

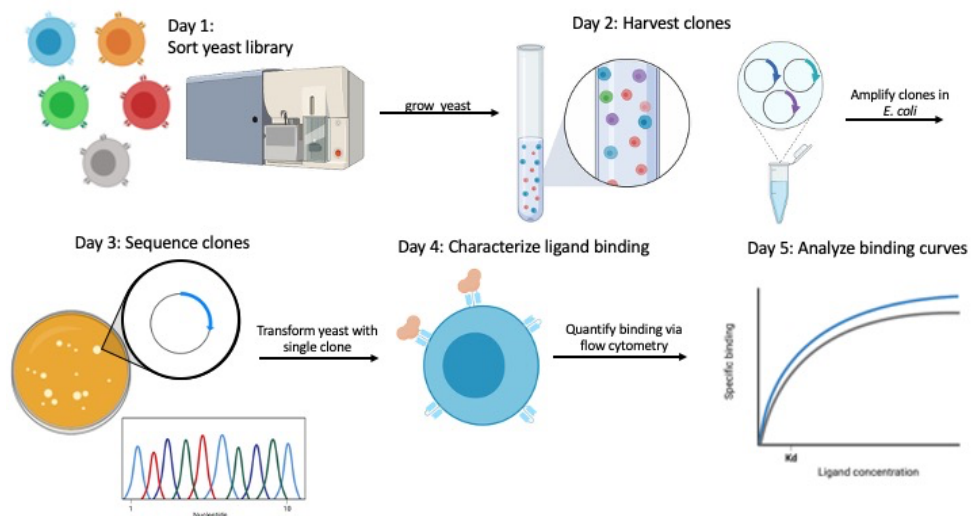
M3D4 (+ D5):

Characterize clone by titration using flow cytometry

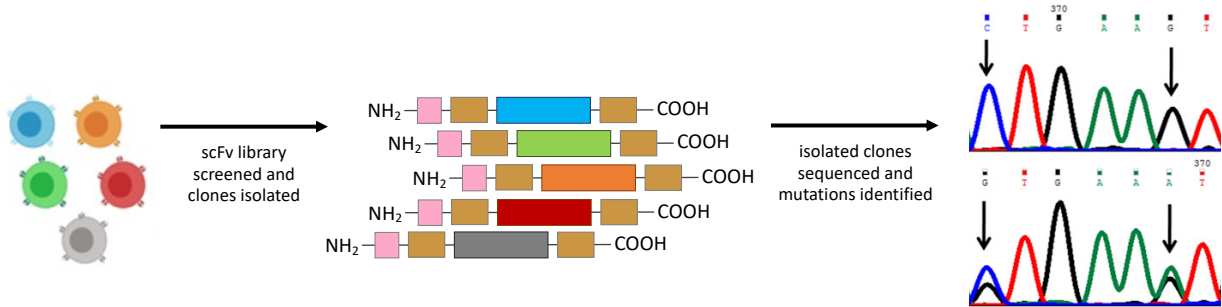
1. Prepare titration that will be used for K_d calculations
2. Use flow cytometer to examine lysozyme binding to clone
3. Generate binding curves



Overview of Mod3 experiments



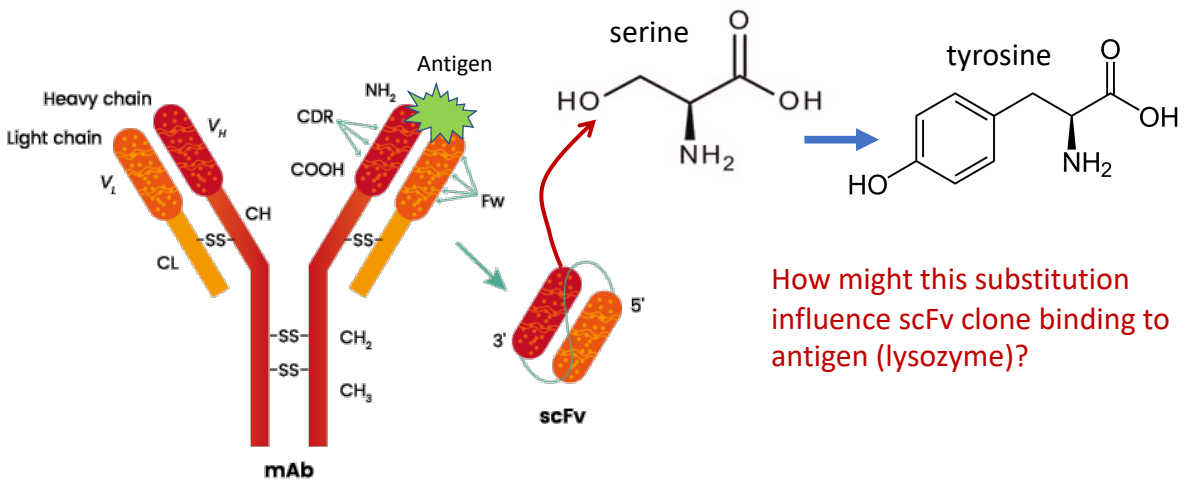
How did we get here?



What mutation(s) occurred in the isolated clones?

