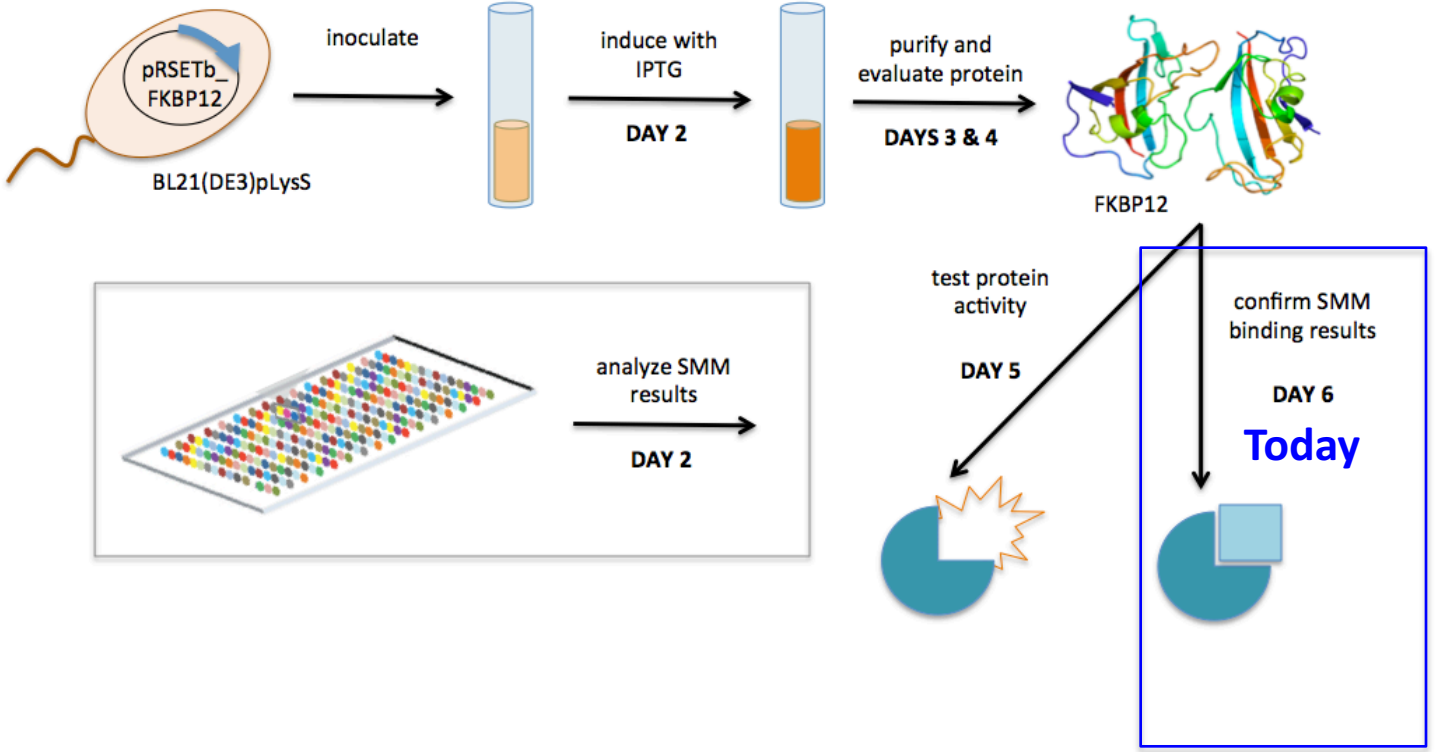
M1D6: Confirm ligand binding using differential scanning fluorimetry (DSF)

Announcements/Reminders:
3/7: M1 Quiz 2
3/12: Data Summary due
3/17: Mini presentation due

