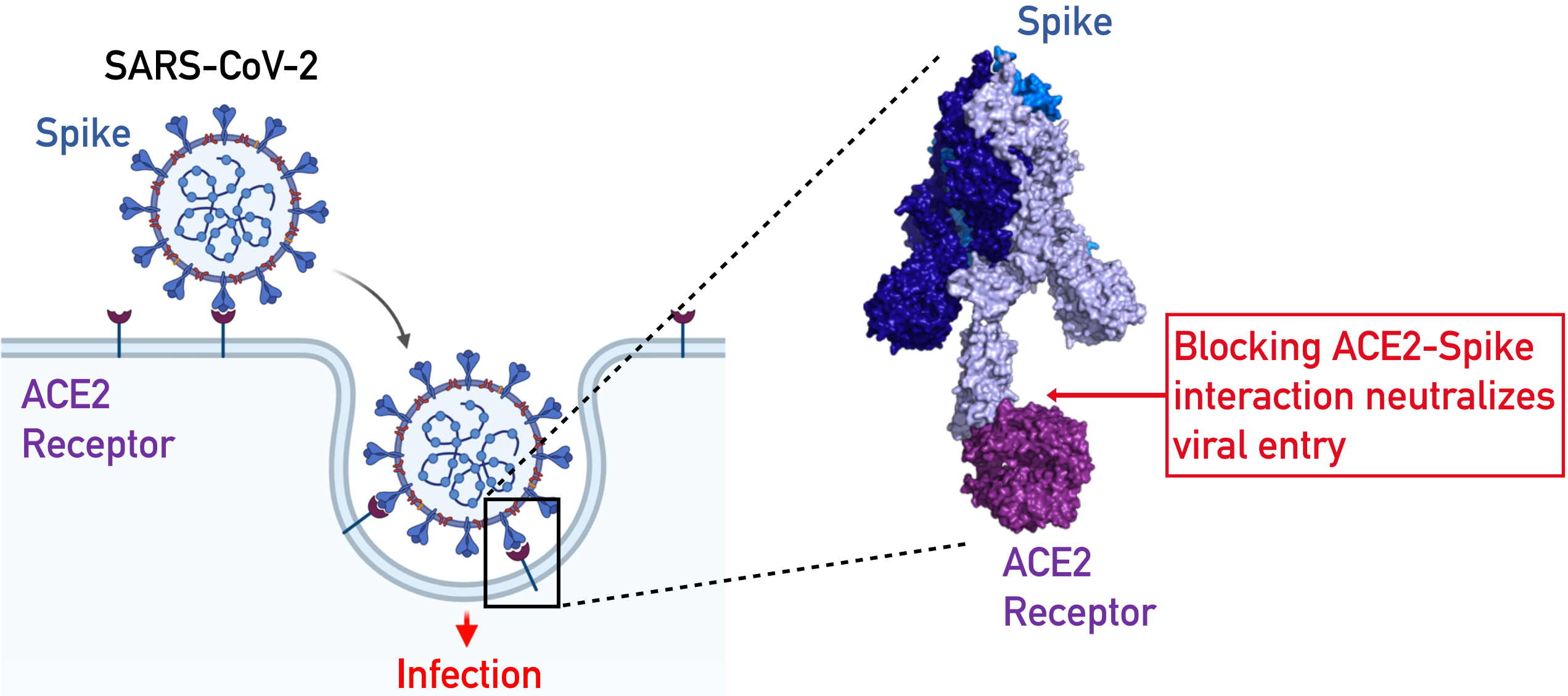
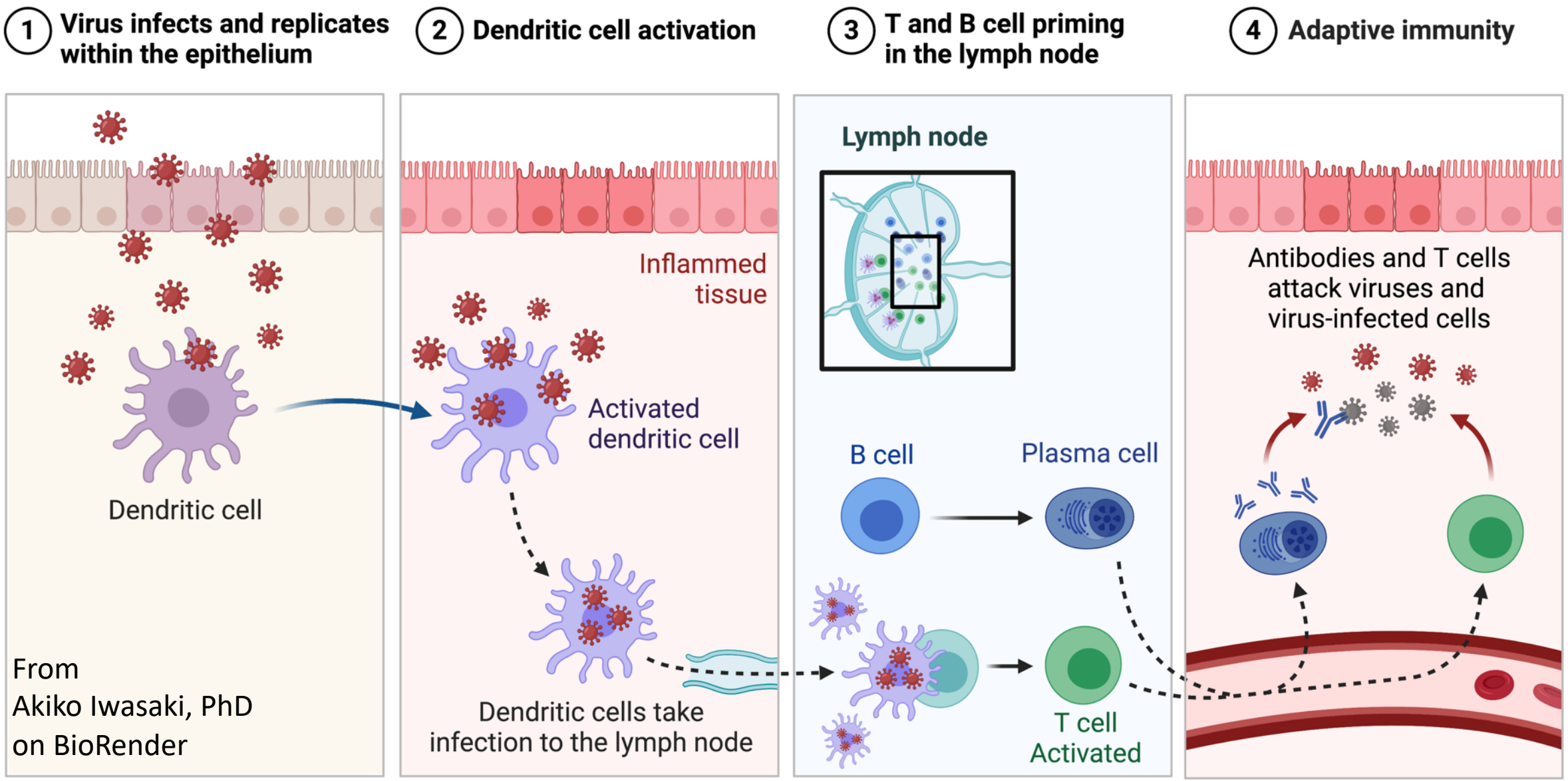
BLOCKING SARS-COV-2 ENTRY

