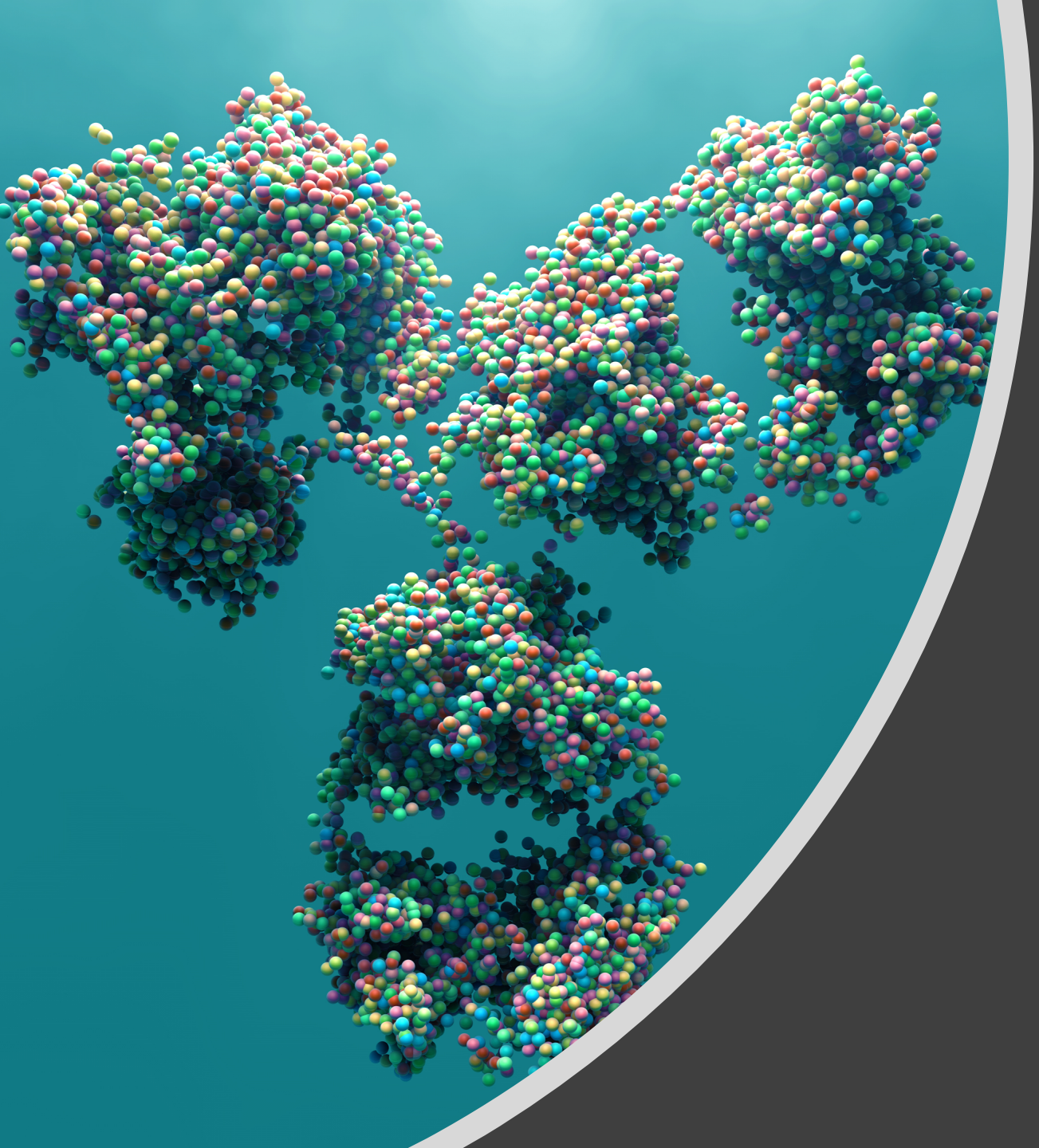
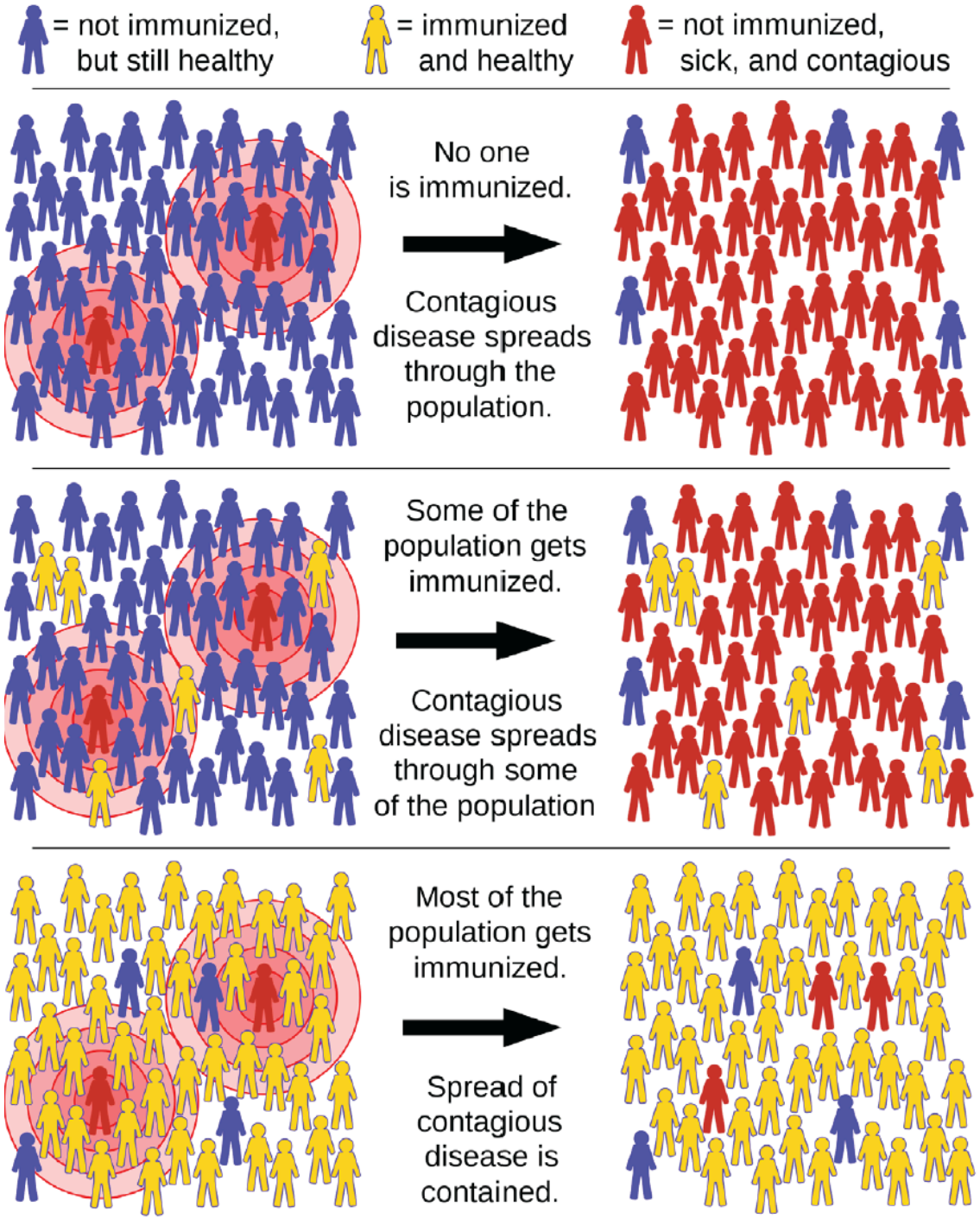


The antigen-antibody interaction

It takes a Herd!

