Last Name (PRINT):

First Name:

Pg	Topic	Pts	Total possible
3	Multiple choice		12
4	Multiple choice		9
5	Multiple choice		12
6	Multiple choice, start T/F		16
7	T/F and Fill in Blank		22
8	Binding problems		12
9	Yakimima, LB equation, rate vs temp for enz		12
10	SS kinetics		7
11	cysteine protease		15
12	$\Delta\Delta G$, regulation and [S] to give ¾ Vmax		14
13	comp. inhibition and TS analog		14
14	S vs TS binding		5
total			150 points

Instructions: READ INSTRUCTIONS BEFORE BEGINNING EXAM.

1) Carefully read question before answering. Often I highlight very important information so please make note when I do so to make sure you are answering the question correctly.

2) Write your FULL name above and *at least your last name or initials* on ***every*** page.

3) Write all of your answers on the exam paper itself in the space provided. If you need additional space, you can write on the back of the SAME page. If you do this, you must write "ON BACK" so that we know where to look for your answer.

4) Your answers should be brief and legible. A correct answer that cannot be read cannot receive full credit. Additionally, extremely lengthy responses containing both correct and incorrect statements will be graded accordingly. Meaning, if you answer the question correctly but if you go on to write a "kitchen sink" response containing incorrect information, you will not receive full credit for that answer.

5) You must write your final answers in pen – no tests taken in pencil will be accepted for a regrade request

6) No use of calculators will be permitted on the exam, no exceptions.

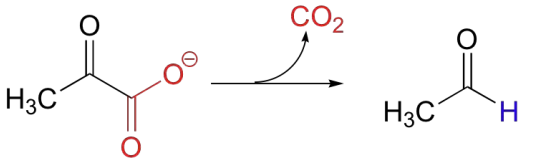
Number	Log	Ln
2	0.30	0.69
3	0.48	1.10
4	0.60	1.39
5	0.70	1.61
6	0.78	1.79
7	0.85	1.95
8	0.90	2.08
9	0.95	2.20
10	1	2.30

$R = 8.314\ J \bullet K^{-1} \bullet mol^{-1} = 1.987\ cal \bullet K^{-1} \bullet mol^{-1}$

Part I: Multiple choice –Circle the choice that *best* answers the question. **Do not** write the letter in the margin to indicate your answer, circle it. 3 points each.

1. Which of the following is the best description of an enzyme (Text, Ch11 ?1)
   a. they allow chemical reactions to proceed very quickly
   b. **they increase the rate at which a chemical reaction approaches equilibrium**
   c. they make a reaction thermodynamically favorable
   d. all of the above
   e. none of the above

2. Pyruvate decarboxylase catalyzes the reaction shown below. Based on this information, you can say this enzyme is a(n) (text, Ch 11, ? 3b)

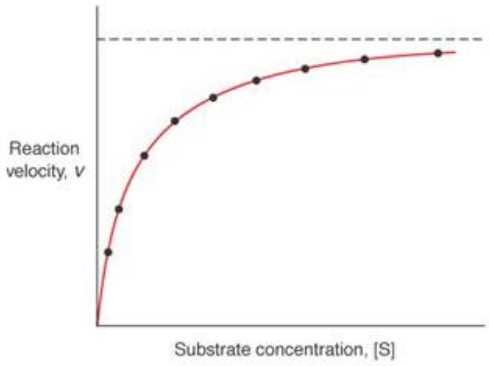


pyruvate acetaldehyde

   a. oxidoreductase
   b. transferase
   c. hydrolase
   d. lyase
   e. ligase

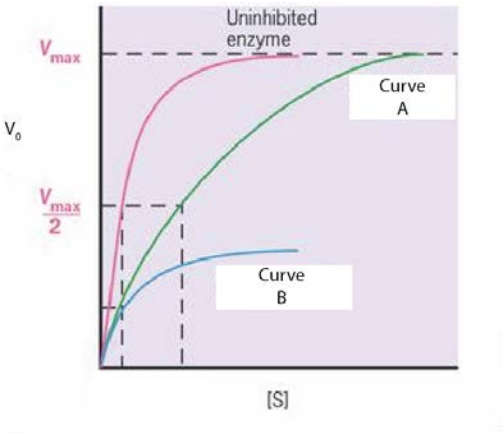
3. Which amino acid below is least likely to participate in general acid-base catalysis (*Companion Ch11, ? 10)*
   a. lysine
   b. cysteine
   c. histidine
   d. aspartate
   e. glycine