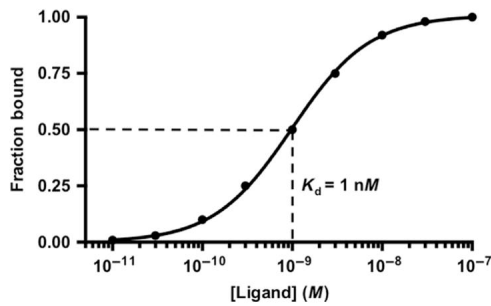
Did the mutation(s) result in an amino acid substitution?

What mutation(s) occurred in your clones?



How will we determine K_d of isolated clones?

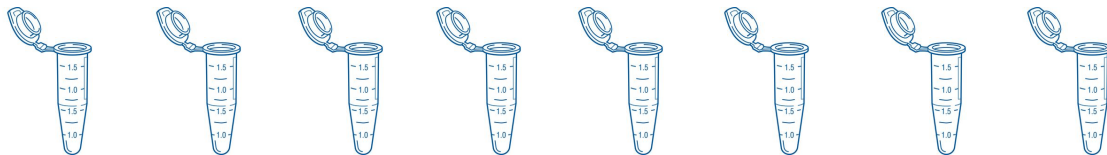
- Titration curves
 - Measure the signal that is proportional to the concentration of bound antigen
 - Plotted against the total concentration of antigen and provides the apparent K_d



To prepare samples for a titration curve, the amount of what should be constant in each tube?

What is the variable that is different between the tubes?

Sample preparation for titration curve



488

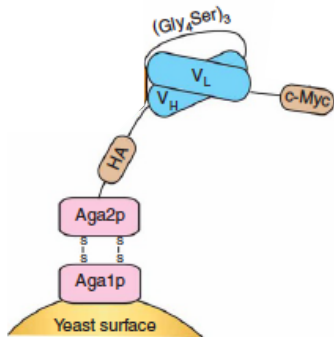
10^6 yeast cells displaying scFv clone

647

increasing concentrations of antigen (lysozyme)

- Antigen range ideally spans two orders of magnitude above / below estimated K_d
- Titrations must be at equilibrium prior to measuring fluorescence

How will you validate / interpret titration experiment?



Negative controls:

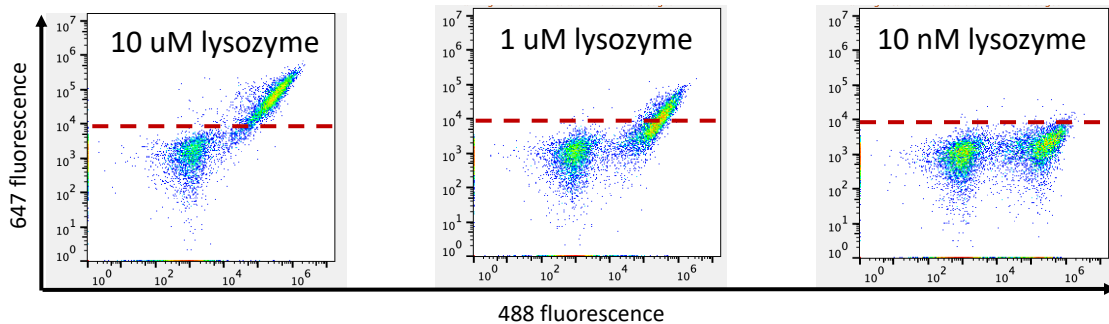
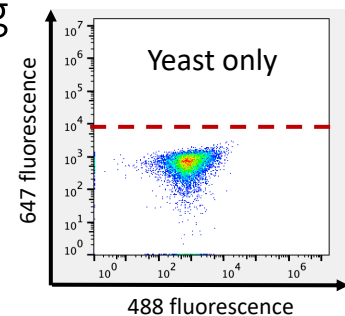
- No lysozyme (antigen), no fluorescent markers
- No lysozyme (antigen), only secondary antibody (Alexa fluor 488)

Positive controls:

- ScFv + primary antibody (anti-Myc) and secondary antibody (Alexa fluor 488)
- Lysozyme w/ marker (Alexa fluor 647) at known binding concentration

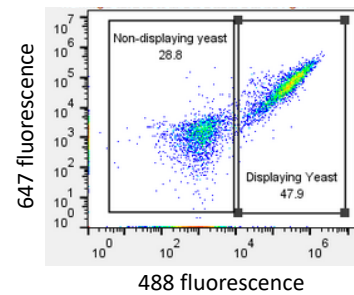
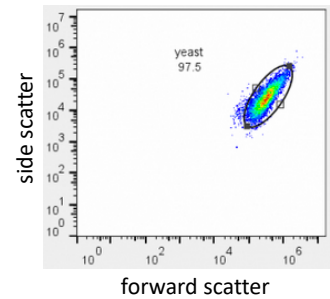
Flow cytometry used to quantify binding of lysozyme to scFv clones

- Higher concentrations of lysozyme show more binding via increase in cells expressing signal in 647 channel



FlowJo used for data analysis

1. Define background fluorescence using unlabeled yeast cells
 - Side scatter = measure of cell complexity
 - Forward scatter = measure of cell size
2. Correct for yeast that are not displaying scFv
3. Determine Median Fluorescence Intensity (MFI) for yeast cells that are displaying scFv
4. Confirm gates are correct using controls
5. Plot titration curves in Excel



For today...

- Read through M3D4 wiki protocol and do M3D5 data analysis!
- Watch flow cytometry video (<https://www.youtube.com/watch?v=5ldYFgYb9ls>)

For M3D5...

- Complete with your lab partner; outline Background & Approach section of Mini-report.