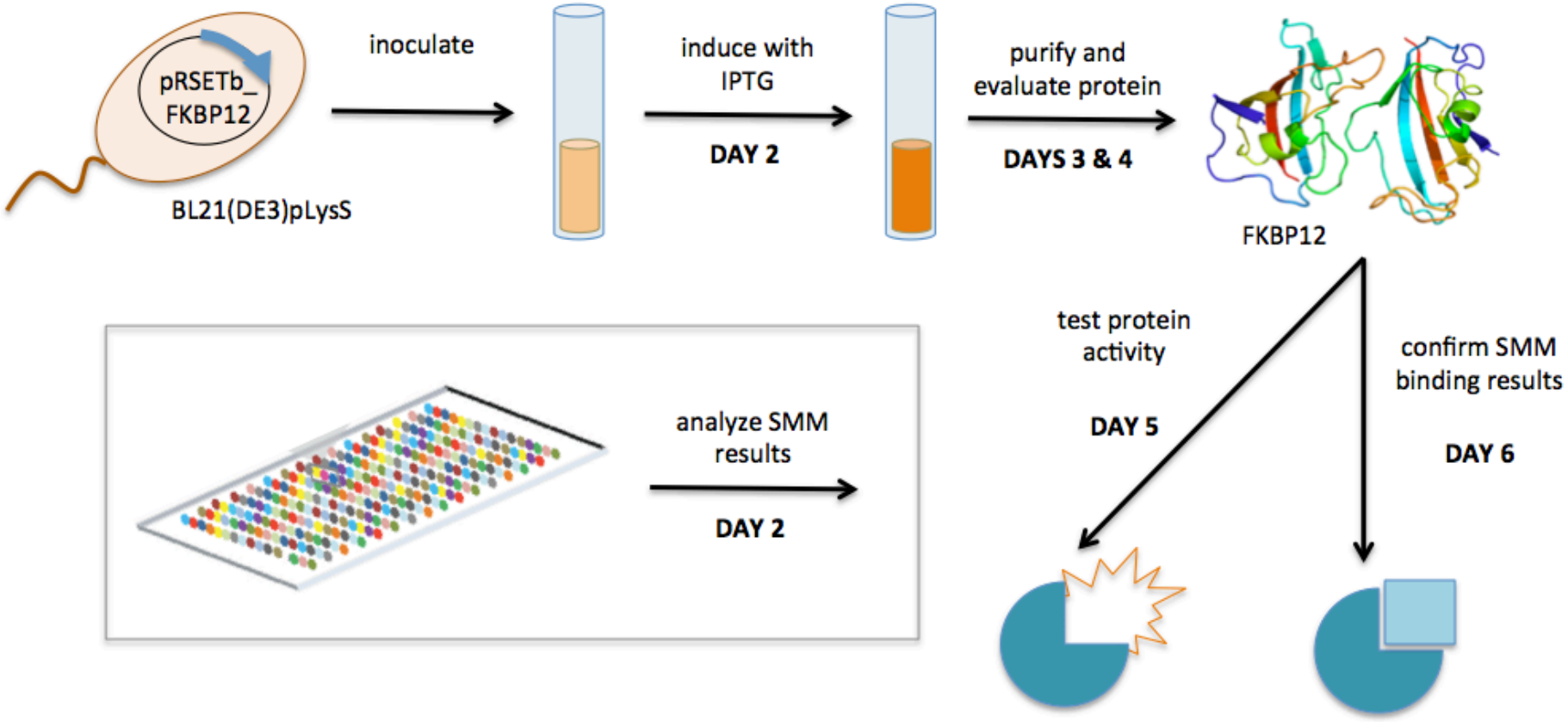
M1D6:
Confirm ligand binding using differential scanning fluorimetry assay

