AEROSOLIZED NANOBODIES FOR SARS-COV-2 PASSIVE IMMUNIZATION

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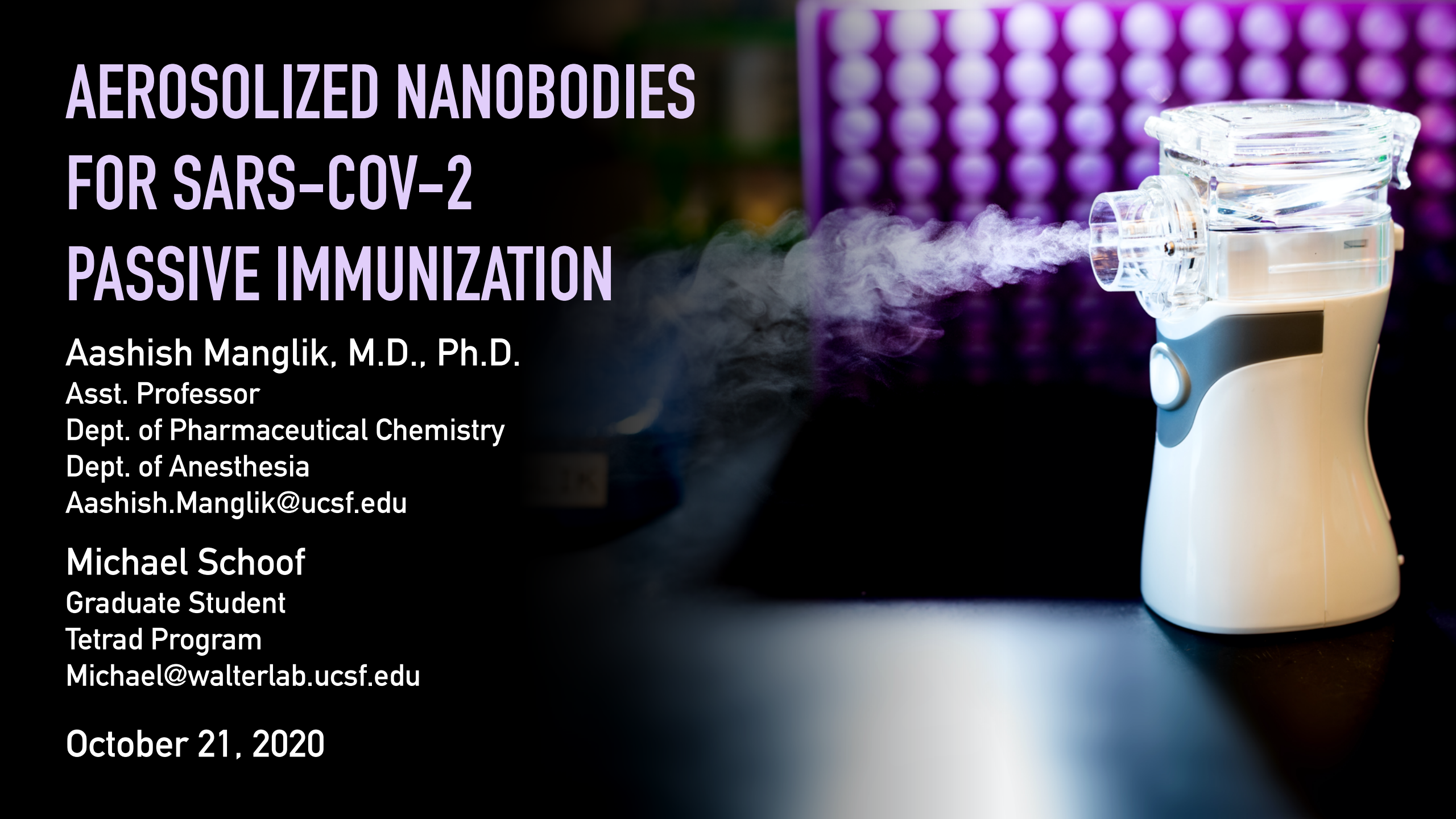
October 21, 2020

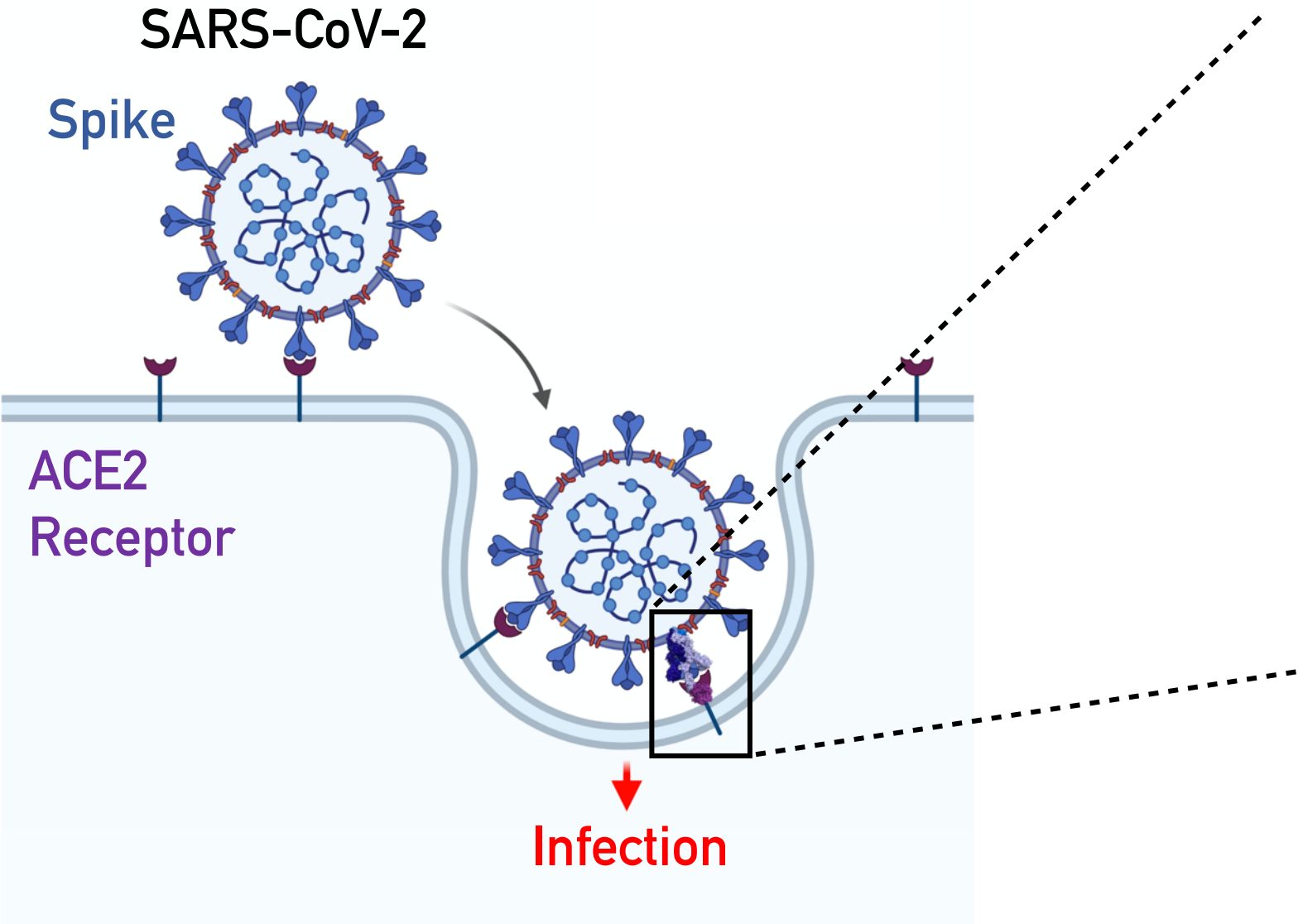


DISCLOSURES

A.M. is a co-founder, stockholder, and consultant for Epiodyne, Inc., a company focused on novel pain therapeutics. He is also a consultant for Third Rock Ventures and Ligand Pharmaceuticals.

A.M. and M.S. are inventors on a patent filed by UCSF pertaining to nanobodies to neutralize SARS-CoV-2

BLOCKING SARS-COV-2 ENTRY



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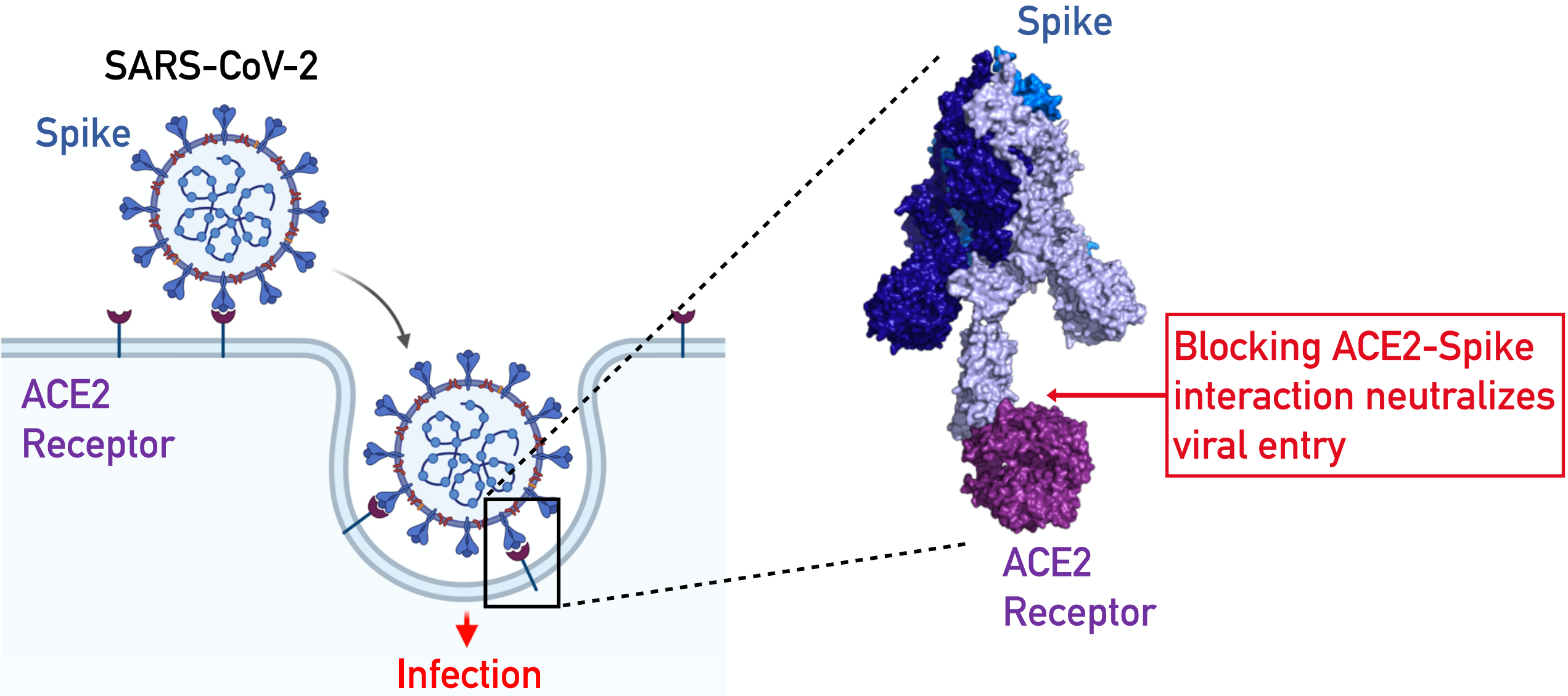
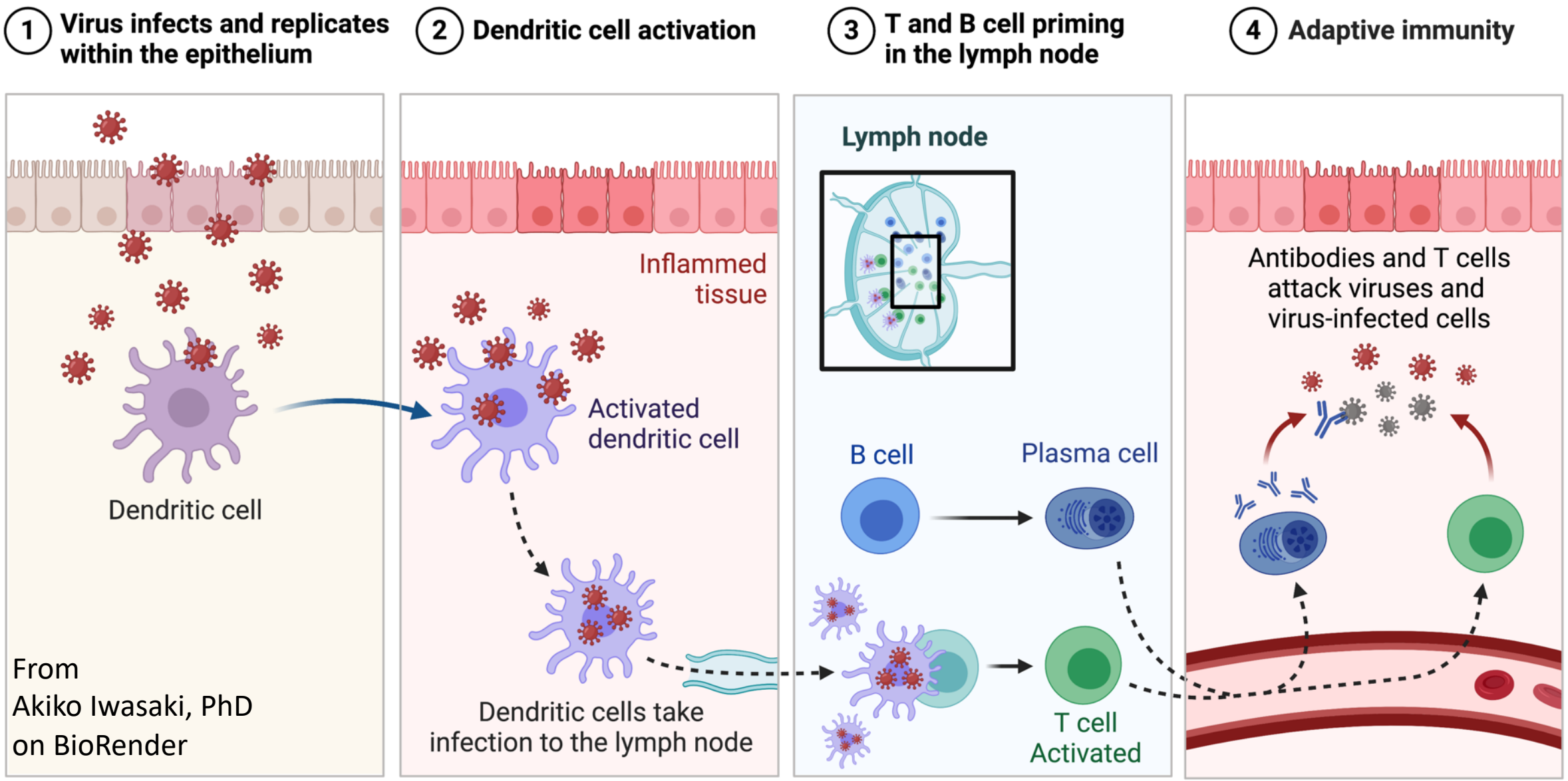
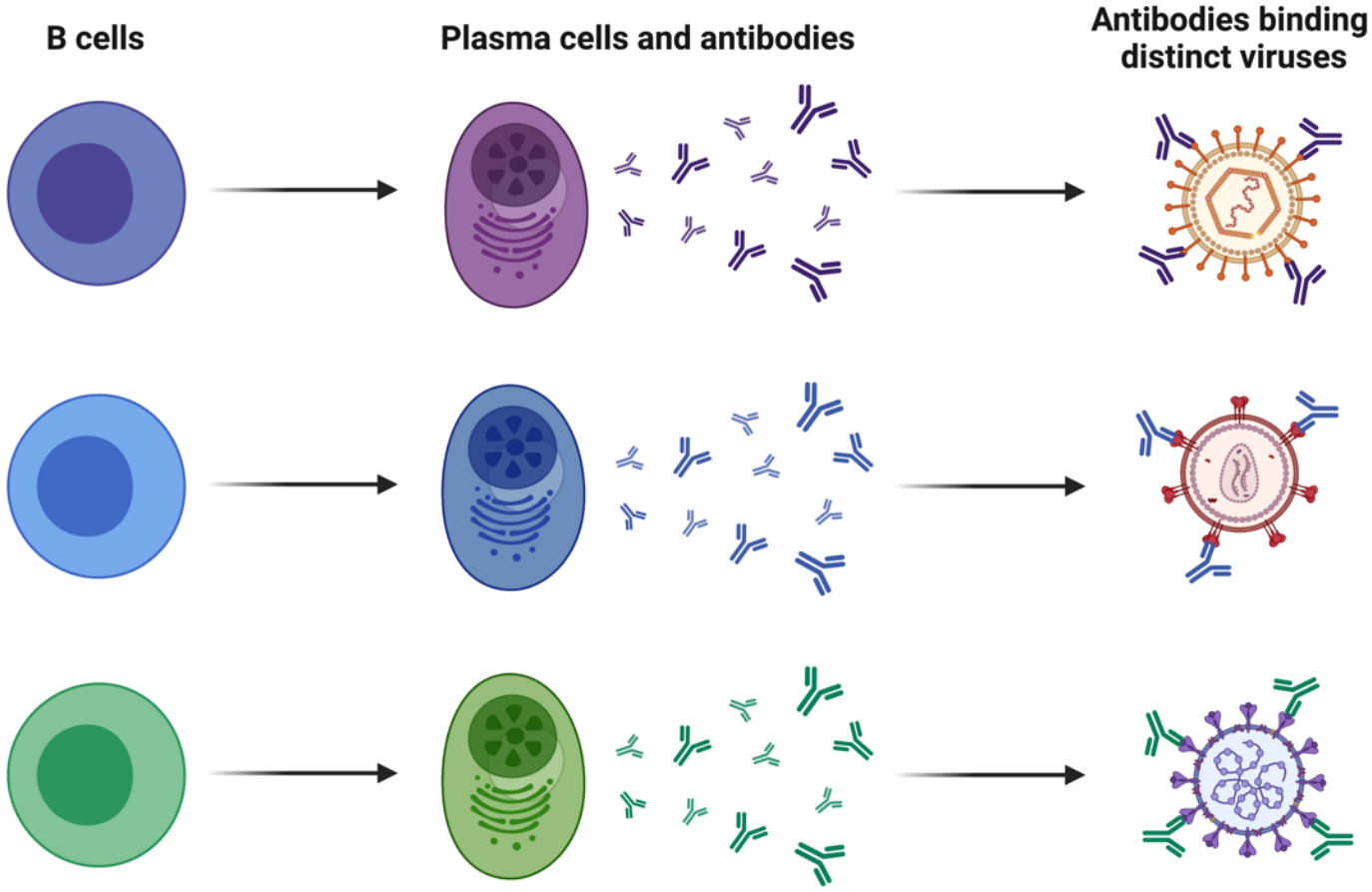


ADAPTIVE IMMUNITY



From
Akiko Iwasaki, PhD
on BioRender

ADAPTIVE IMMUNITY



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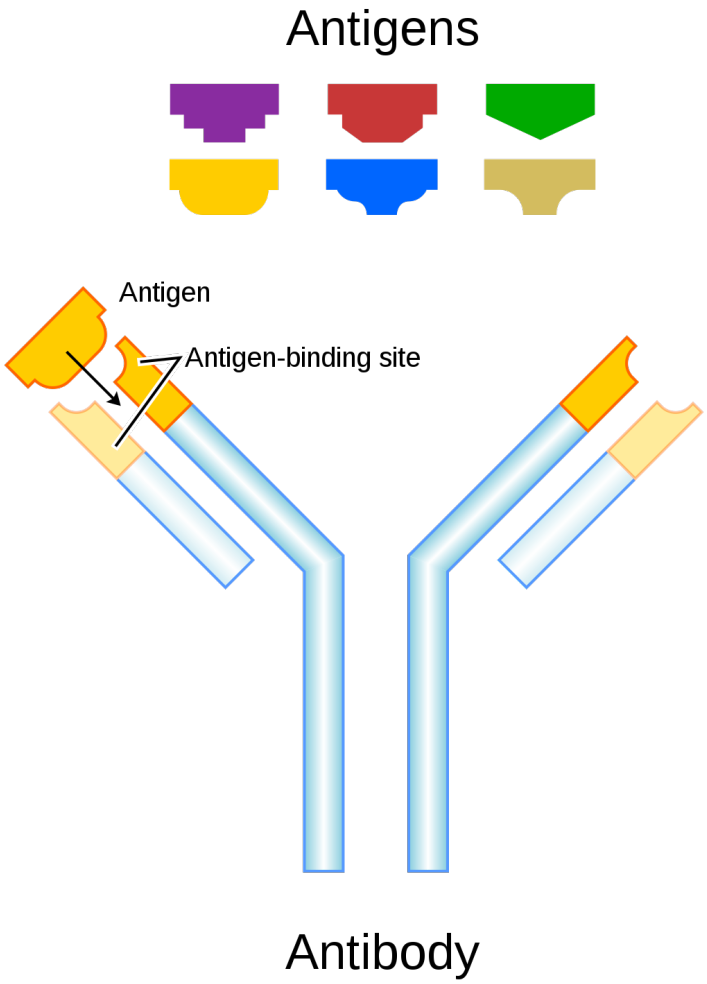
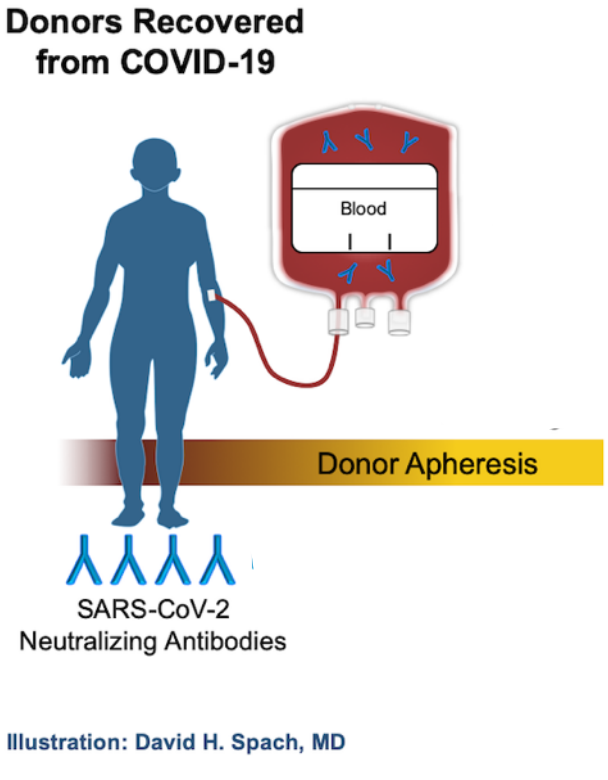


1 trillion possibilities!