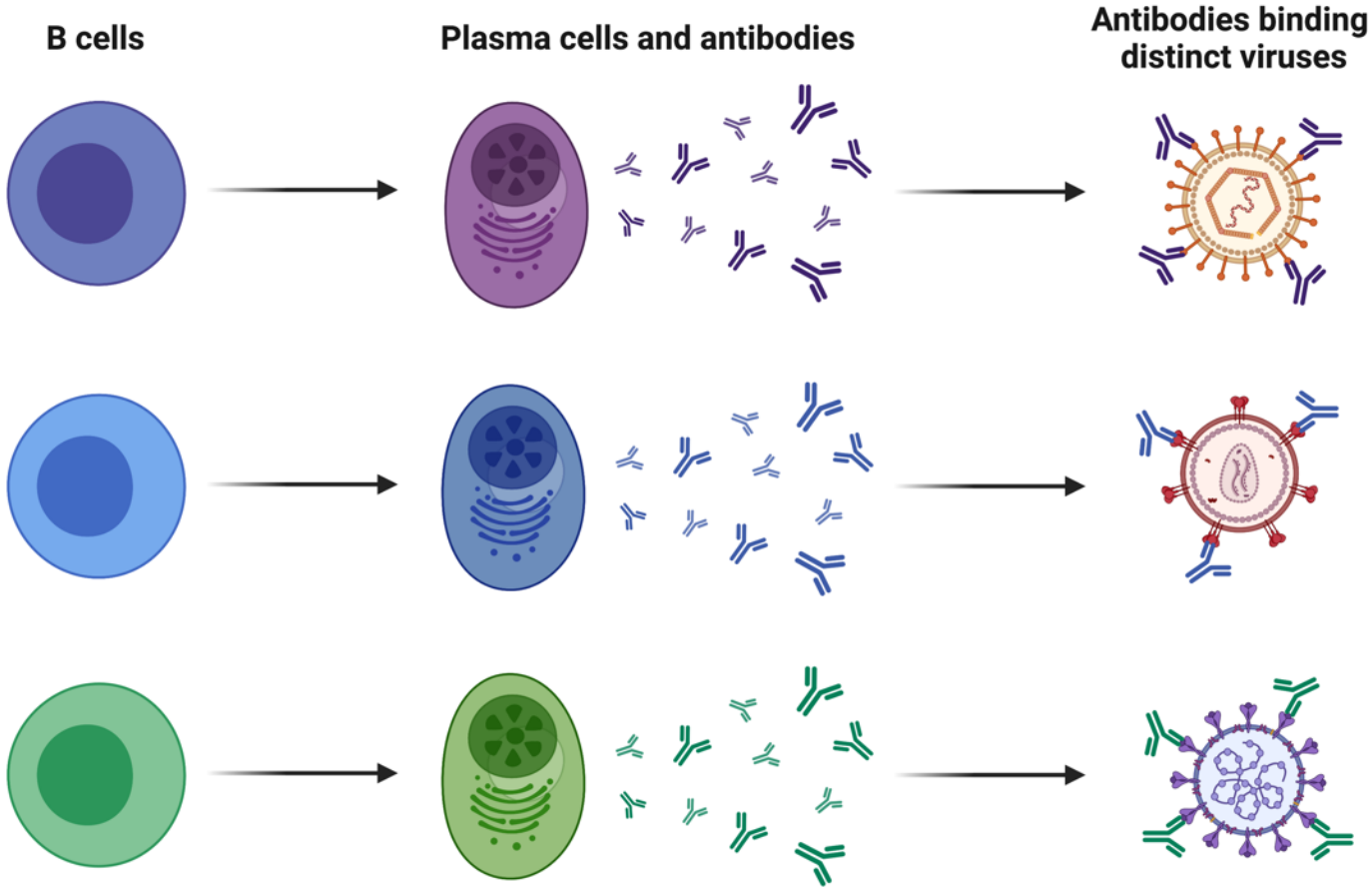


ADAPTIVE IMMUNITY

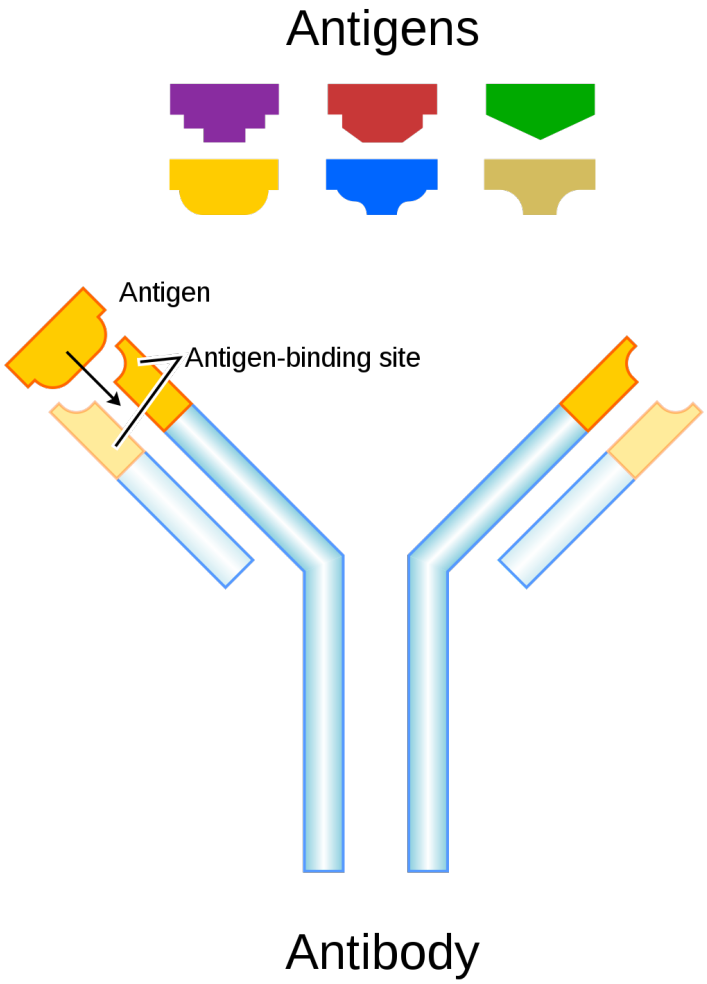


From
Akiko Iwasaki, PhD
on BioRender

ADAPTIVE IMMUNITY

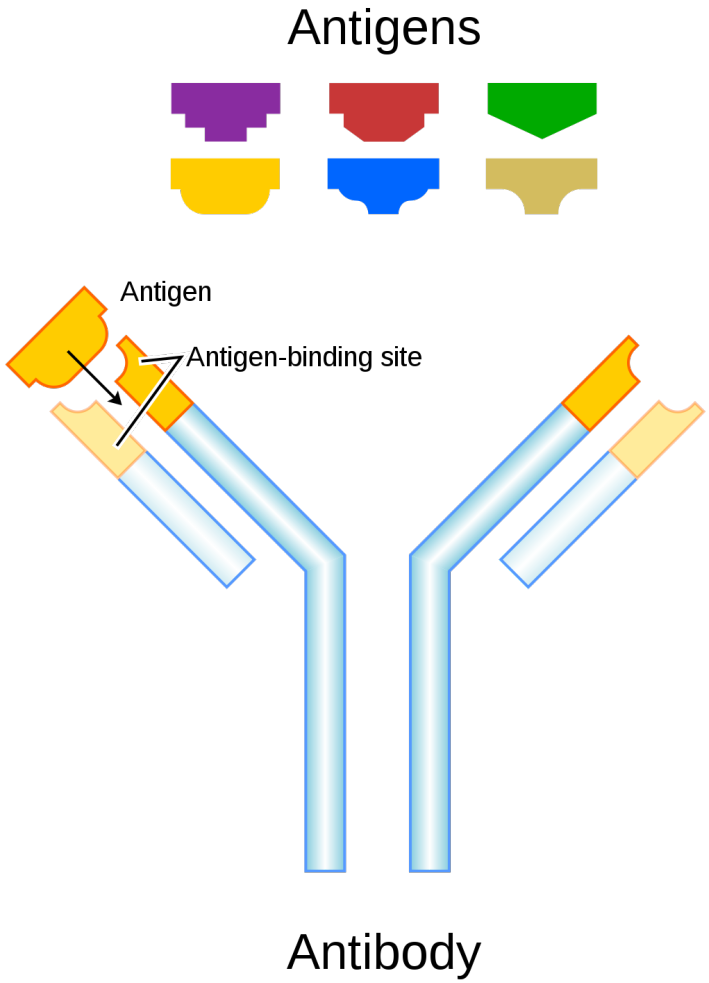


From
Akiko Iwasaki, PhD
on BioRender

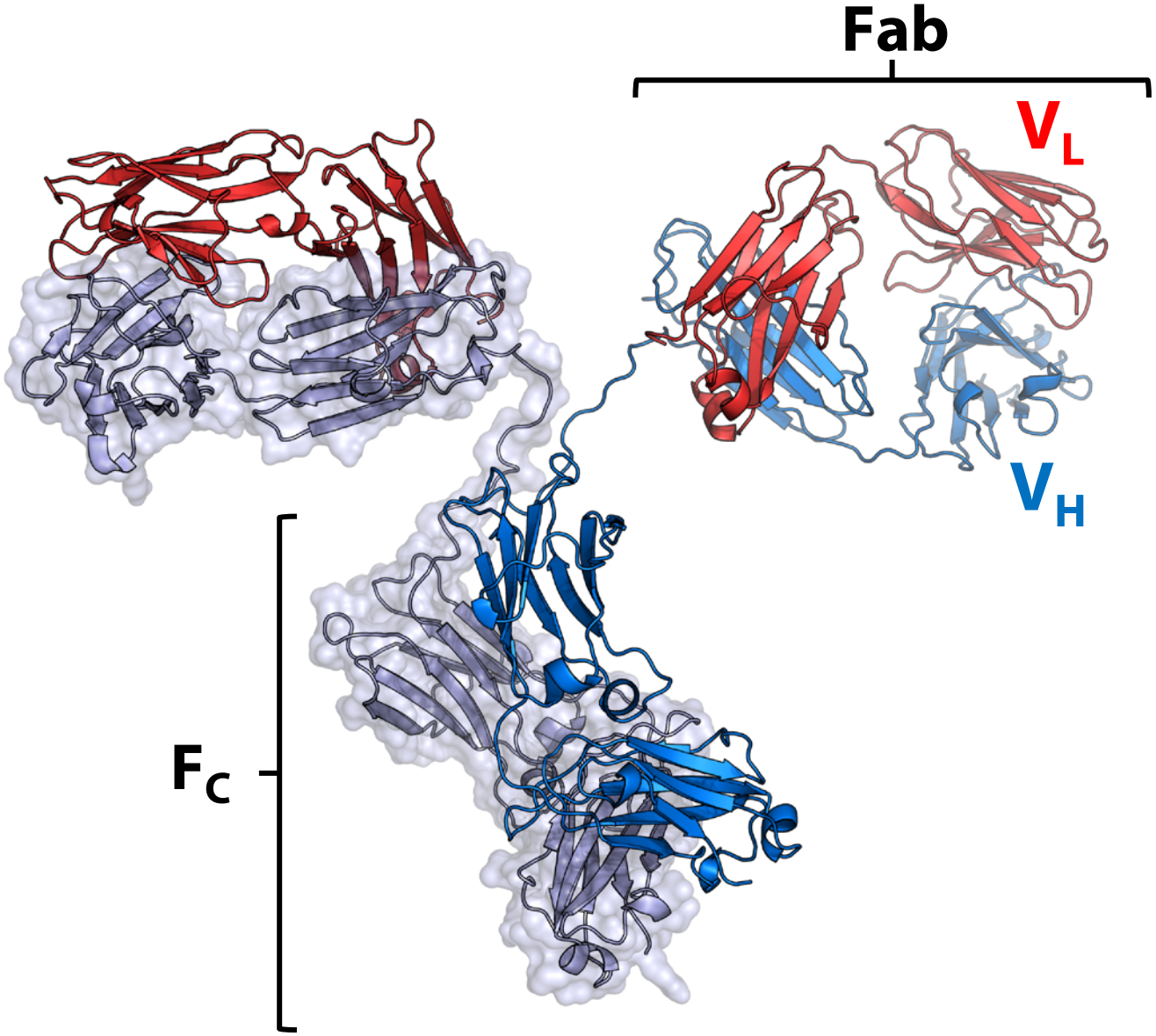


1 trillion possibilities!

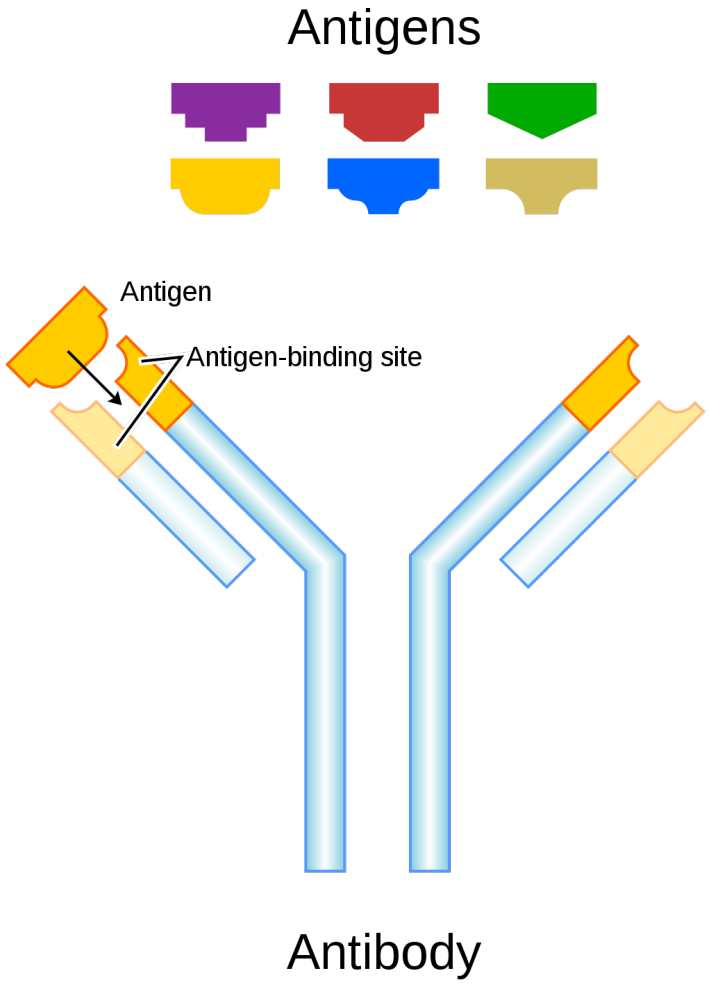
ADAPTIVE IMMUNITY – ANTIBODY STRUCTURE



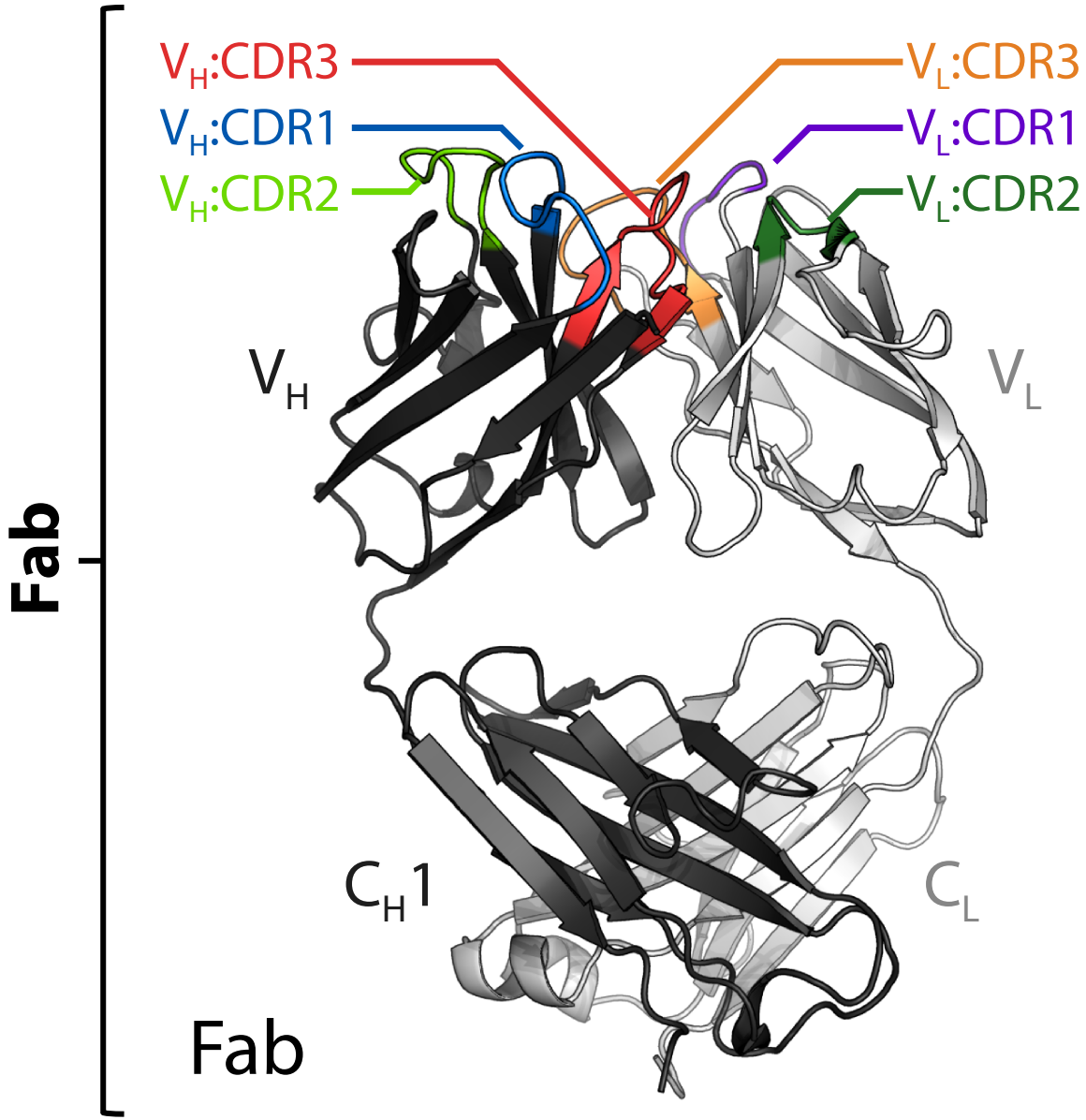
1 trillion possibilities!



ADAPTIVE IMMUNITY – ANTIBODY STRUCTURE

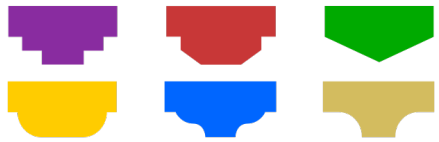


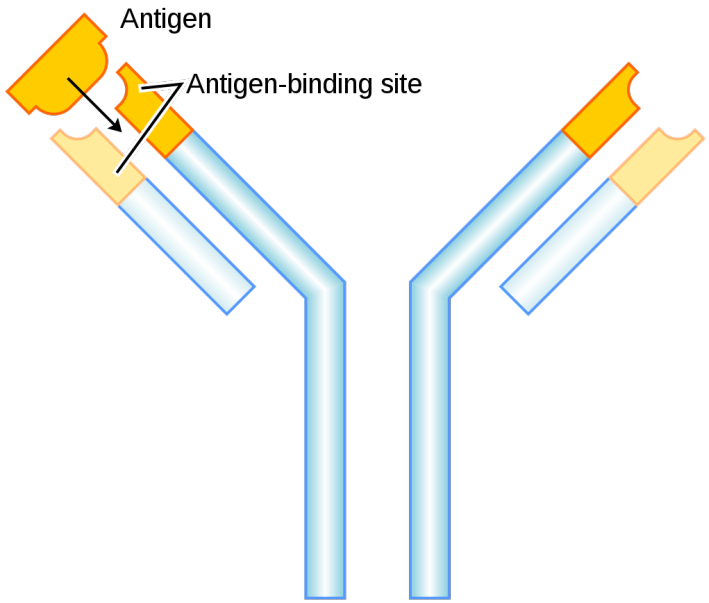
1 trillion possibilities!



JUNCTIONAL DIVERSITY CREATES ENORMOUS DIVERSITY

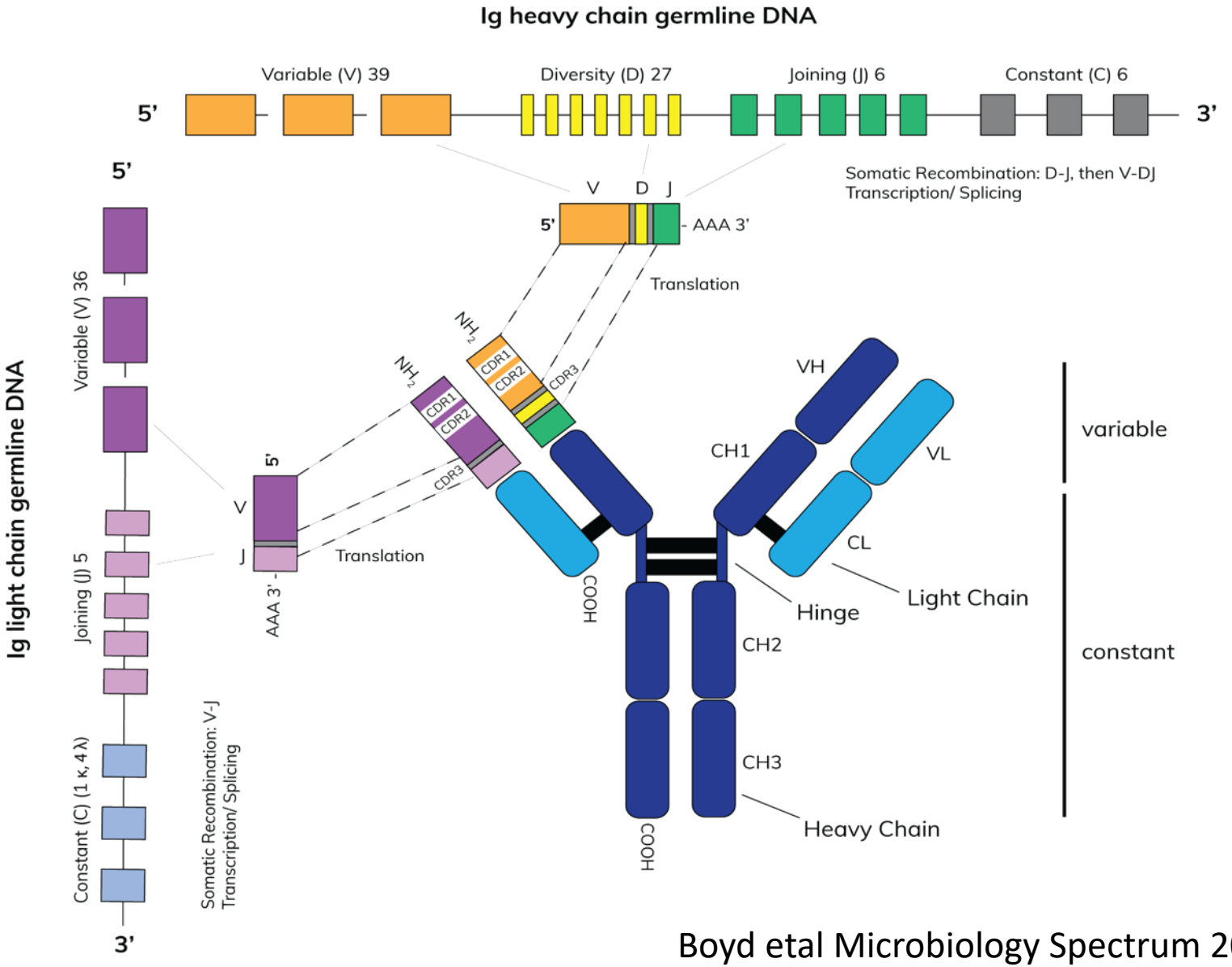
Antigens





Antibody

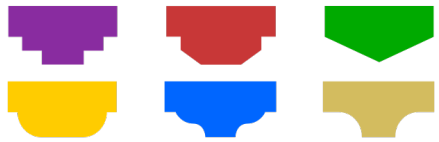
1 trillion possibilities!

