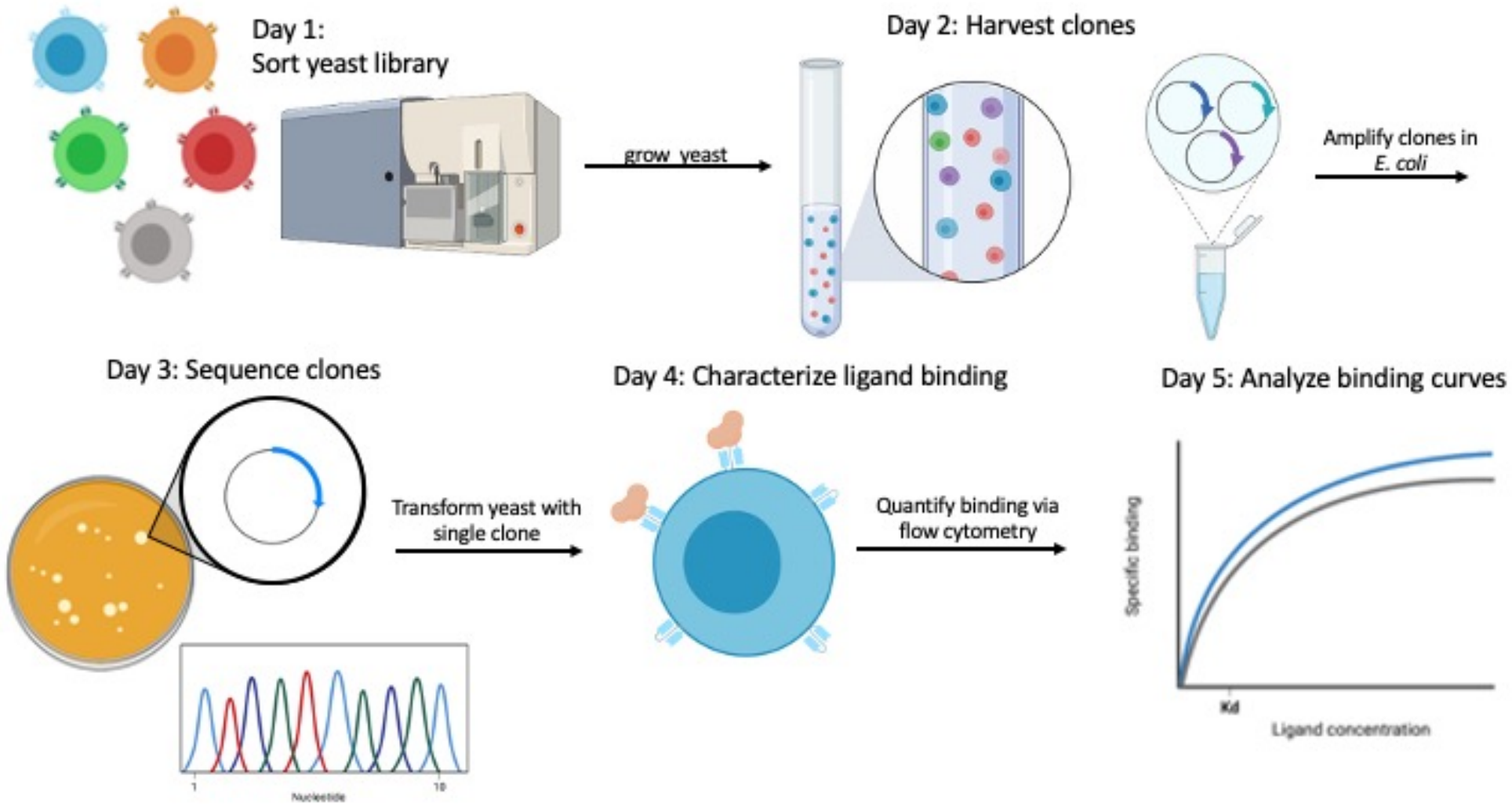
# M3D1:
# Enrich candidate clones using FACS

