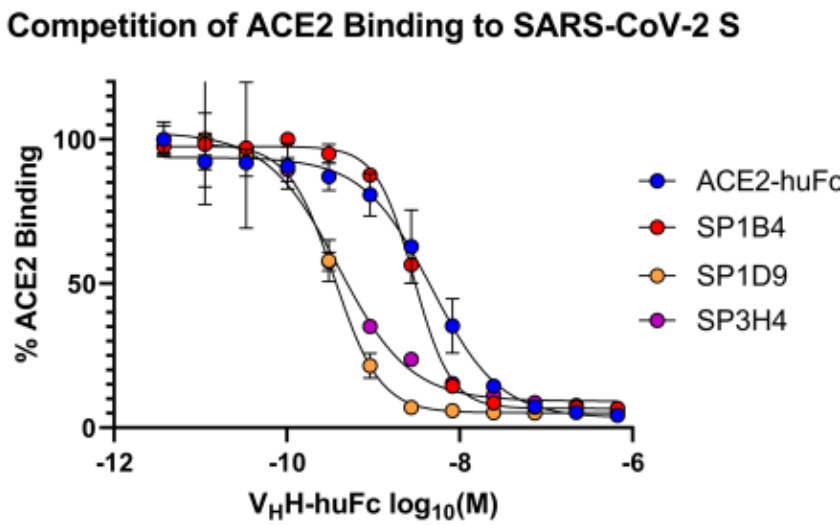
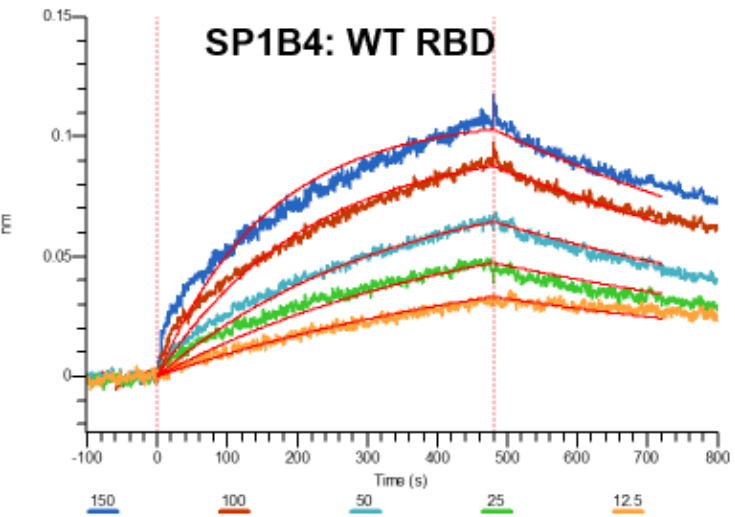
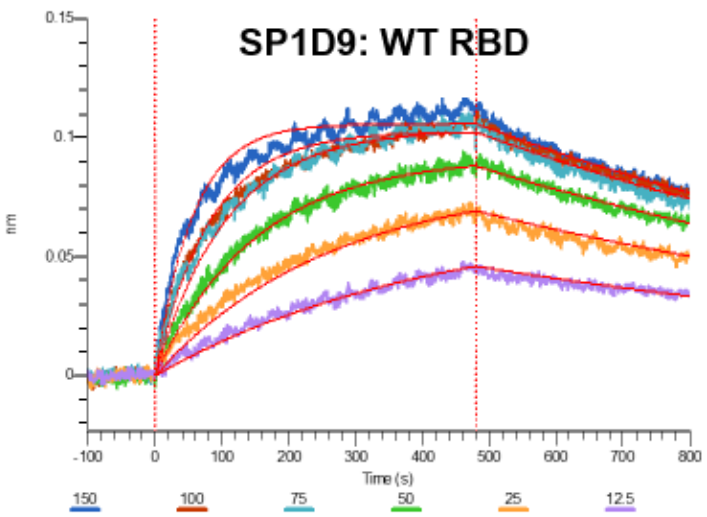
Top Candidates

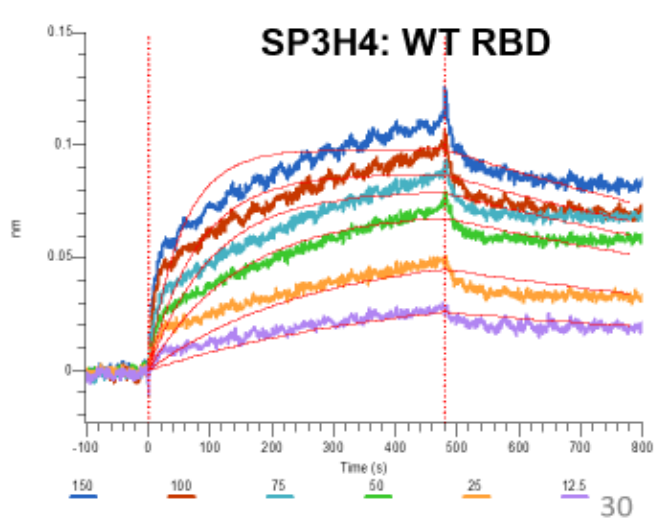












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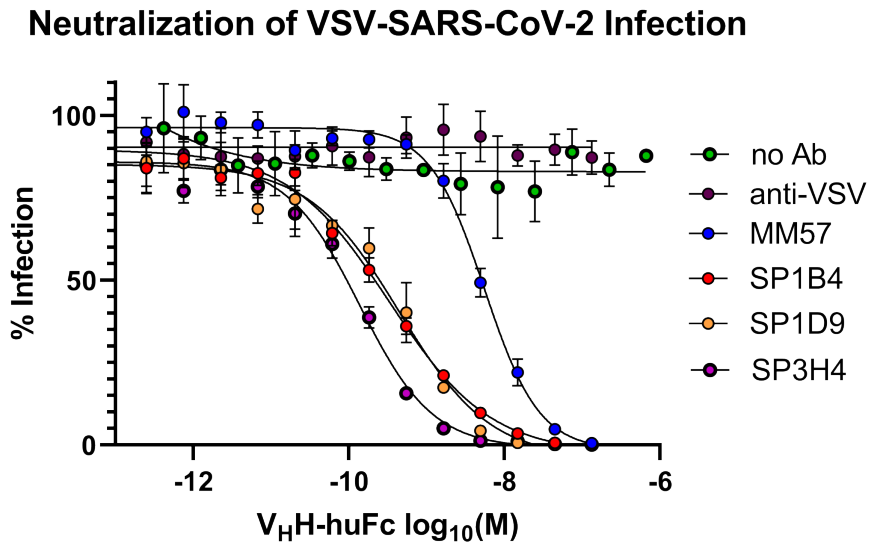
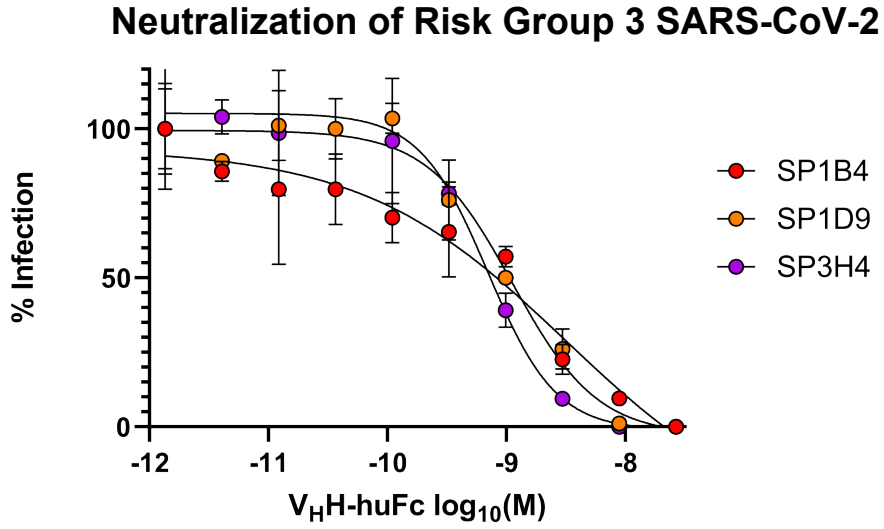
Top V_HH-huFc Candidates





V_HH-huFc	K_D (nM)	K_a ($M^{-1}s^{-1}$)	K_d (s^{-1})	EC_{50} (nM)	$PRNT_{50}$ (nM)	T_m (°C)
SP1B4	39.5	0.34 x 10^5	1.32 x 10^{-3}	0.33	3.14	60.4
SP1D9	8.9	1.12 x 10^5	1 x 10^{-3}	0.45	1.12	64.1
SP3H4	Biphasic	Biphasic	Biphasic	0.14	0.70	61.0
LY-CoV555	3.5Ŧ	-	-	0.080Ŧ	0.133Ŧ	-
REGN10933	3.4‡	30.0 x 10^{5}‡	10.1 x 10^{-3}	0.043‡	0.037‡	-
REGN10987	45.2‡	8.1 x 10^{5}‡	36.5 x 10^{-3}‡	0.041‡	0.042‡	-

Ŧ = DOI: 10.1126/scitranslmed.abf1906
‡ = DOI:10.1126/science.abd0827

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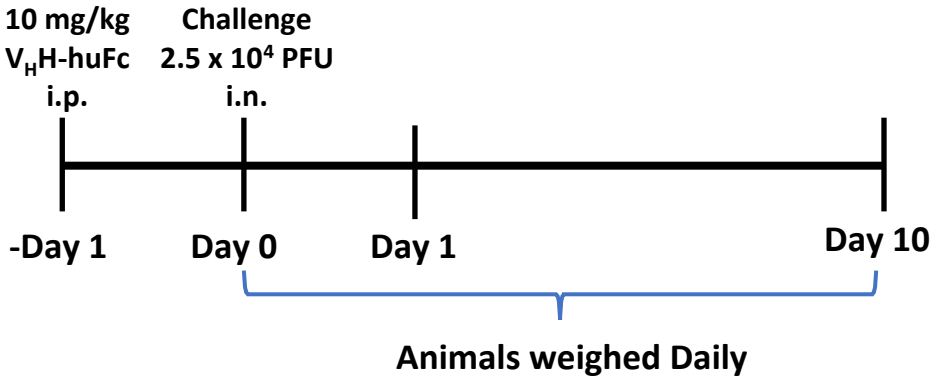
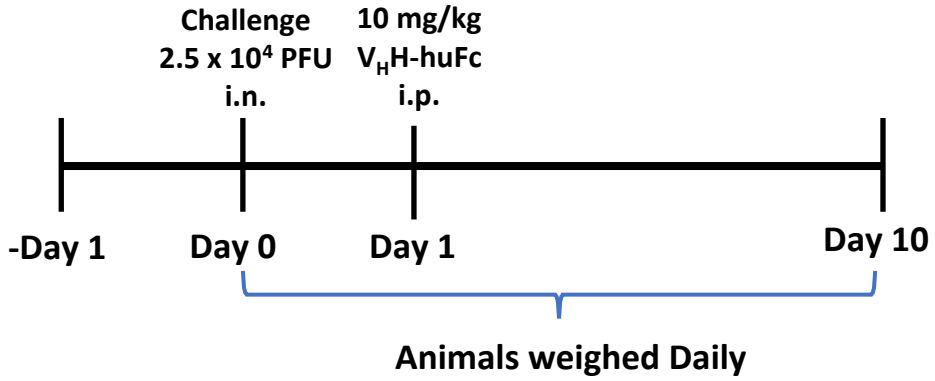
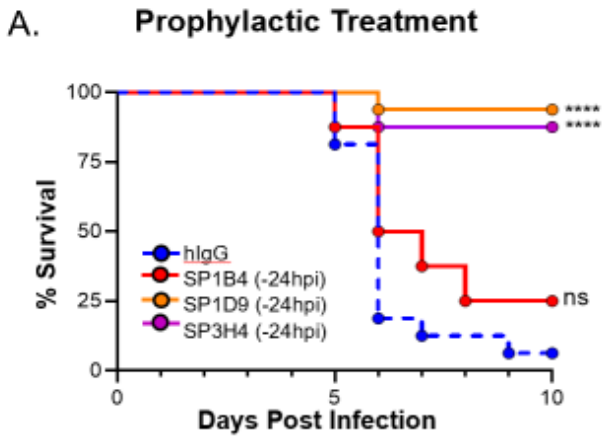
Preclinical Evaluation