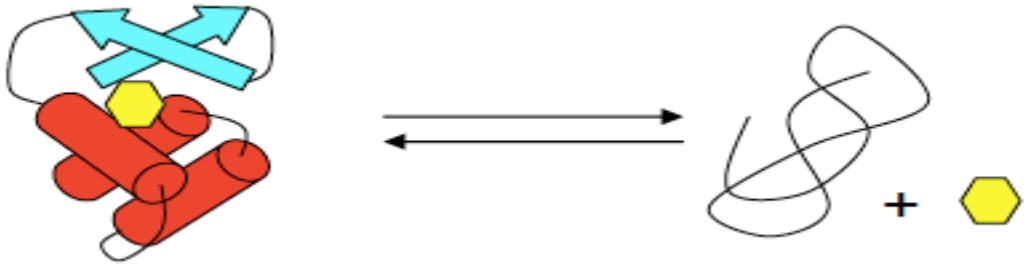
1. Pre-lab discussion
2. Complete calculations
3. Prepare master mixes
4. BE Communication Lab workshop (3 pm)
5. Data analysis

Overview of Mod1 experiments



Protein:ligand interactions increase ΔG_u

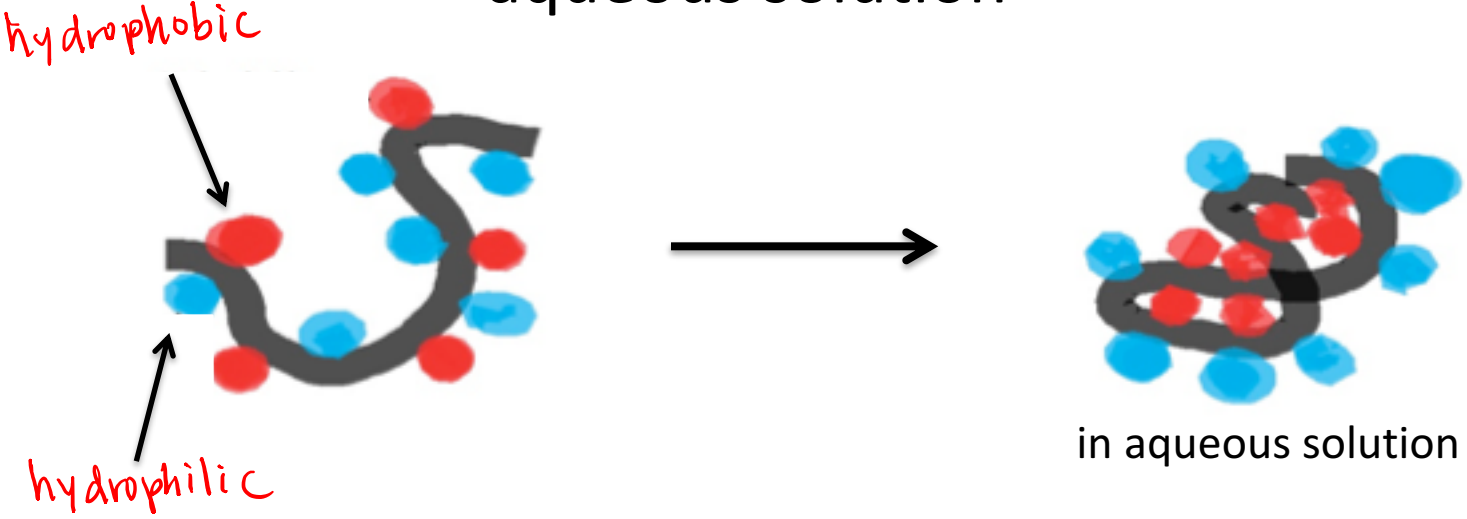
- ΔG_u (Gibbs free energy of folding) = 0; where folded protein is at equilibrium with unfolded protein at T_m
- Increase in ΔG_u may promote increase in T_m