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

# Important M3 due dates

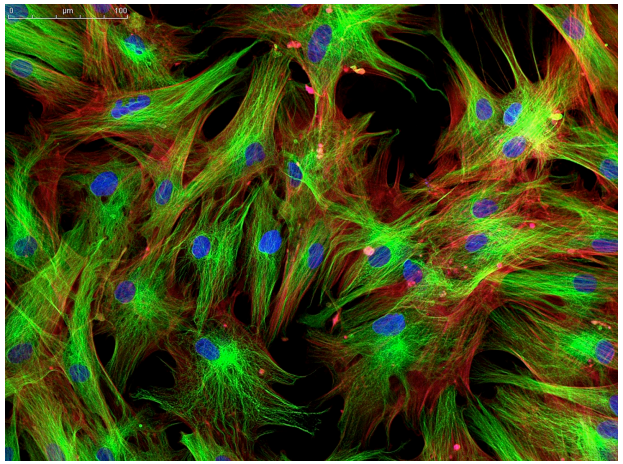
- Research proposal presentation (22.5%)
  - completed in teams and presented via Zoom
  - due 5/8 at 10p
- Mini-report (5%)
  - completed in teams and submitted via Stellar
  - due 5/11 at 10p
- Notebook (part of 10% Homework and Notebook)
  - due 5/6 at 10p via email to Kevin
- Blog (part of 5% Participation)
  - due 5/12 at 10p via Blogspot

