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

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

$$P + L \rightleftharpoons PL$$

$$Y = \frac{[PL]}{[P]+[PL]} \qquad K = \frac{[PL]}{[P][L]} \qquad [PL] = K[P][L]$$

$$Y = \frac{K[P][L]}{[P]+K[P][L]} = \frac{K[P][L]}{[P](1+K[L])} = \frac{K[L] \div K}{1+K[L] \div K}$$

$$Y = \frac{[L]}{\frac{1}{K}+[L]} = \frac{[L]}{K_D+[L]} = Y$$

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%

$$Y = \frac{[L]}{K_D+[L]} \qquad [L]Y + K_D Y = [L]$$

$$K_D Y = [L] - [L]Y = [L](1-Y)$$

$$[L] = \frac{K_D Y}{1-Y}$$

a) $[L] = \frac{0.7(0.25)}{1-0.25} = 0.233$ torr

b) $[L] = \frac{0.7(0.9)}{1-0.9} = 6.3$ torr

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? The structures are, in general, very similar. The biggest difference is in the O_2 binding region – the distal His is modestly moved and the proximal His is pulled closer to the heme in the O_2 bound form. Determine the change in the position of the proximal histidine (report in angstroms) (0.363 angstroms). How does this compare to the ~0.6 A for hemoglobin? It's shorter, but this is not an allosteric protein and the change is completely based on the O_2 binding energy. In Hemoglobin, there are additional protomer contacts that can stabilize a larger distortion. Is the O_2 H-bonded to the distal histidine? Yes) What are the H-bond distances? 2.65 and 2.76 angstroms Do the same comparison for apo and carbonmonoxy (1JW8) myoglobin. Answer all of the above questions. (0.44 Angstroms for the His distortion. And H-bond is 2.81 angstroms. Everything else is essentially the same Additionally, comment on how CO binds to heme and how this differs from O_2 binding. Head on because of hybridization.

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? Carbon has a formal charge of -1 so it is more likely to coordinate to a cation. The oxygen is H-bonded to the imidazole Nitrogen of distal His. This would not be possible if the Carbon were oriented in that direction because carbon can't H-bond.

5. Describe why cyanide ion can inhibit O_2 binding to hemoglobin and myoglobin. Make sure to include atomic orbital hybridization in your answer. CN-, like CO, coordinates in an end on fashion. The carbon directly coordinates to the heme Fe because it carries the negative charge and the N H-bonds with the distal Histidine. The sp hybridization allows the head on binding.

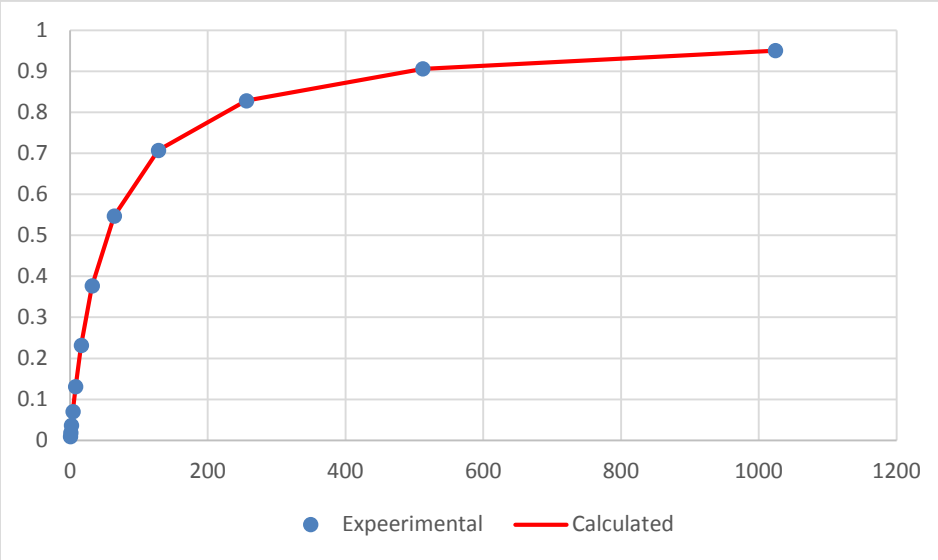
6. From our discussion in class, K_D takes on a unit of concentration. Use a simple equilibrium expression to confirm this. Why is this more useful to biochemists than association constants? Because it has the units of concentration so we can quickly assess the concentration needed to ensure substrate binding.

$K_D = \frac{[P][L]}{[PL]} \rightarrow \frac{M \cdot M}{M} = M$

7. Please summarize the main differences between the KNF and MWC models of allostery. MWC models requires that symmetry is maintained – ALL protomers are in the same form (R or T). The KNF model allows asymmetry; neighboring protomers are induced into an altered conformational state, but not all protomers need to be the same.