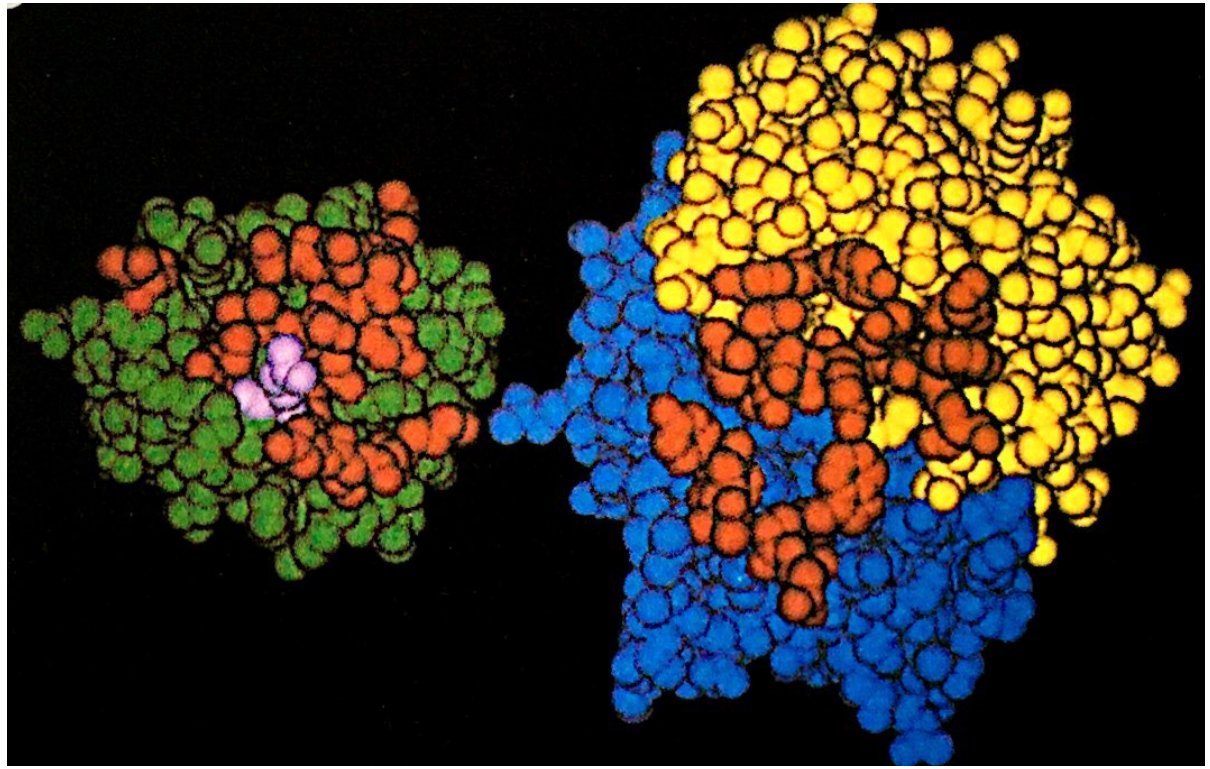
- There is no single policy that will be fully ethical or make everyone happy
- The goal is to be mindful and take different perspectives into account
- With more time to think, does anyone have anything they want to share?

- **Thank you for your thoughtful participation!**



The Antigen - Antibody interaction forms multiple contacts

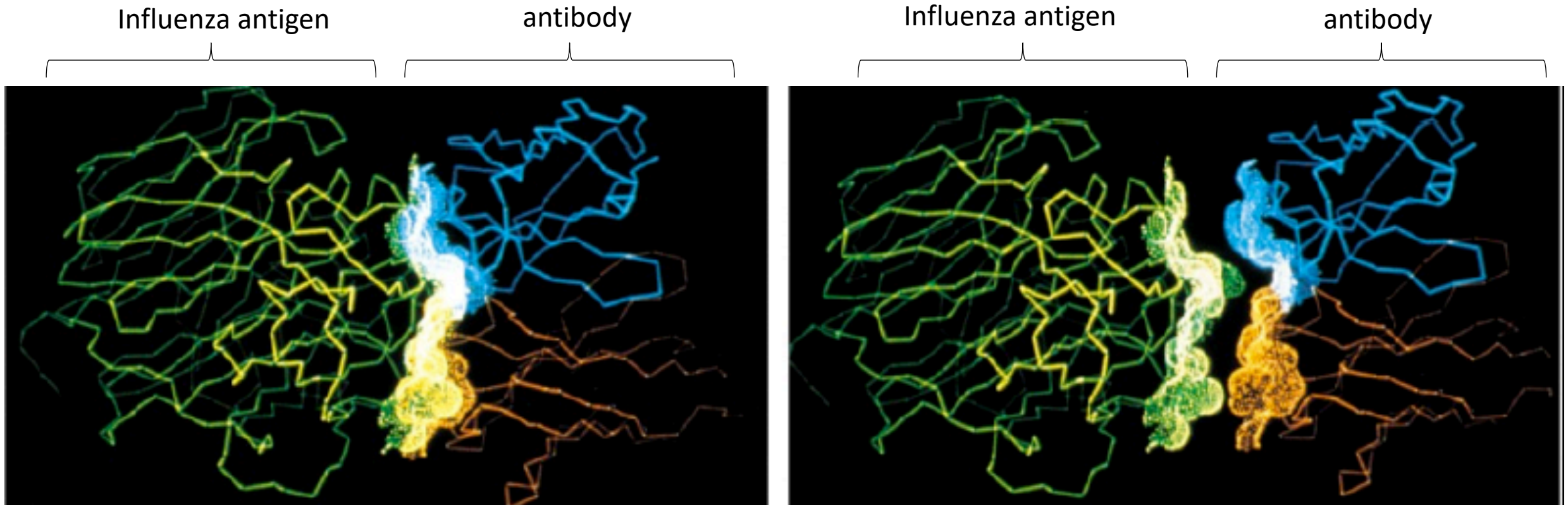
3D: Lysozyme bound to variable region



- Green: lysozyme
- Blue/Yellow: V_L and V_H
- Red amino acids that interact
- Pink critical glutamine reside fits into cleft of CDR

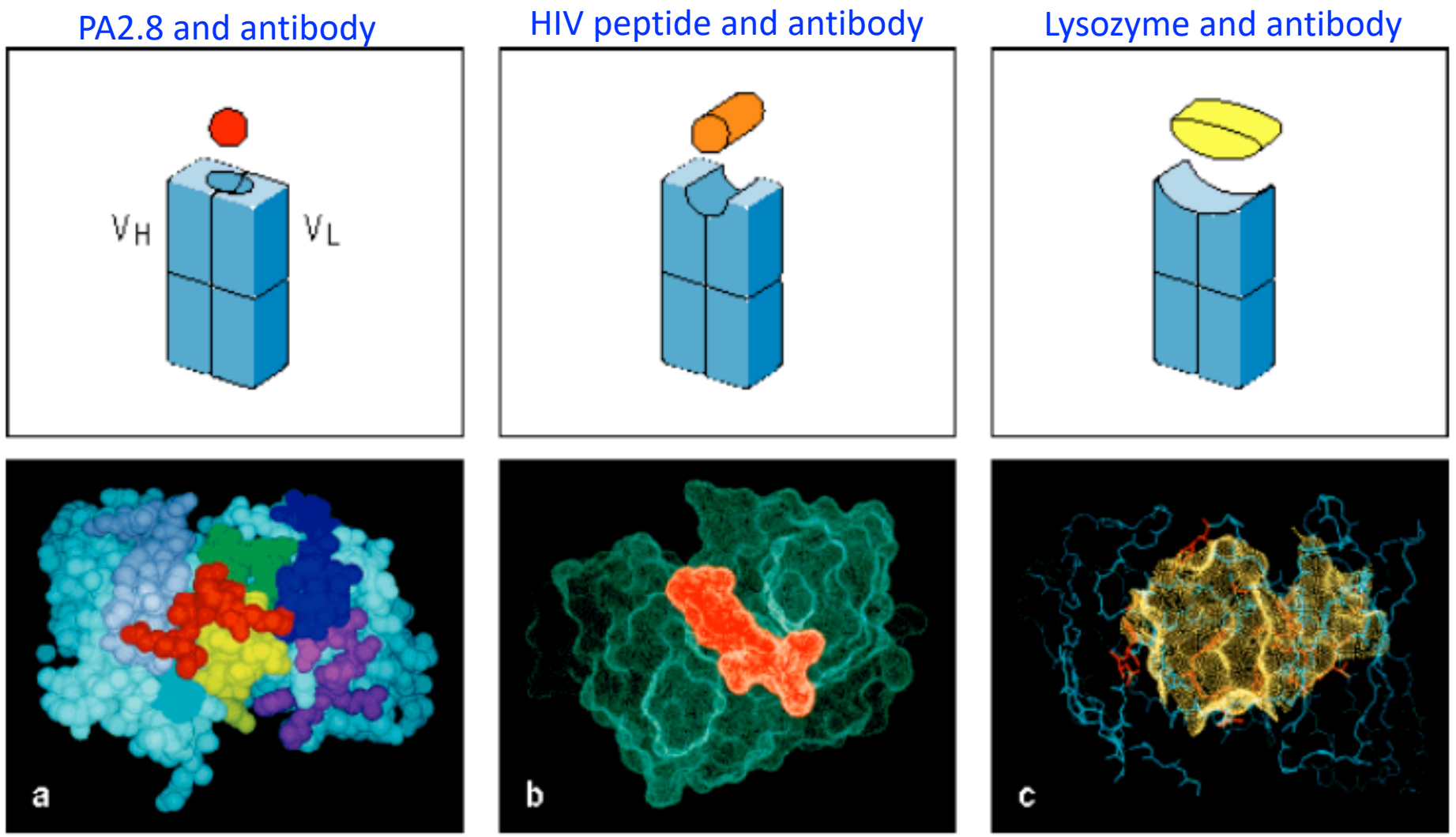
- Antigen-Antibody bind via many non-covalent bonds
- High affinity antibodies evolve to fit the antigen and therefore have complementarity
- Even single amino acid residues in the interacting surfaces between the antigen-antibody (or binding pocket) can be critical for the strength of the interaction