PASSIVE IMMUNITY FOR SARS-COV-2

Convalescent Plasma



Monoclonal antibodies

PASSIVE IMMUNITY FOR SARS-COV-2

Convalescent Plasma

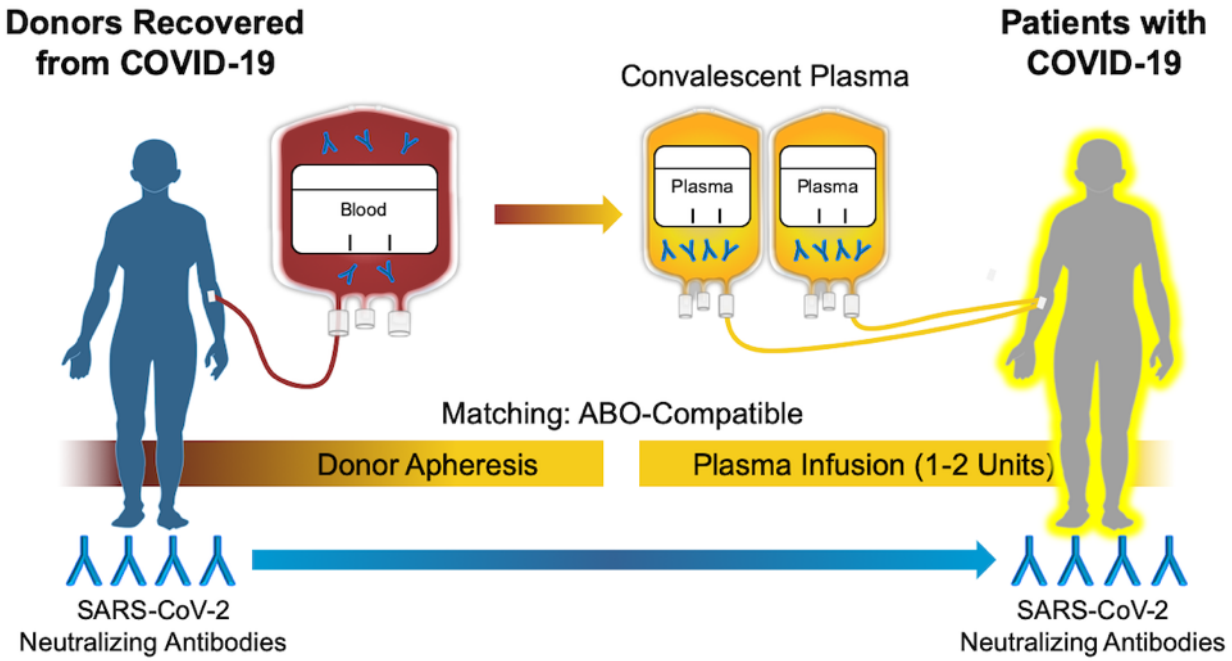


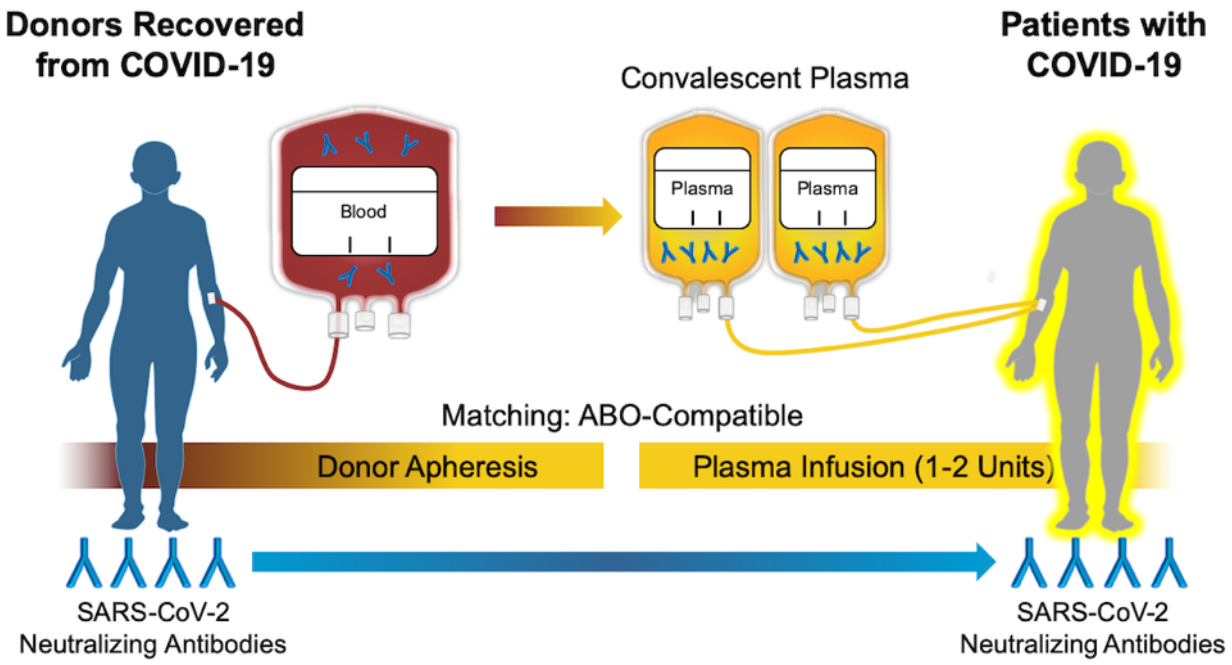
Illustration: David H. Spach, MD

- FDA EUA (8/23) for hospitalized patients with COVID-19
- NIH panel: insufficient data to recommend use
- Unclear safety, non-standardized protocols for titer
- Need prospective randomized trials

Monoclonal antibodies

PASSIVE IMMUNITY FOR SARS-COV-2

Convalescent Plasma



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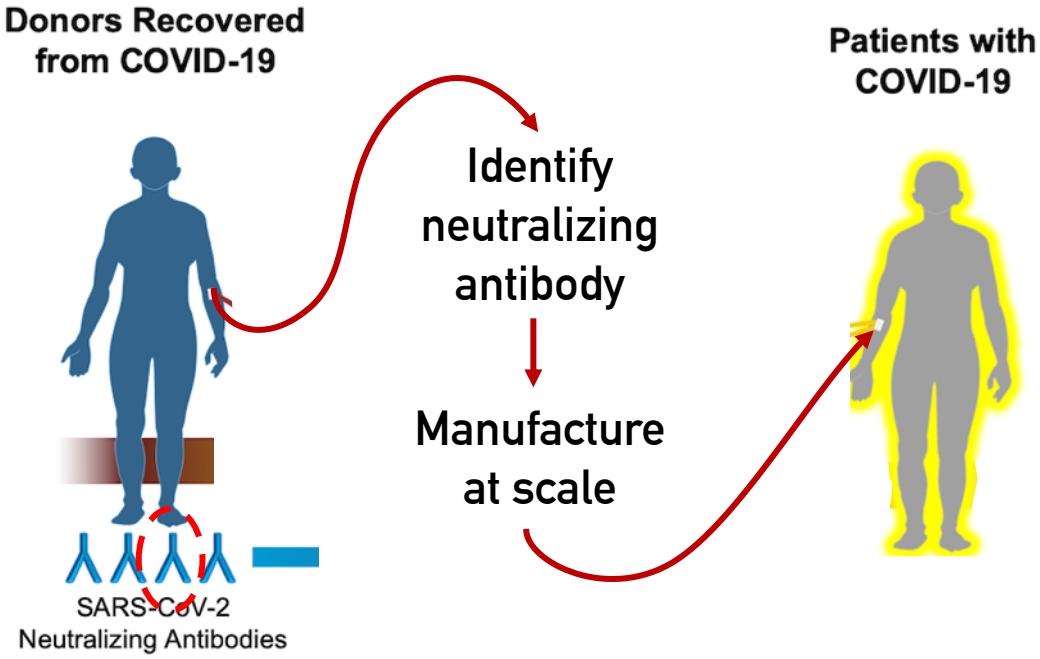


- Multiple candidates in clinical trials
- Intravenous dosing for treatment or prophylaxis
- Require large doses for prophylactic use (50 mg/kg)
- Expensive production

AN ALTERNATIVE APPROACH TO PASSIVE IMMUNITY

Patients with COVID-19

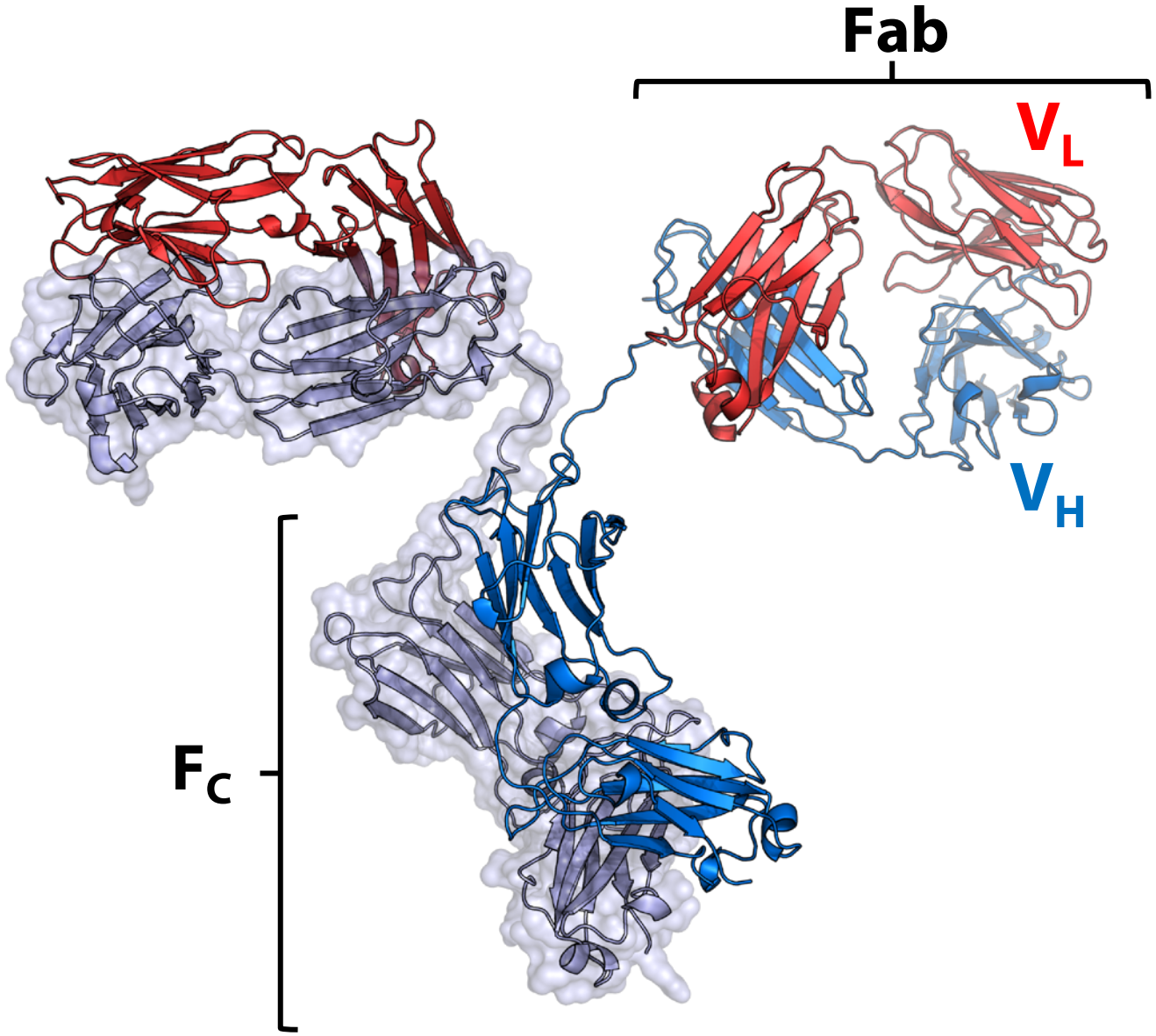
Advantages:

- Self administered
- Direct delivery to site of early infection

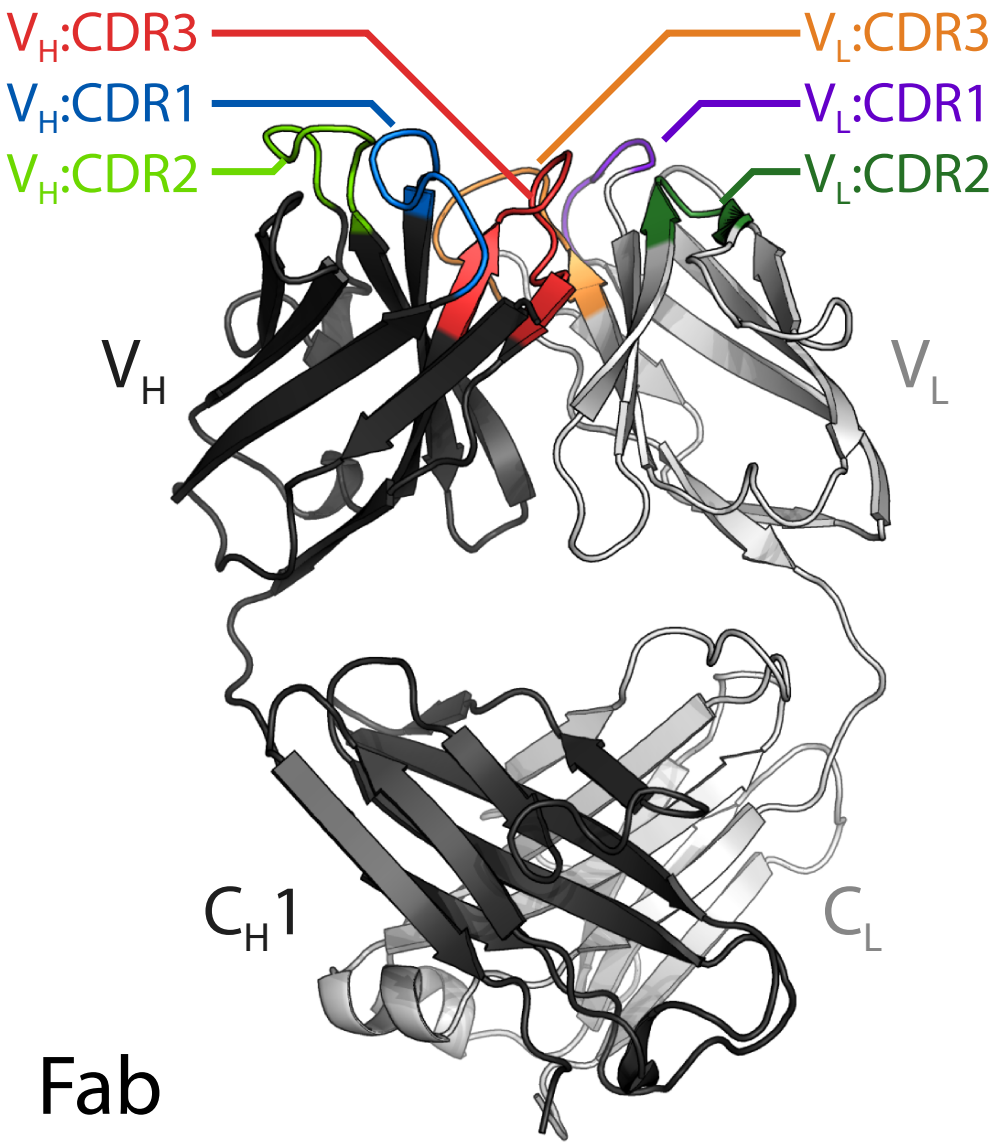
Challenges:

- Ultrastable protein required
- Pharmacokinetics?

ANTIBODIES ARE INCREDIBLY DIVERSE MOLECULES

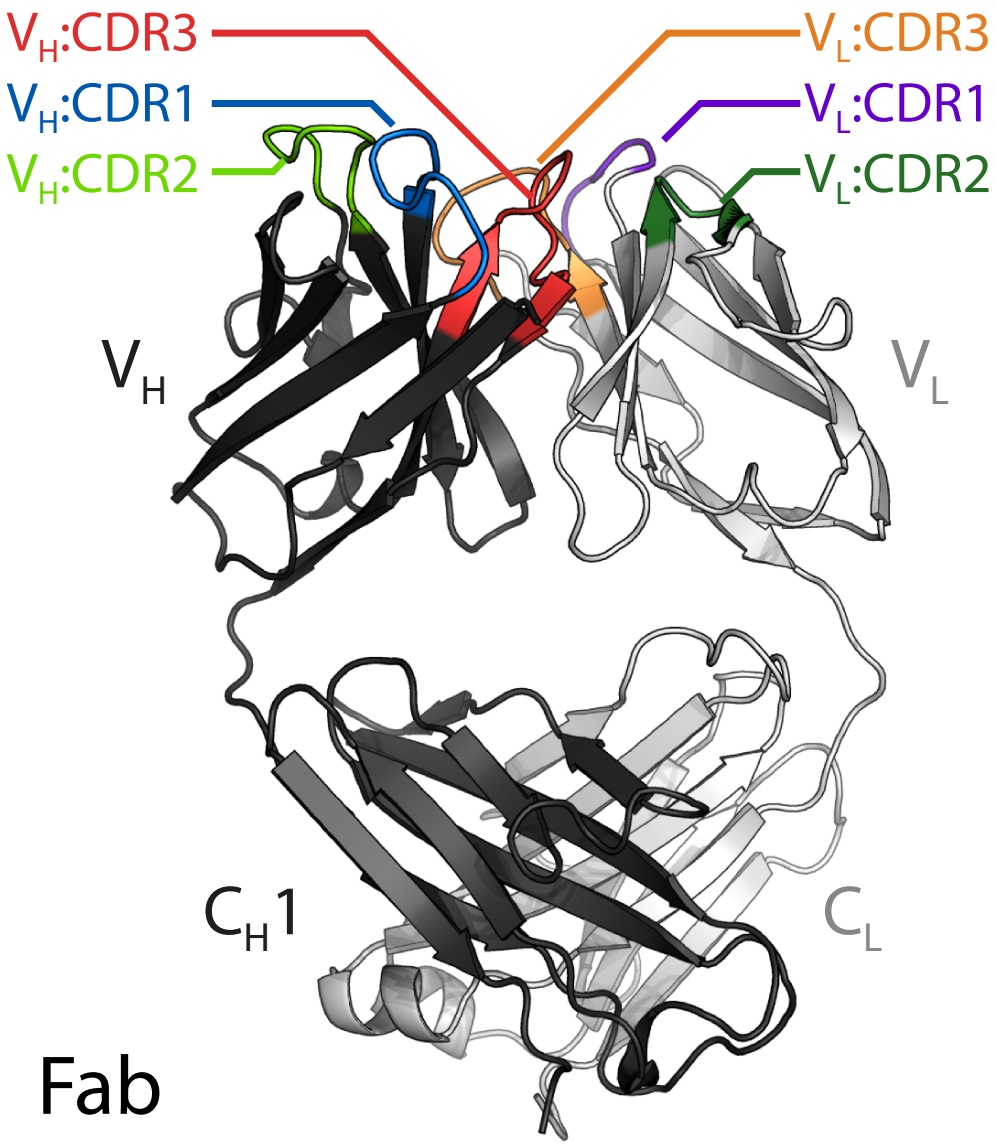


ANTIBODY DIVERSITY ENABLES ACTIVE IMMUNITY



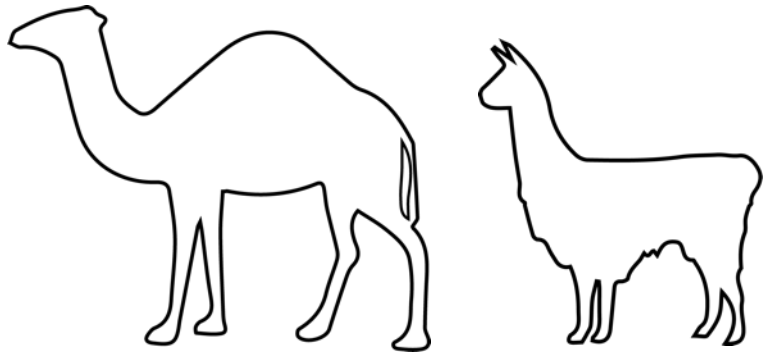
Fab

NANOBODIES – MINIMIZED ANTIBODIES FROM CAMELIDS



Fab

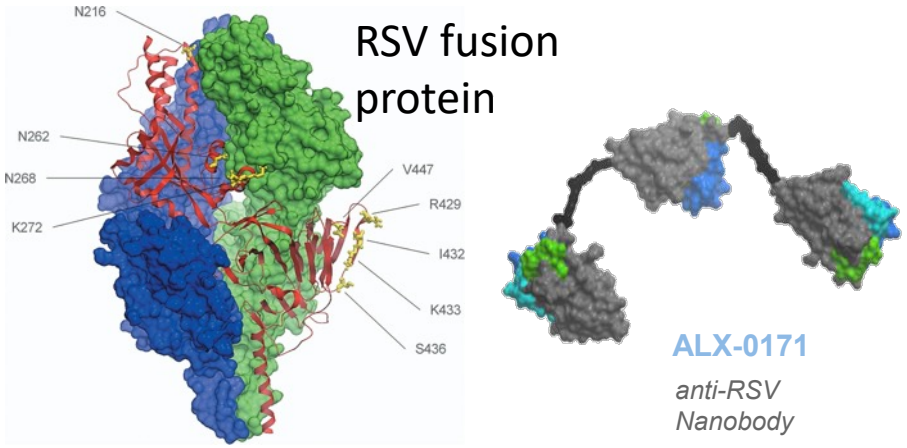
- Small (15 kDa), single chain protein
- Ultra-stable
- Non-glycosylated
- Similar to human antibody heavy chains
- Ease and low expense of rapid mass production



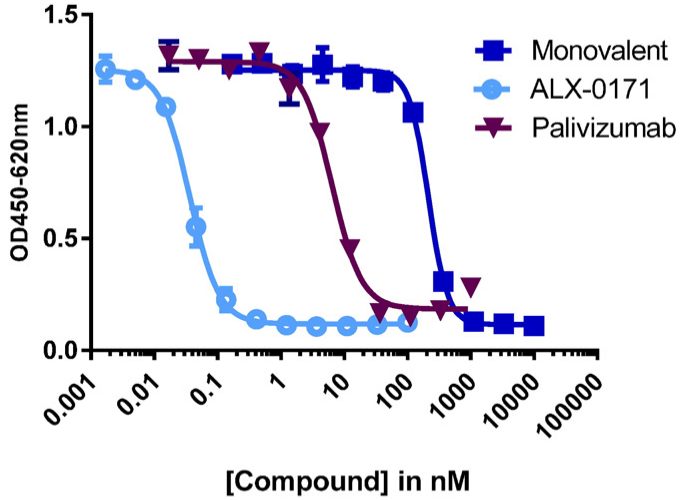
Nanobody (V_{HH})

