

Due Tuesday October 22, 2019

Estimated time: ~ 60 min

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

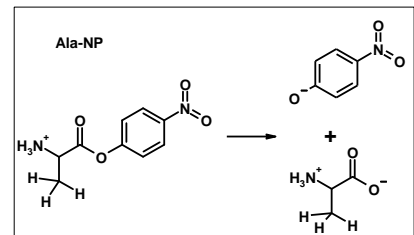
Mushroom		
Yeast	$K_M = 0.1 \text{ mM}$	$k_{cat} = 100 \text{ sec}^{-1}$
	[S] (mM)	V (uM/s)
	0	0
	0.5	90
	1	180
	2	330
	5	660
	10	1000
	20	1300

- 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.

- ii) Why are the K_M and V_{MAX} values obtained directly from the velocity curve unreliable (2 pts)?
 iii) What additional experiments could you have done to make them more reliable (2 pts)?
 iv) Assume that the mushroom enzyme concentration is was 2 uM, calculate k_{CAT} for the mushroom enzyme (2 pts).
 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? ($[S] \ll K_M$)? Briefly justify your answer (4 pts).

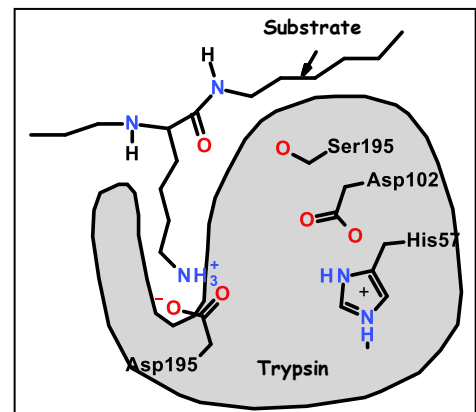
2. (8 pts, 15 min) Steady-state enzyme kinetic data was collected using elastase, which is a serine protease. Three different substrates were used, Ala-NP, Leu-NP, and Phe-NP (reaction with Ala-NP is shown to the right). In all three substrates, the enzyme is recognizing the amino acid to the left of 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	$10^4/\text{sec}$
Leu-NP	10 μM	$10^4/\text{sec}$
Phe-NP	100 μM	$10^2/\text{sec}$

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).



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
Trp1	Asp189 to Ala
Trp2	Asp189 to Glu
Trp3	Asp189 to Lys
Trp4	Asp189 to Met
Trp5	Asp102 to Asn
Trp6	Ser195 to Gly

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?

