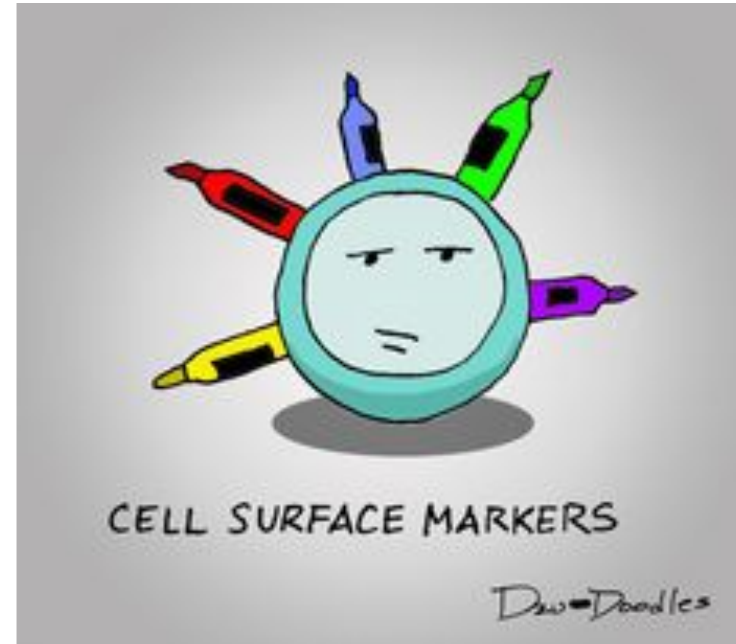
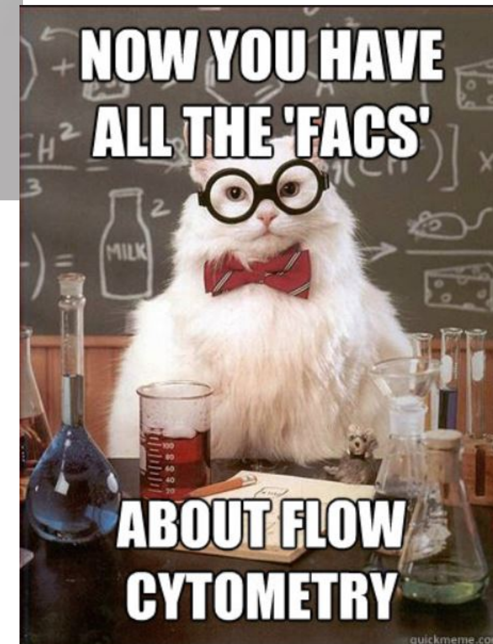
M1D6:
Characterize clone-ligand binding using flow cytometry

