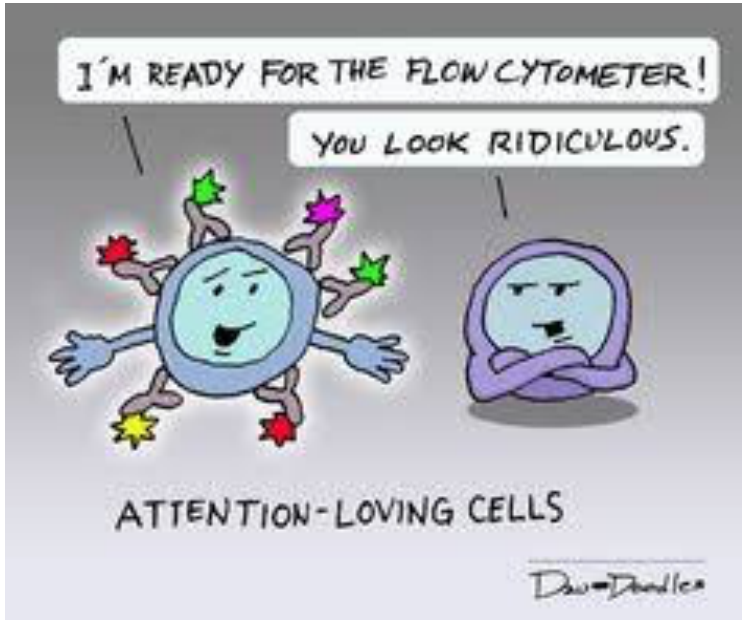
M1D2: Enrich candidate clones using FACS

1. Prelab discussion
2. Complete fluorescence activated cell sorting (FACS) of scFv library
3. Paper discussion



Office Hours	
Leslie:	Sun/Mon 4-5pm
Noreen:	Mon 2-4pm
	Wed/Fri 4-5pm
Becky:	Fri 12-1pm
	Tues/Thurs 4-5pm

Notebook submission and grading details

Daily notebook check:

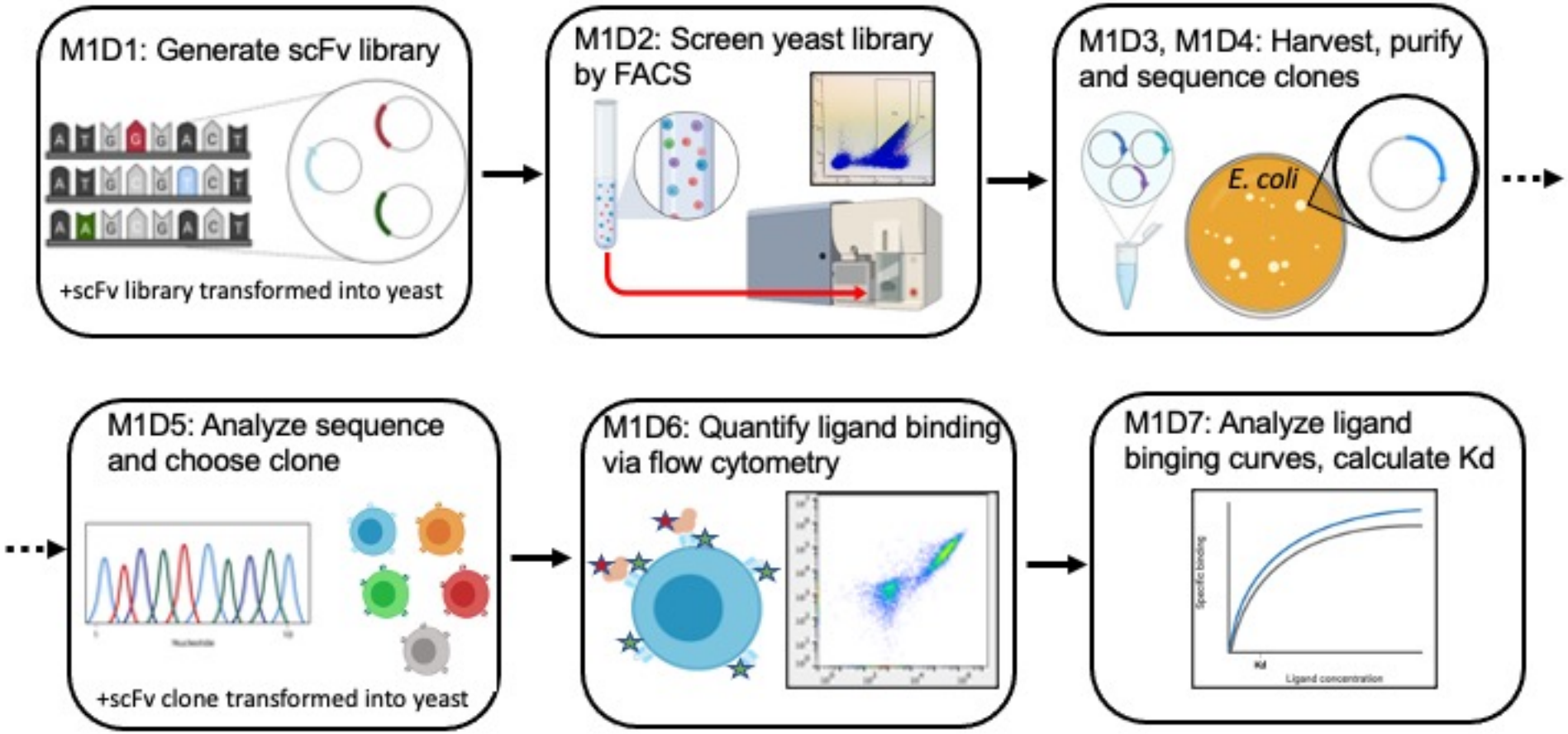
- Submitted to Stellar at the end of every laboratory session
- Graded on attempt to progress through the laboratory exercises (full points for submitted something)
- Scores contribute to ‘Participation’ grade

End-of-module notebook check:

- Submitted to Stellar at the end of every module
- Graded on completeness of notebook entry according to rubric & completeness of all entries for module
- Scores contribute to ‘Laboratory notebook’ grade

Overview of Mod1 experiments

Research goal: Identify and characterize an antibody fragment (scFv) that shows improved binding to the antigen, lysozyme.



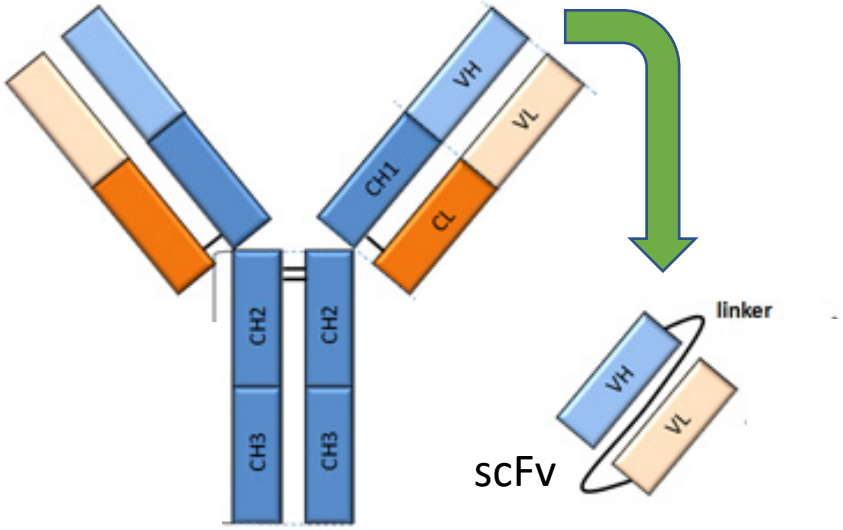
What are your experimental goals?

