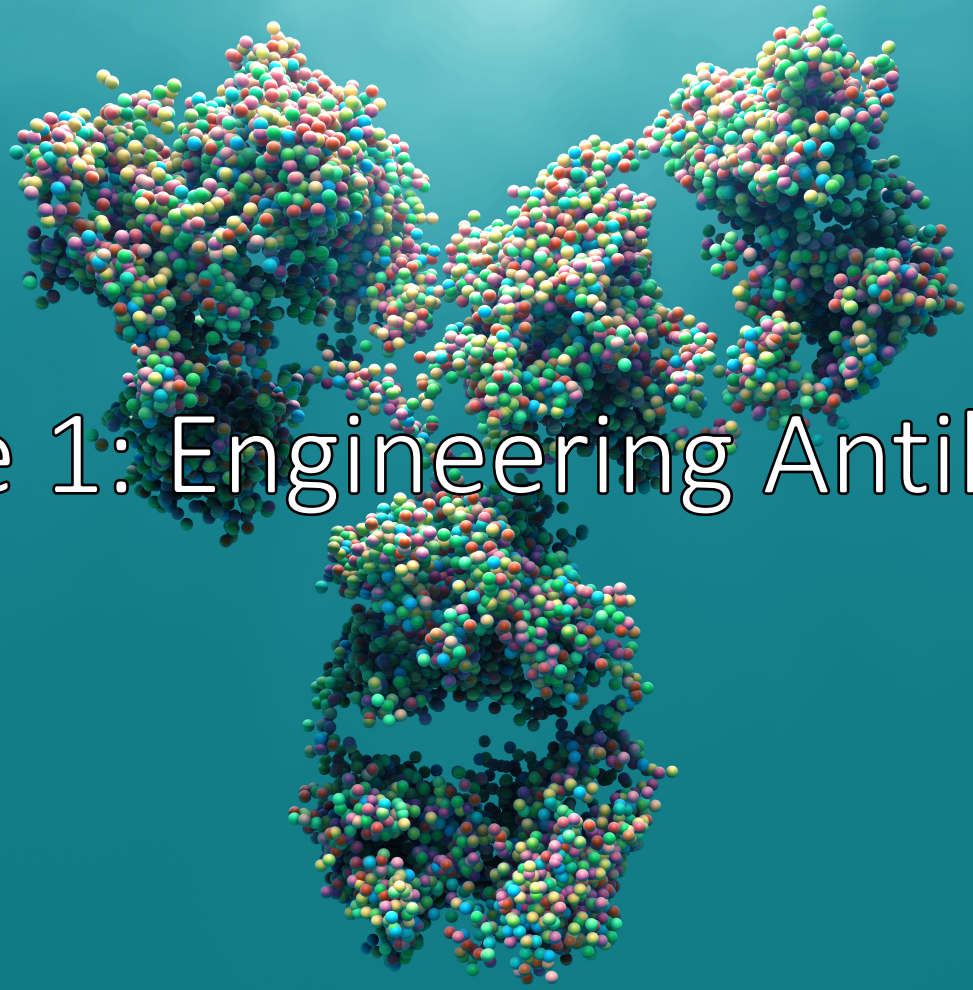


# Module 1: Engineering Antibodies

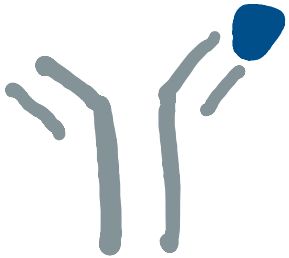
Draw an antibody binding one antigen from memory. (Don’t cheat!)

# What does your antibody drawing look like?

A

C

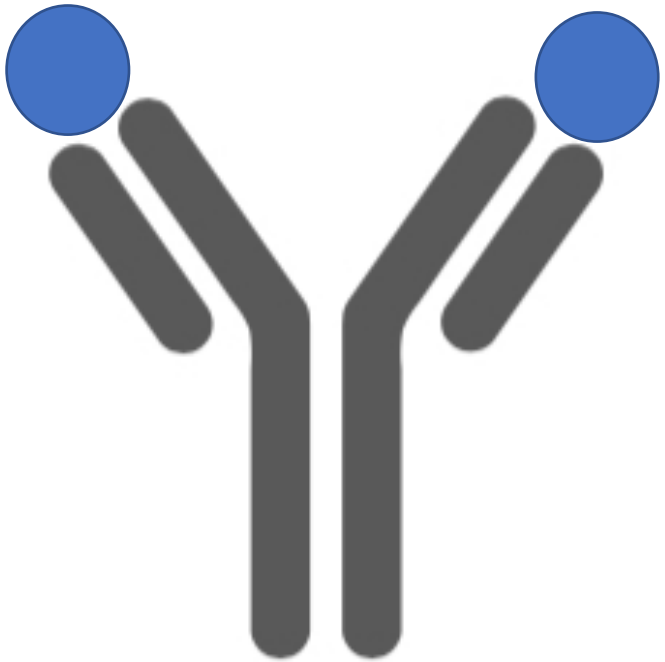
B



D

None of these

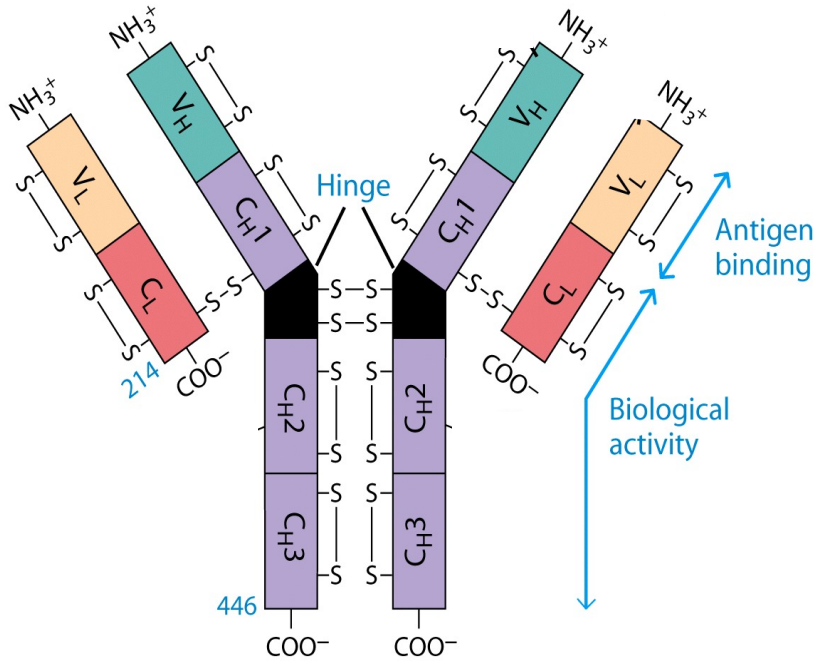
# Antibody common structure: Four peptide chains



- **Antibody shown in grey**
  - Antibody: protein effector molecule in the adaptive immune response
- **Antigen shown in blue**
  - Antigen: any molecule that can bind specifically to an antibody

- Large protein molecule, 150kDa
  - 2 light peptide chains, 25kDa
  - 2 heavy peptide chains, 50kDa
- Each antibody has two identical antigen-binding sites

