Problem Set 7 (**Due Oct 23rd**)

1. Propose a mechanism for the hydrolysis of lactose that retains the stereochemistry at the anomeric carbon but does not transition through a half chair conformation. What enzyme catalyzes this reaction?

2. Biochemists often need to develop synthetic analogues of substrates to see how they interact with an enzyme.
    a. Why is this necessary?
    b. Propose a non-reactive synthetic analogue for the substrate of β galactosidase.
    c. Explain why your proposed analogue is catalytically 'dead'.

3. Propose a mechanism for the $FAD/FADH_2$ dependent conversion of benzene to phenol.

4. Decarboxylation of L-DOPA, produces the neurotransmitter dopamine. Investigate the structure of DOPA Decarboxylase complexed with the substrate analogue carbidopa (1JS3).
    a. What cofactor facilitates this reaction?
    b. Look closely at carbidopa. Why isn't this molecule an active stustrate for DOPA decarboxylase.
    c. Propose a mechanism for DOPA → dopamine.

5. What role does heavy isotope labelling serve in understanding catalytic mechanisms? Propose one example that is not related to hydrolysis of glycosidic bonds.

6. Please explain all pertinent assumptions used in the Michaelis-Menten (M-M) model of enzyme kinetics. How are these assumptions important for the steady state approximation?

7. What is K_M? How does it relate to Kd. Does the meaning of K_M change if the steady state assumptions are not valid? If so, how?

8. Mathematically verify that K_M is the substrate concentration at when $v_o = ½ V_{max}$.

9. What are the units for k_{cat} and catalytic efficiency? Show an enzyme catalyzed reaction that is described by each of these terms (e.g. $E + S \rightleftharpoons [ES]$ is Km).

10. In this exercise, you will use excel to determine the important kinetic values of K_m and V_{max}. Please do these two ways and show me the resulting scatter plot for the data and line plot for the fit.
    a. Use a Lineweaver-Burk plot.
    b. Use the Solver Function in Excel to extract Km and Vmax directly from v_o vs. [S]. If you have never used the Solver Function, please see me for guidance.
    c. Please comment on the two fitting methods. Are you getting the same numbers? Do all numbers make sense? Which is a more reliable method?
    d. Please also determine the turnover number, turnover rate (how long does it take for one turnover?) and catalytic efficiency. The total enzyme concentration in all reactions is 5 pM.

[S], μM	v_o, μM/s
1000	172.9553
500	160.9974
250	141.4395
125	113.7926

62.5	81.81009
31.25	52.37121
15.625	30.4539
7.8125	16.57808
3.90625	8.004
1.953125	5.432
0.976563	2.189
0.488281	0.6286

e. The data below was collected on the same enzyme in the presence of 150 μM of a molecule that is believe to inhibit the enzyme. Determine if this is an inhibitor. If it is, what type of inhibition and what is the K_I?

[S], μM	v_o, μM/s
0.488281	1.07142
0.976563	2.026631
1.953125	3.656645
3.90625	6.116312
7.8125	9.215873
15.625	12.34354
31.25	14.86616
62.5	16.55814
125	17.55727
250	18.10346
500	18.3895
1000	18.53594

11. From Table 12-1 in your book:
    a. Which enzyme has the highest affinity for the indicated substrate? How did you determine this?
    b. Which has the highest 2nd order rate constant?
    c. Which has the highest 1st order rate constant?
    d. Why are these values different?

12. Explain the differences between competitive, uncompetitive and mixed inhibition (make sure to comment on K_M and V_{max}).

13. Sketch Lineweaver-Burk plots for competitive an uncompetitive inhibition.