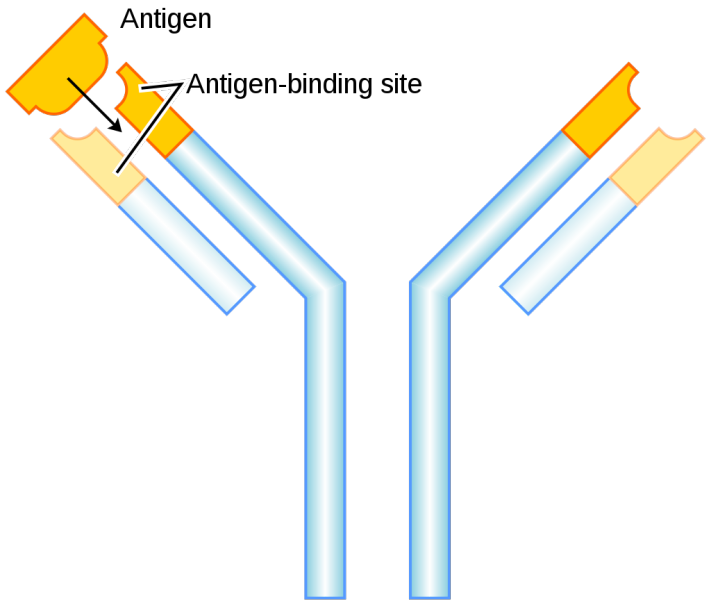


Boyd etal Microbiology Spectrum 2014

SOMATIC HYPERMUTATION TUNES DIVERSITY

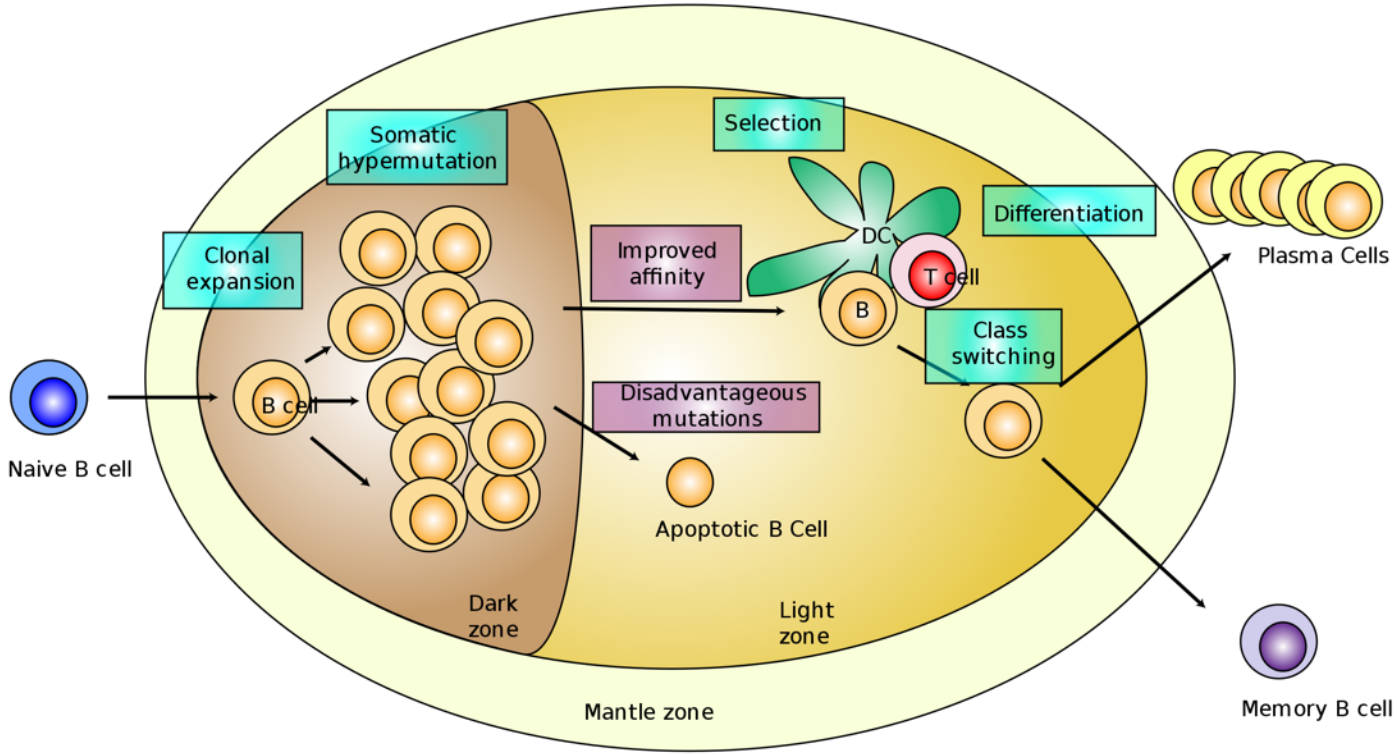
Antigens



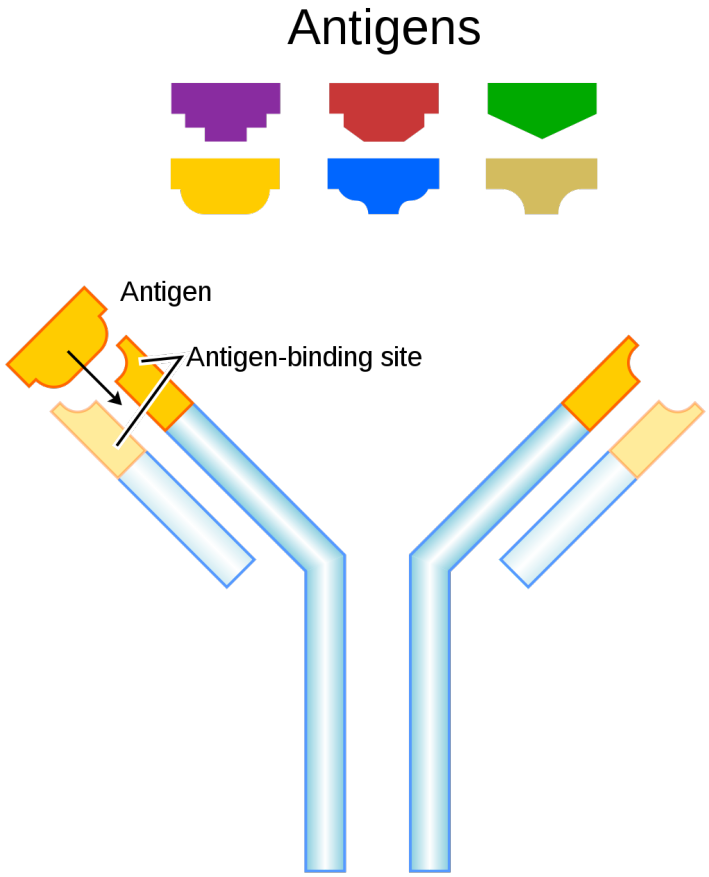


Antibody

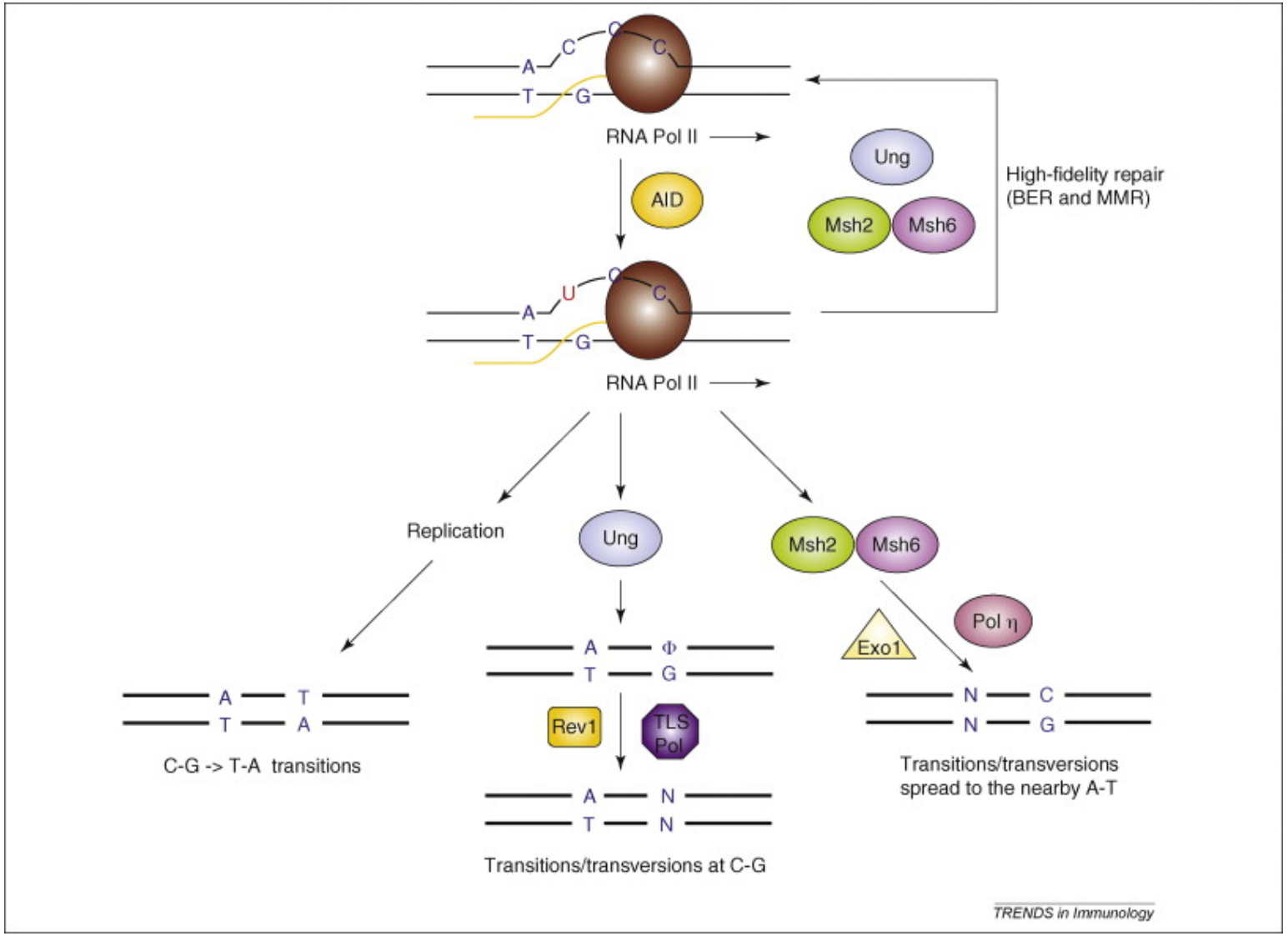
1 trillion possibilities!



SOMATIC HYPERMUTATION TUNES DIVERSITY

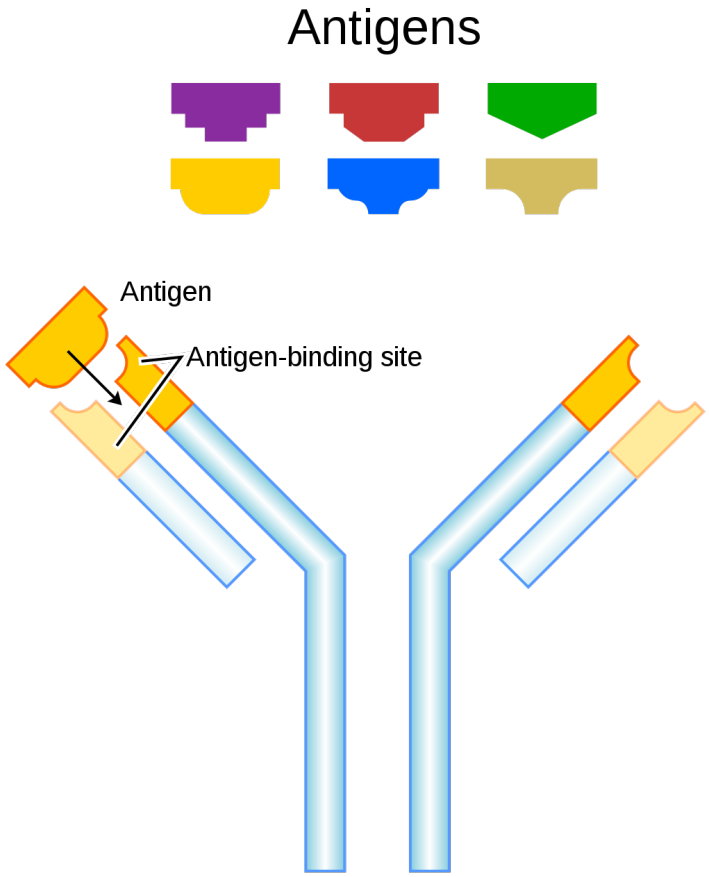


1 trillion possibilities!

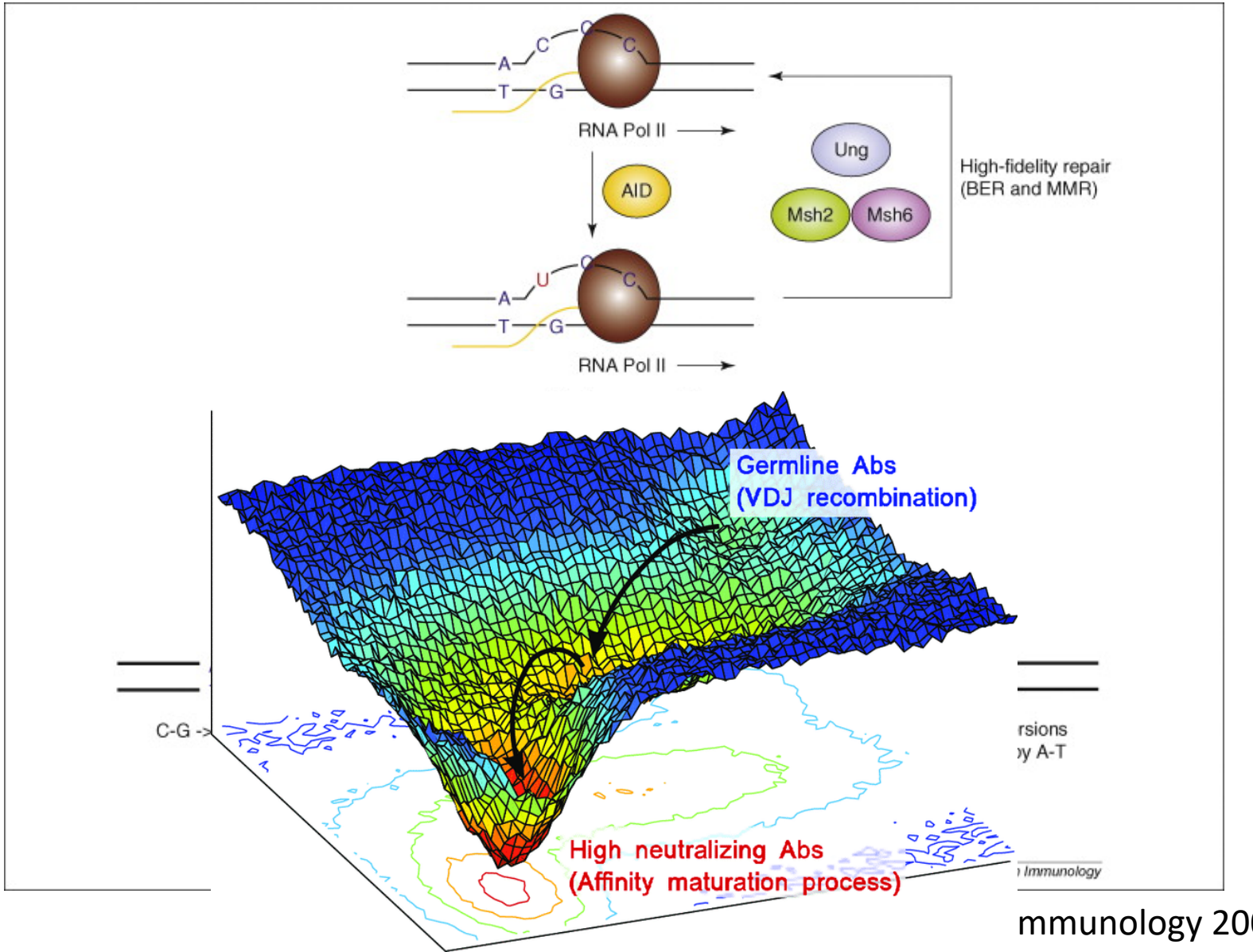


Liu M et al Trends in Immunology 2009

SOMATIC HYPERMUTATION TUNES DIVERSITY

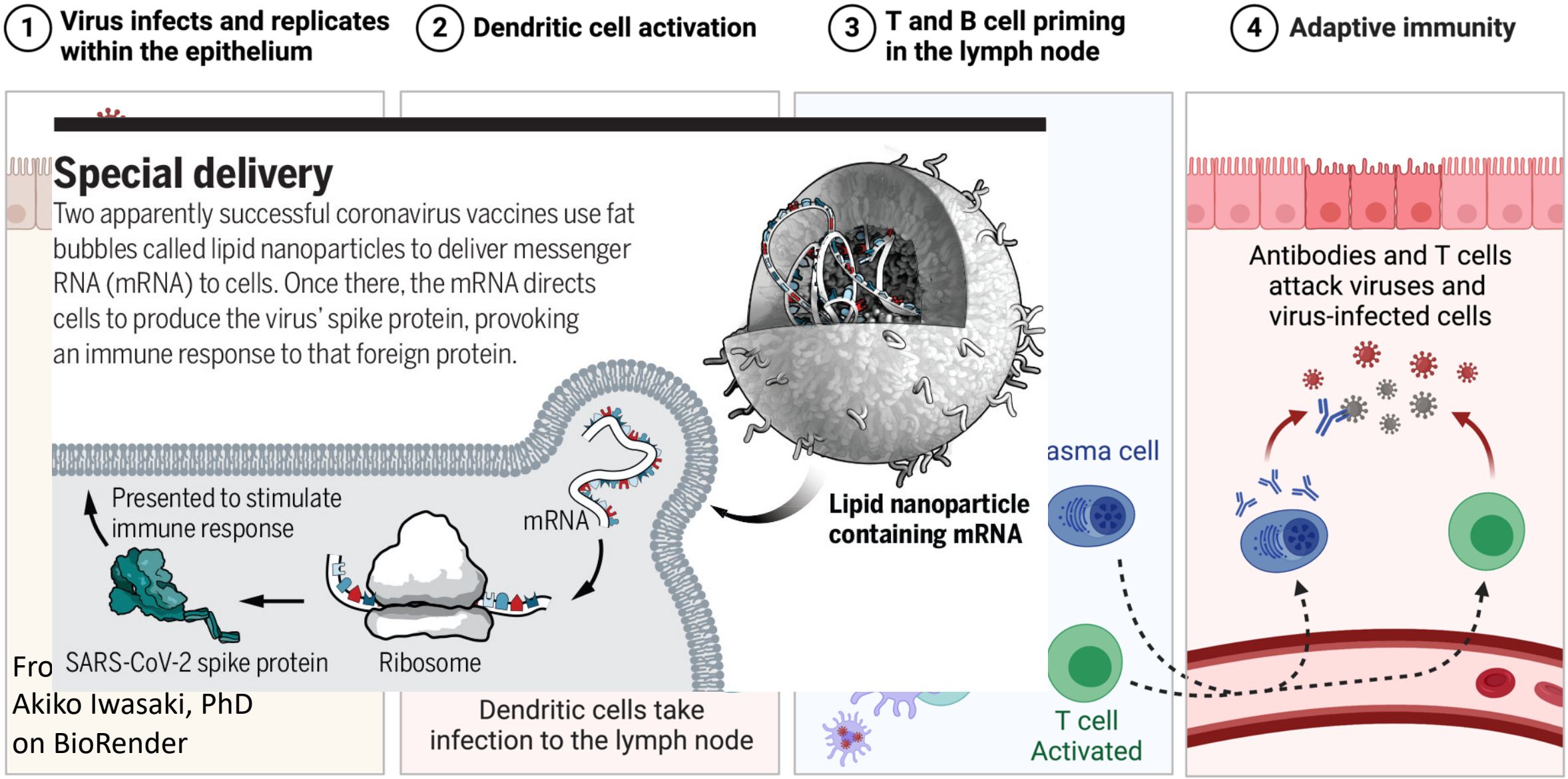


1 trillion possibilities!