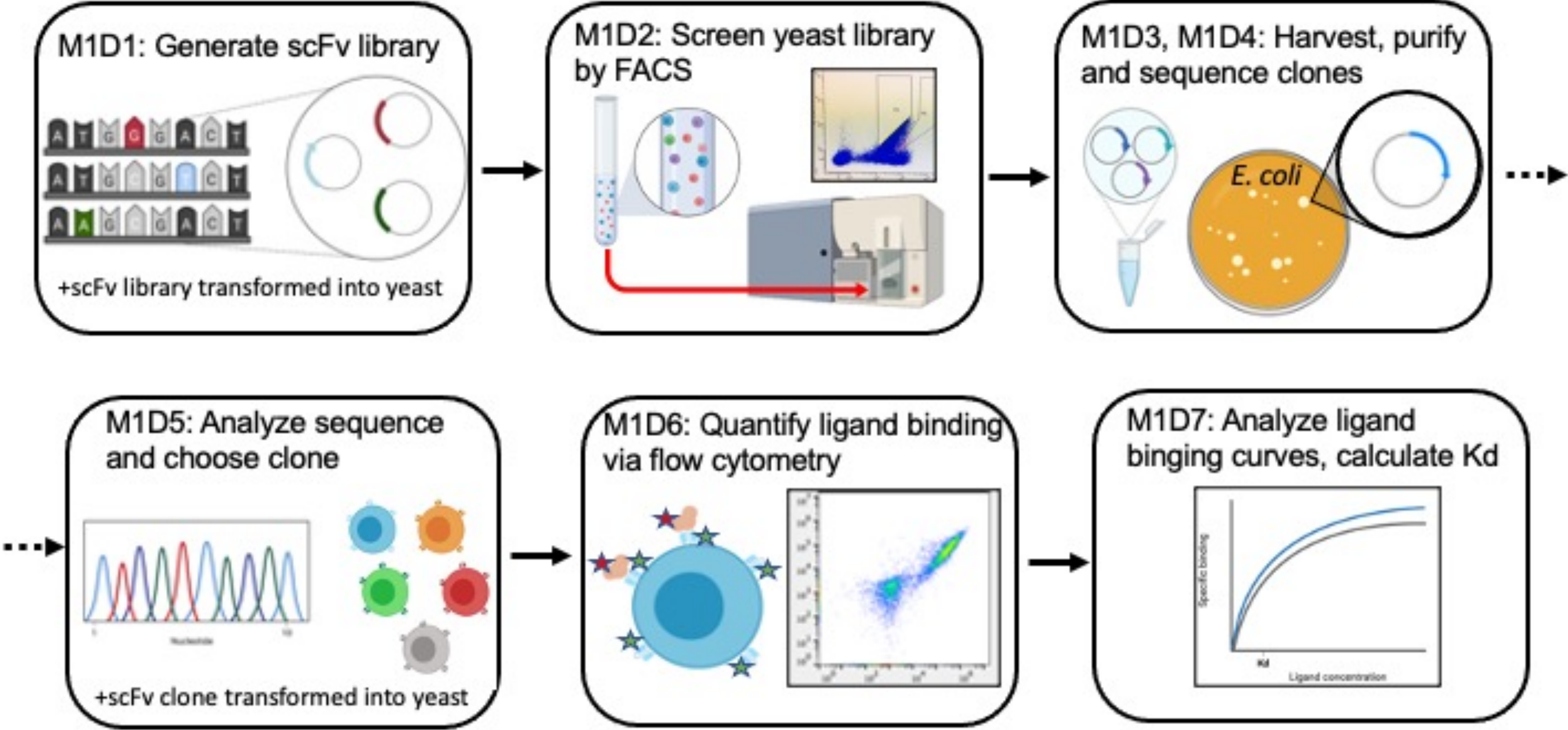
1. Prepare titration that will be used for K_d calculations

2. Use flow cytometer to examine lysozyme binding to clone

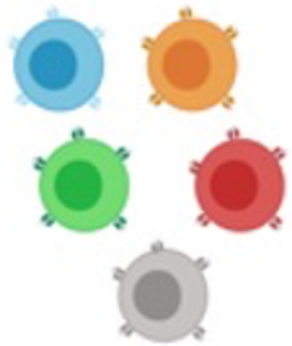




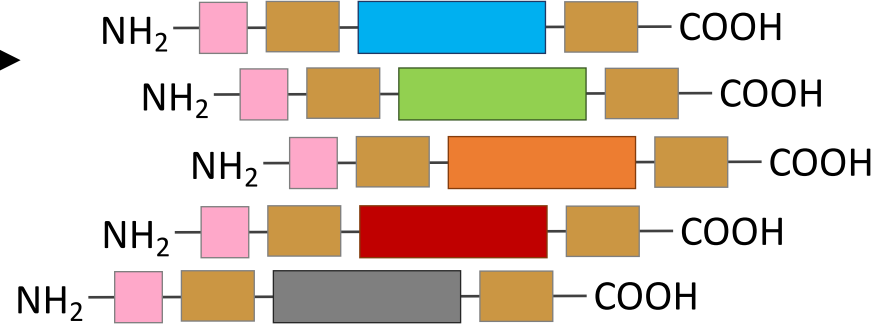
Overview of Mod1 experiments



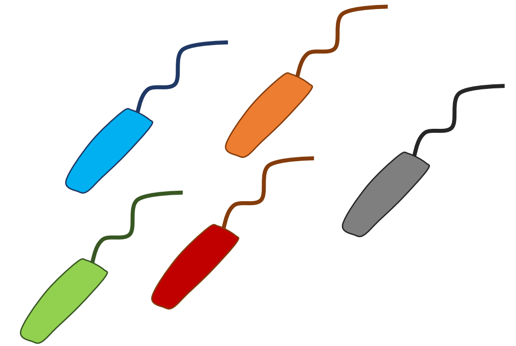
Workflow to get to one scFv clone for flow cytometry