1. Using a parental clone of a single chain variable fragment (scFv) known to bind lysozyme, generate a library of mutant scFv clones

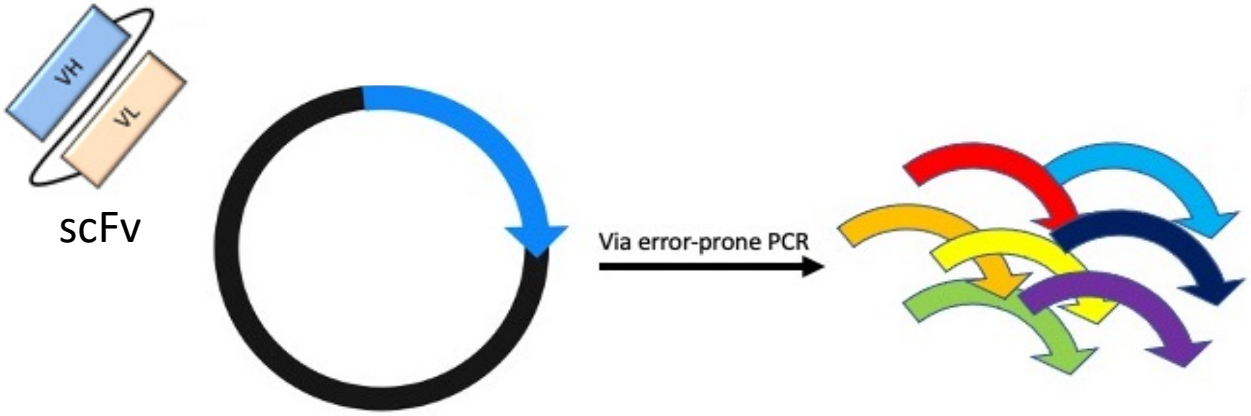
2. Screen that library to identify lysozyme-specific scFv sequences that might bind lysozyme better

3. Characterize binding properties of mutated lysozyme-specific scFv antibodies

Review: Generating the scFv library



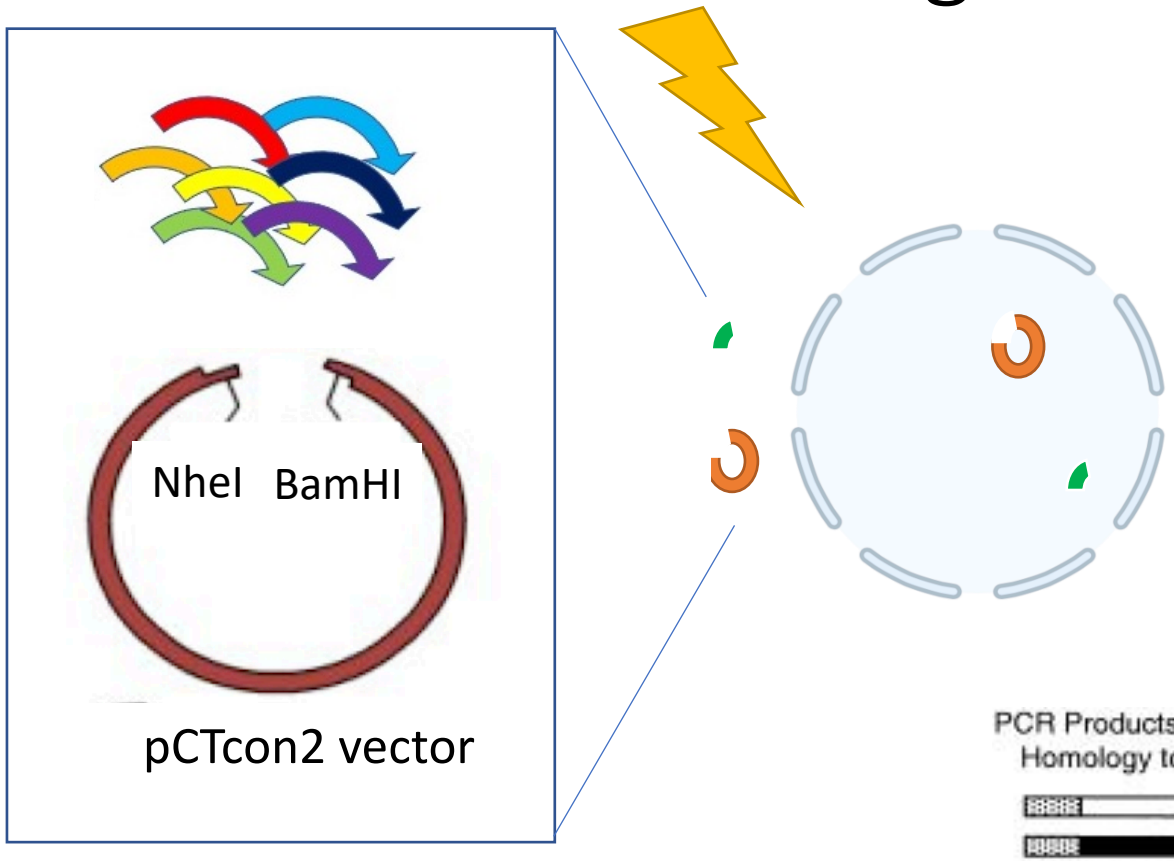
1. Generate the parental scFv



2. Generate the mutant scFv clones with error-prone PCR

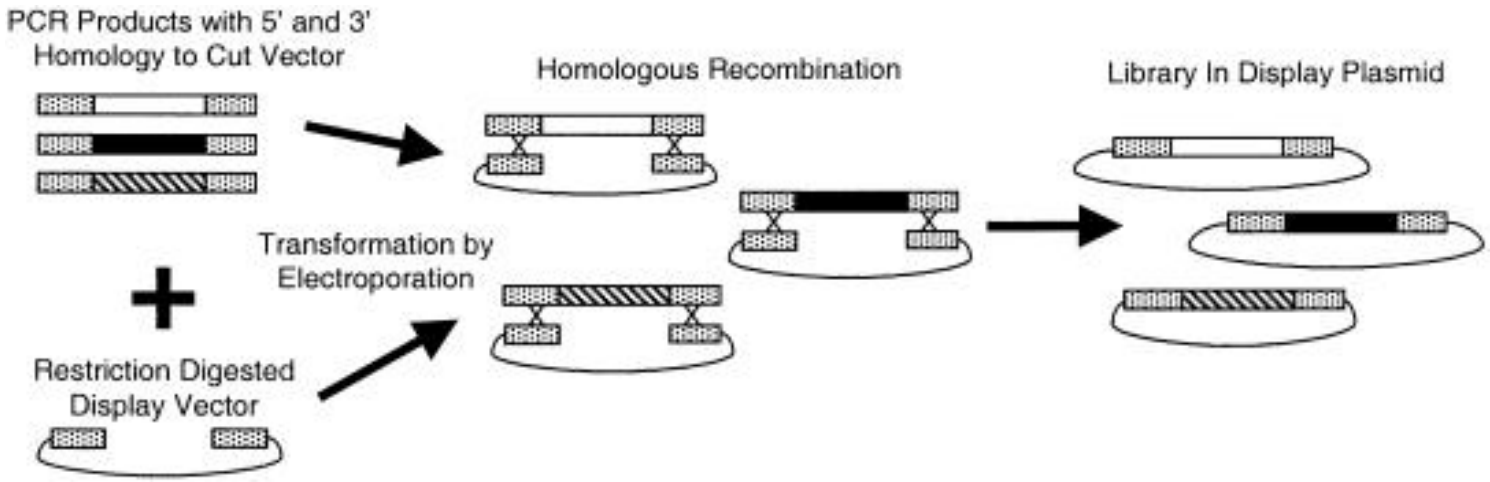
3. Amplify the mutant scFv clones with traditional PCR

