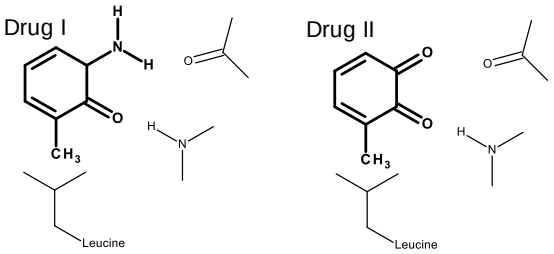
03-232 – Spring 2015 Exam 2 Name:_____

Instructions: This exam consists of 100 pts, 14 questions, 6 pages. On questions with choices all of your attempts will be graded and you will receive the best grade. **Allot 1 min/2 points.**

1. (18 pts) HIV reverse transcriptase can be inhibited by drugs that bind into a nonpolar pocket with hydrogen bond donor/acceptors. Two drugs are shown on the right, drug I and drug II (in bold). The groups on the enzyme are drawn in thin lines. *Both of these drugs are mixed type inhibitors.*



i) (6 pts) Please do **one** of the following choices to obtain fractional saturation.

Choice A: The binding of drug I to the enzyme causes a change in absorption of a near-by tryptophan residue in the enzyme. The absorbance at 280 nM in the absence of drug is 0.1 and the absorbance is 0.2 when the enzyme is fully saturated with drug. When the drug concentration is 1 uM the absorbance is 0.12. What is the fractional saturation at this ligand concentration?

$Y = (A - A_M)/(A_{ML}-A_M) = (0.12-0.1)/(0.2-0.1) = 0.02/.1 = 0.2$

Choice B: Equilibrium dialysis is used to measure the fractional saturation. A solution of 1 uM of reverse transcriptase is placed inside a dialysis bag and drug I is added. After equilibrium the concentration of the drug outside the bag is 1 uM and the total concentration of the drug inside the bag is 1.2 uM. What is the fractional saturation at this ligand concentration?

$Y=[ML]/([M]+[ML])$. $[ML]=L_{IN}-L_{OUT} = 1.2-1.0 = 0.2$ uM. $Y=0.2/1 = 0.2$

Common error – creating additional protein in the bag, i.e. Y=0.2/1.2 -2 pts

ii) (4 pts) The entropic contribution to binding (M + L → ML) is the same for both drugs and is +100 J/mol-K. From a molecular perspective, why is the entropy change favorable when these drugs bind to the enzyme?

The entropy is increasing due to the release of ordered water from the enzyme and drug when binding occurs (hydrophobic effect)

Some students will have discussed entropic stabilization of the transition state in enzyme catalysis. If they have done so, and their answer is correct, give 1 pt.

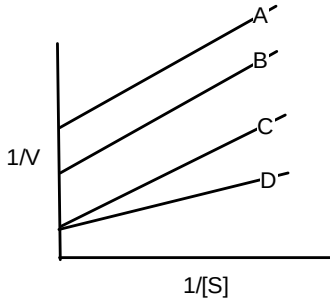
iii) (4 pts) Which of the two drugs would more likely show a smaller K_D? Why?

Drug I because it has one more hydrogen bond to the enzyme than drug II.

iv) (1 pt) How does the inhibition of this enzyme affect the growth of the HIV virus?

The virus requires the enzyme to convert its RNA to DNA before integrating into the chromosome of the infected T-cell.

v) (3 pts) A double reciprocal plot is shown on the right with lines labeled A, B, C, D. Which line corresponds to which condition?



No inhibitor = line __D____ (Highest velocity)

Drug I = line __A____ (Drug I binds better – more effective)

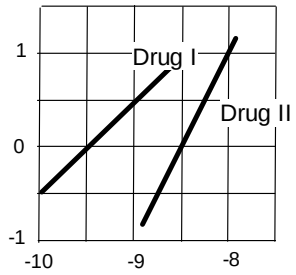
Drug II = line __B____ (Drug II binds worse – less effective)

Either order DAB or DBA is acceptable since it was not possible to determine K_I or K_I' from the information given, some students may have based their conclusion on V_{Max}/K_M differences.