# Overview of Mod3 experiments

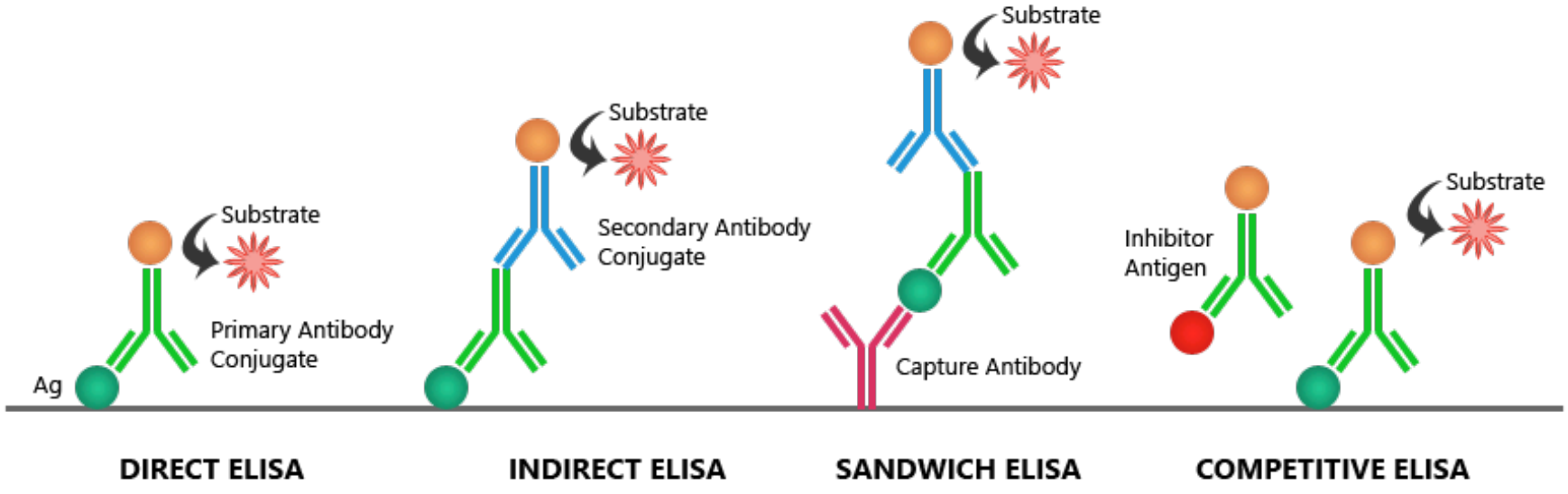


# Antibodies are useful research tools

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

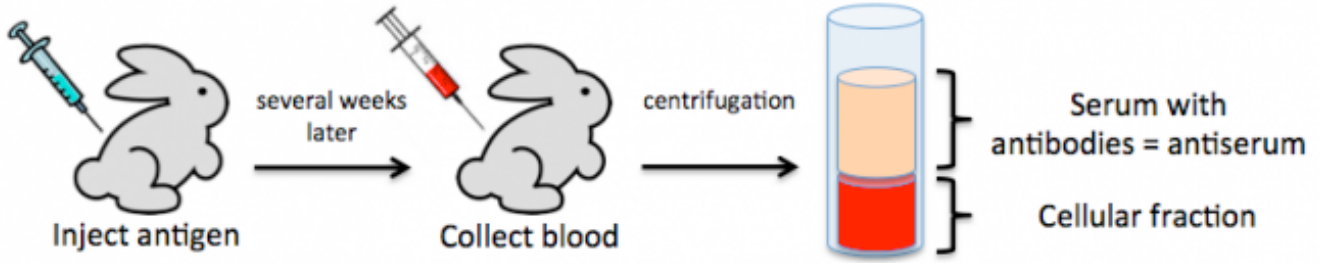


- In assays, antibodies are used for diagnostics

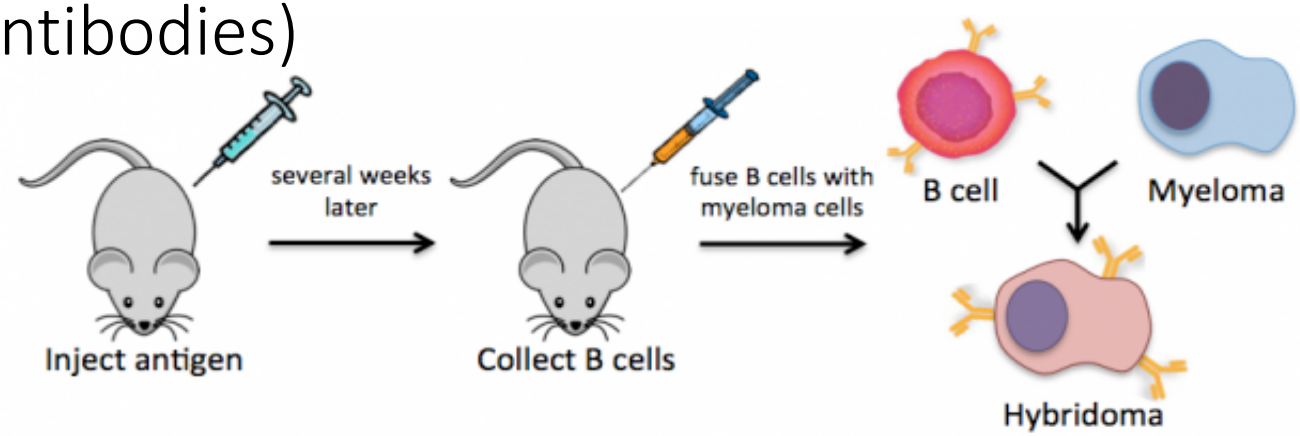


# Polyclonal versus monoclonal antibodies

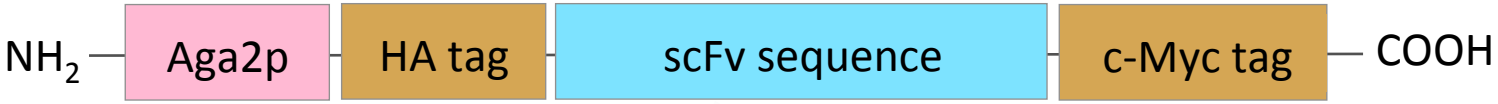
- Antisera contains pool of antibodies (polyclonal antibodies) harvested from animal host

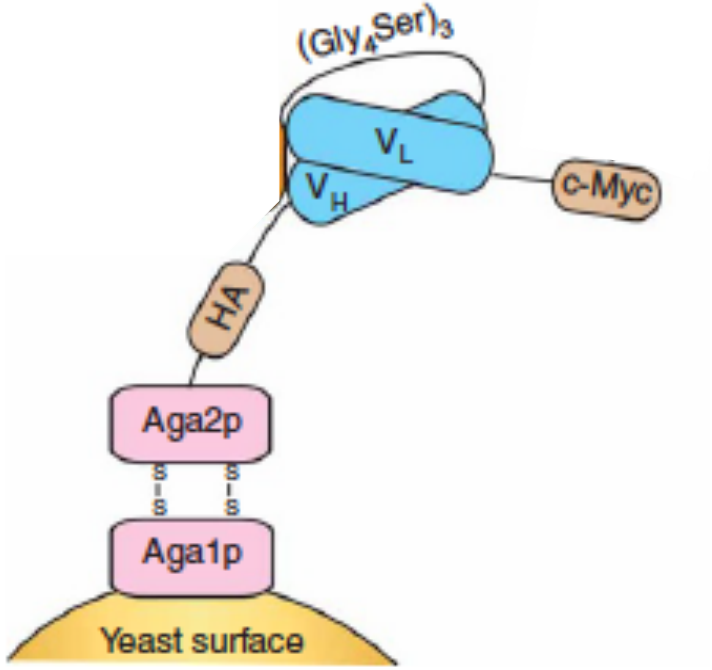



- Hybridomas generate antibodies specific to single epitope on antigen (monoclonal antibodies)



