

Module 3: Engineering Antibodies

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What do you associate antibodies with?
 Draw an antibody binding one antigen from memory.

2

Antibody common structure: Four peptide chains

- Antibody shown in grey
- Antigen shown in blue
 - Antigen: any molecule that can bind specifically to an antibody
- Antibodies also called immunoglobulins
- Large protein molecule, 150kDa
 - 2 light peptide chains, 25kDa
 - 2 heavy peptide chains, 50kDa
- Each antibody has two identical antigen-binding sites

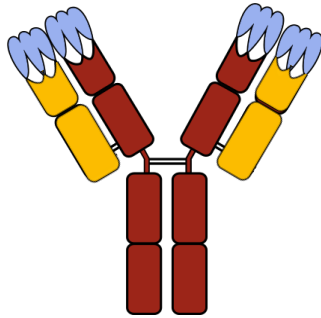
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Peptide sequence distinguishes the variable and constant regions

- 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

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



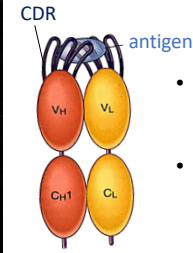
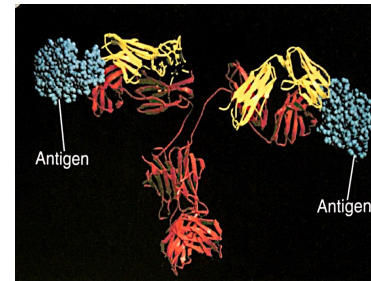
- CDR also called hypervariable regions
 - 3 protruding loops connected to the β -sheets of the V_L and V_H
- Can bind varied macromolecules and small chemicals
 - Protein, peptides, polysaccharides, nucleic acids, or lipids
- Each individual has 10^7 to 10^9 unique antigen receptors
- Diversity created by error prone Non-Homologous End Joining during B cell development (VDJ recombination)

Anypodetos, Antibody CDR, wikipedia

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CDRs generate antigen binding site

Lysozyme bound to antibody

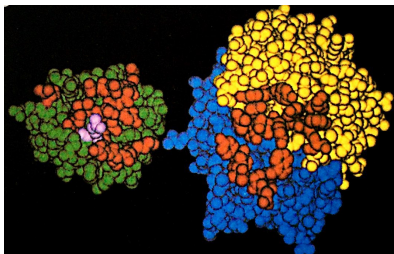


- Finger-like CDRs usually recognize 15-22 amino acids
- Basic antibody structure maintained when variability confined to loops

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The Antigen - Antibody interaction forms multiple contacts

3D: Lysozyme bound to variable region

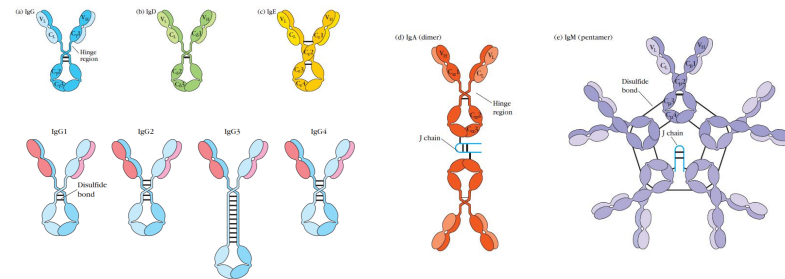


- Green: lysozyme
- Blue/Yellow: V_L and V_H
- Red amino acids that interact
- Pink critical glutamine residue fits into cleft of CDR
- Strong interactions depend on many noncovalent bonds and a high degree of complementarity

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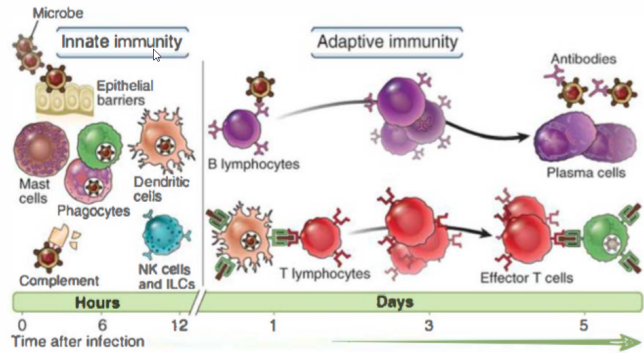
Antibody structure is complex!

