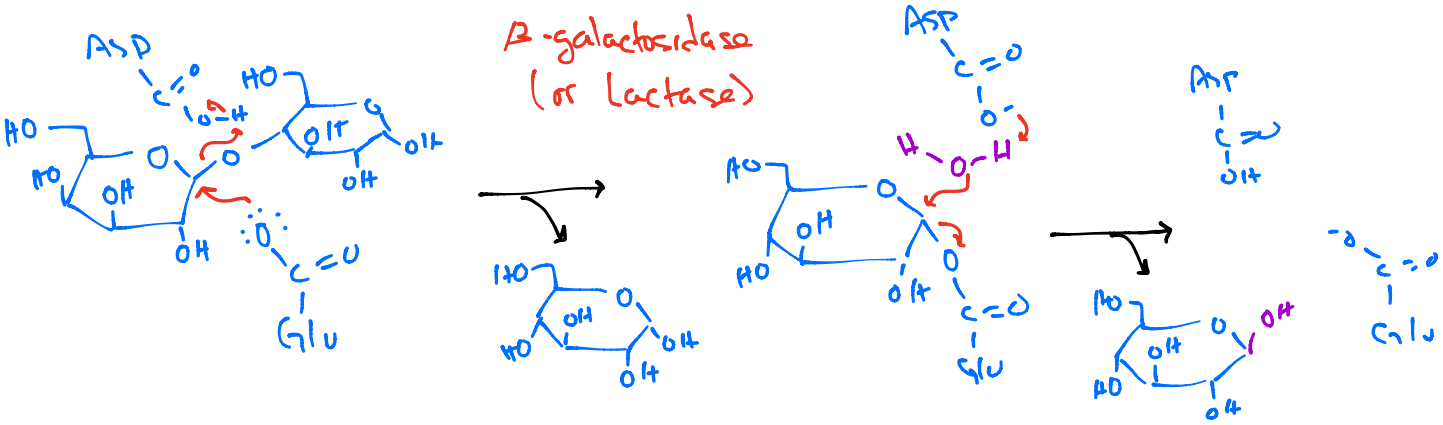
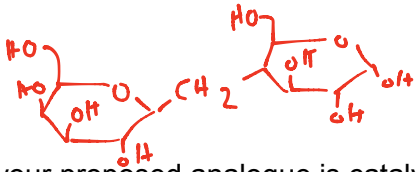
Problem Set 7 **(Due Oct 23rd)**

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?



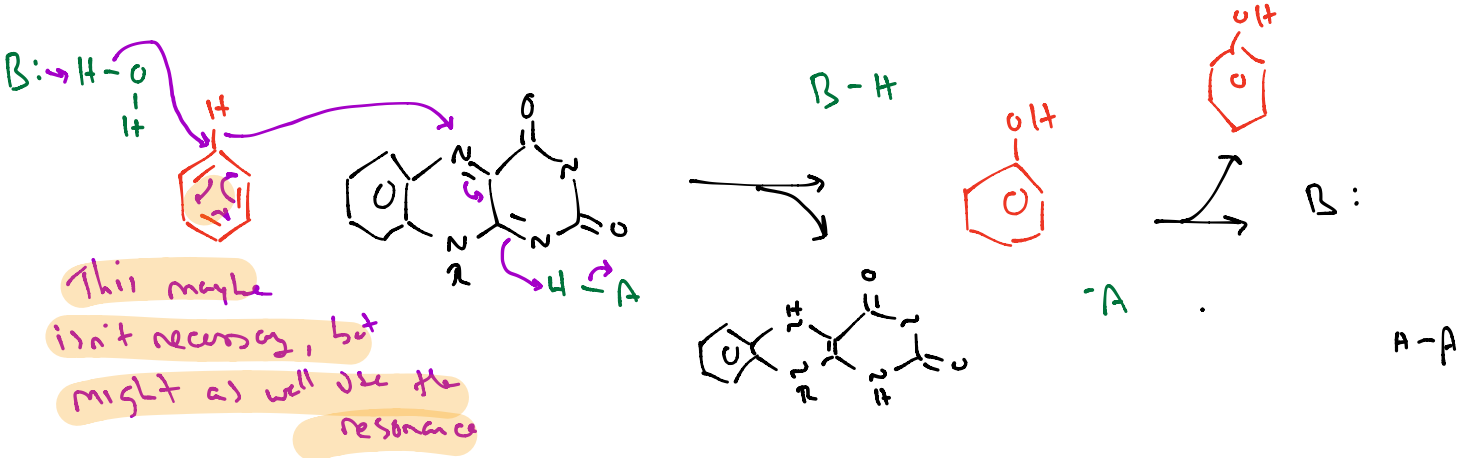
This is a simplified version of the lysozyme mechanism that we discussed in class

