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.

3 Protein structure

1. We're going to consider the structural and energetic effects of mutations to the protein lysozyme. To do so, we'll compare four crystal structures of lysozyme: 2BQA, 2BQC, 2BQM and 2BQI.

   (a) Which residues are within 3 Å of the residue I23 in the wildtype structure (2BQA)? This can be done using the PyMol command *sele byres resi 23 around 3*. This means "create a selection containing all residues that have at least one atom within 3 Å of one atom in residue 23."

   (b) The PDB file contains 2BQC contains the structure for lysozyme with the mutation I23V. Describe how the lysozyme structure responded to the mutation.

   (c) Repeat the analysis you did in steps (a) and (b) for mutations V74A (2BQM) and V121A (2BQI).

   (d) Based on your analysis, is protein structure sensitive or insensitive to sequence?

   (e) The I23V, V74A, and V121A mutations destabilize the protein (i.e. decrease $\Delta G^{\circ}_{unfold}$) by -1.72, -1.80, and $-7.28\ kJ \cdot mol^{-1}$, respectively. Using these structure, come up with hypotheses that explain their different effects on ΔG° of unfolding.

2. 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.

3. Using the information below, answer the following questions. Your arguments should include both thermodynamic and molecular/chemical reasoning. Make sure you state any assumptions you make as part of the analysis.

   (a) What is the average strength of a hydrogen bond in an alanine α helix?

   (b) What is the contribution of the hydrophobic effect to formation of the helix?

   Information for answering the questions:

   - The *md-snapshots* directory contain snapshots at 1.25 ns intervals from a molecular dynamics simulation of an Alanine dodecamer peptide. Three of the snapshots capture the peptide in the unfolded state; three capture the peptide in the unfolded state. All snapshots include water molecules within 5 Å of any atom in the peptide.

     Some analyses that might help you answer the questions above:

     – How many hydrogen bonds are formed between the peptide and water? What is their average length? To as this using PyMol click on the "A" button on the rightmost panel and do the following: $[A] \rightarrow find \rightarrow polar\ contacts \rightarrow involving\ solvent$

     – How many hydrogen bonds are formed between the peptide and itself? What is their average length? To ask this using PyMol, $[A] \rightarrow find \rightarrow polar\ contacts \rightarrow excluding\ solvent$

     – What is the change in the nonpolar and polar surface area between the folded and unfolded structures? To do so, go to $File \rightarrow Run\ script...$ and run "md-snapshots/calc_area.pml". Note that this script assumes you only have one structure loaded at once.

   - Extrapolating from work by Lopez and colleages (https://doi.org/10.1073/pnas.032665199), experimental estimates for the following thermodynamic parameters for helix formation at 298 K are:

- $\Delta H_{F \to U} = 45.2 \pm 0.5 \; kJ \cdot mol^{-1}$
- $\Delta C_{p,F \to U} = 0 \pm 0.1 \; kJ \cdot mol^{-1} \cdot K^{-1}$
- $\Delta G_{F \to U} = 50.2 \pm 0.5 \; kJ \cdot mol^{-1}$