How are antibodies produced in our bodies?



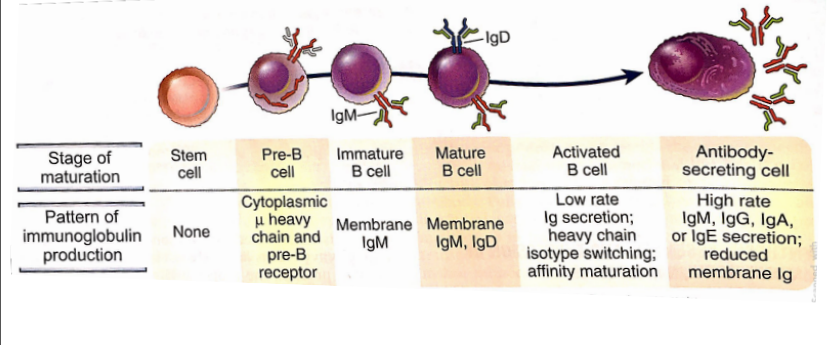
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Innate and Adaptive immunity are sequential



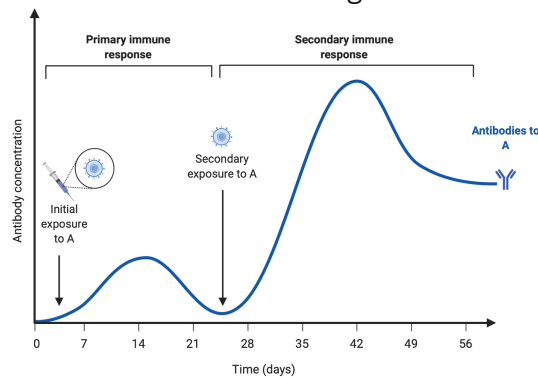
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Antibody secretion occurs during B cell maturation during our adaptive immune response



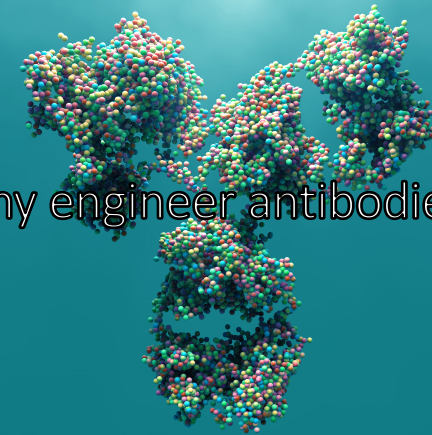
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Adaptive immune response is specific to a distinct antigen



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Why engineer antibodies?



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What types of antibodies are the result of engineering and selection?

- monoclonal antibodies
- bispecific antibodies
- antibody fragments (Fab, scFv)
- antibody drug conjugates

Module 3 adapted from:

PROTOCOL

Isolating and engineering human antibodies using yeast surface display

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Yeast surface display libraries are a powerful platform for antibody discovery

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

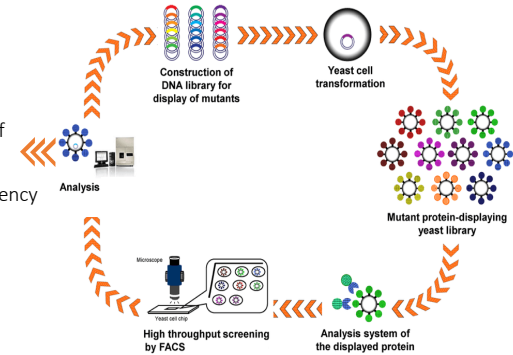
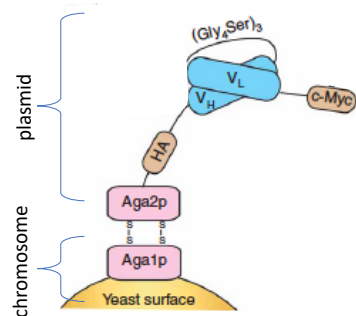