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. Kd = 52.997 nM

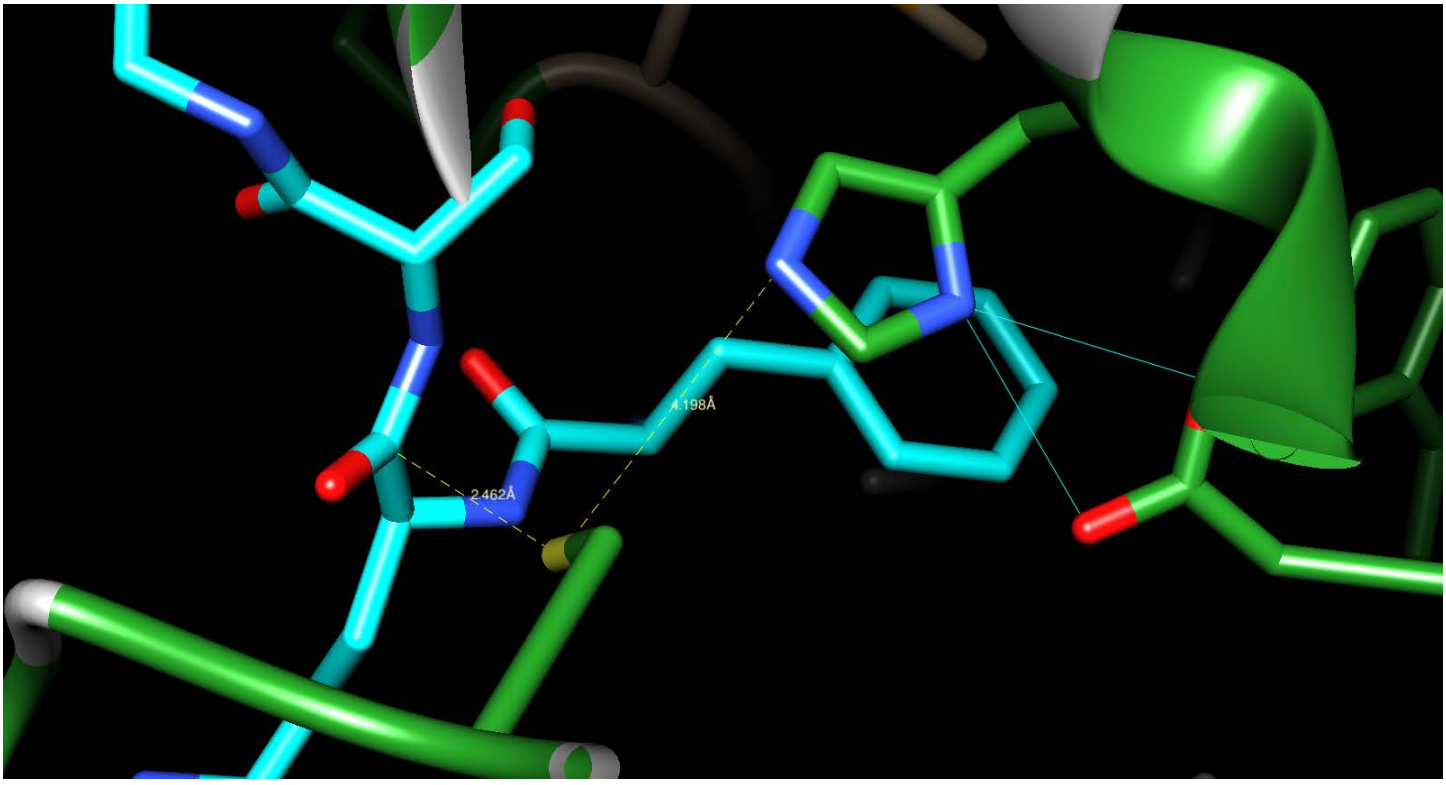
$[O_2]$, nM	Fractional Saturation
0.5	0.0093
1	0.0185
2	0.0364
4	0.0702
8	0.1311
16	0.2319
32	0.3765
64	0.5470
128	0.7072
256	0.8285
512	0.9062
1024	0.9508