2. Biochemists often need to develop synthetic analogues of substrates to see how they interact with an enzyme.
   a. Why is this necessary? It allows us to see how the substrate binds to the active site with converting to products.
   b. Propose a non-reactive synthetic analogue for the substrate of β galactosidase.

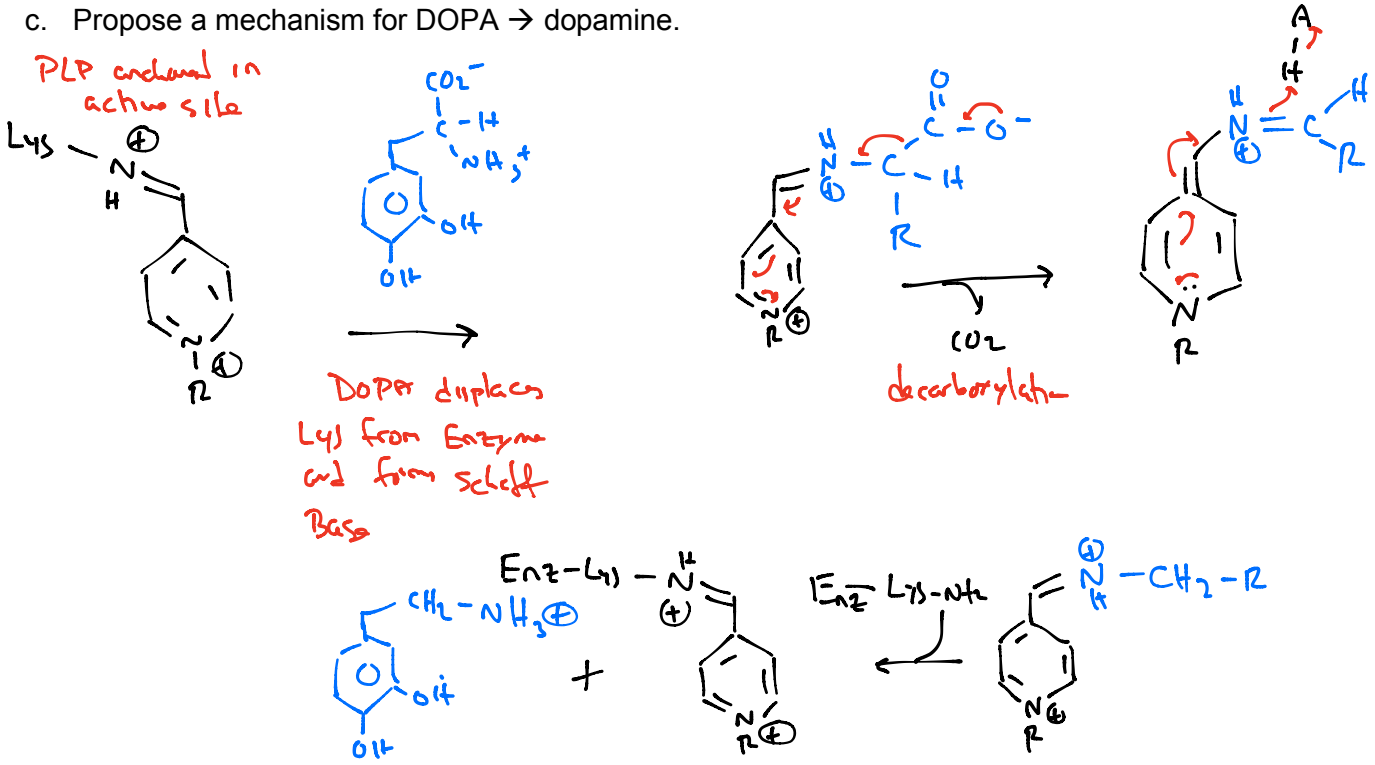


   c. Explain why your proposed analogue is catalytically 'dead'. This molecule looks nearly identical to lactose, so it should interact with the active site in a very similar way. However, replacing the linking oxygen with a less electronegative atom (carbon in this case), will dramatically decreased the electrophilicity of the anomeric carbon.

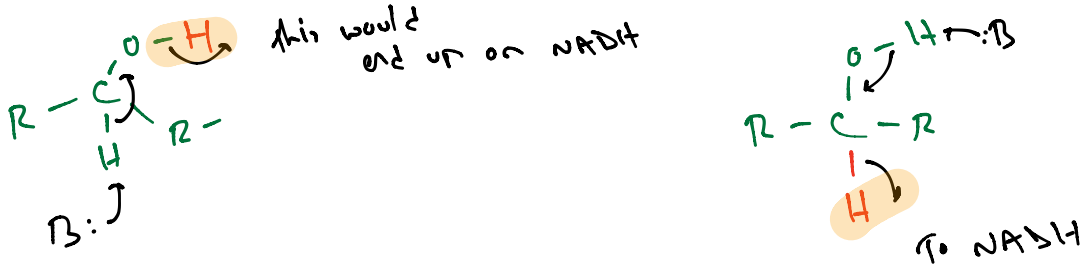
3. Propose a mechanism for the FAD/$FADH_2$ dependent conversion of benzene to phenol.



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? PLP
    b. Look closely at carbidopa. Why isn't this molecule an active stustrate for DOPA decarboxylase.Because is has an extra nitrogen between the Schiff base and the rest of PLP.
    c. Propose a mechanism for DOPA → dopamine.