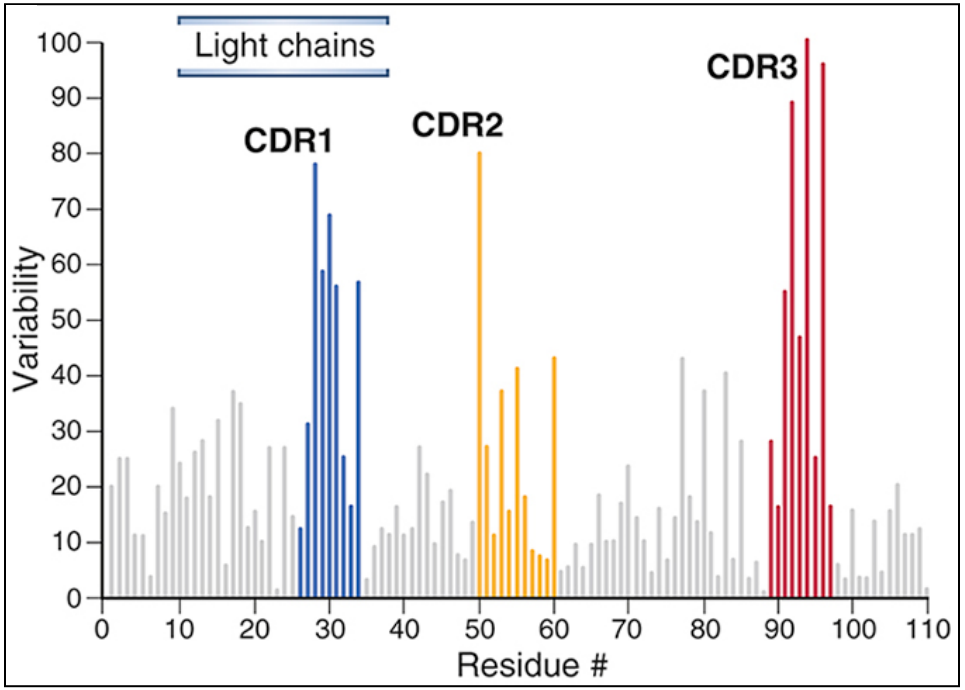
# Peptide sequence distinguishes the variable and constant regions



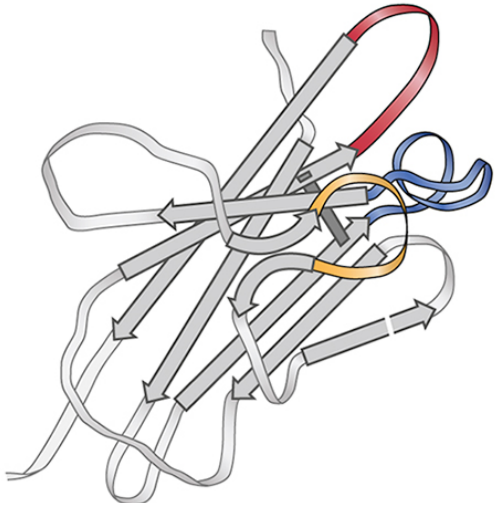

- Antibodies are composed of distinct domains
  - The first domain (~110aa) vary greatly
  - Variable, antigen binding, region
- Peptide chains held by disulfide bonds
- Constant region is essential to the immune response
  - interacts with effector cells and molecules

Kuby's Immunology, Fig 4.2

# There are hypervariable regions within the variable regions of an antibody



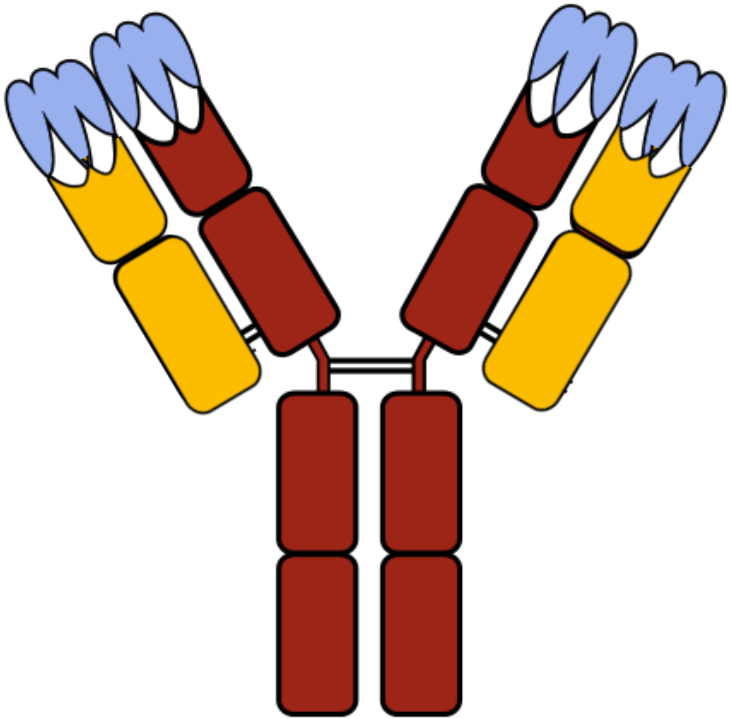

Variable protruding loops, red/blue/yellow



Stable β sheets, grey

Cell and Molecular Immunology, Fig 5.5, adapted from Kabat, E.A. et al.

# Most differences among antibodies fall within the complementarity-determining regions (CDR)

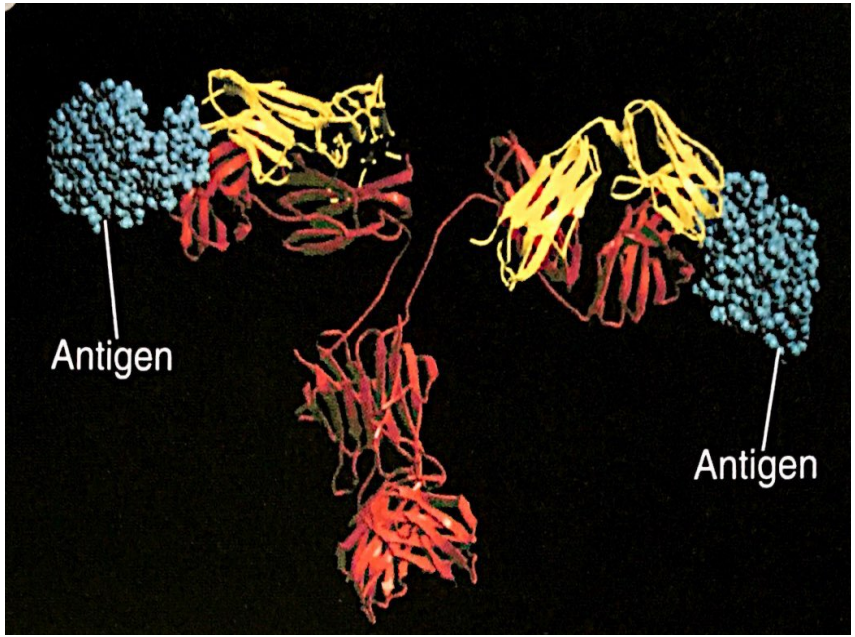


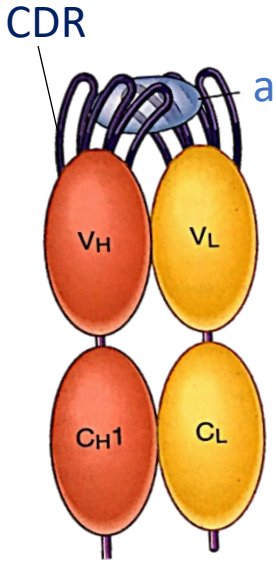
Anypodetos, Antibody CDR, wikipedia

- CDR also called hypervariable regions
  - 3 protruding loops connected to the β-sheets of the $V_L$ and $V_H$
- Can bind varied macromolecules and small chemicals
  - Protein, peptides, polysaccharides, nucleic acids, or lipids
- Each individual has $10^7$ to $10^9$ unique antigen receptors
- Sequence diversity created during somatic mutation of affinity maturation process

