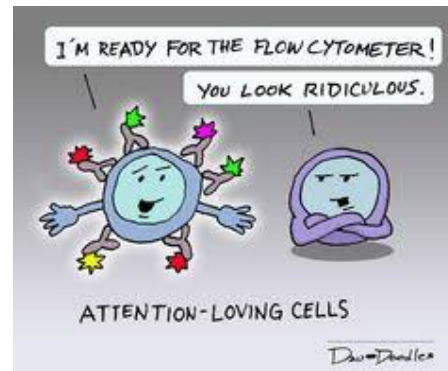


M3D1:

Enrich candidate clones using FACS

1. Prepare cells for sorting
2. Complete fluorescence activated cell sorting (FACS)



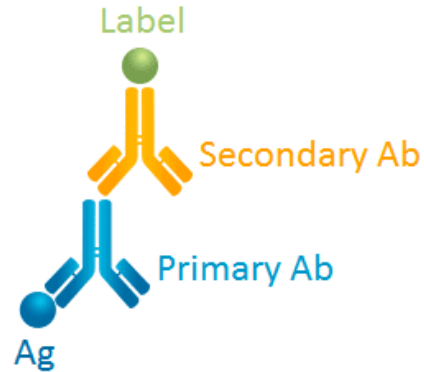
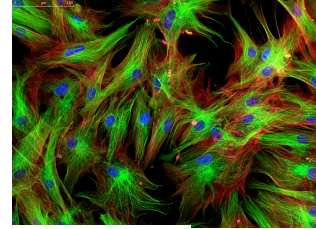
04/14/20

Important M3 due dates

- **Research proposal presentation** (22.5%)
 - completed in teams and presented via Zoom
 - due 5/8
- **Mini-report** (5%)
 - completed in teams and submitted via Stellar
 - due 5/11 at 10p
- **Notebook** (part of 10% Homework and Notebook)
 - due 5/7 at 10p via email to Joe
- **Blog** (part of 5% Participation)
 - due 5/12 at 10p via Blogspot

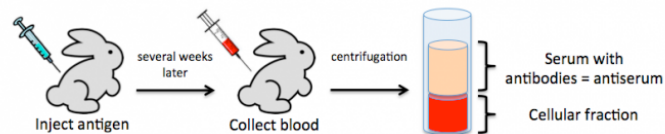
Antibodies are useful research tools

- In immunofluorescence, antibodies are used to label parts of cells
- In assays, antibodies are used for diagnostics

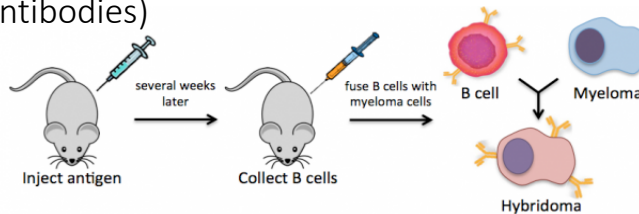


Polyclonal versus monoclonal antibodies

- Antisera contains pool of antibodies (polyclonal antibodies) harvested from animal host



- Hybridomas generate antibodies specific to single epitope on antigen (monoclonal antibodies)



What are your experimental goals?

Engineer an antibody in yeast

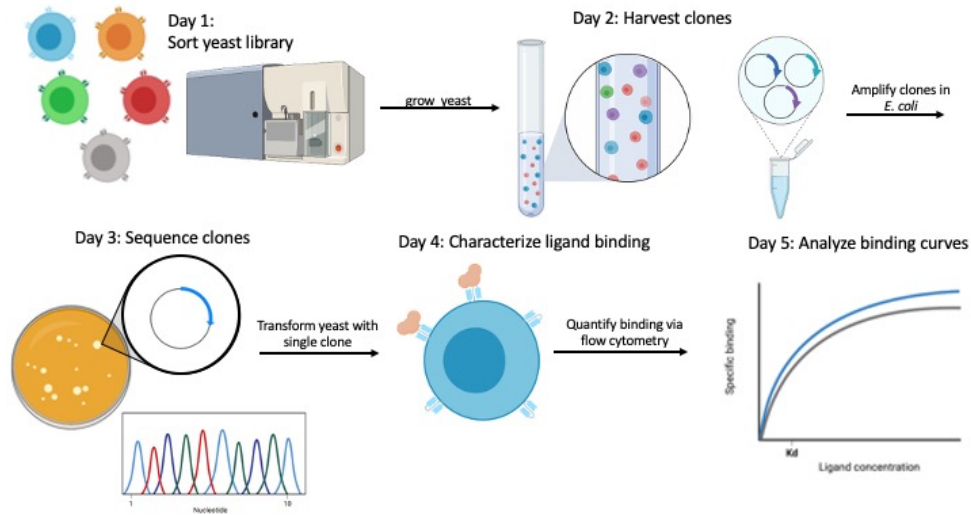
1. Identify lysozyme-specific single chain variable fragment (scFv) sequences that might bind lysozyme better
2. Characterize binding properties of mutated lysozyme-specific scFv antibodies