4. Which of the following statements is true about the region of the graph below *where* $[S] << K_M$?



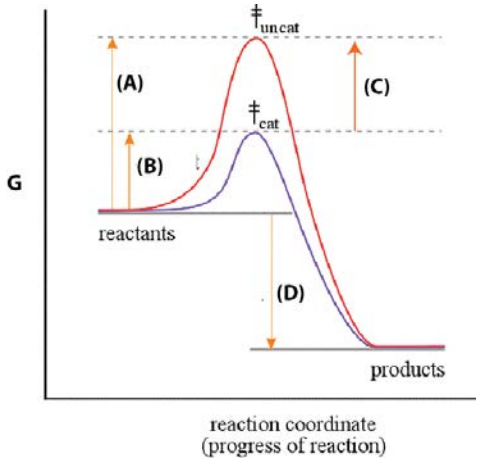
   a) For this part of the graph, [E]>>[S]
   b) When $[S] << K_M$, the initial rate is equal to the catalytic efficiency multiplied by the free enzyme and substrate concentrations.
   c) When $[S] << K_M$, rate is zeroth order with respect to S.
   d) This region (where $[S] << K_M$) allows for determination of the Km
   e) none of the above

5. The graph below represents rate vs substrate plots for an uninhibited enzyme and the same enzyme in the presence of two different inhibitors (A and B). What statements about this data is true?



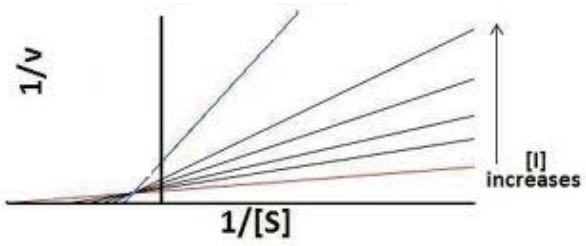
   a. Curve A represents a competitive inhibitor, and Curve B represents an uncompetitive inhibitor
   b. Curve A represents a uncompetitive inhibitor, and Curve B represents an noncompetitive inhibitor
   c. Curve A represents an irreversible inhibitor, and Curve B represents a competitive inhibitor
   d. Curve A represents a mixed inhibitor, and Curve B represents an uncompetitive inhibitor
   e. <u>Curve A represents a competitive inhibitor, and Curve B represents an mixed inhibitor</u>

6. On the diagram shown comparing the same reaction in the presence (cat) and absence (uncat) of a catalyst, which letter is drawn next to the arrow representing the difference in activation energy between the catalyzed and noncatalyzed reaction?



   a. A
   b. B
   c. <u>C</u>
   d. D
   e. none of the above

7. The plot to the right most likely represents



   a. a competitive inhibitor
   b. an uncompetitive inhibitor
   c. a noncompetitive inhibitor
   d. <u>a mixed type inhibitor</u>
   e. an irreversible inhibitor

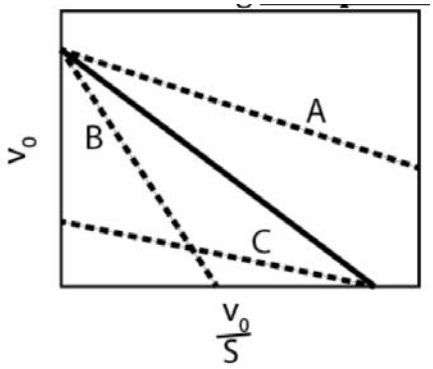
8. In class we discussed aspartate transcarbamylase which has six regulatory subunits which impart ***all*** of the allosteric regulation exhibited by the enzyme. Therefore, the rate of the reaction as a function of substrate concentration would be a _____ curve if we assayed *just* the catalytic subunits, whereas it would be a _____ curve if we assayed a preparation containing *both* the catalytic and regulatory subunits. (*companion ch12, ?15*)
   a) sigmoidal; hyperbolic
   b) hyperbolic; sigmoidal
   c) exponential; hyperbolic
   d) sigmoidal; bell shaped
   e) hyperbolic; concave

