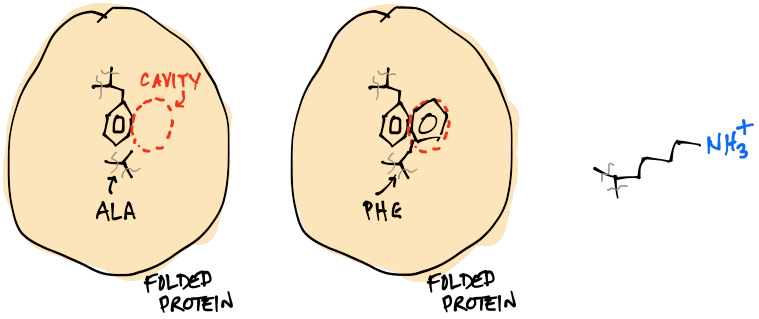
Instructions:

- 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.

2 Differential scanning calorimetry and protein folding

1. Consider a protein that, when folded, has a cavity in its core (denoted with the red circle on the left side of the diagram below). The cavity is not hydrated when the protein is folded. You mutate an alanine near the cavity to a phenylalanine. You then solve the crystal structure of the mutant protein and find that the cavity is perfectly filled by the bulky phenylalanine sidechain (rightmost protein below).

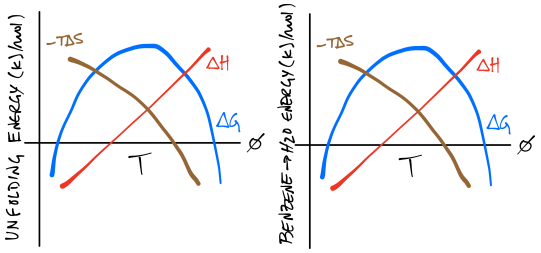


   (a) Do you predict that the *Ala* → *Phe* mutation stabilizes or detabilizes the native state of of the protein? Please justify your answer.

   (b) Predict the effect of the mutation on the *heat capacity change upon unfolding* (ΔC_p). Will it decrease, increase, or stay the same? Please justify your answer.

   (c) Make a prediction: what would the effect on protein stability and ΔC_p be if you substituted a lysine (shown on the right) instead of the phenylalanine? Please justify your answer.

2. The two graphs below show the temperature dependence of the unfolding free energy of a protein (left) and the transfer free energy of benezene into water (right). Why are these results interpreted as evidence that the hydrophobic effect dominates the energetics of protein folding?



3. 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:

$$T_m = 326\ K$$

$$\Delta H^\circ_m = 350.7\ kJ \cdot mol^{-1}$$

$$\Delta C_p = 7.99\ kJ \cdot mol^{-1} \cdot K^{-1}$$

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

   (a) Calculate the standard entropy change at T_m.

   (b) 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.

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

(d) You make a mutation that decreases ΔH_m° to 99.7 $kcal \cdot mol^{-1}$ but does not affect T_m or ΔC_p. Which of the following mutations would most likely lead to this drop in ΔH_m°? Please justify your answer.

i. Leucine in the hydrophobic core to an alanine
ii. Lysine in a salt bridge to an aspartic acid.
iii. Lysine in the hydrophobic core to an aspartic acid.
iv. Leucine on the surface of the protein to an arginine.
v. Leucine on the surface of the protein to an alanine.

(e) *Thermotoga maritima* is a thermophilic bacterium that lives in hydrothermal vents, where water temperatures can be near 90 $^\circ C$. 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). The curves are for wildtype (black), mutant 1 (red), mutant 2 (blue), and mutant 3 (green). The dashed vertical lines indicate the growth temperatures of *Bacillus* and *Thermotoga*, respectively.

i. Which mutant(s) 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.