mmunology 2009

ADAPTIVE IMMUNITY



Fro
Akiko Iwasaki, PhD
on BioRender

PASSIVE IMMUNITY FOR SARS-COV-2

Convalescent Plasma

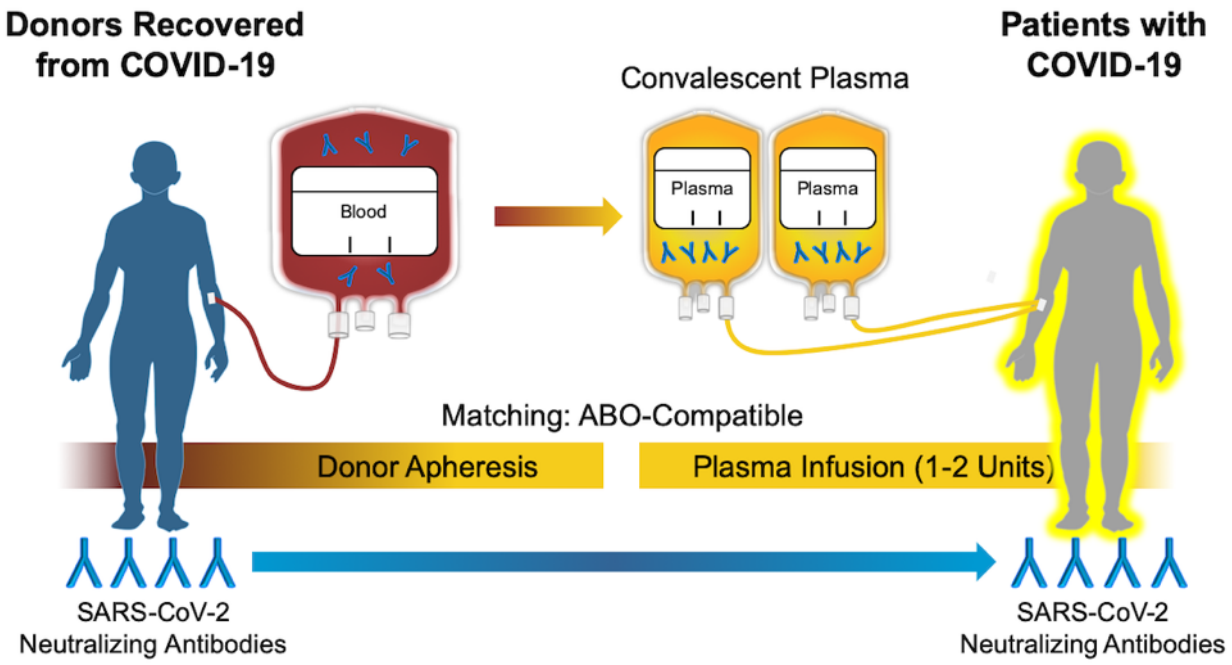
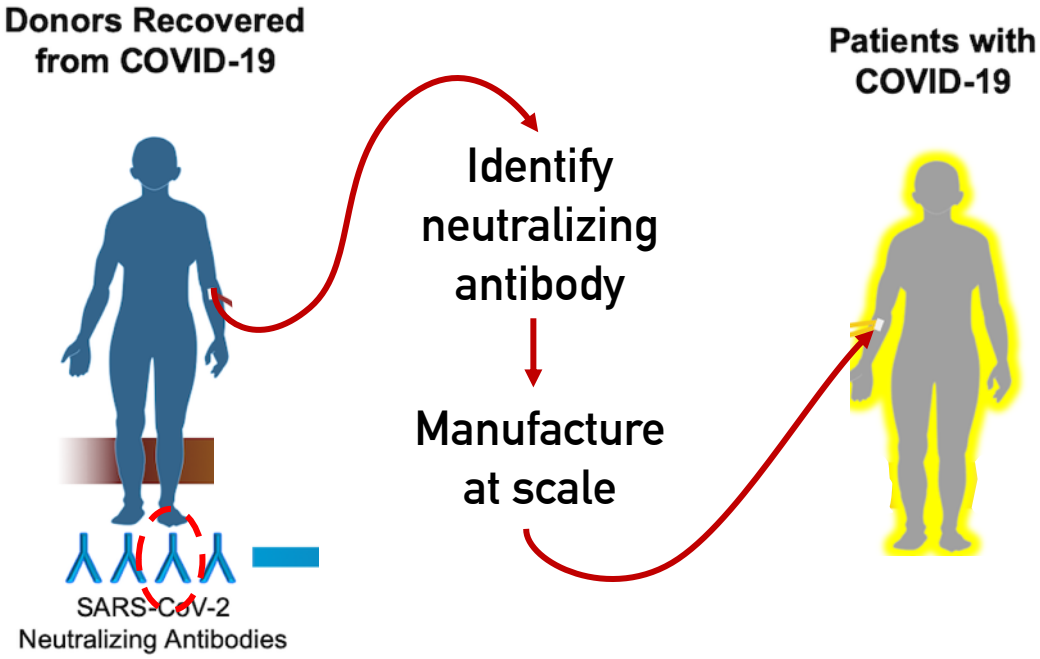


Illustration: David H. Spach, MD

- 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



- 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:

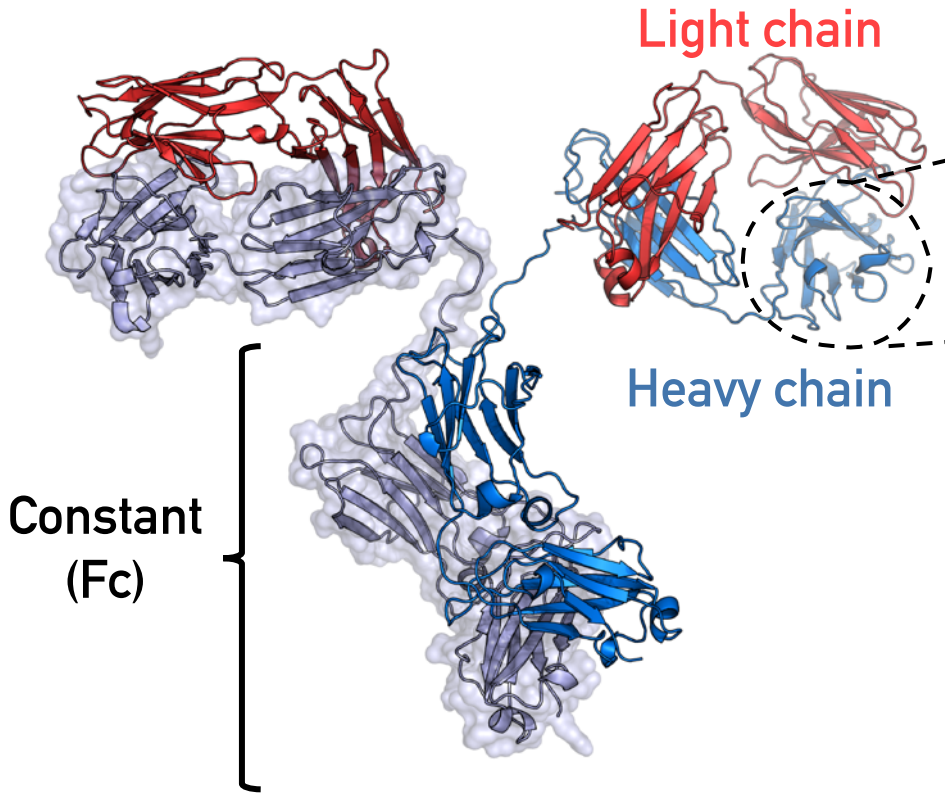
- Self administered
- Direct delivery to site of early infection

Challenges:

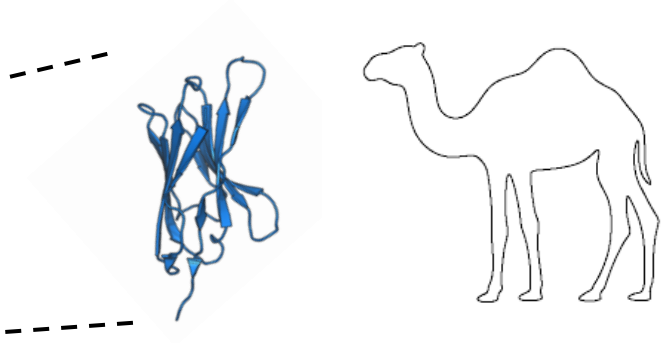
- Ultrastable protein required
- Pharmacokinetics?

NANOBODIES VS. MONOCLONAL ANTIBODIES

Conventional Antibody

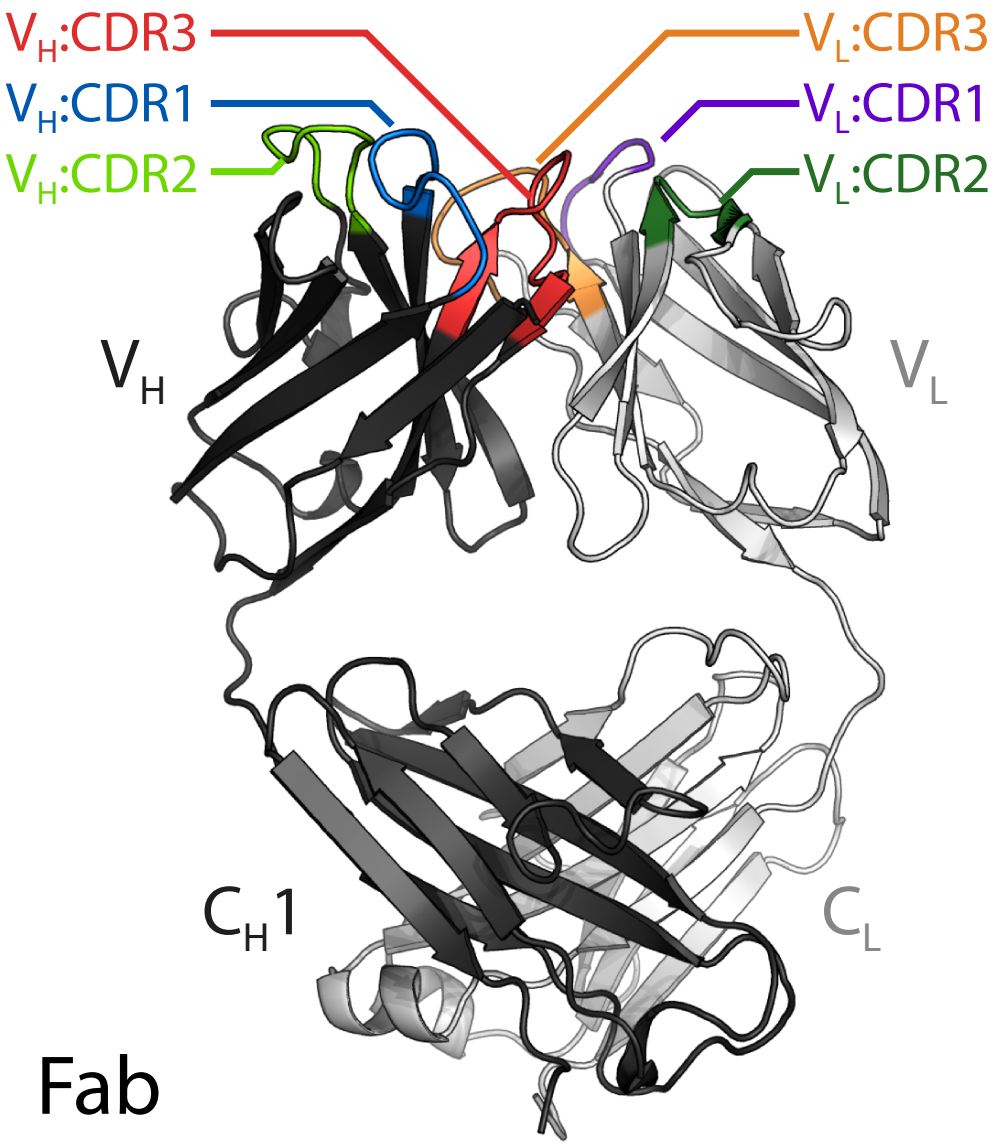


Nanobody

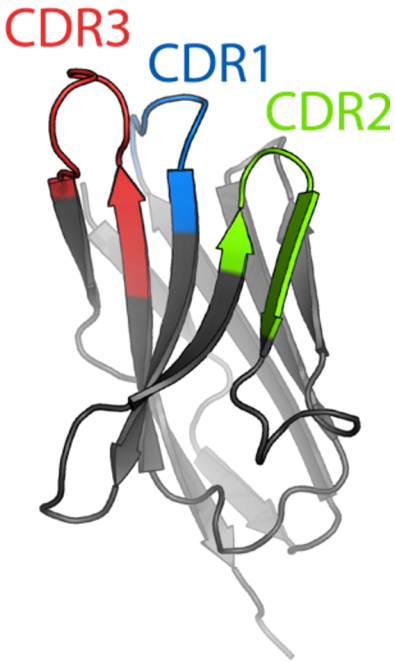


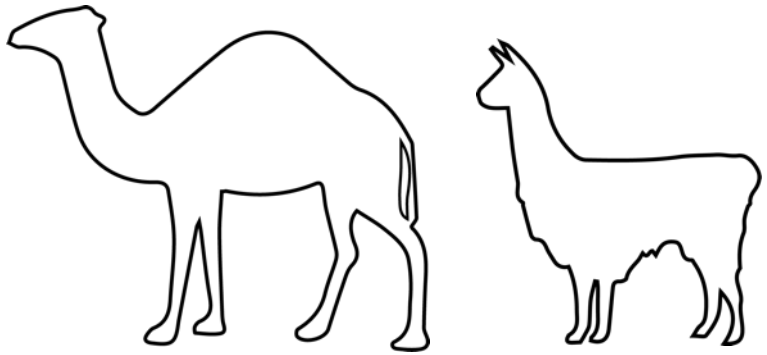
- Small (15 kDa), single chain protein
- Ultra-stable
- Non-glycosylated
- Similar to human antibody heavy chains
- Ease and low expense of rapid mass production