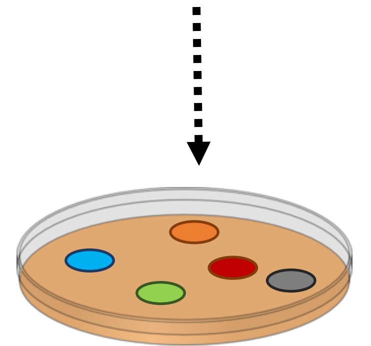


scFv library in yeast screened with FACS and better / worse binding clones isolated

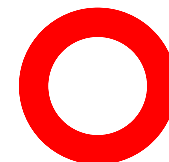


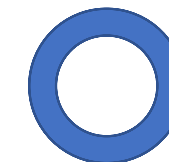
isolated clones transformed in *E. coli*

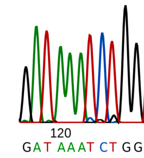


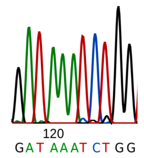


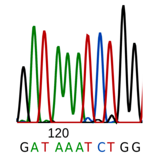
clone plasmids isolated for sequencing

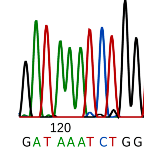




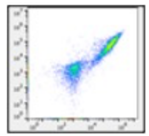


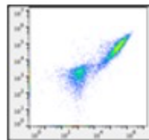


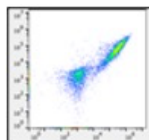


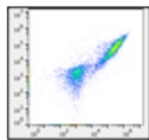


Yeast transformed with individual scFv clone for analysis with flow cytometry



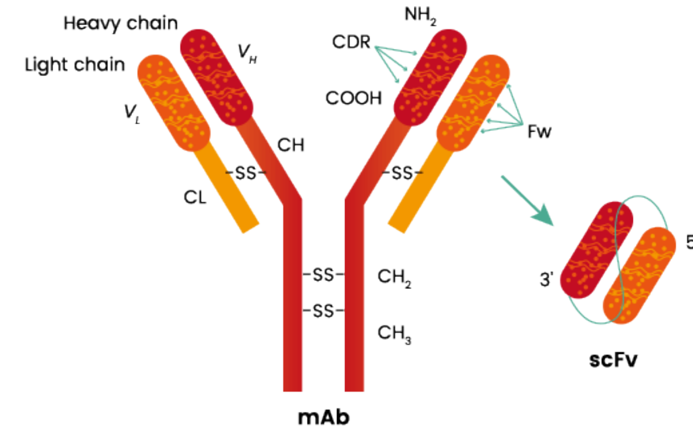






How did we get here? Where are we going?

- What is our project goal?



- What techniques did we utilize? Why?

- What could we do if there is no difference between the parental and mutant scFvs?