5. What role does heavy isotope labelling serve in understanding catalytic mechanisms? It allows us to understand enzyme catalyzed reaction mechanisms. Propose one example that is not related to hydrolysis of glycosidic bonds. There are limitless possibilities that you could propose. One example: if you are looking at the NAD+ dependent oxidation of an alcohol to a ketone and you wanted to know if the hydride anion comes from the carbon or the oxygen, you could produce a ^{2}H labelled alcohol and see if it ends up in H_2O/H_3O+ or on the cofactor.



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?
    - The concentration of ES is constant
    - ES → E + P is irreversible
    - $k_2 << k_{-1}$
    - $V_{max} = k_2[E]_{total}$
    - $[E]_{total} = [ES] + [E]$ There are no other forms of the enzyme (e.g. EP or an intermediate EQ)

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? Yes, the meaning changes. K_M is derived assuming the steady state model. If [ES] is not constant, the ratio of rate constants cannot be easily determined.

$K_D = \frac{k_{-1}}{k_1}$ $\quad K_M = \frac{k_{-1} + k_2}{k_1} \simeq \frac{k_{-1}}{k_1}$ IF $k_{-1} >> k_2$

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

$V_0 = \frac{V_{max}[S]}{K_m + [S]}$ $\quad \frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]}$ $\quad \frac{1}{2} = \frac{[S]}{K_m + [S]}$ $\quad K_M + [S] = 2[S]$