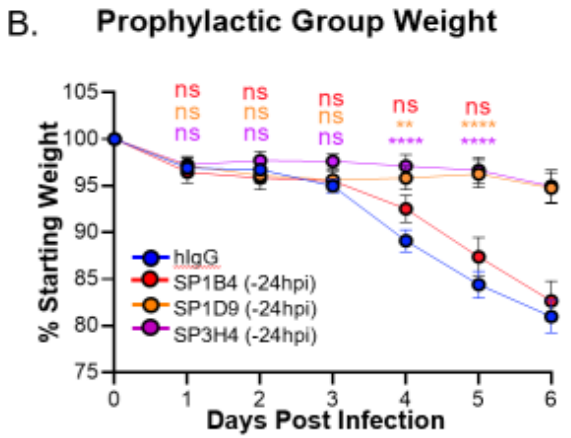
Prophylactic Treatment



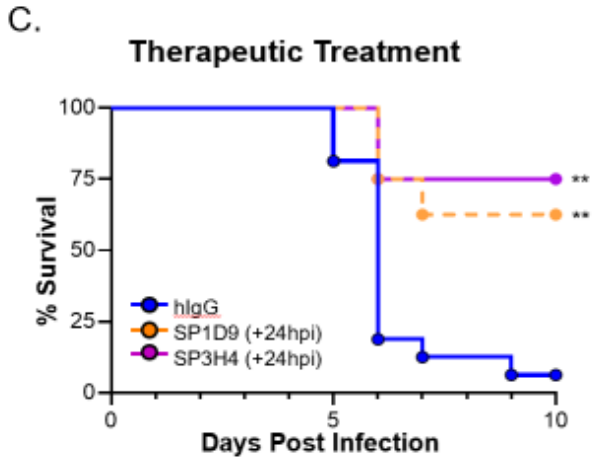
Therapeutic Treatment

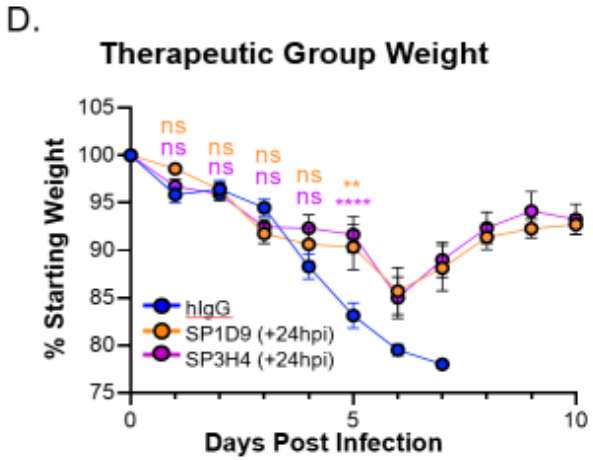






Isotype control n=16
SP1B4 (-24 hpi) n=8
SP1D9 (-24 hpi) n=16
SP3H4 (-24 hpi) n=16





Isotype control n=8
SP1D9 (-24 hpi) n=8
SP3H4 (-24 hpi) n=8

K18-hACE2 mice

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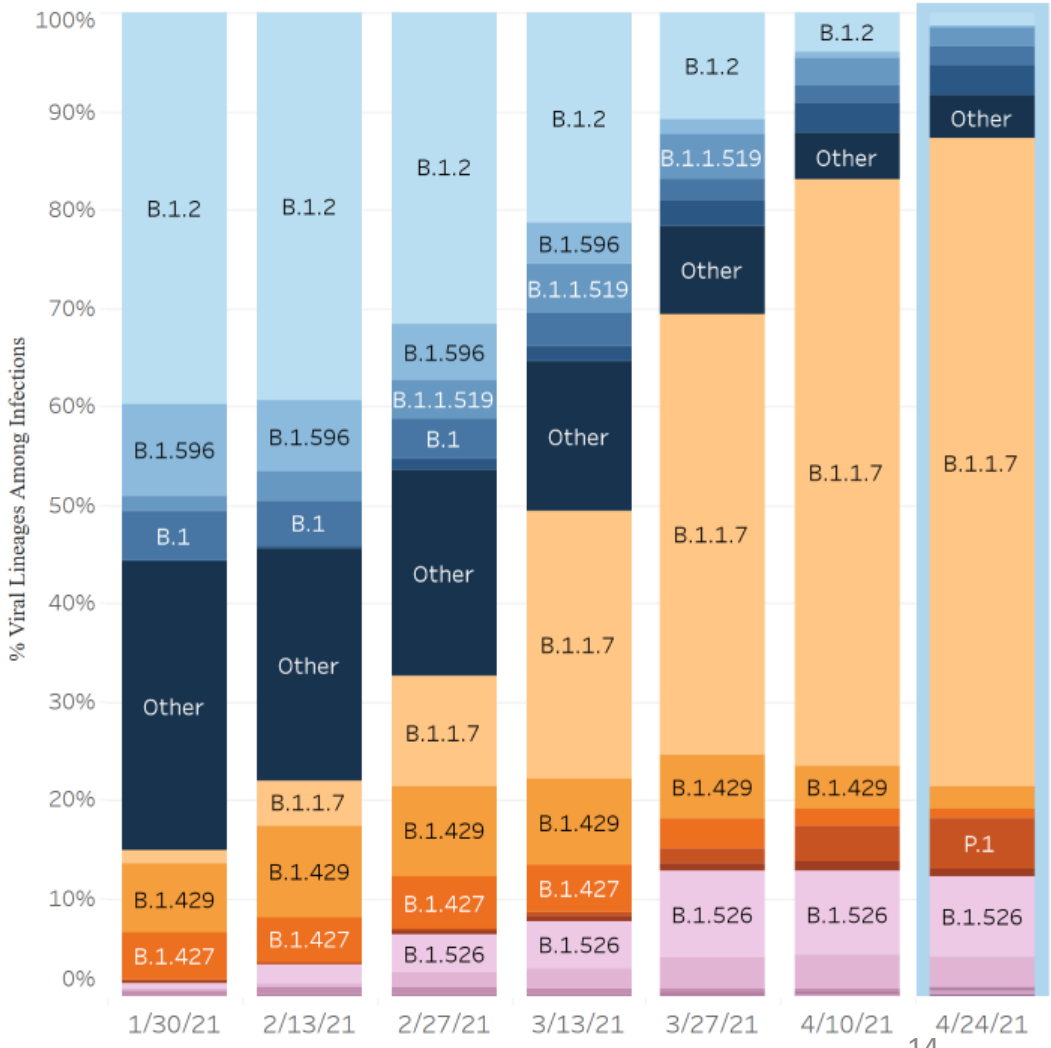
(**** $p < 0.0001$; ** $p < 0.01$; ns=not significant)

SARS-CoV-2 Variants Represent Future Threat

- SARS-CoV-2 RNA virus
  - Prone to mutation and antibody escape
  - Several variants circulating cause reduction in efficacy of antibody therapeutics and vaccines
  - Major variants of concern
    - B.1.1.7 (UK)
    - B.1.351 (South Africa)
    - B.1.526 (NY)
    - B.1.429 (CA)
    - P.1 (Brazil)
    - B.1.617.2 (India)



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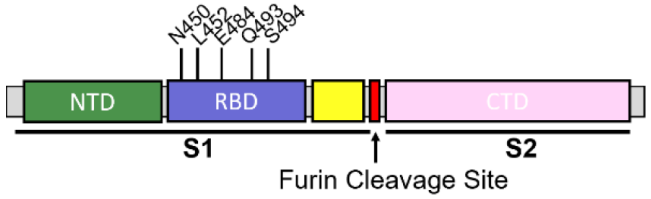
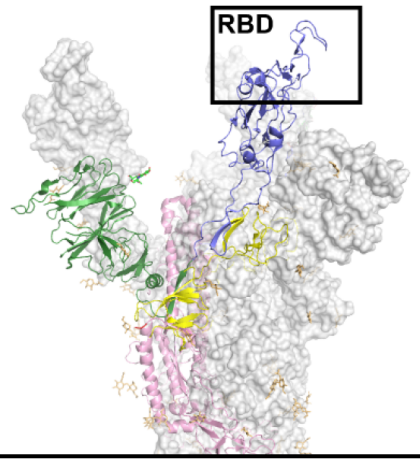
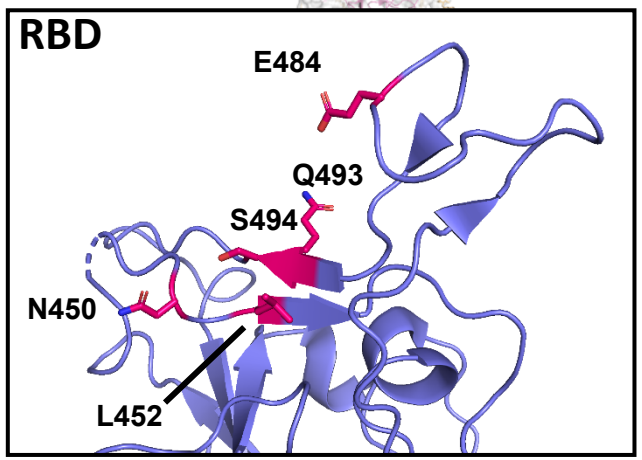
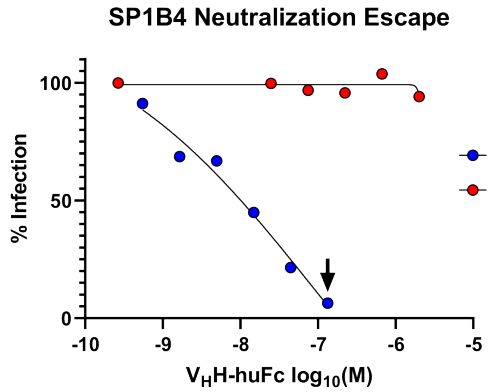
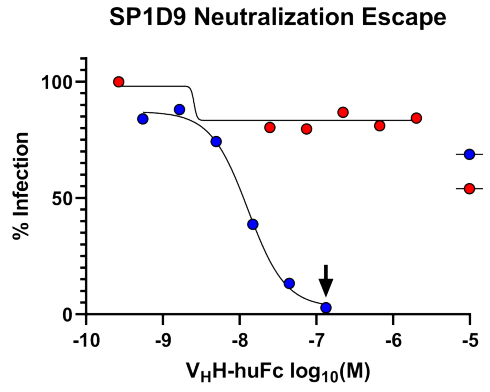
Epitope Identification via Escape Mutation