Lysozyme is the target for the scFv you will study

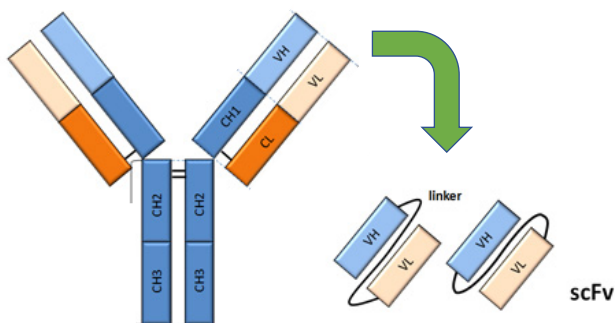
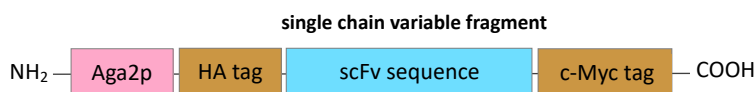
- Antimicrobial enzyme produced by animals
 - Part of the innate immune system, present in tears
- Catalyzes the breakdown of bacterial cell membranes
 - Glycoside hydrolase that hydrolyzes 1, 4-beta linkages in peptidoglycan



Overview of Mod3 experiments



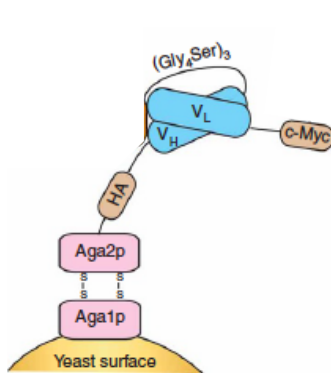
Yeast display can be used to engineer antibodies



- Sequence for antibody of interest is cloned into the yeast display plasmid (=scFv sequence)
- scFv sequence is then mutated in effort to improve affinity or specificity for target
 - Error prone PCR
- Mutated sequence is produced by the yeast cell and displayed on the surface
- Binding to target then characterized

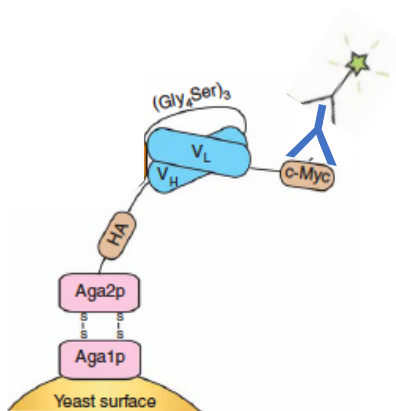
Yeast display used to express antibodies of interest

NH₂ — Aga2p — HA tag — scFv sequence — c-Myc tag — COOH



- Single chain variable antibody fragments (scFv) displayed on cell surface
- Aga2p attaches to yeast cell wall via disulfide bonding to Aga1p
 - Aga1p expressed from yeast chromosome
 - Aga2p (and associated sequences) expressed from yeast display plasmid

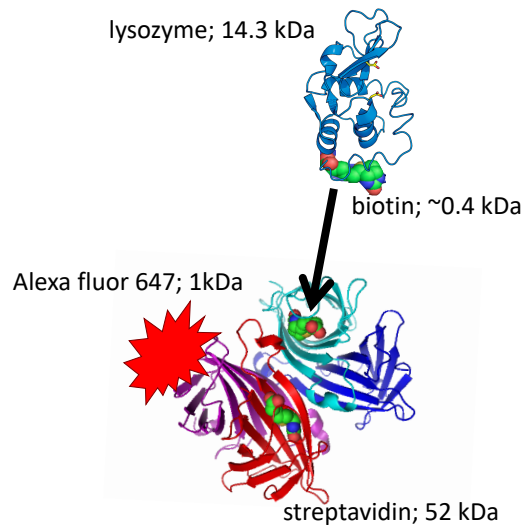
Antibodies used to confirm scFv expression



- Primary antibody = anti-cMyc, chicken IgY fraction
- Secondary antibody = anti-chicken IgG, goat
 - Alexa fluor 488 covalently linked
- This is how we recognize protein (antibody) on the yeast cell surface, but why would we want to express it there?