Fluorescence Activated Cell Sorting vs Flow Cytometry

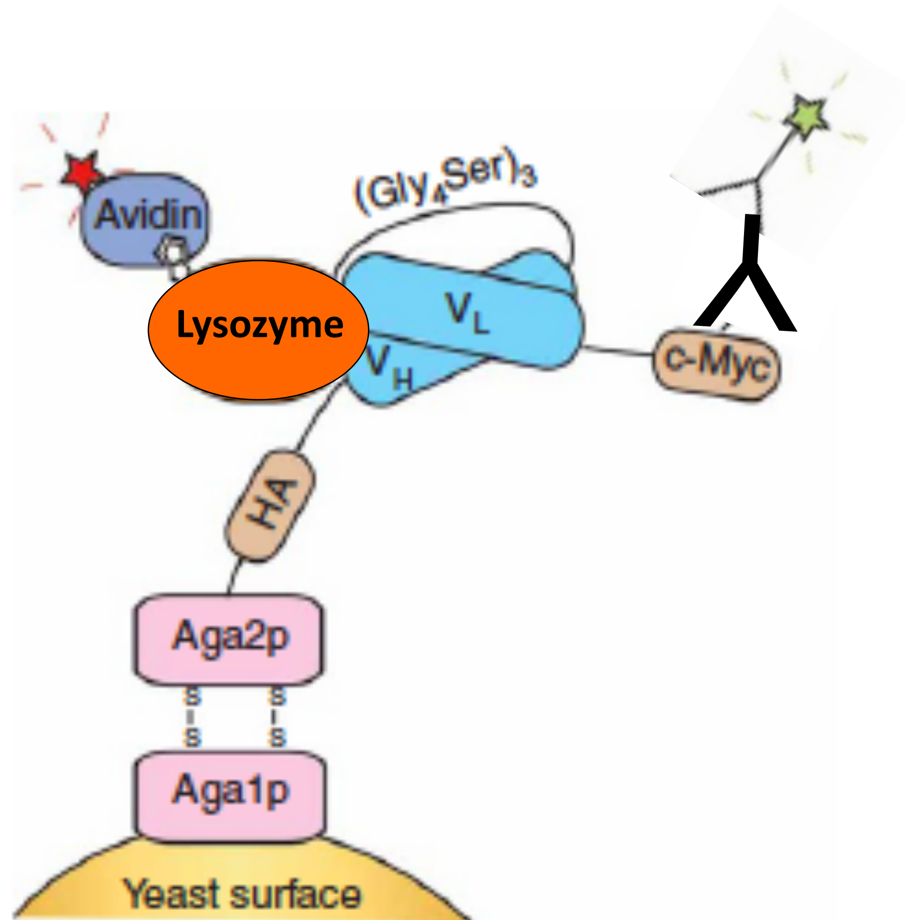
FACS

- Physically sorts cells from heterogeneous population
  - Based on light scattering and fluorescent labeling
- Used as a first step to isolate cell populations for further analysis
- Electromagnetic sorts cells

Flow Cytometry

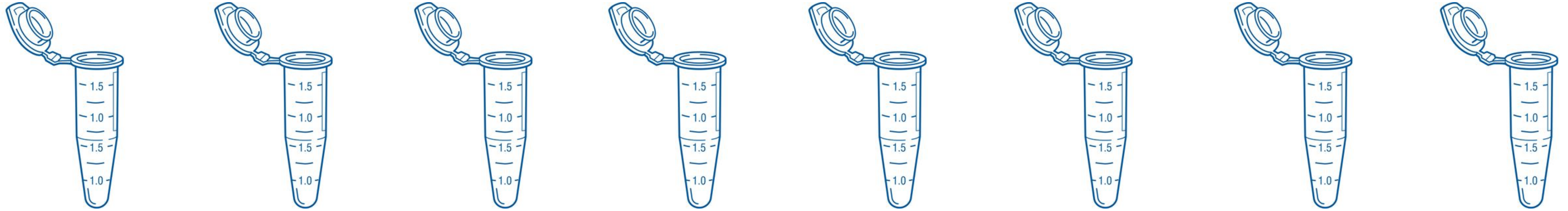
- Collect data about cell population for analysis
  - Based on light scattering and fluorescent labeling
- Follows FACS
- Sensor records cell information for multiple characteristics simultaneously

How are we identifying our binding partners for flow cytometry?



What are our controls?