Influenza antigen and antibody binding illustrates complementary when separated by 8 Å



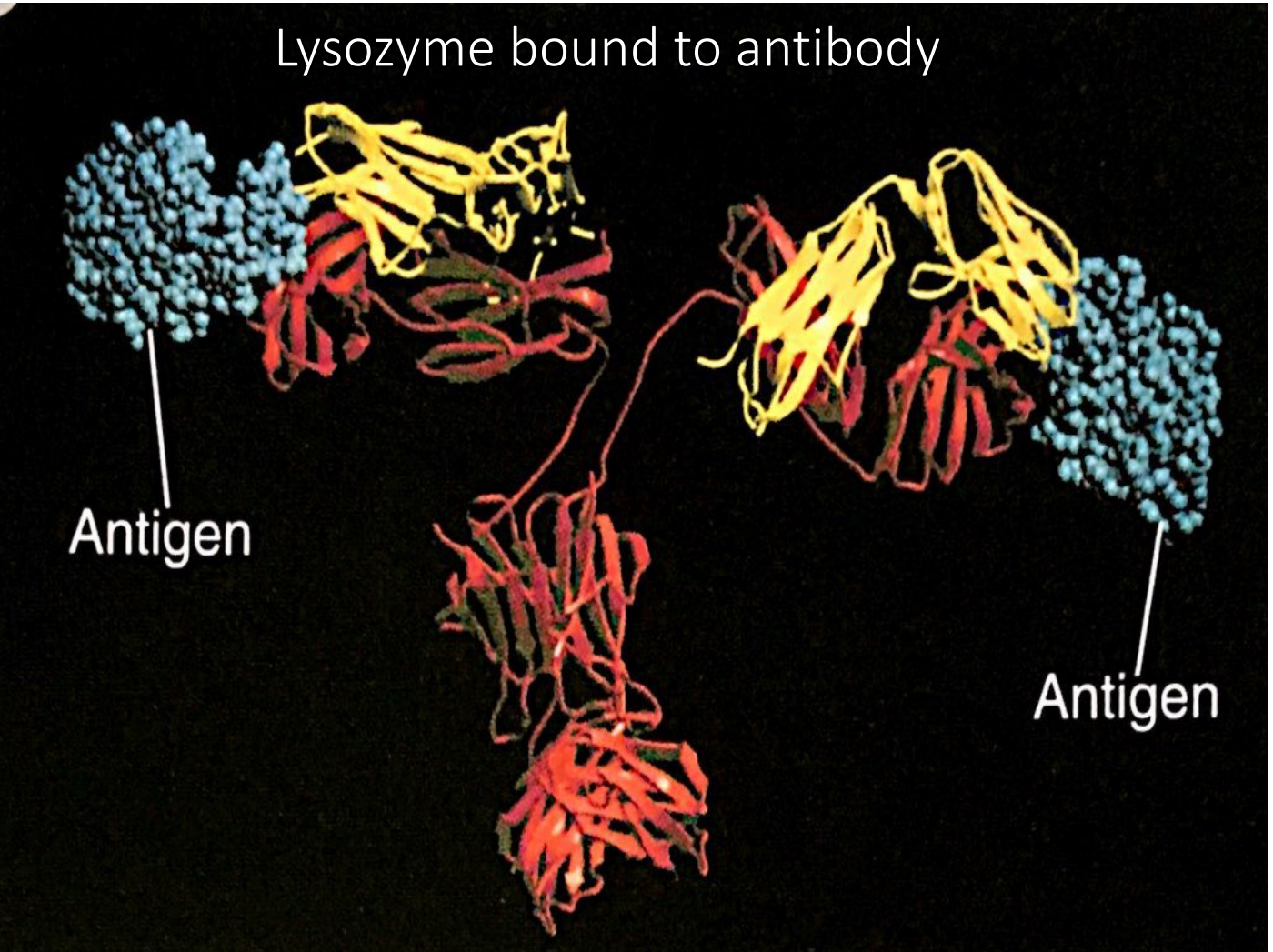
Immunology 5th ed. Kuby et al. W. H. Freeman and Company; 2000.

Large variation in antibody binding pockets due to the structural variability of the V_H and V_L domains



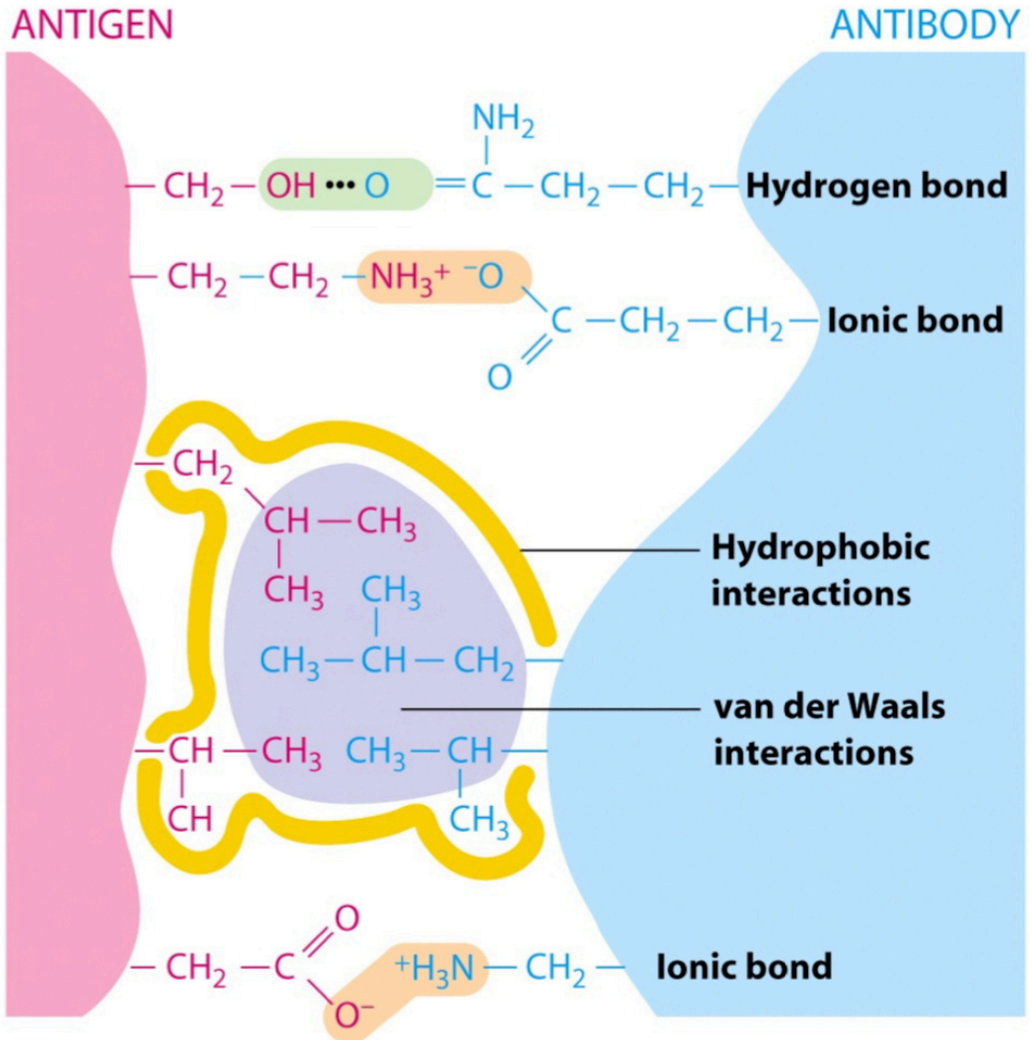
Immunobiology: The Immune System in Health and Disease 5th ed. Janeway CA Jr, Travers P, Walport M, et al. New York: Garland Science; 2001.