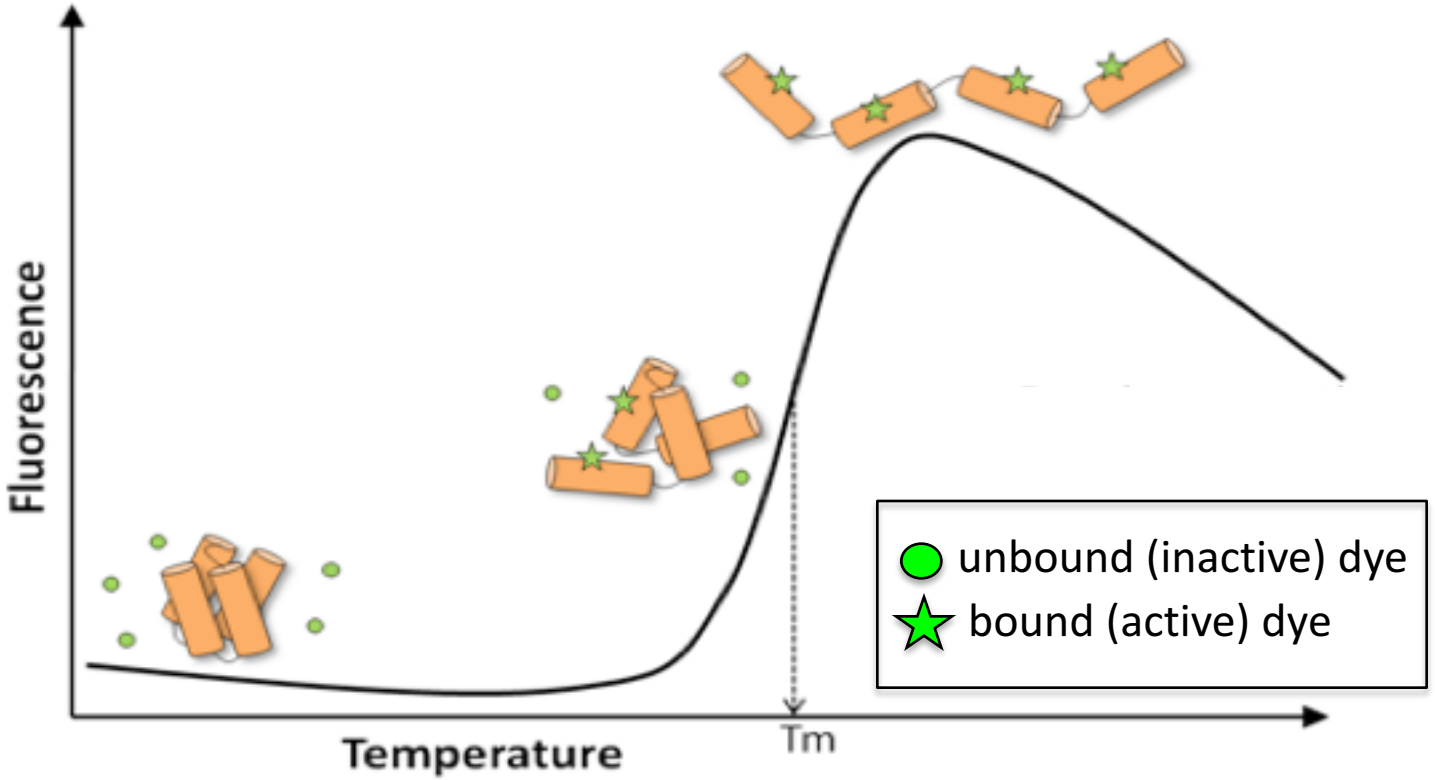
Differential scanning fluorimetry (DSF)

