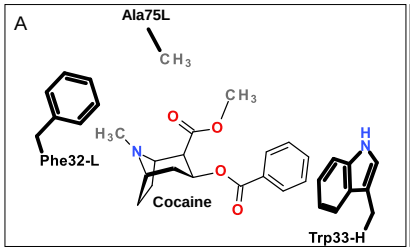
03-232 S2016 Exam II Name:_____

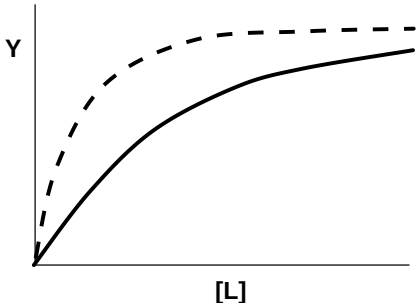
Instructions: this exam consists of 11 questions on 5 pages, for a total of 100 points. On questions with choices, all of your attempts will be graded and you will be awarded the highest grade. Please use the space provided or the back of the preceding page.

1. (10 pts) The binding of cocaine to two different Fab fragments (from antibodies) was measured using equilibrium dialysis and absorption spectroscopy. The data for one concentration of cocaine is shown for both of these Fabs in the table on the right. Binding curves are also shown for these two Fab fragments.



	$[L]_{Free}=[L]_{OUT}$	$[L]_{IN}$	A_{280}
A	10 uM	11.25	0.25
B	10 uM	12.27	0.37

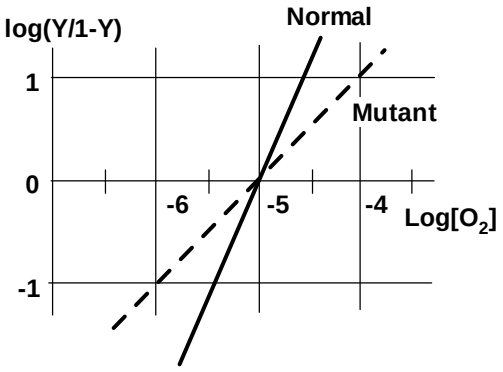
i) (5 pts) Determine the fractional saturation, Y for **both** antibodies for [L]=10 uM, using **either** method. *You **must** show your work for full credit*. The following information is required to solve this problem. [Fab]=2.5 uM, A_M=0.1, A_{ML}=0.4.



ii) (2 pt) Indicate which binding curve corresponds to which antibody, based on your answer to part i and with reference to the structures above.

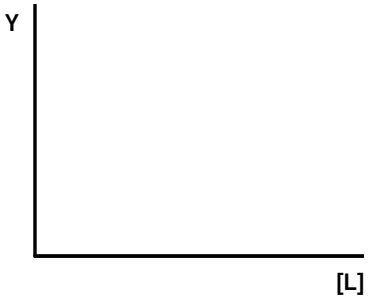
iii) (3 pts) Indicate how you would obtain the K_D for both antibodies from the binding curves. It is not necessary to give numerical values.

2. (16 pts) The binding of oxygen to two different hemoglobins is being studied. The Hill plots for these two proteins are shown on the right. One hemoglobin is normal hemoglobin (solid line) while the second is a mutation (dashed line), where the distal histidine has been replaced by glycine (glycine lacks a sidechain). (*Part of this question is on pg 2*).



i) What are the K_D values for each of these proteins (2 pts)?

ii) Assess the cooperativity of each of these proteins, using the Hill plot, i.e. is the binding cooperative, and if cooperative, weakly or strongly (4 pts).



iii) Sketch the binding curve for these two proteins in the graph on the right. Take care to indicate accurate K_D values (3 pts).

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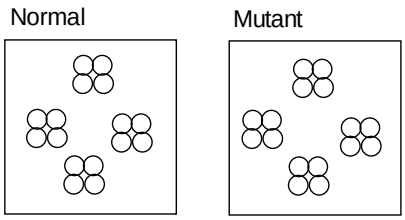
Question 2, continued

iv) Individuals that have the **<u>mutant</u>** hemoglobin suffer from poor oxygen delivery. Explain why this is the case (5 pts).

v) Indicate the distribution of bound oxygen on the normal and mutant hemoglobins using the diagrams by shading a circle if oxygen is bound. Assume Y=0.25, i.e. ¼ of the binding sites are occupied. Briefly justify your answer (2 pts).



3. (10 pts) Please do **<u>one</u>** of the following choices:

Choice A: Discuss how BPG (bisphosphoglycerate) is used to increase oxygen delivery at high altitudes.

Choice B: Briefly discuss the general framework of allosteric effects, your answer should compare and contrast tense and relaxed states, homotropic and heterotropic compounds.

4. (12 pts) Briefly describe why enzymes increase the rate of reactions. Feel free to illustrate your answer with a diagram. Your answer should indicate factor(s) that enhance the rate of all enzymes and factor(s) that are only found with some enzymes.

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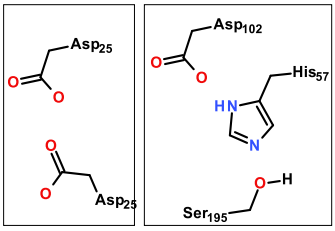
5. (12 pts) Amino acid residues in the active site of enzymes are involved in substrate binding or catalysis. Pick any **one** of the enzymes that we have discussed in the class thus far (trypsin, chymotrypsin, elastase, HIV protease) and answer all of the following questions. You need not use the same enzyme for parts i and ii. The chemical structures on the right may be helpful.



i) (5 pts) Discuss how the amino acid sidechains are responsible for peptide bond hydrolysis. You need not give the complete mechanism for serine proteases.

ii) (5 pts) Discuss how residues in the active site define substrate specificity. Your answer should discuss **molecular complementarity** (e.g. molecular details of the interaction between S and E) and how this affects K_M.

iii) (2 pts) For **either** part i **or** part ii, briefly discuss how pH would affect **either** k_{CAT} (part i) **or** K_M (part ii).