AEROSOLIZED NANOBODIES FOR VIRAL DISEASES

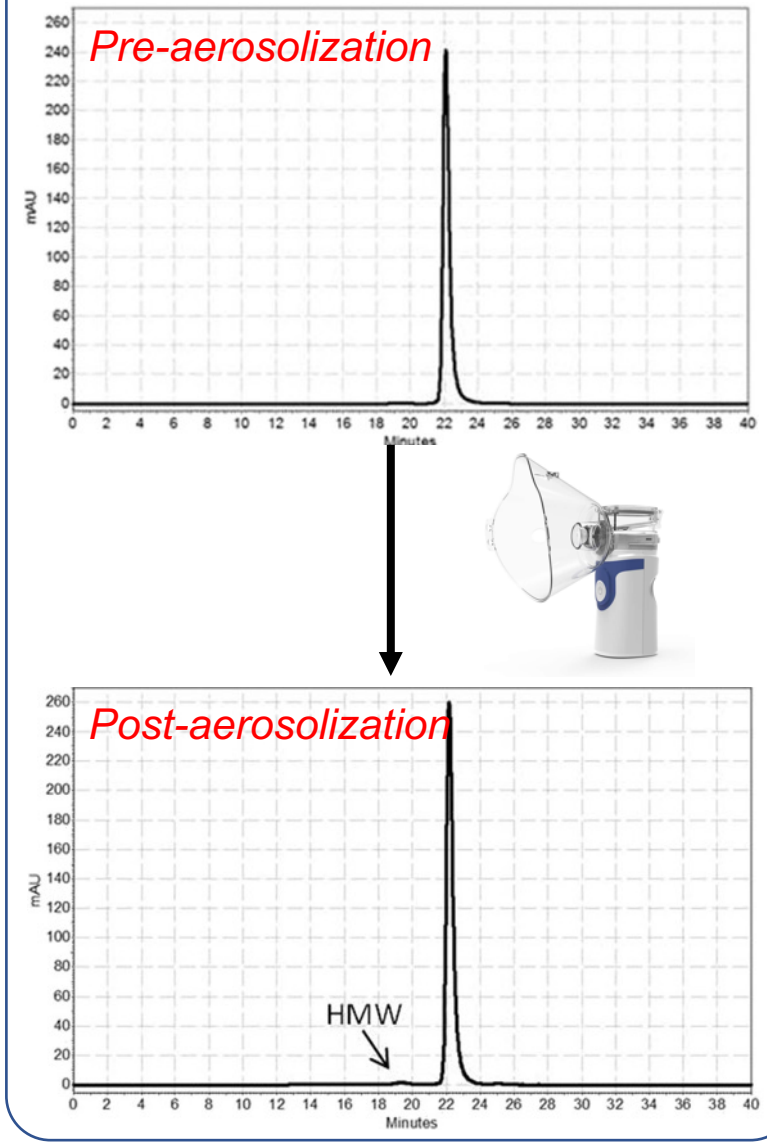
ALX-0171: trivalent RSV nanobody



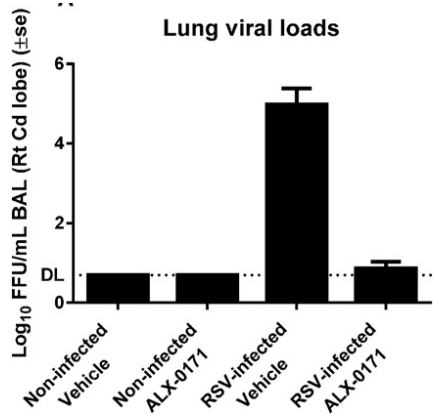
Potent in vitro viral neutralization



Stable to aerosolization



Preclinical efficacy



Human safety and PK

Phase 1 (adults): tolerated

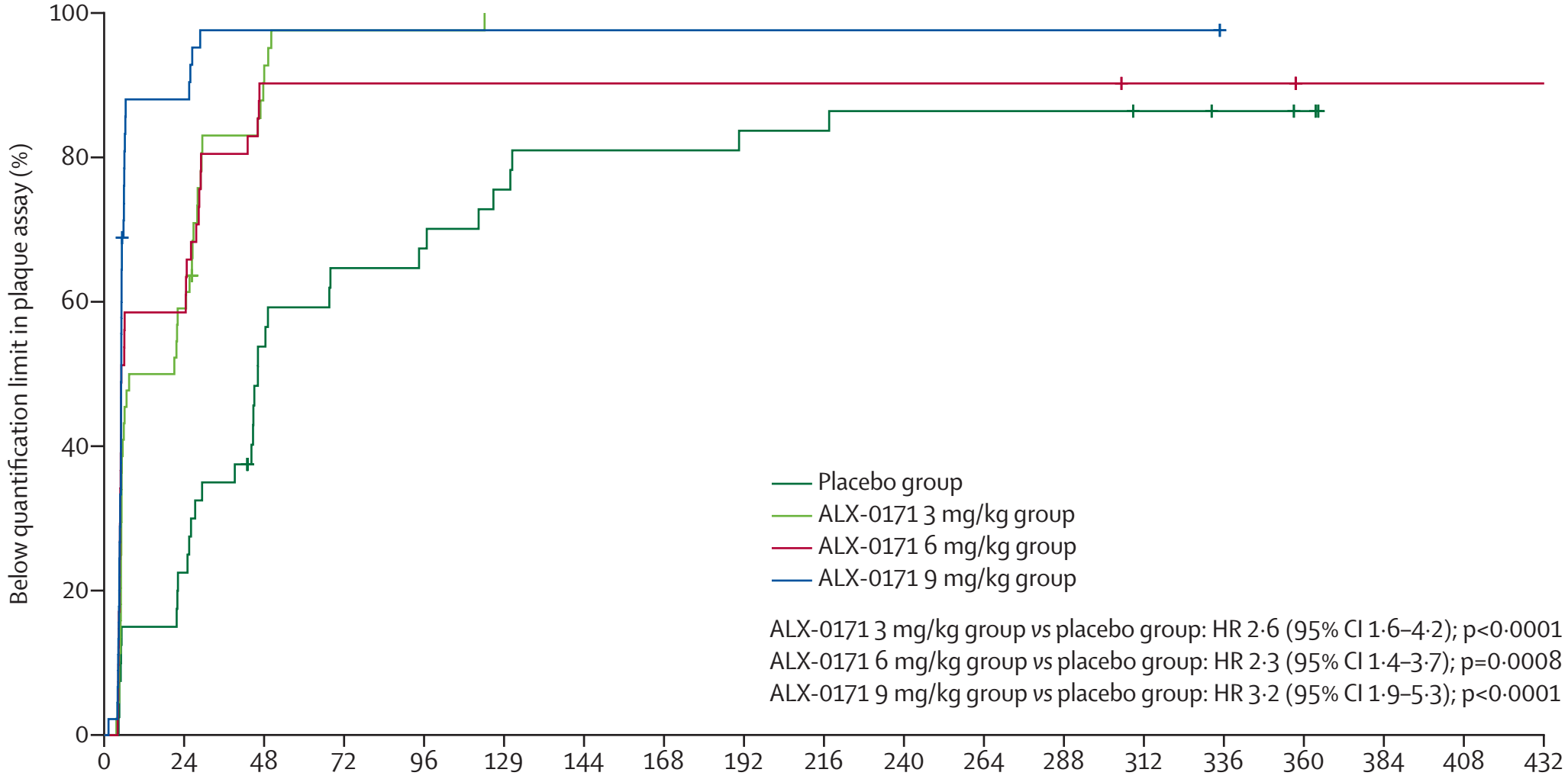
Parameter	21 mg	70 mg	140 mg	210 mg
t_{max} (h)	10.5	14.0	10.5	11.2
$t_{1/2}$ (h)	NC	18.6	21.0	19.1
C_{max} (ng/mL)	24.8	73.9	151.5	275.9
AUC_{inf} (h*ng/mL)	NC	2859	5141	10419
AUC_{tau}* (h*ng/mL)	-	-	-	-

Phase 2 (infants): tolerated

Once daily dosing based on BAL

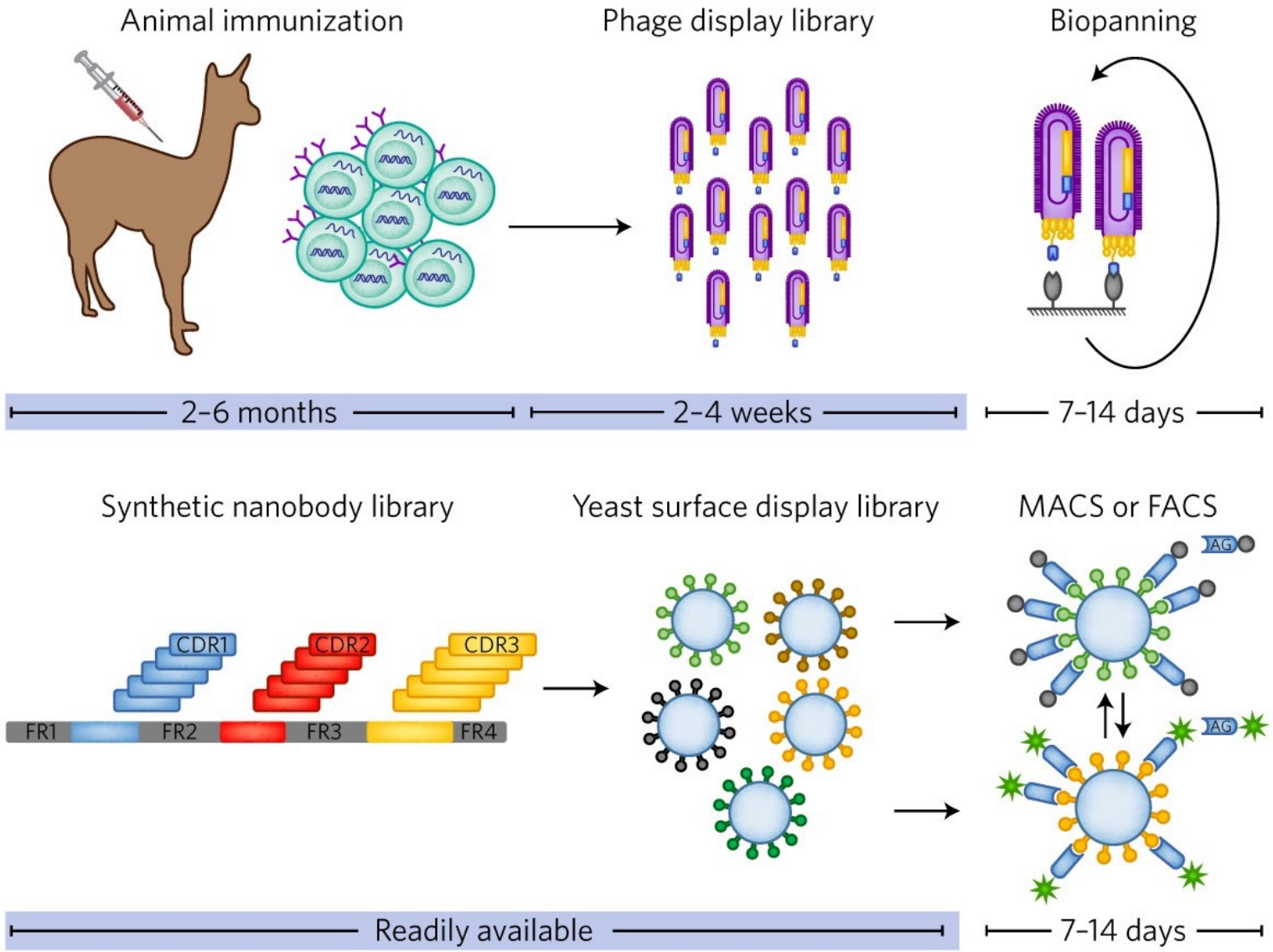
Laurent et al. Antimicrob. Agents Chemother. 2016
De Bruyn et al. Resp. Drug. Delivery. 2015

AEROSOLIZED NANOBODIES FOR VIRAL DISEASES



Cunningham S et al, Lancet Respiratory Medicine 2020

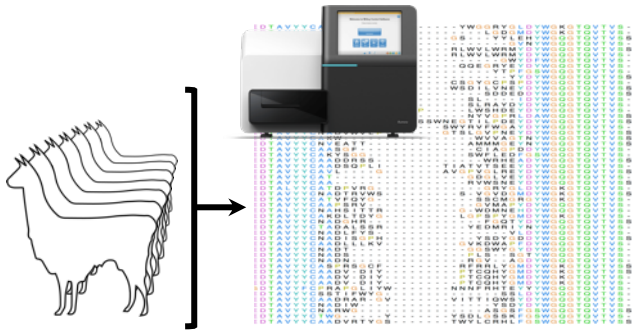
DIFFERENT APPROACHES TO NANOBODY DISCOVERY



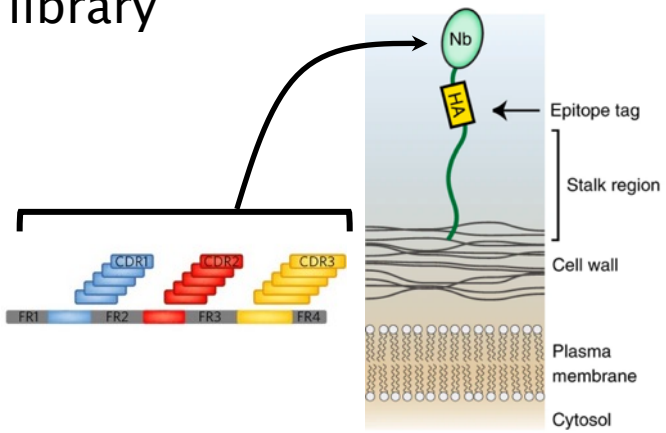
Rothbauer U, Nat Struct Mol Biol. 2018

A RAPID PLATFORM FOR NANOBODY DISCOVERY

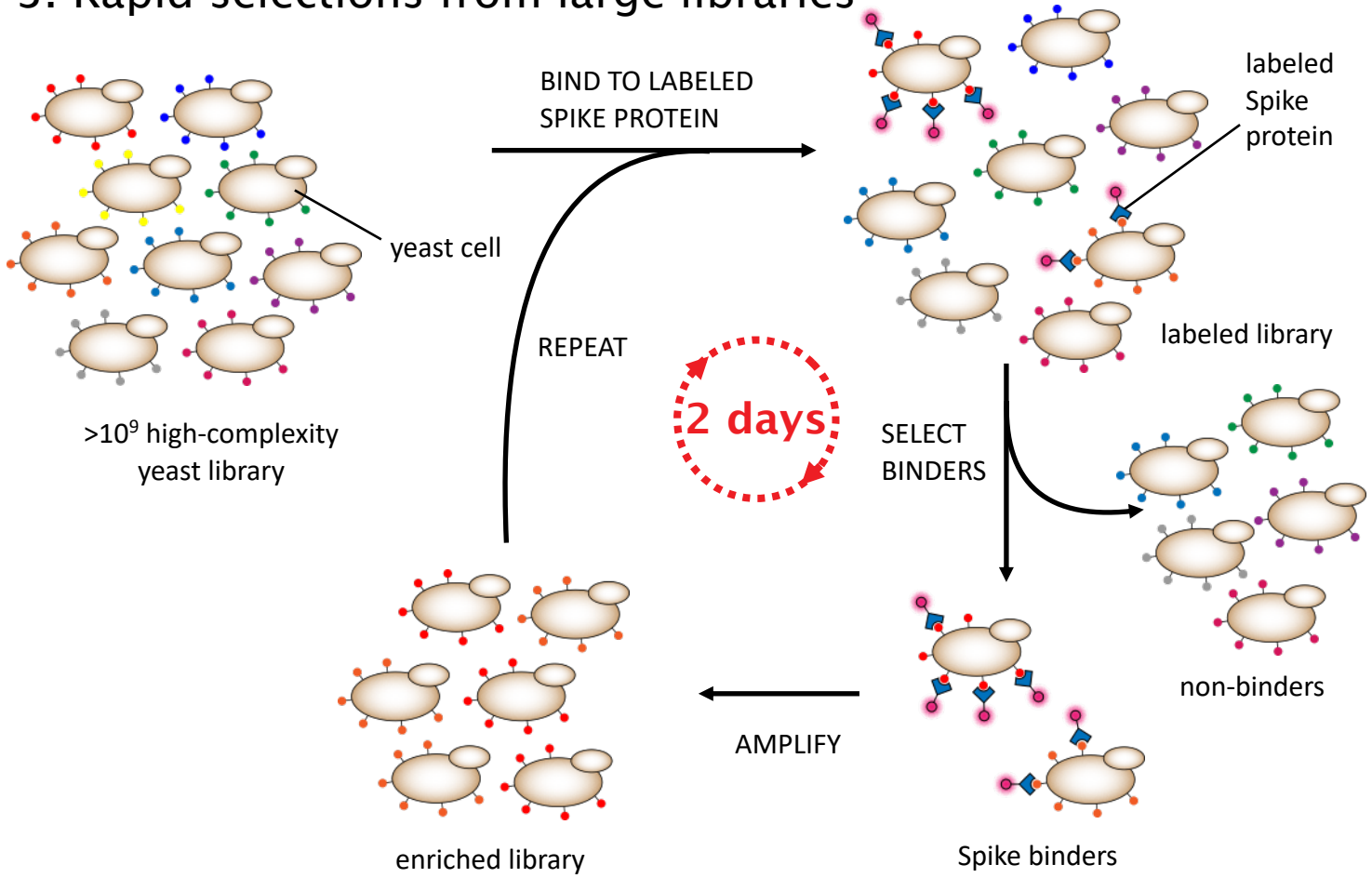
1. Bioinformatic analysis of natural camelid repertoire



2. Synthesis of precision proprietary library



3. Rapid selections from large libraries



A TEAM TO MEET THE MOMENT

AERONAB TEAM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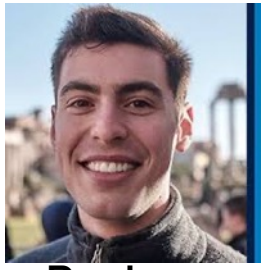
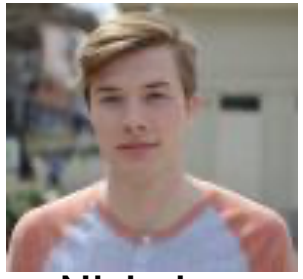
Michael Schoof

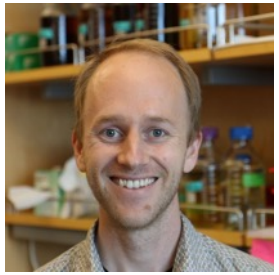

Bryan Faust


Reuben Saunders


Smriti Sangwan


Veronica Rezelj


Nicholas Hoppe


Christian Billesbølle


Morgane Boone

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Oren Rosenberg

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Yanxin Liu
Kaihua Zhang
David Bulkley
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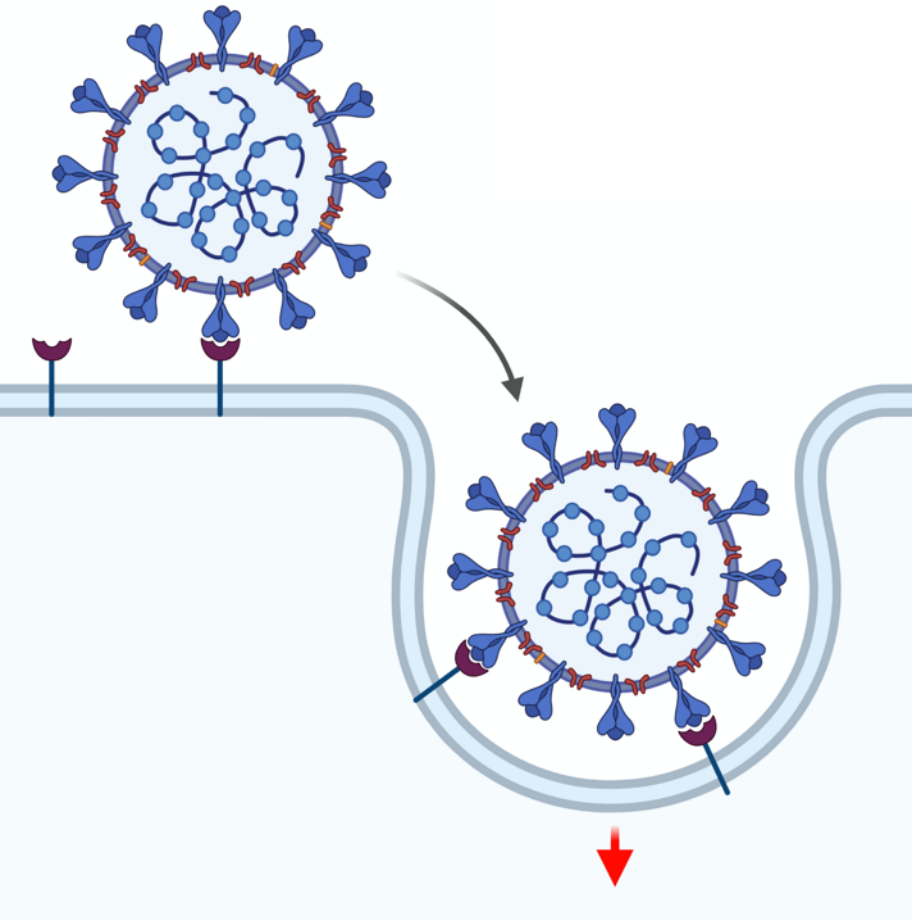
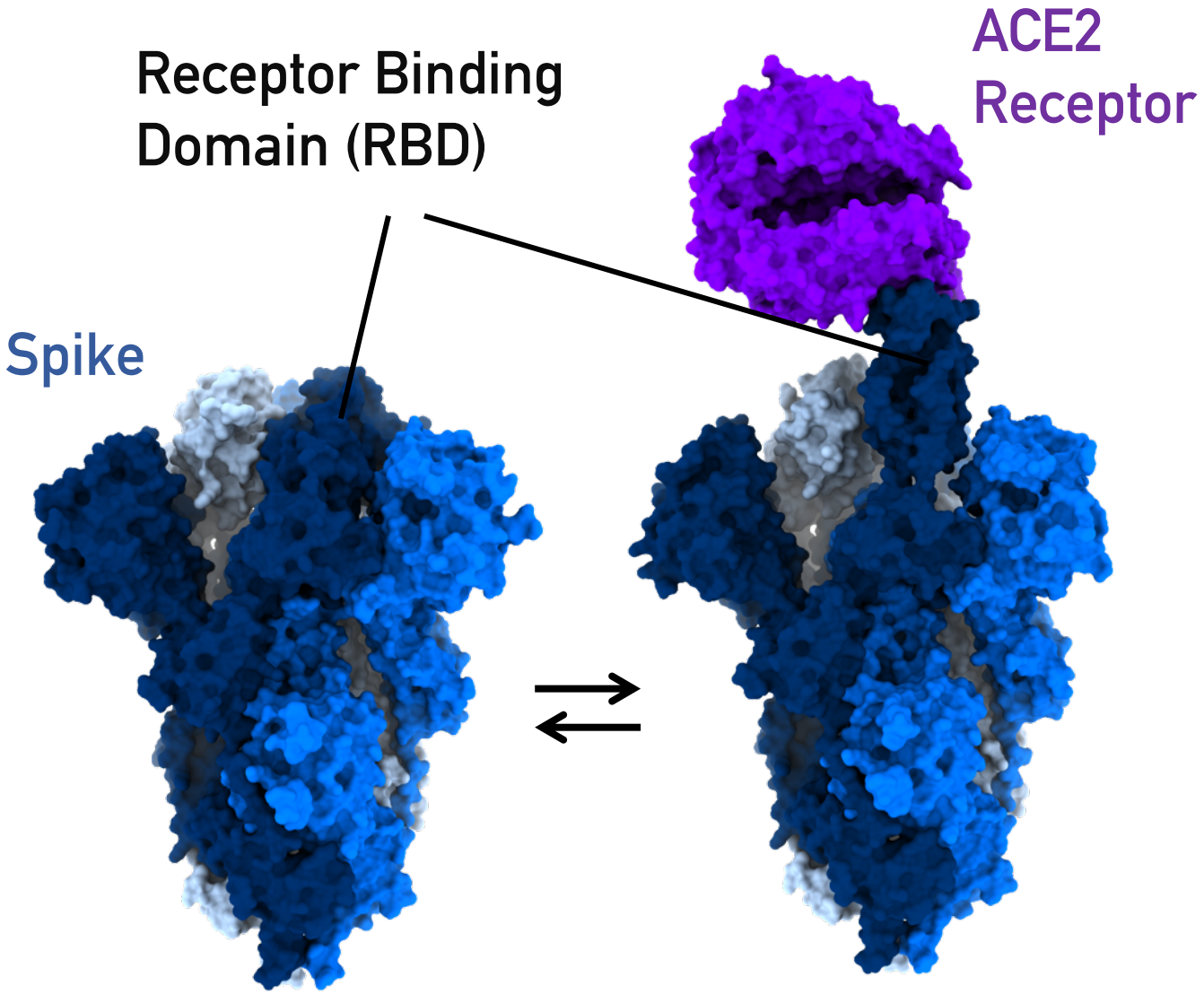
Amber Smith
Alexandrea N. Rizo
Frank Moss
Axel Brilot
Sergei Pourmal
Raphael Trenker
Thomas Pospiech
+50 other trainees