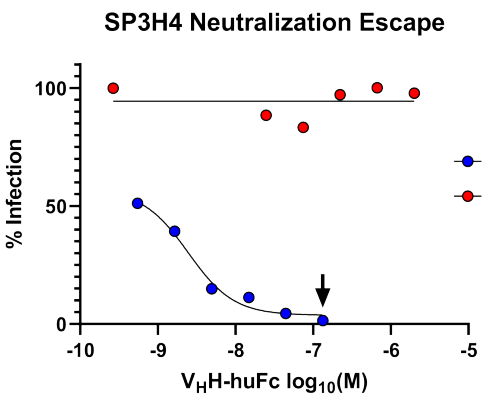








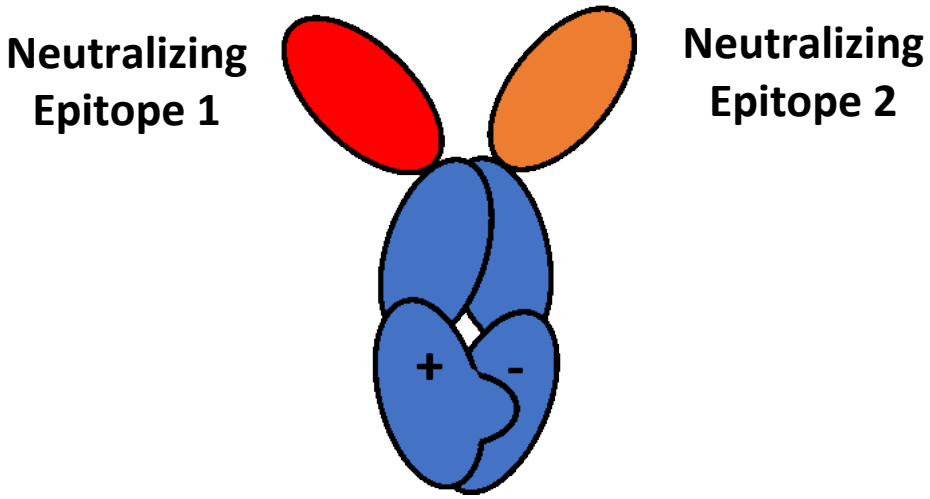


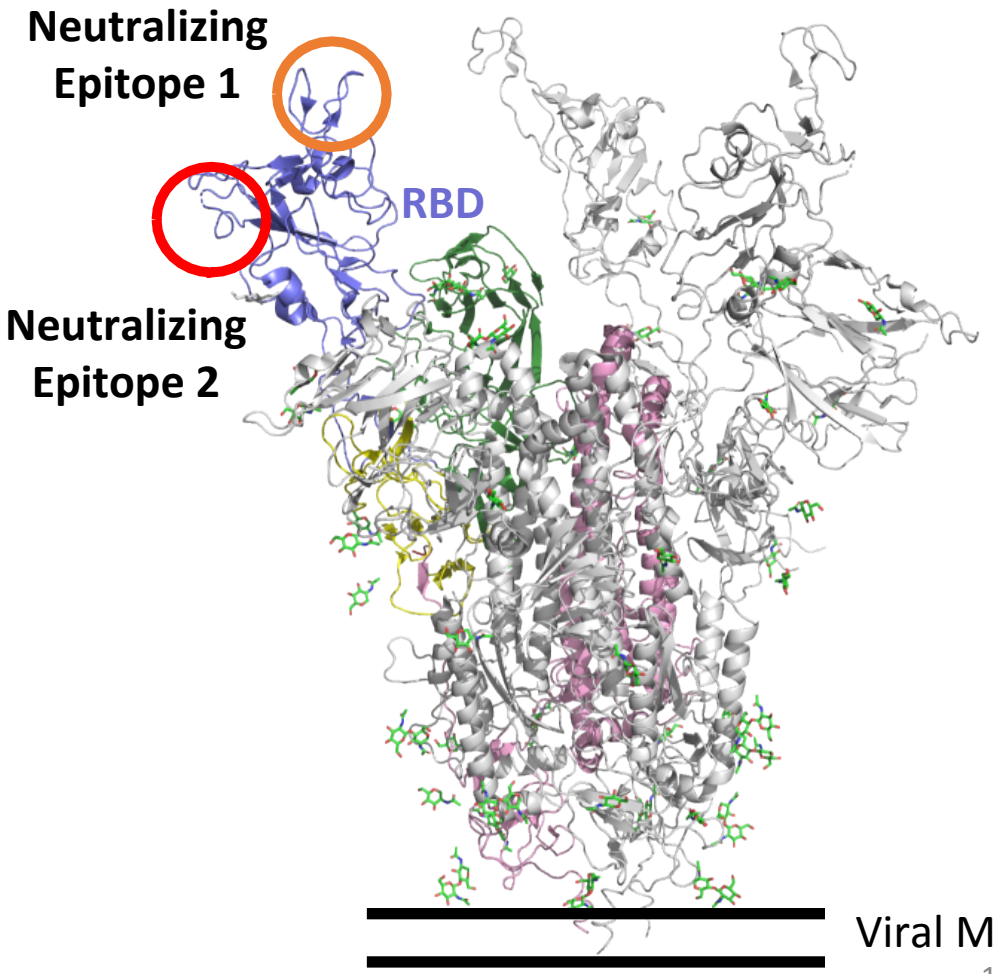


		WT	N450K	L452R	E484K	E484A	Q493R	S494P	B.1.1.7 (UK)	B.1.351 (South Africa)
ACE2	K_D (nM)	13.3	19.8	16.3	25.6	13.5	17.4	16.9	2.5	10.1
SP1B4	Passage 1	-	30%	0%	30%	3%	14%	10%	-	-
	Passage 2	-	10%	0%	17%	19%	46%	43%	-	-
	K_D (nM)	16	N.B	N.B.	N.B.	N.B.	N.B.	N.B.	97.7	N.B.
SP1D9	Passage 1	-	0%	0%	44%	0%	6%	26%	-	-
	Passage 2	-	0%	0%	10%	0%	23%	63%	-	-
	K_D (nM)	8.9	10.7	51.8	N.B.	32.8	N.B.	N.B.	49.7	N.B.
SP3H4	Passage 1	-	24%	23%	12%	21%	0%	0%	-	-
	Passage 2	-	10%	59%	4%	19%	0%	0%		
	K_D (nM)	Biphasic	N.B.	64.7	N.B.	N.B.	N.B.	N.B.	Biphasic	N.B.

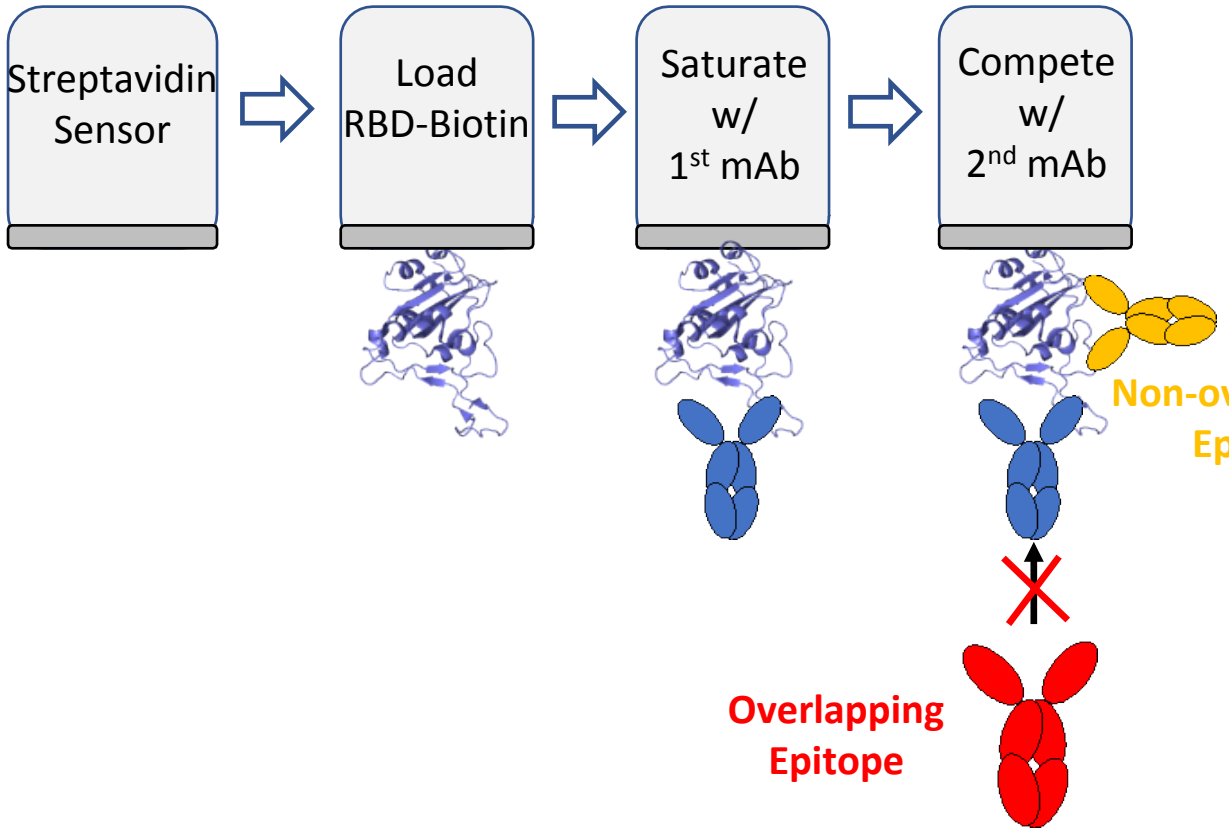
Improving Therapeutic Potential by Targeting Multiple Non-overlapping Epitopes

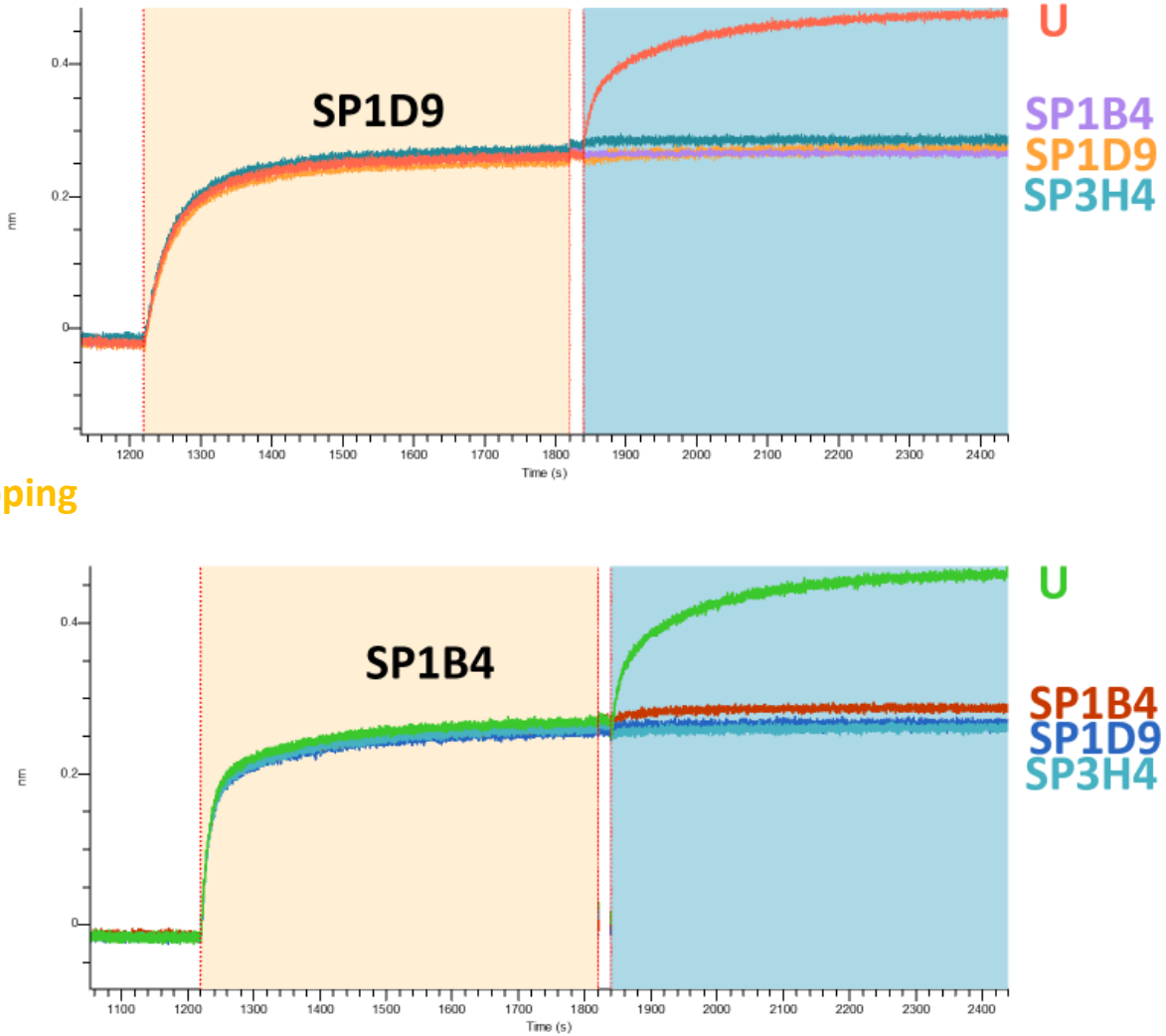
- Vaccines elicit antibody response which target many epitopes
- Combination therapy can reduce formation of escape
- “Biparatopic” antibodies - Bispecific Antibodies which target two independent epitopes on the same antigen





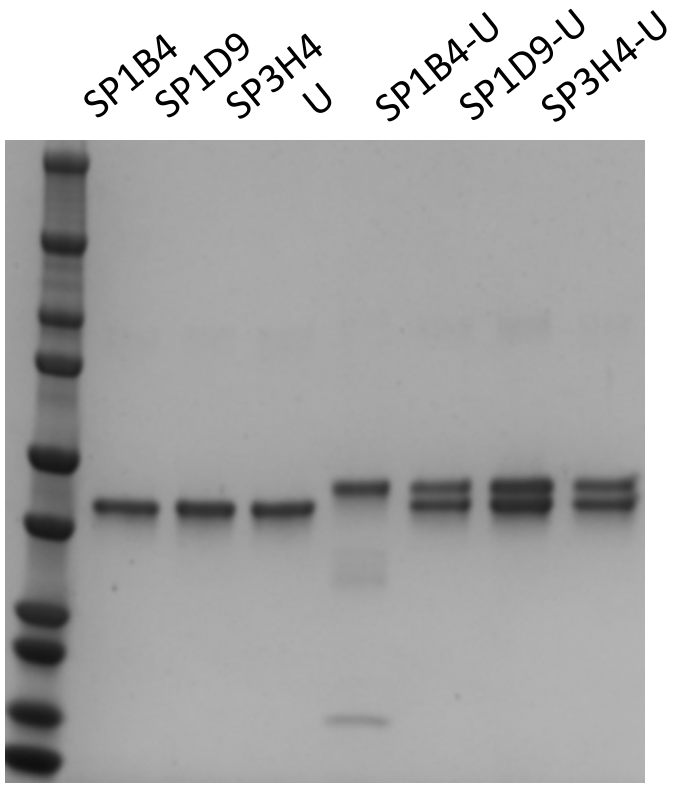
SNL Nanobodies all have Overlapping Epitopes