NANOBODIES – MINIMIZED ANTIBODIES FROM CAMELIDS



Fab

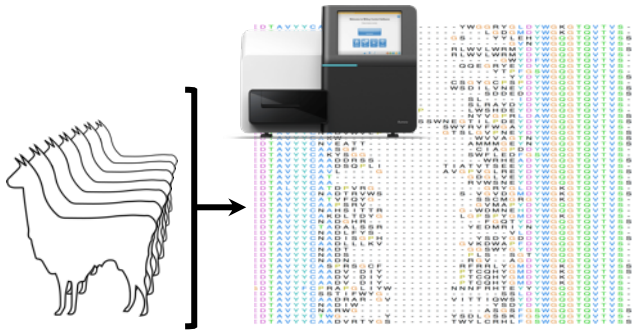




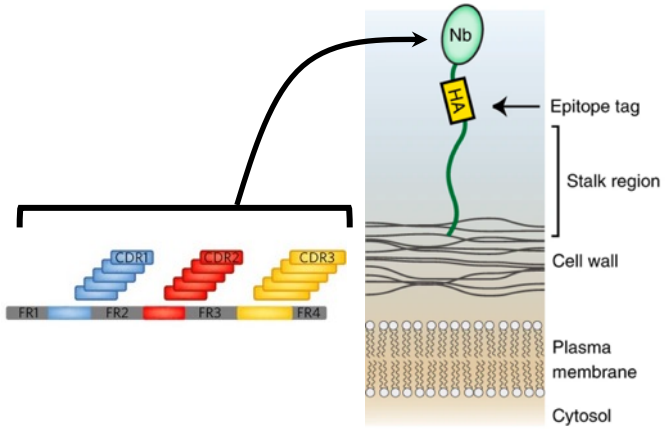
Nanobody (V_{HH})

A RAPID PLATFORM FOR NANOBODY DISCOVERY

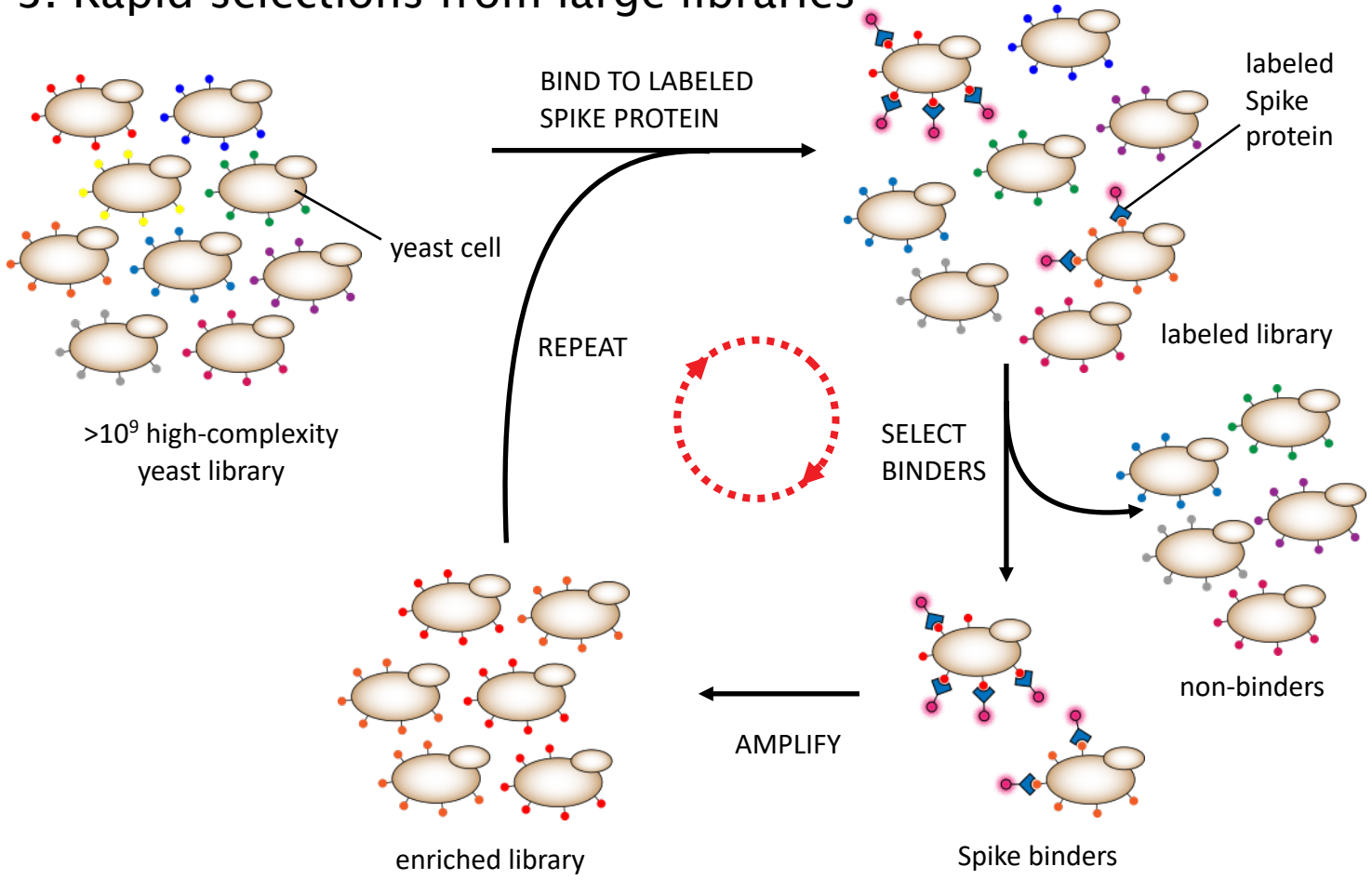
1. Bioinformatic analysis of natural camelid repertoire



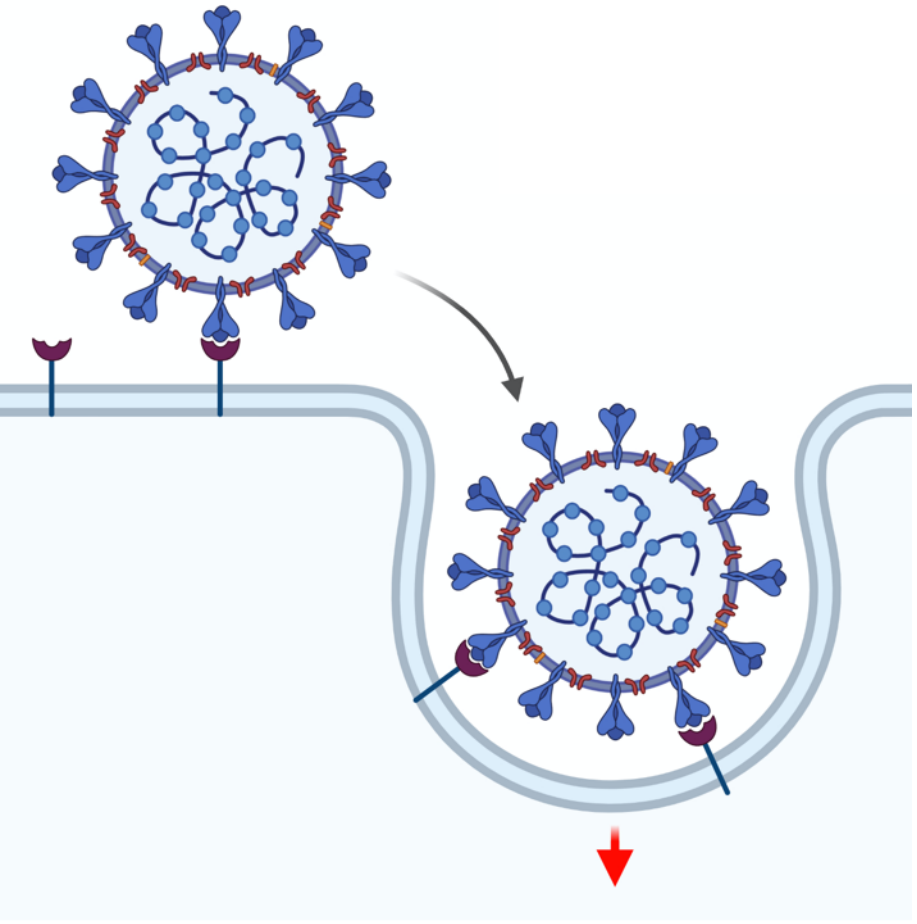
2. Synthesis of precision library

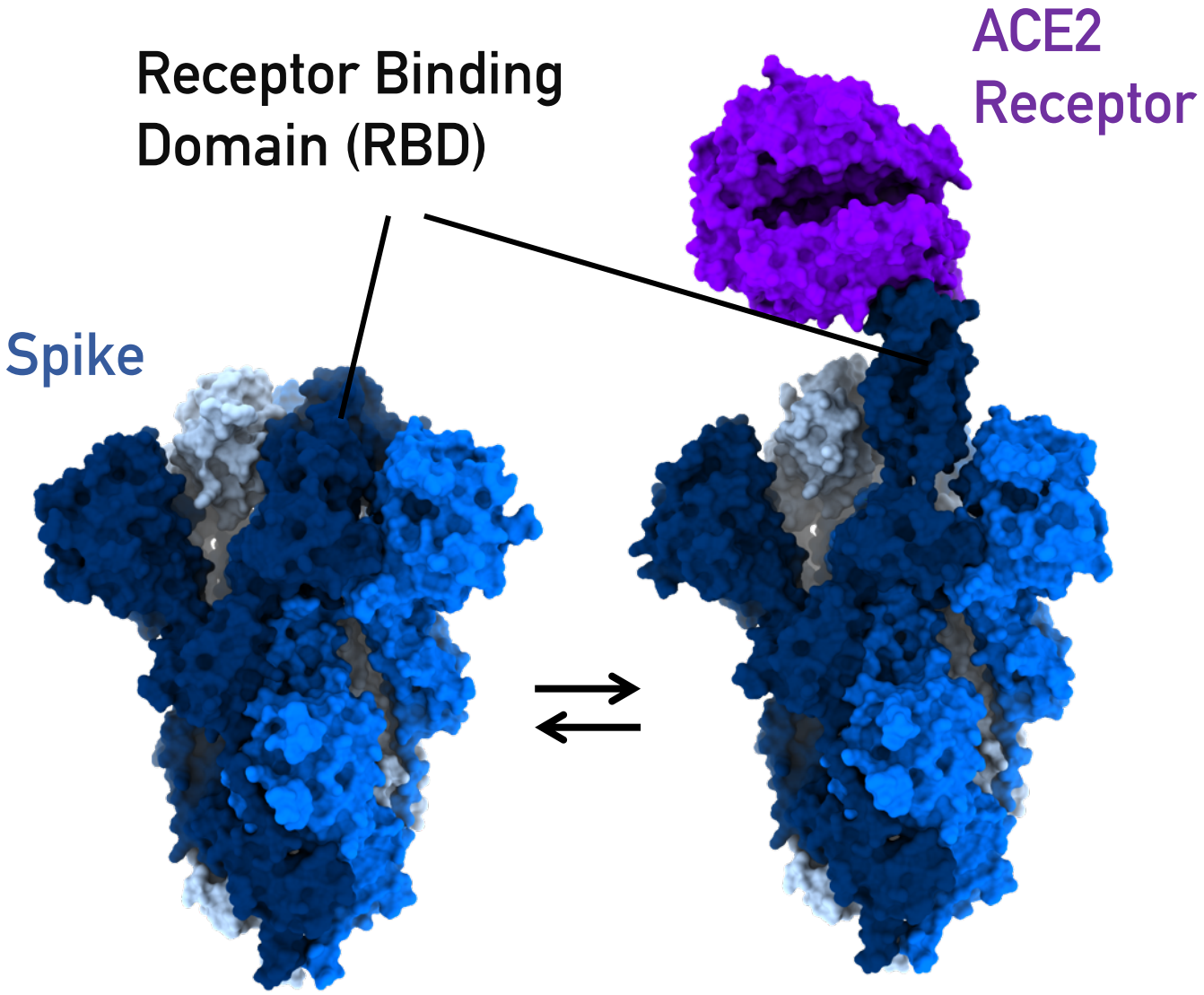


3. Rapid selections from large libraries



FULL SPIKE ECTODOMAIN FOR NANOBODY DISCOVERY





UNDERSTANDING BINDING

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

R binds L to make RL

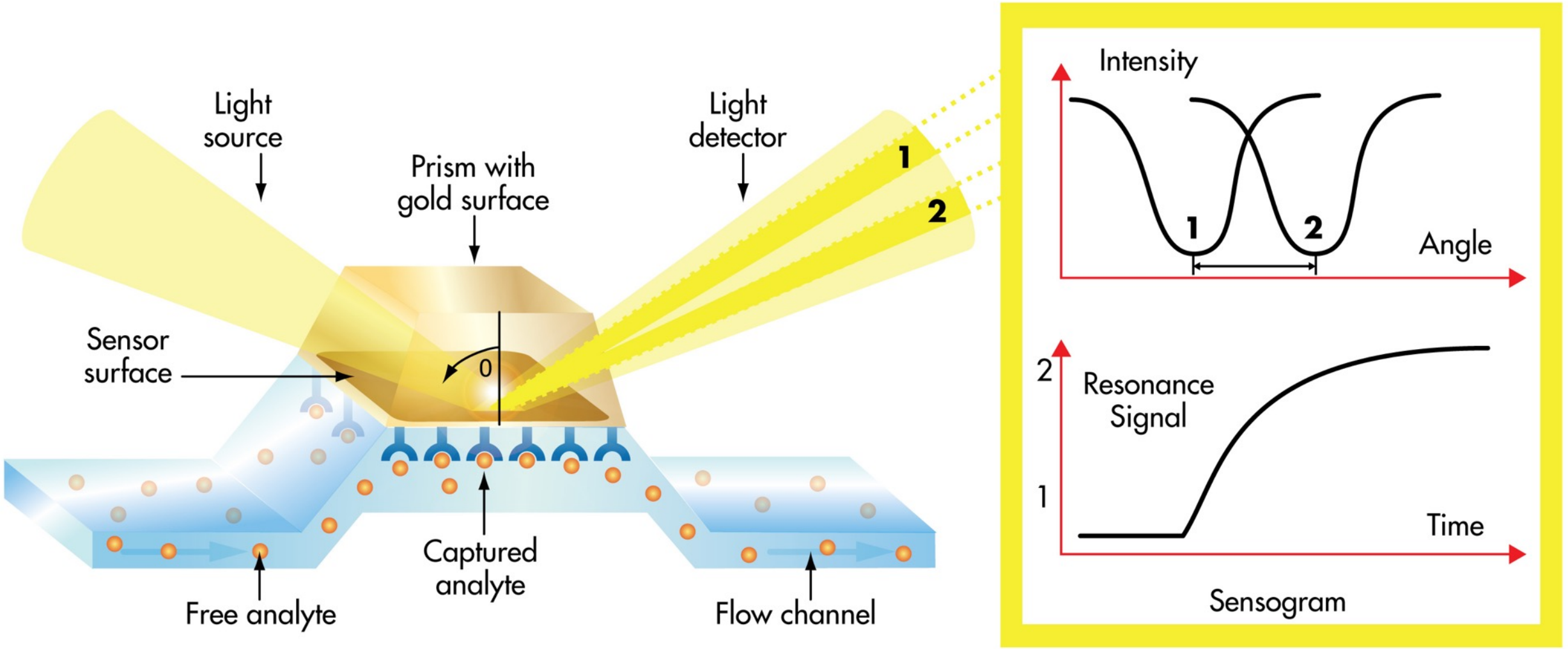
$$K_\mathrm{a} = \frac{k_\mathrm{on}}{k_\mathrm{off}} = \frac{[\mathrm{RL}]}{[\mathrm{R}]\,[\mathrm{L}]}.$$

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

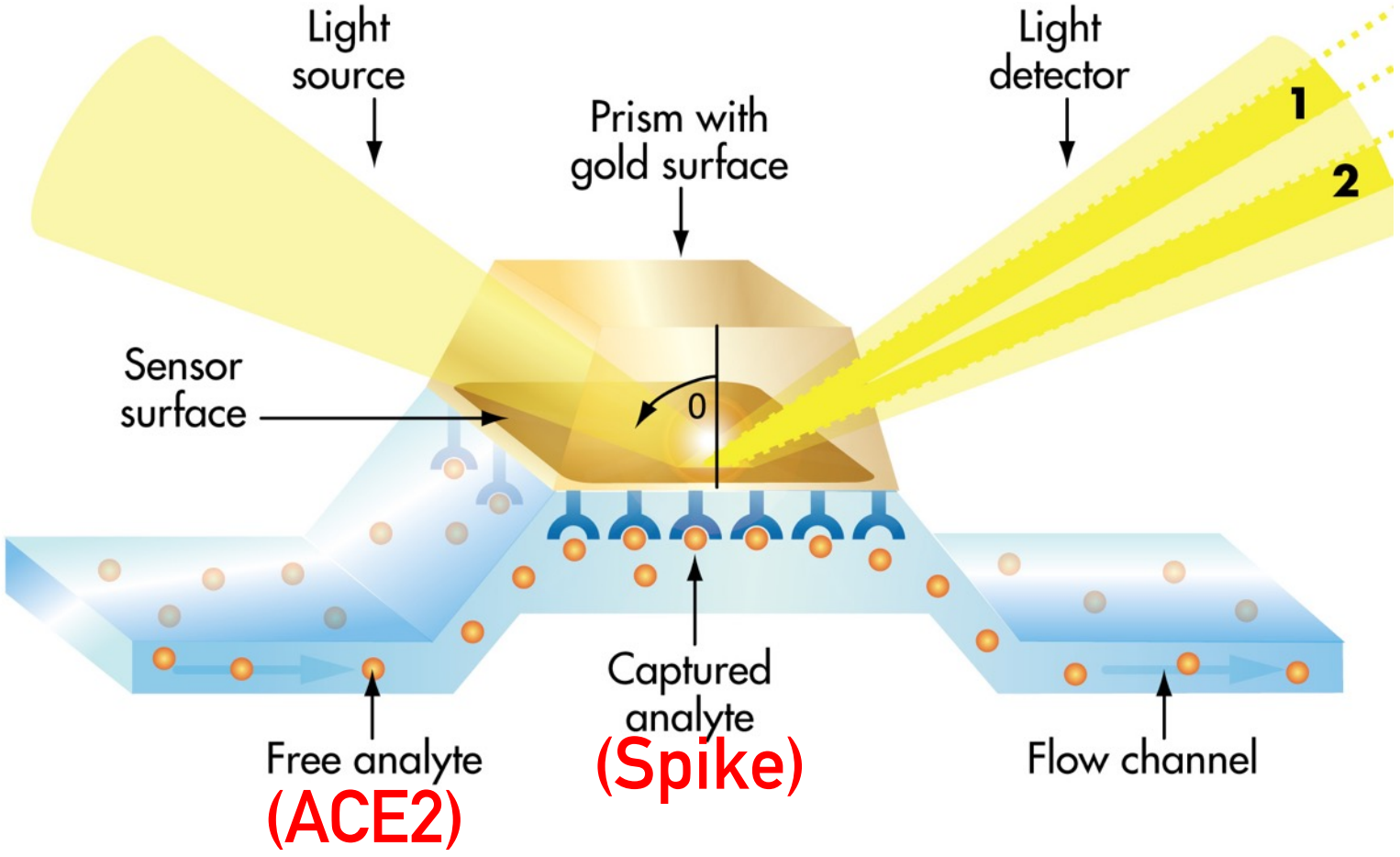
If things bind tight: more RL, less R and L.