(line C is for a competitive inhibitor)

Points on Page:_____

2. (6 pts) The Hill plots for the binding of two drugs to a protein are shown on the right (these are not necessarily the same drugs from Q1).
   i) (4 pts) How does the binding of drug I differ from drug II? You need to compare **both** K_D and cooperativity. Specifically state how the affinity and cooperativity of drug I is different from drug II.
   ii) (2 pts) Based on the Hill plot, what can you say about the number of binding sites for drug I and drug II?



i)

Drug I is non-cooperative with a K_D of $10^{-9.5}$ (Hill coefficient is one)

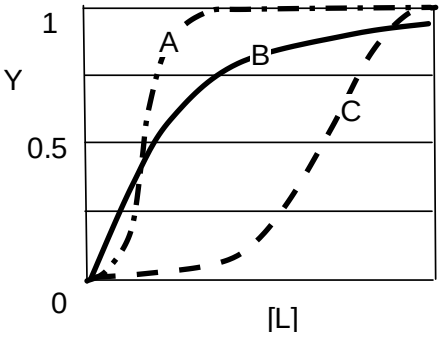
Drug II is positivitely cooperative with a K_D of $10^{-8.5}$ (Hill coefficient is >1)

ii)

Drug 1 has either one site, or many non-interacting sites. (-1/2 pt if just one site is stated)

Drug 2 has at least two sites.

3. (6 pts) The graph on the right shows three binding curves, A, B, and C. Based on the Hill plot from **question 2**, indicate which curve belongs to which drug. *Briefly justify your answer.*



Drug I = Curve __B____ (non-cooperative hyperbolic shape)

Drug II = Curve __C_____(pos-cooperative, higher K_I than drug I)

If curve A was selected for drug II -1 pt because the K_D is wrong.

4. (10 pts) Please do **one** of the following choices. Please answer both a) and b) within a choice.
   Choice A: Bis phosphoglycerate is a heterotropic allosteric inhibitor of oxygen binding.
      a) Briefly explain how it affects oxygen binding.
      b) Why is this effect important in adaptation to high altitudes?
   Choice B: Oxygen is a homotropic allosteric activator of oxygen binding.
      a) Briefly explain how the binding of one oxygen affects the binding of another.
      b) Why is this effect important in the effective delivery of oxygen from the lungs to the tissues?

Choice A:

a) Bis-phosphglycerate stabilizes the tense form of hemoglobin, decreasing oxygen affinity.

b) BPG changes the shape of the binding curve, to enhance the release of oxygen in the tissues, the system becomes more cooperative with respect to oxygen binding.

Choice B:

a) The binding of one oxygen enhances the binding of subsequent oxygen molecules

b) Causes full saturation of Hemoglobin in the lungs

As oxygen is lost in the tissues, the affinity decreases, enhancing release.

Points on Page:_____

5. (6 pts) Please do **one** of the following choices. *Briefly justify your answer in the space below.*

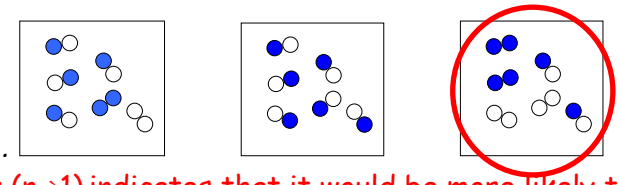
Choice A: The following ***microscopic*** dissociation constants (box, on right) were measured for a system that binds two ligands. The binding is (*circle correct answer*):

K_{1D} = 2.0 x 10^{-6} M
K_{2D} = 0.5 x 10^{-6} M

Neg. Coop Non-Coop Pos. Coop

K_D values are decreasing – binding is getting better.

Choice B: Which diagram more closely represents a system with a Hill coefficient of 1.5? (*circle correct diagram*).



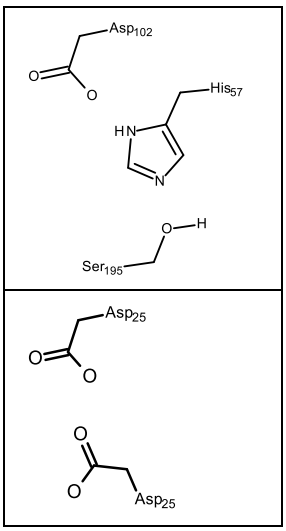
The positive coopertivity (n_h>1) indicates that it would be more likely to have fully saturated (two ligands bound) molecules.

6. (6 pts) Enzymes increase the rate of reaction by lowering the energy of the transition state, Briefly discuss **one** method by which this is accomplished and clearly state whether this method applies to almost all enzymes, or a select number of enzymes.

- The transition state is lowered by the pre-ordering of functional groups in the active site. Therefore there is no decrease in the entropy of the system (unfavorable) in going from the (ES) complex to (EX). This is common to all enzymes.

- Unique interactions occur between the enzyme and the transition state, such as the formation of H-bonds to just the transition state. This only occurs for a small number of enzymes.

7. (10 pts) Briefly describe the role, or function, of amino acid side chains in the mechanism of either serine proteases or aspartyl (HIV) protease. The diagrams on the right may be helpful.