Image:Yeast Display Library Construction Services, creative-biolabs.com

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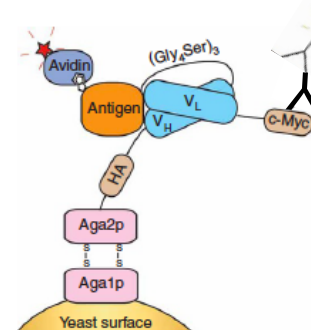
Yeast display single chain variable antibody fragments (scFv)



- 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'

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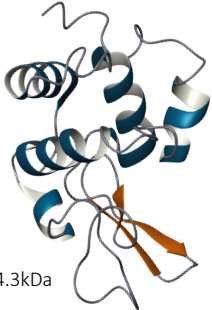
Fluorescently labeled antibodies and streptavidin identify scFv expression and antigen binding respectively



- Antigen= Biotinylated lysozyme
- Fluorescently labeled streptavidin to label antigen binding
 - Streptavidin binds biotin tightly
 - affinity is the strongest noncovalent biological interaction known
- Primary antibody to c-MYC and fluorescently labeled secondary antibody against primary antibody

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Mod3 Antigen: Lysozyme, antimicrobial enzyme found in plants and animals

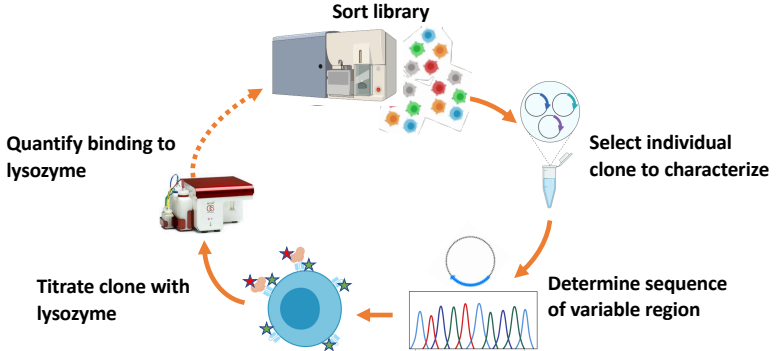


14.3kDa

- 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
- Today: commercially valuable enzyme for the food and pharmaceutical industry

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Mod 3 Workflow: Selection and characterization of lysozyme binding scFvs



Sort library

Quantify binding to lysozyme

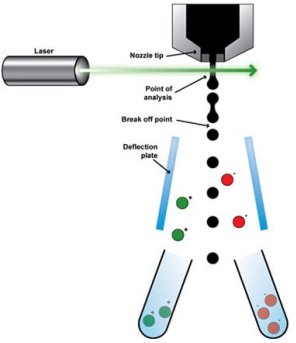
Titrate clone with lysozyme

Determine sequence of variable region

Select individual clone to characterize

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Today: Fluorescence activated cell sorting (FACS)

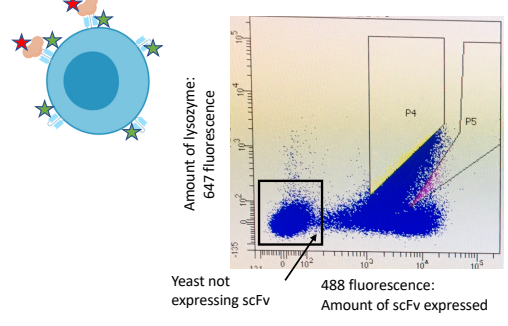


- Incubate biotinylated-lysozyme and library of yeast expressing scFv, allow time for binding
- Incubate with primary antibody (anti-c-MYC) to scFv
 - Secondary antibody anti-constant region of MYC antibody conjugated to AF488
 - streptavidin conjugated at AF647 binds biotin on lysozyme
- What yeast population do we want to sort out if our goal is to find an improved scFv binder?**

Image: Abcam

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FACS Scatterplot of yeast library during sorting