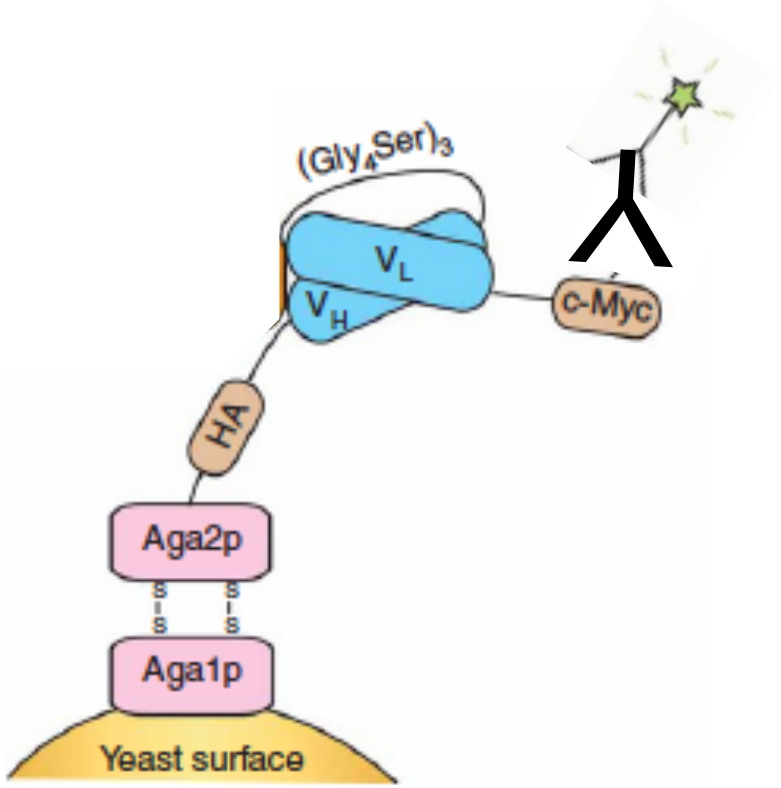
# Yeast display used to express antibodies of interest




- 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

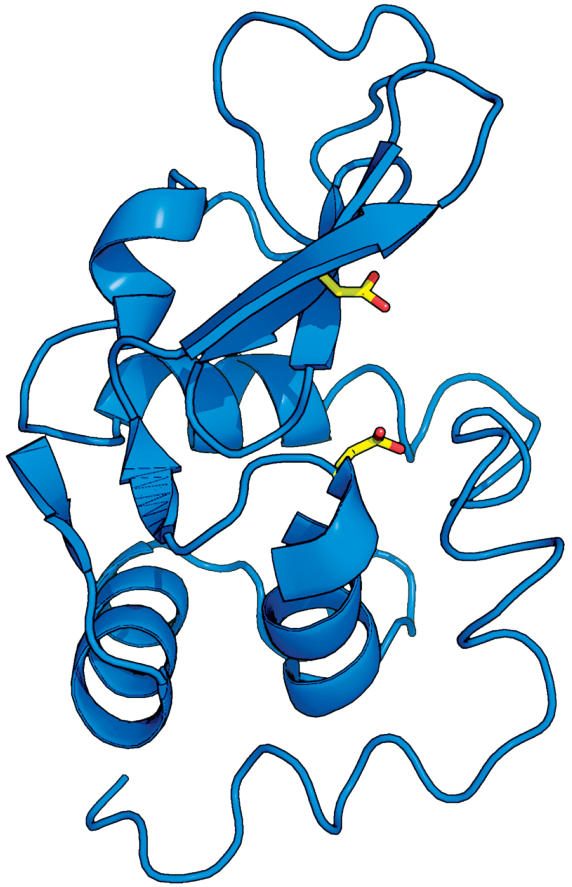
- Now what? Why might expressing proteins / antibodies on the surface of yeast cells be useful?