a.k.a. Thermal Shift Assay

Fluorescent dye binds hydrophobic regions of protein in aqueous solution

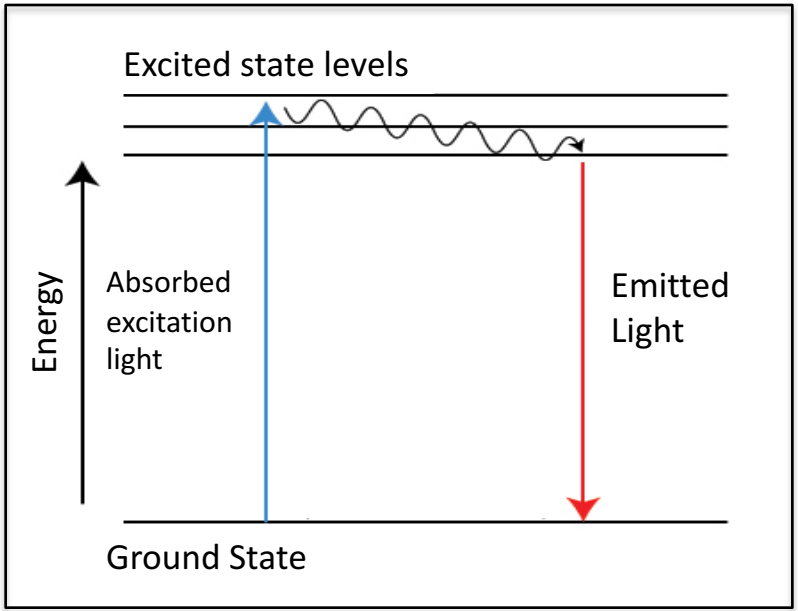


Fluorescence measured as Temperature increases

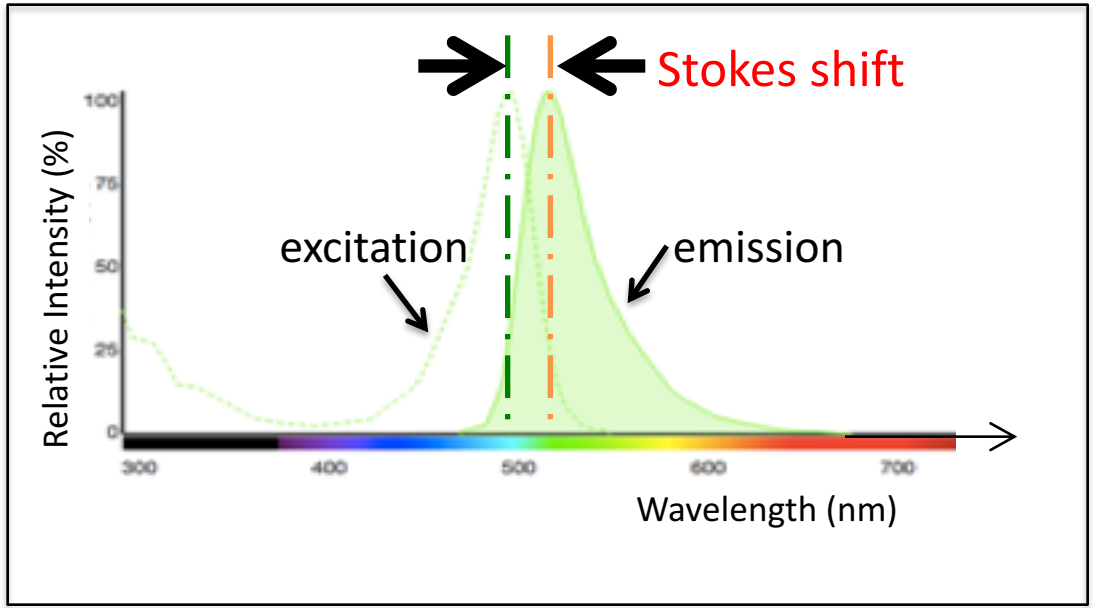


Physical principles of fluorescence

Jablonski diagram