Review: Transforming the scFv library into yeast

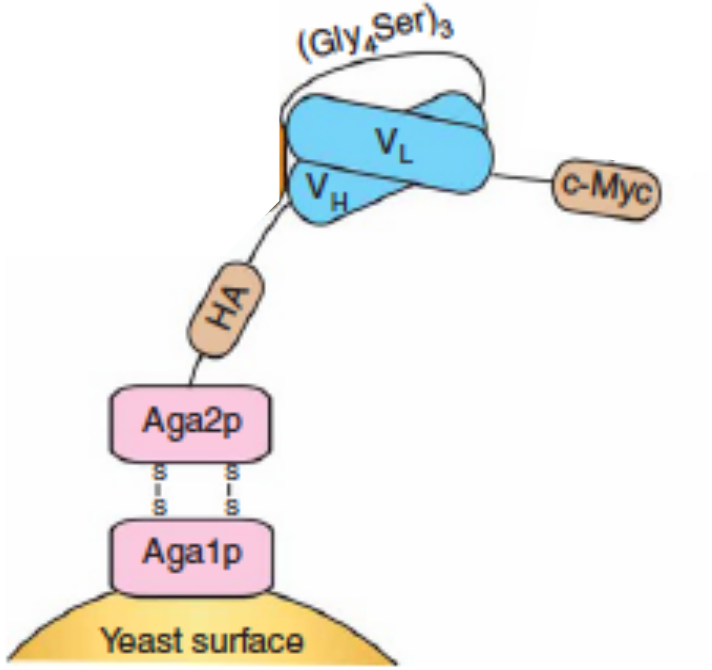


4. Introduce mutant scFvs and digested backbone into yeast

5. Yeast generate scFv plasmids for expression

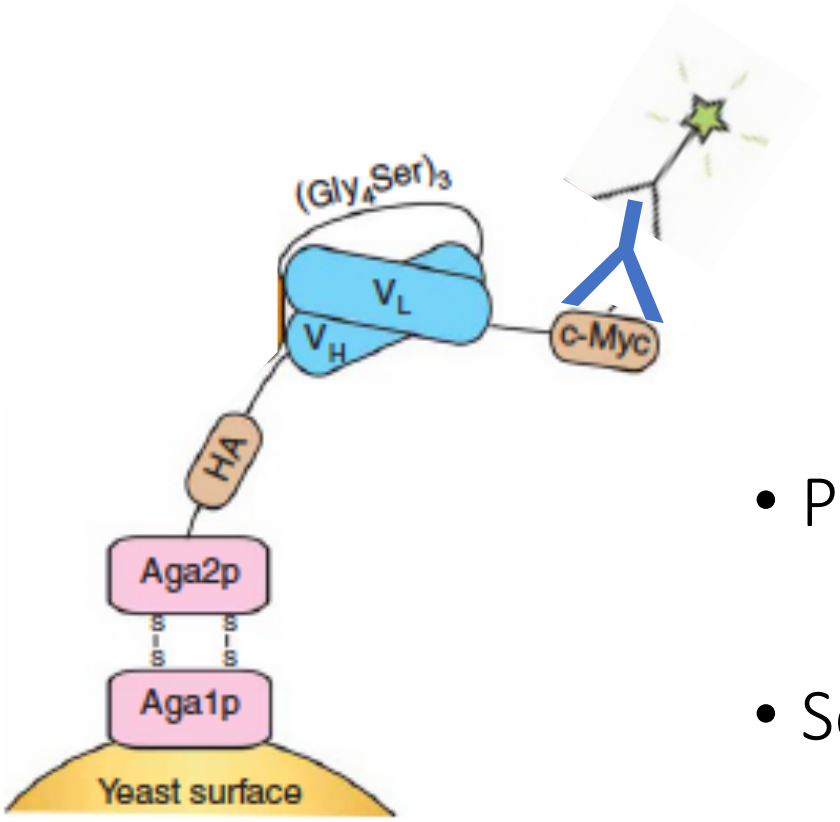


Yeast display used to express scFvs of interest



- 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

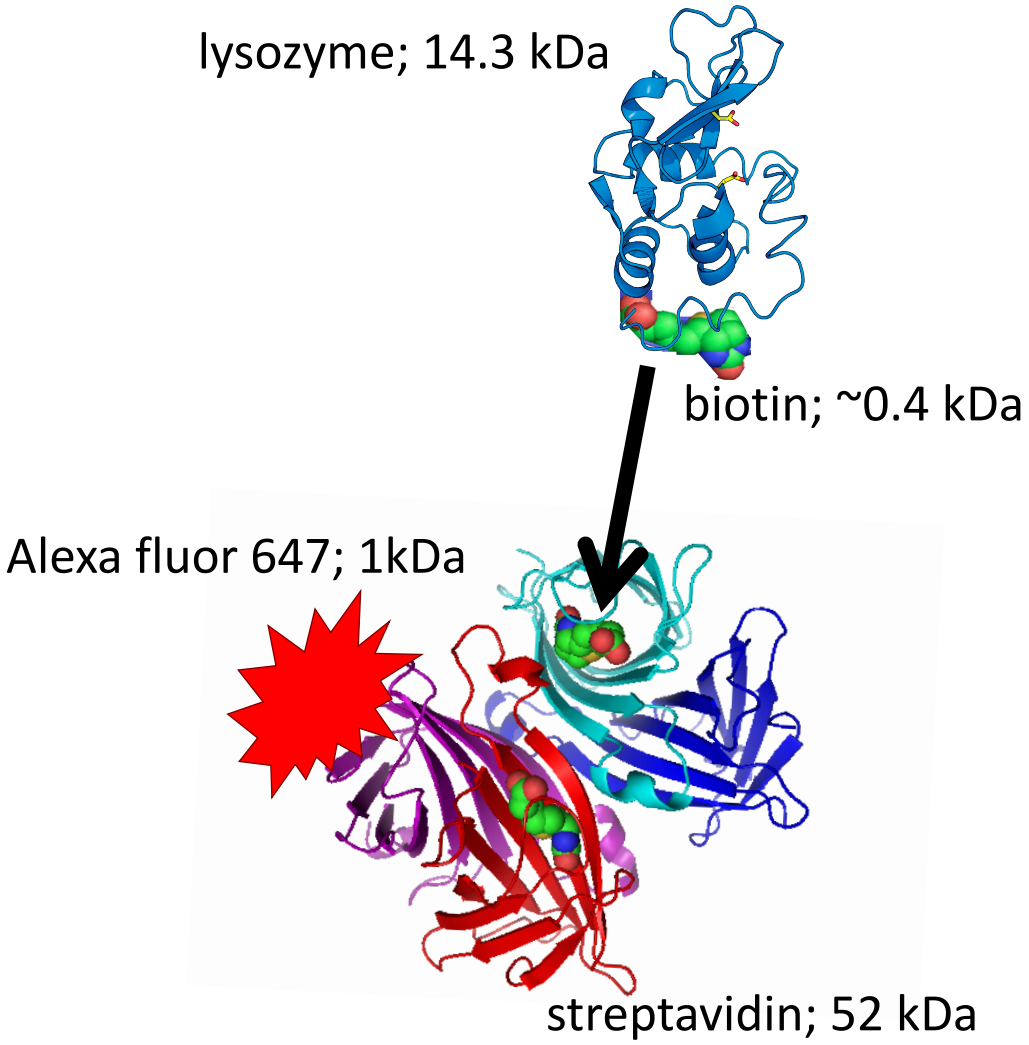