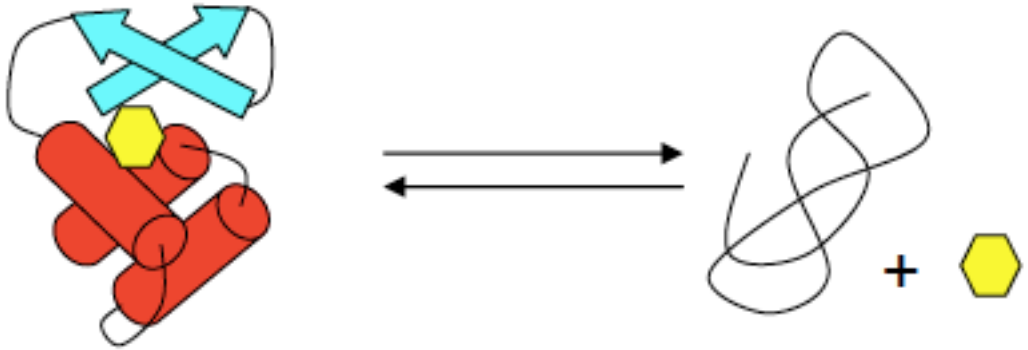
1. Pre-lab discussion
2. Complete calculations
3. Prepare master mixes
4. BE Communication Lab workshop
5. Data analysis

Overview of Mod1 experiments



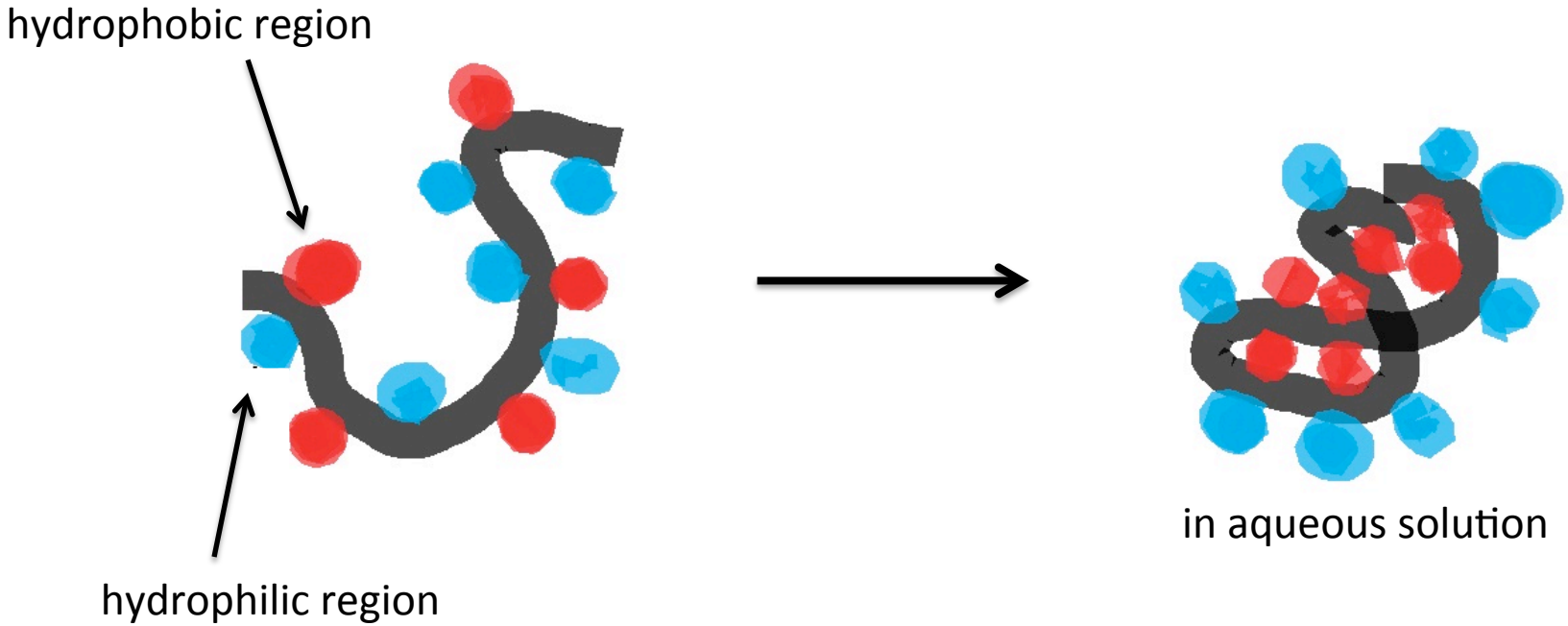
Protein:ligand interactions increase ΔG_u