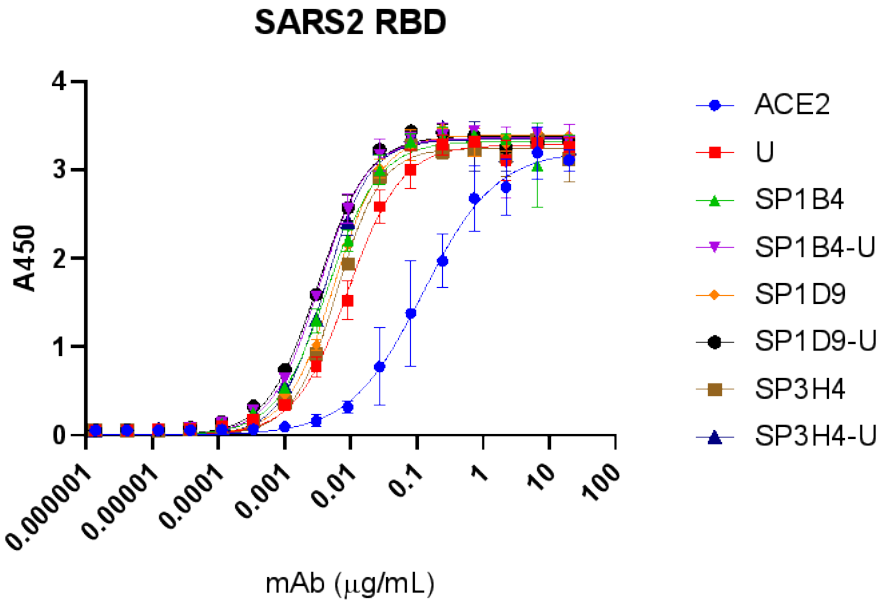


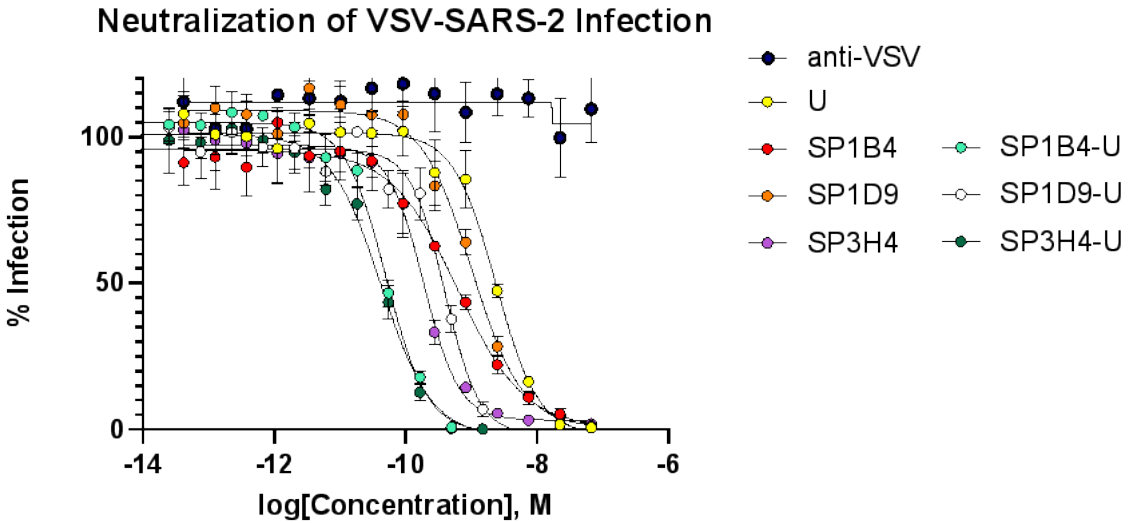


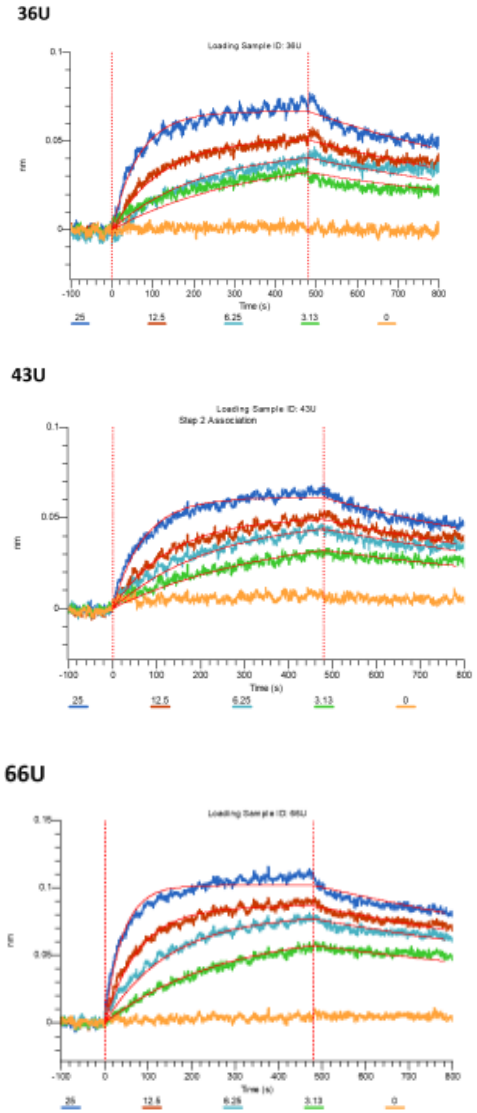
PDB: 6M0J

Biparatopic bsAb Characterization







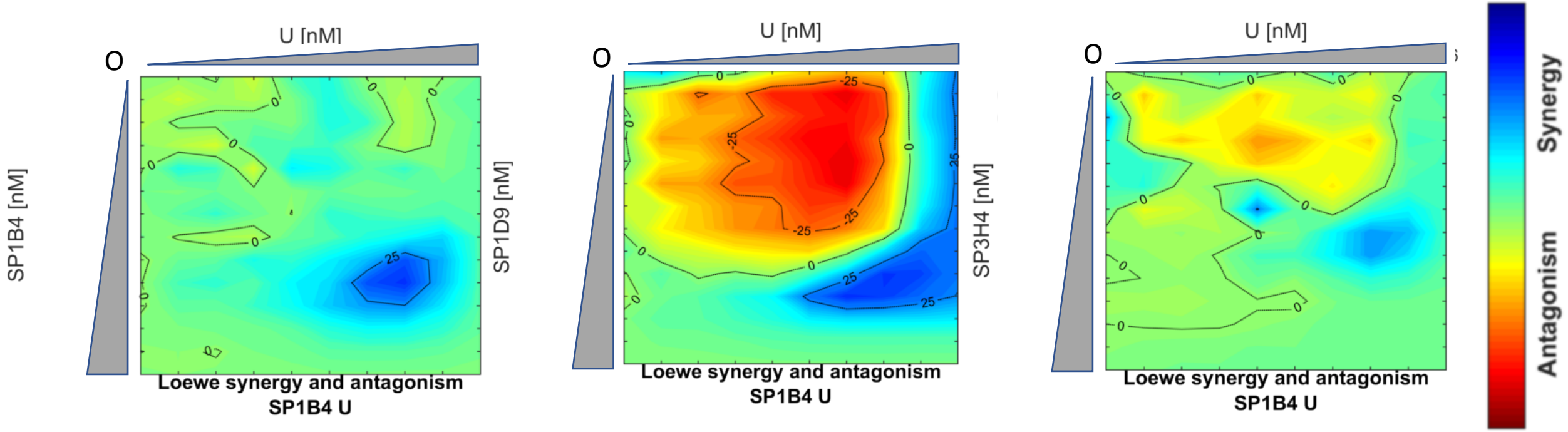


Asymmetric Bispecific Antibodies Comparable to Approved Antibody Therapeutics

V_HH-huFc	K_D (nM)	K_a ($M^{-1}s^{-1}$)	K_d (s^{-1})	EC_{50} (nM)	$PRNT_{50}$ (nM)	T_m (°C)	EC_{50} Fold Change
SP1B4	39.5	0.34×10^5	1.32×10^{-3}	0.33	3.14	60.4	-
SP1D9	8.9	1.12×10^5	1×10^{-3}	0.45	1.12	64.1	-
SP3H4	Biphasic	Biphasic	Biphasic	0.14	0.70	61.0	-
U	14.4	4.4×10^5	6.3×10^{-3}	2.36	N.T	N.T	-
SP1B4-U	2.2	5.5×10^5	1.2×10^{-3}	0.05	N.T.	N.T.	6.6
SP1D9-U	2.0	5.2×10^5	1.1×10^{-3}	0.39	N.T	N.T	1.1
SP3H4-U	0.79	9.4×10^5	0.74×10^{-3}	0.04	N.T.	N.T.	3.5
LY-CoV555	3.5Ŧ	-	-	0.080Ŧ	0.133Ŧ	-	-
REGN10933	3.4‡	30.0×10^5‡	10.1×10^{-3}	0.043‡	0.037‡	-	-
REGN10987	45.2‡	8.1×10^5‡	36.5×10^{-3}‡	0.041‡	0.042‡	-	-

Ŧ = DOI: 10.1126/scitranslmed.abf1906
‡ = DOI:10.1126/science.abd0827

SP1B4 Shows Synergy; SP1D9 Show Antagonism and Synergy

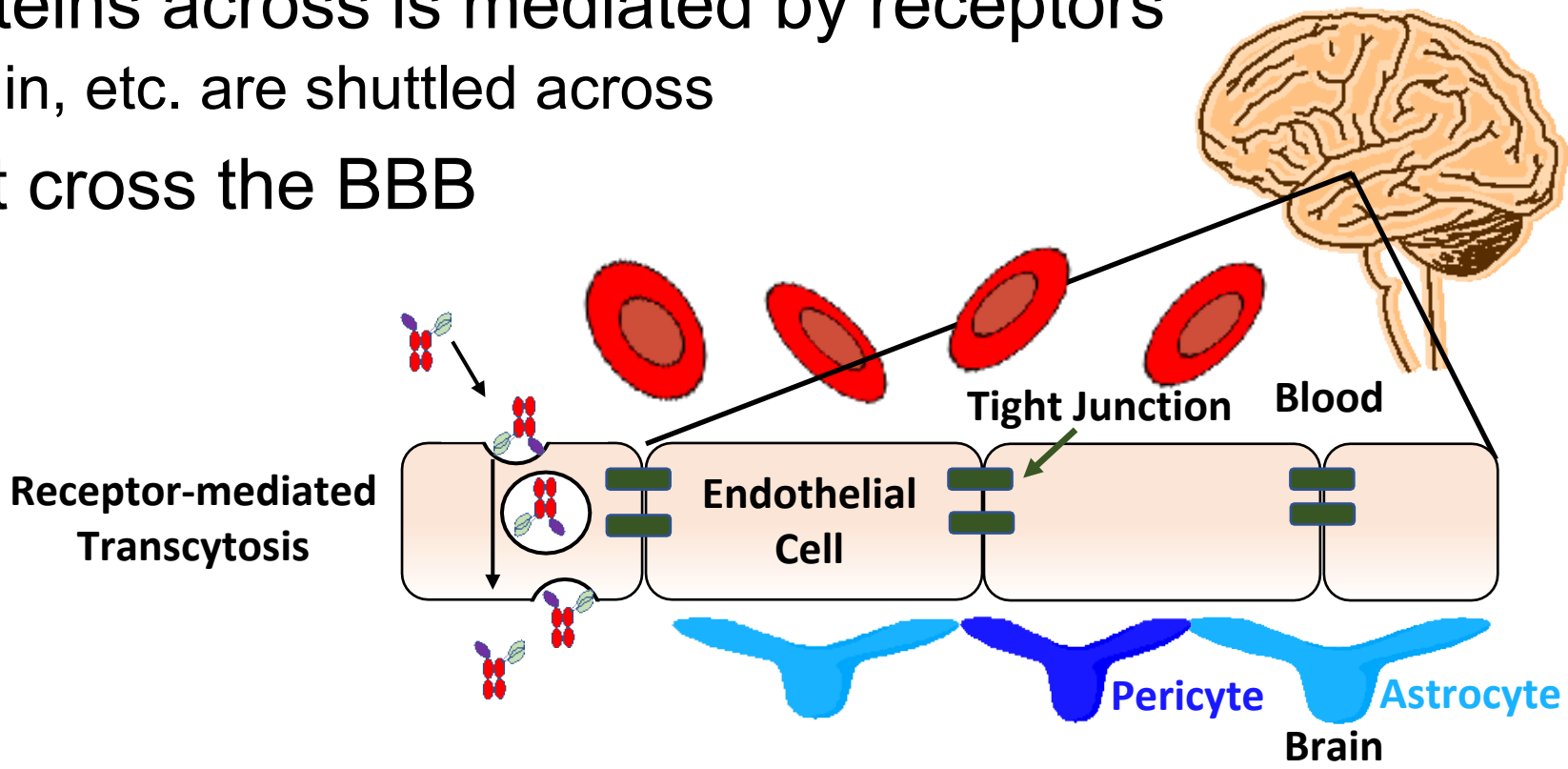


Plots are processed with Combenefit software based of relative infection with VSV-SARS-2 GFP