# 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 / altered in effort to improve affinity or specificity for target
  - Mutated / altered sequence is produced by the yeast cell and displayed on the surface
- Binding to target then characterized

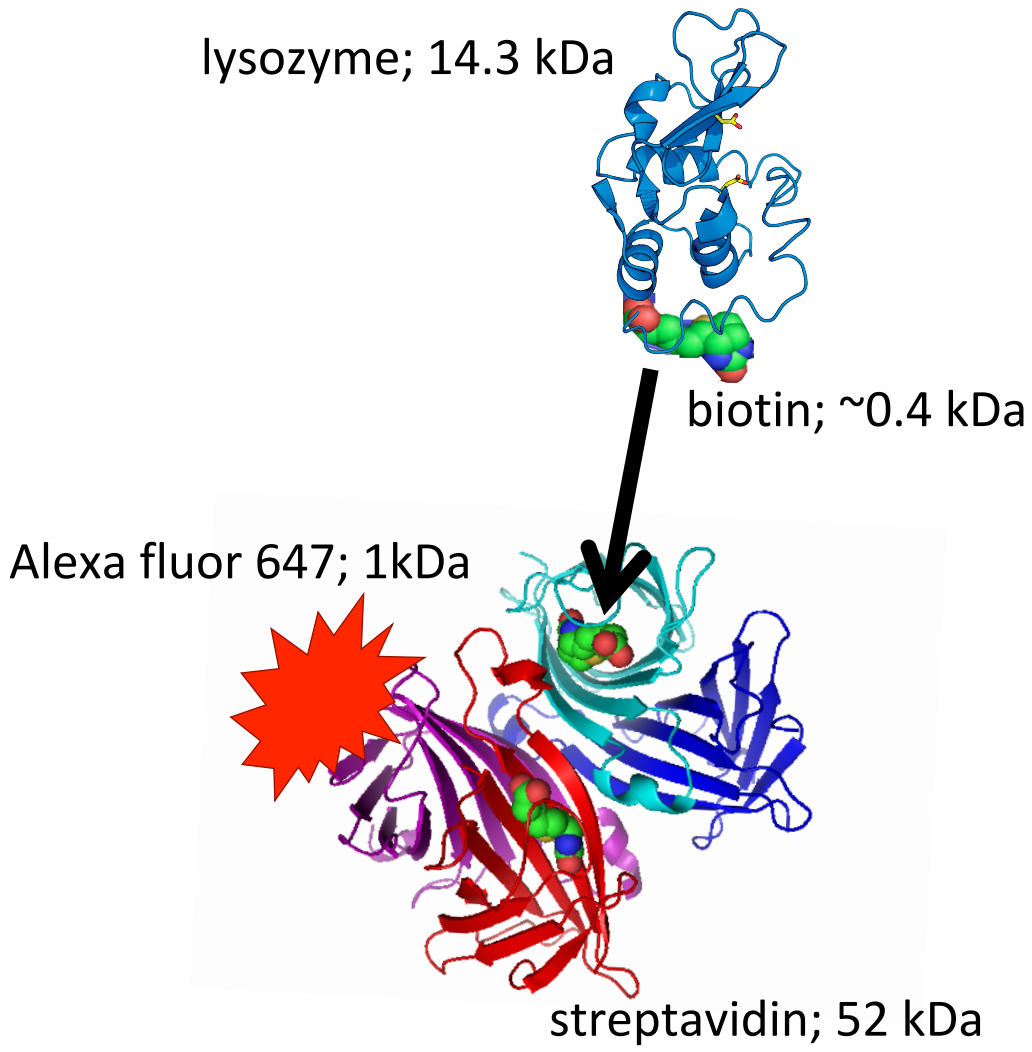
# Lysozyme is the target for the scFv you will study