# CDRs generate antigen binding site

Lysozyme bound to antibody




- Finger-like CDRs usually recognize 15-22 amino acids
- Basic antibody structure maintained when variability confined to loops

Cell and Molecular Immunology, Fig 5.6

# The Antigen - Antibody interaction forms multiple contacts

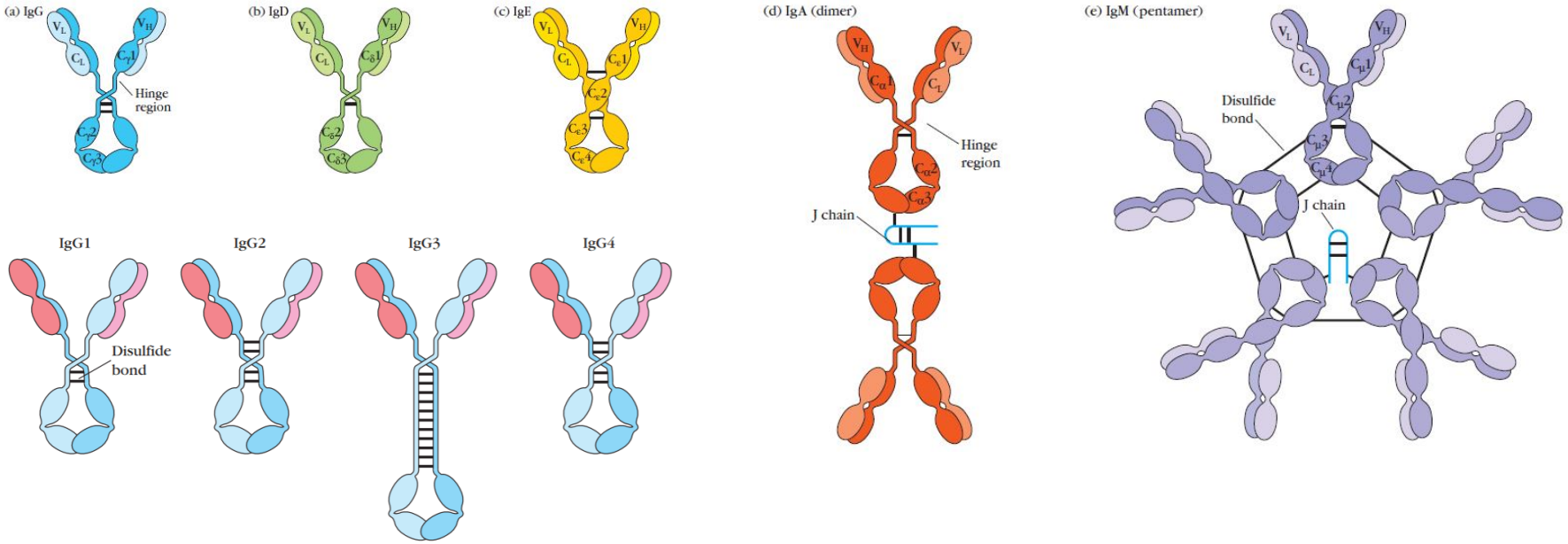
3D: Lysozyme bound to variable region

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

- Strong interactions depend on many noncovalent bonds and a high degree of complementarity