Sample preparation for titration curve



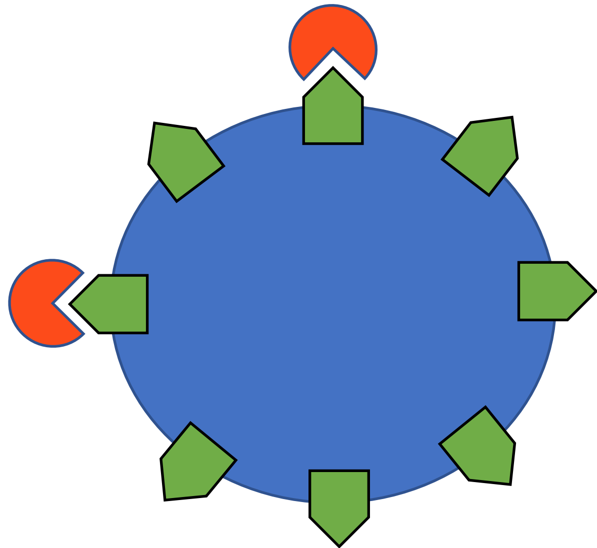
488 | 10^6 yeast cells displaying scFv clone

647 | increasing concentrations of antigen (lysozyme)

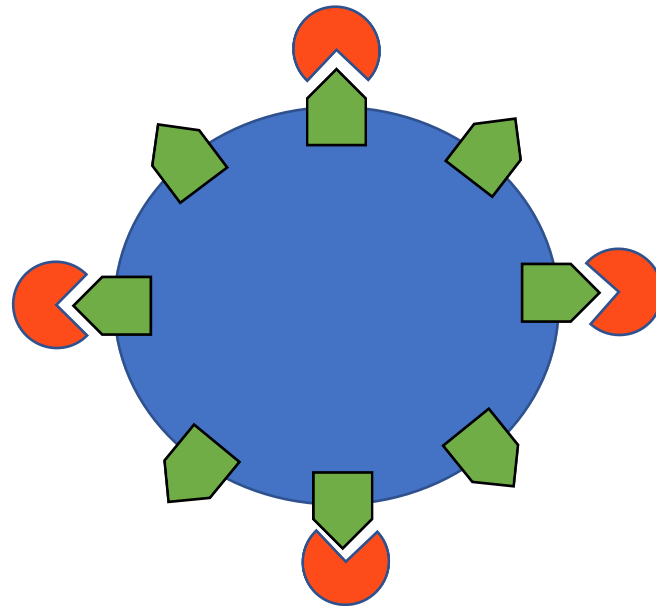
- Antigen range ideally spans two orders of magnitude above / below estimated K_d
- Titrations must be at equilibrium prior to measuring fluorescence

Equilibrium binding and ”receptor availability”

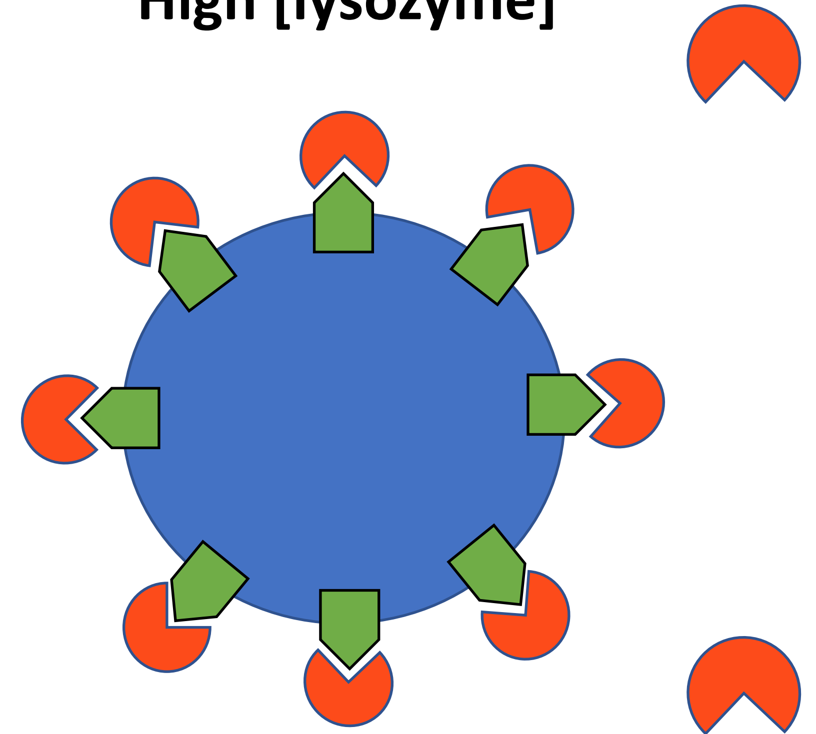
Low [lysozyme]