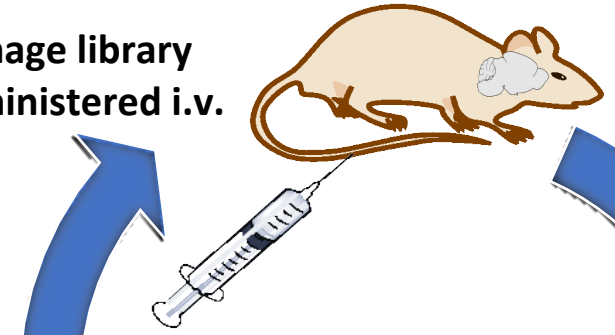
In vivo biopanning to identify novel receptor-mediated transcytosis targets

The Blood-Brain Barrier (BBB)

- BBB is a semipermeable barrier, tight junctions prevent leakage
  - protects the brain from pathogens and other neurotoxins
- Movement of proteins across is mediated by receptors
  - Transferrin, insulin, etc. are shuttled across
- Antibodies do not cross the BBB

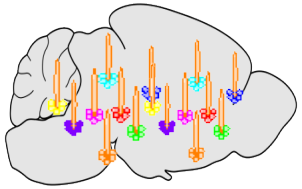


Screening for Functional Tissue Targeting Nanobodies *in vivo*



Phage library administered i.v.

1. **Mice are perfused**
2. **Tissue is harvested**

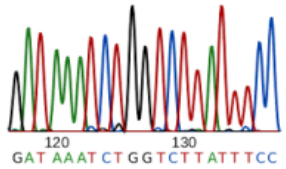


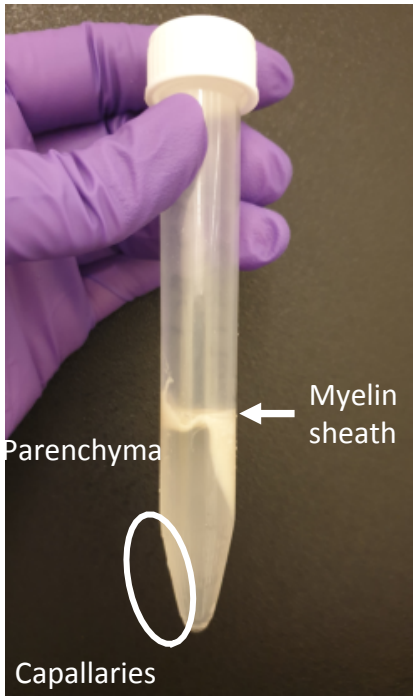
3 rounds in vivo Biopanning

1. **Tissues are fractionated**
2. **Phage extracted**

Phage are cultured for re-administration

IHC-based Detection & Localization of Injectables in Mouse Lung





Round 1
Input Phage: 5.0 x10^{12} CFU
Output Phage: 6.2 x 10^{5} CFU
Output/Input (%): 0.00001%
NGS Read Count: 18,273,372
Cp > 5: 319,200

Round 2
Input Phage: 5.0 x10^{11} CFU
Output Phage: 8.5 x 10^{4} CFU
Output/Input(%): 0.00002%
NGS Read Count: 4,011,902
Cp > 5 and EF > 1: 26,006

Round 3
Input Phage: 5.0 x10^{11} CFU
Output Phage: 6.0 x 10^{4} CFU
Output/Input(%): 0.00001%
NGS Read Count: 6,012,748
Cp > 5 and EF > 1: 3,717

Triage of Phage Sequences

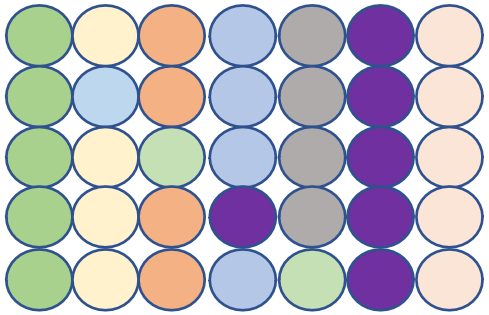
Round 1
Input Phage: 5.0 x10^{12} CFU
Output Phage: 6.2 x 10^{5} CFU
Output/Input (%): 0.00001%
NGS Read Count: 18,273,372
Cp > 5: 319,200

Round 2
Input Phage: 5.0 x10^{11} CFU
Output Phage: 8.5 x 10^{4} CFU
Output/Input(%): 0.00002%
NGS Read Count: 4,011,902
Cp > 5 and EF > 1: 26,006

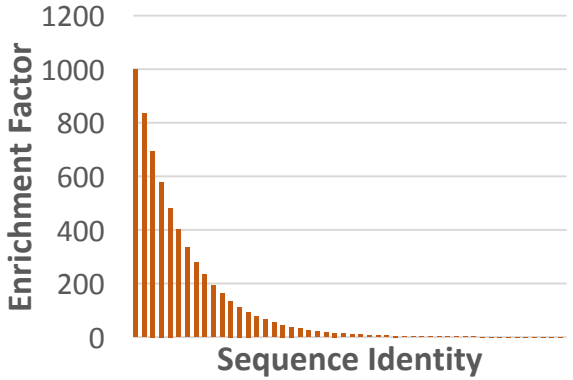
Round 3
Input Phage: 5.0 x10^{11} CFU
Output Phage: 6.0 x 10^{4} CFU
Output/Input(%): 0.00001%
NGS Read Count: 6,012,748
Cp > 5 and EF > 1: 3,717

3,717 Unique Sequences

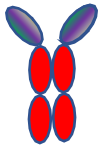
CDR3 Sequence Cluster Analysis



NGS Enrichment Analysis



25 Candidates Selected



Brain Targeting V_HH Facilitates Significant Accumulation in the Brain

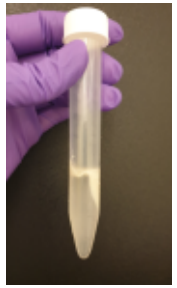
Triage of Candidates

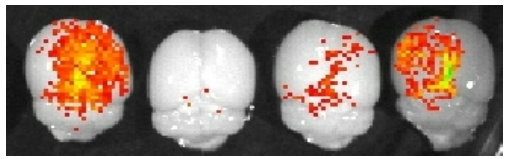
Quantitative ELISA/FACS

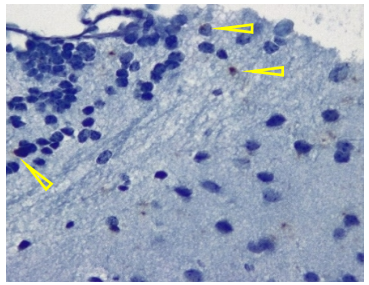
Real Time Monitoring using PerkinElmer *IVIS*

IHC-based Detection

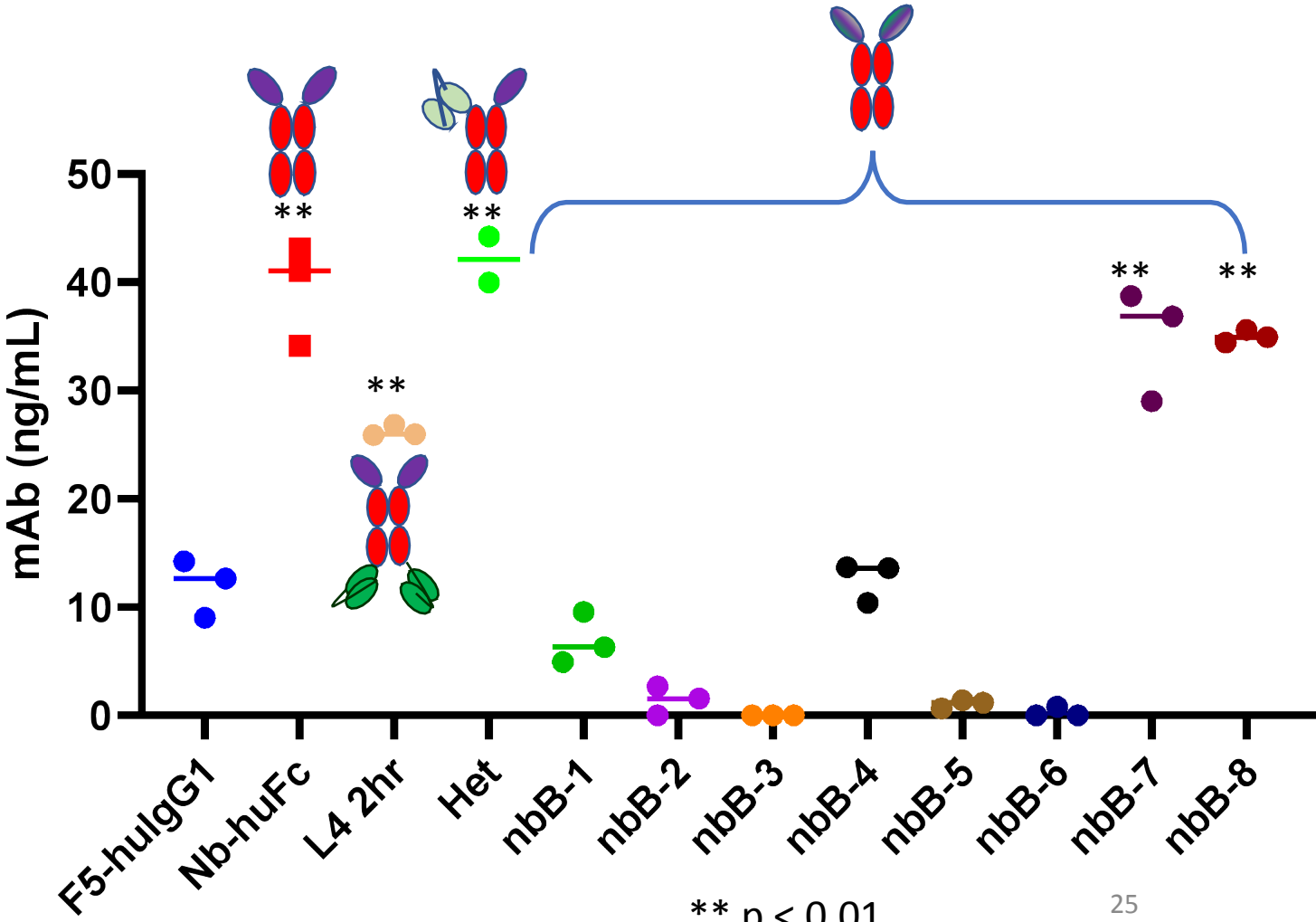
Target Identification
Immunoprecipitation
Proteomics







Tissue ELISA To Quantitate Antibody in the Brain



** $p < 0.01$

Other Organs were Collected for Future Studies

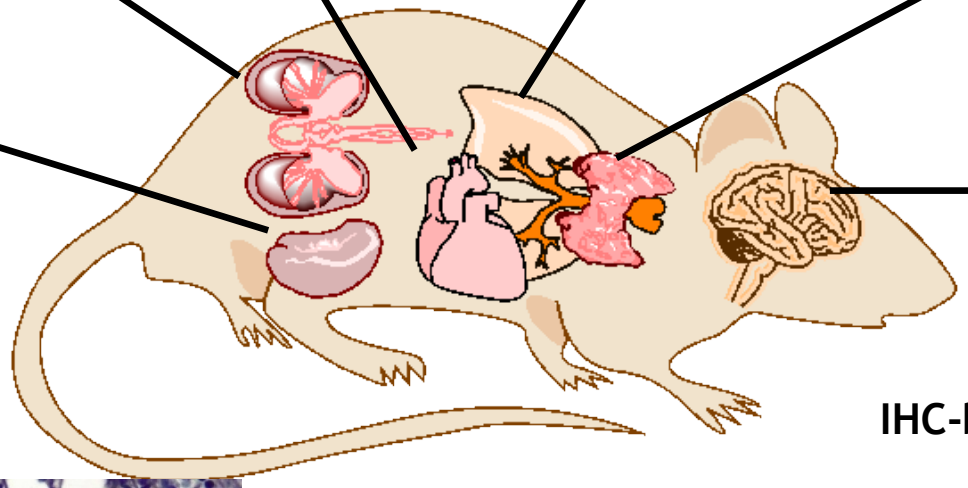
Kidney
Total CFU: 1.4×10^{10}
CFU/mg: 7.3×10^{6}
% Library Admin: 0.3%