Complementarity Determining Regions (CDRs) generate antigen binding site specificity



- Specificity, degree to which an antibody differentiates between different antigens
- Finger-like CDRs usually recognize 15-22 amino acids
- Basic antibody structure maintained (β strands) when variability confined to CDR loops

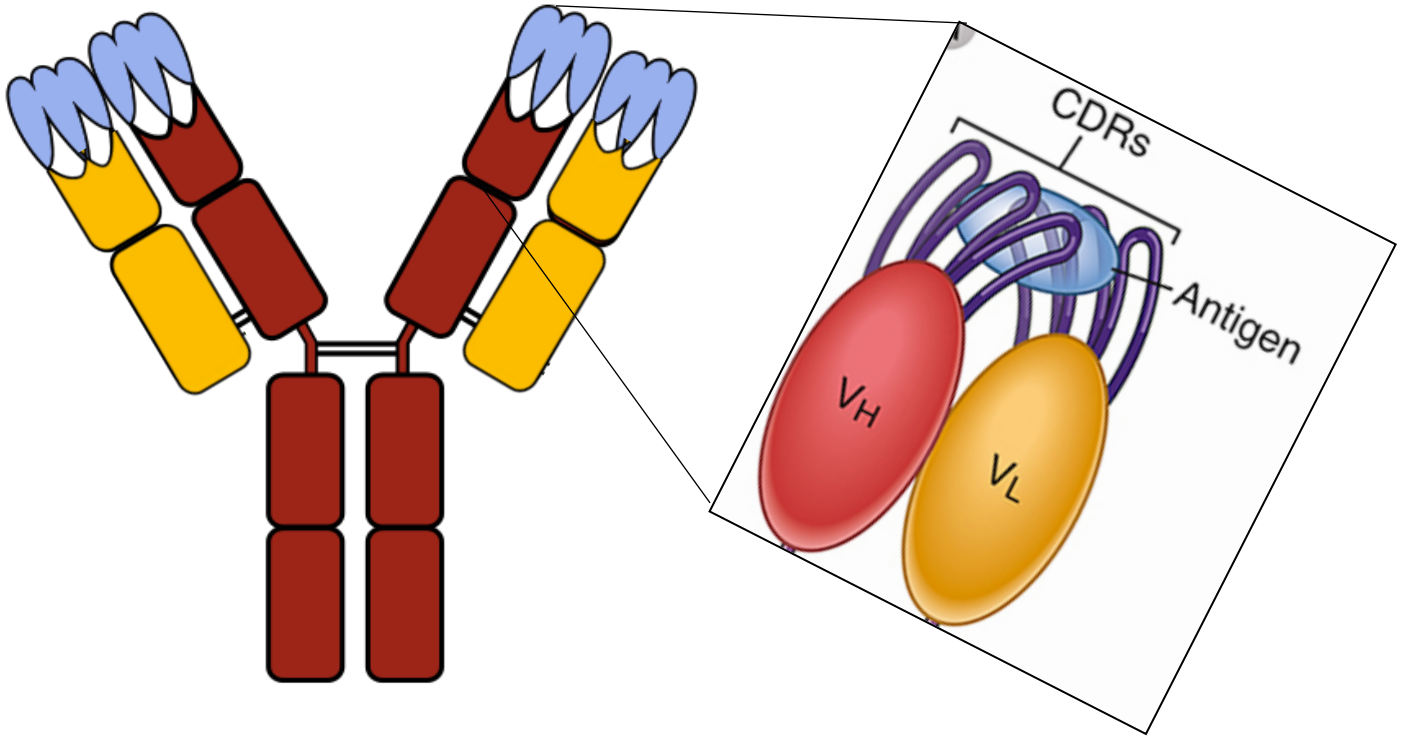
Noncovalent bonds form the basis of the antibody binding site



- Strength of each of these noncovalent interactions is weak
  - Many noncovalent bonds are required to form a strong interaction
- Each of these interactions operates over a very small distance (~1 Å)
- This requires a high degree of complementarity between the CDR of the antibody and the antigen

Immunology 4th ed. Kuby et al. W. H. Freeman and Company; 2000.

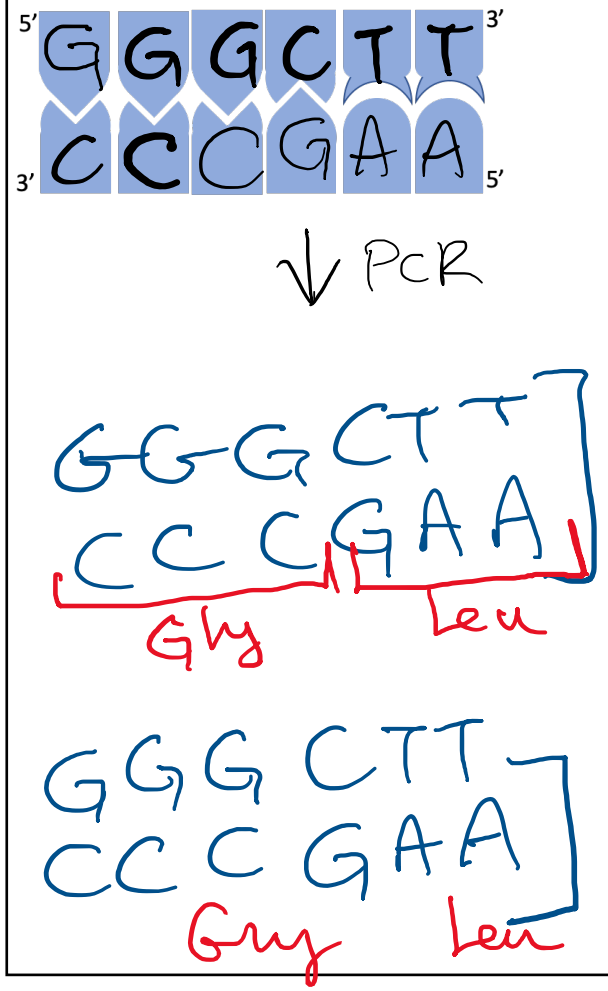
Mod1: Characterization of scFvs that bind lysozyme



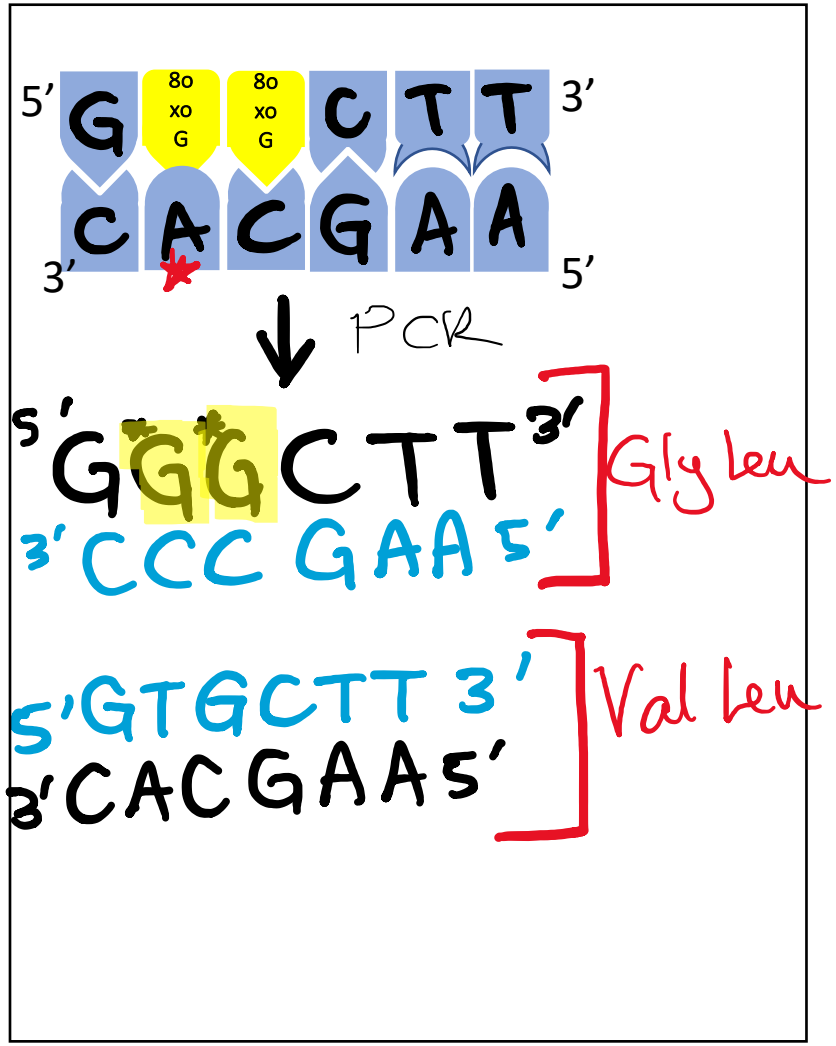
- The goal of this screen is to find a scFv clone with stronger binding to lysozyme
- Antibody with a lower K_d for its antigen means a more stable interaction and a higher affinity (stronger)
- We sorted a library of scFv yeast that bind to lysozyme
- Today will determine the DNA sequence of those mutants and later measure binding strength