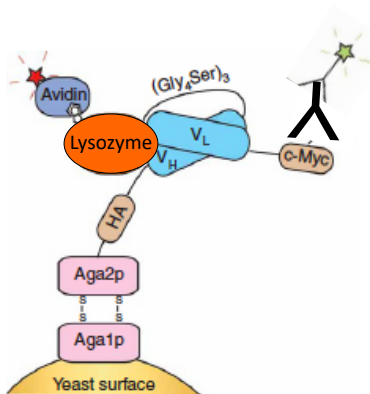
Streptavidin / biotin used to confirm lysozyme binding

- Lysozyme was biotinylated
 - Biotin (vitamin B7 / H) covalently attached
 - Small size unlikely to interfere with function or activity of enzyme
- Alexa fluor 647 tagged streptavidin used to label lysozyme
 - Streptavidin:biotin are high affinity binding partners, strongest non-covalent association in nature



How do we identify which yeast cells are expressing scFv that is bound to lysozyme?

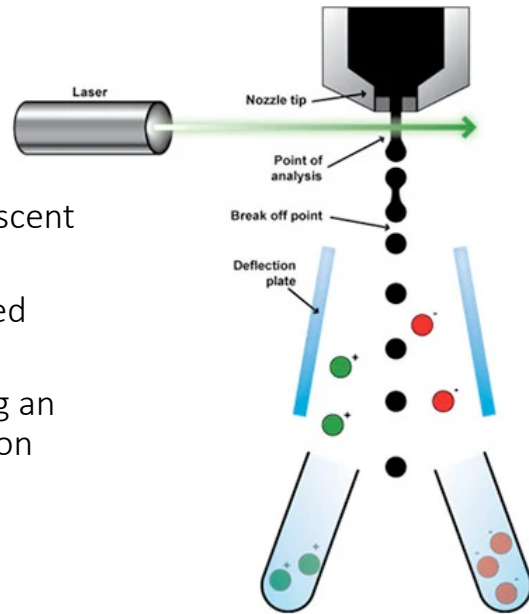
Review questions!



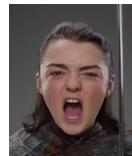
- What is the scFv?
- What is the binding partner for scFv of interest in your experiment?
- How will you identify expression of ScFv?
- How will you identify binding of the binding partner?

FACS used to sort cells based on fluorescent signal(s)

- Fluorescence activated cell sorting separates live cells based on fluorescent signals
- Cells are 'read' by laser then charged based on fluorescent signal
- Charged cells are sorted using an electric field established by detection plates



FACS completed using Aria 4

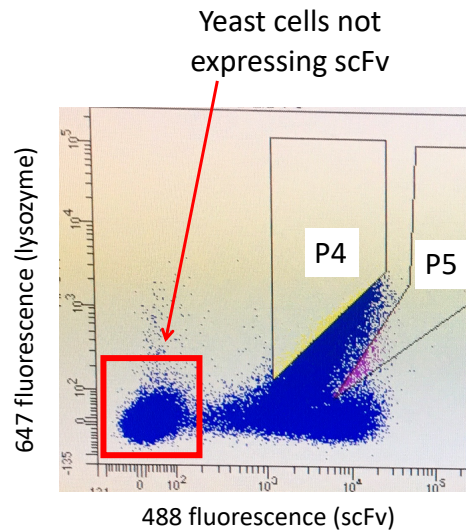


laser used to read fluorescent signal
 ↓
 charge applied to cell in droplet
 ↓
 cell sorted based on charge

- Able to visualize real-time fluid stream and droplet formation

Cells sorted and collected based on intensity of fluorescent signals

- Gates used to identify cells with specific fluorescence signature
 - P4 = yeast cells expressing scFv variant that is potentially a better lysozyme binder
 - P5 = yeast cells expressing scFv variant that is potentially a worse lysozyme binder
- Gates established experimentally



For today...

- Read through wiki information!
- Yeast display: <https://www.youtube.com/watch?v=UgG6xANt5ok>
 - Just the first ~3 minutes
- FACS: <https://www.youtube.com/watch?v=7bCZx5xPwt0>

For M3D2...

- Complete individually; read through literature and identify 5 topics / papers that you find interesting.
 - Involve biological engineering and at least tangentially related to a 109 topic/technique
 - Include full citation for articles
 - Write short summary of the information