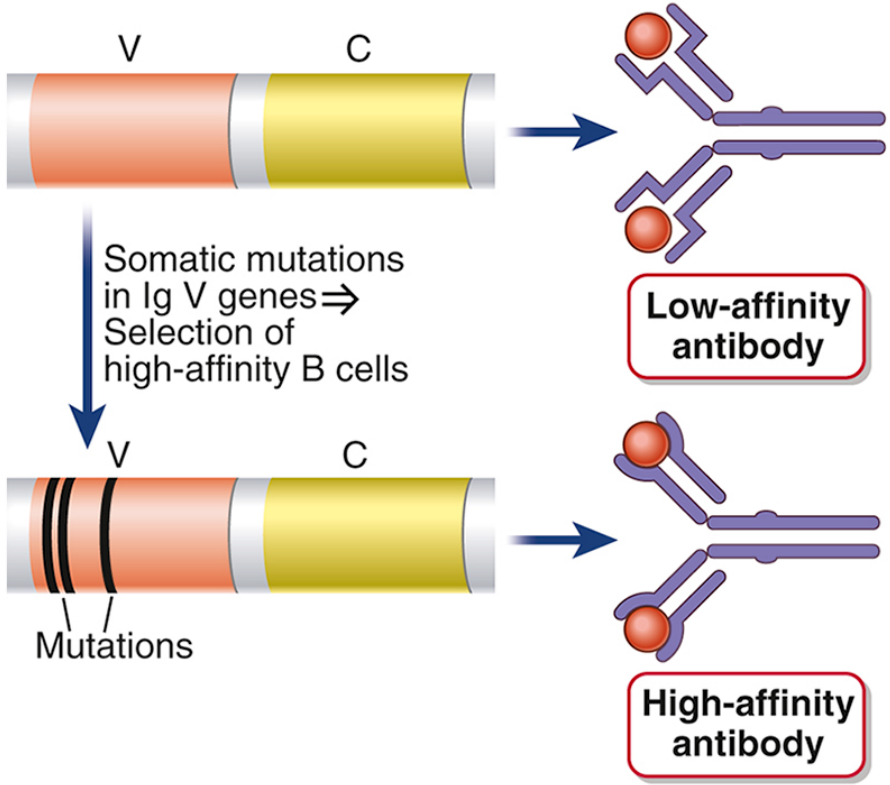
Cell and Molecular Immunology, Fig 5.6

# Antibody sequence and structure is diverse!



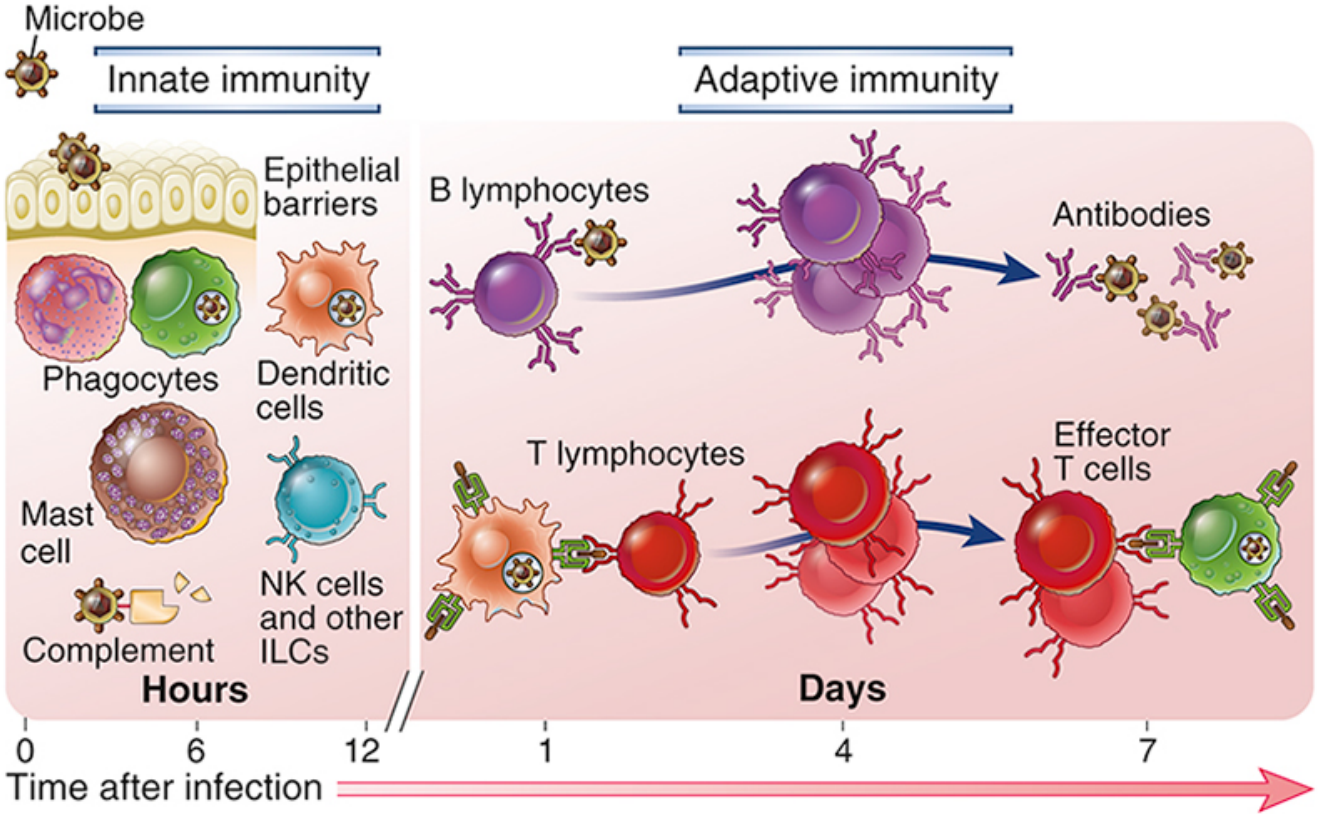

Cell and Molecular Immunology, Fig

# How does antibody diversity arise?



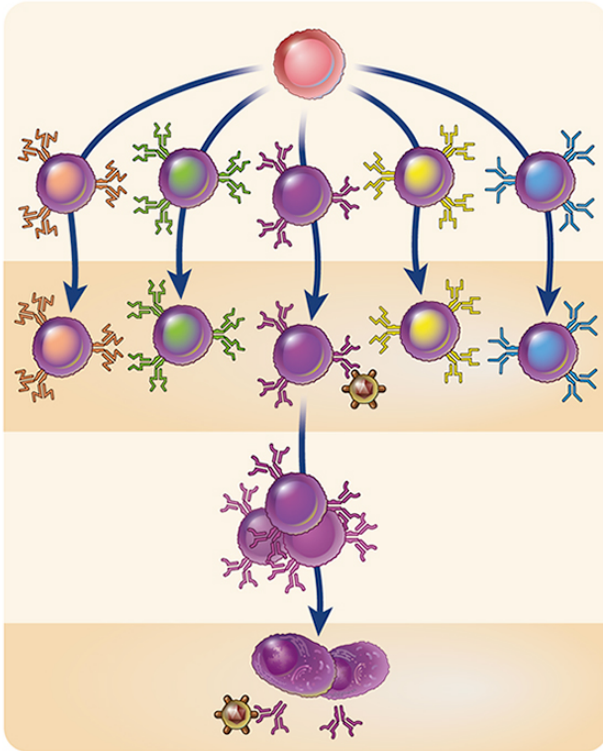

Cell and Molecular Immunology, Fig 12.16

# Innate and Adaptive immunity are sequential




Cell and Molecular Immunology, Fig 1.1

# Antibody secretion occurs as a result of B cell maturation, during the adaptive immune response



1) Lymphocyte clones mature in absence of antigens

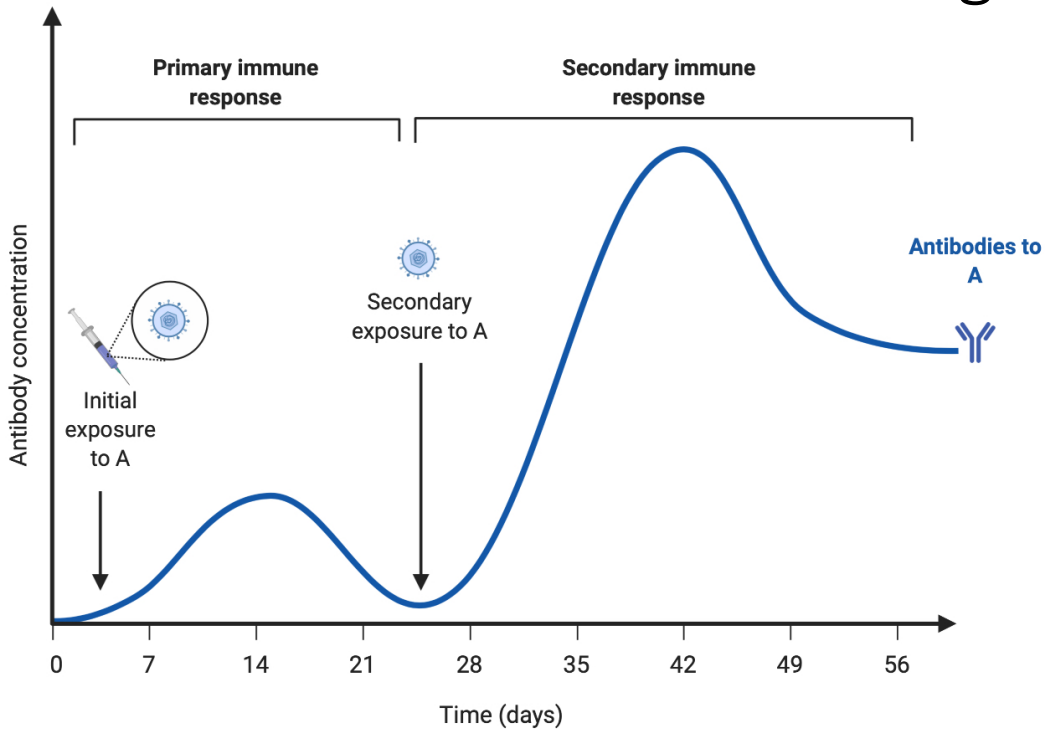
2) Mature lymphocytes enter lymphoid tissue and can bind antigens

3) Antigen binding activates clonal expansion

4) Antigen specific immune response occurs

Cell and Molecular Immunology, Fig 1.3

# Adaptive immune response is specific to a distinct antigen




- Secondary responses create a ”stronger” response because of memory cells specific to an antigen
- Memory cells can react more rapidly and vigorously to its antigen than a naïve lymphocyte