Heart
Total CFU: 3.73×10^{9}
CFU/mg: 5.3×10^{6}
% Library Admin: 0.07%

Lung
Total CFU: 1.13×10^{9}
CFU/mg: 1.1×10^{6}
% Library Admin: 0.02%

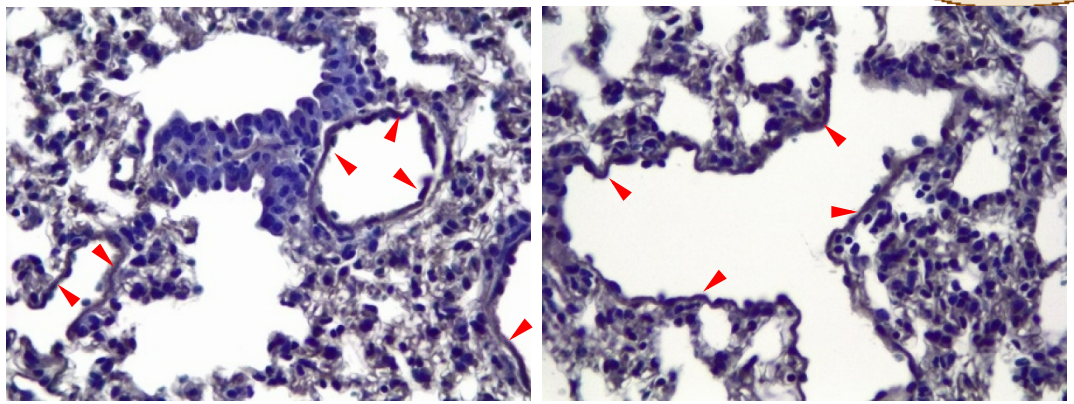
Thymus
Total CFU: 1.42×10^{8}
CFU/mg: 4.48×10^{5}
% Library Admin: 0.003%

Spleen
Total CFU: 9.98×10^{9}
CFU/mg: 1.2×10^{7}
% Library Admin: 0.2%

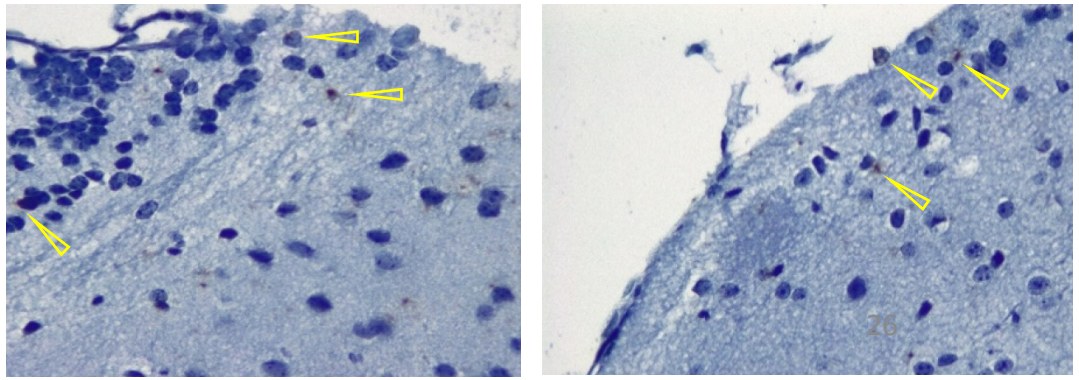


Brain
Total CFU: 6.17×10^{5}
CFU/mg: 274
% Library Admin: 0.00001%

IHC-based Detection & Localization of Injectables in Mouse Lung



IHC-based Detection & Localization of Injectables in Mouse Brain



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- Yooli Light
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- High-throughput Discovery of Tools for Brain Protection LDRD
- Department of Energy (DOE) Office of Science through the National Virtual Biotechnology Laboratory (NVBL), a consortium of DOE national laboratories focused on response to COVID-19, with funding provided by the Coronavirus CARES Act.
- COVID Late Start LDRD
- DTRA (AA number DTRA1002727600)

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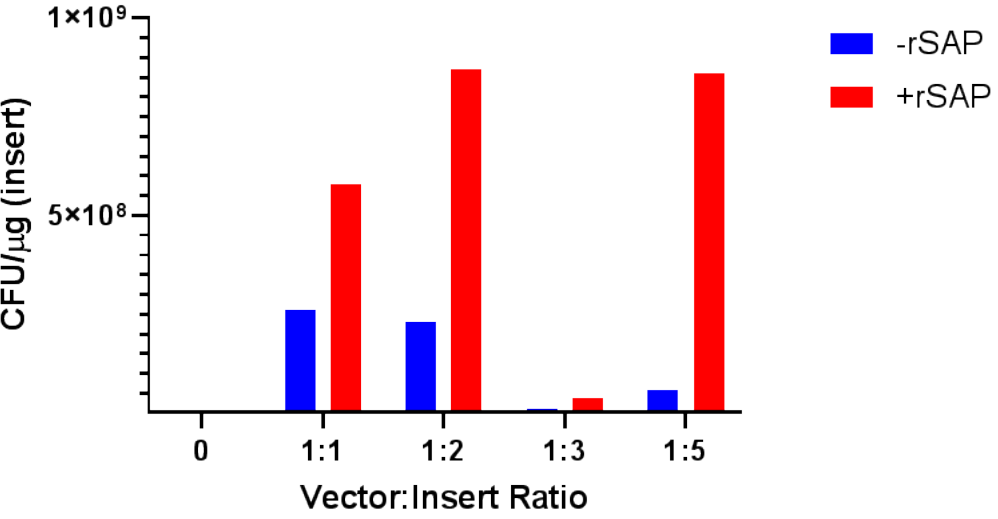
Supplementary Slides

Library Construction Optimization

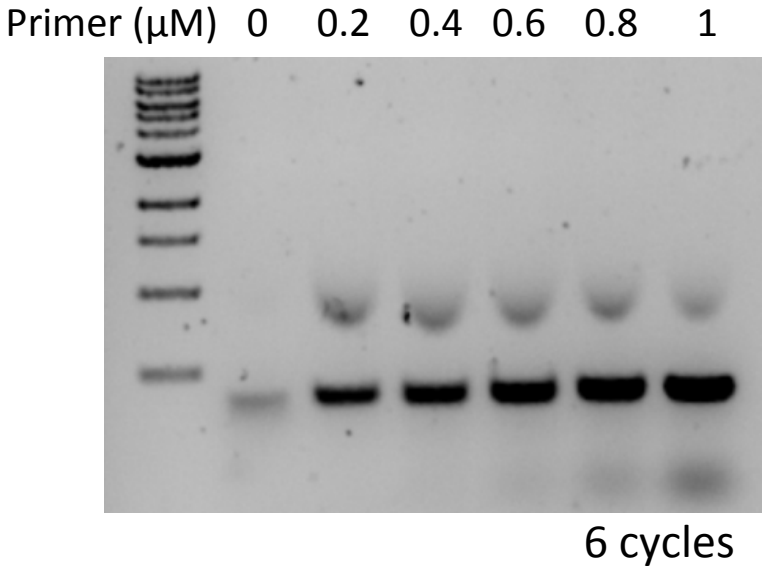
Ligation Optimization for Nanobody Library



For 2.5x10^{10} colonies: V:I = 1:2 = 205 µg: 42 µg
~100 electroporations

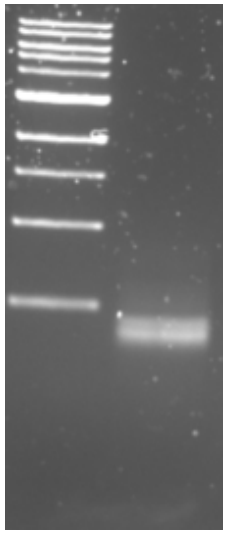
Background: 0.5% for both –rSAP and +rSAP

PCR Amplification of Mock Library Insert



6 cycles

Test Amplification on Twist Library



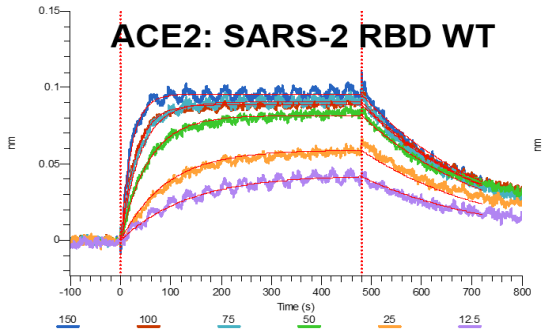
Multiple bands observed which is expected as the library has CDRs with 3 different lengths

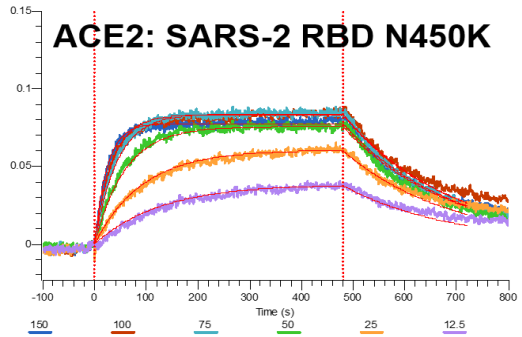
NGS Validation of V_HH Library

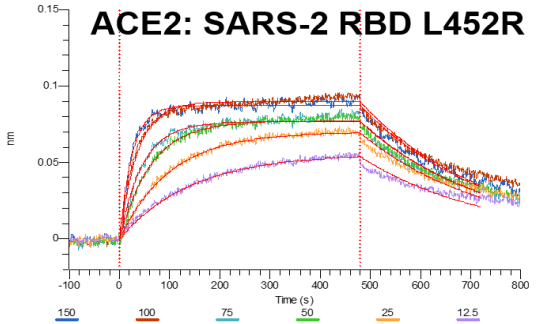
V_HH Library Diversity		
Sequence Replicate	Number of Reads	Percent of Whole
Unique	38,592,027	99%
Duplicate	402,415	1%
Triplicate	5,133	0.01%
Greater than 3	1,095	<0.01%
Total	39,870,360	

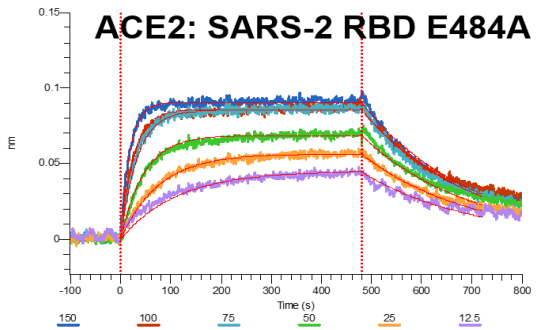
V_HH Library CDR Distribution		
CRD3 Length (AA)	Number of Reads	Percent of Whole
9	15,830,200	40%
12	13,623,399	34%
15	9,932,942	25%
With Stop Codon	450,602	~1%