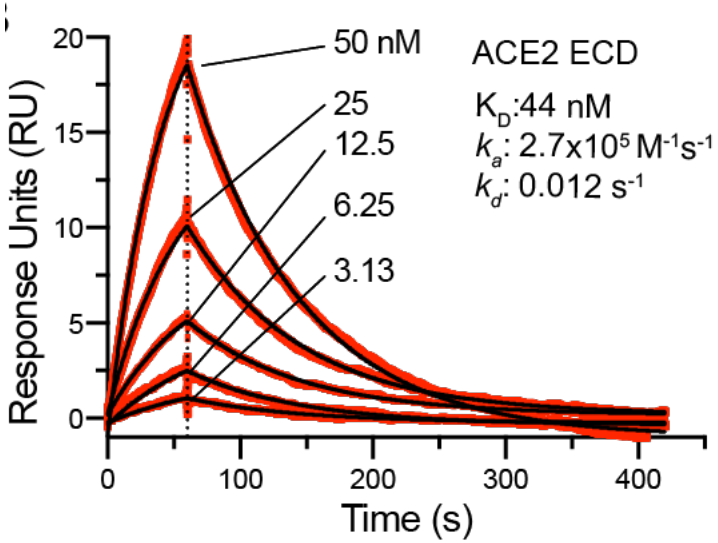
Rate forward (k_{on}) is faster than rate backward (k_{off})