6. (5 pts) Please do **one** of the following choices:

Choice A: 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}?

Choice B: Explain why it is preferable to experimentally obtain the velocity of enzyme catalyzed reactions as soon as possible after substrate is added.

7. (4 pts) Compare and contrast competitive and suicide inhibitors. You answer should discuss the following: i) the location of the binding site on the enzyme, ii) whether the binding is reversible.

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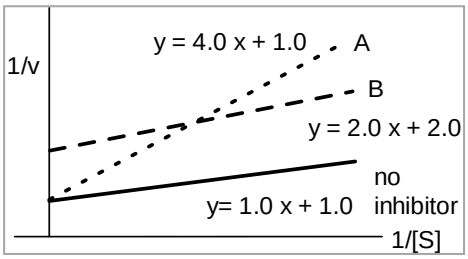
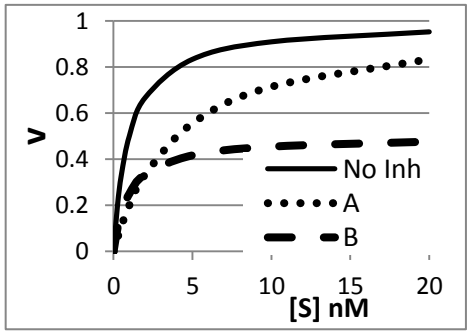
8. (16 pts) A new class of HIV protease inhibitors has been invented. You perform enzyme kinetic measurement using 1 nM of the new inhibitor to evaluate its effectiveness. For comparison purposes you also collect data using the existing drug (also at 1nM). These data are plotted on a double reciprocal plot, (A=existing drug, B=new drug). The equations of the lines are also given. The data are also shown in a plot of v versus [S]. Please answer all of the following questions.



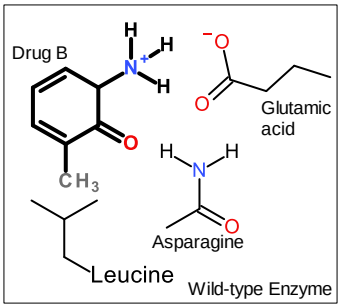
i) Which drug is a competitive inhibitor and which is a mixed inhibitor? *Justify your answer* (4 pts).

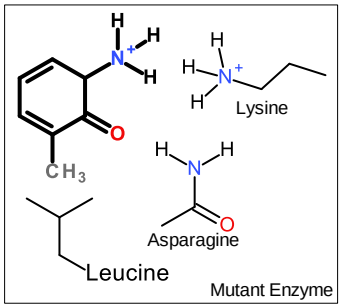


ii) Which drug binds more tightly to the **free enzyme**? You answer should be based on a calculation of K_I or K_I', whichever is appropriate (5 pts).

iii) Which is the better drug when the substrate concentration is 10 nM, A or B? Why? Your answer should consider the type of inhibitor (comp/mixed) and how the type of inhibitor affects its sensitivity to the substrate concentration (3 pts).

iv) The new drug is initially quite successful, but then the drug becomes ineffective due to mutations in the HIV protease. The structures of the enzyme-drug complex for the wild-type (left) and mutant enzyme (right) are shown. The binding pocket for drug consists of a Leu, Asp, and Glu residue. The mutation is Glu to Lys. Indicate how you would modify the inhibitor to increase its affinity for the mutant protease. Cross out the group(s) on the drug on the right panel and indicate their replacement. *Briefly explain your approach (use the back of the prev. page if necessary)* (4 pts).





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Question 8 continues:

v) (Bonus 2 pts) What aspect of the HIV life-cycle is responsible for the generation of mutations in the protease? *Use the back of the preceding page for your answer.*

9. (6 pts) Pick any **one** of the following purification techniques and describe how it works to separate proteins. Your answer should include a description of the resin (beads), how proteins interact with the resin, and how the proteins are removed (eluted) from the resin:

Choice A: Size exclusion (gel filtration)
Choice B: Anion exchange
Choice C: Cation exchange
Choice D: Affinity chromatography

10. (4 pts) You are performing a purification scheme and measure the specific activity after each step. How should you modify your purification scheme? Suggest **one** step that you should change? *Justify your answer*.

Sample	Specific Activity
Lysate	13.5 units/mg
After step 1	12.0 units/mg
After step 2	50.0 units/mg
After step 3	50.0 units/mg

11. (5 pts) You are purifying a glucose binding protein from an E. coli lysate. The target protein has six histidine residues at its amino-terminus. Describe a purification scheme that will separate the target protein from the other four proteins. The properties of all of the proteins are shown in the table. You can assume pKa values of 4.0 and 9.0. Values of ammonium sulfate 1M below the listed solubility will not precipitate any protein, values 1 M above will precipitate all of the protein). Points: One step=6 pts (1 point bonus), Two steps=5 pts, Three steps=3 pts

Protein	Sol. Amm. Sulfate	Molecular Wt	# Asp Residues	# Lys Residues
Target	**2.0**	**15,000**	**5**	**5**
A	2.0	25,000	10	15
B	2.5	15,000	5	10
C	6.0	15,000	15	5
D	6.0	30,000	20	10

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