9. For serine proteases, the first step of the mechanism in which the serine side chain attacks the scissile peptide bond is *best described* as an example of
   a. general acid catalysis
   b. electrophilic catalysis
   c. electrostatic catalysis
   d. covalent catalysis
   e. transition state binding

10. k_{cat} is
   a) a measure of the catalytic efficiency of the enzyme
   b) ½ V_{max}
   c) the rate constant for the reaction ES → E + P
   d) the [S] that half saturates the enzyme
   e) A and C above

11. Lineweaver-Burk isn't the only way to linearize the Michaelis-Menten equation. For example, the Eadie-Hoffstee plot shown to the right can be fit with the equation

$$v = -K_m \frac{v}{[S]} + V_{\max}$$

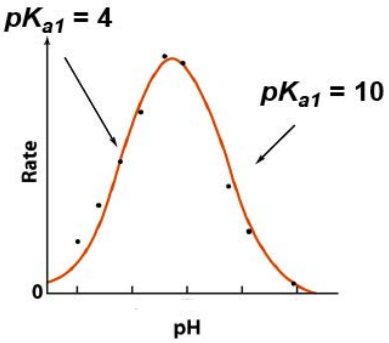


If the solid line in the plot shown represents the enzyme in the absence of the inhibitor, which line, A, B, or C, represents what you would observe for a competitive inhibitor?
   a. A
   b. B
   c. C
   d. it could be any of these depending on the K_I
   e. none of the above

12. Why is histidine unlikely to act as a general base in the catalytic mechanism of enzymes localized to the lysosome, an organelle where the pH is close to 4.5?
    a. His has a positive charge at pH 4.5
    b. His side chain has a pKa close to 6
    c. His would be in its acid form, not basic form at pH 4.5
    d. all of the above (a, b, and c)
    e. none of the above.

13. You are working at a company evaluating compounds with the potential to become new medications. Your evaluate several inhibitors of an enzyme you are studying and get the data below, which would be the most promising lead compound?
    a. $K_I = 4.7 \times 10^5$ M
    b. $K_I = 1.5 \times 10^8$ M
    c. $K_I = 1.5 \times 10^{-8}$ M
    d. $K_I = 4.7 \times 10^{-5}$ M
    e. $K_M = 4.7 \times 10^5$ M

14. Based on the plot shown (rate = k_{cat}), which of the following statements is ***NOT*** true?
    a. The optimal pH of this enzyme would be around 7
    b. The residue with a pKa of 4 is likely acting as a general base in catalysis
    c. At the optimal pH, the residue with a pKa of 10 is in its conjugate base form.
    d. The residue with a pKa of 4 could be a Asp or Glu residue
    e. all of the above are true statements



15. Which of the following side chains is *least* likely to function as a nucleophilic catalyst
    a. cysteine
    b. serine
    c. histidine
    d. alanine
    e. tyrosine

Part II: True False (2 pts each) Circle T or F to indicate if the statement is true or false.

16. **T** F The turnover number of an enzyme is calculated by dividing the maximal velocity by the enzyme concentration.

17. T **F** In a Lineweaver-burk plot, the Y-intercept is $-1/K_M$.

18. T **F** The reaction shown in question 6 will proceed spontaneously *only* in the presence of the catalyst.

19. T **F** If an enzyme has a hill constant that is less than zero we say it is a negatively cooperative enzyme.

20. **T** F The T state of hemoglobin is most similar to the deoxy conformation.

21. T **F** Only the symmetry model of allosterism can be used describe a negatively cooperativite enzyme.

22. **T** F A Lineweaver-Burk plot for an uncompetitive inhibitor has parallel lines with the Y intercept increasing as the concentration of the inhibitor increases.