FULL SPIKE ECTODOMAIN FOR NANOBODY DISCOVERY





UNDERSTANDING BINDING

$$\mathrm{R} + \mathrm{L} \rightleftharpoons \mathrm{RL}.$$

R binds L to make RL

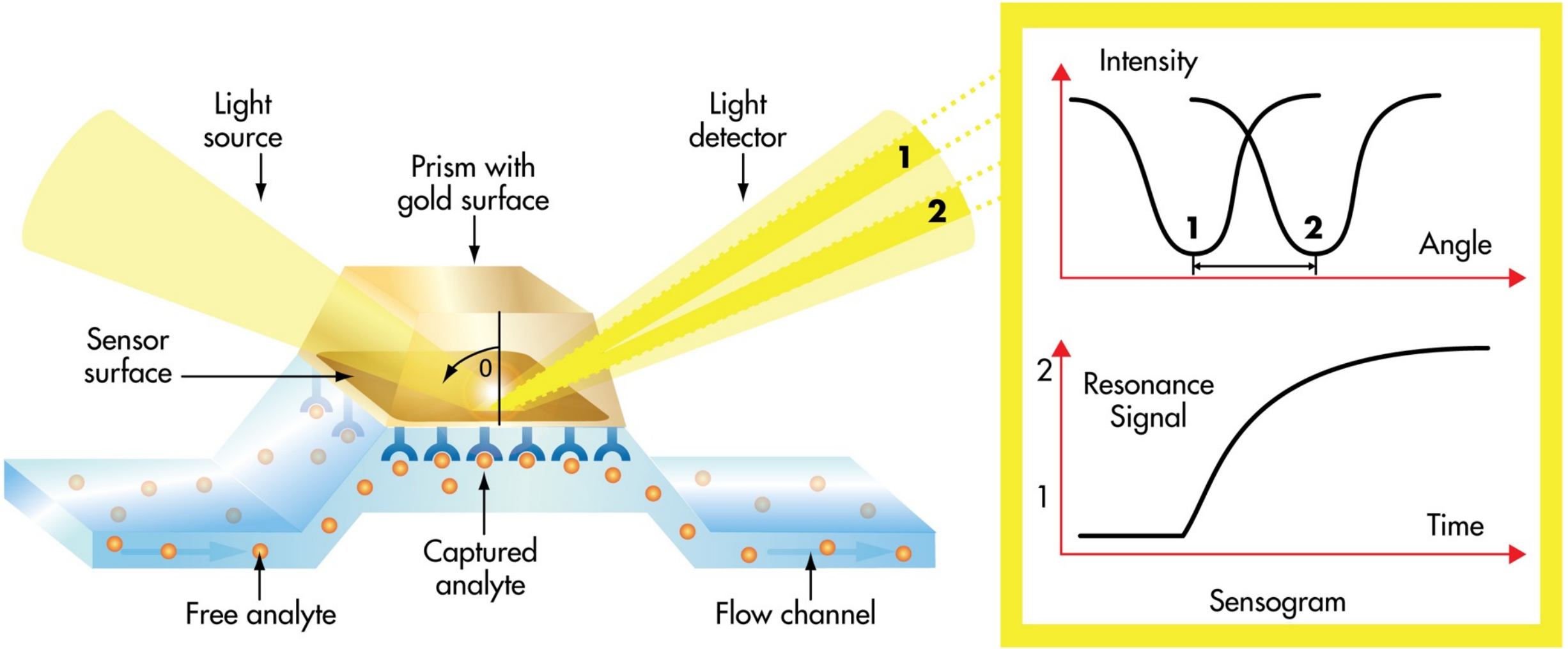
$$K_\mathrm{a} = \frac{k_\mathrm{on}}{k_\mathrm{off}} = \frac{[\mathrm{RL}]}{[\mathrm{R}]\,[\mathrm{L}]}.$$

At equilibrium (Ka), forward and reverse reactions are equal

If things bind tight: more RL, less R and L.

Rate forward (k_{on}) is faster than rate backward (k_{off})

PROTEIN INTERACTIONS BY SURFACE PLASMON RESONANCE



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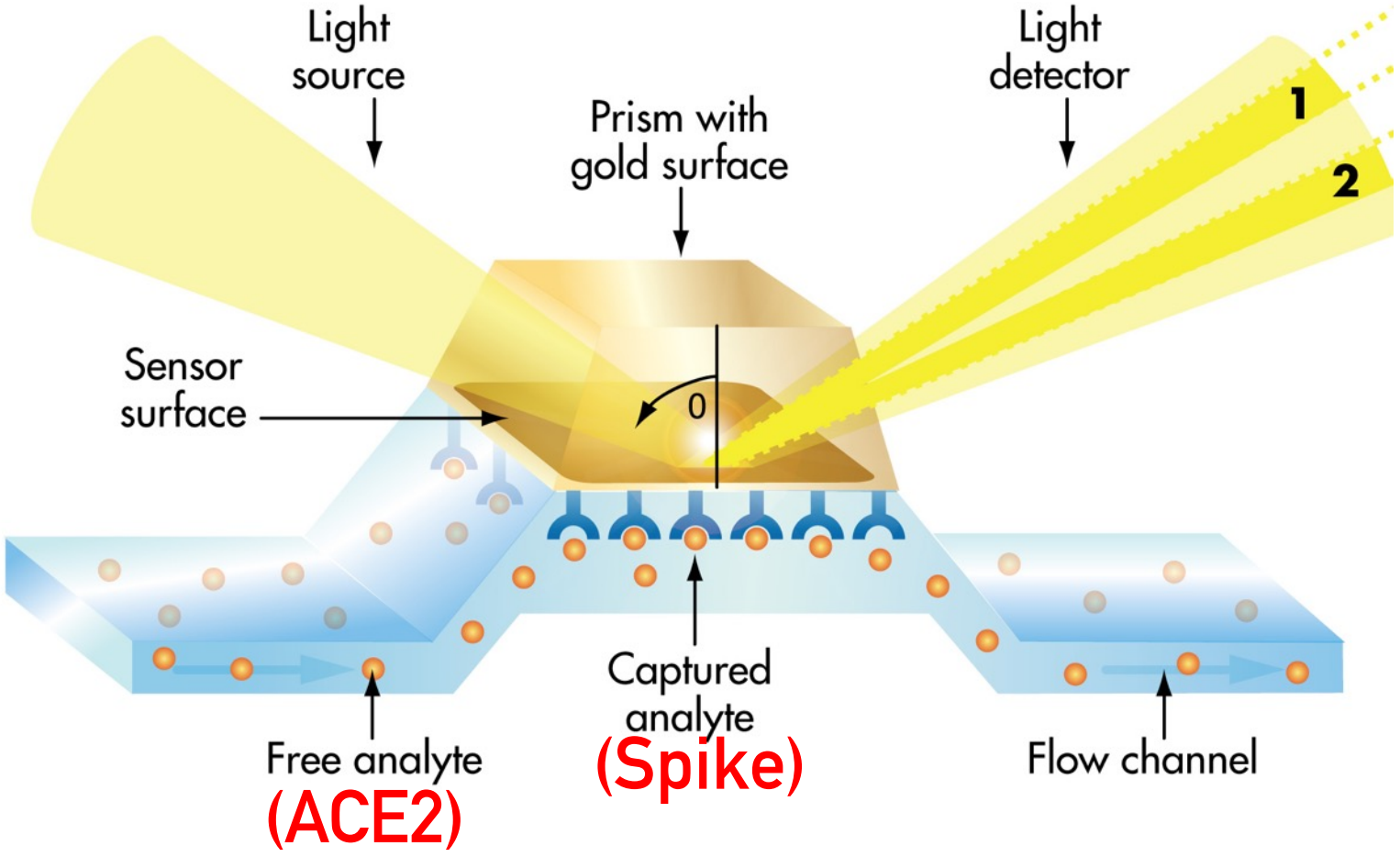
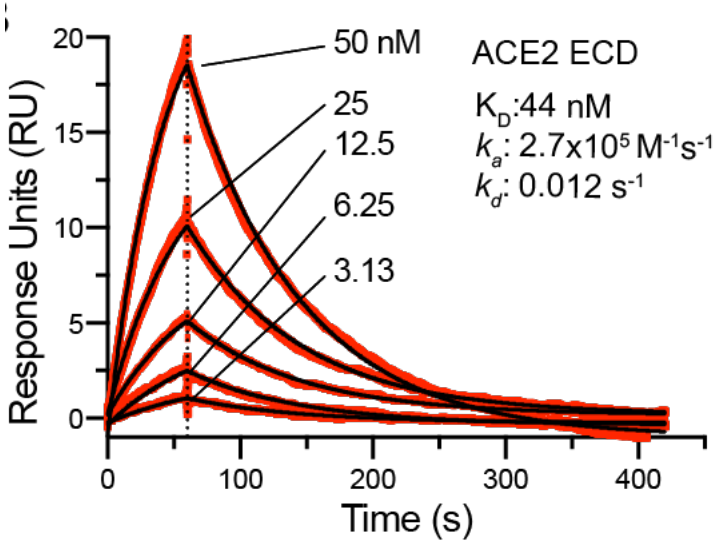




K_D: 44 nM

ka: 2.7×10^5 $M^{-1}s^{-1}$

kd: 0.012 s^{-1}

FINDING NANOBODIES THAT BLOCK ACE2

Synthetic library with
$2x10^9$ variants

↓ 200 nM Spike

Round 1

↓ 100 nM Spike

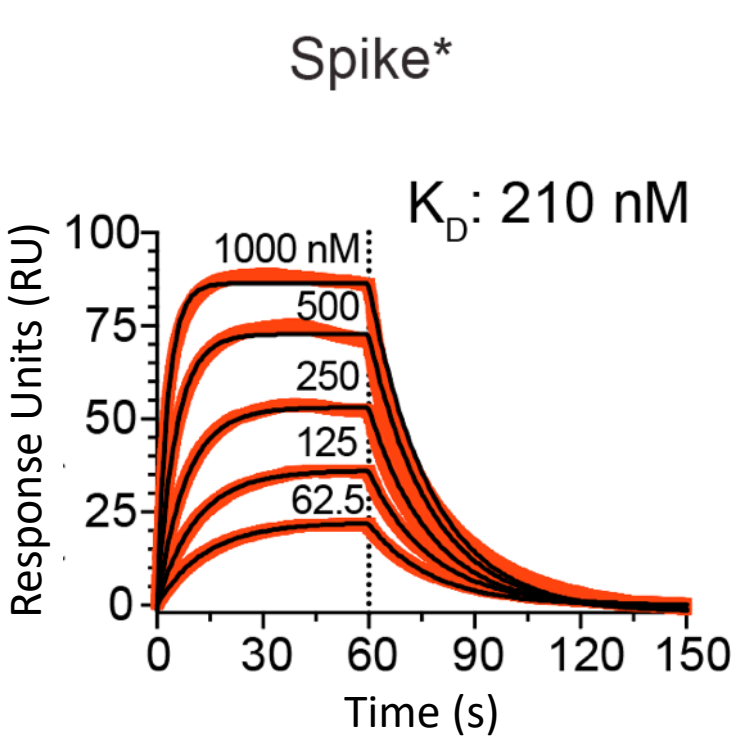
Round 2

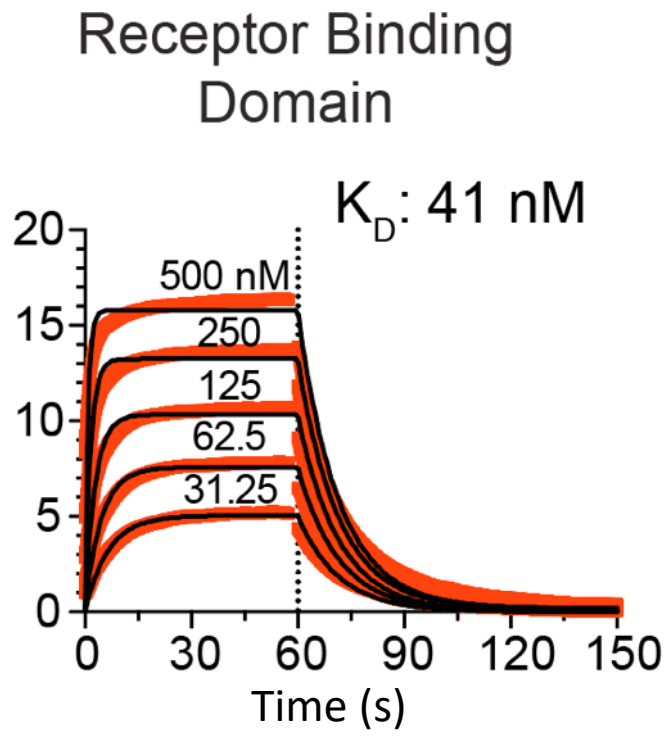
↓ 10 nM Spike

Round 3

↓ Bind Spike
Blocked by ACE2

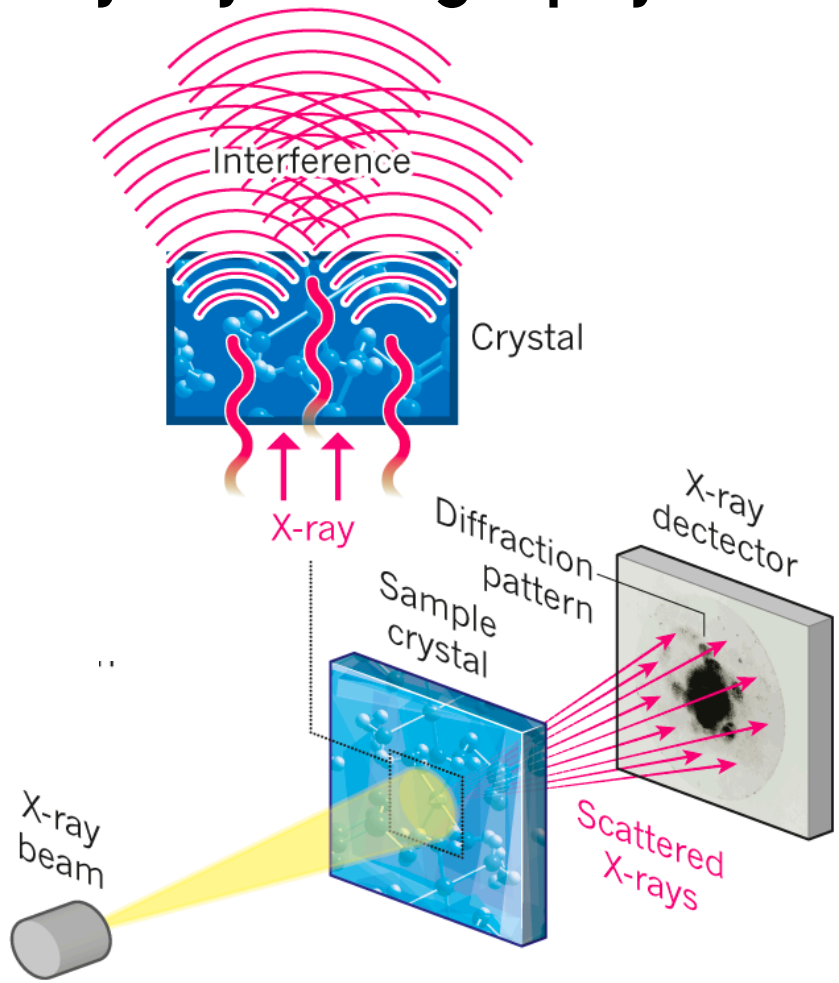
21 clones



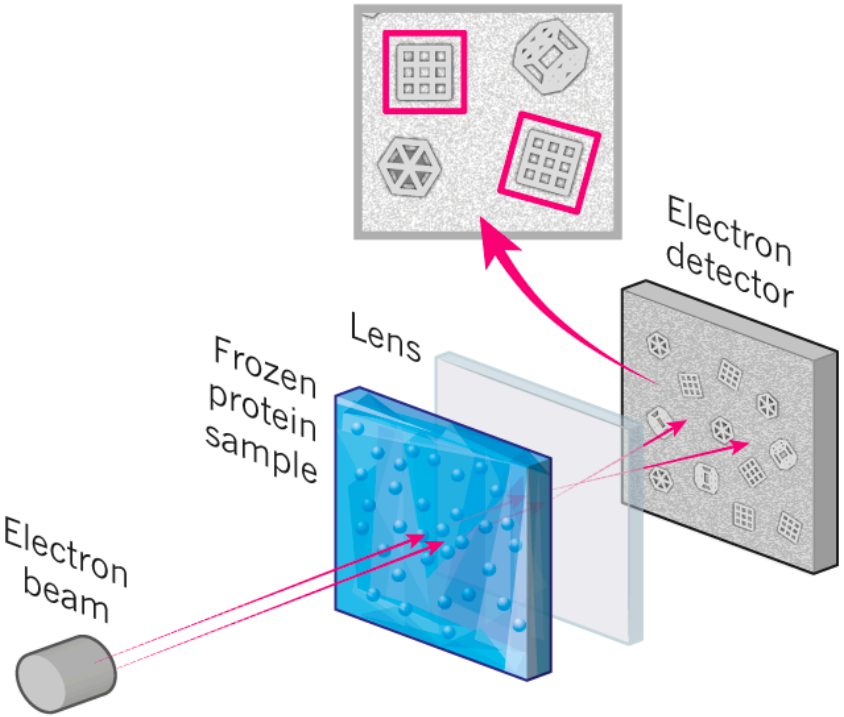


SEEING THE SMALLEST UNITS OF LIFE

X-ray crystallography



Cryo-electron microscopy

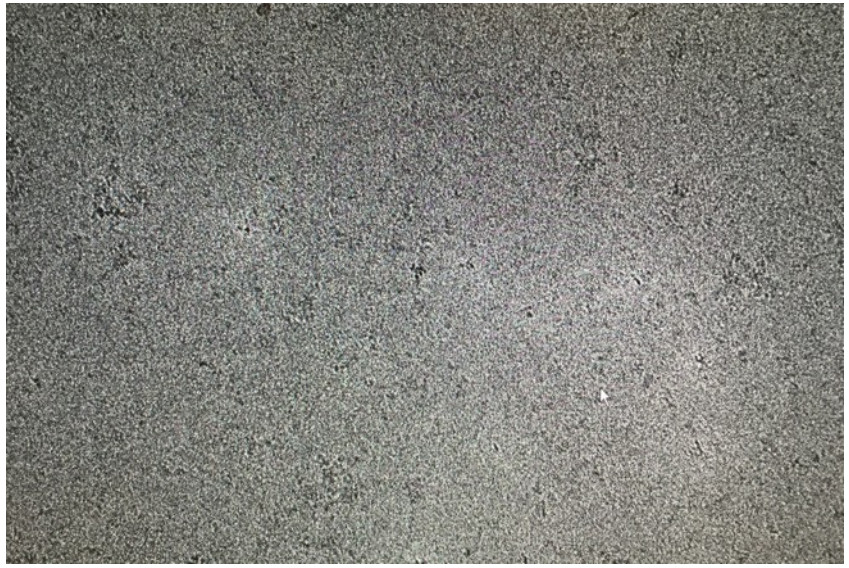


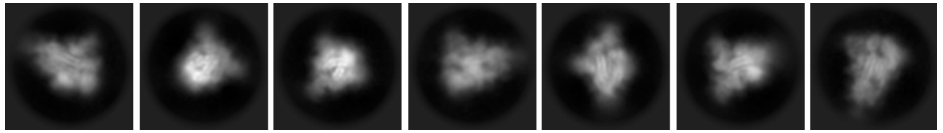
Callaway E, *Nature* 2015; 525

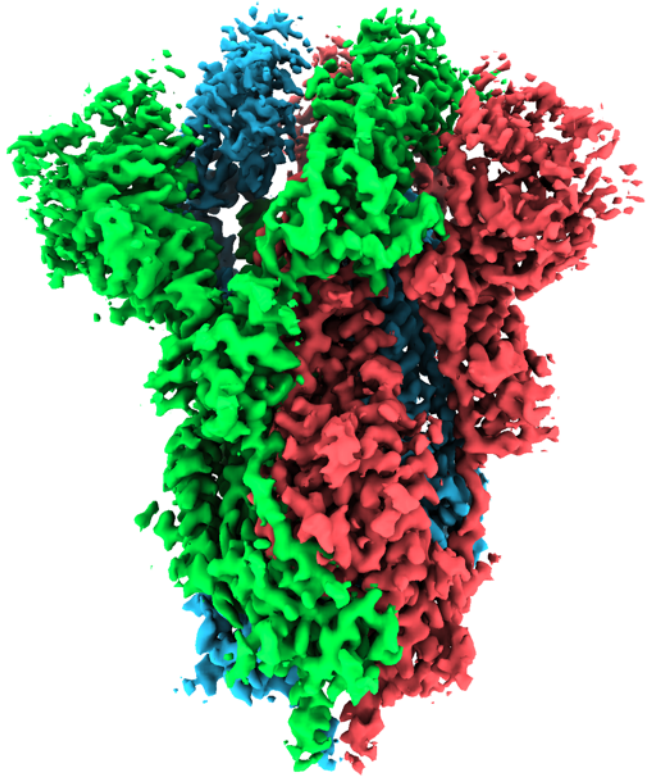
STRUCTURE OF SPIKE ECTODOMAIN

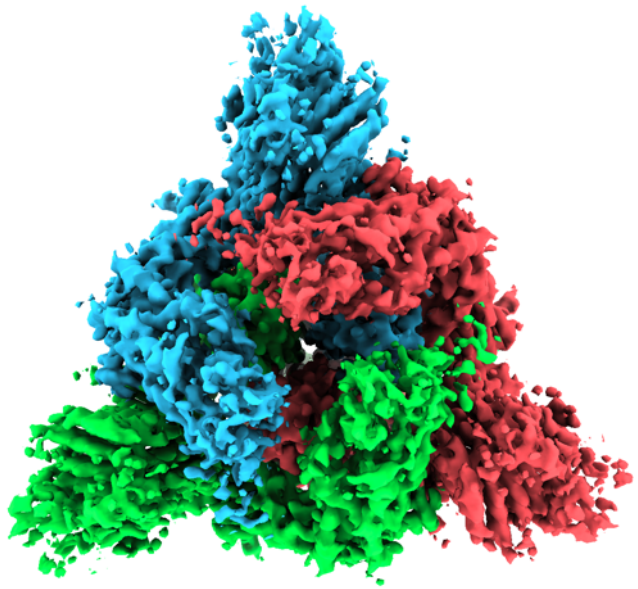
David Bulkley
Yifan Cheng
UCSF CryoEM facility