- ΔG_u (Gibbs free energy of folding) = 0; where folded protein is at equilibrium with unfolded protein
- Increase in ΔG_u may promote increase in T_m

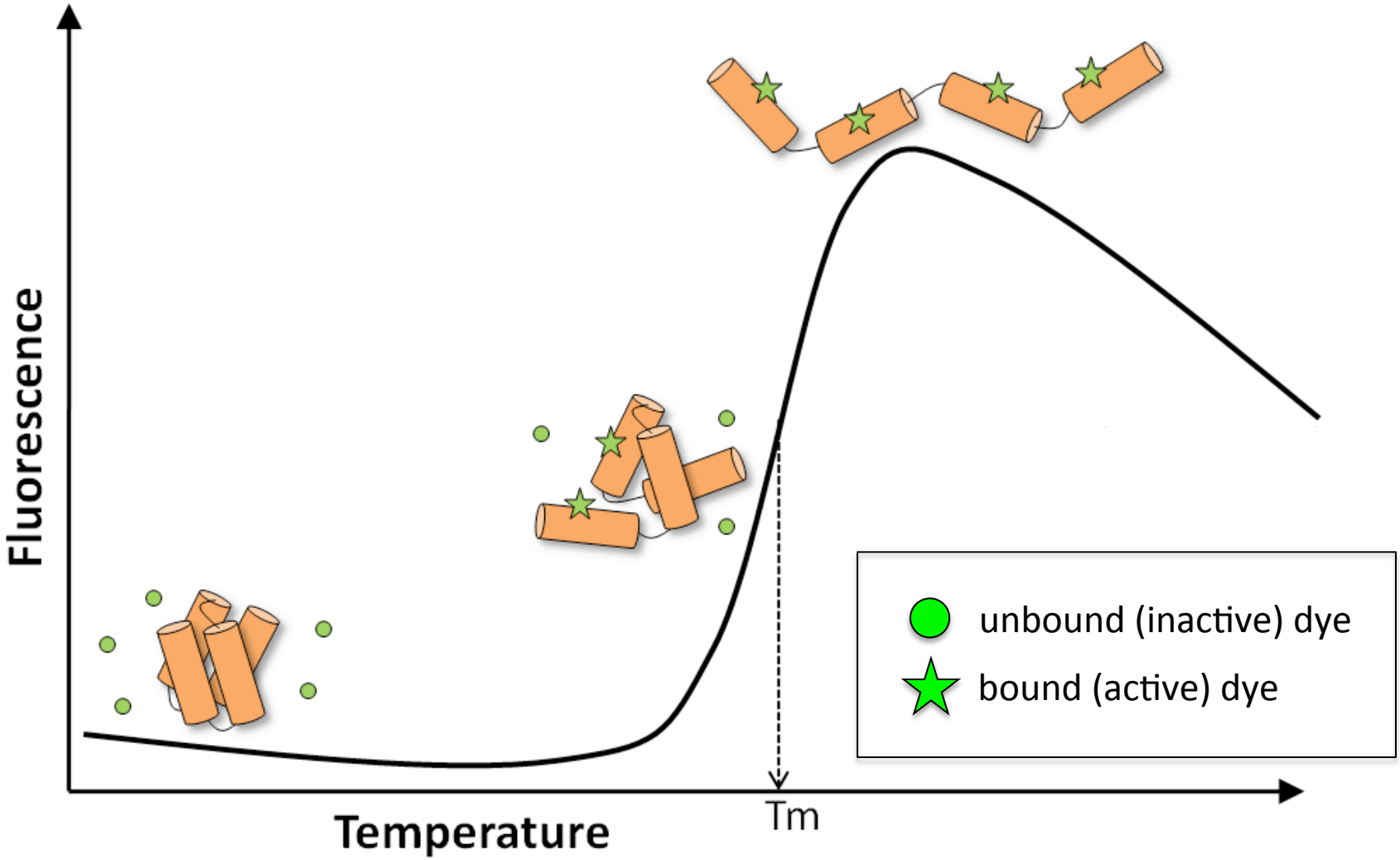