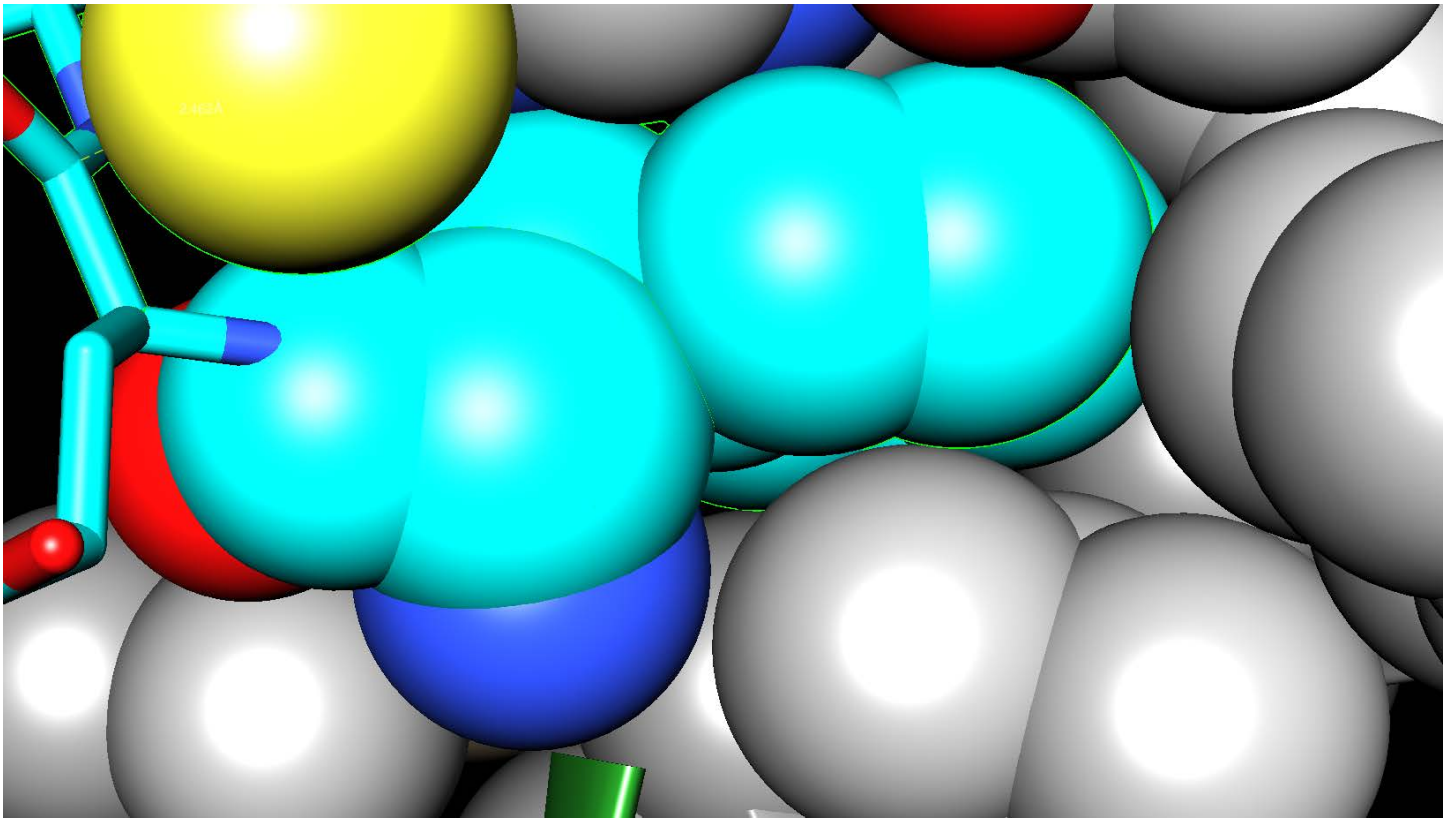
9. Briefly summarize how O_2 binding is allosterically communicated to other subunits of hemoglobin. Prior to O_2 binding, the Fe is positioned close to the proximal histidine. When O_2 binds, the Fe is pulled back into the plane of the heme, which pulls the proximal histidine with it. This causes a shift in the F-helix which propagates into a major

10. CO_2, pH and BPG all influence the affinity of hemoglobin for oxygen.
    a. Can these be considered allosteric modifiers? Yes. The influence the 'activity' (or O_2 binding) of hemoglobin by an interaction at a spatially distinct site.
    b. Clearly discuss what role each play. CO_2 and pH both affect the salt bridges formed at the N and C-termini. CO_2 reacts with the N terminus to make a carbabmate which promotes ion pairing in the T state. pH has the same affect; lowering the pH stabilizes the T state by protonating residues in the T state which favors ion pairing. BPG binds at the protomer interface and stabilizes the T state. In all cases, the T-state is stabilized and more O_2 is needed to shift to the R state.

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? It allows the structure to be solved with the substrate in the active site. An active form of the enzyme would end up hydrolyzing the peptide bond (as you see in the 1LVM structure) and the active site would be representative of the Enzyme-Product complex, not the active configuration.
    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? Asp81, His46, Cys151
    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.