- 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

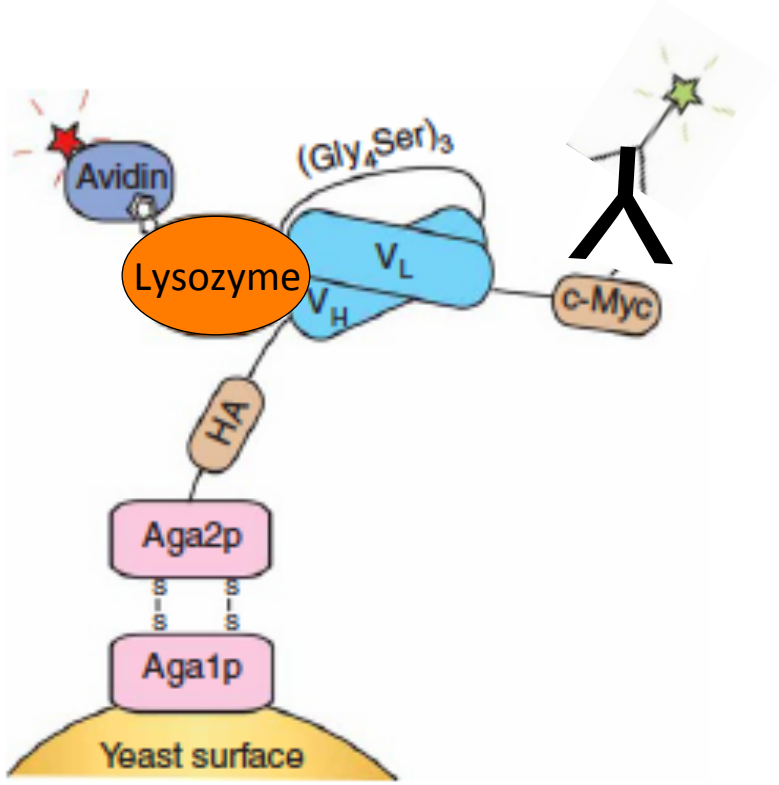


# Streptavidin / biotin used to confirm lysozyme binding

- 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 thought to function as antibiotic
  - 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?




Let's review the key players:

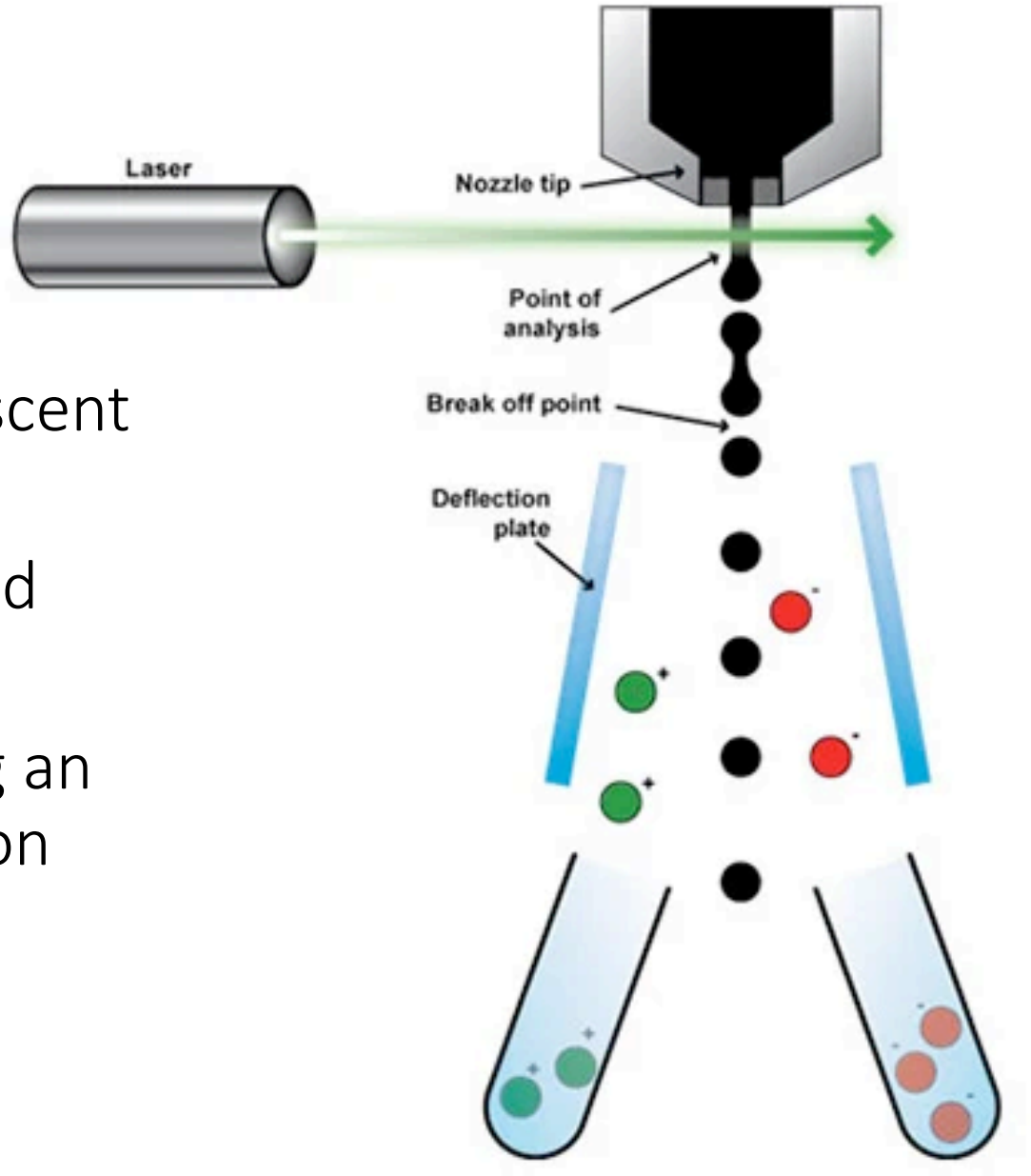
- What is the scFv?
- What is the binding partner for scFv of interest in your experiment?