Cryo-electron microscopy



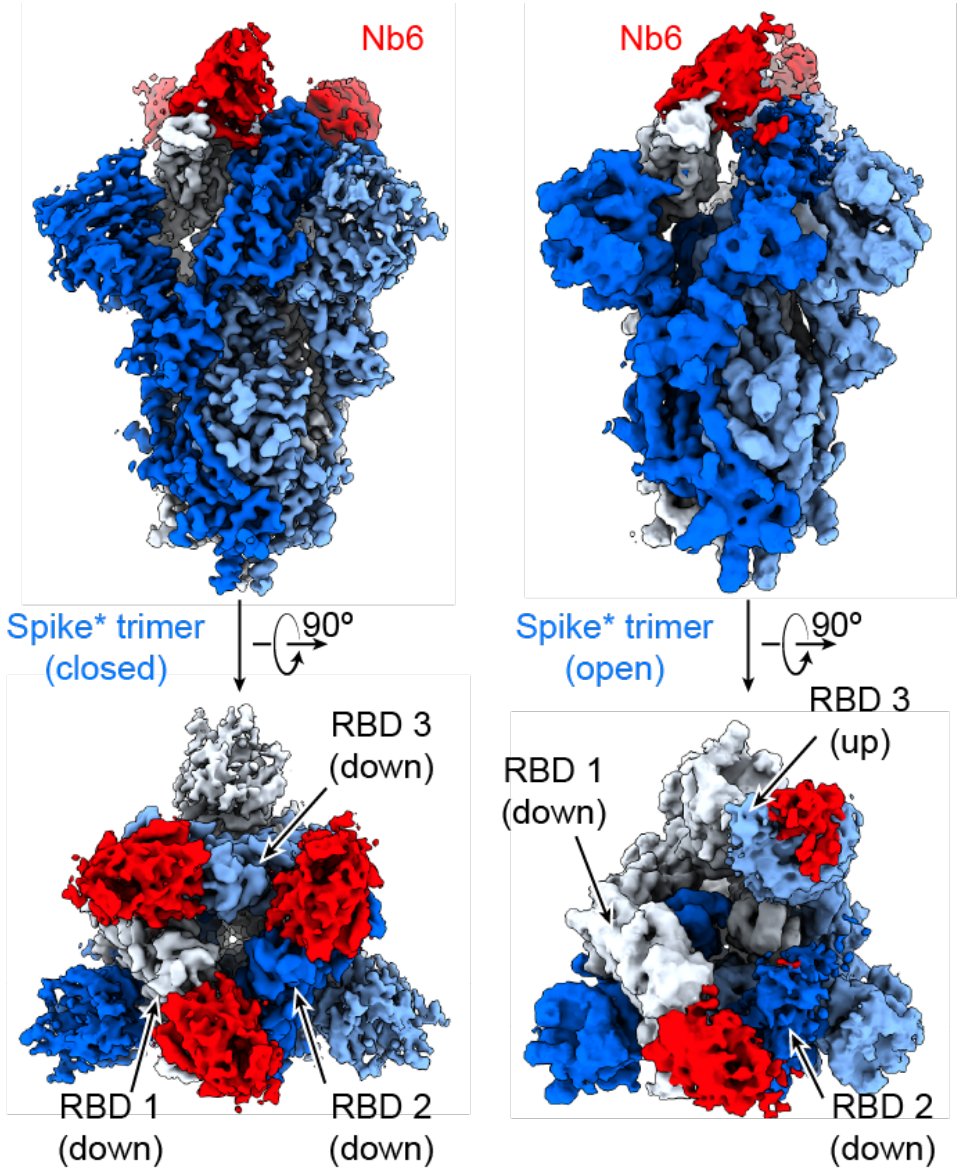




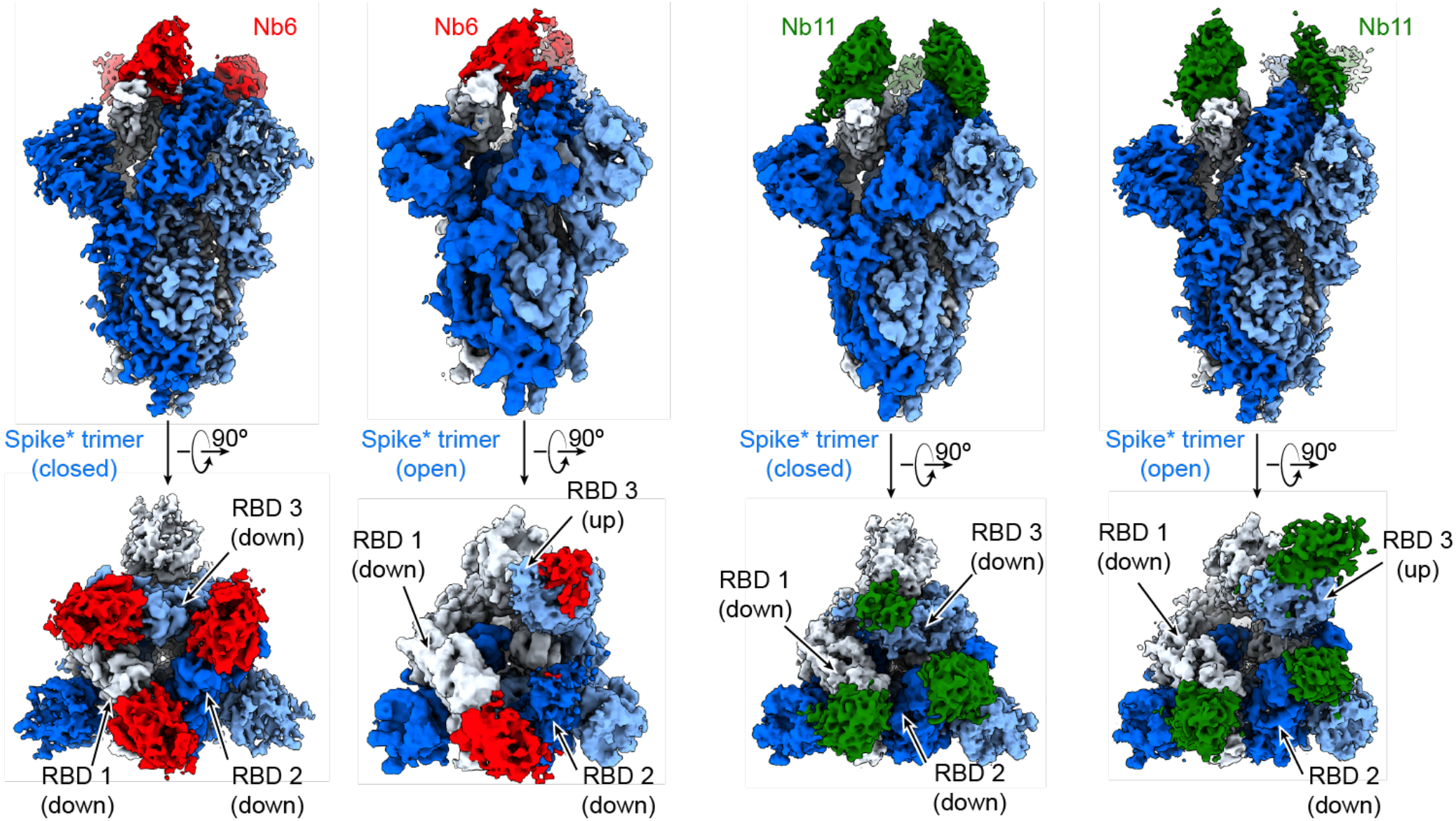


Preliminary reconstruction at ~2.5 Å

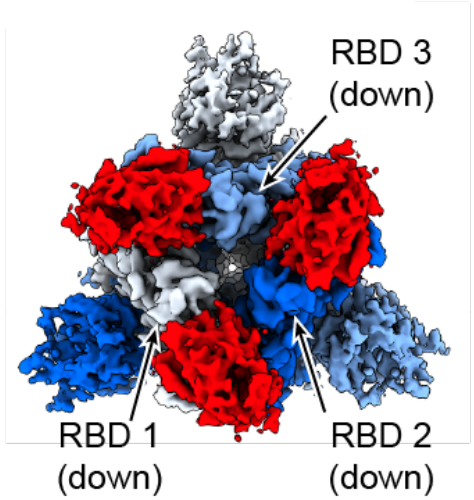
STRUCTURES OF ANTI-SPIKE NANOBODIES

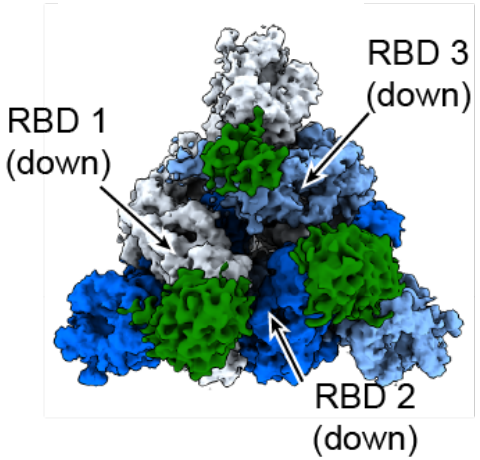


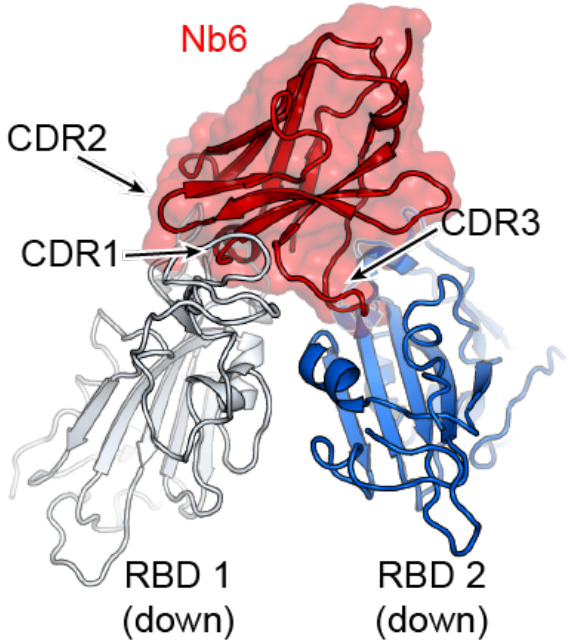
STRUCTURES OF ANTI-SPIKE NANOBODIES

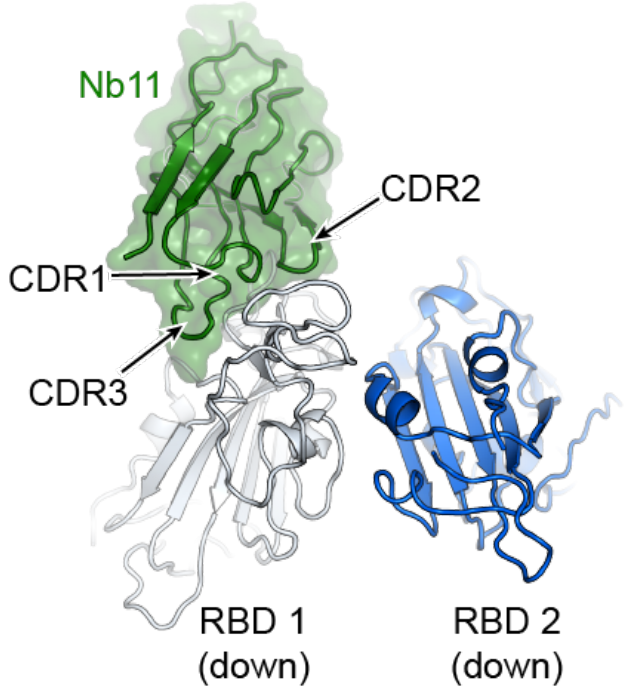


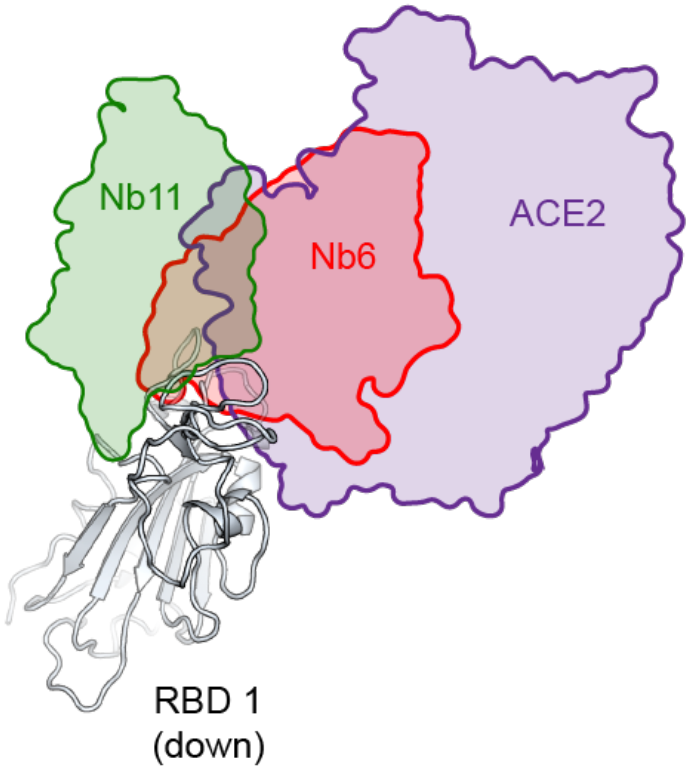
NB6 INHIBITS ACE2 BINDING BY A DUAL MECHANISM



