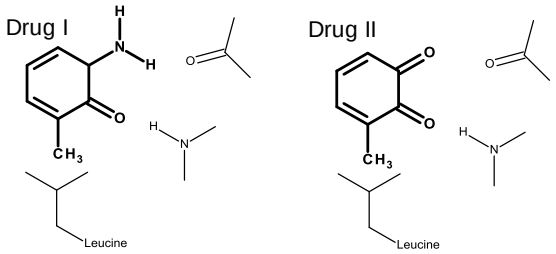
03-232 – Spring 2015 Exam 2 Name:_____

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 **<u>one</u>** 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?

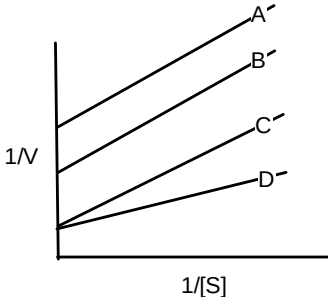
Choice B: Equilibrium dialysis is used to measure the fractional saturation. A solution of 1 uM of reverse transcriptase is placed inside a dialysis bag and drug I is added. After equilibrium the concentration of the drug outside the bag is 1 uM and the total concentration of the ligand inside the bag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

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?

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

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

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?



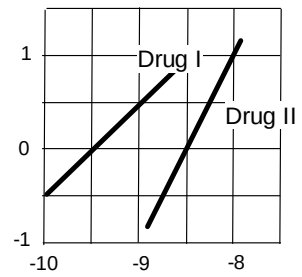
No inhibitor = line _____

Drug I = line _____

Drug II = line _____

Points on Page:_____

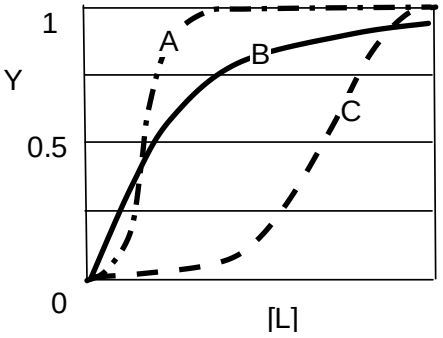
2. (6 pts) The Hill plots for the binding of two drugs to a protein are shown on the right (these are not necessarily the same drugs from Q1).



   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?

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 _____

   Drug II = Curve _____

4. (10 pts) Please do **<u>one</u>** 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?

Points on Page:_____

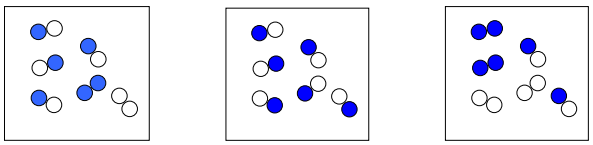
5. (6 pts) Please do **one** of the following choices. *Briefly justify your answer in the space below.*

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*):

$K_{1D} = 2.0 \times 10^{-6}$ M
$K_{2D} = 0.5 \times 10^{-6}$ M

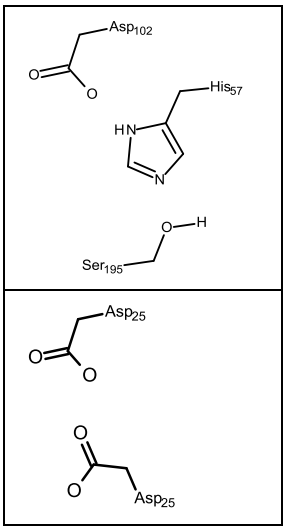
Neg. Coop Non-Coop Pos. Coop

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

. 

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.

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.



