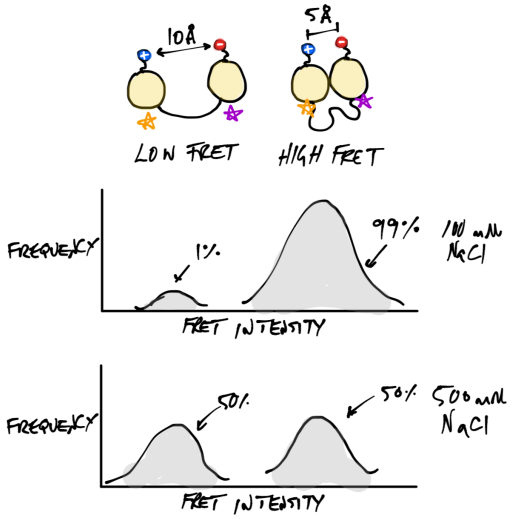
Instructions:

- Turn in your results in some kind of clear form (handwritten, typed, pdf).
- Feel free to work in groups.
- I have tried to be consistent in my language in my prompts if I want something specific.
  - *Sketch:* Hand draw a plot. Axes should be labeled conceptually, with key features indicated and explained.
  - *Generate a plot:* Plot using software. Label axes, use appropriate significant figures, etc.
  - *Calculate:* Actually calculate numbers using math. Report units and uncertainty, as appropriate.
  - *Describe/Argue:* Use any combination of writing, sketching, plotting, and calculation to argue for an interpretation.
  - Finally, I will sometimes specify the sort of explanation I want.
    - *Molecular*: Describe what the atoms and molecules are doing in space and time. Depending on the context of the question, this might also involve explaining the result in terms of atomic properties like hydrophobicity.
    - *Energetic*: Describe in energetic terms (entropy, free energy, statistics).
    - *Mathematical*: Answer in terms of how the functions behave. For example, if I asked "mathematically, why does $Kx/(1+Kx)$ saturate with increasing x" the answer would be: "because as $x \to \infty$, $Kx \gg 1$ and the function tends to 1."
- Some of the questions may require you to play with math and/or ideas we did not explicitly discuss in class. This is intentional. Many times in science you will be faced with a paper that uses an approach you are not familiar with. Learning how to gather enough information to critically evaluate their findings, as well as understand any mathematical models employed, is important.
- Some questions are listed as **GRAD STUDENT** questions. Undergrads are free to do those questions, but it is not required.

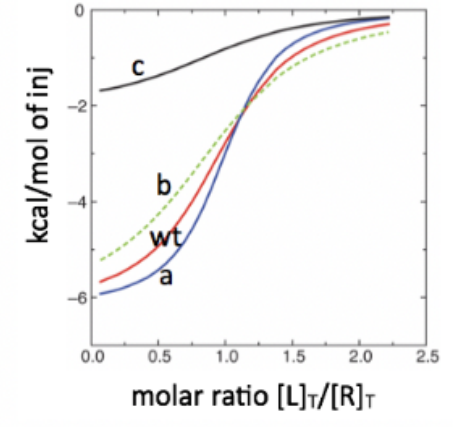
5 Binding interactions

1. Consider a protein in which the carboxylic acid of an asparate sidechain forms an ion pair with the amine of a lysine sidechain. The interaction is favorable by $-15\ kJ \cdot mol^{-1}$. If the pK_a of the aspartate carboxylic acid in water is 4.0, what is its pK_a value in this protein?

2. You are studying a protein that consists of two domains connected by a flexible linker. Using FRET, you can distinguish between a closed conformation and an open conformation. In the open conformation, two charged residues are 10 Å apart. In the closed conformation, the same two residues are 5 Å apart (see diagram). Using single-molecule FRET, you measure the relative populations of the open and closed conformations. In 100 mM $NaCl$, the protein is closed 99% of the time. In 500 mM $NaCl$, the protein is closed 50% of the time. Can this result be explained by salt screening of the two charged residues? Please justify your answer.



3. You are studying the interaction between a protein kinase and its peptide substrate by ITC. The interaction is 1:1 and the affinity is 2.2 μM. You make several mutations in the substrate that influence the interaction as described below. Based on this information, indicate which trace corresponds to which mutation. Explain your answers.



   (a) Mutation #1 creates a disulfide bond in the peptide that reduces its configurational entropy without influencing any of its interactions with the kinase.

(b) Mutation #2 reduces the amount of non-polar surface area buried when the substrate peptide binds, but does not influence the net contribution from van der Waal's interactions or hydrogen bonds.

(c) Mutation #3 replaces the Asp in an Asp-Lys salt bridge with an Arg.

4. Baran et al. set out to measure the effective dielectric constant for Coulomb interactions between charged amino acids on the surface of a protein (Baran et al. *JMB* 379(5):1045-1062). To do so, they measured changes in the pK_a values of histidine residues when they mutated acidic residues throughout the protein. They then measured the distance between the acidic residue and histidine in the crystal structure of the protein. The experiments were done at 298 K using $I = 0.1$ M.

   (a) Their results are a .csv file that should have come with this homework. Use this information to determine the apparent dielectric constant for these Coulomb interactions.

   (b) Does this result match what you expect? How might you explain this result, mechanistically?

5. Using Isothermal Titration Calorimetry, you measure the enthalpy for a binding reaction at multiple different temperatures:

T ($^\circ C$)	$\Delta H_{dissociation}$ ($kJ \cdot mol^{-1}$)
15	11.0
20	13.5
25	16.0
30	18.5
35	21.0

   (a) What is the heat capacity change associated with this reaction?

   (b) Given this information, make a hypothesis about the molecular basis for the binding interaction. Please justify your hypothesis.

   (c) Design an experiment to test your hypothesis, describing how the possible outcomes will support or refute your hypothesis.

6. You are studying the function of the cytoskeletal protein actin and want to set use ITC to measure how tightly it binds to an actin regulatory protein called Scar. You titrate a 1.3 mL solution of 25 μM actin with a series of 5 μL titrations of 450 μM Scar. From another experiment (using a method other than ITC), you determined that Scar binds to actin with a one-to-one stoichiometry at a K_D of 0.9 μM ($T = 298K$) and that the standard enthalpy change for the binding reaction is -42.3 $kJ \cdot mol^{-1}$.

   (a) From the information given, use a spreadsheet to generate the expected plot of the heat ($kJ \cdot mol^{-1}$ of injectant) versus the molar ratio of Scar to actin.

   (b) Are these conditions under which you could reliably determine the value of K_D by ITC? Explain your answer.

   (c) How much heat is released when the first injection is made? (Show your work)

   (d) For the same reaction, plot $[Scar]_T$ versus $[Scar \cdot actin]/[actin]_T$ and $[Scar]_{free}$ versus $[Scar \cdot actin]/[actin]_T$. Based on your plots, are the reaction conditions here considered "excess ligand conditions"? Explain your answer.

   (e) You use a fitting program to determine the K_D from your ITC data and you find that it exactly matches the binding affinity you measured using the other technique, 0.9 μM. What is the standard free energy change for the binding reaction?