PROTEIN INTERACTIONS BY SURFACE PLASMON RESONANCE



PROTEIN INTERACTIONS BY SURFACE PLASMON RESONANCE





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

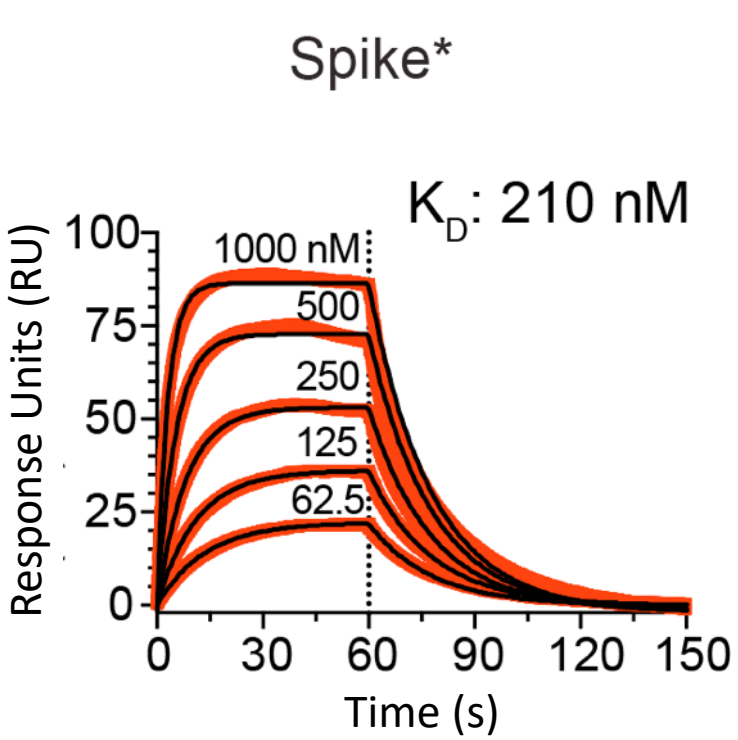
Round 2

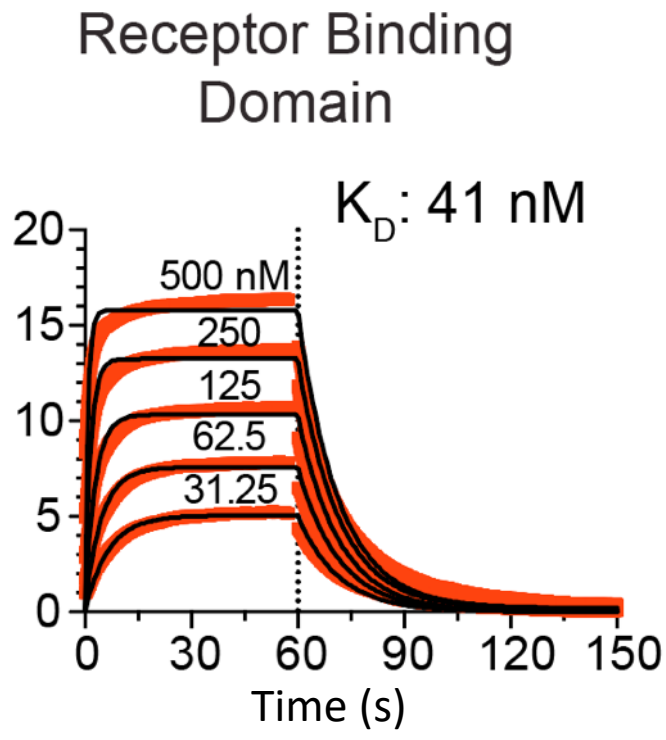
↓ 10 nM Spike

Round 3

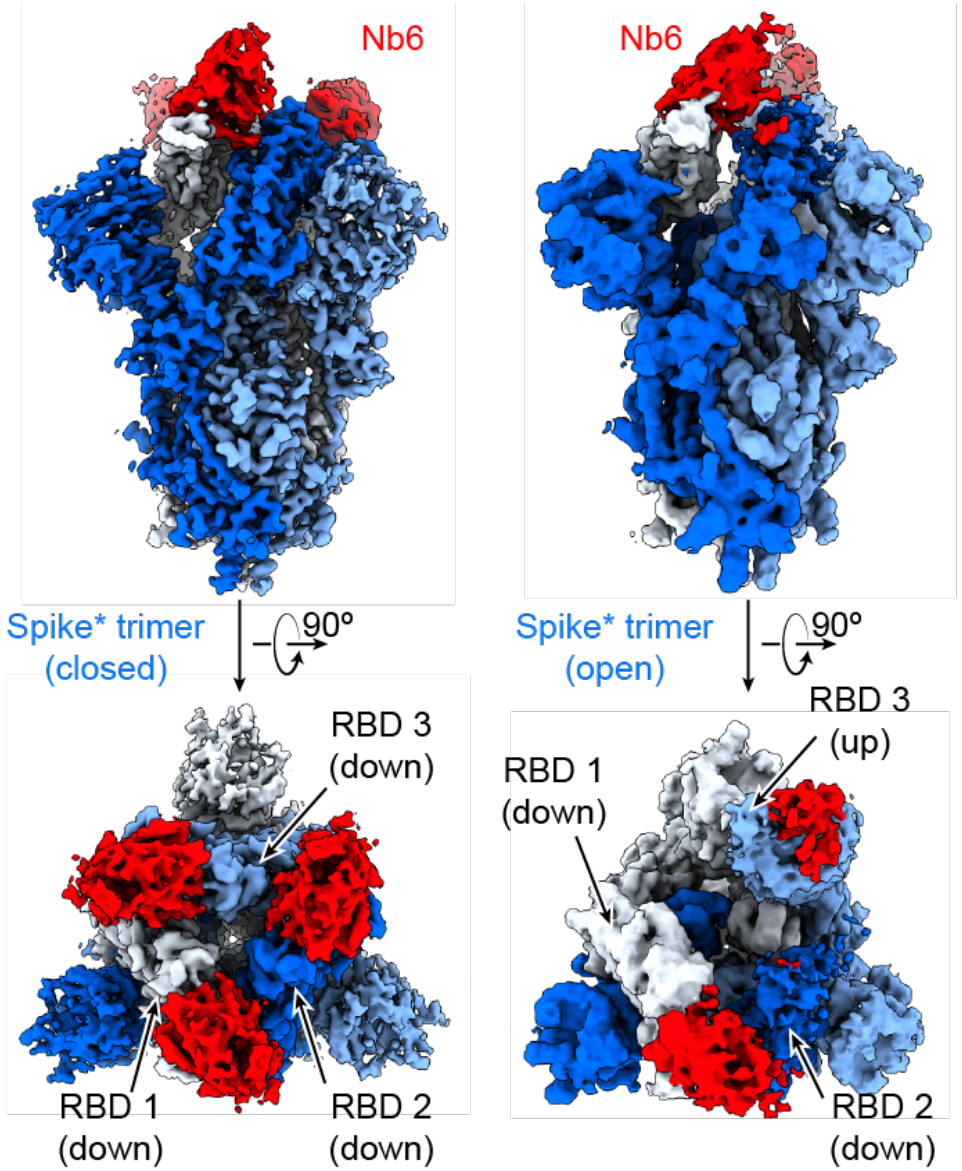
↓ Bind Spike
Blocked by ACE2

21 clones

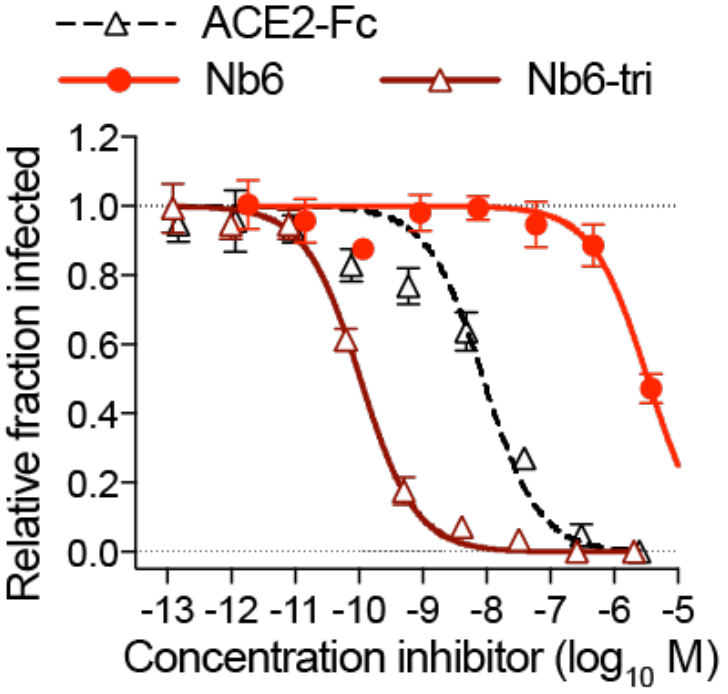




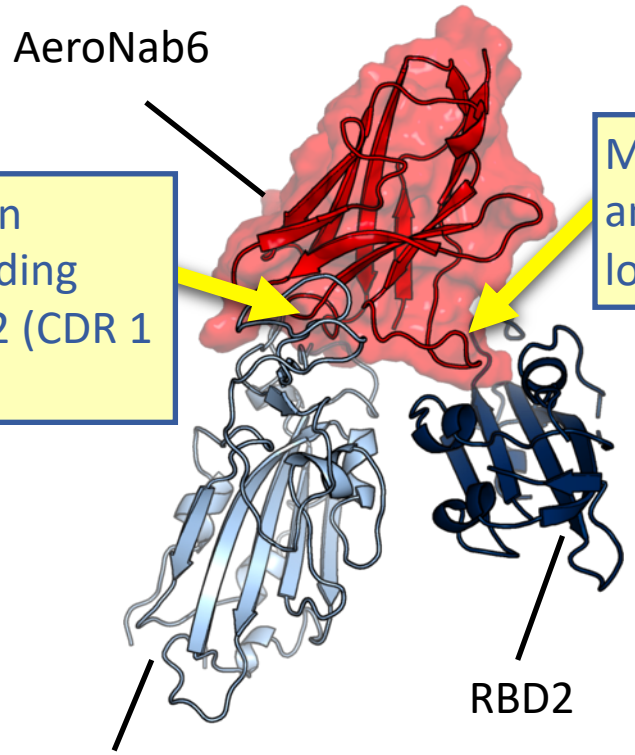
STRUCTURES OF ANTI-SPIKE NANOBODIES



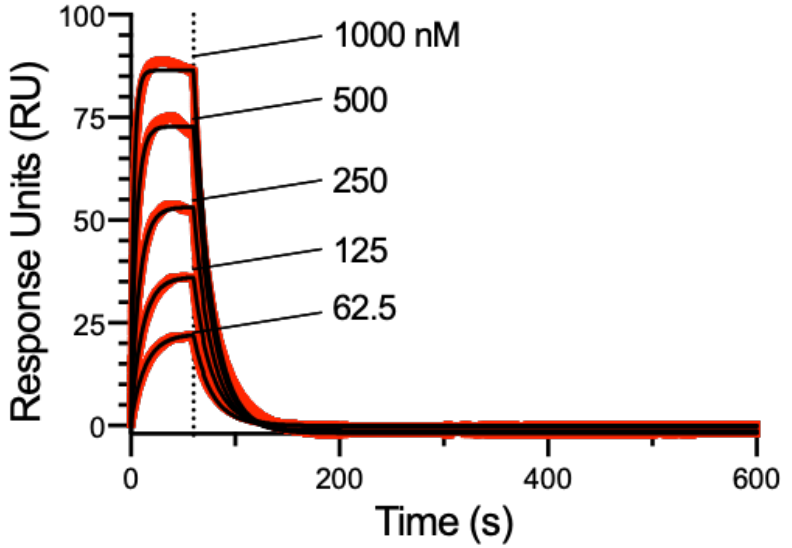
Live-virus Neutralization

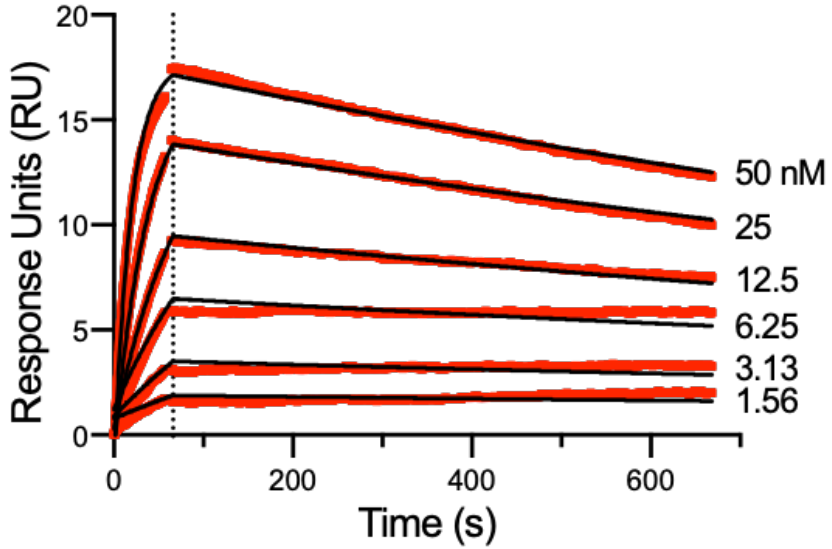


AFFINITY MATURATION OF NB6



"CDR" = Complementarity-Determining Regions in AeroNab





Nb6

K_D: 210 nM

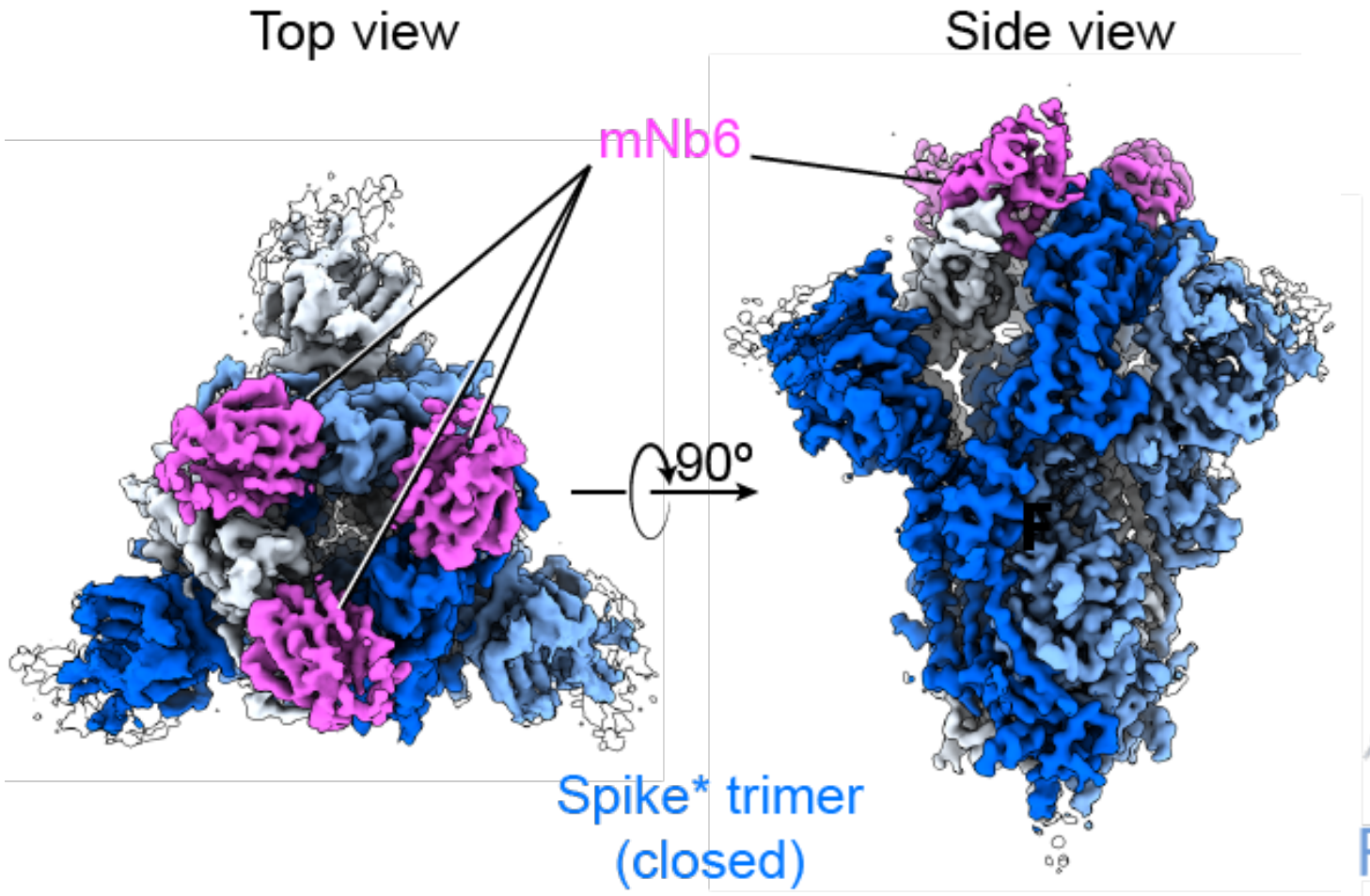
500x

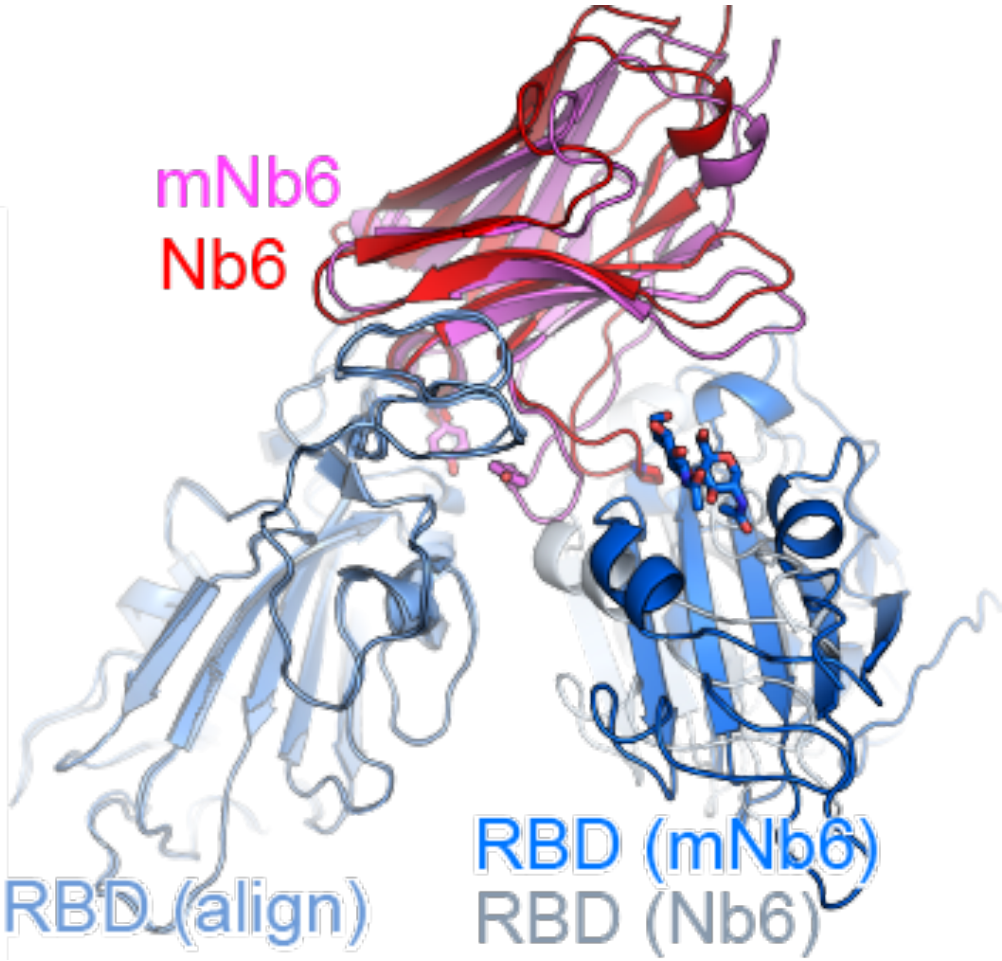
mNb6

K_D: 0.45 nM

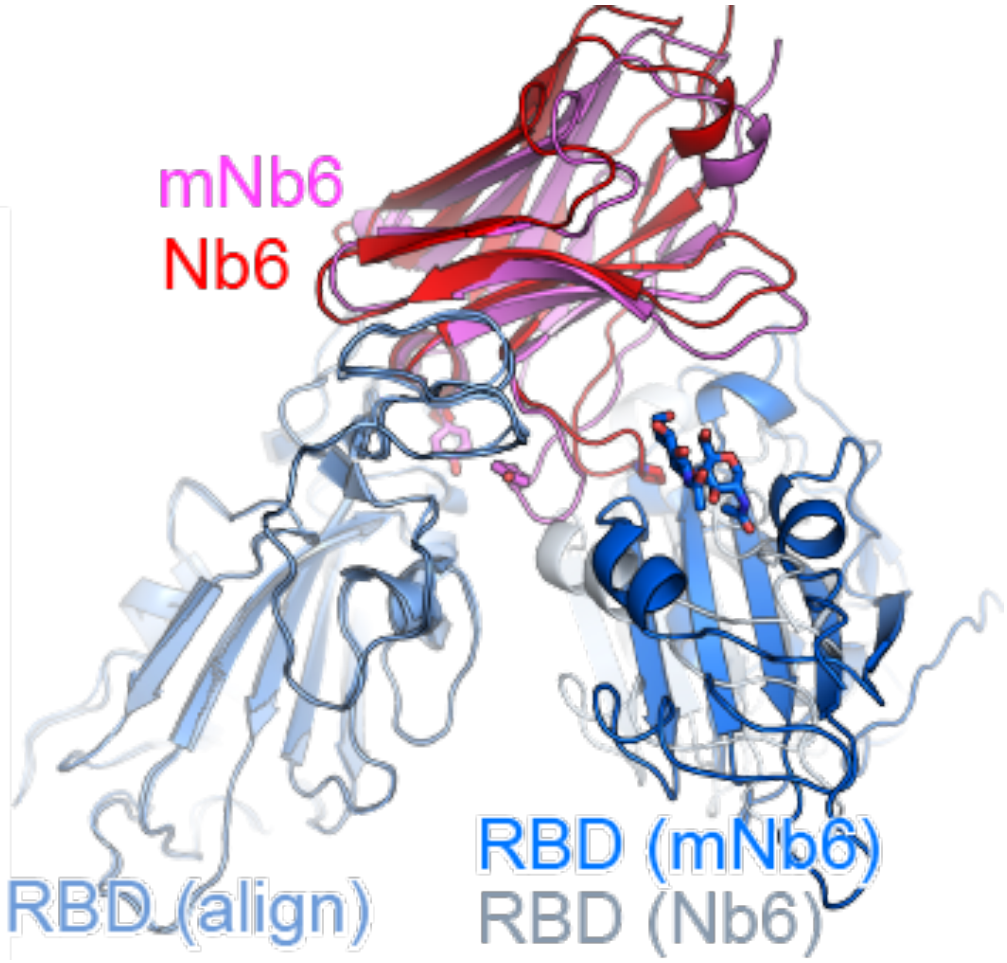
2 mutations
(CDR1: I27Y
(CDR3: P105Y)

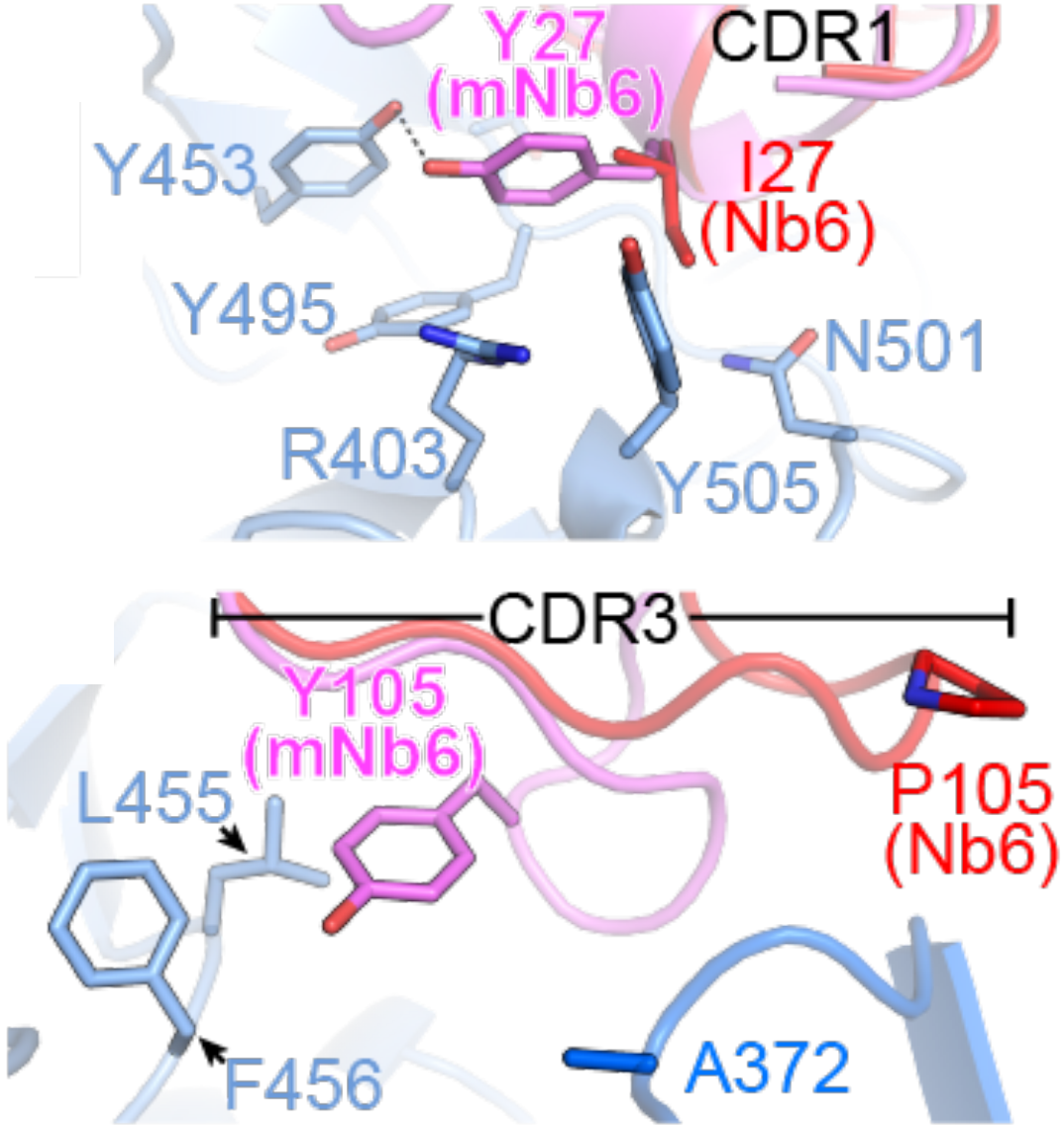
AFFINITY MATURATION OF NB6



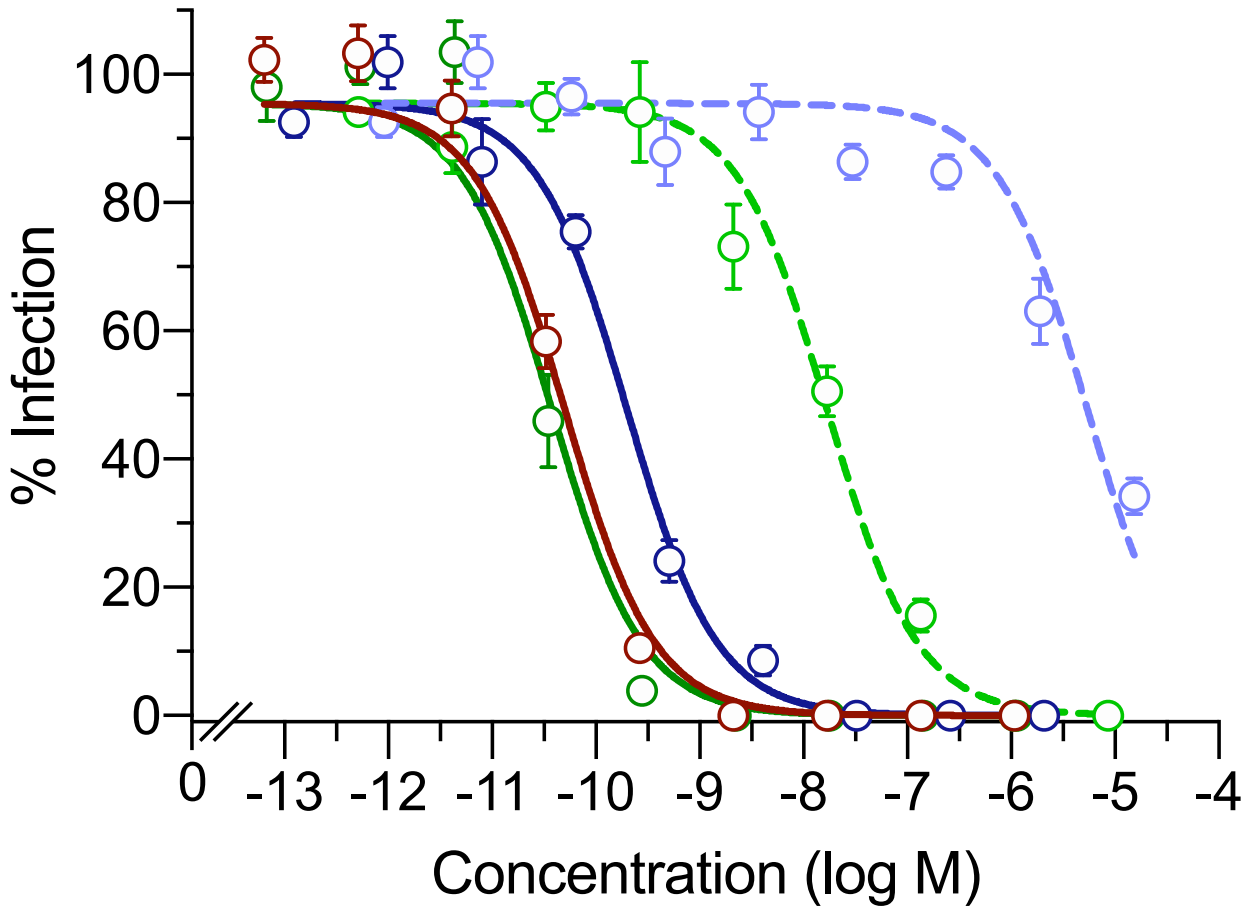


AFFINITY MATURATION OF NB6





NEUTRALIZATION ACTIIVTY OF DESIGNED NANOBODIES



Nb6 (5500 nM; 70 µg/mL)