23. **T** F The IC_{50} of an inhibitor is the concentration of inhibitor that results in 50% inhibition.

24. T **F** Two proteases that are unrelated in their primary and secondary structure yet have very similar chemical mechanisms are good examples of divergent evolution.

25. **T** F Since K_M is an intrinsic property of an enzyme, its value does not depend on the enzyme concentration.

Part III: Fill in the blank. For the following sentences, fill in the blank with the word(s) that best complete the sentence. (2 pt each blank)

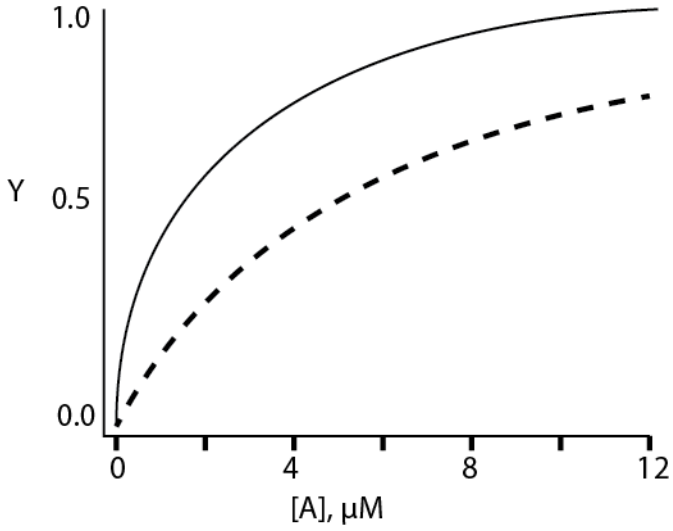
26. During a reaction, the reactants pass through a short lived high energy state that is structurally intermediate to the reactants and products called the transition state__.

27. The Michaelis-Menten equation is: $$v = \frac{V_{max}[S]}{K_M + [S]}$$ or $k_{cat}[E][S]$ / Km + [S]

28. An enzyme utilizing a __*ping pong*_ kinetic mechanism is one in which one or more products are released before all the substrates bind (*Essential concept Ch 12 # 13).*

Part V: Short answer

30. You are studying two enzymes X and Y that utilize the same substrate A and collect the following data.



a. Estimate the K_d of enzyme X (solid line). (3 points)

read to Y=0.5 and down to X axis
in this case somewhere in the range of 1.5-2.0 µM (I see it as ~1.7 µM)

b. Which enzyme X (solid line) or Y (dashed line) has a higher affinity for the substrate? (2 points)

X has higher affinity because it has the lower Kd

c. the Y axis of the plot is "Y", what is this? Answer this question by writing an equation for Y in terms of the protein (P), ligand (A) and/or P•A complex (PA) concentrations (3 points)

Y = *fractional saturation* = $\frac{[PA]}{[PA]+[P]}$

31. Use the Hill equation to estimate the fractional saturation of a new tetrameric oxygen carrier that was just identified that has an n = 2 and a p50 of 26 torr and a pO_2 of 50 torr. <u>You don't have to solve for the fractional saturation</u>, just set up the equation and plug the right numbers into the right places. (4 points)

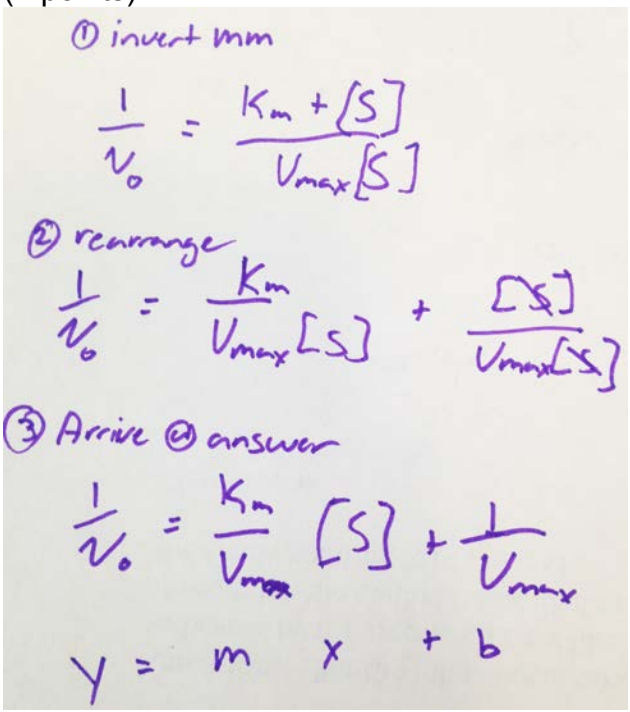
$Y_{O2} = \frac{(pO_2)^n}{(p50)^n+(pO_2)^2} = 50^2/\{26^2+50^2)$