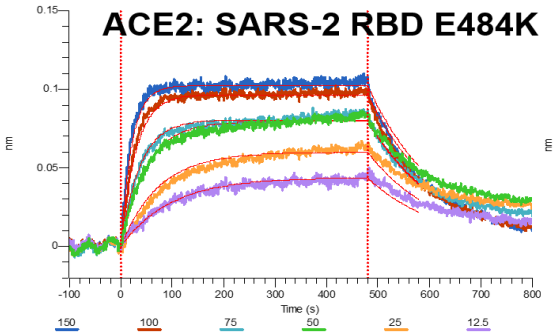
ACE2-huFc Binding to SARS-2 RBD

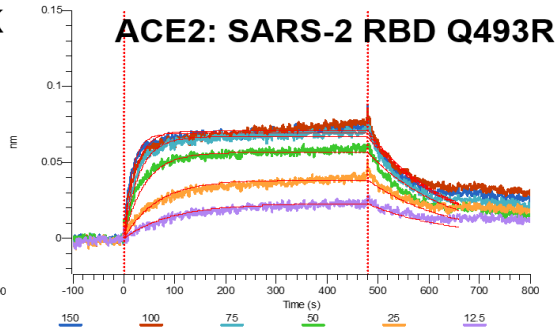


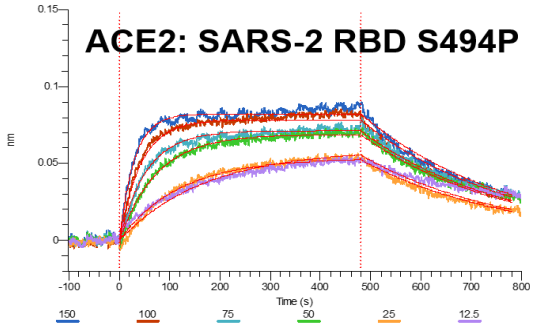


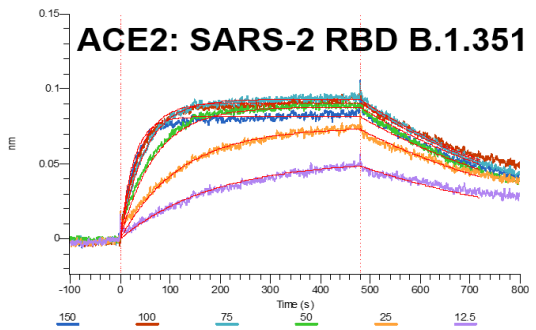


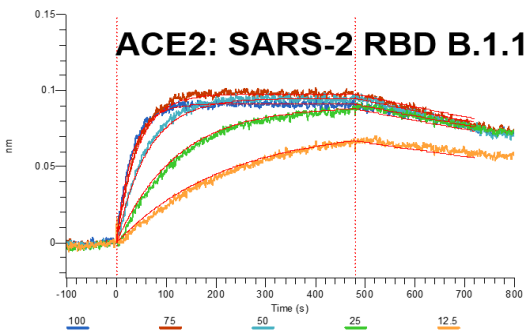






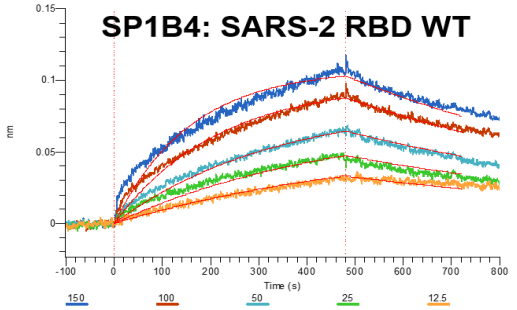


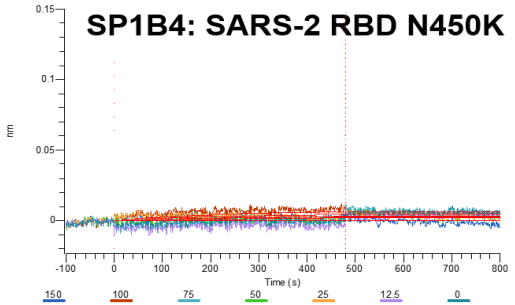


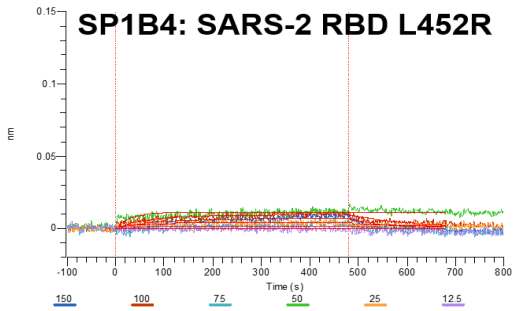


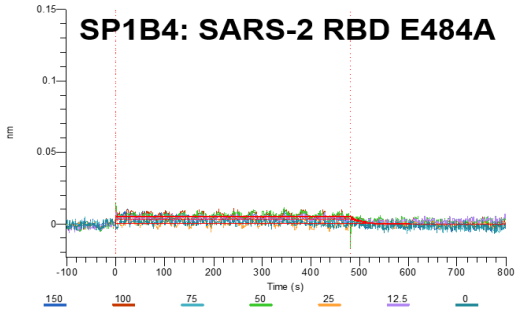
SARS-CoV-2 RBD	K_D (nM)
WT/Wuhan	13.3
N450K	19.8
L452R	16.3
E484A	13.5
E484K	25.6
Q493R	17.4
S494P	16.9
B.1.351/South Africa	10.1
B.1.1.7/UK	2.5

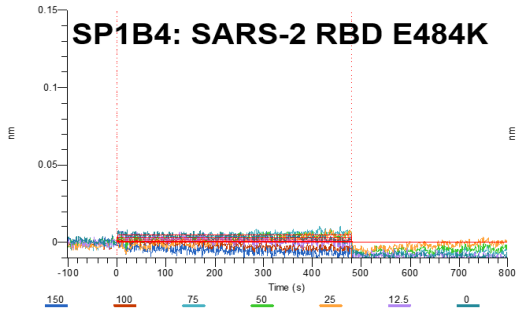
SP1B4 Binding to SARS-2 RBD

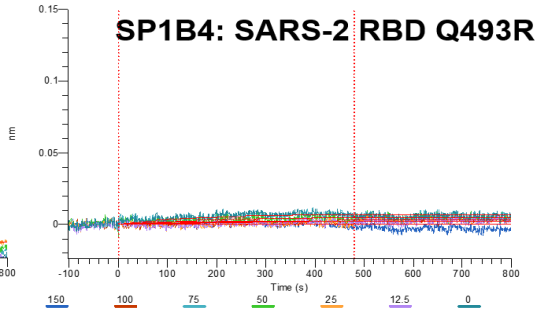


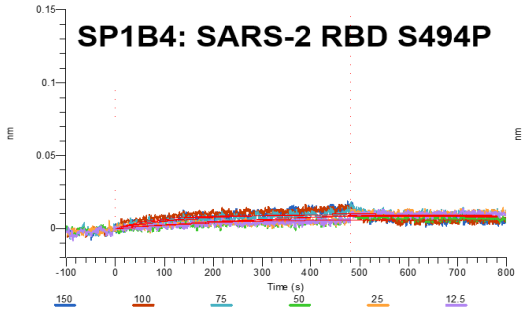


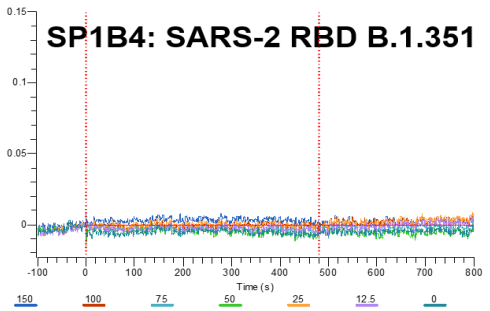


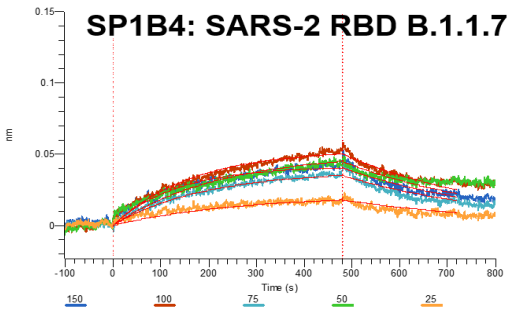


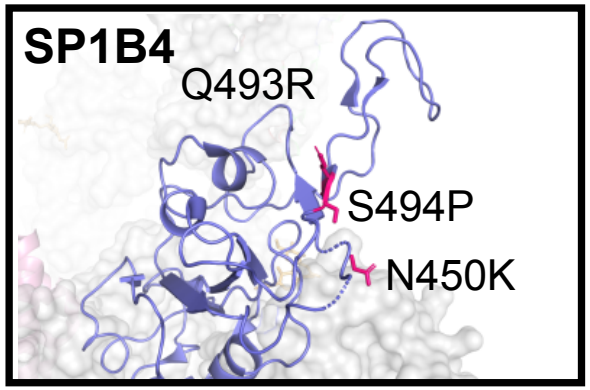






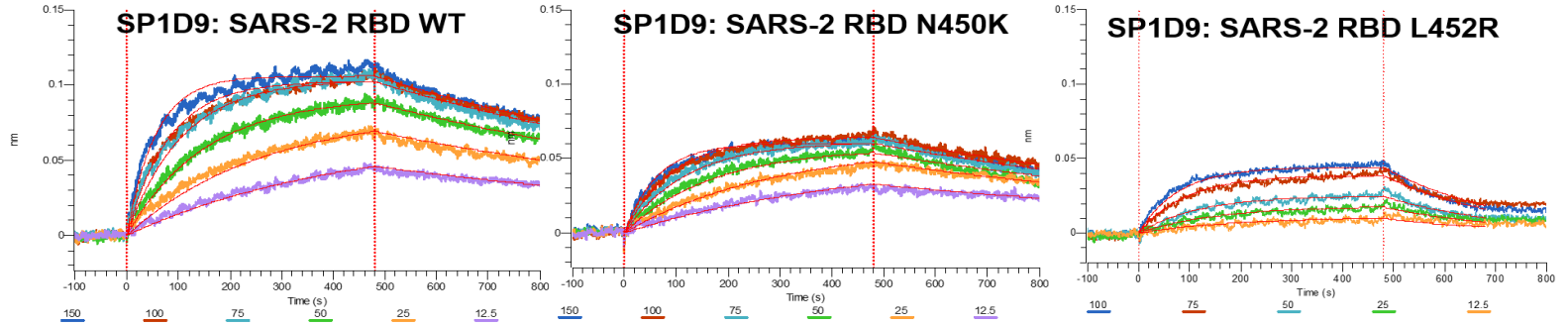


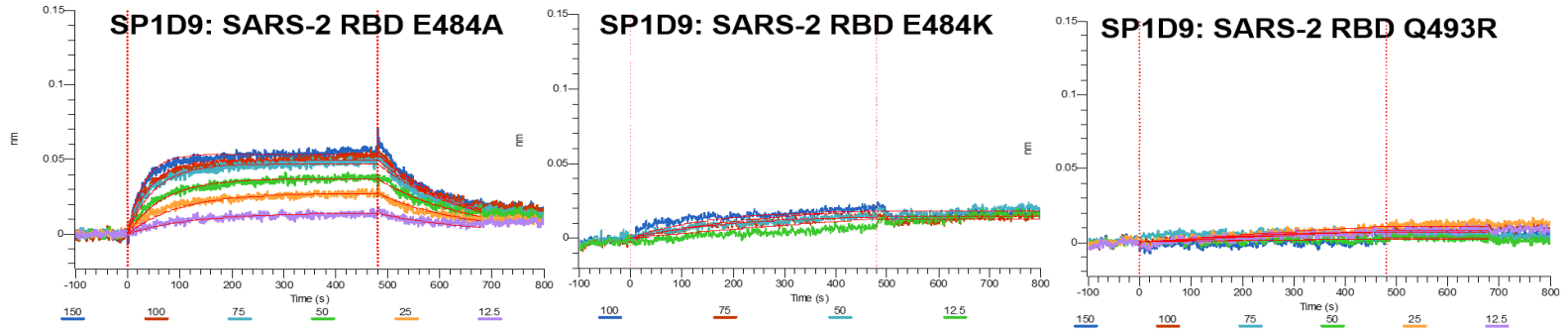


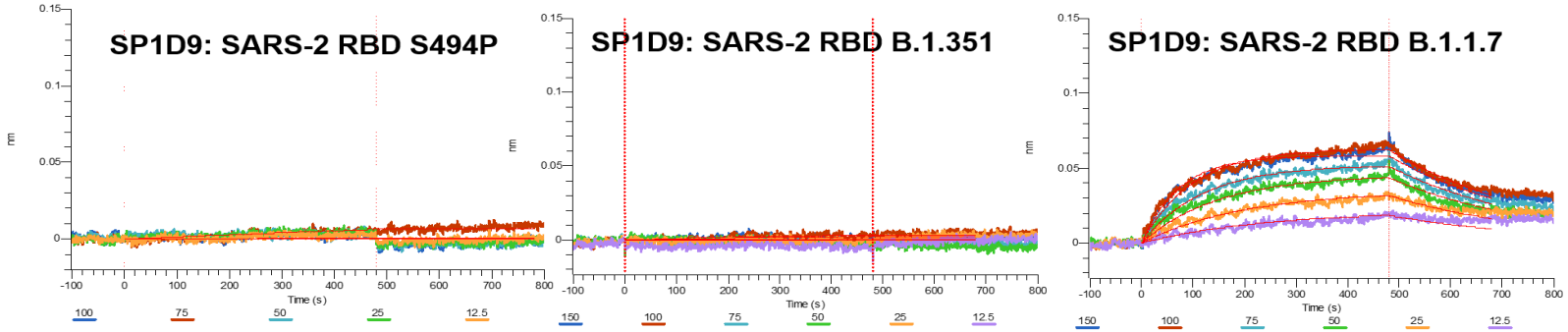


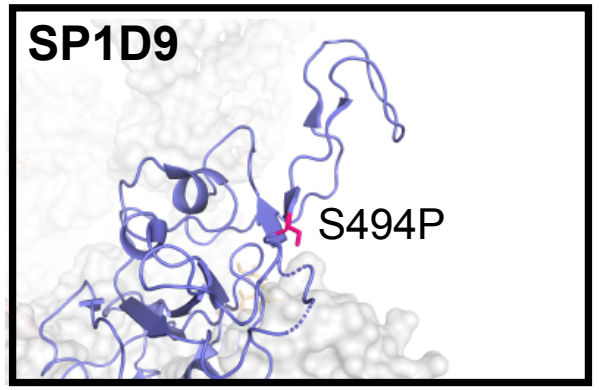
SARS-CoV-2 RBD	K_D (nM)
WT/Wuhan	39.5
N450K	N.B.
L452R	N.B.
E484A	N.B.
E484K	N.B.
Q493R	N.B.
S494P	N.B.
B.1.351/South Africa	N.B.
B.1.1.7/UK	97.7

