Homework 2

1. In a sentence, how does the hydrophobic effect stabilize protein structures?
2. Why are the ionic interactions in macromolecules generally much weaker than ionic interactions in something like a salt crystal?
3. How does ion release lead to affinity between a positively charged protein and DNA?
4. How do unfulfilled interactions lead to binding specificity for proteins that bind DNA?
5. HIV protease is an enzyme required for HIV maturation. It recognizes specific sequences of amino acids and then cleaves peptide bonds, yielding the final protein components of a mature viron. It has also been an important HIV drug target. One early drug was Ritonavir. This is a peptide mimic that binds at the enzyme active site and thus blocks the ability of the enzyme to bind to peptide targets. Unfortunately, HIV has acquired resistance to this drug by acquiring mutations in the active site.
   (a) Load the structure of the protease-drug complex (1HXW) in PyMol.
   (b) What is the distance between Val-82 and the drug? Does Val-82 interact physically with the drug? If so, what sort of interaction is formed? Is it favorable?
   (c) One of the first mutations that occurred to HIV protease to evolve resistance to ritonavir was V82A. Introduce this mutation into the structure. What happened to the interaction you observed above?
   (d) Other mutations might conceivably have occurred besides V82A. Use the mutagenesis tool to introduce the V82W mutation. After you select TRP, but before you hit "apply," use your arrow keys to sample different possible conformations for tryptophan-82. Is this a feasible mutation for HIV protease to evolve? Why or why not?
6. Phosphorylation is a key method of protein regulation. A class of enzymes called *kinases* can pull a phosphate group (PO_4^-, often abbreviated to P) from ATP and link it to a specific amino acid on a specific protein. This change in the chemistry of a protein can, in turn, modulate its function. A general phosphorylation reaction is something like:

$$protein + ATP \rightleftharpoons protein - P + ADP$$

   (a) Write out an equation for the ΔG of this reaction as a function of the concentrations of all of the species.
   (b) ΔG° for this reaction is $-20\ kJ \cdot mol^{-1}$. If all species are at 1 M (at 300 K), what is the ΔG for the reaction? Which direction is favored?
   (c) Let's drop to more biologically reasonable concentrations. Given the table below, what is the ΔG to phosphorylate the protein?

species	concentration
$protein$	1 nM
$protein - P$	1 fM
ATP	3 mM
ADP	0.3 mM

(d) $[ATP]$ and $[ADP]$ are held roughly constant in normal cells. If $[ATP]$ and $[ADP]$ are held constant at the concentrations above, what is the ratio of $\frac{protein-P}{protein}$ at equilibrium (again, 300 K)?

7. Protein kinases must recognize a specific protein substrate that they will then phosphorylate.

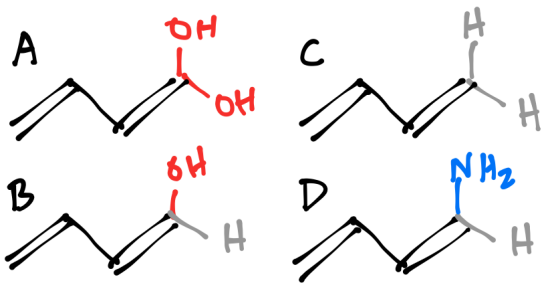
   (a) 1ATP is a structure of a kinase bound to its substrate. Look at the structure and determine which residues from the substrate are important for specific recognition. Use the nomenclature P_i where i is the position relative to the phosphorylated residue (e.g. P_{i-1} is the residue directly N-terminal to the phosphorylated residue, while P_{i+1} is the residue directly C-terminal to the phosphorylated residue).

   (b) The structure in 1ATP does not actually lead to lead to phosphorylated substrate. Why, structurally, might this be?

   (c) An inactive kinase is found in the 1IRK PDB file. Describe conformational differences between an active and inactive kinase that may be important for determining activity.

8. Calculate ΔG° for the protonation of the aspartic acid side chain for a solution with a total amino acid concentration of 1 mM at 25 $^\circ C$ and pH 7.

9. You have a protein that binds to ligands that look like those shown below. You find that A binds with the same K_D as B, but that both bind more tightly than C. You are interested in figuring out the molecular basis for this recognition, so you start changing amino acids in the binding pocket and re-measuring binding.



   (a) You change an Ala to a Leu in the binding pocket and find that it improves the binding of A.

      i. What part of the small molecule do you think this amino acid touches?

      ii. What effect would you predict for ligands B and C? Why?

   (b) You change a different site from a Ser to Thr, and find that it also improves the binding of A.

      i. What part of the small molecule do you think this amino acid touches?

      ii. What effect would you predict for ligands B and C? Why?

   (c) You identify a new ligand, D, and measure its binding. You find that it binds poorly. What sort of recognition "rule" do you think the protein is using to recognize A and B?

10. Now, we'll consider the contributions of the hydrophobic effect and hydrogen bonds to the binding free energy (ΔG_{bind}) for ligand B and ligand C. Use the information in the problem to populate the table below. Assume that $\Delta G_{Hbond} = -20\ kJ/mol$ and $T = 300\ K$.

	ligand B		ligand C	
	$\Delta G_{H2O\ release}$	ΔG_{Hbond}	$\Delta G_{H2O\ release}$	ΔG_{Hbond}
ligand				
pocket				

(a) First, consider hydrophobic effect:

i. Burying the hydrophobic portion of both B and C in the protein increases the entropy of the system by $0.06\ kJ/mol/K$. What is their contribution to ΔG_{bind}?

ii. The hydrophobic pocket on the protein that binds these ligands sequesters water molecules away from "bulk" solvent. Release of these waters on ligand binding increases entropy by $0.12\ kJ/mol/K$. What is their contribution to ΔG_{bind}?

(b) Next, consider the hydrogen bonds for ligand B:

i. B is able to form 2 hydrogen bonds to water when free in solution and 2 to the protein in the binding pocket. To a first approximation, what are the contributions of these hydrogen bonds to the ΔG of binding?

ii. The pocket contains polar groups that form 2 hydrogen bonds to water in the absence of B and 2 hydrogen bonds to B when it's bound. To a first approximation, what are the contributions of these hydrogen bonds to the ΔG of ligand binding?

(c) Next, consider the hydrogen bonds for ligand C:

i. C is able to form 0 hydrogen bonds to water when free in solution and 0 to the protein in the binding pocket. To a first approximation, what are the contributions of these hydrogen bonds to the ΔG of binding?

ii. The pocket contains polar groups that form 2 hydrogen bonds to water in the absence of C and 0 hydrogen bonds to C when it's bound. To a first approximation, what are the contributions of these hydrogen bonds to the ΔG of ligand binding?

(d) Given the values in your table above, what is the free energy difference for binding B versus binding C to this protein?

(e) If the K_D for binding B is $0.4\ nM$, estimate the K_D for binding C?

(f) Why, given your calculation above, is the hydrophobic effect considered the driver for binding, while the polar contacts determine specificity?