Differential scanning fluorimetry (DSF)

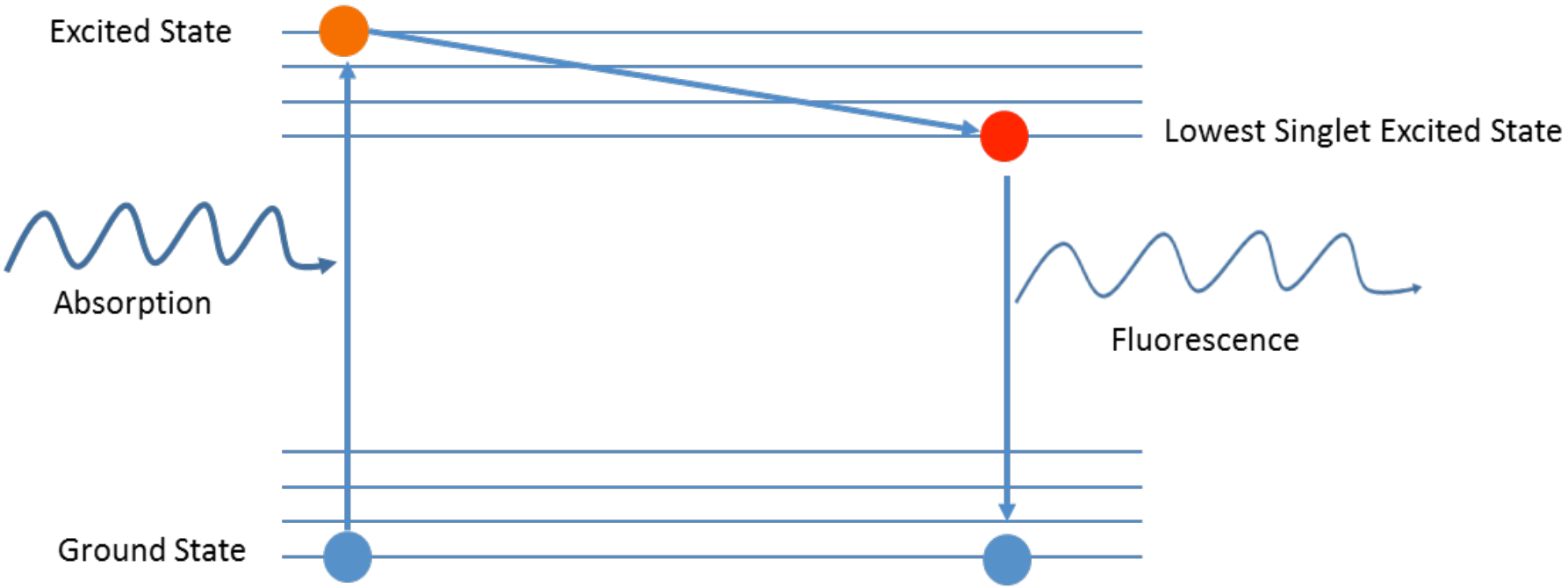
Fluorescent dye binds hydrophobic regions of protein in aqueous solution