Keep track of the antibody players!

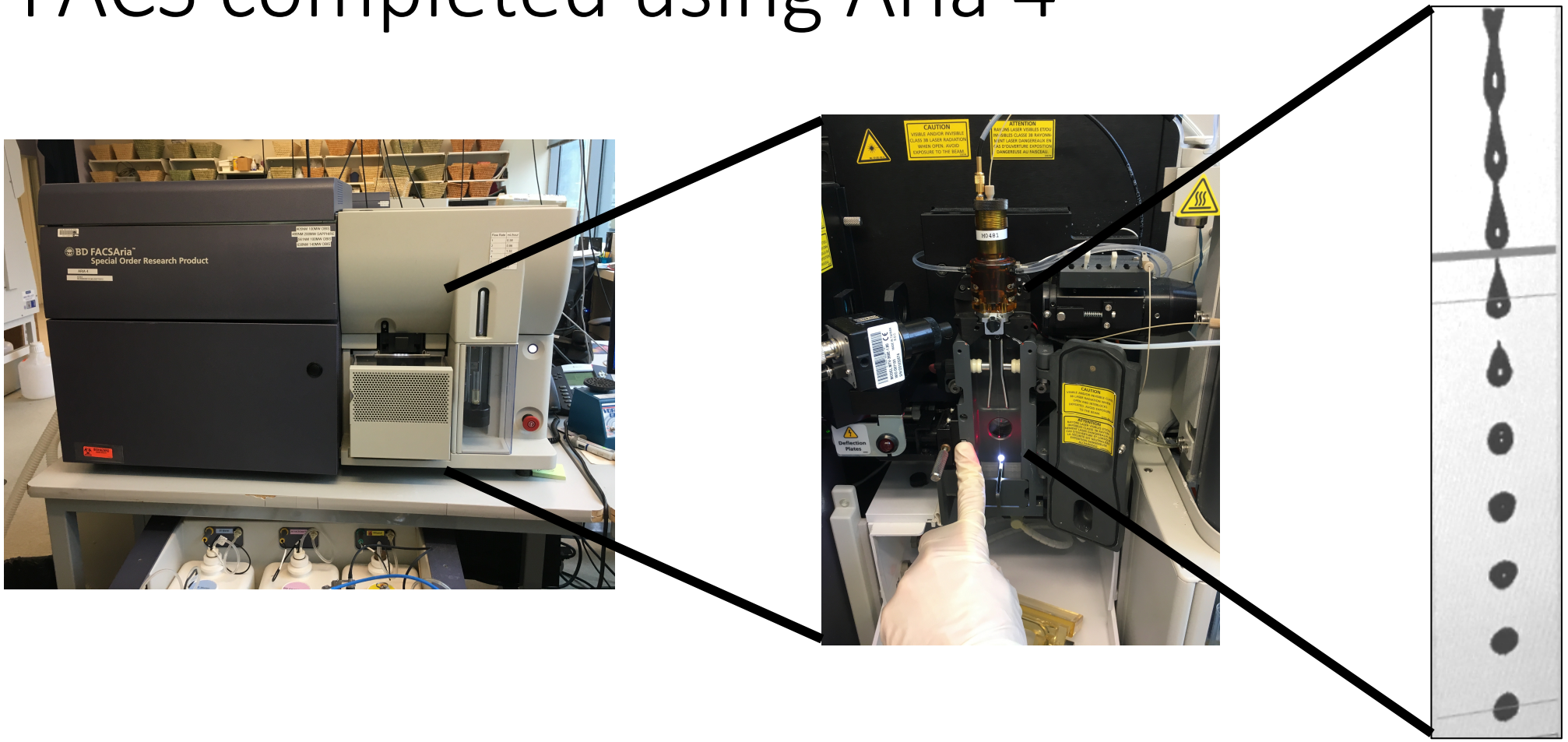
- What antibodies other that the scFv are involved? How?