Mispaired bases during PCR amplification steps results in changes to the DNA sequence and protein sequence

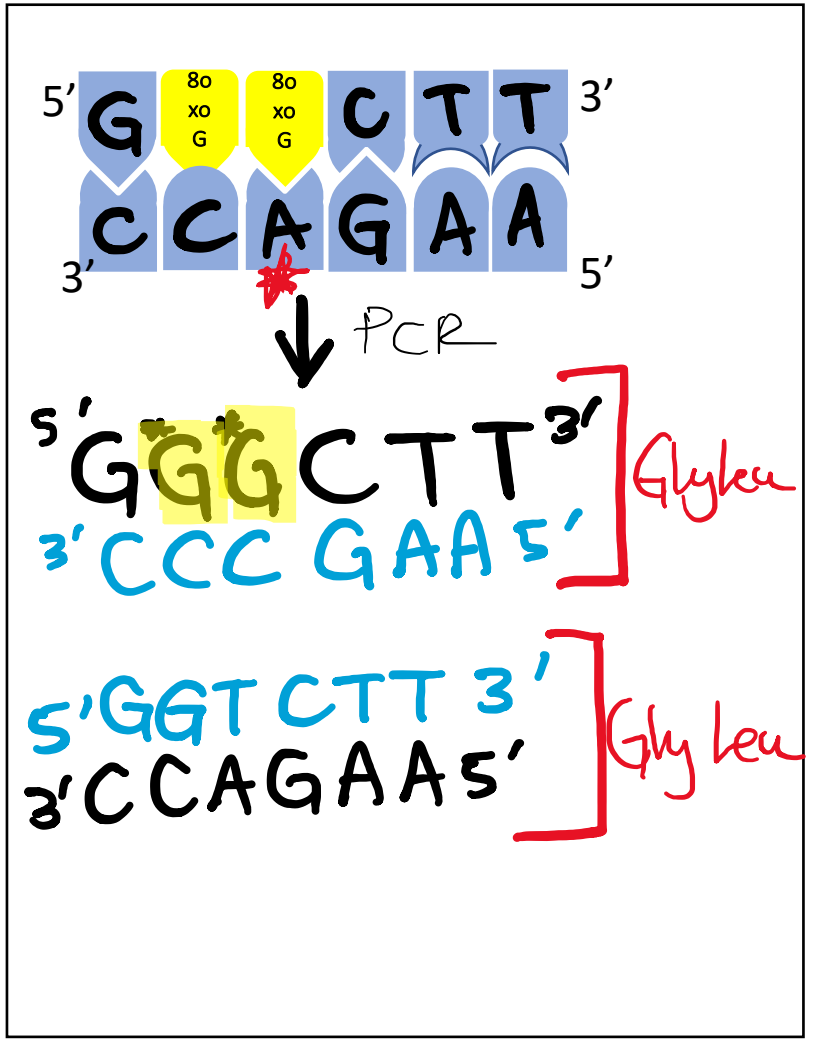
Parental Sequence: No mutations



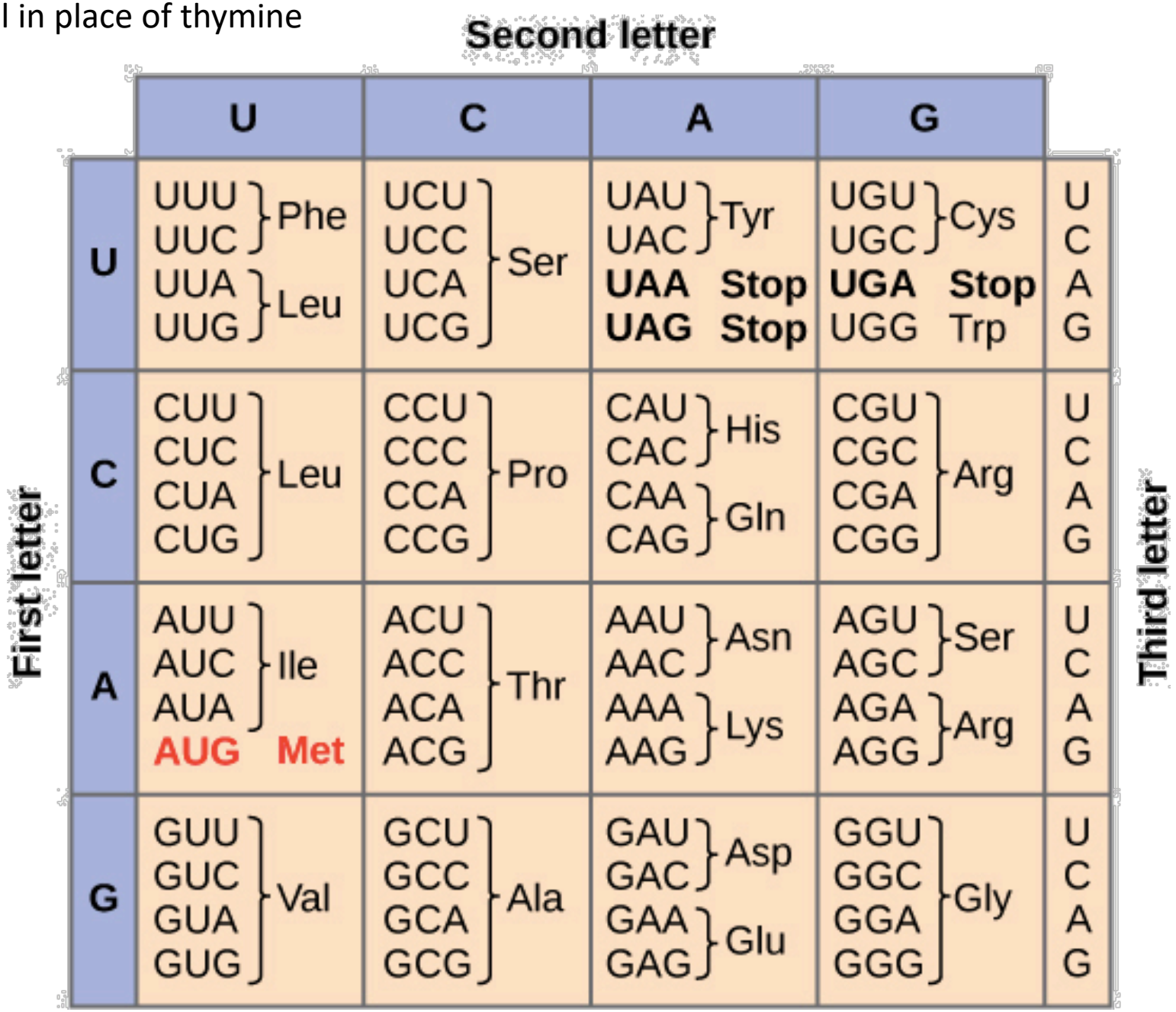
Mutant DNA sequence, mutant protein sequence



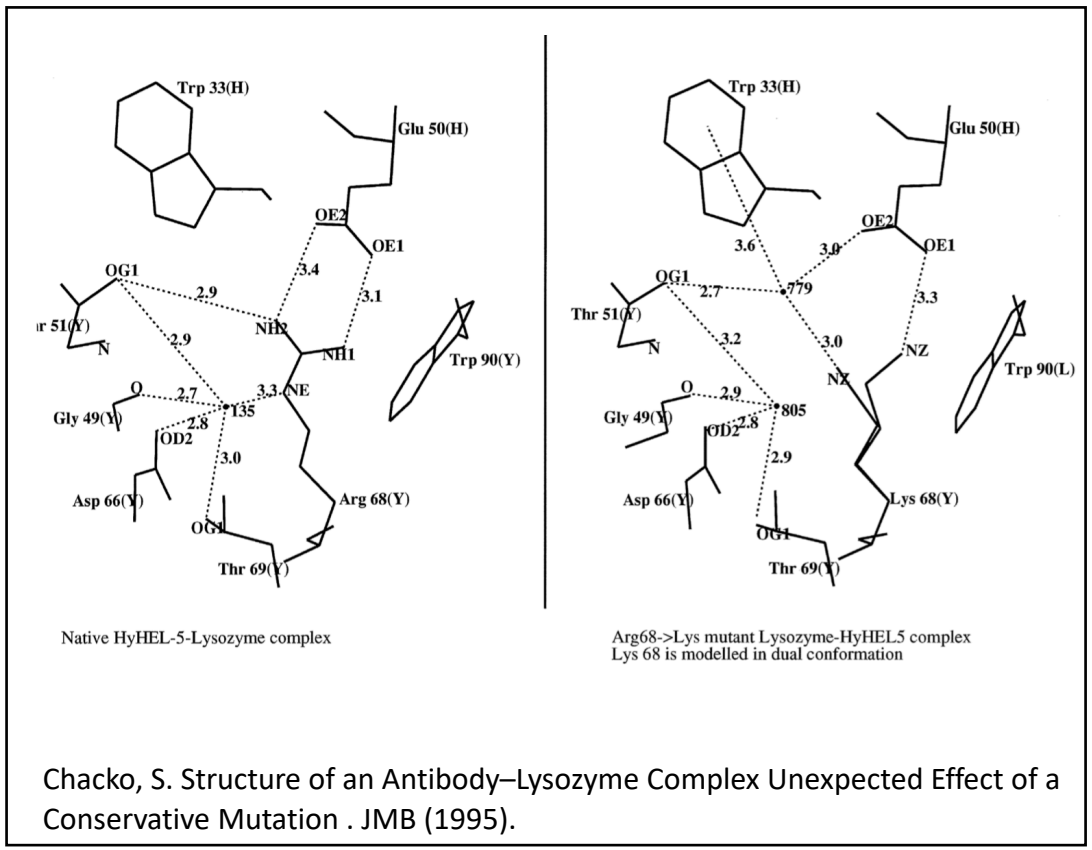
Mutant DNA sequence, silent mutation in protein sequence



