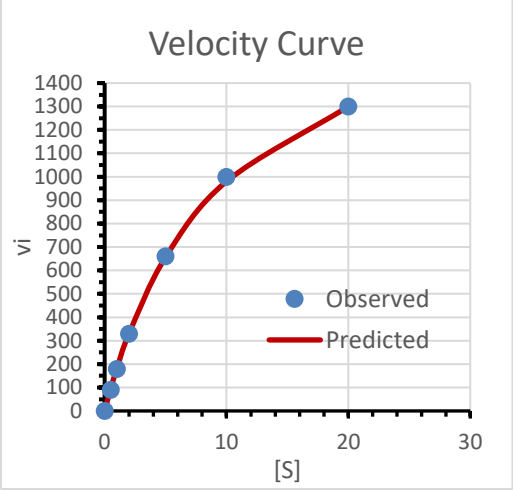
1. (16 pts, 20 min) You work for a pharmaceutical company that produces a key synthetic intermediate by enzymatic means. This process uses an enzyme called alcohol dehydrogenase. You have two sources of this enzyme, from yeast or from mushrooms. The kinetic parameters for the yeast enzyme are provided for you. The following values were obtained for the mushroom enzyme (right hand table). A spread sheet is provided that you can use to analyze this data

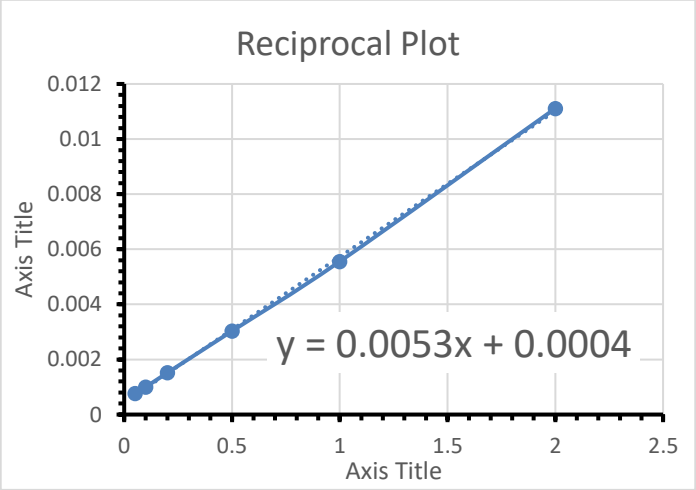
Mushroom		
Yeast	K_M = 0.1 mM	k_{cat} = 100 sec^{-1}

[S] (mM)	V (uM/s)
0	0
0.5	90
1	180
2	330
5	660
10	1000
20	1300

i) Obtain K_M and V_{MAX} values by each of the following approaches (6 pts):
   a) directly from the velocity curve,
   b) from a double reciprocal plot,
   c) Using Solver, using your answer to a) as the initial guess.

Include the velocity plot with the best fit line and double-reciprocal plot in your answer.





a) The estimated V_{MAX} is 1600 uM/s, the substrate concentration to give ½ V_{MAX} is ~ 7 mM.
b) V_{MAX} = 1/0.0004 = 2500 uM/s. K_M = slope x V_{MAX} = 0.0053 x 2500 = 13.25.
c) The best fit values are 9.7 mM for K_M and 1930 uM/sec for Vmax.

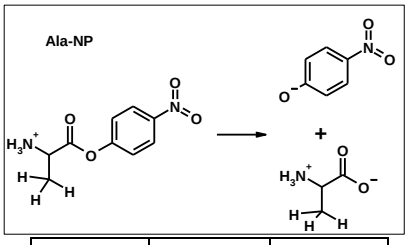
ii) Why are the K_M and V_{MAX} values obtained directly from the velocity curve unreliable (2 pts)?
The substrate was not saturating, so it was not possible to get an accurate Vmax or K_M

iii) What additional experiments could you have done to make them more reliable (2 pts)?
Take data at higher substrate.

iv) Assume that the mushroom enzyme concentration is was 2 uM, calculate k_{CAT} for the mushroom enzyme (2 pts).
$k_{CAT} = V_{max}/E_{total}$ = 1930 uM-s^{-1}/ 2 uM = 965 s^{-1}

v) Your supervisor tells you to use the yeast enzyme for the reaction, is this the correct choice assuming that the substrate concentration is 1 uM? ([S]<<K_M)? *Briefly* justify your answer (4 pts).
Yes, because at low substrate the overall rate is kCAT / KM. This is ~100 for the mushroom enzyme and 1000 for the enzyme from yeast.