Amount of lysozyme: 647 fluorescence

Yeast not expressing scFv

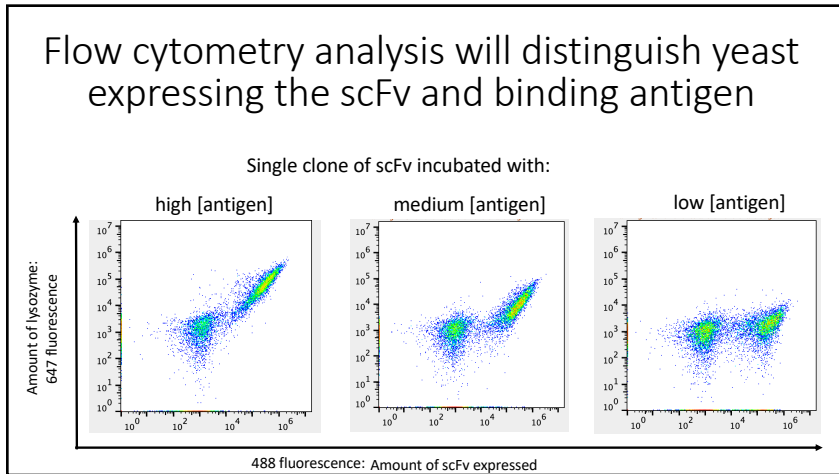
488 fluorescence: Amount of scFv expressed

Dot= event/cell

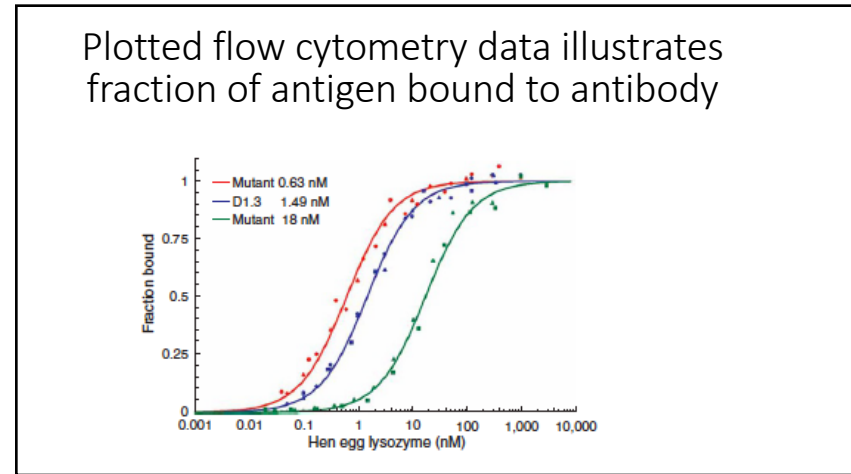
P4 Gate : yeast sorted as potentially better binders compared to a characterized clone

P5 Gate: yeast sorted as worse binders compared to a characterized clone

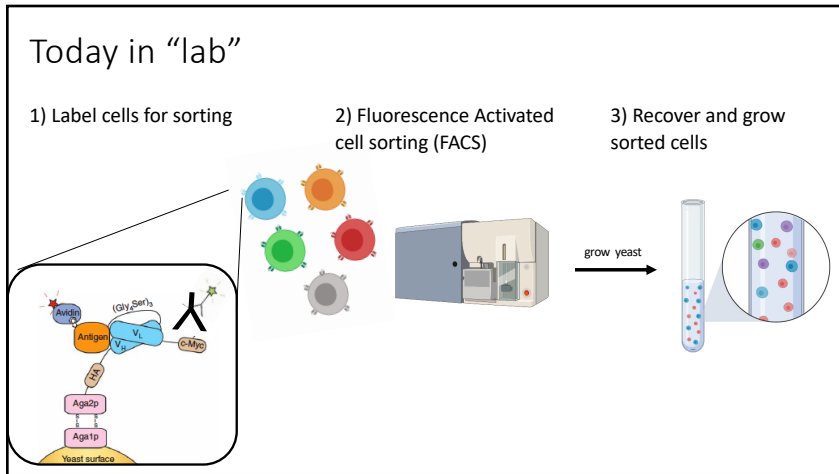
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