*In mRNA, Uracil in place of thymine

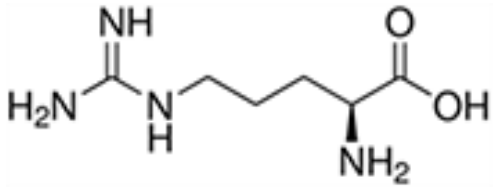


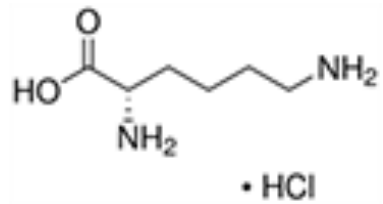
Effects of amino acid mutations on hydrogen bonding within the binding pocket of anti-lysozyme antibody



Chacko, S. Structure of an Antibody–Lysozyme Complex Unexpected Effect of a Conservative Mutation . JMB (1995).

- Arginine to lysine is a conservative mutation



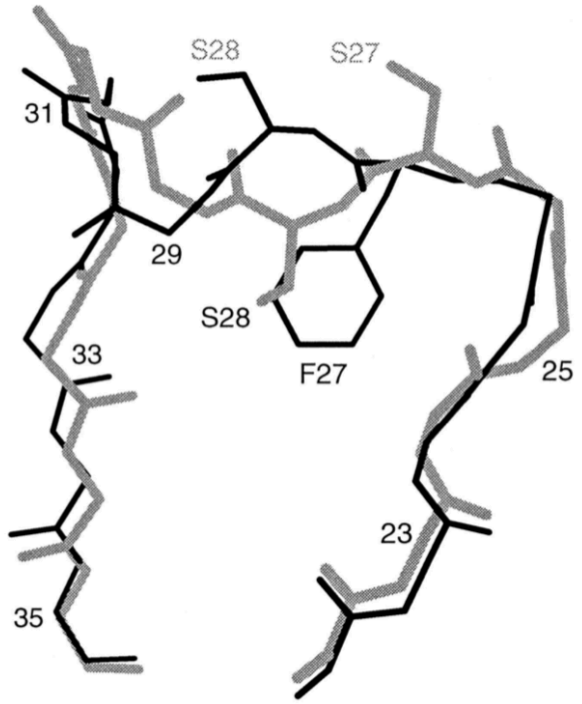


- A conservative replacement is an amino acid replacement in a protein that changes a given amino acid to a different amino acid with similar biochemical properties.
- The opposite is **radical replacement**, is an amino acid replacement that exchanges an initial amino acid by a final amino acid with different physicochemical properties.

Effects of amino acid mutations on anti-lysozyme antibody structure of a V_H CDR folding

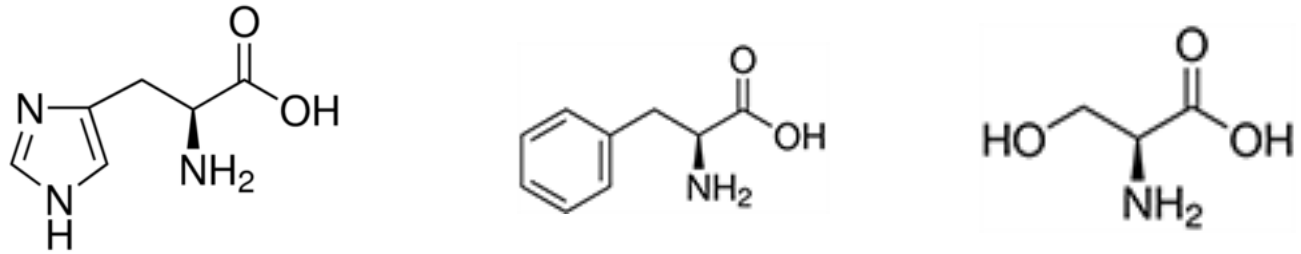
Mut1: H27F black

Mut2: H27S grey



Holmes, M. Structural Effects of Framework Mutations on a Humanized Anti-Lysozyme Antibody . J Immunology (2001).

- Left: Histidine 27 to Phenylalanine or Serine



- Changes in amino acid sequence can also affect the folding or structure of several amino acids in a peptide chain
- Mut1 and Mut2 create a pocket like structure instead of an exposed charge

Antibody K_d (dissociation constant) is equated strength of the interaction

- Dissociation constant= K_d
- Lower K_d= stronger interaction

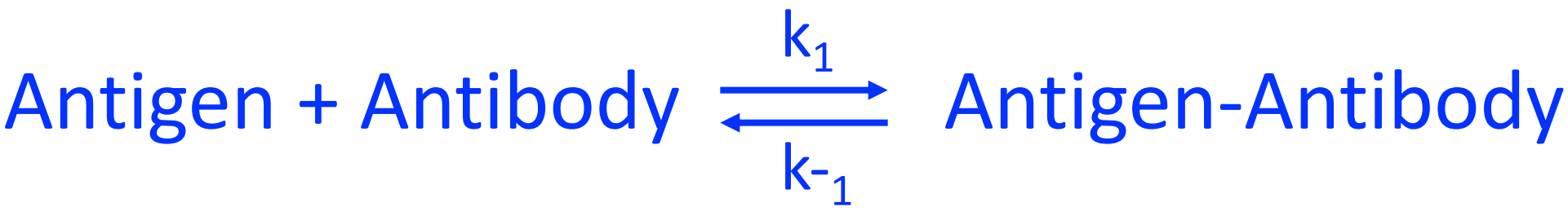
TABLE 6-1 Forward and reverse rate constants (k_1 and k_{-1}) and association and dissociation constants (K_a and K_d) for three ligand-antibody interactions

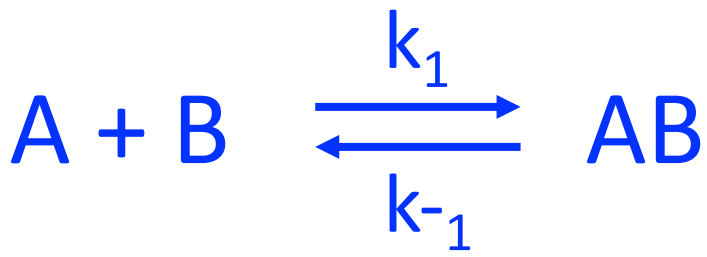
Antibody	Ligand	k_1	k_{-1}	K_a	K_d
Anti-DNP	ϵ-DNP-L-lysine	8×10^7	1	1×10^8	1×10^{-8}
Anti-fluorescein	Fluorescein	4×10^8	5×10^{-3}	1×10^{11}	1×10^{-11}
Anti-bovine serum albumin (BSA)	Dansyl-BSA	3×10^5	2×10^{-3}	1.7×10^8	5.9×10^{-9}

SOURCE: Adapted from H. N. Eisen, 1990, *Immunology*, 3rd ed., Harper & Row, Publishers.