Serine Protease:

- The His activates the Ser by deprotonation.
- Transfer of the proton to His is enhanced by electrostatic stabilization of the neg. charged Asp residue.
- Ser acts as a nucleophile and attacks C=O in peptide bond.

HIV Protease:

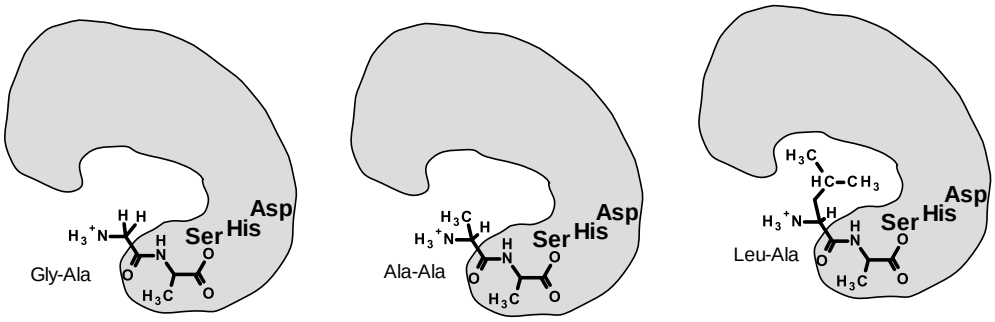
- One Asp is protonated, other deprotonated.
- Deprotonated Asp activates water as a nucleophile by removing a proton.
- OH^- acts as a nucleophile and attacks C=O in peptide bond.

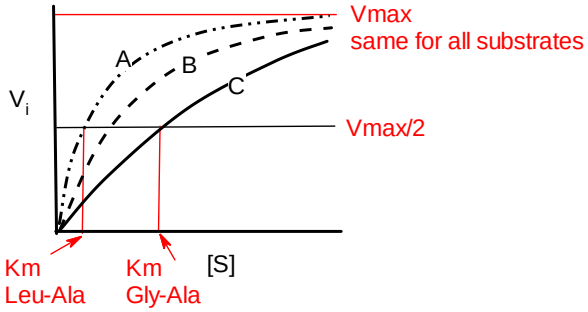
Points on Page:_____

8. (4 pts) What is the "steady-state assumption" and what is its importance in the measurement of enzyme kinetic parameters, such as K_M and V_{MAX}?

- The assumption is that during the measurement the concentration of (ES) is not changing (3 ½ pts)
- It results in a very simple form of the kinetic equation: v=Vmax [S]/(Km +[S]) (½ pt)

9. (10 pts) The diagram on the right shows the serine protease elastase, bound to three different substrates: Gly-Ala, Ala-Ala, and Leu-Ala. Steady-state reaction velocities were measured for each substrate and the resultant data is shown on the graph on the right. Which curve is associated with which substrate? **(3 pts)**





Gly-Ala = curve ___C___ (high K_M)

Ala-Ala = curve ___B___

Leu-Ala = curve ___A___ (low K_M)

Briefly justify your answer with reference to possible interactions between the substrate and the enzyme and how this interaction would affect the appropriate steady-state kinetic parameter (K_M or V_{MAX}) **(7 pts for justification)**

The different substrates would have different K_M values:

Gly-Ala has the weakest interaction with the enzyme, and would have the **highest K_M** (remember you can consider $K_M \approx K_D$).

Leu-Ala interacts more strongly with the enzyme (van der Waals and hydrophobic effect) and would have the **lowest K_M**.

Ala-Ala would be intermediate in terms of its interaction and K_M.

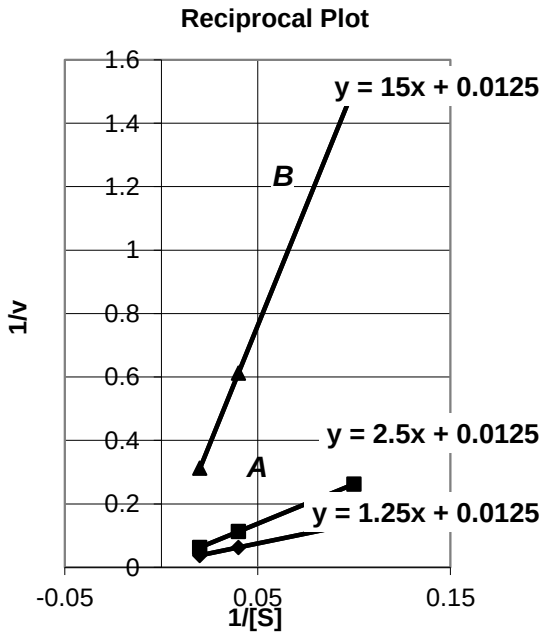
Vmax is the same because the same chemical reaction is occurring for all three substrates, and both Ala and Gly are smaller and therefore would not distort the enzyme upon binding, thus the triad would be the same in all cases.