Why do we want our scFv expressed on the cell surface?

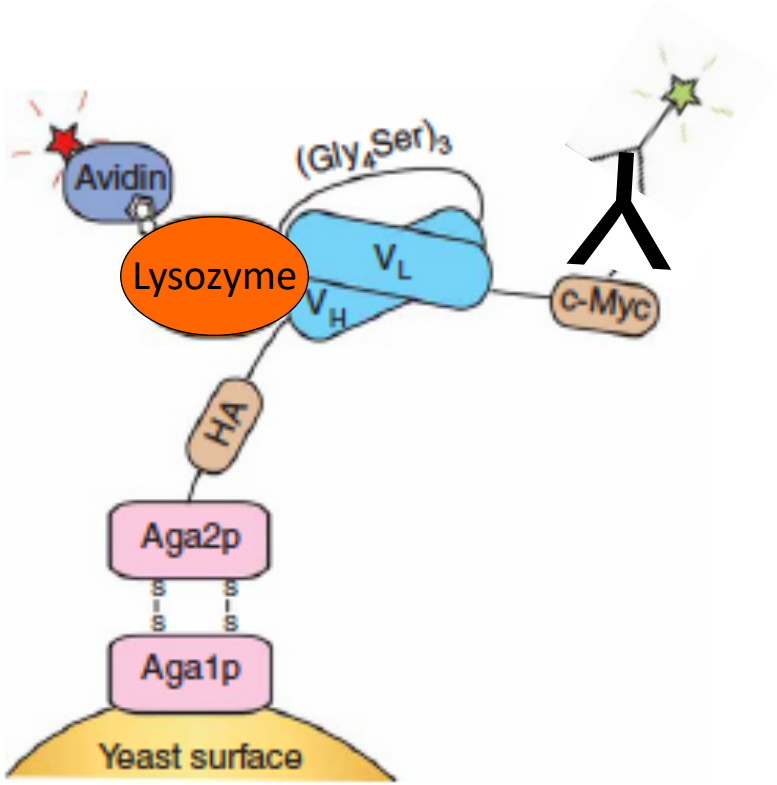
- Primary antibody = anti-cMyc, chicken IgY
- Secondary antibody = anti-chicken IgG, goat
  - Alexa fluor 488 covalently linked

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



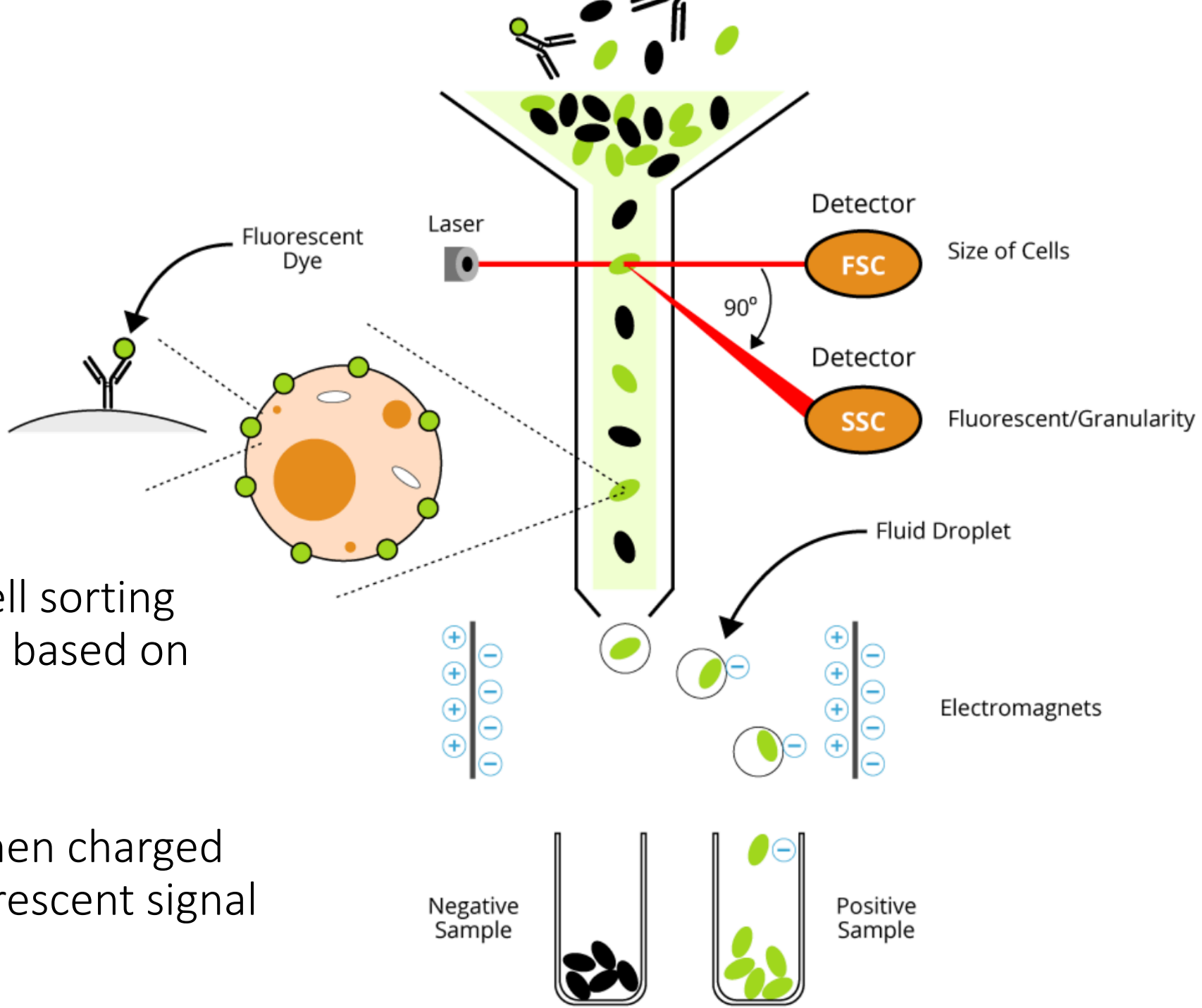
How do we identify which yeast cells are expressing scFv that is bound to lysozyme?