you didn't have to multiply it out but it is ~79% saturated

32. Many mutant hemoglobins (hemoglobin variants) are known to exist. One called "Yakimima" harbors a Asp→His mutation in the beta chain. This mutation disrupts a hydrogen bond that stabilizes the T conformation. What effect, if any, would you predict this mutation would have on the p50 as compared to the wild type protein (normal hemoglobin without the mutation)? (4 points). Briefly justify your answer. (Related to *Companion Ch7 ?14c)*

If you had a mutation that removes a H-bond that stabilizes T conformation, then you might expect the p50 to go down (become more saturated at lower O_2 concentration) because it would be easier for the enzyme to be in the high affinity state or more of the deoxyhemoglobin could exist in the high affinity state

33. The Lineweaver burk is a linearization of the Michaelis Menten equation. In the space below, derive the lineweaver burk relationship starting from Michaelis Menten. (4 points)



34. Explain why if you measured the rate of an enzyme catalyzed reaction at different temperatures and plotted your results, you would get a bell shaped curve where the rate increases with temperature up to a point then rapidly falls off. (*text ch 11? 11).* (4 points)

as the temperature increases, the thermal energy boosts the portion of reactants that can achieve the transition state. Or mathematically (see box 12-2 in text)

$$k = \frac{k_B T}{h} e^{-\Delta G‡/RT}$$

For an enzyme catalyst, the rate will increase with temperature to a point then at higher temps, the enzyme becomes denatured and unfolds.

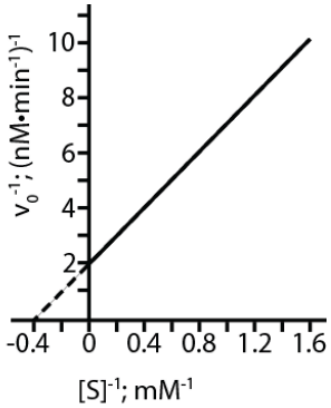
35. You are a graduate student working in the Perlstein Lab and you collect the following set of data for the enzyme that you are studying:

[S]	v_o
0.12	0.17
0.10	0.15
0.08	0.13
0.07	0.11

During your weekly meeting with me, I tell you that you have to go back and redo your kinetic assays but change a key crucial parameter to obtain the k_{cat} and K_M for your enzyme, what do I tell you to change and why? 3 points (text Ch12 ?12)

You can see from this data that you are no where close to saturating the enzyme (all points are below K_M) and so you cannot determine the V_{max} (and therefore kcat) or K_m from this data. You would have to go back and collect rate vs S data where you have [S] on either side of the K_m, typically 0.5-5 K_M is a good range