Table 6-1
Kuby IMMUNOLOGY, Sixth Edition

Binding a monovalent antigen by an antibody can be described by a bimolecular equation





K_1=rate of association

K_{-1}=rate of disassociation

The equilibrium association constant (K_a) is a good indicator for antibody affinity

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AB$$

$$K_a = \frac{[AB]}{[A][B]}$$

- Ratio of products to reactants
- Affinity, the strength of the total noncovalent interactions between one antigen and antibody
- Units of K_a are concentration^{-1}
- Example: nM^{-1}

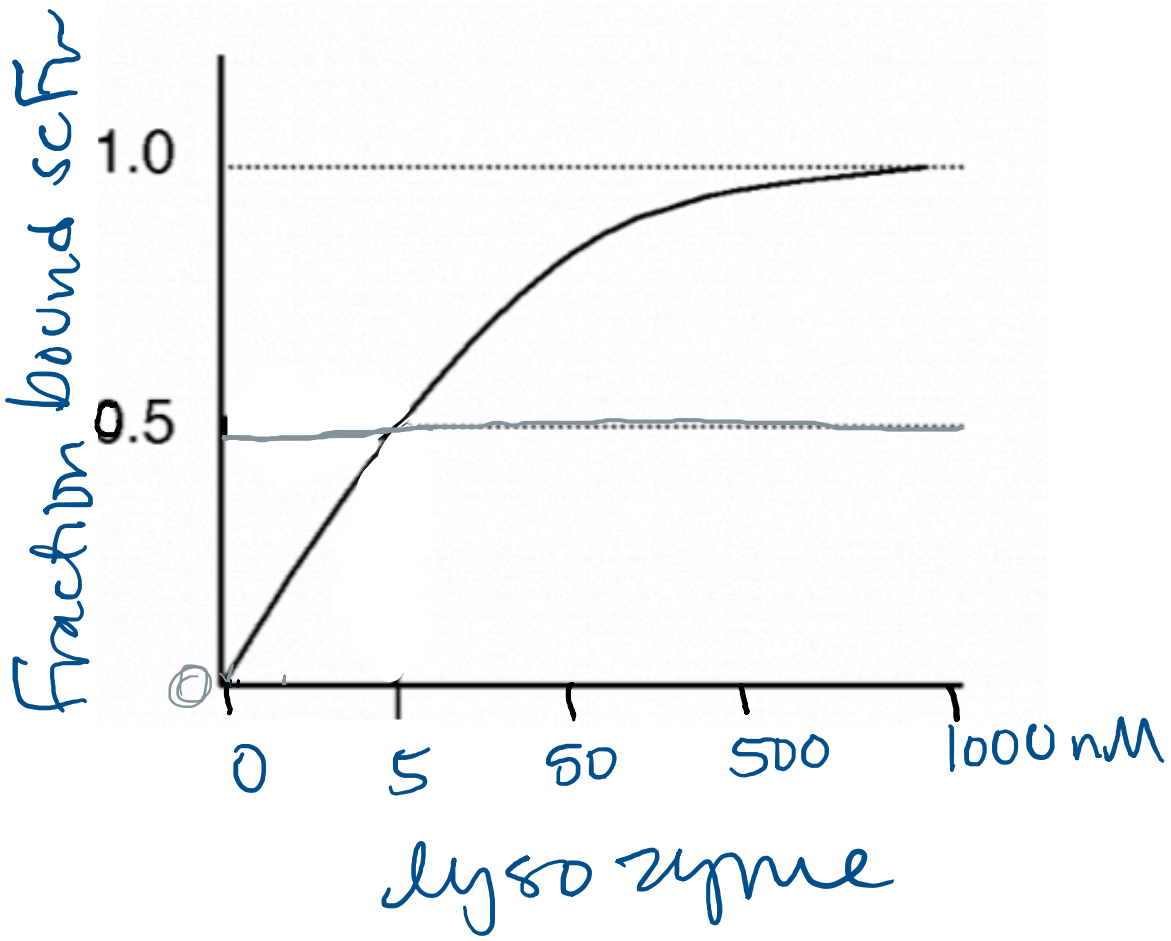
Equilibrium dissociation constant (K_d) is an indicator of the stability of a complex

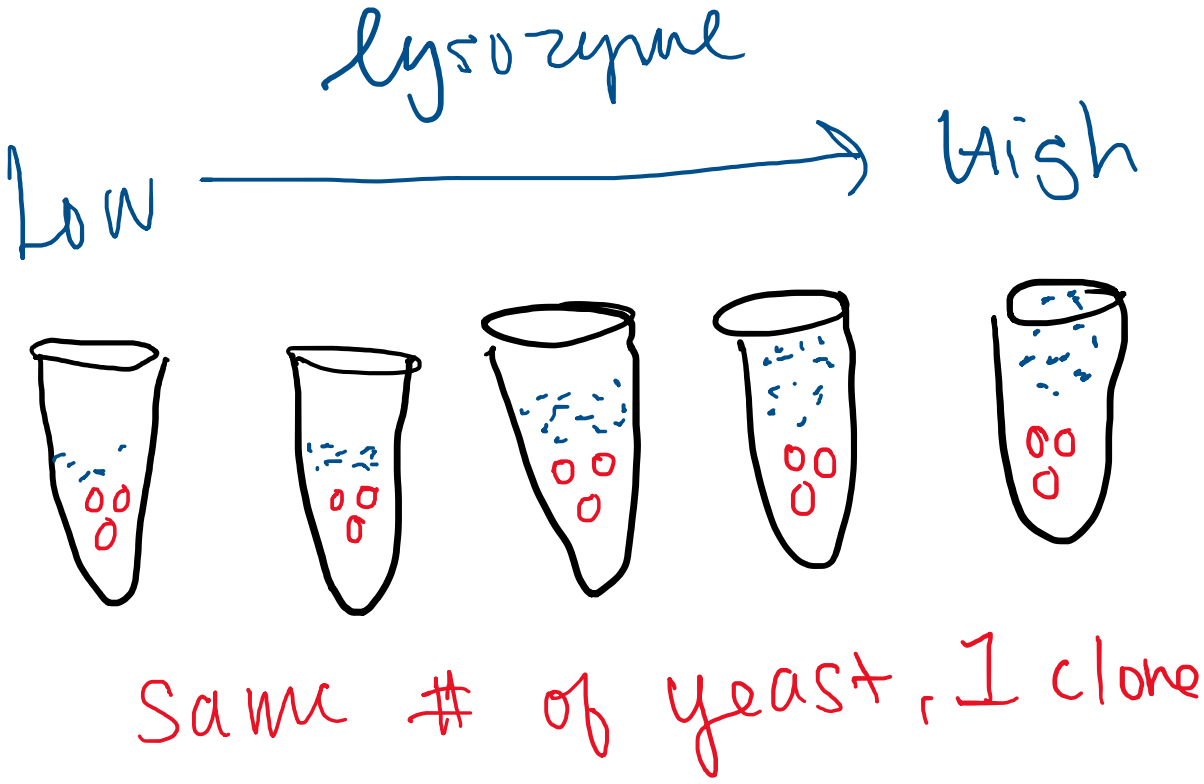
$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AB$$

$$K_d = \frac{[A][B]}{[AB]}$$

- Ratio of reactants to products
- Antibodies produced in a typical immune response usually varied from $K_d = 10^{-7}$ (~100nM) to 10^{-9} (~1nM)
- Units of K_d are concentration
- The smaller the K_d the more stable the interaction

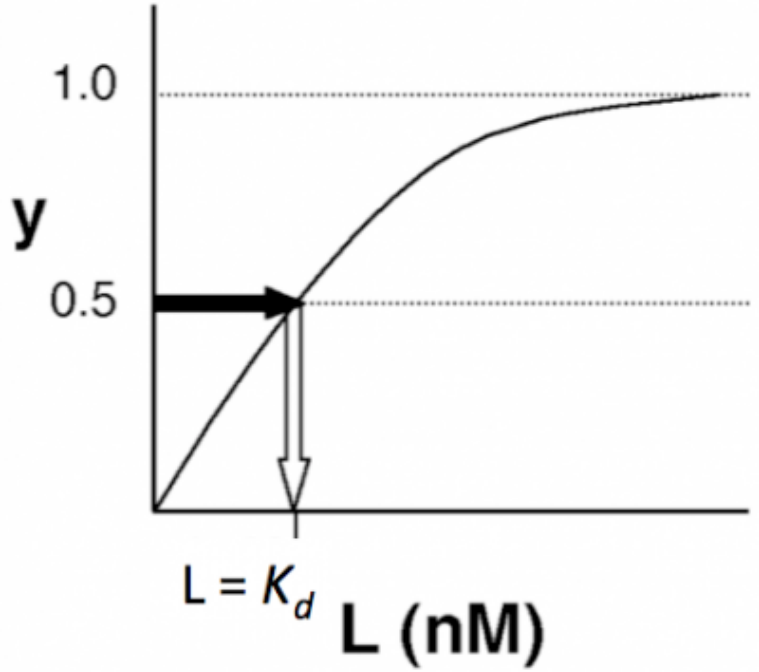
Practically how will we measure the strength of our lysozyme and scFv interaction





Biomolecular binding interaction at equilibrium:

Why is antibody dissociation constant (K_d) equal to the antigen concentration at which 50% antibody is bound to antigen?



