Problem Set 6 Partial (**Due Oct 14th**)

1. Derive an expression that allows us to determine a dissociation constant for the protein/ligand equilibrium shown below.

$$P + L \rightleftharpoons PL$$

2. If myoglobin is 50% saturated at a pO_2 of 0.7 torr, calculate the pressure of oxygen needed to reach a fractional saturation of
   a. 25%
   b. 90%

3. In Chimera, superimpose the structures of oxy (2Z6S) and apo (1MBW) myoglobin (do this through the matchmaker tool under Structural Comparison). What differences do you observe? Determine the change in the position of the proximal histidine (report in angstroms). How does this compare to the ~0.6 A for hemoglobin? Is the O_2 H-bonded to the distal histidine? What are the H-bond distances? Do the same comparison for apo and carbonmonoxy (1JW8) myoglobin. Answer all of the above questions. Additionally, comment on how CO binds to heme and how this differs from O_2 binding.

4. In problem 3, you should have noted that CO binds to the protoporphryn IX in myoglobin through the Carbon, not the Oxygen atom. There are two good reasons for this. What are they?

5. Describe why cyanide ion can inhibit O_2 binding to hemoglobin and myoglobin. Make sure to include atomic orbital hybridization in your answer.

6. From our discussion in class, K_D takes on a unit of concentration. Use a simple equilibrium expression to confirm this. Why is this more useful to biochemists than association constants?

7. Please summarize the main differences between the KNF and MWC models of allostery.

8. Give the following data for O_2 binding to an O_2-binding protein isolated from *E. coli*, please approximate the K_D for O_2 binding directly from a graph. Determine the exact value of K_D using the Solver function of Excel. There is a tutorial video on the course homepage.

$[O_2]$, nM	Fractional Saturation
0.5	0.0093
1	0.0185
2	0.0364
4	0.0702
8	0.1311
16	0.2319
32	0.3765
64	0.5470
128	0.7072
256	0.8285
512	0.9062
1024	0.9508

9. Briefly summarize how O_2 binding is allosterically communicated to other subunits of hemoglobin.

10. CO_2, pH and BPG all influence the affinity of hemoglobin for oxygen.
    a. Can these be considered allosteric modifiers?
    b. Clearly discuss what role each play.

11. Cysteine proteases catalyze the hydrolysis of peptide bonds. These enzymes are quite similar to serine proteases; the main difference is that the nucleophile is a cysteine instead of a serine. One of the critical steps in Tobacco Etch Virus infection is harnessing the host machinery to express one of these enzymes. Please access pdbID 1LVB and address the following questions. It may be beneficial to refer back to the non-mutated structure (1LVM) for guidance. Please submit at least one image that supports your answers to c and d.
    a. This structure is a mutated protein (C151A) that abolishes catalytic activity. Why is this a beneficial mutation?
    b. Use Chimera to mutate the alanine back to a cysteine. Select the rotamer that makes the most sense when considering the function of the protein.
    c. Does this enzyme contain a catalytic triad like serine proteases? If so, what amino acids (with numbers) are involved?
    d. The recognition sequence for this enzyme is ENLYFQS. Using your structure (with the cysteine present), predict where this enzyme will cleave the peptide
    e. Based on this structure, do you think that any other amino acid could replace the Phe? The binding pocket looks perfectly suited for Phe (spacefill blue) – not very likely that another amino acid could replace it.
    f. Propose a mechanism for this enzyme.

12. Can you recognize the type/class of enzymes?