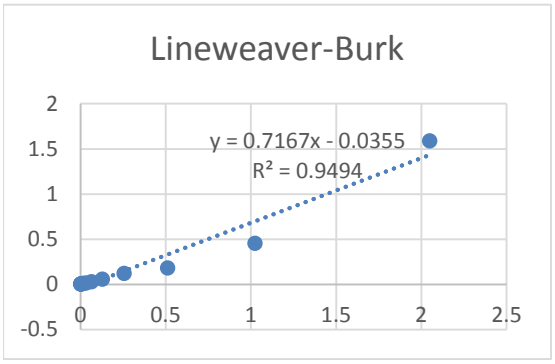
$V_0 = \frac{V_{max}}{2}$ $\quad K_m = [S]$

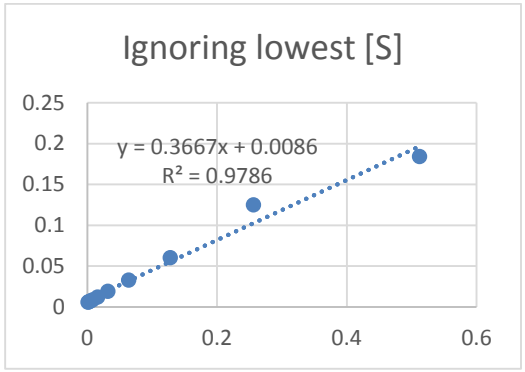
9. What are the units for k_{cat} and catalytic efficiency? Show an enzyme catalyzed reaction that is described by each of these terms (e.g. E + S ⇌ [ES] is Km).

$k_{cat} = \frac{1}{s}$ $\quad ES \xrightarrow{k_2} E + P$

cat. eff $= \frac{k_{cat}}{K_m} = M^{-1}s^{-1}$ $\quad E + S \rightarrow E + P$

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.



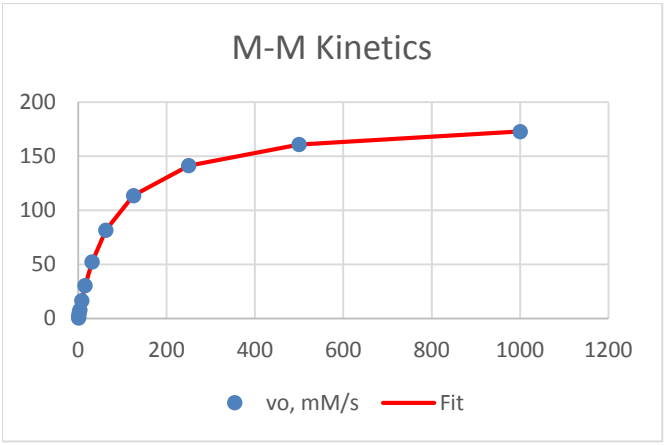


Note the issue here – the y-int is negative. This is because of the huge error in the first couple of points. SOOO - if you ignore the first couple, you get the 2nd graph. In this case, K_M = 42.6 μM V_{max} = 116.3 μM s^{-1} These values are still pretty wrong, but at least they are closer. The point I'm trying to demonstrate here is that using the double reciprocal approach has a lot of problems that can come from the V_0 measured at the smallest concentrations.

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? No. there is error in the Lineweaver-Burk plot

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.
5pM = $5x10^{-6}$ μM Turnover = k_{cat} = $V_{max}/[E]_{Tot}$ = $3.736x10^{7}$ s^{-1}
Catalytic efficieny = k_{cat}/K_m = 4.65 x 10^5 $M^{-1}s^{-1}$