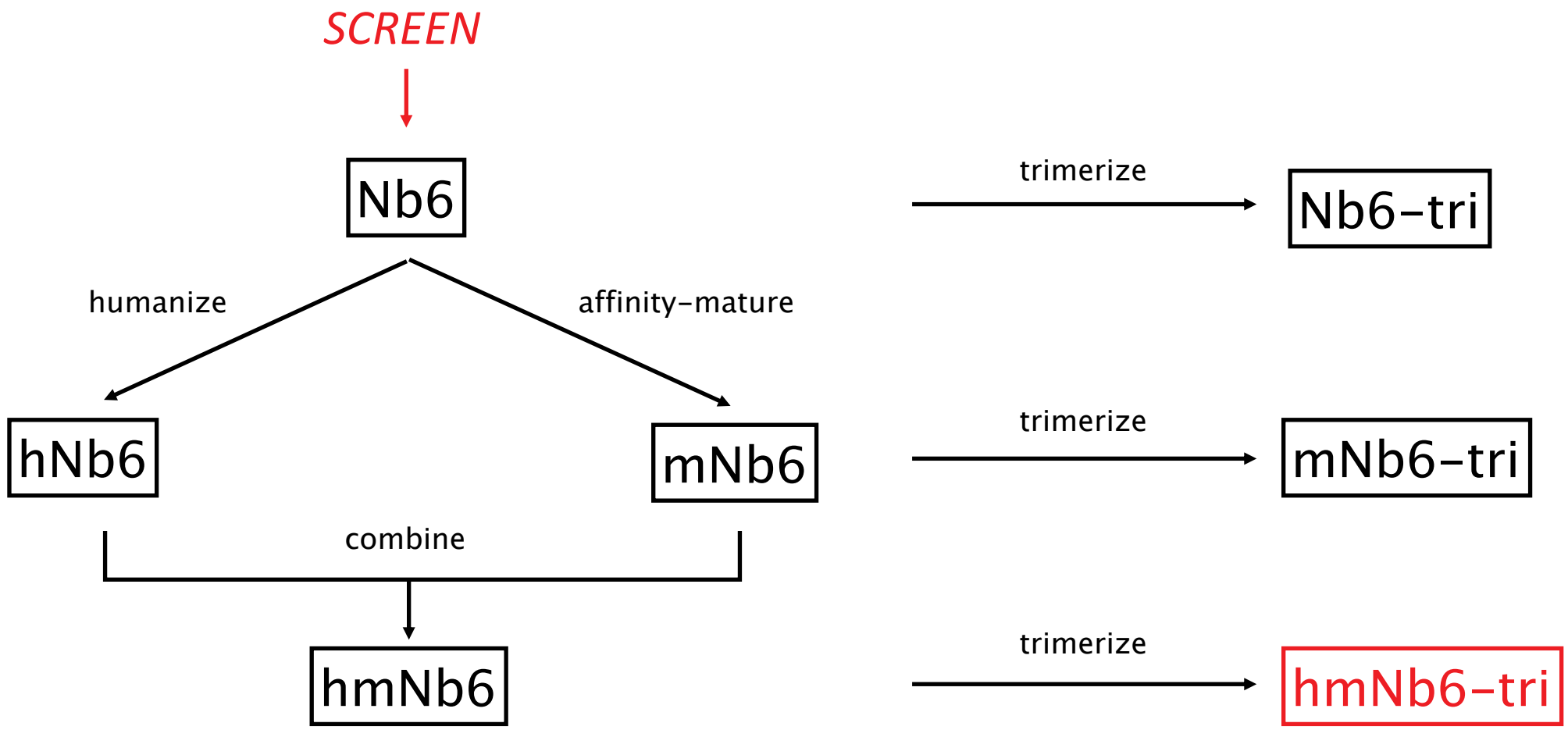






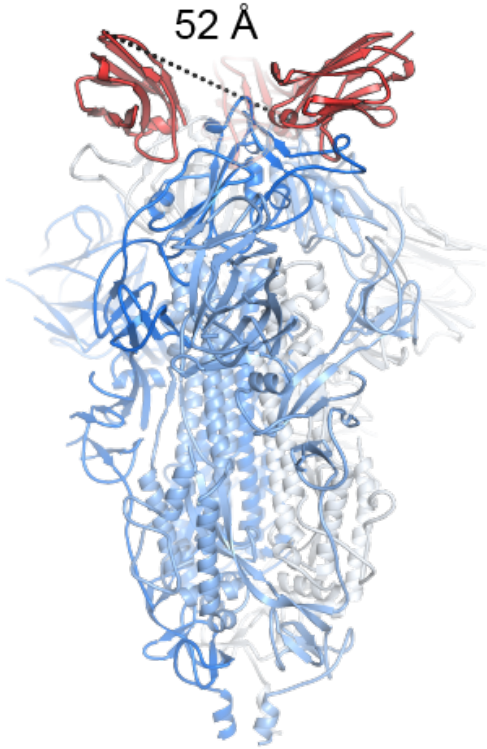


HARDER, FASTER, BETTER, STRONGER

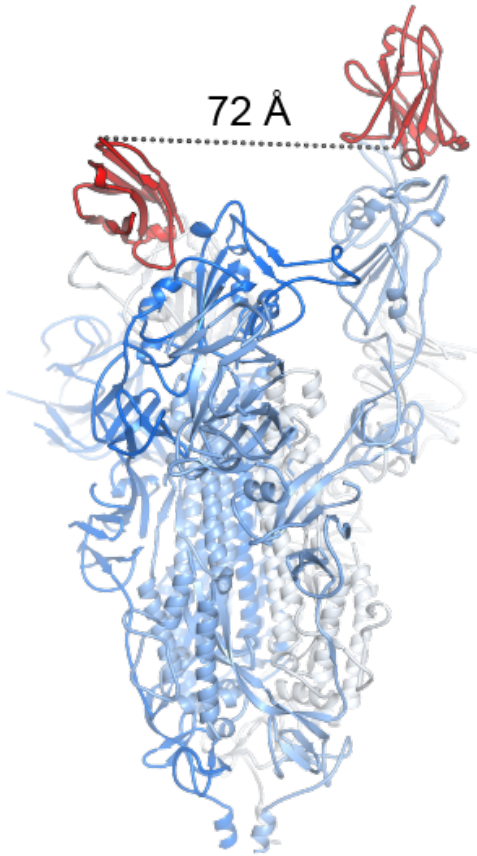


DESIGN OF MULTIVALENT NB6

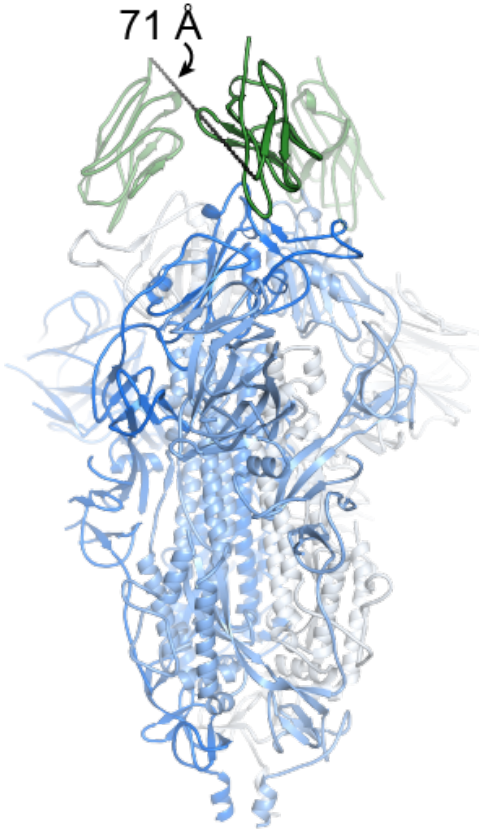
Spike* (closed)
Nb6



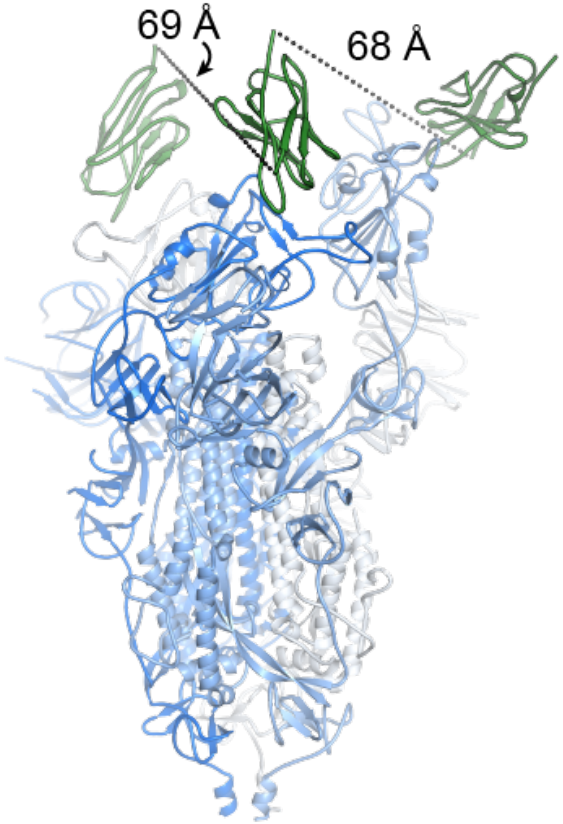
Spike* (open)
Nb6



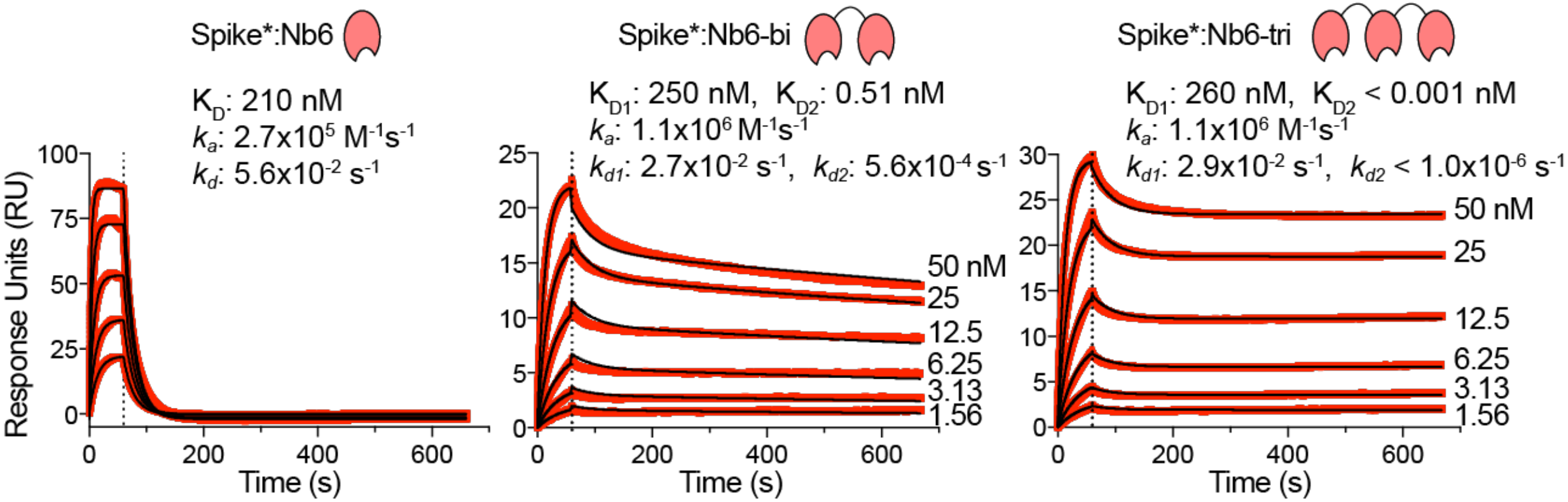
Spike* (closed)
Nb11



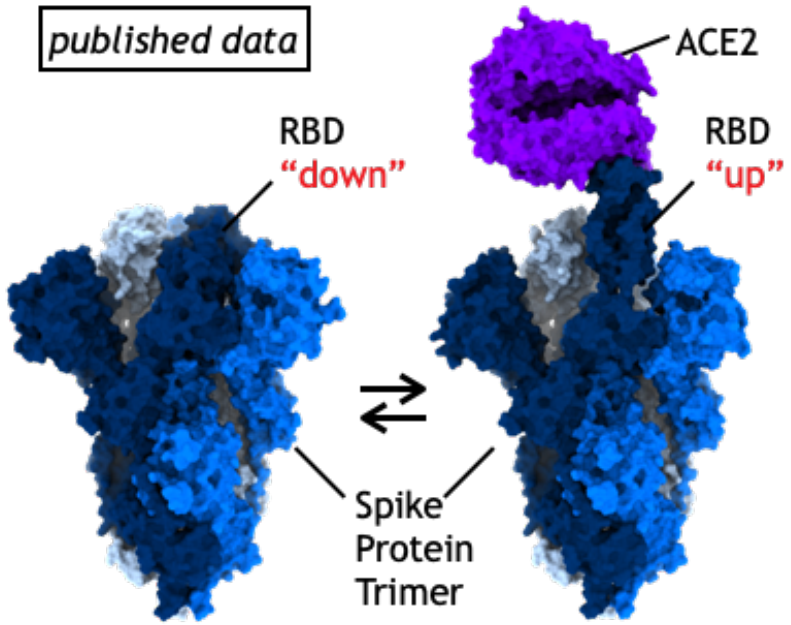
Spike* (open)
Nb11

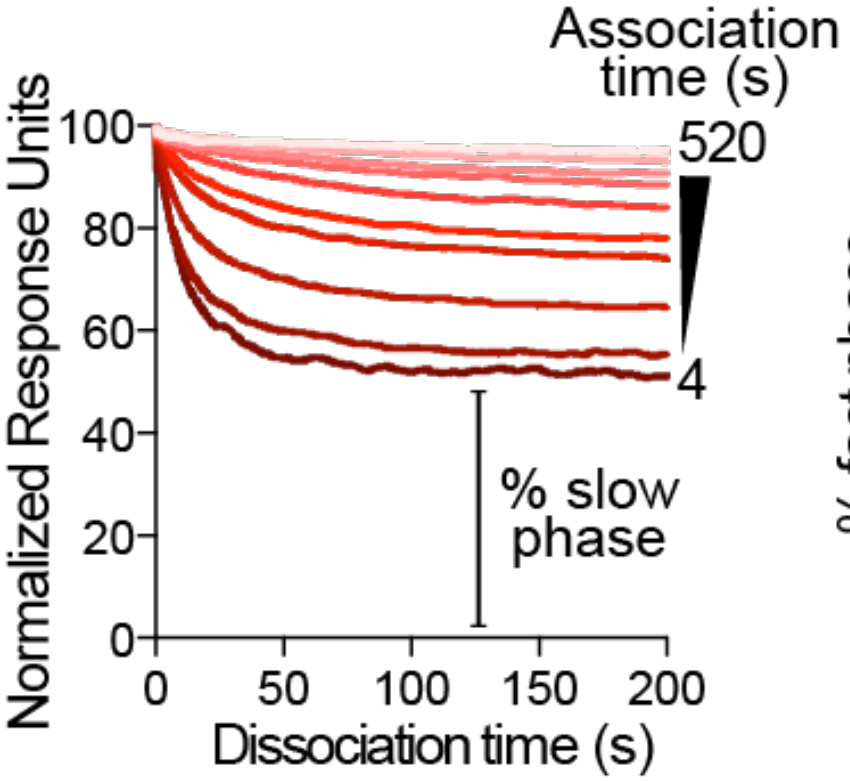


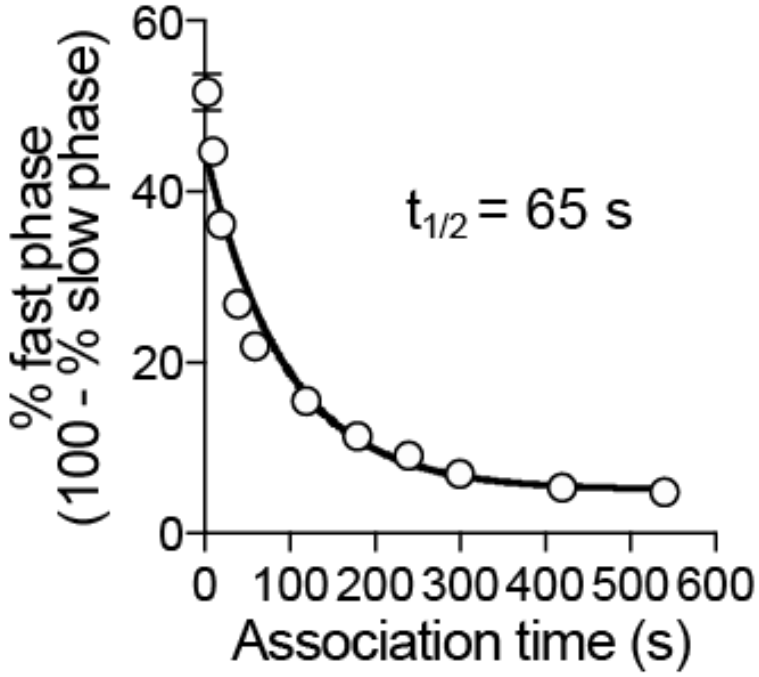
MULTIVALENCY-BASED GAINS IN POTENCY



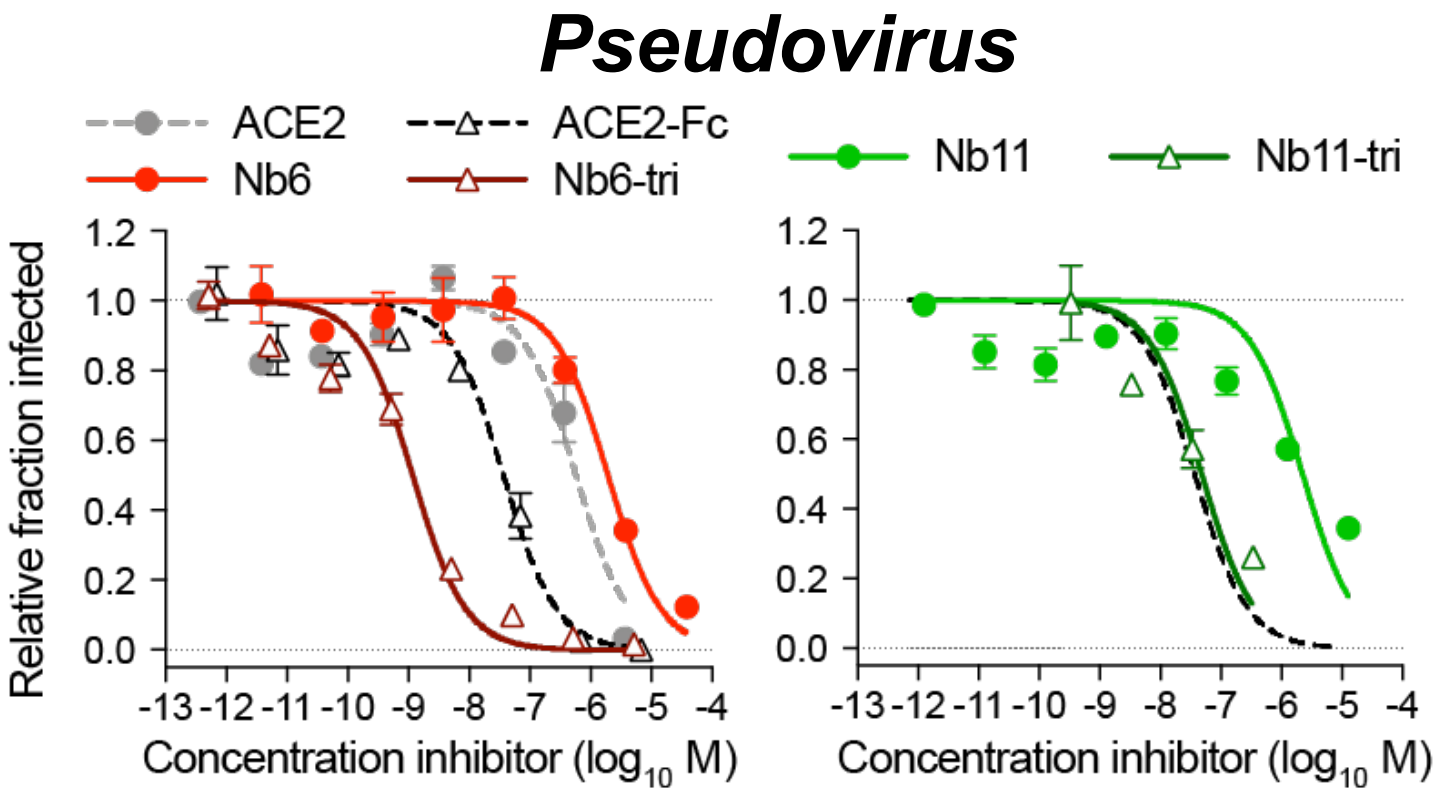
MULTIVALENT NB6 LOCKS SPIKE IN INACTIVE STATE

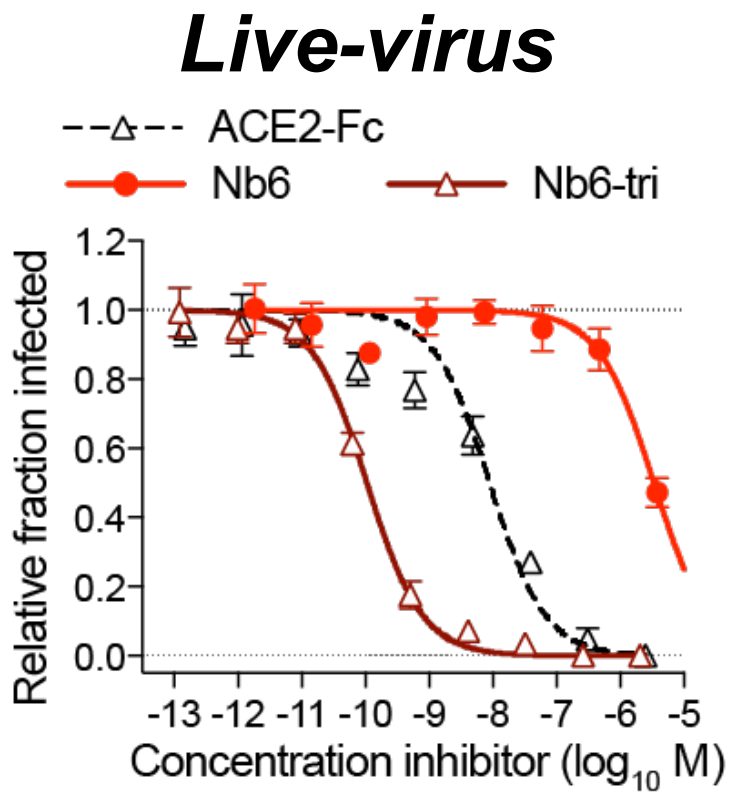




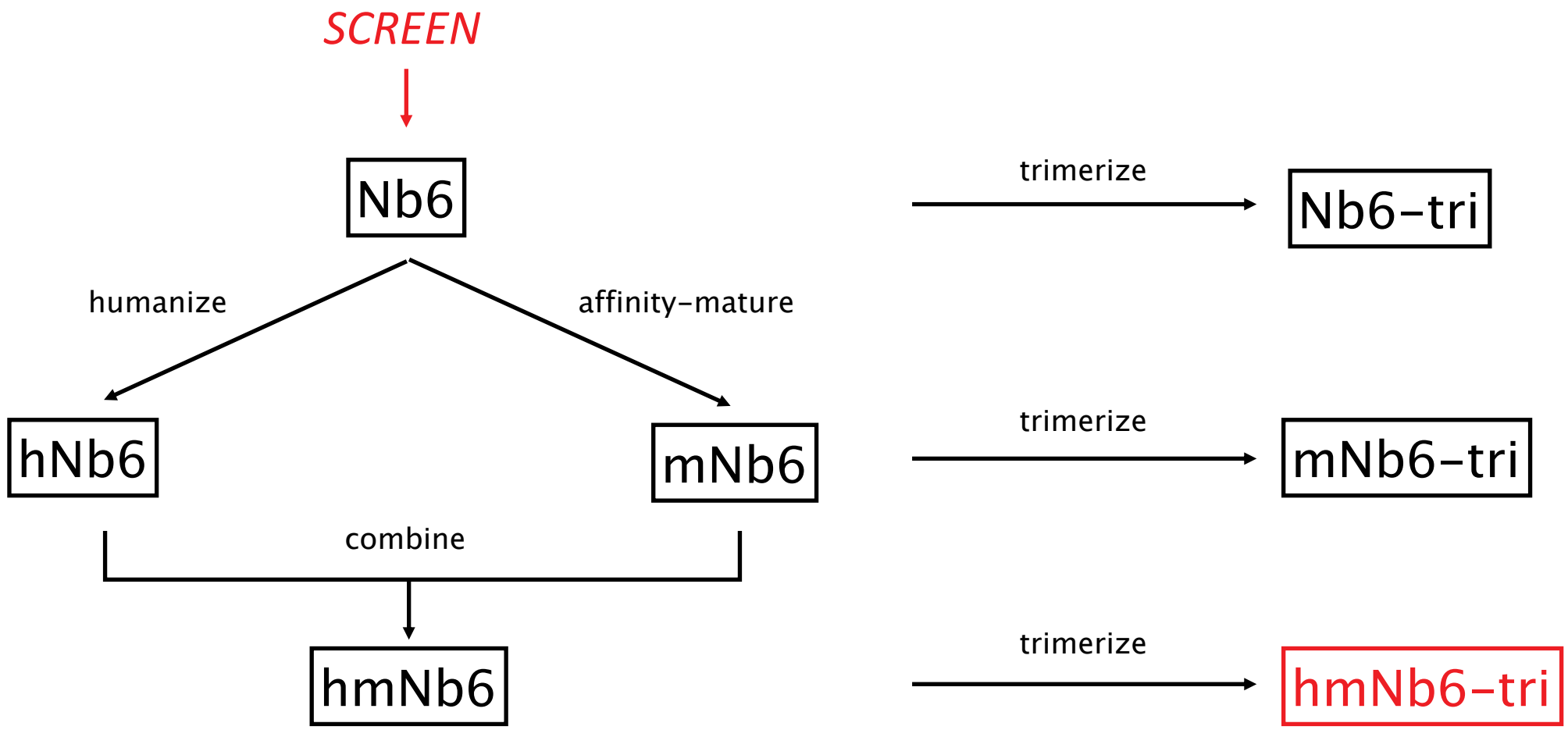


POTENT INHIBITION OF VIRAL ENTRY

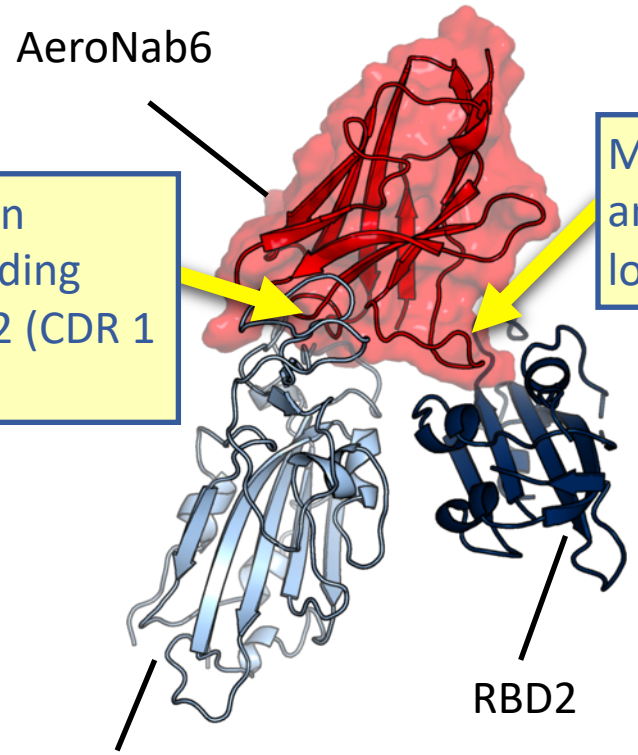




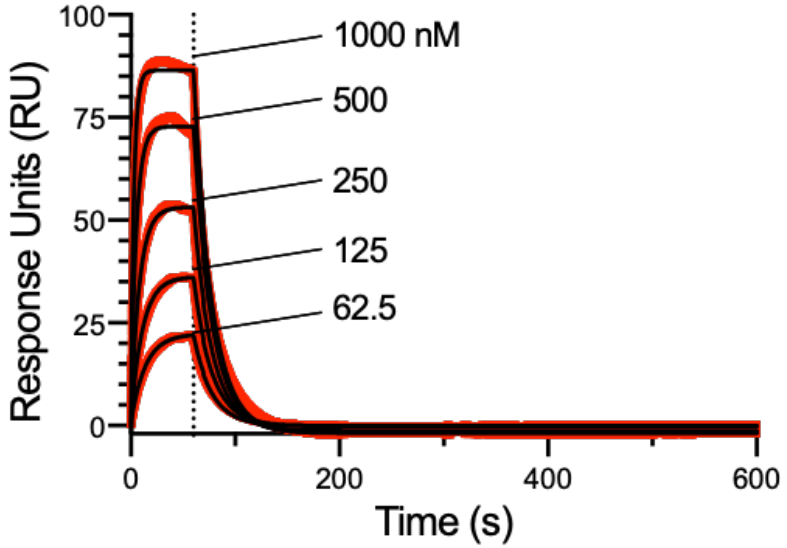
HARDER, FASTER, BETTER, STRONGER



AFFINITY MATURATION OF NB6



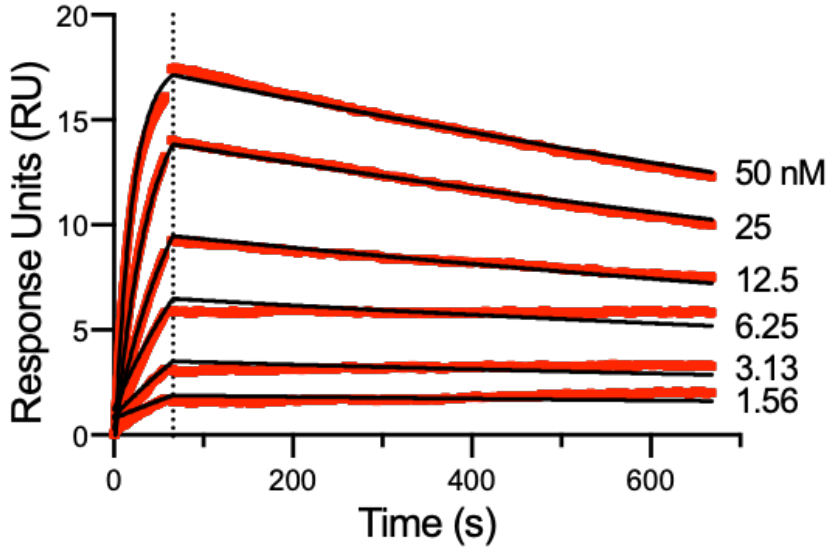
"CDR" = Complementarity-Determining Regions in AeroNab