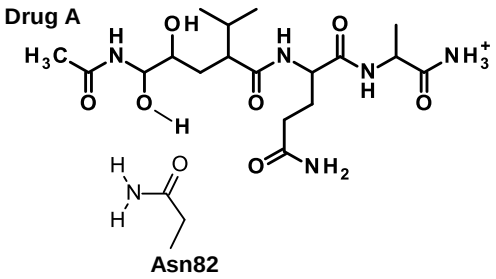
10. (2 pts) Would you expect the K_M for substrate binding to elastase to be affected by pH? *Briefly justify your answer.*

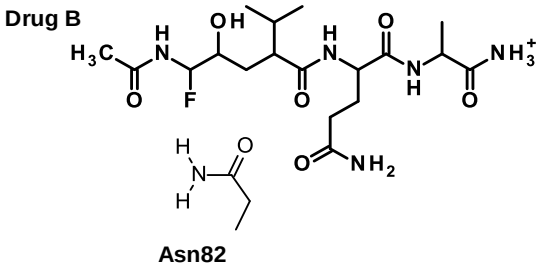
No, since the specificity pocket does not have any charged residues.

Points on Page:_____

11. (12 pts) A mutation has occurred in the HIV virus such that a patient is no longer resistant to the normal inhibitor. The amino acid change in the protein is such that Val82 is changed to Asn82. Two drugs have been designed that should inhibit the mutant HIV protease. The structures of these drugs are shown below. Steady-state enzyme data, on a double reciprocal plot, is shown on the right.
    i) Briefly explain why these molecules are competitive inhibitors (2 pts)
    ii) Obtain the K_I for each inhibitor. Assume [I] = 1nM (4 pts)
    iii) Which inhibitor is more effective, A or B, based on the K_I value? (4 pts)
    iv) Explain the differences in K_I due to differences in the interaction between the drug and Asn82 on the enzyme. (2 pts)







i) These are competitive inhibitors because they are similar to the substrate, but they lack a peptide bond – they bind in the active site, but can't be cleaved.
Acceptable answer – y-intercept is the same +/- inhibitor, a signature of a competitive inhibitor.

ii) $K_I = [I]/(\alpha-1)$

Drug A:	$\alpha = 2.5/1.25 = 2$	$K_I = 1\ nM/(2-1) = 1\ nM$
Drug B:	$\alpha = 15/1.25 = 12$	$K_I = 1\ nM/(12-1) = \sim 0.1\ nM$

iii) Drug B, because its K_I is lower.

iv) Both hydrogen bond to Asn82, apparently the hydrogen bond involving a fluorine is stronger – not surprising since fluorine is very electronegative.

12. (2 pts) Briefly define specific activity and describe its usefulness in protein purification.

- It is the ratio of the activity of the target protein/total amount of protein
- The specific activity should increase after each purification step since the amount of target protein is approximately the same, but the total amount of protein will decrease since we have discarded some of the impurities.

Points on Page:_____

13. (6 pts) Devise a purification scheme to purify **protein D** from a mixture of A, B, C, and D.

Protein	[Ammonium Sulfate] that precipitates 50% of protein*	# Residues (Mol Wt)	Charge at pH=6.0
A	1.0 M	120 (13,200 Da)	+8.0
B	1.5 M	120 (13,200 Da)	+10.0
C	4.0 M	120 (13,200 Da)	+9.5
D	6.0 M	240 (26,400 Da)	+8.0

*Concentrations 1 M below will leave all of the protein in solution. Concentrations 1M above will ppte all the protein.

Ammonium sulfate – 5 M. This will precipitate A, B, and C, leaving pure D in solution.

Gel filtration. Protein D, being larger, will elute from the column first, separated from A, B, and C.

Cation exchange. Cannot be used as a single step because A and D have the same charge and will elute at the same time. This could be used after the above two steps (since A would be removed), but a two step purification isn't necessary.

No penalty for more than one step, as long as the scheme will work to purify protein D.

14. (2 pts) Pick any **one** of your steps and briefly describe why proteins are separated by that technique.

Ammonium Sulfate: Proteins have different solubilities in ammonium sulfate solutions.

Gel Filtration. Resin consists of beads with small pores. The smaller proteins (A, B, C) enter the beads and take longer to run down the column. Protein D can't enter the beads as effectively, so it will elute first.

Cation Exchange. Resin beads have a negative charge such that the positively charged proteins will bind. The stronger the charge, the more tightly they bind, ie. Protein A and D would elute first, followed by C and then D.

Total: _____

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