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

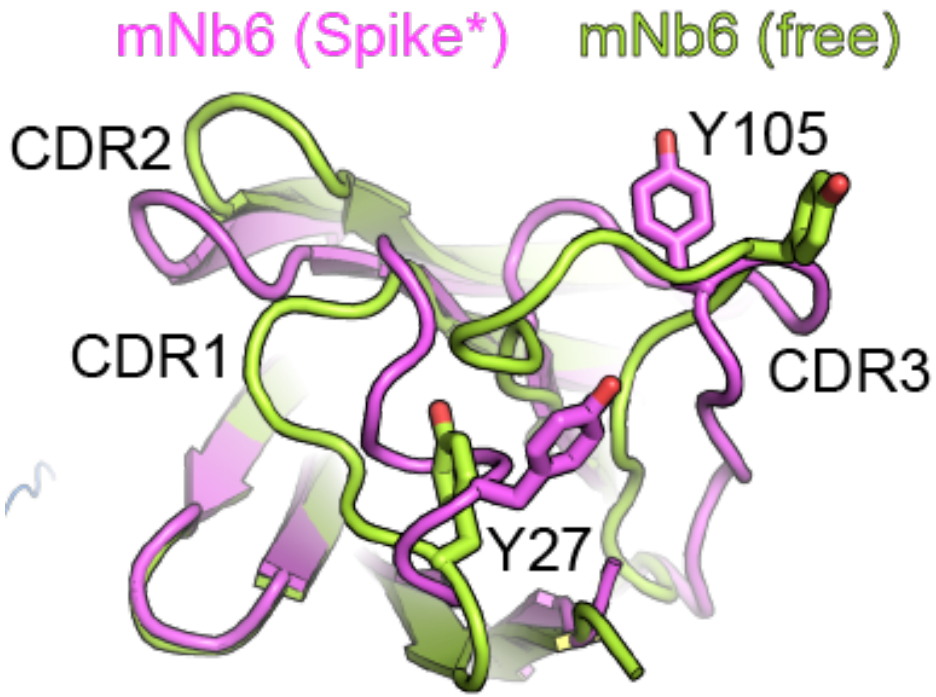
mNb6 (17 nM; 215 ng/mL)

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

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

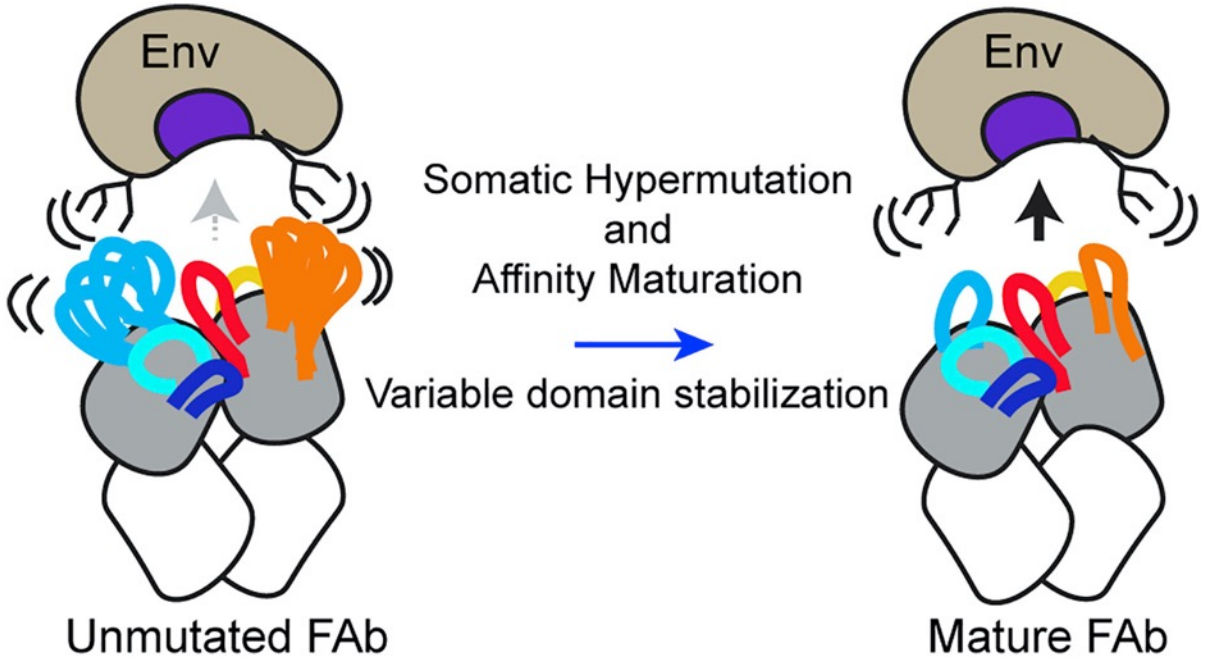
LOOP CONFORMATIONAL PLASTICITY

Bound vs unbound mNb6:



2 different conformations!

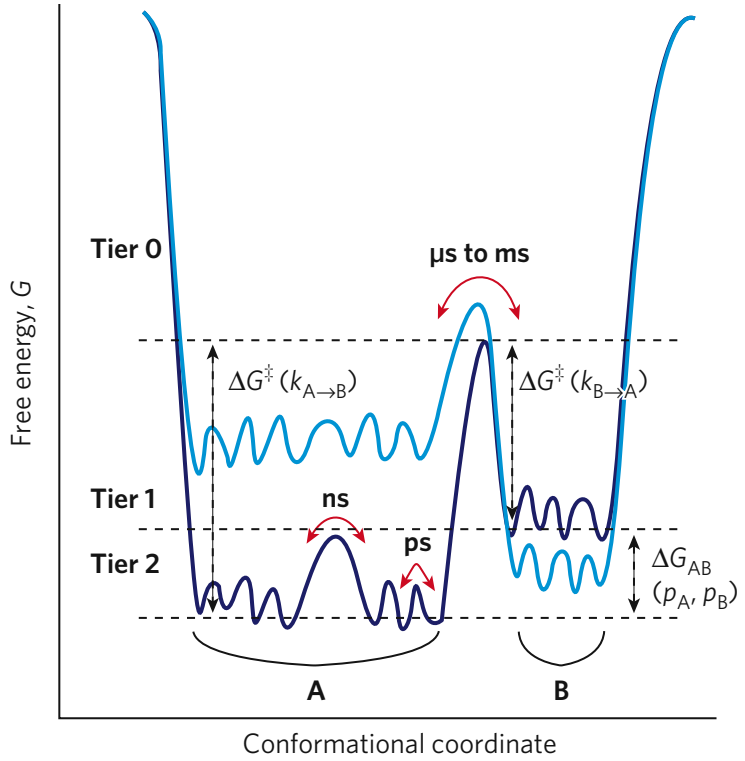
The usual case with antibodies:

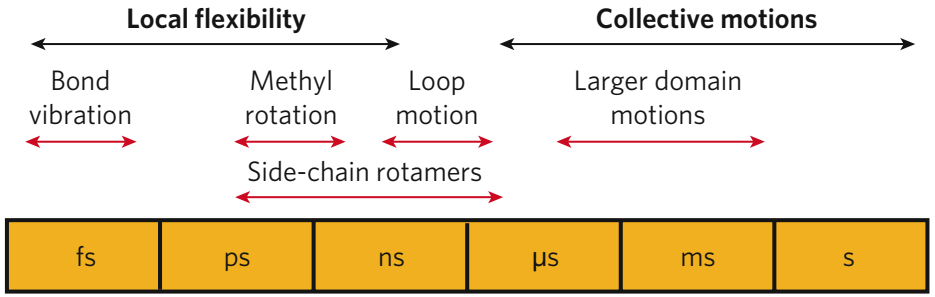


Davenport TM et al Structure 2016

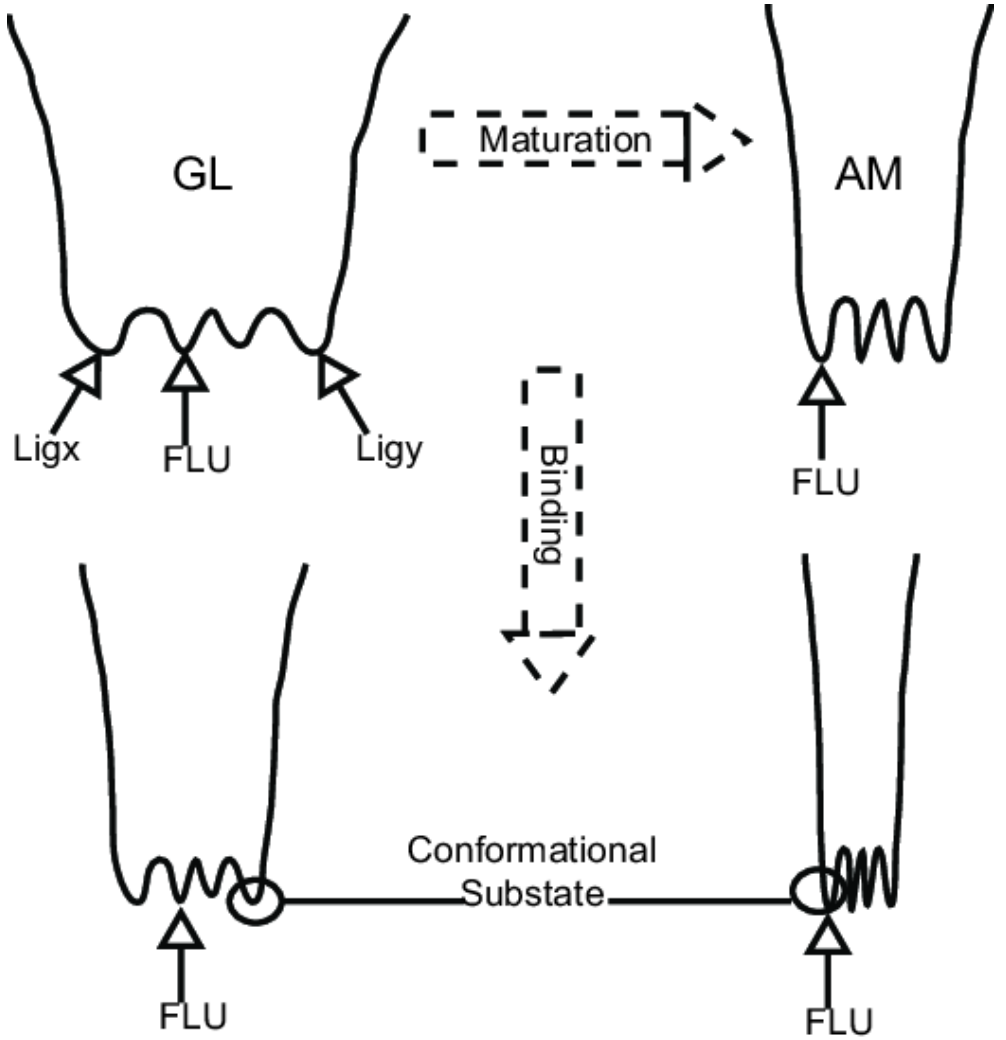
Maturation rigidifies loops

PROTEIN DYNAMICS SHAPES ANTIBODY FUNCTION





Henzler-Wildman, K and Kern, D NSMB 2007



Thorpe I et al PNAS 2007

RECAP and "Mini-Quals"

Normally, antibody affinity maturation leads to conformational rigidification of loops (decreased entropic penalty for binding)

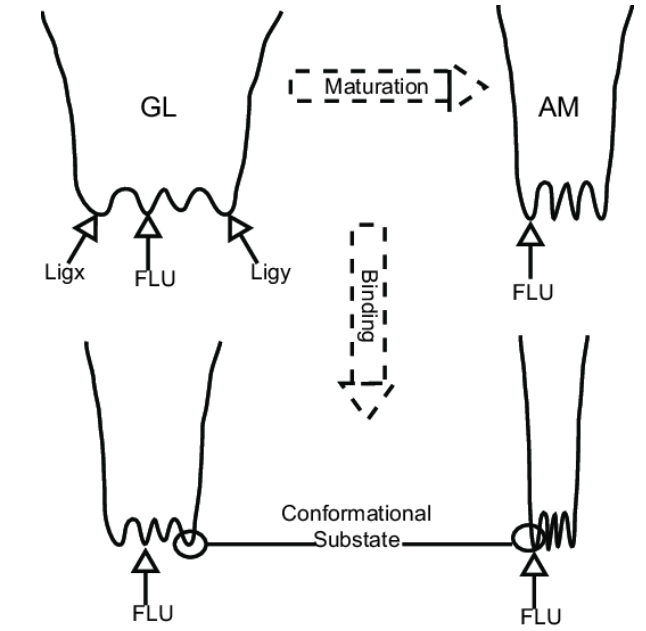
We engineered an initial nanobody against Spike (Nb6)

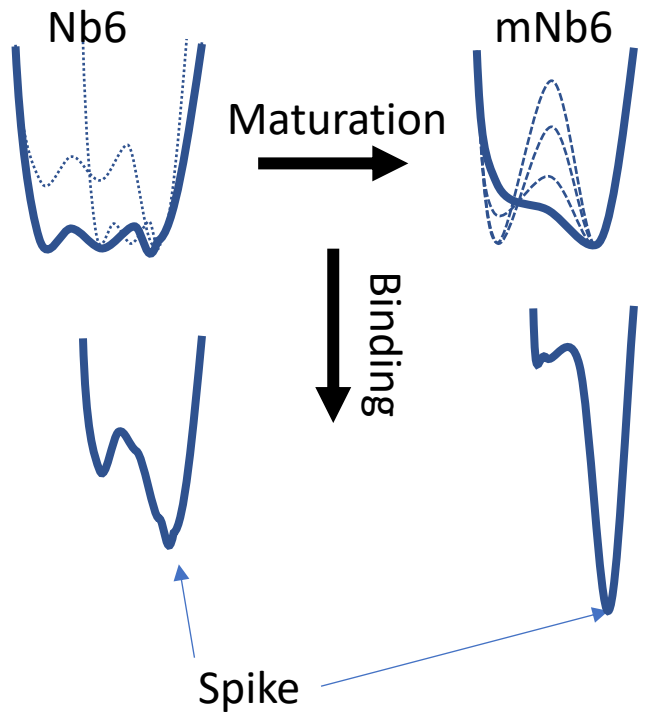
- Failed to get good quality crystals (maybe too flexible?)
- Got cryo-EM structure with Spike

We affinity matured to get mNb6 (500x increase in potency with only 2 mutations!)

- Cryo-EM structure shows some improved contacts, probably not sufficient to explain 500x gain
- Crystal structure of unbound mNb6 shows huge loop conformational differences, contrary to "conventional wisdom" for affinity maturation of antibodies

Question: Are loop conformational dynamics a key driver of exceptional potency gain from Nb6 to mNb6?





"Mini-Quals"

Question: Are loop conformational dynamics a key driver of exceptional potency gain from Nb6 to mNb6?

1) How "rigidified" are these loops in the bound state?
   - Refine our EM structures – how confident are we in the loop conformations modeled?
   - Use NMR to see if Nb6 and mNb6 really bind in the same way in solution.

2) How much disorder is there in the unbound states?
   - How confident are we in our X-ray structure of mNb6 – are there regions that are dynamic? How can we estimate disorder?
   - Can we see other conformation of mNb6 loops in other X-ray structures?
   - Are there differences in loop conformations between Nb6 and mNb6 by NMR? Can we quantify these motions?