Image adapted from Biorender

# How can we engineer antibody diversity?

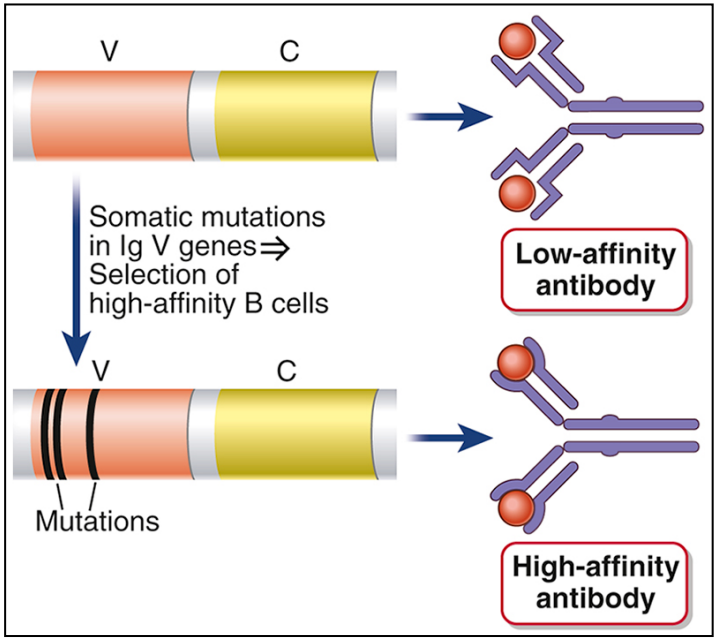
Module 1 adapted from:

PROTOCOL

## Isolating and engineering human antibodies using yeast surface display

Ginger Chao, Wai L Lau, Benjamin J Hackel, Stephen L Sazinsky, Shaun M Lippow & K Dane Wittrup

Department of Chemical Engineering and Biological Engineering Division, Massachusetts Institute of Technology, 77 Massachusetts Avenue E19-563, Cambridge, Massachusetts 02139, USA. Correspondence should be addressed to K.D.W. (wittrup@mit.edu).



# Yeast surface display libraries are a powerful platform for antibody discovery

- Mutagenesis of clone(s)
- Increase stringency of screen

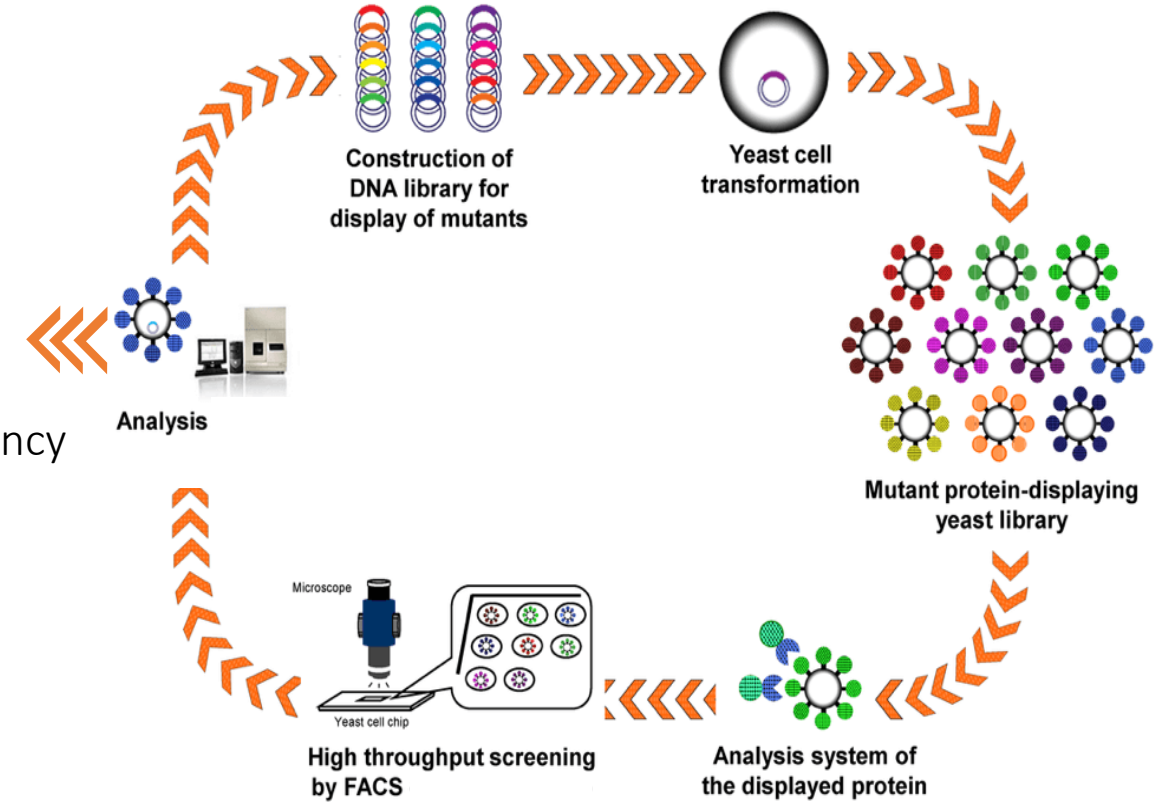
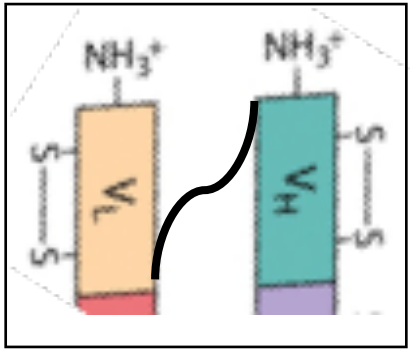



Image:Yeast Display Library Construction Services, creative-biolabs.com

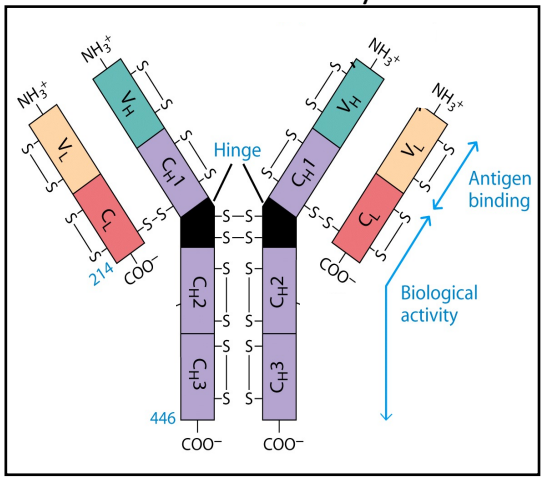
# Module 1 Experimental Goal: Identify an antibody fragment (scFv) that shows improved binding to an antigen.