Medium [lysozyme]



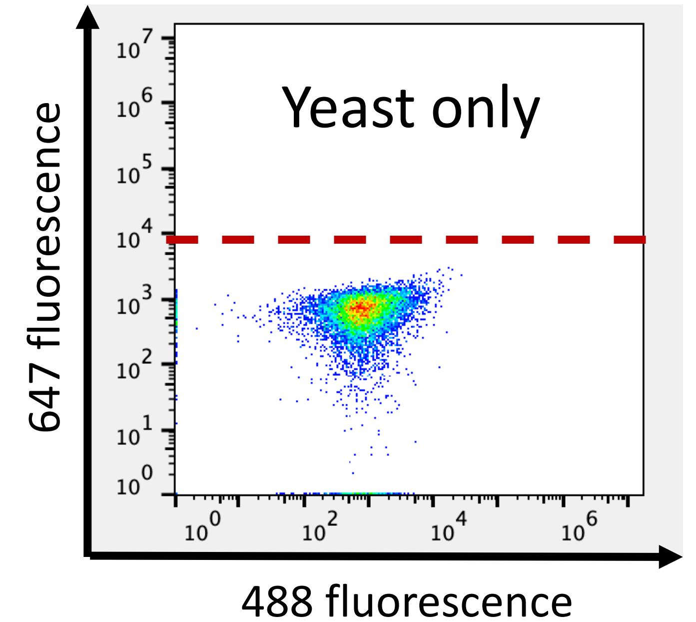
High [lysozyme]



Signal intensity:

Flow cytometry used to assess binding of lysozyme to scFv clones

- Higher concentrations of lysozyme show more binding via increase in 647 fluorescent intensity



10 nM lysozyme

1 uM lysozyme

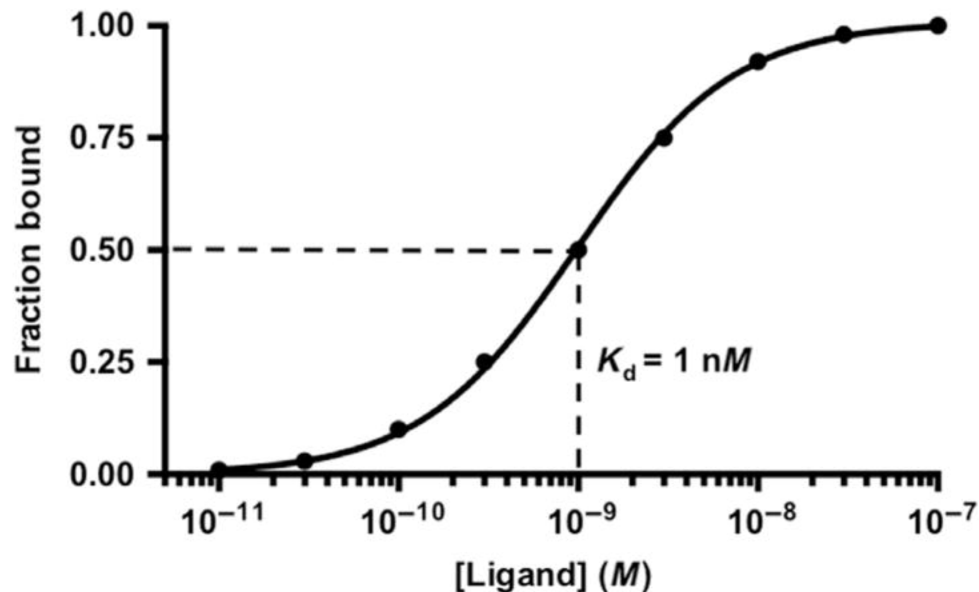
10 uM lysozyme

647 fluorescence

488 fluorescence

How will we use a titration curve to assess binding?