Stokes (red) shift of emission wavelength



Emitted light is at Longer wavelength than excitation wavelength

Example of fluorescence detection

Thermal shift dye:
Excitation ~580 nm
Emission ~623 nm

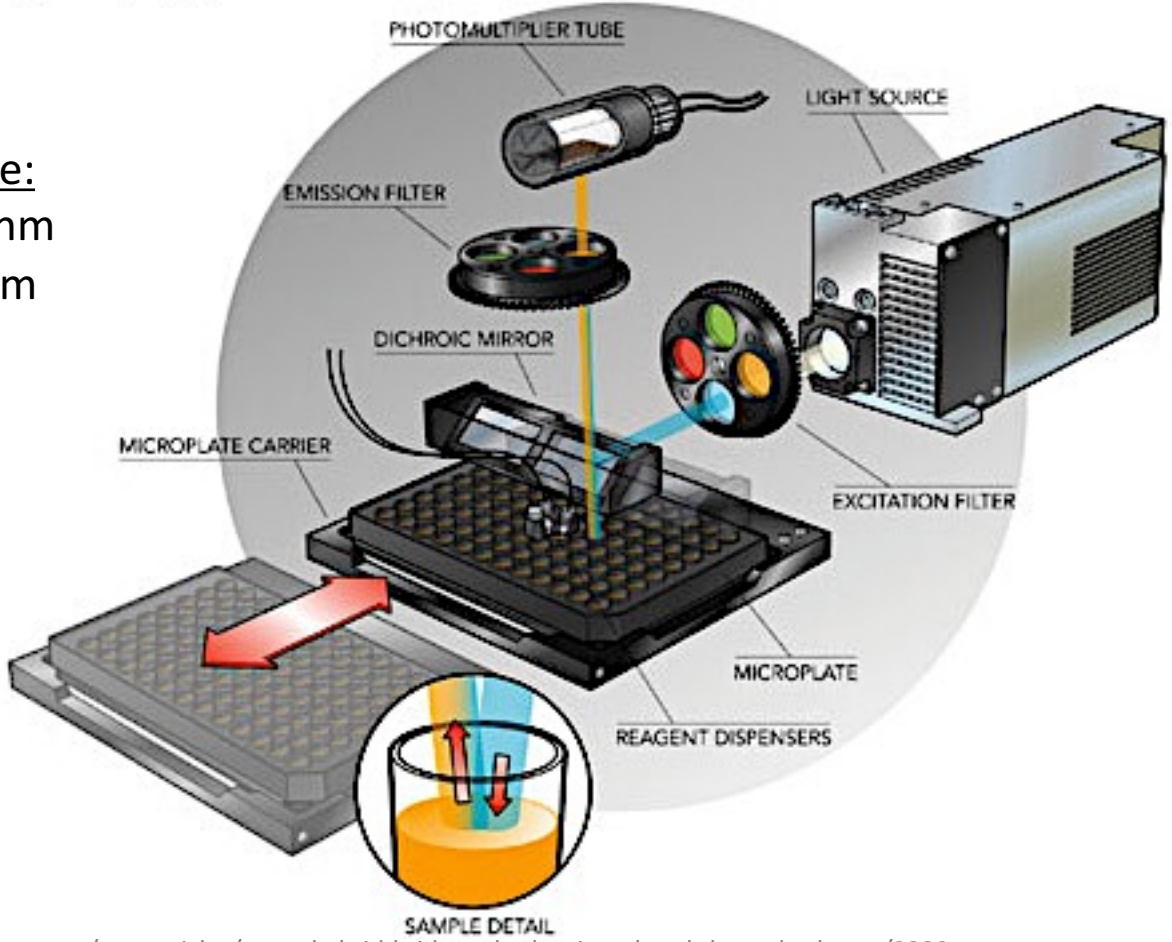
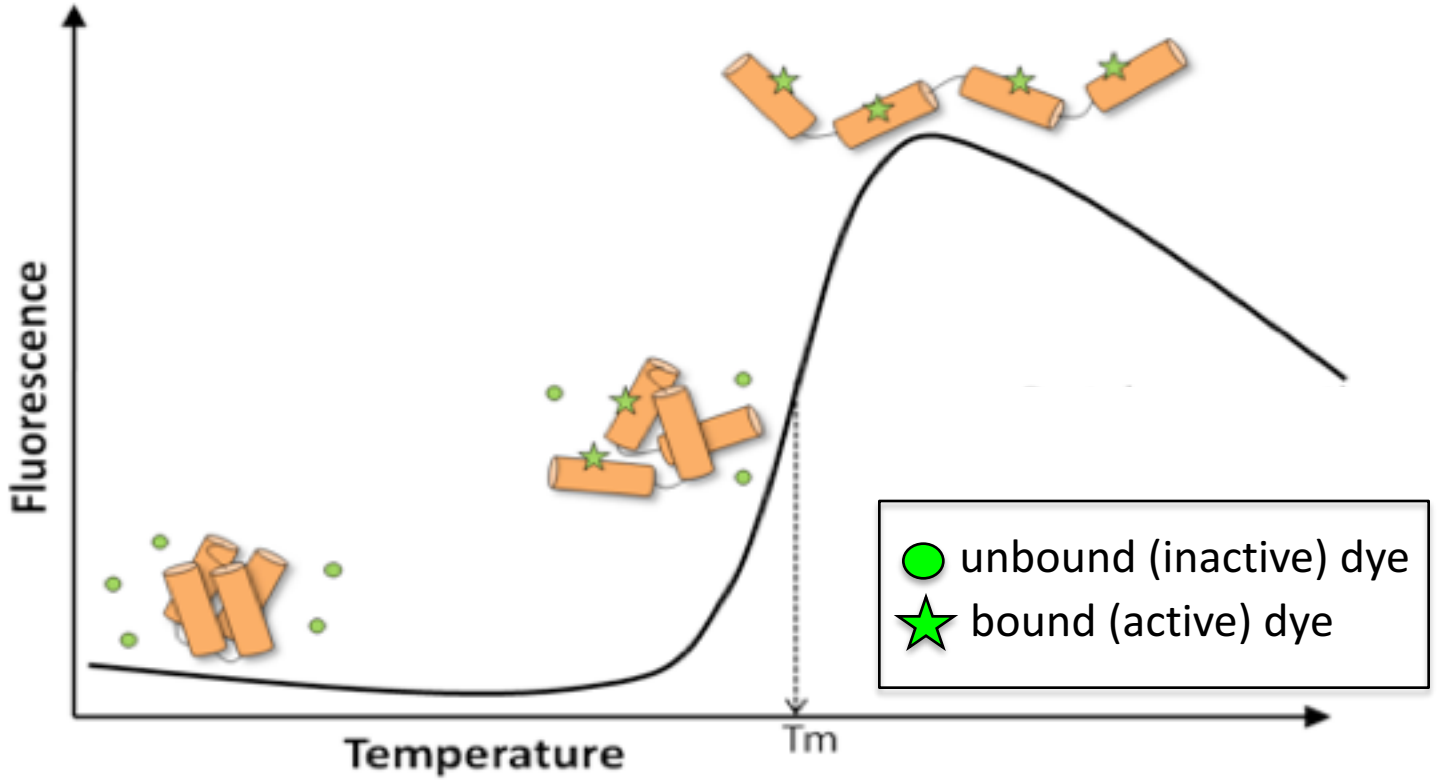