Lysozyme

$$A + B \rightleftharpoons AB$$

A: lysozyme-scFv

B: scFv

$$\text{equilibrium constant } K_d = \frac{[A][B]}{[AB]}$$

Reactants over products

fraction B bound $= \dfrac{[AB]/[AB]}{[B]+[AB]} = \dfrac{1}{\frac{[B]}{[AB]}+1}$

replace

$\dfrac{[B]}{[AB]}$, $K_d = \dfrac{[A][B]}{[AB]} \div \dfrac{1}{[A]} = \dfrac{[B]}{[AB]}$

$=$

$$= \frac{1}{\frac{K_d}{[A]} + 1} \times \frac{[A]}{[A]} = \frac{[A]}{K_d + [A]}$$

lets plug in some numbers

fraction B bound $= \frac{[A]}{K_d + [A]}$; so when $K_d = [A]$; $\frac{1}{1+1} = \frac{1}{2}$

$1 = 1$

so...

So when $K_d = [A]$

fraction bound $B = \frac{1}{2}$

Experimentally find 50% bound antibody, then antigen concentration used for this condition = K_d

Mathematical relationship between fraction bound and free reactant makes estimations easy

$$L + Ab \rightleftharpoons_{k_r}^{k_f} C$$

$$y = \frac{[L]}{[L] + K_d}$$

- at $L = K_d$ $y = 0.5$

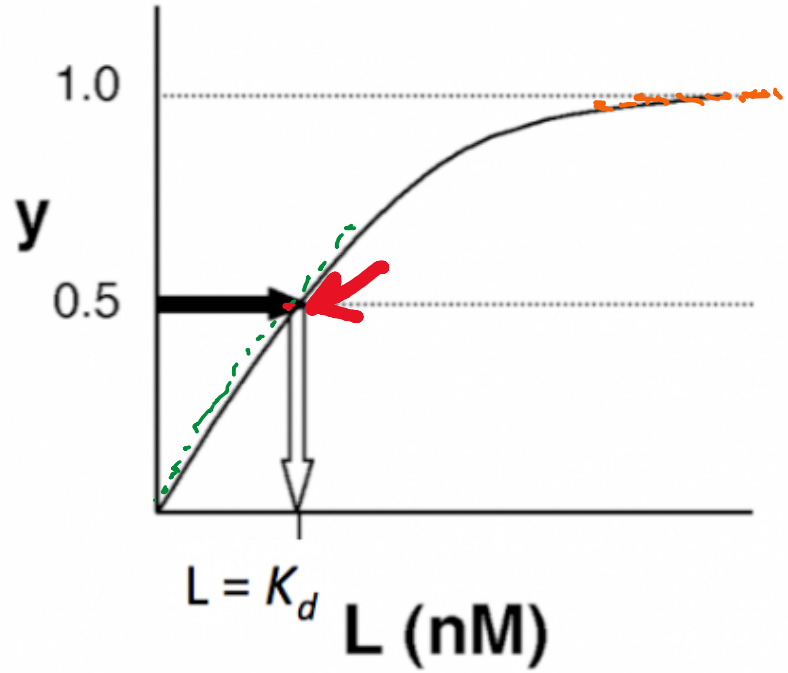
equal

- if $L << K_d$ then $y \approx \frac{[L]}{K_d}$ (linear relationship)

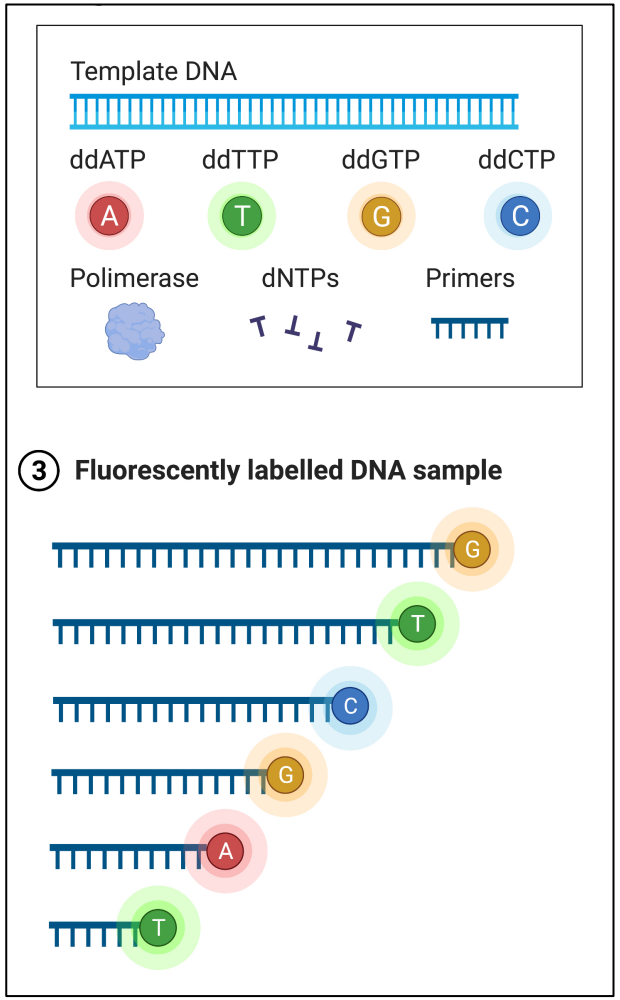
lower

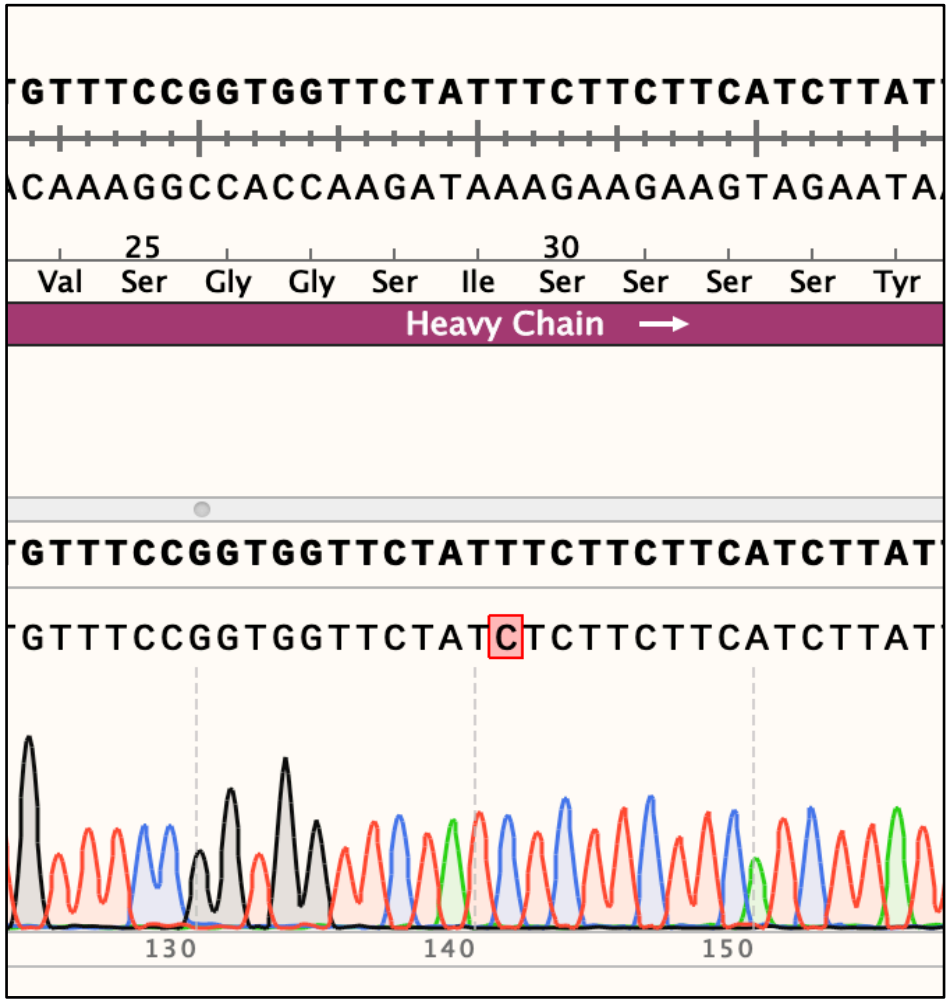
- if $L >> K_d$ then $y \approx 1$ (at saturation)

higher



Today in lab, M1D5: Analyze clone sequences





THE CENTRAL DOGMA