36. Using the data shown, calculate the Vmax and K_M for this enzyme (4 points)

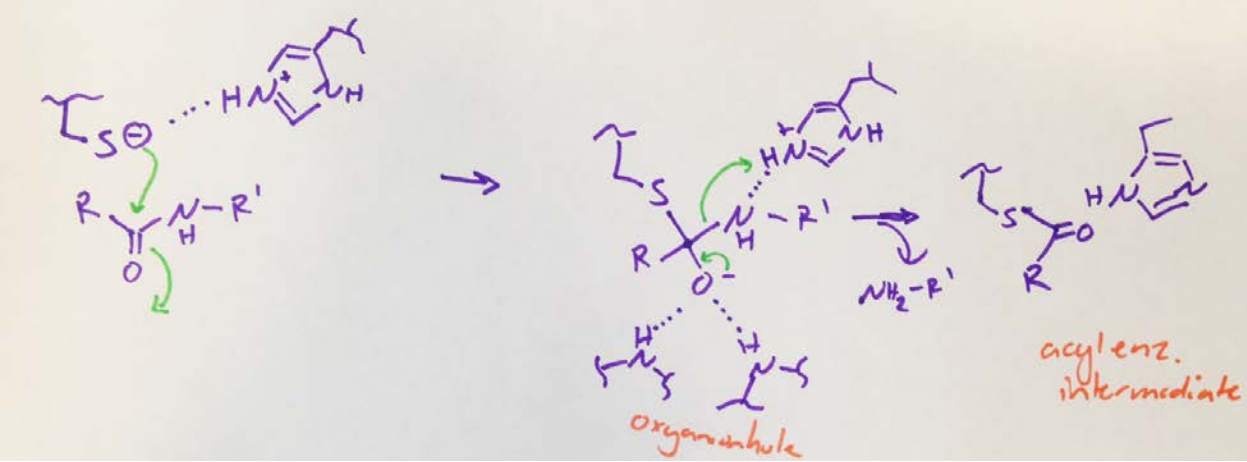


Y intercept is 1/Vmax = ½ = 0.5 nM/min

X intercept is -1/Km = 1/0.4 = 2.5 mM

37. In class we learned about serine proteases, which use a serine nucleophile to cleave a peptide bond. There are also cysteine proteases that utilize a thiolate nucleophile that is activated by its proximity to a histidine residue. Therefore, these enzymes have a catalytic diad, not triad like the serine proteases .

a. propose a mechanism by which cysteine proteases work. Draw your proposed mechanism through formation of the acyl-enzyme intermediate (STOP at this point, you do not need to draw hydrolysis of the intermediate to reform the free enzyme). Please also include in your drawing the tetrahedral intermediate being stabilized by an oxyanion hole as we saw with serine proteases. 9 points



b. What would you observe if you monitored the k_{cat} for the cysteine protease as a function of pH between pHs of 3 and 12? For each inflection point in your graph, indicate what ionization is giving rise to that inflection point. In other words, hypothesize what the pH rate profile would look like and then specifically tell me why you think it looks like that. 6 points

Should be a bell shaped curve with an acidic arm attributed to pKa of cys (needs to be deprotonated for enzyme to be active) and the basic arm with a pKa of His (needs to be protonated to be + so it can activate Cys).

*If instead you drew the His deprotonating the Cys in the first step concerted with the S- attacking the peptide bond, then this looks just like serine protease mechanism. therefore, you would expect the kcat vs pH plot to look exactly like a serine protease, with **one** titratable group, the His where it would be inactive at low pH and active at high. If the S gets deprotonated at high pH, then this should be fine because the thiolate is the active form.*

38. What concentration of substrate (in terms of the K_M value) is required to achieve ¾ of the maximal velocity. Show your work for credit. (4 points)

v_0/V_{max} = [S]/{Km+[S]}

.75{Km+[S]} = [S]
0.75Km = .25[S]
3Km = [S] @ which enzyme will run at 75% of its maximal velocity

39. Name the three distinct mechanisms used inside the cell to regulate enzyme activity (6 points).

1. control of [enzyme] via synthesis or degradation
2. covalent modification
3. allosteric regulation (could also say feedback inhibition or similar but cannot get credit for both allosteric regulation and feedback since these are not really two distinct mechanisms, just a more specific form of the other).