Review!

- 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?

How do we isolate yeast expressing our scFv?

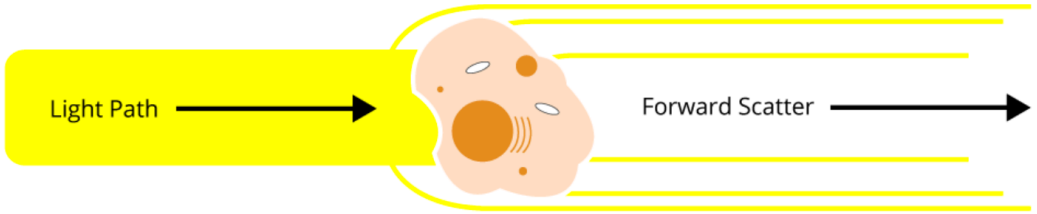


- Fluorescence activated cell sorting (FACS) separates live cells based on fluorescent signal
- Cells are ‘read’ by laser then charged and sorted based on fluorescent signal

Forward and side light scatter provide valuable information about cell population

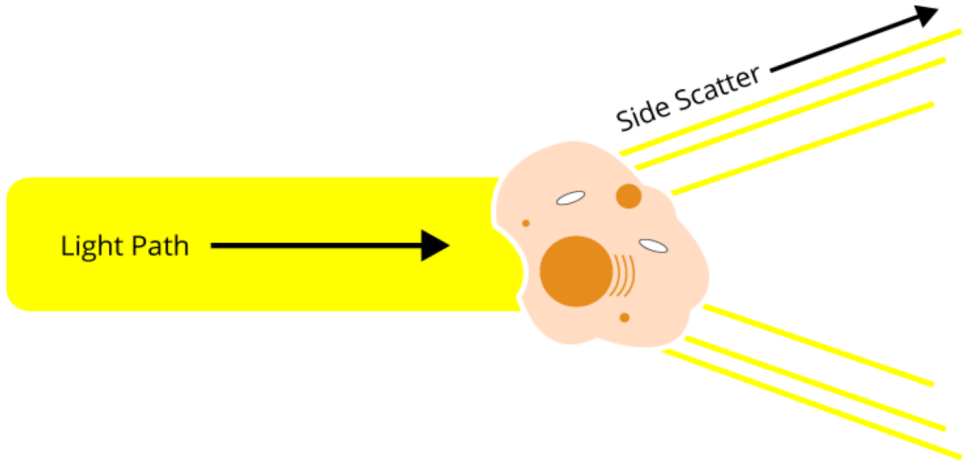
Forward light scatter

- Collected by forward scatter channel (FSC)
- Provides information about particle size
- Usually, bigger particles will produce more forward scattered light than smaller ones, and larger cells will have a stronger forward scatter signal



Side light scatter

- Collected by side scatter channel (SSC)
- Provides information about the granularity and complexity of the cells
- Cells with a low granularity and complexity will produce less side scattered light, while highly granular cells with a high degree of internal complexity will have a higher side scatter signal

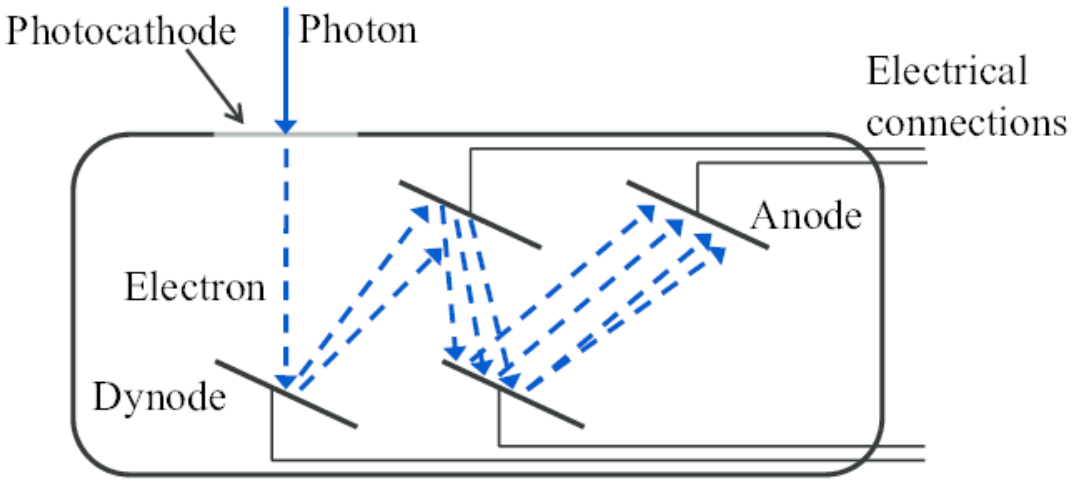


How does a flow cytometer use light information?

- Photomultiplier tubes (PMT) in each channel convert photon emission to voltage pulse, called an “event”

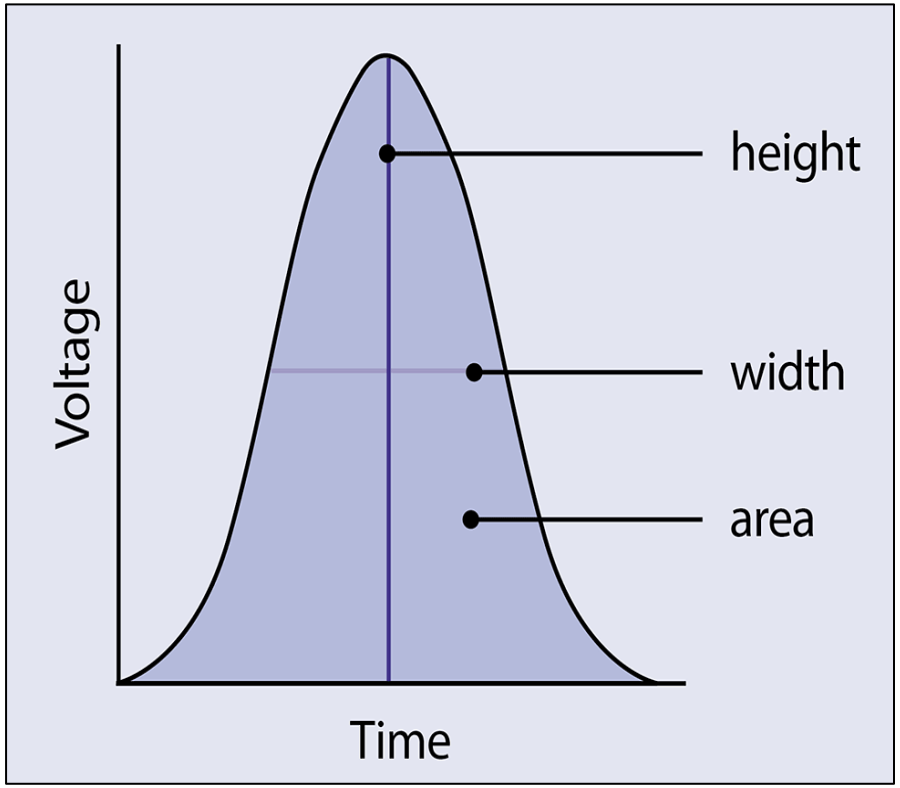
- As cell passes through laser beam, photons are detected as forward scatter and side scatter