Nb6

K_D: 210 nM

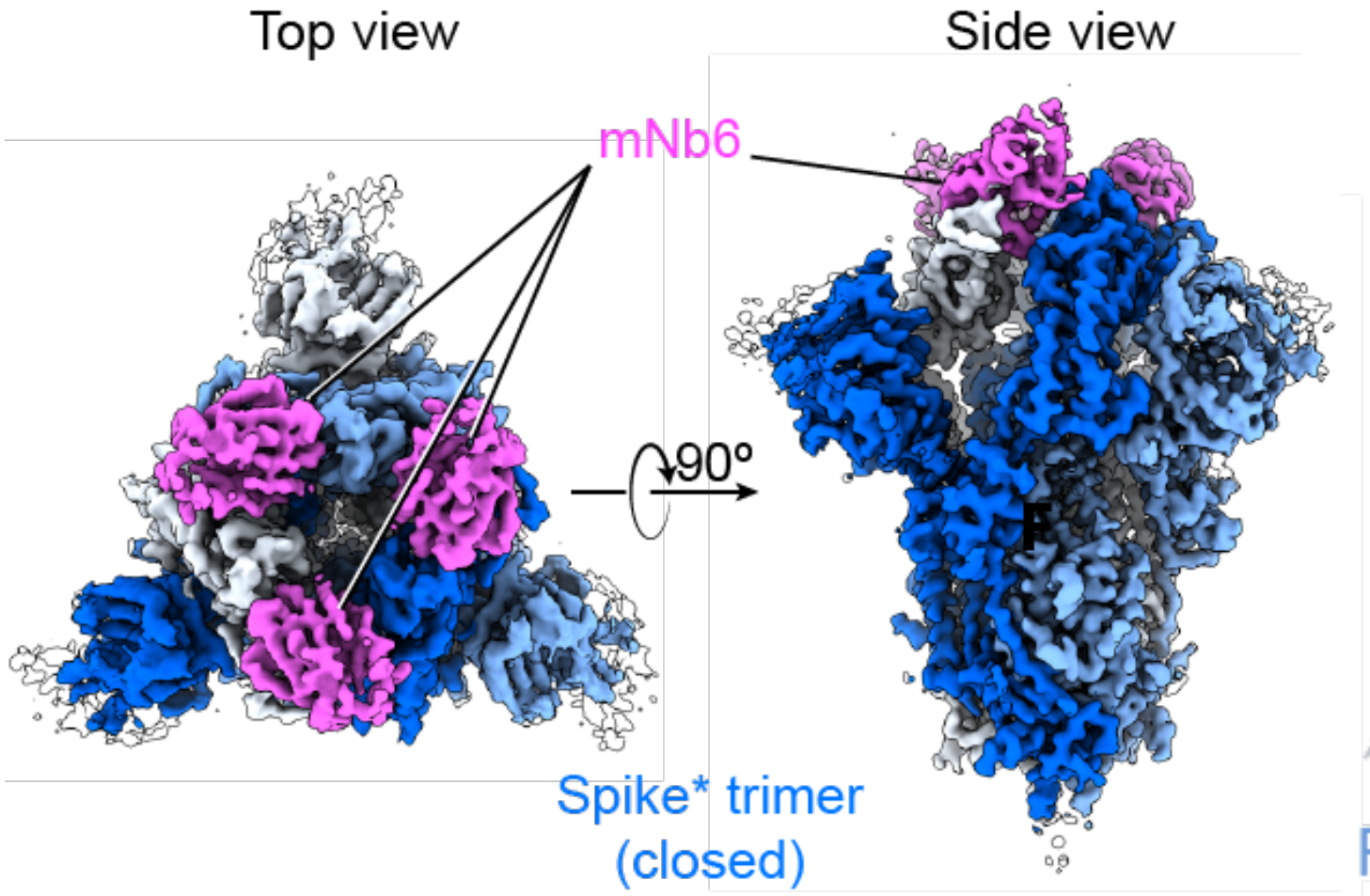
500x

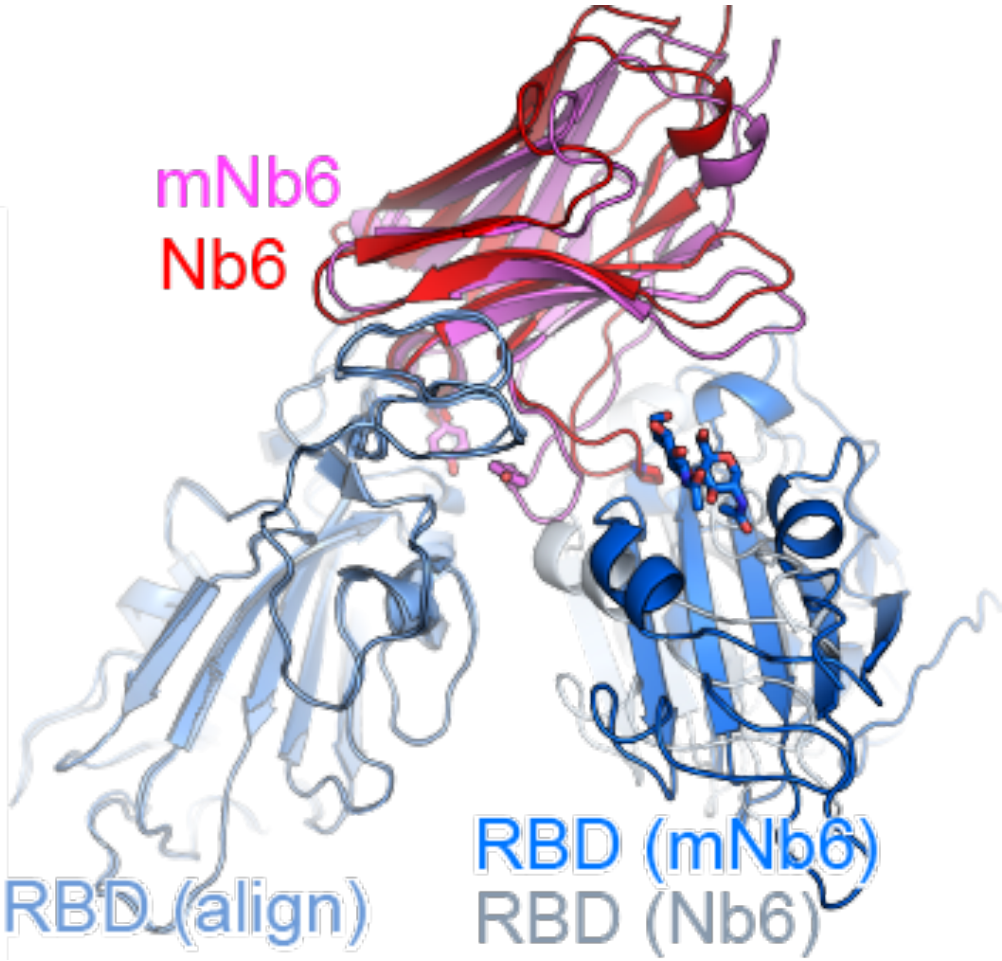


mNb6

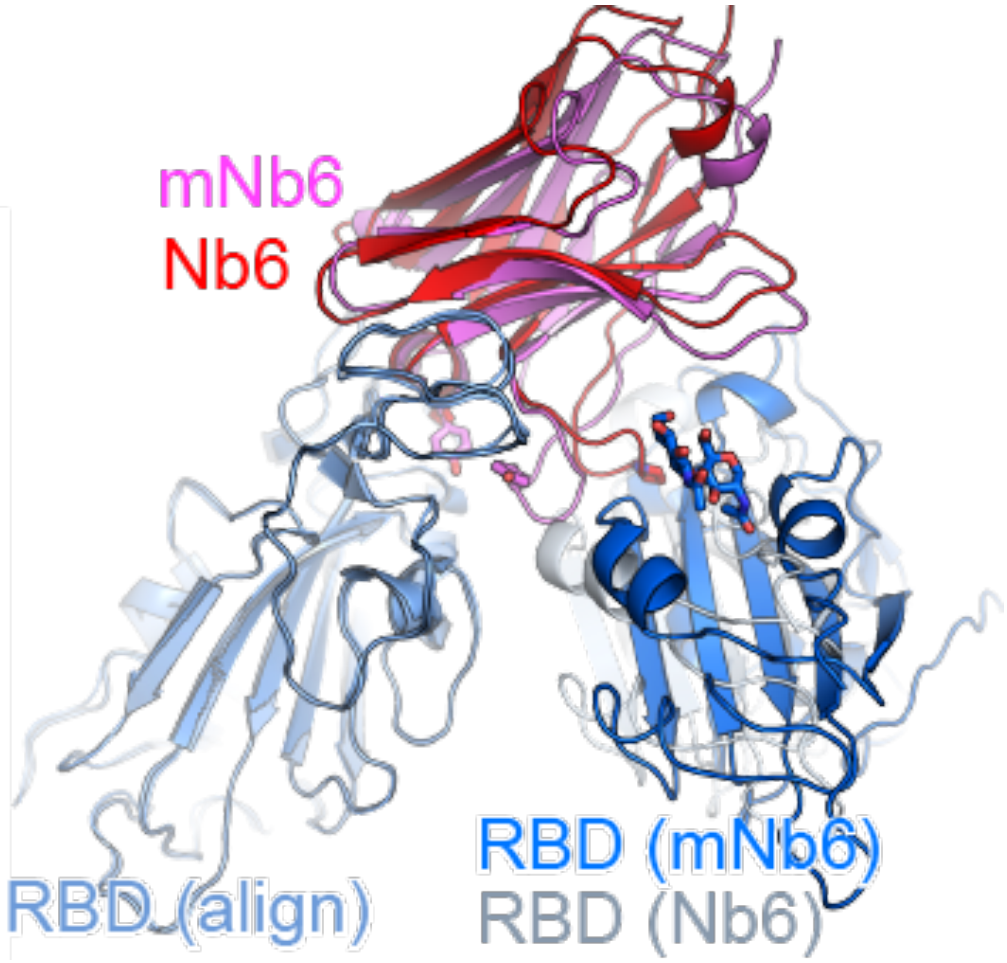
K_D: 0.45 nM

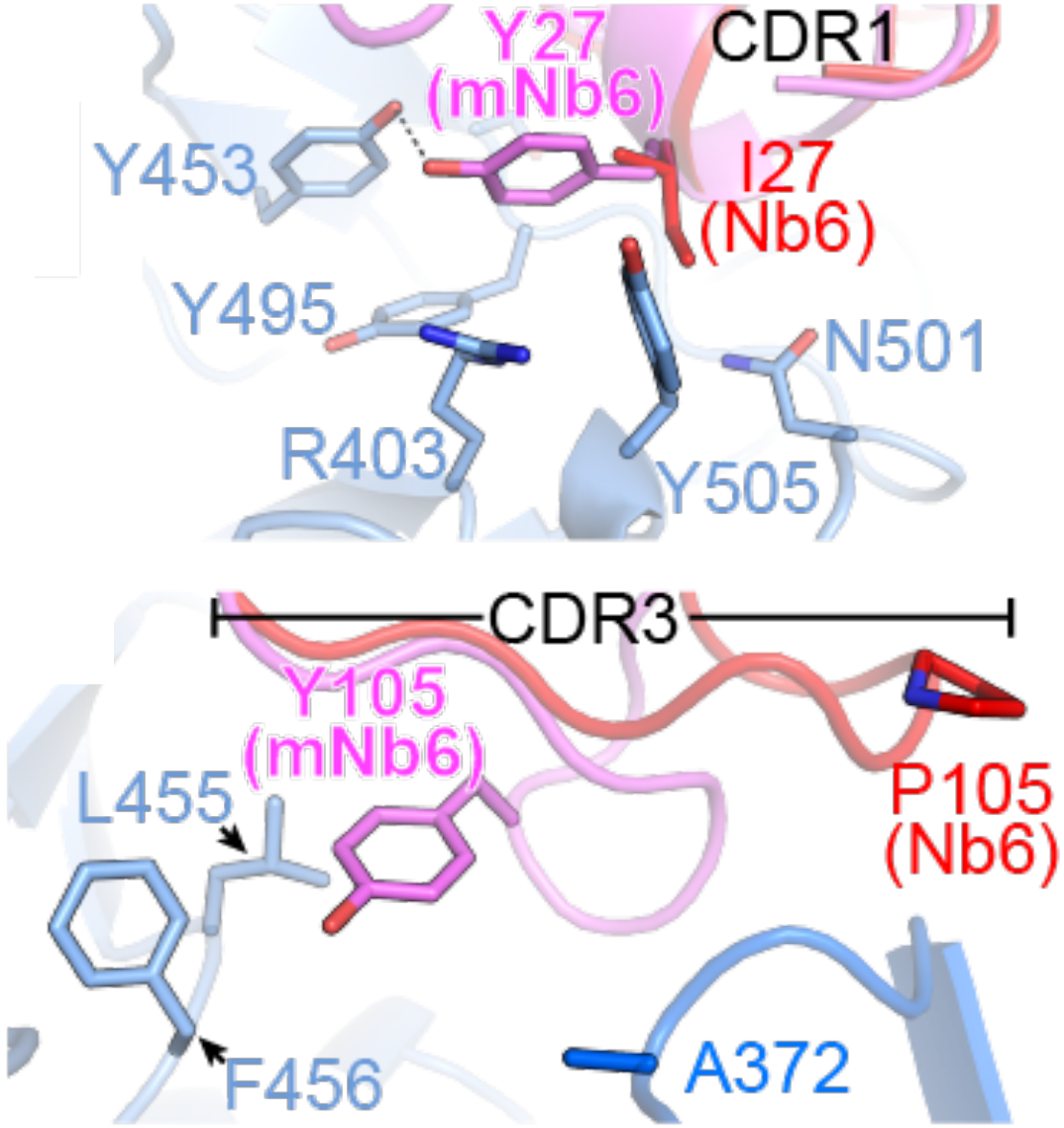
AFFINITY MATURATION OF NB6



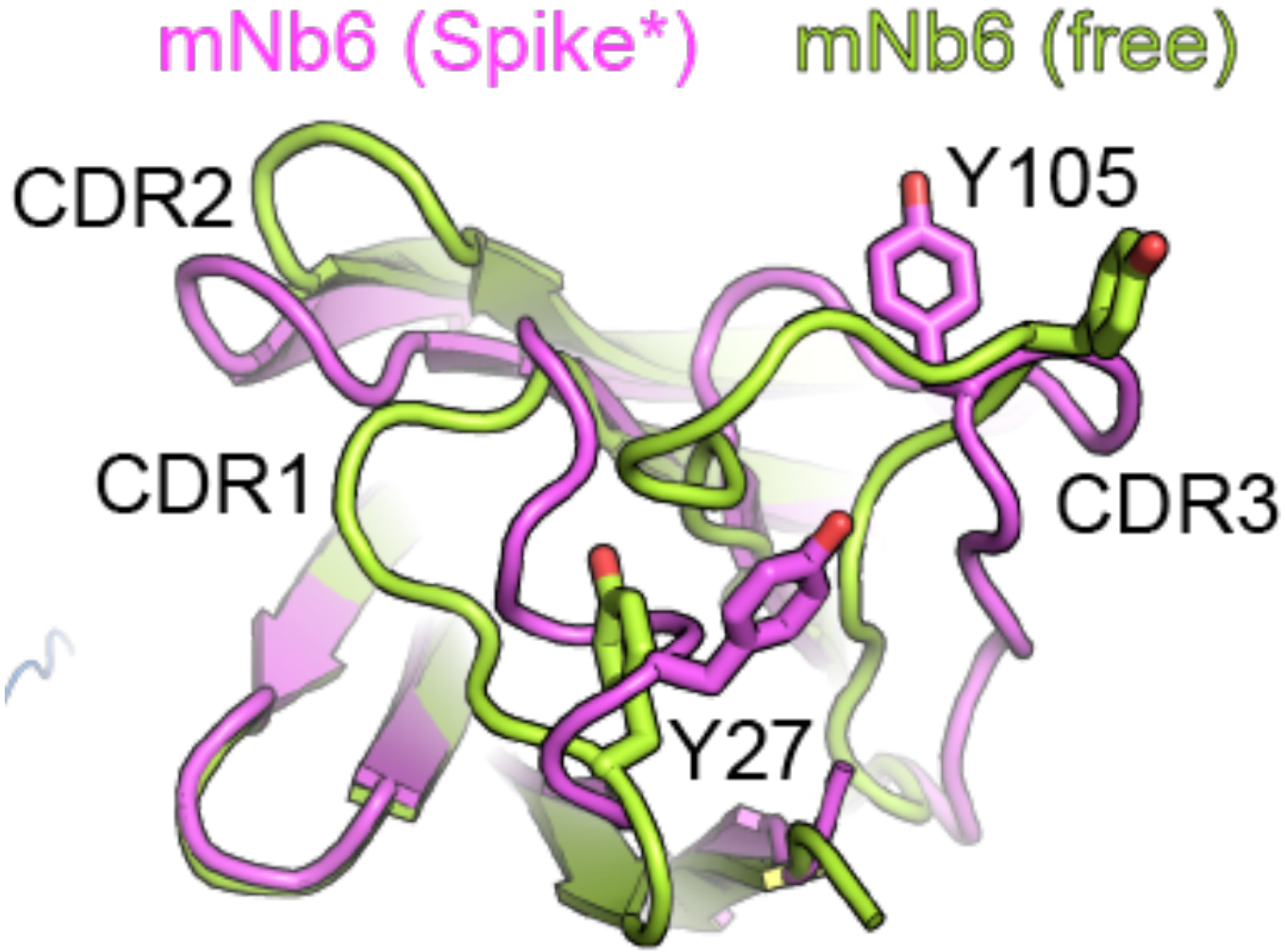


AFFINITY MATURATION OF NB6

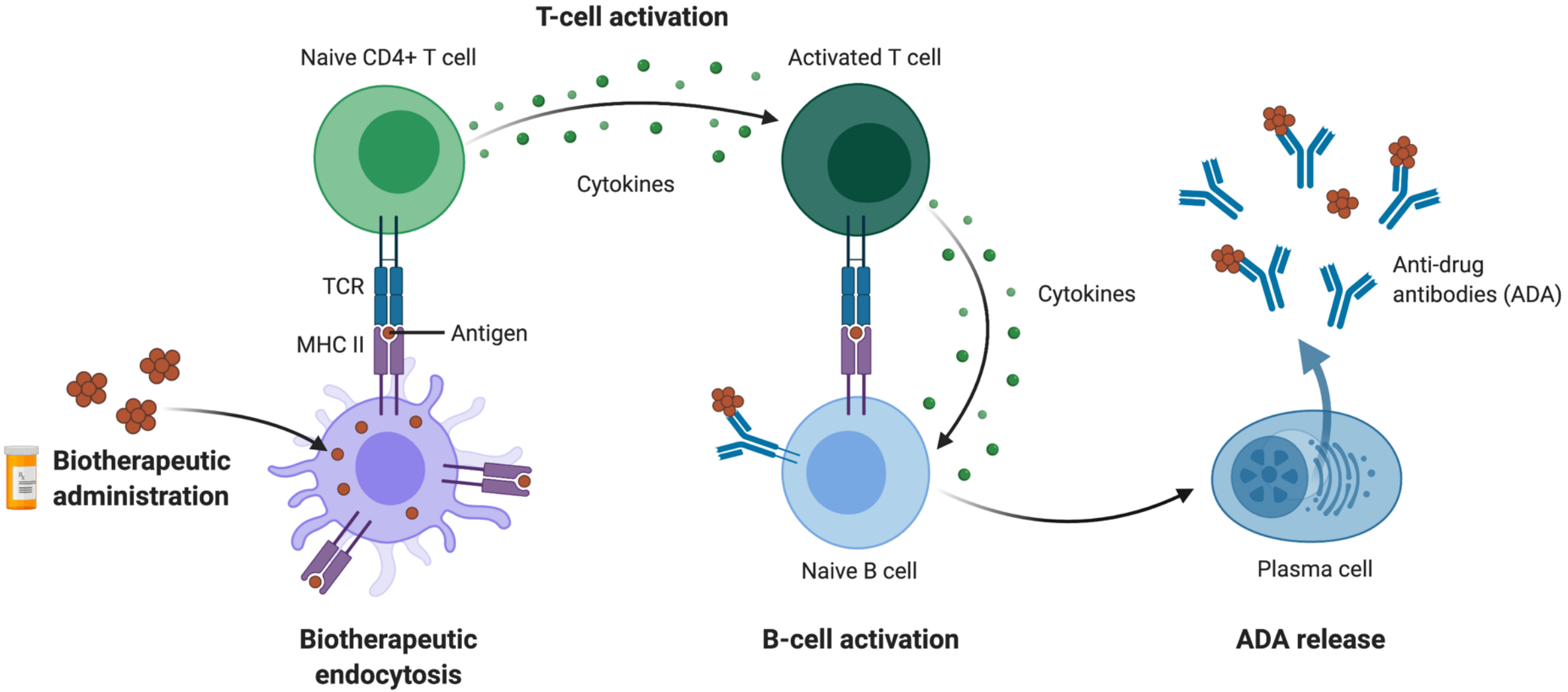




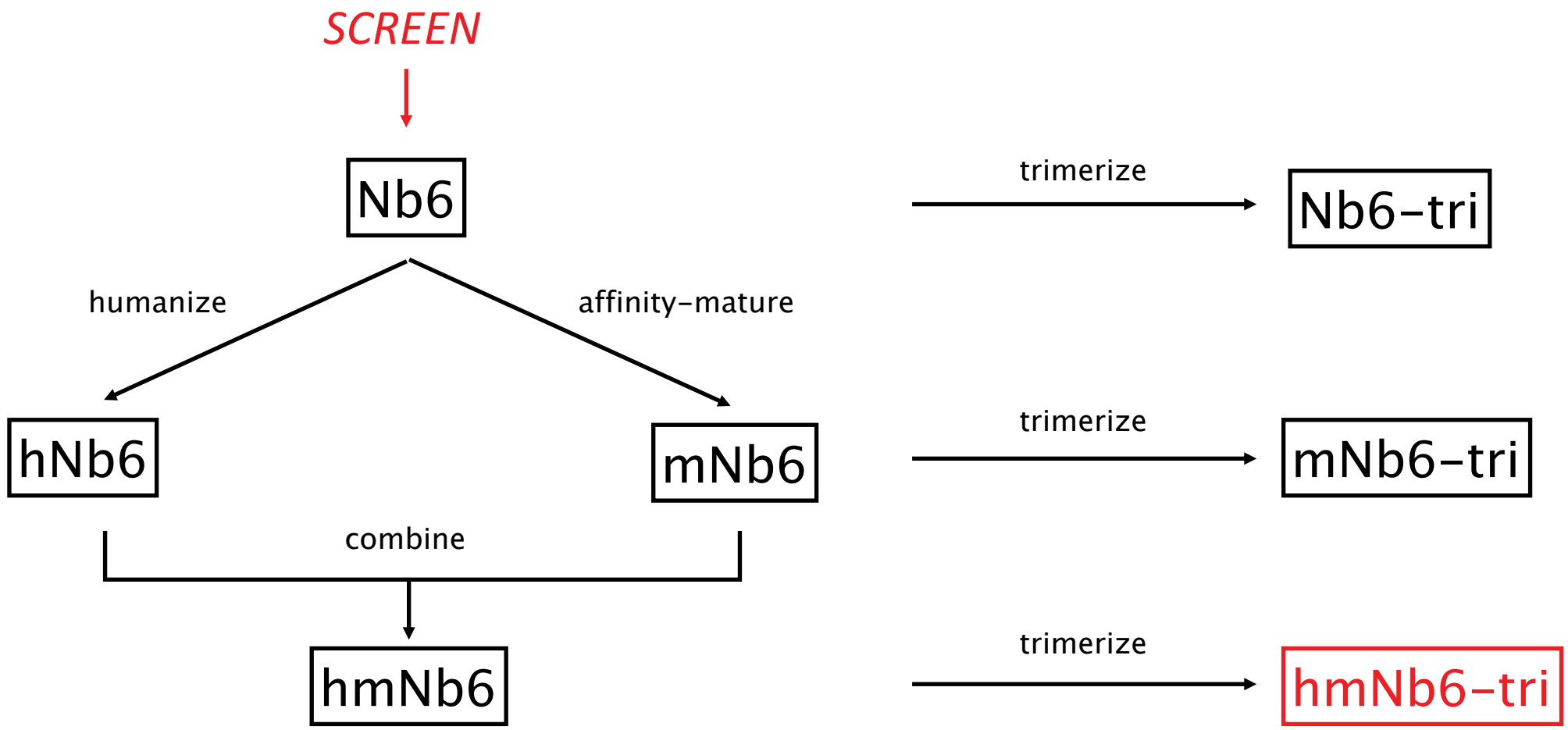
LOOP CONFORMATIONAL PLASTICITY



PROTEIN-BASED DRUGS CAN BE IMMUNOGENIC

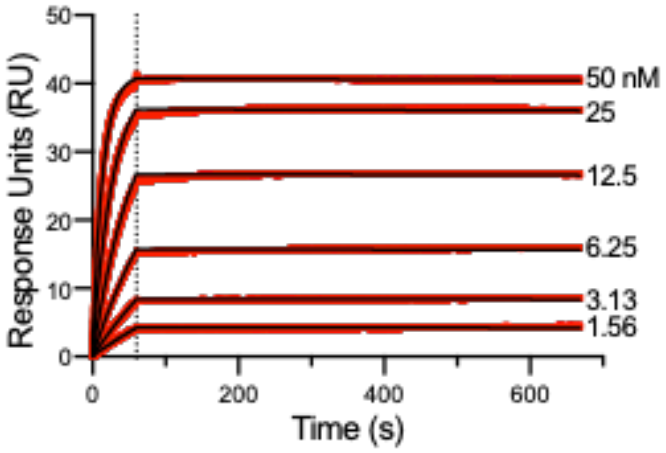


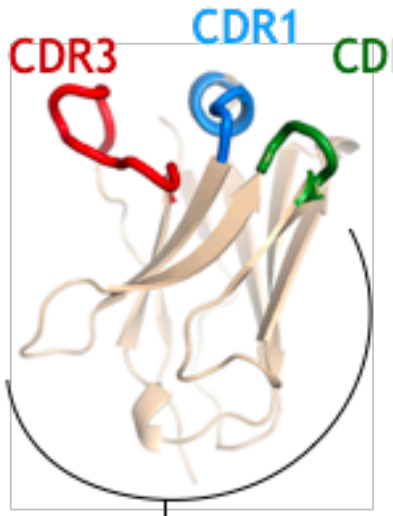
HARDER, FASTER, BETTER, STRONGER



NANOBODY "HUMANIZATION"

mNb6-tri

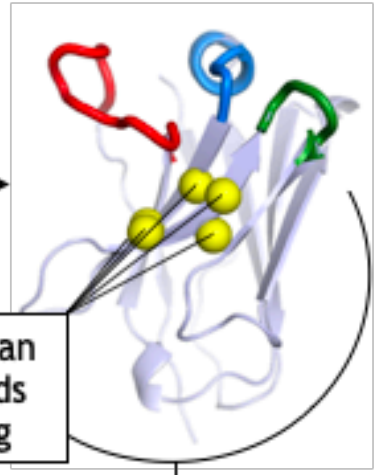




Llama nanobody Framework

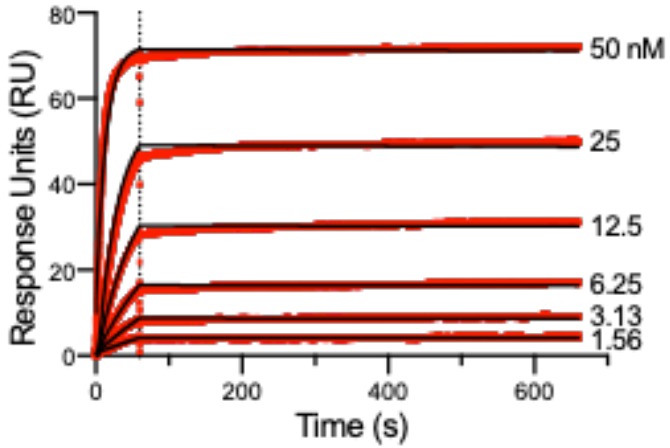
Transplant CDRs

5 non-human amino acids remaining



Human heavy chain Framework (IGHV3-66)

Humanized mNb6-tri



K_D: <0.001 nM

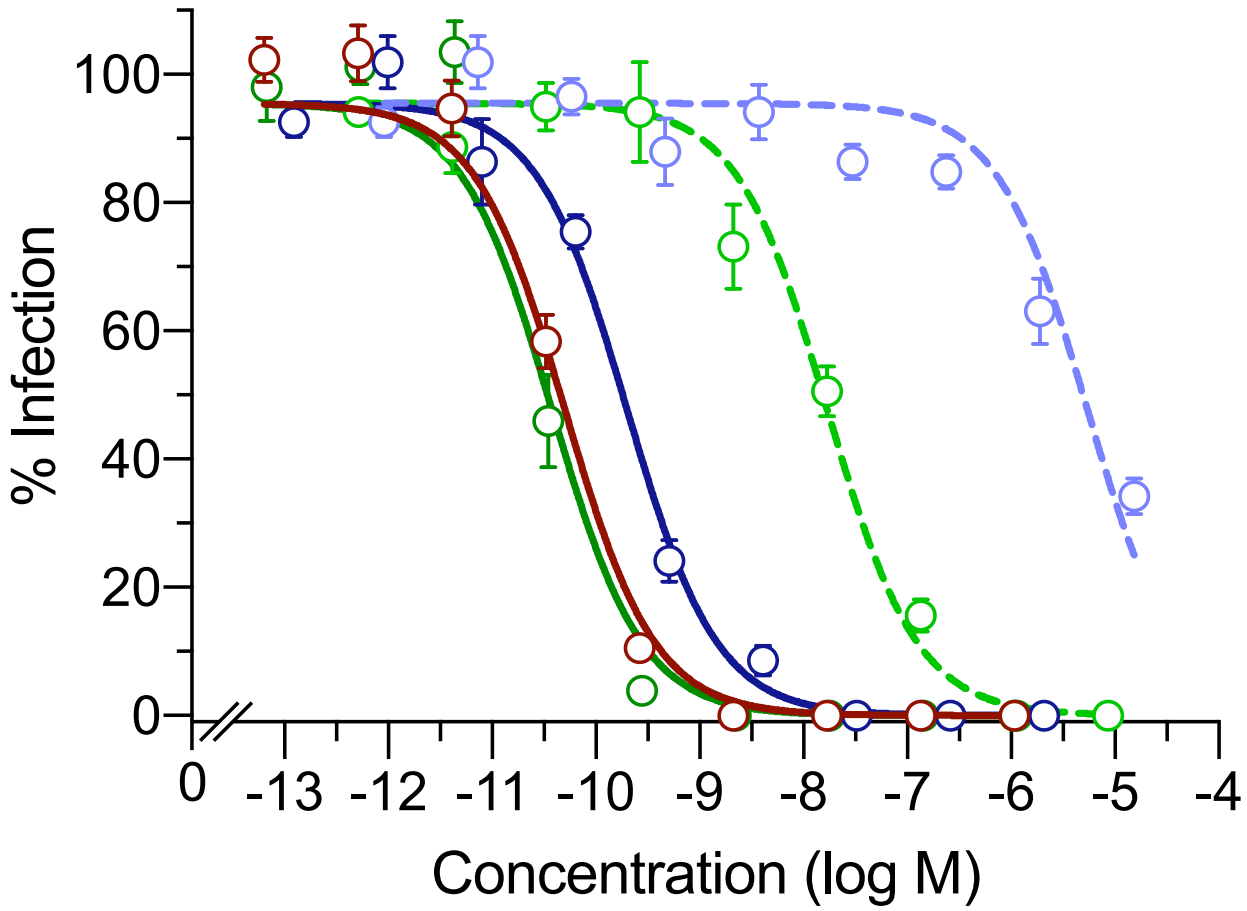
$t_{1/2}$ = >8 *days*

Humanized AeroNab6$_{mh}$x3 is only five amino acids different from fully human heavy chain gene IGHV3-66.

K_D: <0.001 nM

$t_{1/2}$ = >8 *days*

NEUTRALIZATION ACTIIVTY OF DESIGNED NANOBODIES



Nb6 (5500 nM; 70 µg/mL)