**What's a scFv?** single-chain variable fragment; small, stable, fully functional fusion protein that contain the variable domains of an antibody

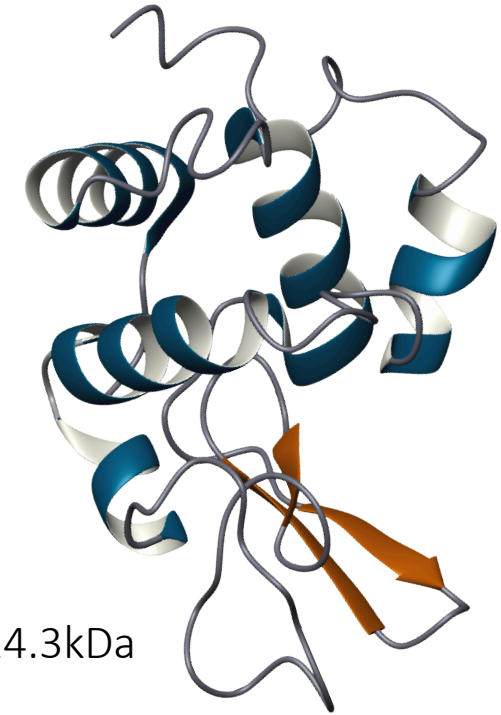
scFv




Full antibody



# Our Antigen: Lysozyme, antimicrobial enzyme found in plants and animals

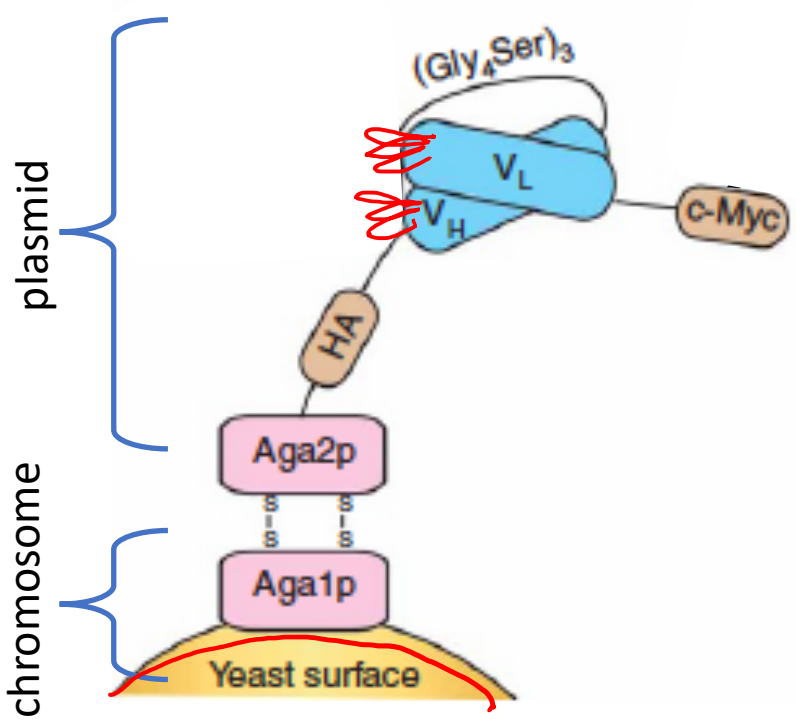


14.3kDa

- Hydrolyzes the bond between peptidoglycan amino sugars resulting in bacterial cell wall lysis
- Expressed in human mucosal membranes and tears
- 1966: lysozyme was the first enzyme crystalized and the mechanism of catalytic activity was elucidated
- **Used extensively in studies of antibody-antigen binding**
- commercially valuable enzyme for the food and pharmaceutical industry

Image: Protein Data Bank archive

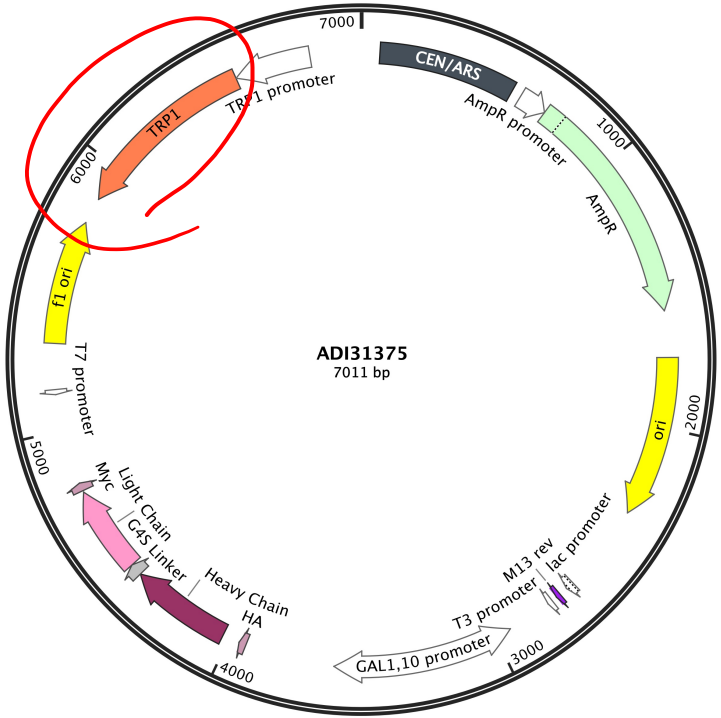
# Yeast display of single-chain variable antibody fragments (scFv)




- Single chain= single fusion peptide encodes variable region of light and heavy chain, connected by linker
- scFv fused to Aga2p which attaches to yeast cell wall by Aga1p
- Yeast express $10^4$ to $10^6$ copies of scFv on a cell
- Folded in the endoplasmic reticulum taking advantage of the chaperones and quality-control 'machinery'

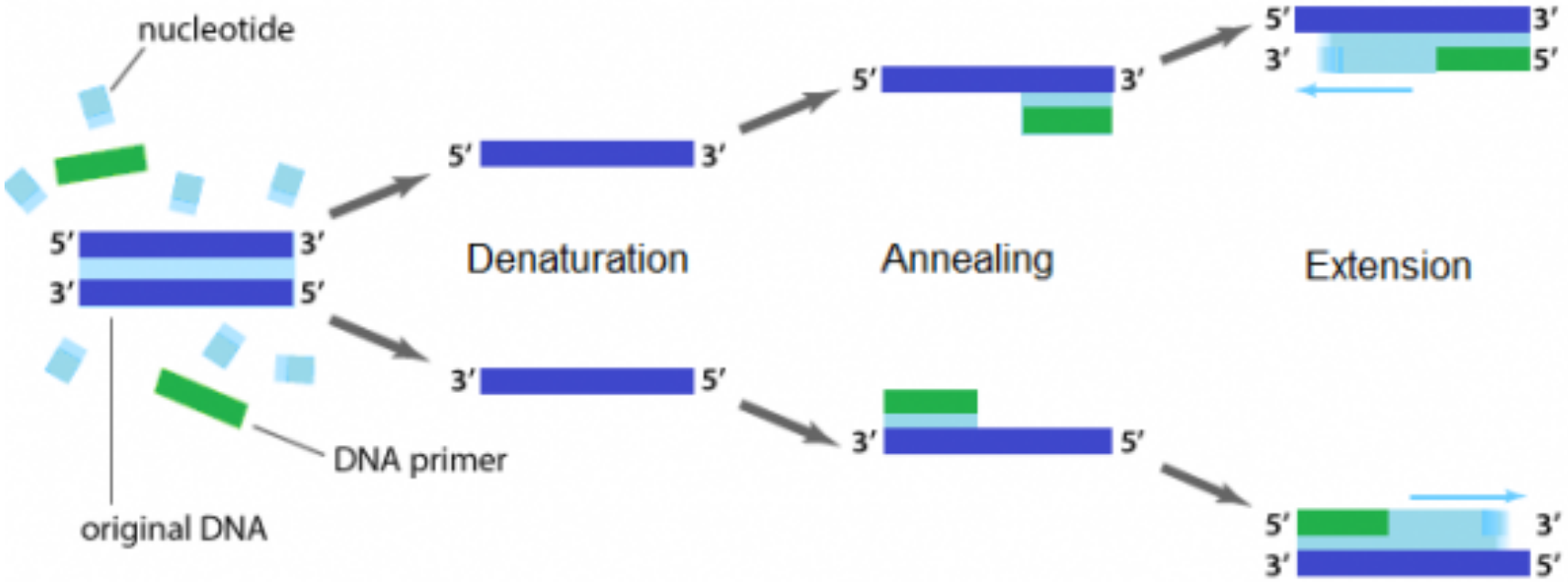
Chao et al. Nature Protocols 2006.