# 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 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

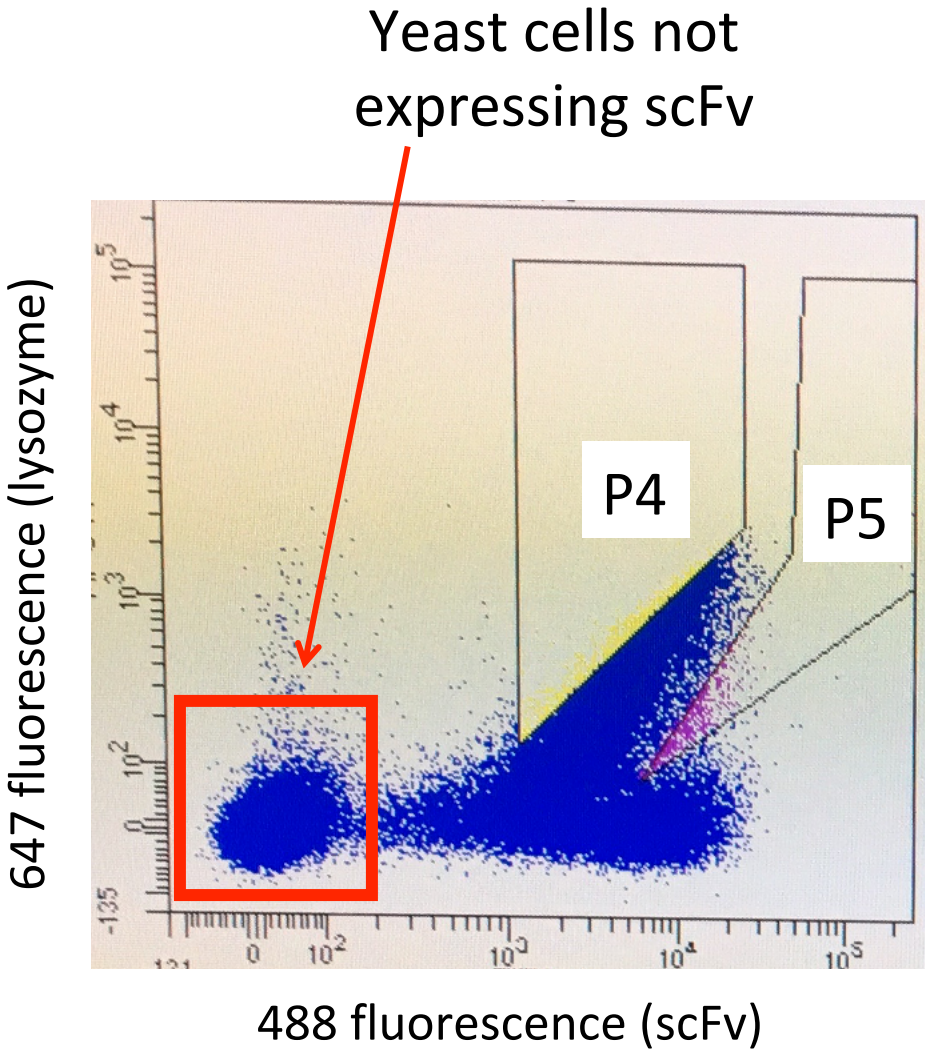
↓

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



# What is your experiment?

- Background: scFv sequence specific to lysozyme was cloned into yeast display plasmid and then error-prone PCR was used to randomly mutate the sequence

- Your goals:

1. Identify lysozyme-specific scFv sequences that might bind lysozyme better
2. Characterize binding properties of mutated lysozyme-specific scFv antibodies

# For today…

- Read through wiki information!
- Yeast display: https://www.youtube.com/watch?v=UgG6xANt5ok&t=217s
  - 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.
  - Include full citation for articles
  - Write short summary of the information