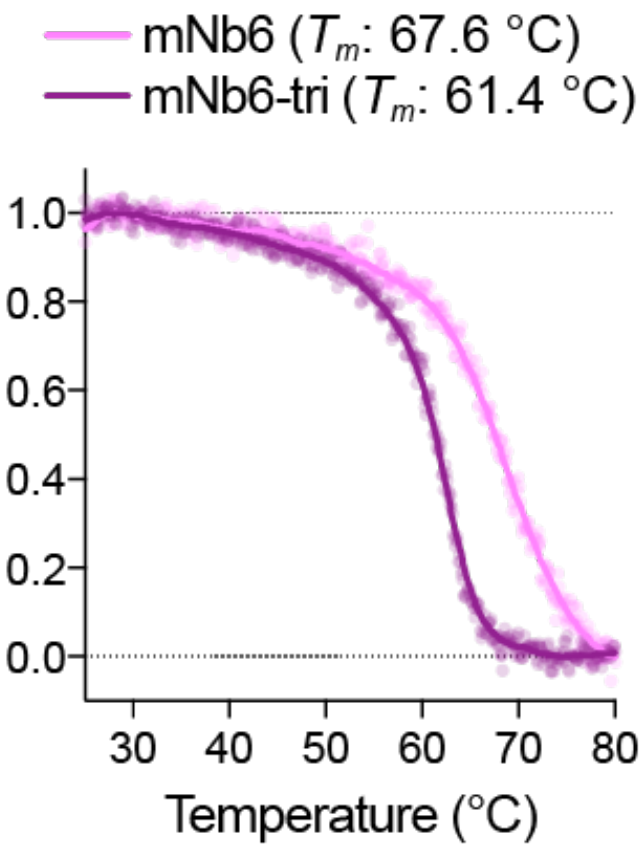
Nb6-tri (0.2 nM; 7.6 ng/mL)

mNb6 (17 nM; 215 ng/mL)

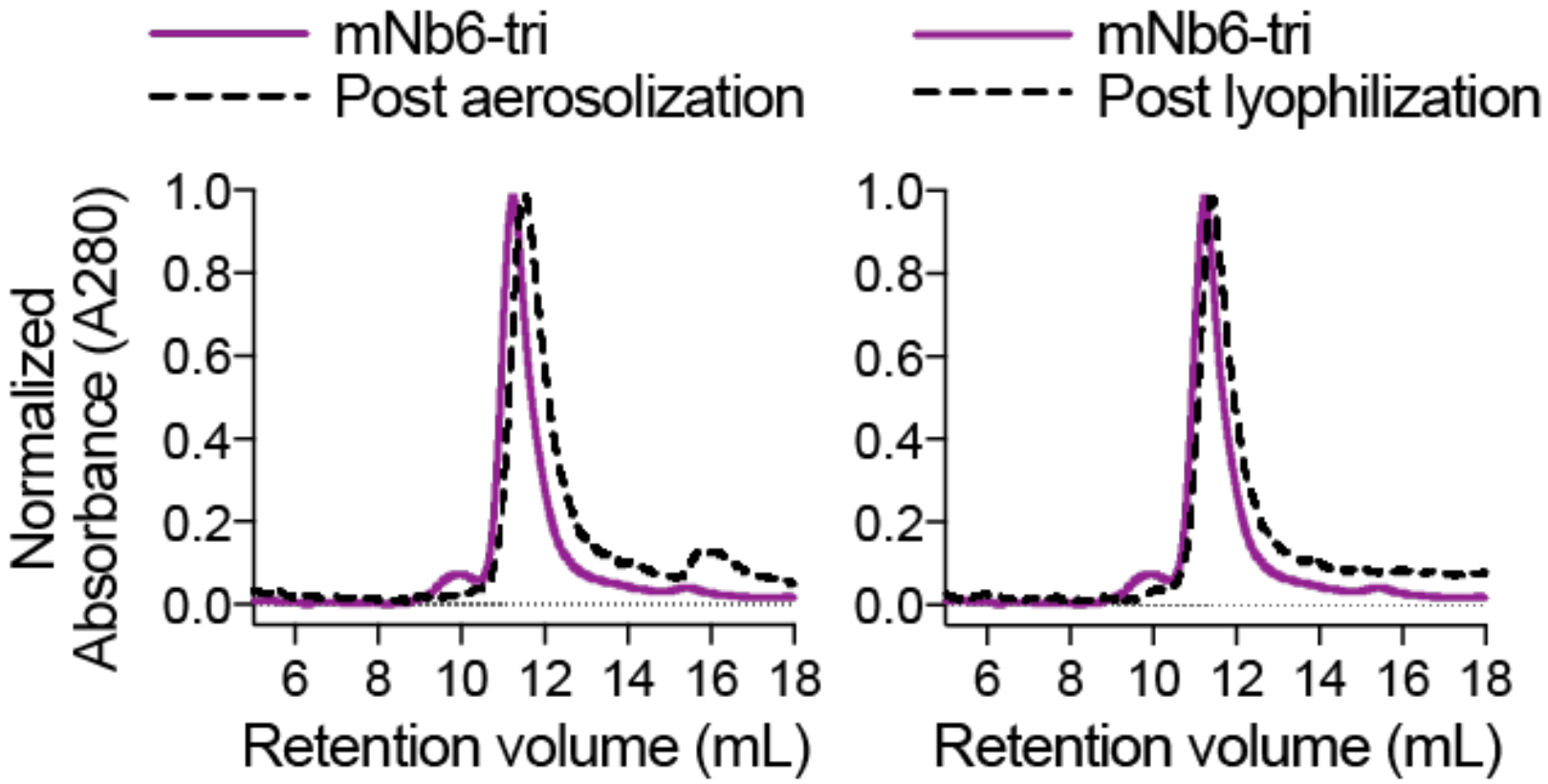
mNb6-tri (0.03 nM; 1.1 ng/mL)

hmNb6-tri (0.05 nM; 2.0 ng/mL)

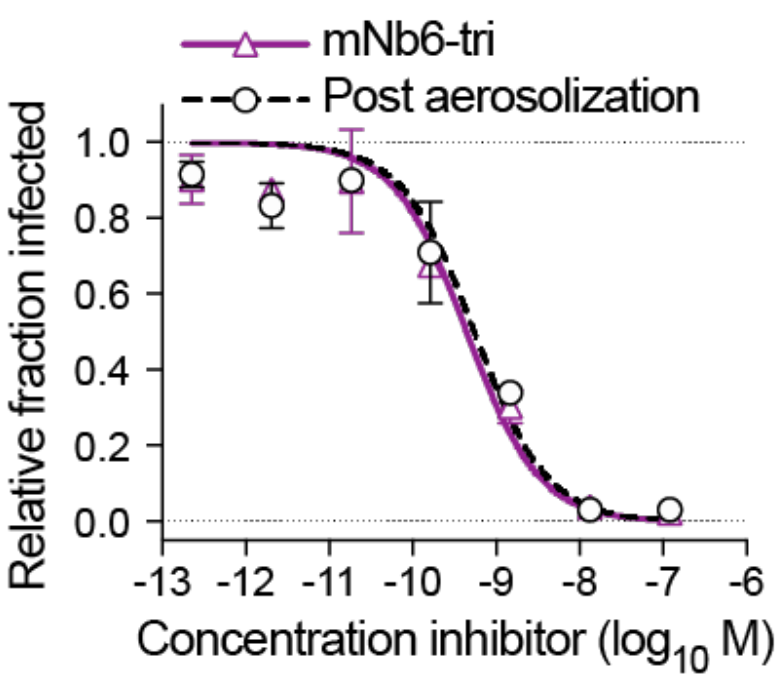
NANOBODIES ARE STABLE FOR AEROSOL DELIVERY

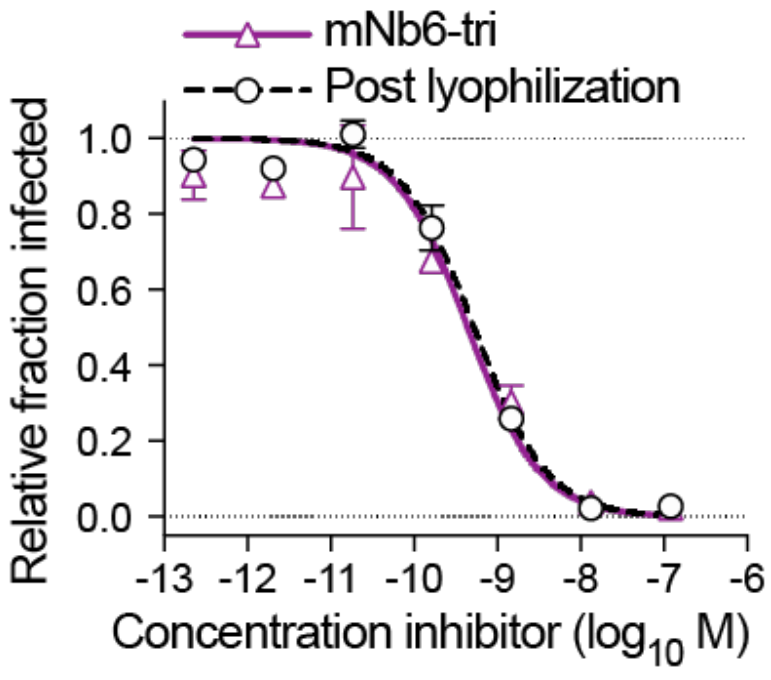


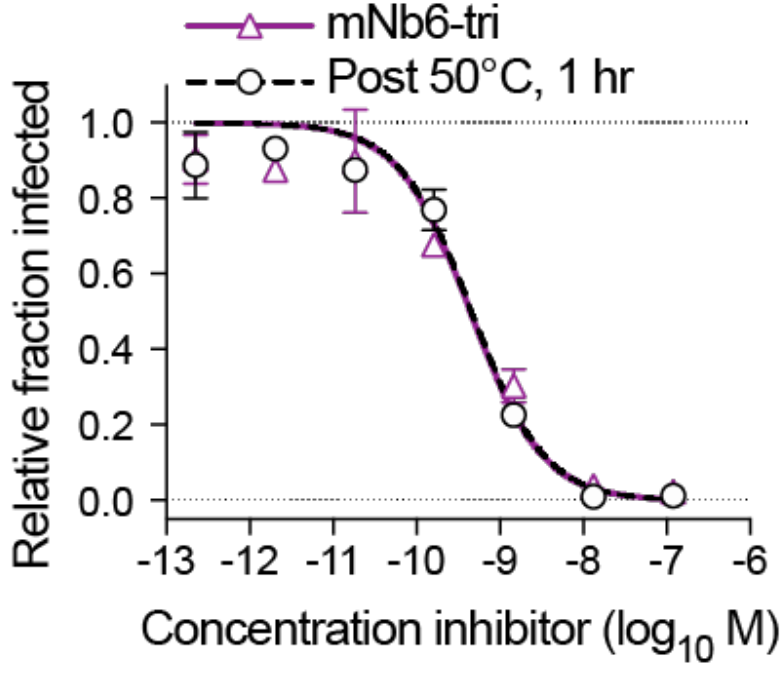
NANOBODIES ARE STABLE FOR AEROSOL DELIVERY



NANOBODIES ARE STABLE FOR AEROSOL DELIVERY







AN ALTERNATIVE APPROACH TO PASSIVE IMMUNITY

Patients with COVID-19

Advantages:

- Self administered
- Direct delivery to site of early infection

Challenges:

- Ultrastable protein required
- Pharmacokinetics?

CROSSING THE TRANSLATIONAL VALLEY OF DEATH

OUR TEAM

AERONAB TEAM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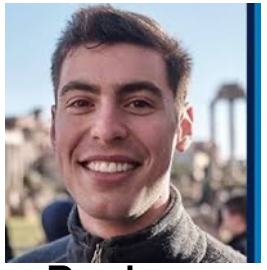
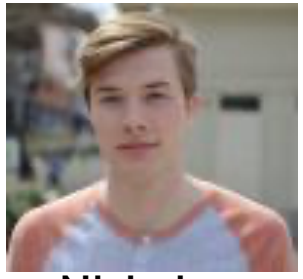
Michael Schoof

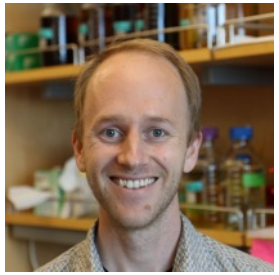

Bryan Faust


Reuben Saunders


Smriti Sangwan


Veronica Rezelj


Nicholas Hoppe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Morgane Boone

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