- Photomultiplier tube (PMT) detects photons and converts to photoelectrons that are multiplied to amplify the signal



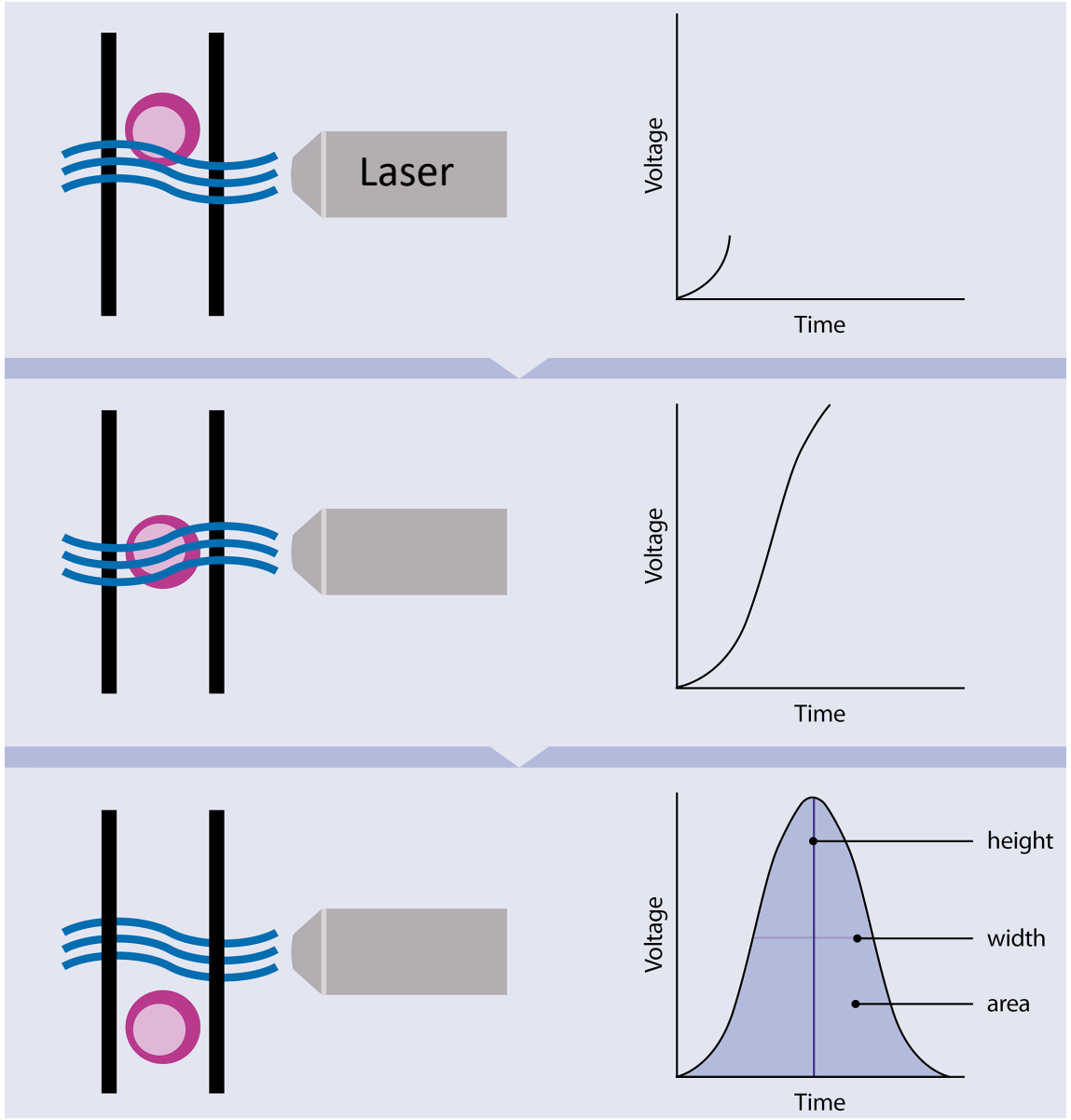
Pulse characteristics provide details for each event

- The total pulse height, width, and area is measured by the flow cytometer instrument
- Voltage pulse area will correlate directly to the signal intensity for that individual event.



Pulse characteristics provide details for each event

- Height at maximum when entire object is illuminated
  - i.e. at center of cell
- Width corresponds to length of time required for cell to pass through laser beam

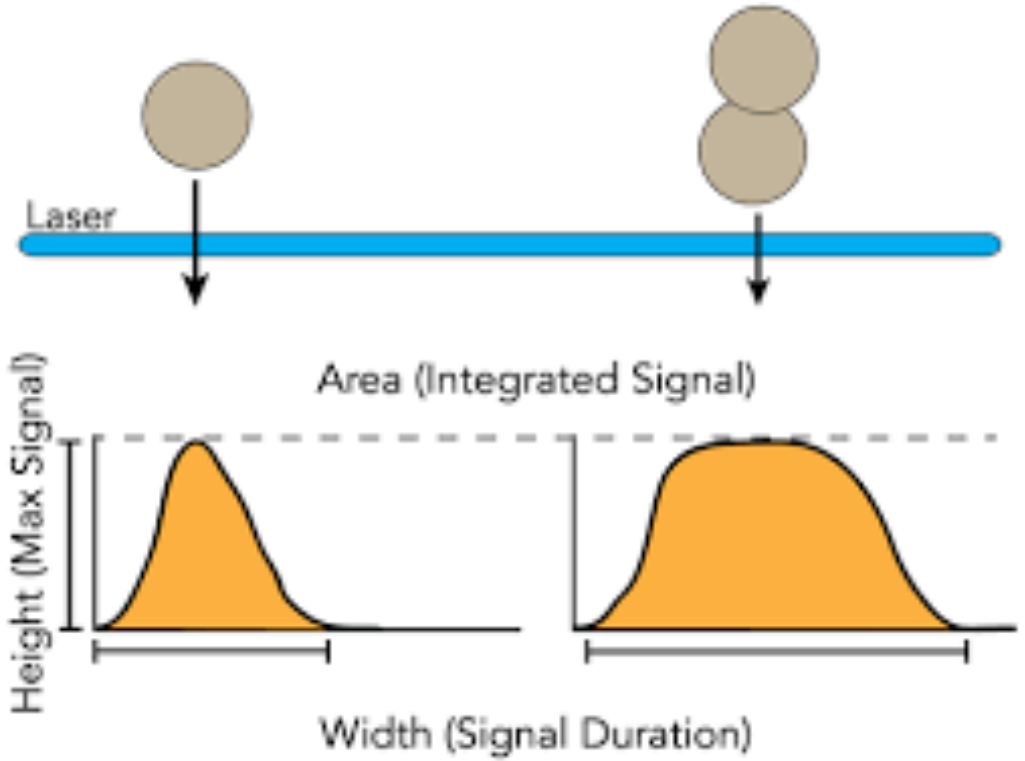


How can the pulse indicate information about cell population?