# The yeast display plasmid is maintained episomally with nutritional selection, TRP1




- The  strain of yeast we use are incapable of making tryptophan and the media is carefully made to exclude this amino acid
  - The TRP1 gene synthesizes this AA and allows yeast that maintain the plasmid to survive
- Our library was made using  error prone PCR specific to the scFv sequence

# Mutagenesis of the scFv gene will be carried out by error-prone Polymerase Chain Reaction (PCR)

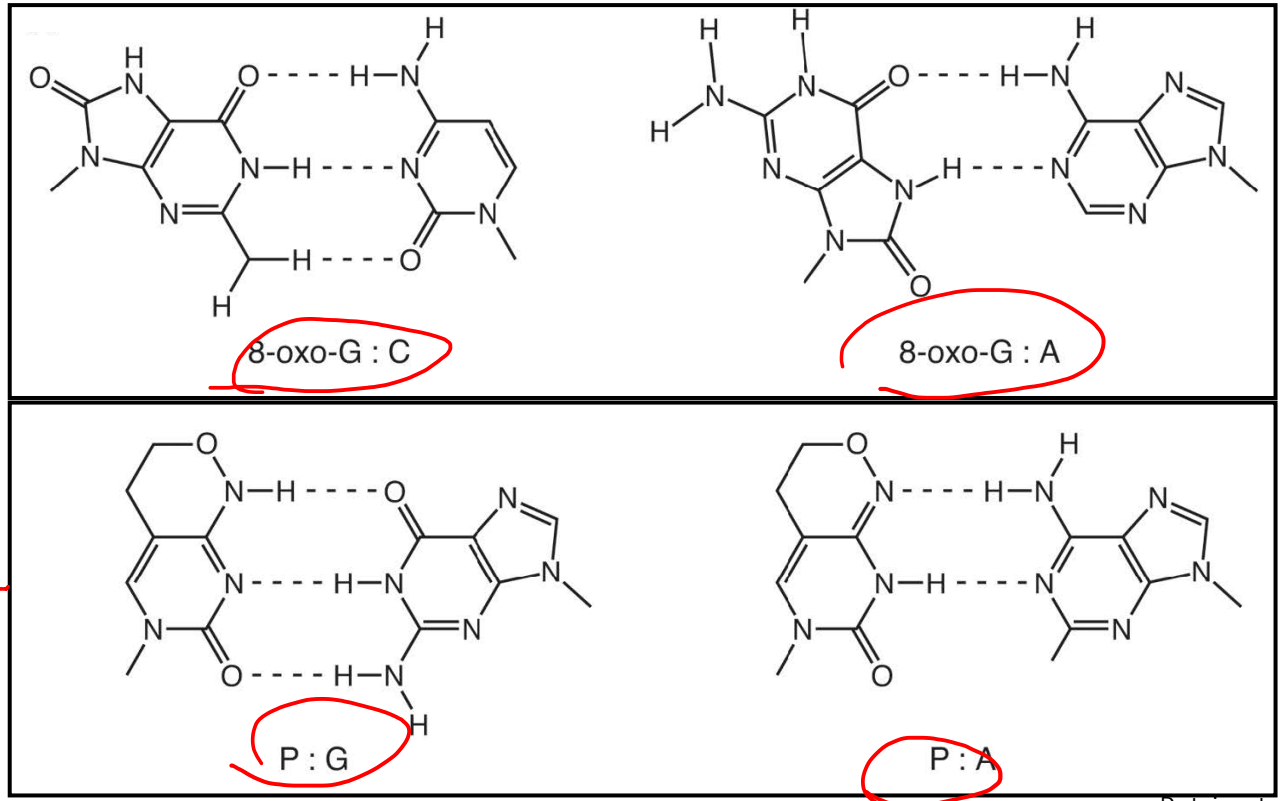



https://www.xxpresspcr.com

# Incorporation of mutant bases into the sequence of a scFv results in permanent sequence changes

8-oxo-dGTP

dPTP

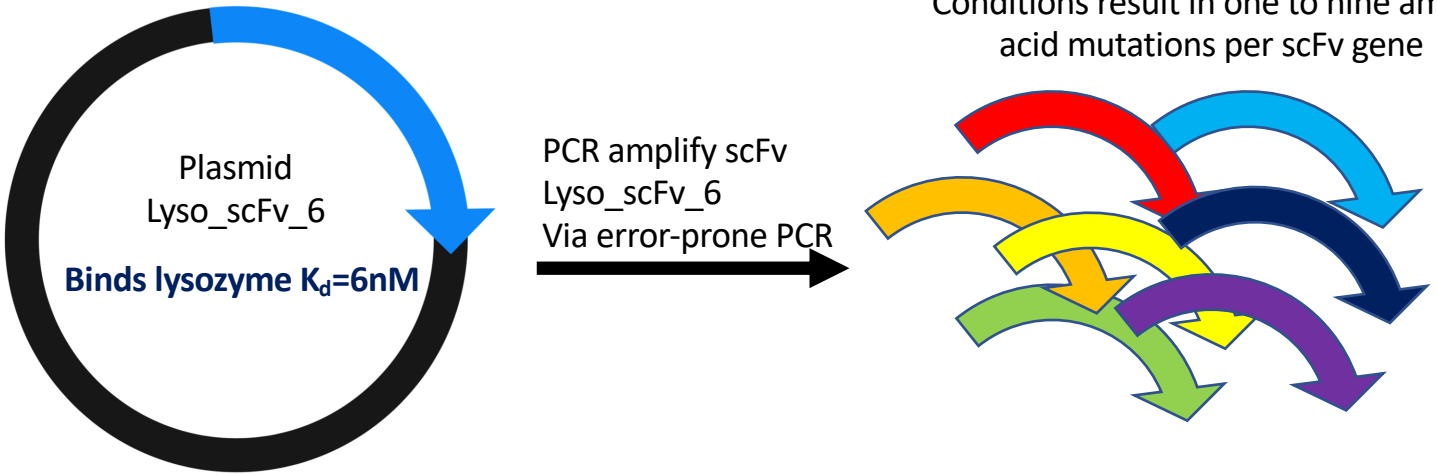



G–C

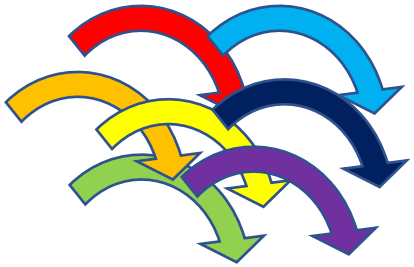
A–T

Petrie et al. Nucleic Acids Res 2010.