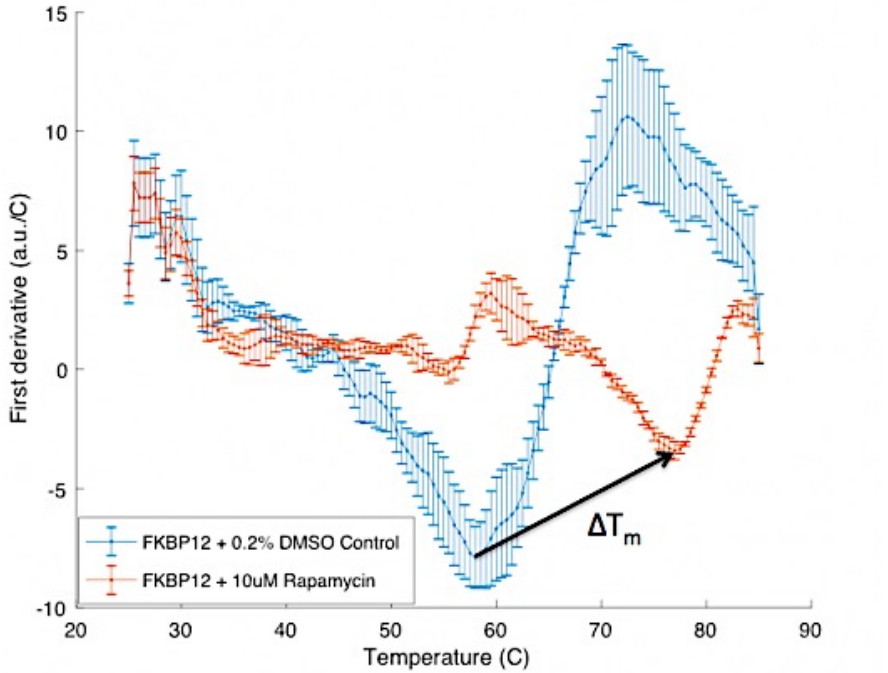
Image: https://www.genengnews.com/gen-articles/assay-hybrid-bridges-the-b-microplate-b-b-reader-b-gap/2332

How will you analyze the data?



How will you analyze the data?

1. T_m = First derivative peak
2. Find ΔT_m compared to control and graph to validate by eye
3. Use class data to calculate apparent K_d
   - Each group will prepare one additional concentration of rapamycin



Data acquired by Becky Leifer

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 hypothesis
  - Include ‘here we show…’ statement with preview of key results