2. (8 pts, 15 min) Steady-state enzyme kinetic data was collected using elastase, which is a serine protease. Three different substrates were used, Ala-NP, Leu-NP, and Phe-NP (reaction with Ala-NP is shown to the right). In all three substrates, the enzyme is recognizing the amino acid to the left of the ester and cleaving the ester. The released nitrophenol ion is bright yellow, allowing measurement of the rate of product formation. The K_M and k_{CAT} parameters for these three substrates are shown in the table on the right.



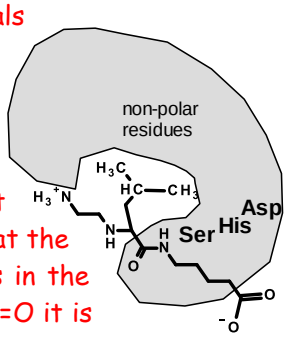
	K_M	k_{CAT}
Ala-NP	100 µM	10^4/sec
Leu-NP	10 µM	10^4/sec
Phe-NP	100 µM	10^2/sec

Explain the trend in both K_M and k_{CAT} for the different substrates (2 pts for each x 3 substrates). Illustrate your answer with a simple cartoon like drawing (similar to that shown, but with corrections), that illustrates the

interaction of elastase with peptide substrates (2 pts). Your diagram should include some information regarding the specificity pocket (e.g. large or small, non-polar or polar) and the three residues in the catalytic triad, in an appropriate location with respect to the bound substrate. You may find it helpful to visit the Jmol page on Elastase to gain an understanding of the interaction with the sidechain of each substrate with the elastase's specificity pocket (see problem set links).

K_M values indicate that leucine containing substrate bind best. Alanine with a smaller sidechain, and Phenylalanine with a larger substrate do not bind as well. Consequently, the specificity pocket in elastase is just the right size to bind Leucine (see Jmol). The methyl sidechain of alanine can't form as productive van der Waals interactions with the pocket. The larger phenylalanine must distort the pocket to fit, this distortion is energetically unfavorable (van der Waals repulsion), also increasing the K_M.

k_{CAT} values reflect the rate at which *bound* substrate is converted to product, [ES]→[EP]. Both Ala and Leu have the same k_{cat} values, so either substrate is hydrolyzed at the same rate on the enzyme, once the substrate is bound. The k_{cat} for Phe is smaller, meaning that the enzyme is less efficient with at converting this substrate to product. One possible explanation is that the distortion of the enzyme that occurred during binding has caused changes in the catalytic triad, i.e moving the serine a few 1/10ths of an Å away from the C=O it is attacking, would make the enzyme less efficient.

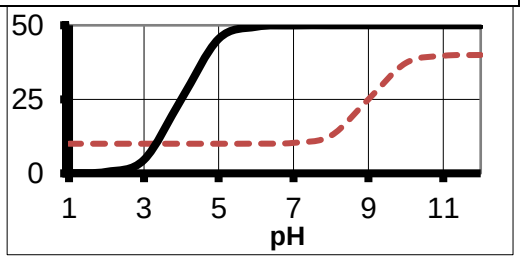


Elasatase +Leu substrate

3. (8 points, 15 min) In the previous problem set you viewed some mutations of trypsin. For each of the following, indicate:
   i) Whether the mutation would affect primarily K_M or k_{CAT} (6 pts).
   ii) For mutant Trp1, how might the mutation affect the specificity (2 pts).

	Residue Altered	Effect Vmax ?	Effect specificity ?	
Trp1	Asp189 to Ala	no	yes	Methionine (similar to Lys, but non-polar)
Trp2	Asp189 to Glu	no	yes	
Trp3	Asp189 to Lys	no	yes	
Trp4	Asp189 to Met	no	yes	
Trp5	Asp102 to Asn	yes	no	
Trp6	Ser195 to Gly	yes	no	

4. (6 pts, 10 min) You measure the K_M (dotted red line) and V_{MAX} (solid black line) for an enzyme as a function of pH and obtain the data shown in the graph on the right. What can you say about the active site, in terms of the residues involved in the mechanism and substrate binding?



Mechanism:

The highest Vmax is seen at pH values > 7. One half of the maximum value is seen at pH=4. Therefore, at pH=4 50% of the enzyme is inactive and 50% in active. The active form is deprotonated since Vmax is higher at higher pH values. The pKa value of 4.0 suggests a glutamic acid or an aspartic acid. The deprotonated acid could either stabilize a + charge (like serine proteases) or abstract a proton (like HIV protease).

Substrate binding: The Km is low at low pH values and increases at high pH values. The ½ way point is at pH=9, suggesting a Lysine residue is involved in substrate binding and that the + charge on the lysine is required for good binding (lower Km).