# Our scFv library is a collection of mutants from the **Lyso_scFv_6** plasmid

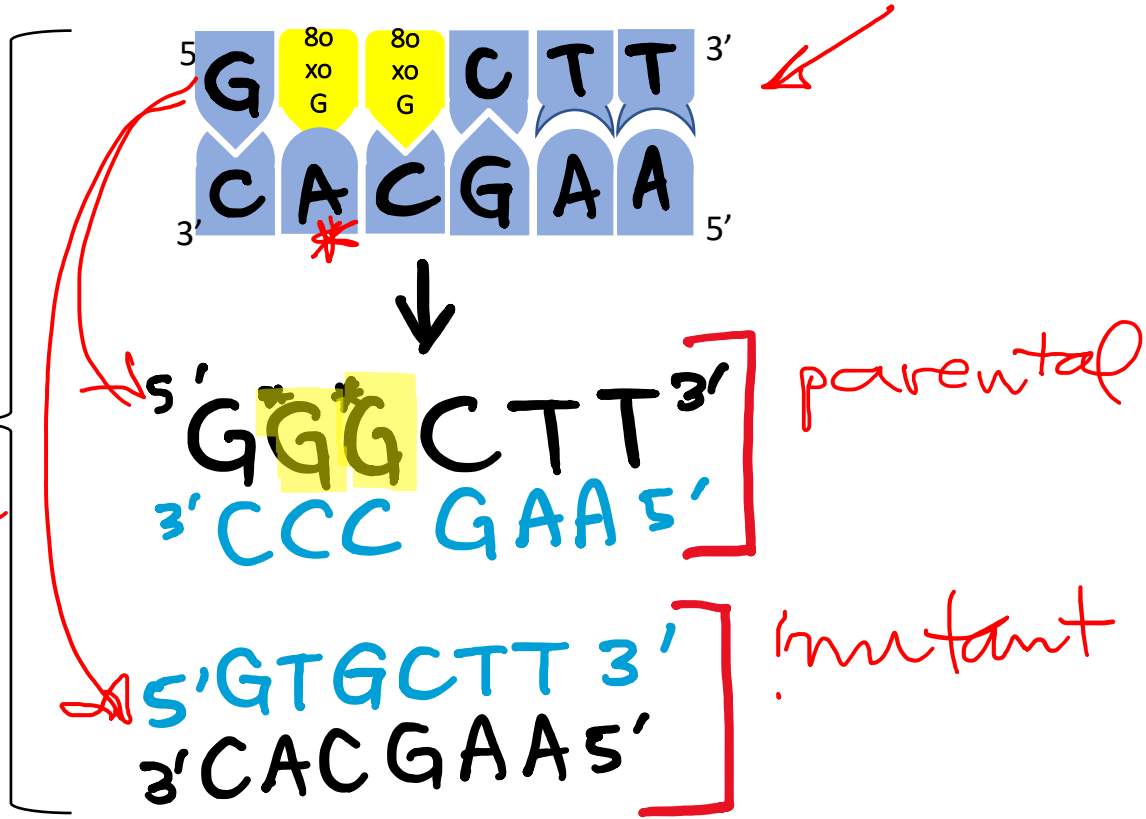


# Mispaired bases during PCR amplification steps results in changes to the sequence of the scFv gene

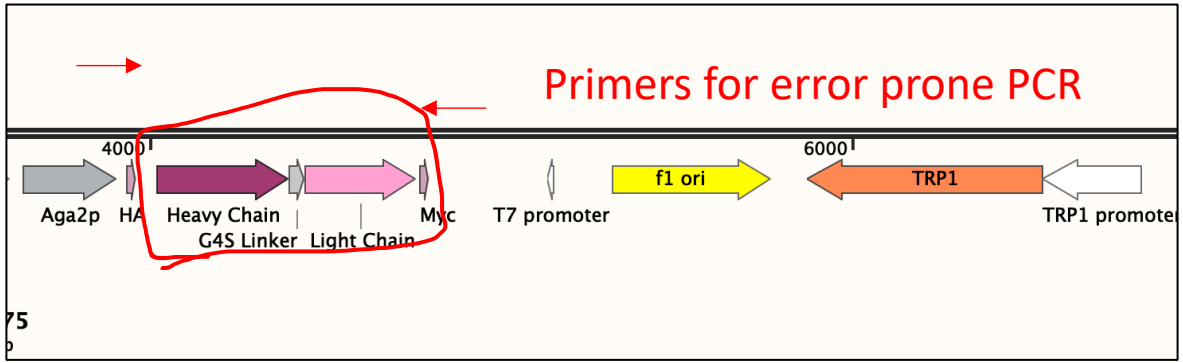


PCR Amplification of scFv genes with mutant bases results in permanent sequence change

PCR Round 1



# Design of scFv library from Lyso_scFv_6 clone

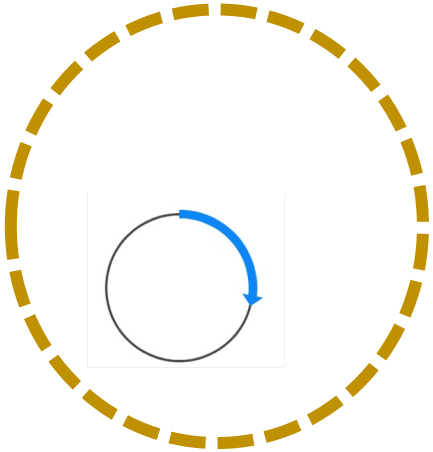



- Clone Lyso_scFv_6 bind lysozyme with high affinity (measured $K_d$ 6nM)
- Modified dNTPs introduce mutations
- 10 cycles of PCR result in 1-9 amino acid changes in most PCR products

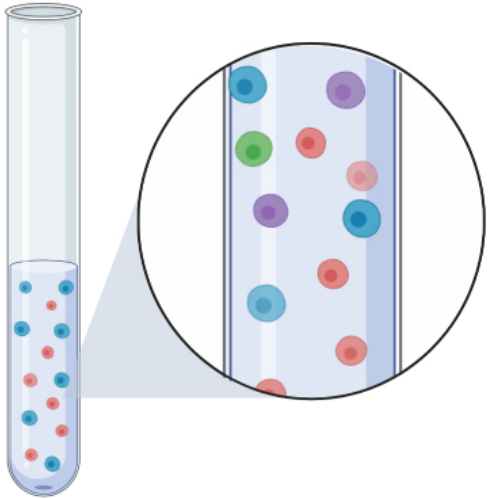
- This product (~900bps) was gel purified, amplified and incorporated into the backbone plasmid
- The DNA to create this plasmid was electroporated into a yeast strain that cannot make the amino acid Trp
  - Only yeast that have taken up a plasmid will grow on our Trp- selective media

# Today in lab, M1D1 Generate scFv library

Transform DNA into yeast, recombination results in stable plasmid



Library of ~$10^8$ scFv mutants expressed on yeast



# Getting to know each other!

- Create a google slide so we can get to know each other.
  - What do you want us to know about you?
  - Info appropriate for class
  - Examples: favorite food, hobbies, **pets** (*please*), secret powers, or favorite places to visit or dream travel
- Link in the chat, https://docs.google.com/presentation/d/1H_8I4a1MIKqEEJYtGGdiGJwteOC8LB9KrqYC8Mw11e0/edit?usp=sharing