SP1D9 Binding to SARS-2 RBD



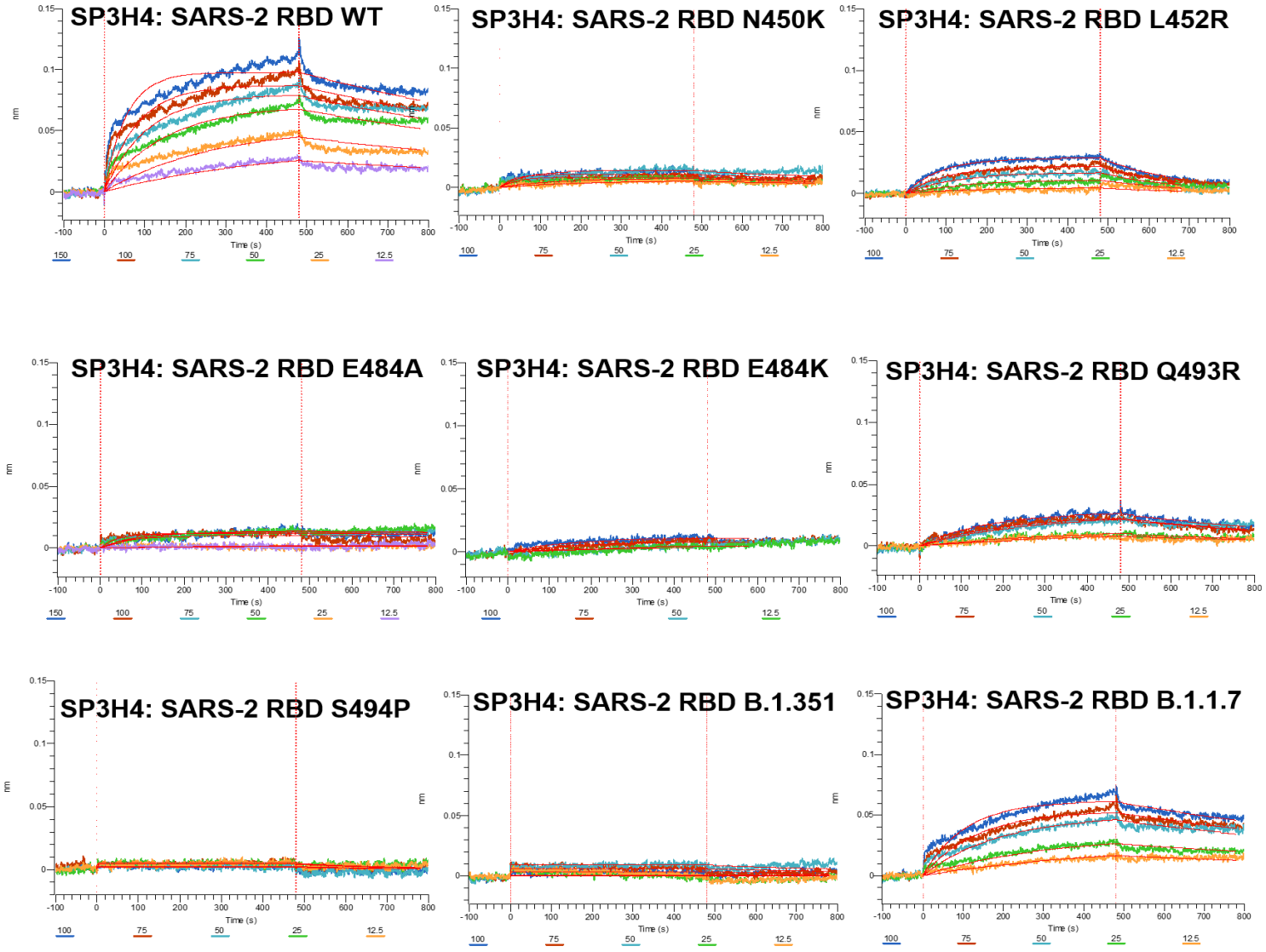


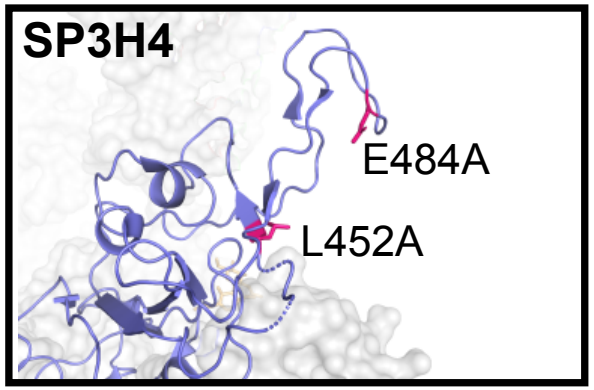




SARS-CoV-2 RBD	K_D (nM)
WT/Wuhan	8.9
N450K	10.7
L452R	51.8
E484A	32.8
E484K	N.B.
Q493R	N.B.
S494P	N.B.
B.1.351/South Africa	N.B.
B.1.1.7/UK	49.7

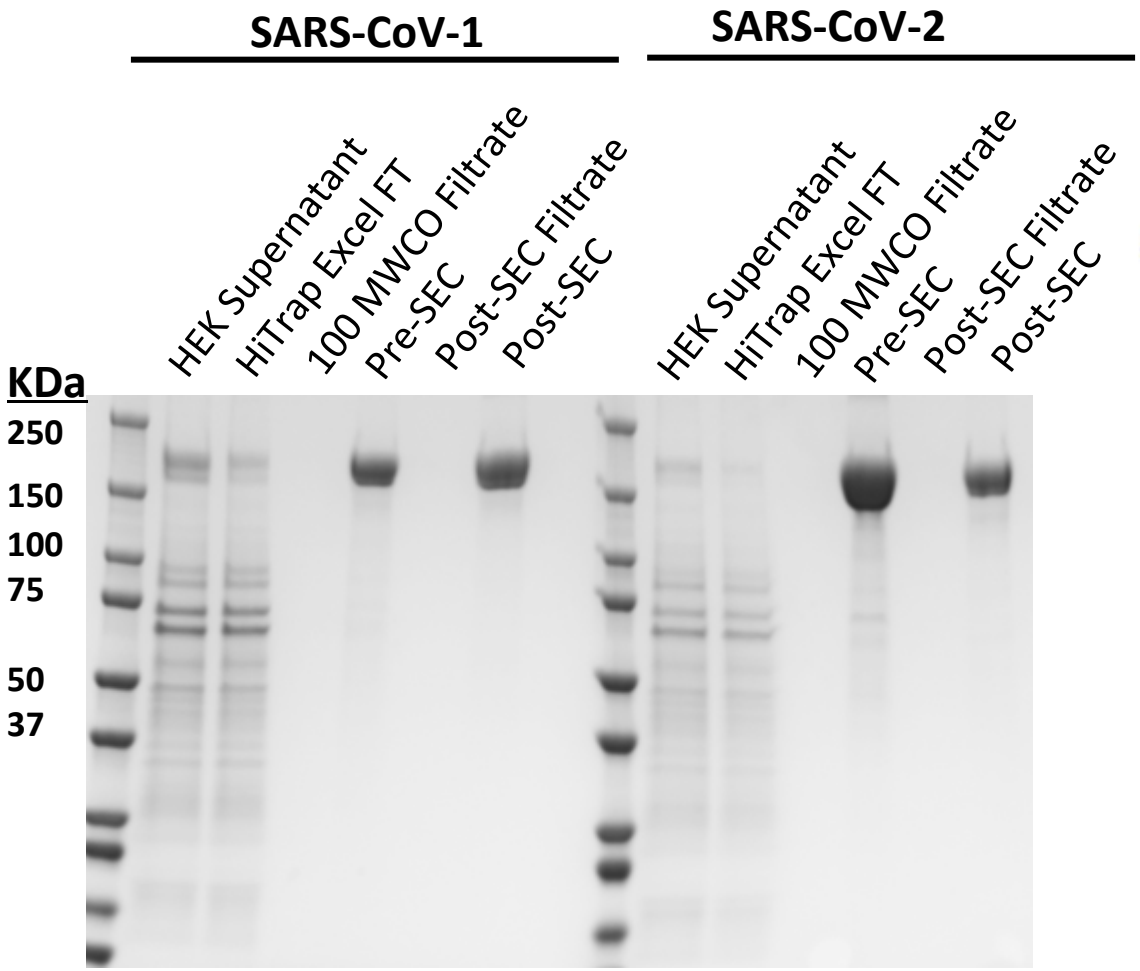
SP3H4 Binding to SARS-2 RBD

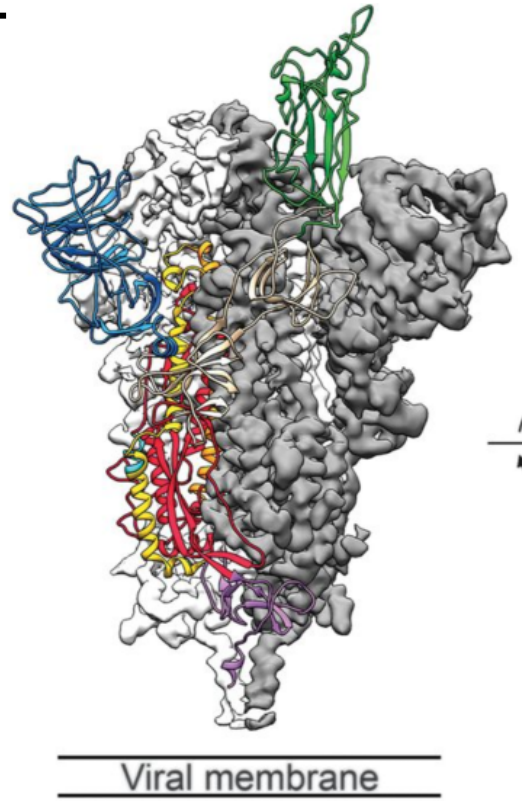


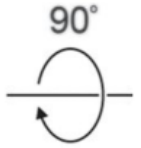


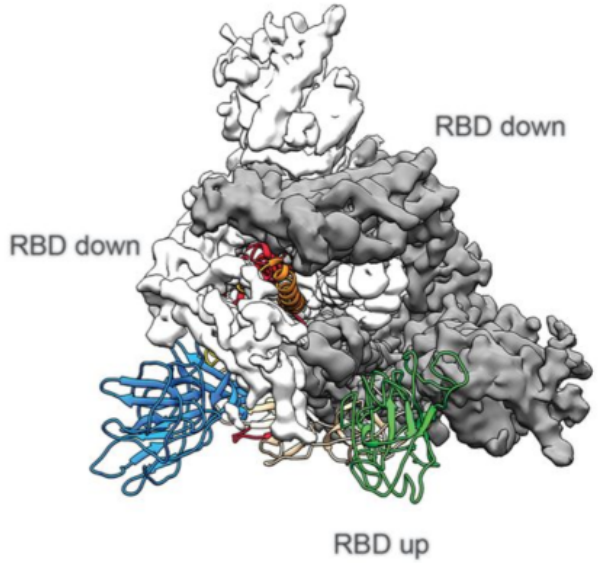
SARS-CoV-2 RBD	K_D (nM)
WT/Wuhan	Biphasic
N450K	N.B.
L452R	64.7
E484A	N.B.
E484K	N.B.
Q493R	Biphasic
S494P	N.B.
B.1.351/South Africa	N.B.
B.1.1.7/UK	Biphasic

SARS-CoV-1 and SARS-CoV-2 Spike Trimer









BBB Penetrant Bispecific Antibodies