For example:

- Pulse width can be used to identify doublets

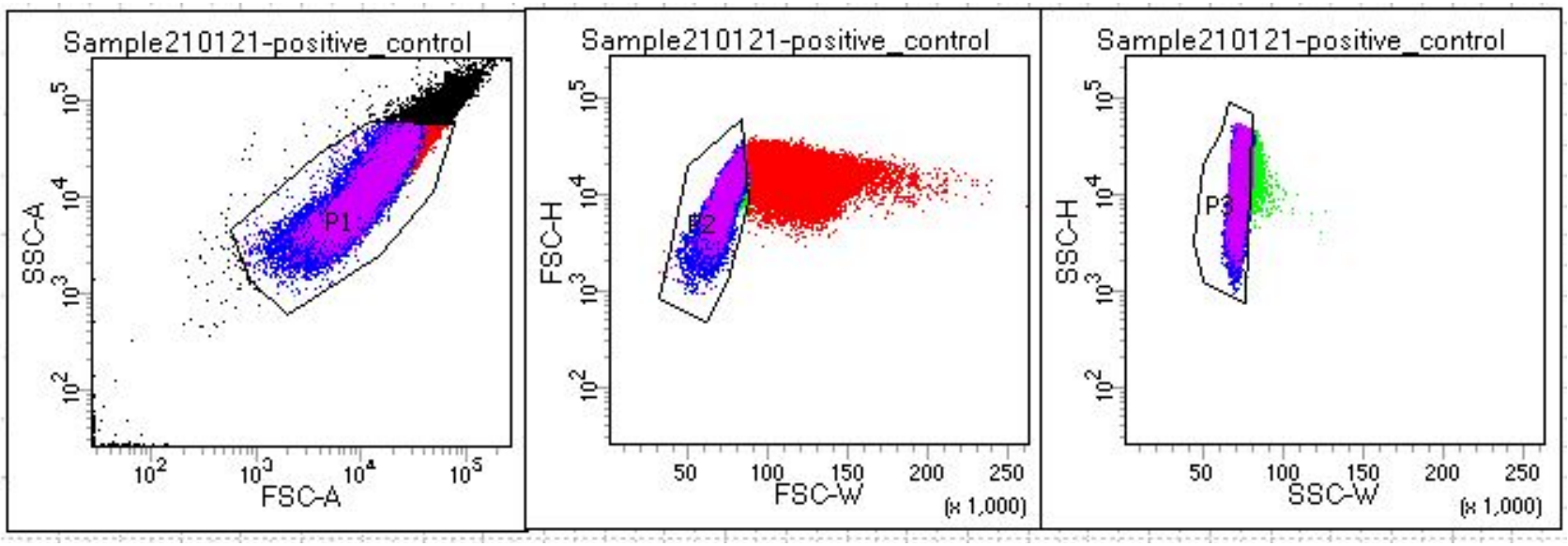
- Why would it be important to identify doublets/aggregates in our population?

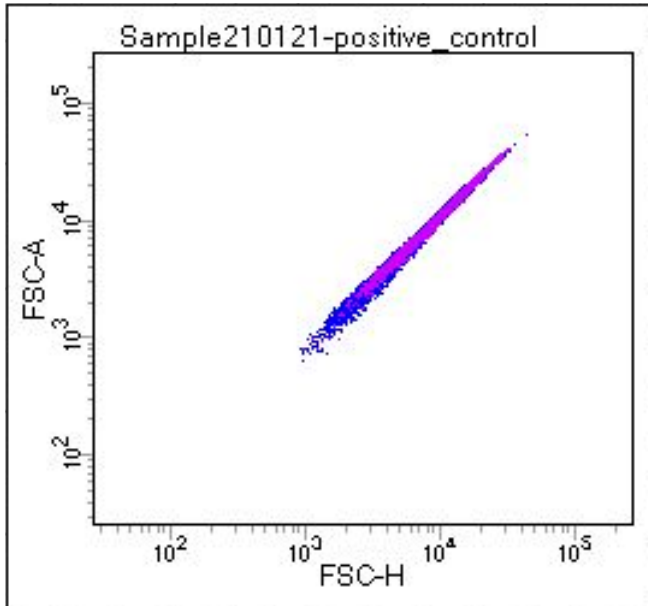


How can we use FSC and SSC in our experiment?

- Before you can assess binding of scFv, you need to define the cell population that should be sorted

- Cell populations are defined using gates



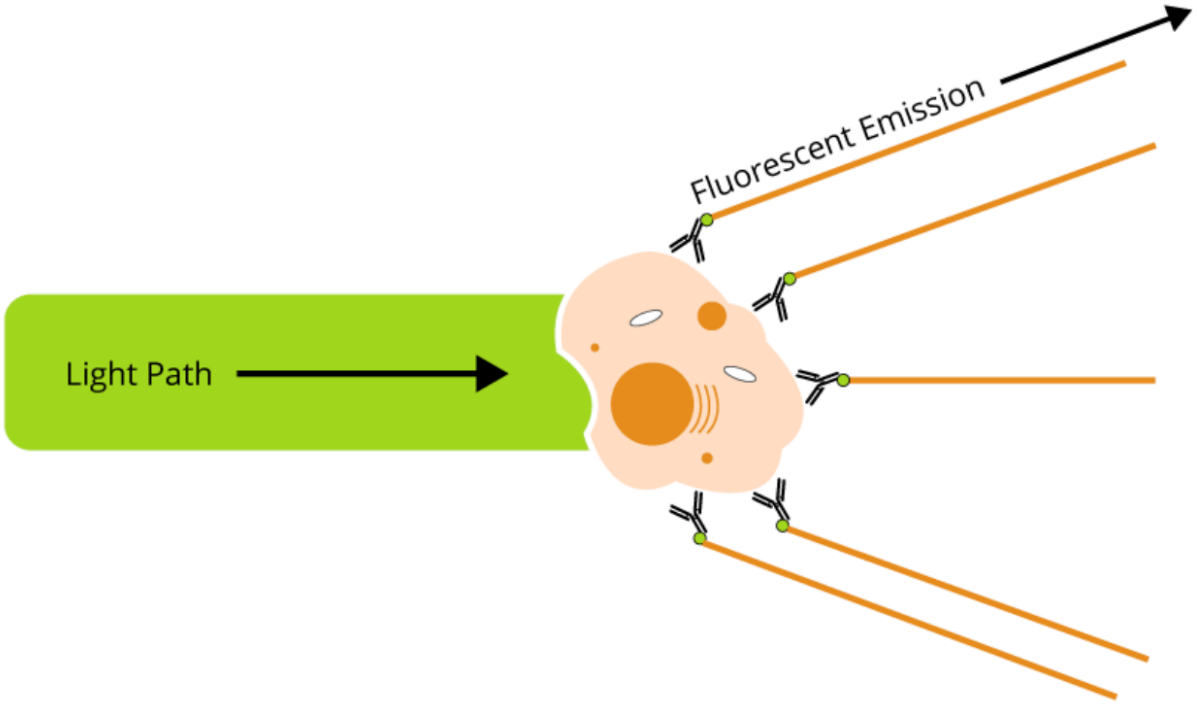


Fluorescent light emission allows us to identify cells with our scFvs bound to lysozyme

- In addition to information about the cell population from FSC and SSC, we can use our fluorescent labels to identify a cell population that meets our experimental criteria

- 488 fluroescence:

- 647 fluorescence:



How do we use fluorescent signal to assess our scFV population?