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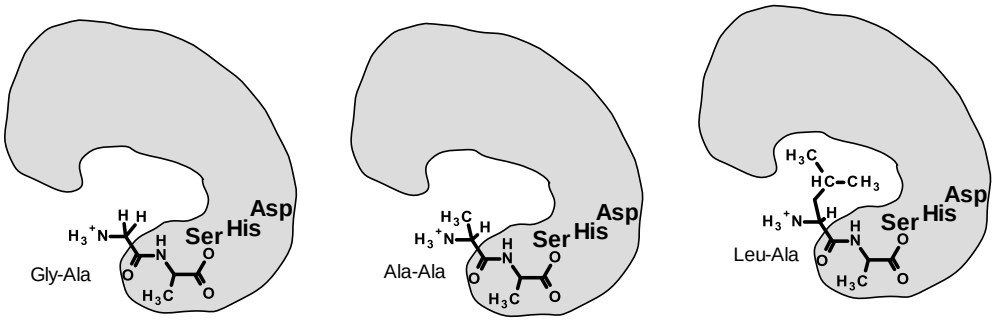
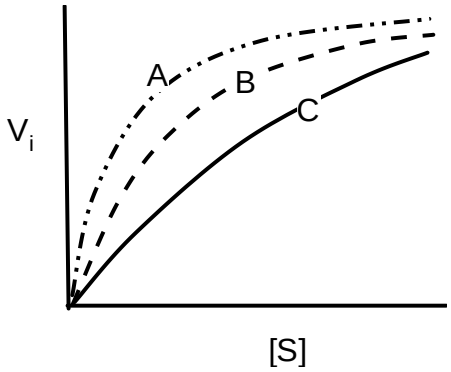
8. (4 pts) What is the "steady-state assumption" and what is its importance in the measurement of enzyme kinetic parameters, such as K_M and V_{MAX}?

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?





Gly-Ala = curve _____

Ala-Ala = curve _____

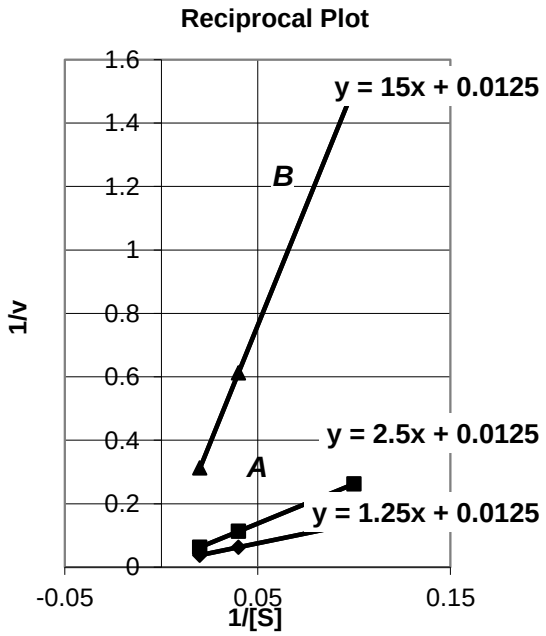
Leu-Ala = curve _____

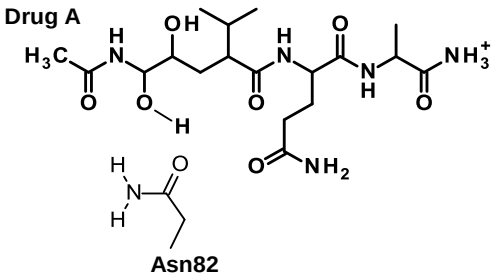
Briefly justify your answer with reference to possible interactions between the substrate and the enzyme and how this interaction would affect the appropriate steady-state kinetic parameter (K_M or V_{MAX})

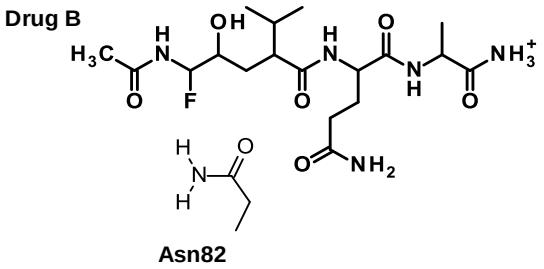
10. (2 pts) Would you expect the K_M for substrate binding to elastase to be affected by pH? *Briefly justify your answer.*

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)







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

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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.

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

Total: _____

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