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 error bars, 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.

2 Potentials, entropy, and calorimetry

1. I bought a used copy of Dill and Bromberg's "Molecular Driving Forces." On the very first page, they write:

   "Entropy is one of the most fundamental concepts in statistical thermodynamics. It describes the tendency of matter toward disorder."

   Whoever owned the book before me scratched out the word "disorder" and wrote "NO!"—with no other explanation.

   Make a case defending either Dill and Bromberg or my anonymous book vandal.

2. What, conceptually, do changes in free energy, enthalpy, and entropy measure?

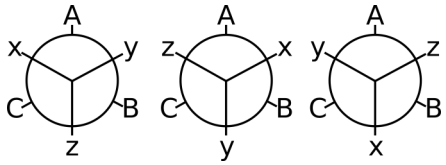
3. You are studying a protein that favors the folded state by $-32.3\ kJ \cdot mol^{-1}$. You introduce a mutation that makes a new ion pair worth $-11\ kJ \cdot mol^{-1}$ formed only in the unfolded state.

   (a) What fraction of protein molecules are in the folded state for the wildtype protein?

   (b) What fraction of the protein molecules are in the folded state for the mutant protein?

   (c) The ion pair involves a histidine residue. Do you predict its pK_a value will be elevated, unchanged, or depressed? Please justify your answer.

4. A protein with 50 amino acids has 98 rotatable bonds in its backbone. To a first approximation, each bond can populate one of three configurations:

   The folded state is one unique combination of bond configurations; the unfolded state consists of all other combinations.

   (a) Given our definition of entropy from class, estimate the change in entropy for the folding of the protein backbone.

   (b) What assumptions did you have to make to allow this calculation?

   (c) Do you think these assumptions lead to an overestimate or understimate of the entropy change? Why or why not?

5. Pentose sugars pucker to relieve steric strain. Ribose and deoxyribose are pentoses that differ only in a single moeity at position C2. In ribose, this is a hydroxyl group; in deoxyribose, it is a hydrogen atom. Relative to hydrogen, the bulkier hydroxyl restricts the number of puckering conformations that the ring can adopt. As a result, ribose adopts only 4 puckered conformations, while deoxyribose populates 8. An enzyme can bind both ribose and deoxyribose with identical interactions and, thus, identical binding enthalpy.

   (a) Calculate the difference in the standard entropy of binding (ΔS°_{bind}) for the two sugars. Assume that except for differences in the configurational entropy of the sugar, all other contributions to the binding entropy are the same for the two sugars and can be neglected.

   (b) If the K_D for the ribose binding to the enzyme at 25 $^{\circ}C$ is 3.4 μM, calculate the K_D of deoxyribose binding to the enzyme.

6. The lipid bilayers that form cellular membranes are composed of two leaflets with different lipid compositions. In blood cells, for example, phosphotidylcholine is present in the outer but not the inner leaflet.

This lipid is initially inserted into the inner membrane, and is then flipped into the outer membrane by enzymes called flippases. Phosphotidylcholines are zwitterionic (one +1 and one −1 charge), and you would like to understand how passing a zwitterion through a membrane would be different than passing a singly charged species though the membrane.

(a) Download the pdb file 4BES from the protein data bank and measure the distance between the nitrogen and phosphorus atoms in phosphocholine. Take this distance to be the diameter of a charged sphere that must be passed through hydrophobic layer of a lipid bilayer membrane ($\varepsilon = 2$) when phosphotidyl choline lipids flip into the outer membrane. The energy of activation (i.e., the energy of passing the charged head group) through the aliphatic portion of the membrane has been experimentally measured to be 105 $kJ \cdot mol^{-1}$ for the uncatalyzed reaction. Assume that the energy of transfer of the uncharged "sphere" into the membrane is zero ($\Delta G^{\circ}_{trans,uncharged} = 0$).

(b) Given this information, calculate the apparent "self-charge" (q) on the phosphotidylcholine head group.

(c) What does the result of your calculation tell you about how passing the zwitterion through the membrane together influences the desolvation energy?

i. It experiences the exact same energetic penalty as passing a single charged species with the same radius through the membrane.

ii. It experiences almost no energetic penalty in passing through the membrane.

iii. It experiences a greater energetic penalty in passing through the membrane compared to a singly charged species with the same radius.

iv. It experiences a slightly smaller energetic penalty in passing through the membrane compared to a singly charged species with the same radius.

7. You are studying the thermodynamics of folding for the enzyme ferredoxin from the bacterium *Bacillus subtilis*. Using differential scanning calorimetry, folding appears reversible and apparently two-state. You find the following parameters:

$$T_m = 326\ K$$

$$\Delta H^{\circ}_m = 83.83\ kcal \cdot mol^{-1}$$

$$\Delta C_p = 1.91\ kcal \cdot mol^{-1} \cdot K^{-1}$$

where ΔH°_m is the standard enthalpy change at $T = T_m$.