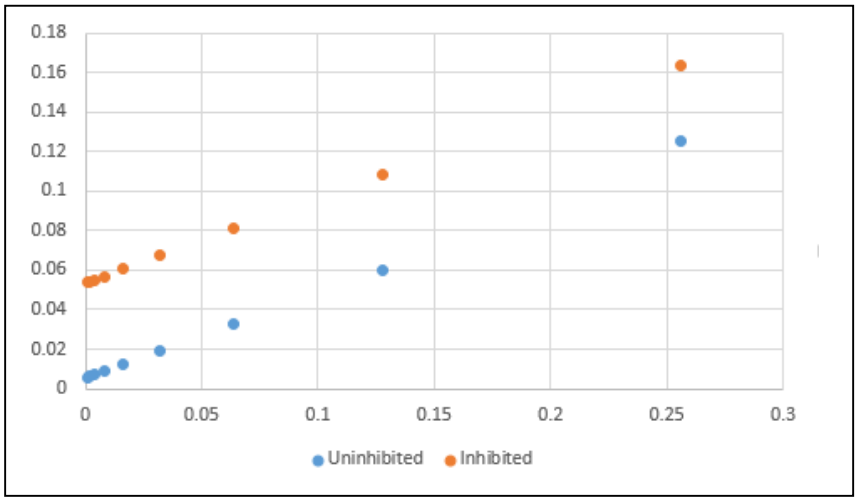
[S], μM	v_o, μM/s
1000	172.9553
500	160.9974
250	141.4395
125	113.7926
62.5	81.81009
31.25	52.37121
15.625	30.4539
7.8125	16.57808
3.90625	8.004
1.953125	5.432
0.976563	2.189
0.488281	0.6286



K_M = 80.3 μM V_{max} = 186.8 μM s^{-1}

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? This is an uncompetitive inhibitor (parallel lines)

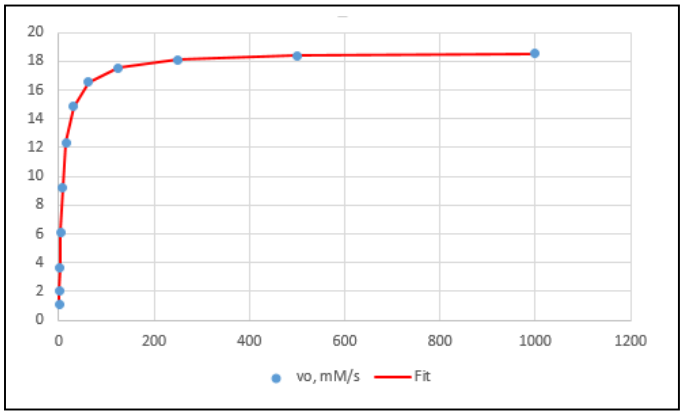
[S], μM	v_o, μM/s
0.488281	1.07142
0.976563	2.026631
1.953125	3.656645
3.90625	6.116312
7.8125	9.215873
15.625	12.34354
31.25	14.86616
62.5	16.55814
125	17.55727
250	18.10346
500	18.3895
1000	18.53594



When fitting this data, $K_{m,app}$ = 8.03 μM and $V_{max,app}$ = 18.68 μM s^{-1}. Table 12.2 in your book shows the relationship between Km and $K_{m,app}$ for uncompetitive inhibition. $K_{m,app} = \frac{K_m}{a'} = \frac{K_m}{\left(1+{}^{[I]}/_{K_I}\right)}$

$$8.03\left(1+{}^{150\mu M}/_{K_I}\right) = 80.3$$

K_I = 16.7 μM



You can arrive at the same number if you use the V_{max} and $V_{max,app}$

11. From Table 12-1 in your book:
    a. Which enzyme has the highest affinity for the indicated substrate? Fumarase How did you determine this? It has the lowest K_M.
    b. Which has the highest 2nd order rate constant? k_{cat}/K_M Catalase
    c. Which has the highest 1st order rate constant? k_{cat} Catalase
    d. Why are these values different? The describe different processes. See problem 9.

12. Explain the differences between competitive, uncompetitive and mixed inhibition (make sure to comment on K_M and V_{max}). Competetive inhibitors bind to the active site and prevent the substrate from binding. This lowers the observed Km but does not change V_{max}. Uncompetetive inhibitors bind to an allosteric site and influence the observed Km and V_{max}. Mixed inhibitors (e.g. non-competetive inhibitors) change the apparent V_{max} and may (or may not) change Km.

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