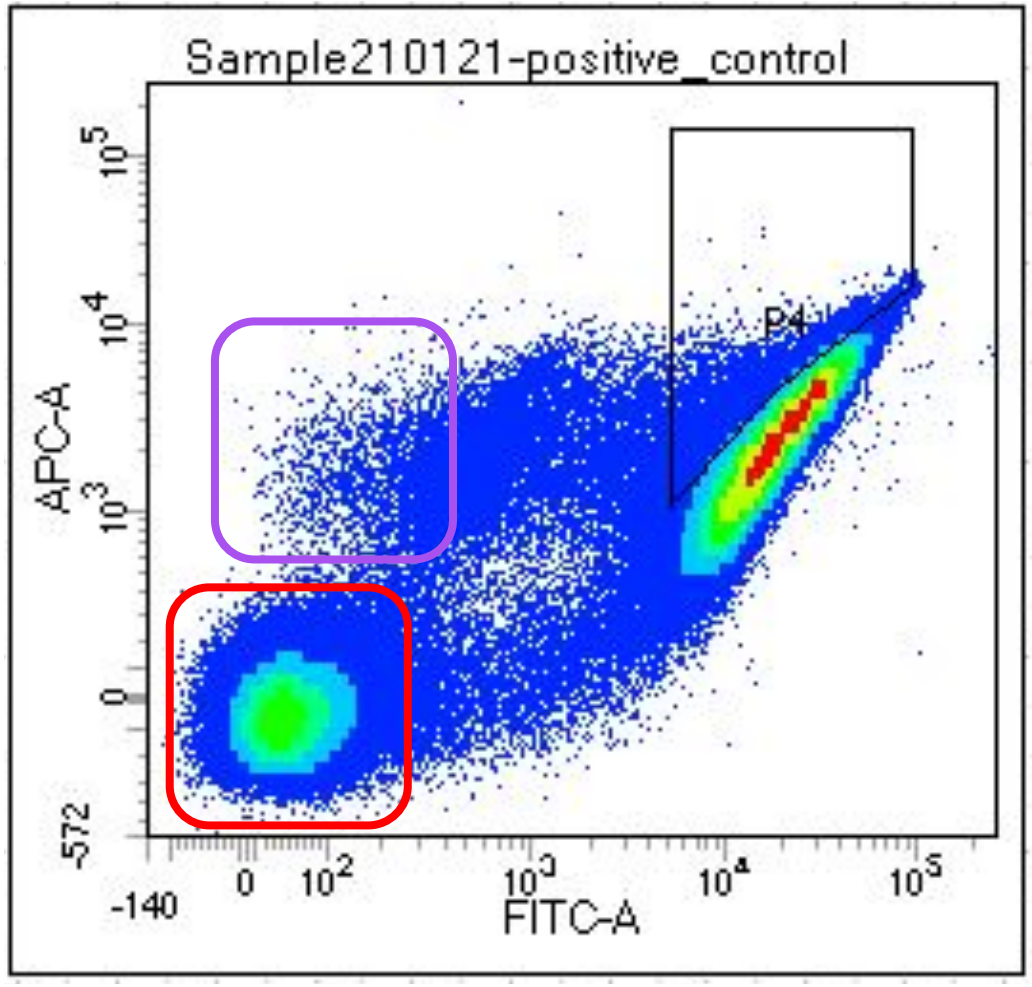
FITC-A=

APC-A=

Red circle=

Purple circle=

Gate P4=



Notes on figure making:

- Image should not be the entire page
  - Only needs to be large enough to be clear
  - 1/3 page is a good start
- Title should be conclusive
  - Don't include what you did, rather include what you found / discovered
- Caption should not include methods details
  - Define abbreviations, symbols, etc.

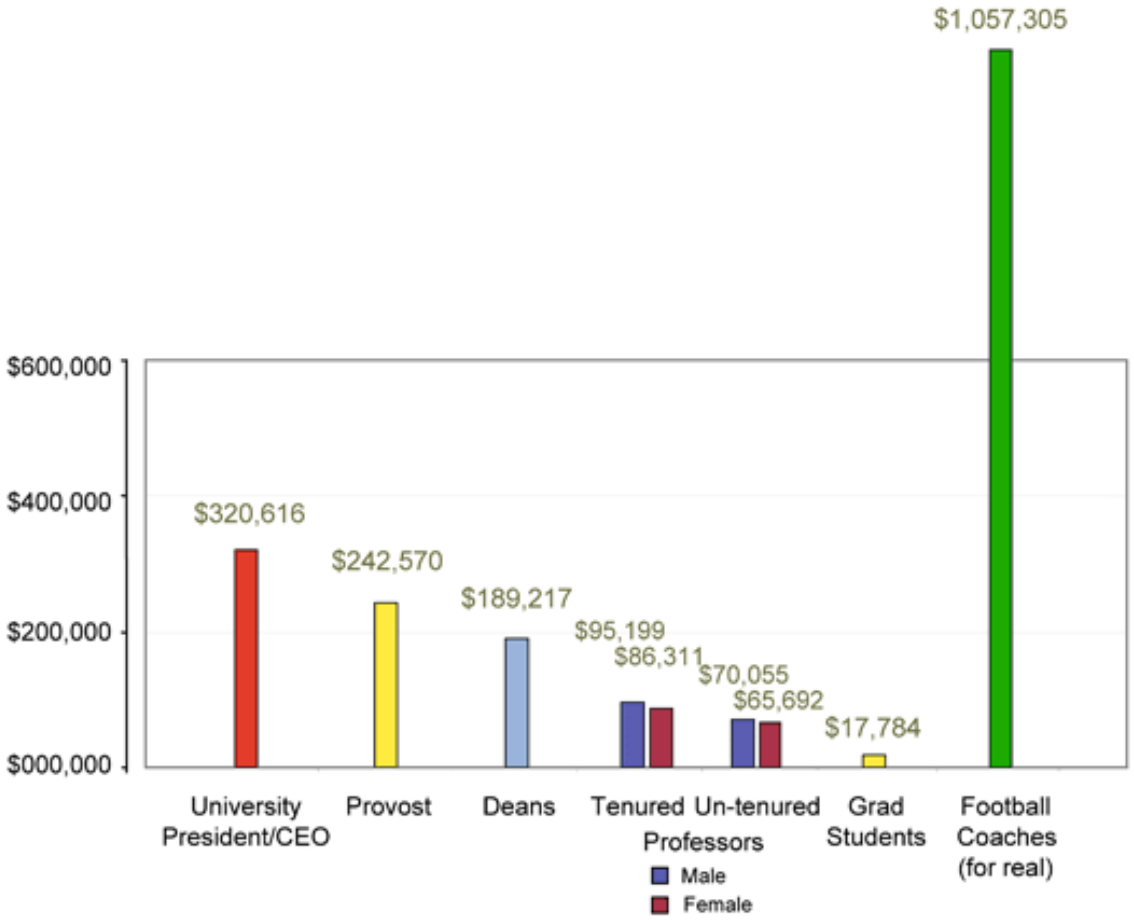


Figure X: Title is the take-home message of the experimental data.
Caption includes all of the details necessary to understand the data presented in the figure…not methods!!

For today…

- Work through wiki

- Paper discussion
  - First discussion group: Red, Orange, Yellow, Green
  - Second discussion group: Blue, Pink, Purple, Teal

For M1D3…

- Make figure of scatterplot data (see wiki Homework for specifics)
  - All figures must include a title and caption

- Make an appointment with the BE Comm lab and visit before M1D5