(a) Generate a plot of the standard free energy change for the unfolding reaction as a function of temperature, from 250 to 380 K. Label axes, including units.

(b) What is the temperature at which ferredoxin is most stable?

(c) Calculate the standard entropy change at T_m.

(d) Calculate the standard entropy change at 298 K.

(e) When ferredoxin unfolds, its intrinsic tryptophan fluorescence increases. You find that the thermally unfolded protein has a signal of 4.7 units, while the folded protein signal is 1.2 units. Plot the fluorescence signal you expect as a function of temperature (250 to 380 K).

i. Why does this plot have the shape it does?

ii. If the protein folded irreversibly, rather than reversibly, would the shape of the plot change? Please justify your answer.

(f) You make a mutation that decreases ΔH°_m to 23.83 $kcal \cdot mol^{-1}$ but does not affect T_m or ΔC_p.

i. Assuming the same signal for folded and unfolded protein as above, plot the expected fluorescence versus temperature.

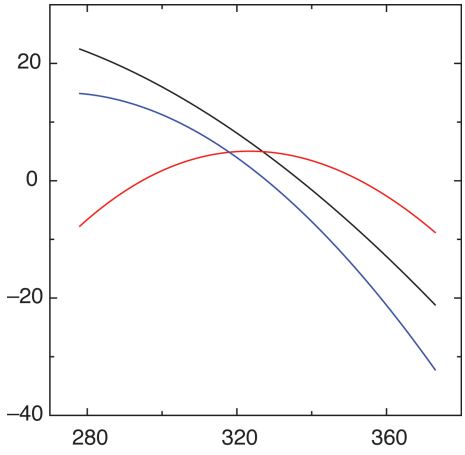
ii. Which of the following mutations would most likely lead to this drop in ΔH°_m? Please justify your answer.

A. Leucine in the hydrophobic core to an alanine

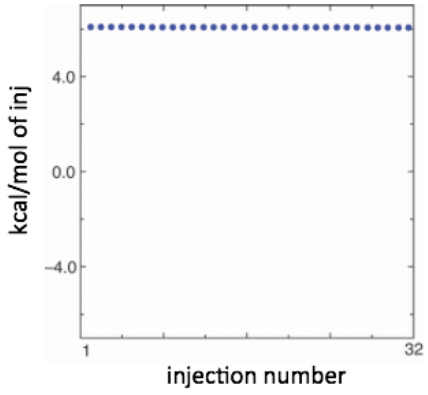
B. Lysine in a salt bridge to an aspartic acid.
C. Lysine with a decreased pK_a to an aspartic acid.
D. Leucine on the surface of the protein to an arginine.
E. Leucine on the surface of the protein to an alanine.

(g) *Thermotoga maritima* is a thermophilic bacterium that lives in hydrothermal vents, where water temperatures can be near 90 $°C$. You are studying how ferredoxin has evolved to be stable at such high temperatures. You create three mutant version of *Bacillus* ferredoxin in which amino acids have been changed to match those in *Thermotoga*. You then measure the stability of each mutant, plot and fit the data using the Gibbs-Hemholtz equation (see graph below).

i. Which of three mutants would be the most likely to be able to replace the wild type ferredoxin in Thermatoga? Please justify your answer.
ii. In which of the three mutants does the hydrophobic effect contribute the least to the overall stability of the protein? Please justify your answer.



8. Shown below is an ITC experiment you ran to measure the affinity of protein A to ligand, L. You performed 8 μL injections of a 1 mM solution of L into a 500 μM solution of protein A. In a separate experiment, you titrated 8 μL injections of 1 mM L into buffer and observe that there is no heat change.



(a) Based on your initial results, which of the following statements about the reaction between Protein A and L can you make?

i. Protein A binds to L very tightly.

ii. Protein A does not bind L at all.
iii. Binding of L to Protein A is an exothermic reaction.
iv. Binding of L to Protein A is an endothermic reaction.
v. Binding of L to Protein A is enthalpically driven.
vi. Binding of L to Protein A is entropically driven.

(b) It is not possible to determine the binding affinity or thermodynamic parameters of the binding reaction from the data to the right. Which of the following changes to the experimental setup would potentially improve the experiment so that these parameters can be determined? (Circle all that are correct and explain your answer)

i. Increase the concentration of Protein A.
ii. Increase the concentration of L.
iii. Decrease the concentration of Protein A.
iv. Increase the injection volume.

9. 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?