Fluorescence measured as T increases



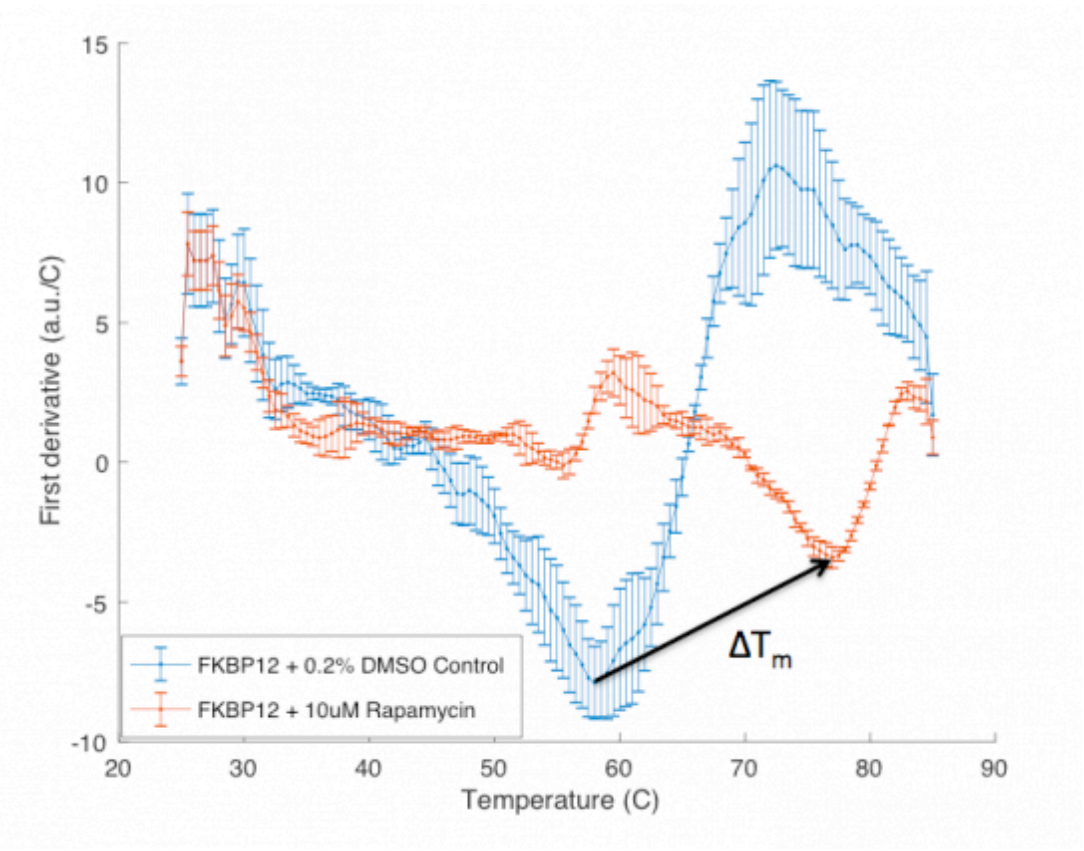
Dye emits fluorescent signal



Emitted light is at longer wavelength than excitation wavelength

How will you analyze the data?

1. Graph data to identify ΔT_m
2. Use class data to calculate apparent K_d
   - Each group will prepare one additional concentration of rapamycin



In lab today…

- Keep track of your tubes and the time!

For next time…

- Draft Implications & Future works
- Revise Methods
  - Include protein induction information

Notes on implications & future works:

Implications and Future Work: potential topics [edit]

- ***Topic***: What is the positive hit rate? Is this consistent with similar research?
- ***Topic***: Do your hits, or confirmed binders, share any common chemical structures?
  - If no, provide a putative explanation. If yes, how can you further test if this structure is important in binding?
- ***Topic***: How can you use your FKBP12 binders to further research focused on this protein?
- ***Topic***: How might the methods be improved?
- ***Topic***: How might your results be used in the clinic? in industry?

Be sure the implications addressed are in line with the problems / goals in your introduction

Quick review of previous assignments

- Schematic diagram
  - Figure rules concerning size apply
  - Be mindful of methods details
- Topic sentences
  - Follow ‘funnel’ structure
  - Include ‘here we show…’ statement with preview of key results