- 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



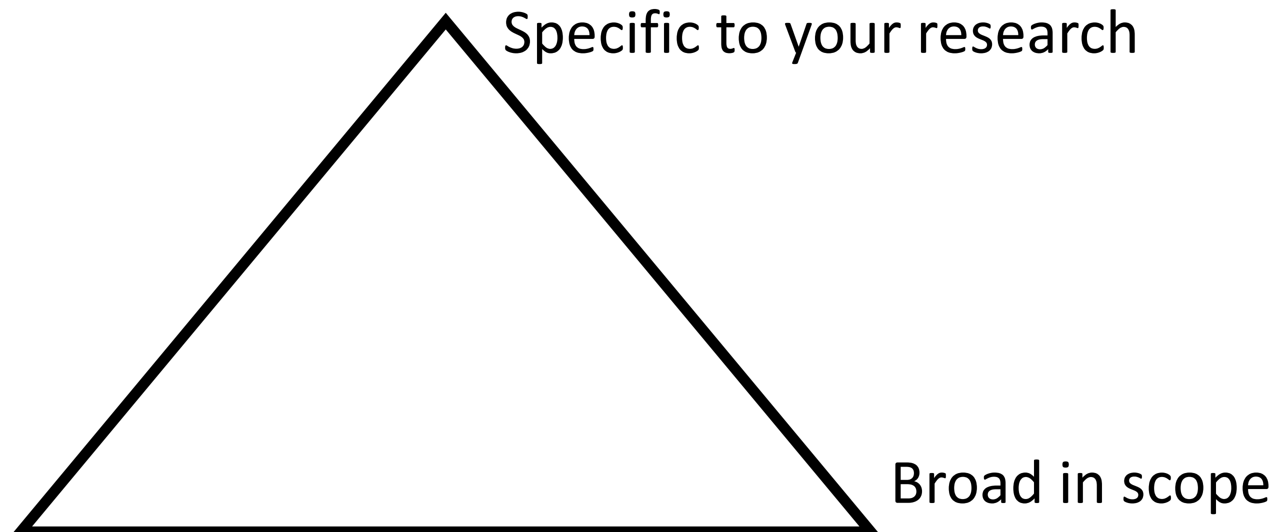
What will we keep constant in our titration curve experiment?

What will be variable in this experiment?

Implications & Future Works

Implications and Future Work: potential topics [edit]

- ***Topic***: Did this experimental approach result in a scFv clone with an increased or decreased Kd?
  - If no, provide a putative explanation. If yes, how can you further test this approach?
- ***Topic***: Did the characterization of the scFv clones add new knowledge to what's known about the antibody-antigen interaction?
- ***Topic***: Based on the results, whether they matched your expectations or not, what experiments might you recommend next?
  - Follow-up experiments could distinguish between competing explanations of a given outcome, broaden the sample set with optimized screen conditions, or provide further characterization of clones.
- ***Topic***: How might this approach be improved?
- ***Topic***: Discuss a novel way this approach can be used in research, medicine or industry.
- ***Topic***: What are the broader implications of this experiment and approach?
  - Don't overreach. Suggest impact within the field of antibody engineering.



Your Implications & Future Works should:

1. Tie back to your Background & Motivation
2. Synthesize the results such that the hypothesis / research question is answered

Notes on Implications & Future Works…

- Start with a very similar paragraph to the last paragraph in your Background/Motivation (restate major results and broad implications)

- Follow same order as in Figures/Results
  - Tie together the conclusions from your data
  - If necessary, describe caveats of experiment and suggest improvements
  - Identify unknowns and speculate (within reason)
  - Don’t make huge generalizations or overreach the results shown

- Propose future experiments, identify new questions that arise
  - Incremental next steps that can be tested / measured

- Come back to the big picture / impact statement topic introduced in background

For today…

- Work through M1D6 wiki
- Work with partner(s) on Methods revision homework

For M1D7…

- Outline Implications and Future Works section of Data Summary

- Work with your lab partner(s) to revise the Methods homework and write additional methods for M1D4-M1D6