40. Calculate the $\Delta\Delta G^{\ddagger}$ of an enzyme catalyzed reaction at 25˚C if the enzyme catalyzed reaction proceeds with a k_{cat} of 600 s^{-1} and the k_{non} (the rate constant for the nonenzyme catalyzed reaction) is 6 x10^{-4} s^{-1}. You <u>do not</u> need to solve the equation, just set it up and plug in the right numbers in the right places so you could easily solve it if you had a calculator. 4 points *(similar to companion ch11, ?9b and the problem worked in class).*

$k_{cat}/k_{non} = e^{\Delta\Delta G/RT}$

kcat = 600 s^{-1}
knon = 6x10^{-4} s^{-1}
R = 8.314J $K^{-1}mol^{-1}$
T = 298 K

could have gone in to plug it in and begin to solve but didn't have to
ln(10^6) = $\Delta\Delta G^{\ddagger}$/(8.314•298)

<u>ln(10^6)</u> = $\Delta\Delta G^{\ddagger}$ in J mol^{-1}
2,477

41. Researchers have identified an enzyme that converts SLEEPY to PEPPY. They call it Sleepase (SPase).

a. When the monomeric [SPase] = 10 nM, the V_{max} is 100 μM s^{-1}, what is the k_{cat}? (show your work for credit) 4 points

$k_{cat} = V_{max}/E_T = 100\ \mu M\ s^{-1}/0.01\ \mu M = 1x10^4\ s^{-1}$

b. MIDTERM is a competitive inhibitor of SPase. In the presence of 1 μM MIDTERM the apparent K_M for SLEEPY is 10 μM whereas the K_M is 5.0 μM in the absence of MIDTERM., what is the K_I for MIDTERM? 4 points

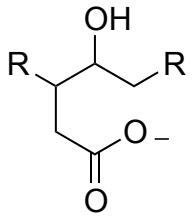
$K_M^{APP} = \{1+[I]/K_I\}K_M$

10 μM = {1+1μM/K_I}5 μM
2= 1 + 1/K_I
1= 1/K_I
K_I = 1 μM

c. Given in (b) above that the K_M for SLEEPY appears to increase 2-fold in the presence of 1μM of the competitive inhibitor MIDTERM, what effect would be observed on the apparent V_{max}? It would: (circle one): (2 points)

Increase ***remain the same*** Decrease not enough info to answer

42. You discover a new serine protease that is a paralog of trypsin, chymotrypsin and elastase except it has a specificity that allows it to cleave after negatively charged amino acids. Design a stable transition state analog that would be a competitive inhibitor of your newly discovered enzyme. (4 points)



this could have been almost anything but it had to have
1) something that was negatively charged and has right geometry to fit in specificity pocket (like a D or E sidechain but it didn't have to be as long as reasonable)
2) had to be tetrahedral (since the intermediate/TS is tetrahedral)
3) be something that was stable – often the N of the scissile bond is replaced with methylene or similar substitution at this position.
4) it had to be something that would reversibly interact with the enzyme, covalent modifiers would not be a competitive inhibitor

43. You are studying a collection of orthologous enzymes (Enz 1, Enz 2, and Enz 3) all capable of converting A → P. Your spectrophotometer is broken and the company can't come service it for the next two weeks but you are dying to know which one is the *BEST* catalyst. Although you cannot assay your enzymes, you do have a method to measure the K_d for substrate A and you have access to I, a stable analog that approximates the geometry and charge distribution of the transition state for the reaction you are studying (A→P). You can also measure the K_d for this transition state analog.

Enzyme	K_d for A (nM)	K_d for I (nM)
Enz 1	500	5
Enz 2	50	50
Enz 3	5.0	500

Based on this information, which one is likely the *best* enzyme (the one capable of accelerating the rate of its reaction the most vs the rate of the non enzyme catalyzed reaction). Briefly justify your answer. if you want to draw something to help you explain, please feel free to do so. (5 points)

the best enzyme catalysts preferentially bind the TS relative to the substrate ground state. Therefore, Enz 1 would be your best choice since it selectively binds/stabilizes the transition state by having a lower Kd for TS analog vs the substrate. This serves to decrease the activation energy by selectively lowering the energy of the TS and therefore the reaction goes faster because the rate constant is inversely related to the size of the activation energy barrier.

Enzyme 2 binds substrate and TS with roughly equal affinity => therefore not likely a great catalyst.

Enzyme 3 binds substrate tightly and TS relatively weakly, => therefore it will bind substrate but not likely activate it for transformation to P since it does not preferentially bind the TS