Biochemistry I Exam II - 2006 Name:_____

This exam consists of 8 pages and 90 points. Allot 1 min/2 points.

Part A: Please circle the best answer (2 pts each/16 pts total).

1. A protein that is infinitely positive cooperative for the binding of *n* ligands will
   a) show a Hill coefficient (n_h) of 1/*n*
   b) show a linear Scatchard plot.
   c) show a Hill coefficient (n_h) of *n*
   d) show a Hill coefficient (n_h) of 1.

2. If an antibody binds specifically to the _____ of a reaction it will have catalytic activity and can therefore act as an enzyme.
   a) substrate.
   b) transition state.
   c) product.
   d) none of the above, antibodies cannot function as catalysts.

3. Which of the following *best* describes the assumption made in steady-state kinetic measurements
   a) The concentration of [S] is constant.
   b) The concentration of [ES] is constant.
   c) The concentration of [E] is constant.
   d) The concentration of [EP] is constant.

4. k_{cat} or the turn-over number, is a measure of
   a) how fast the substrate binds to the enzyme.
   b) how fast the product leaves the enzyme.
   c) the number of products a single enzyme molecule produces/unit time.
   d) the off-rate of the substrate.

5. A non-competitive inhibitor effects _____ of an enzyme catalyzed reaction which always changes the _____ on a double reciprocal (Lineweaver-Burk Plot).
   a) V_{MAX}, slope.
   b) V_{MAX}, *y*-intercept.
   c) K_M, slope.
   d) K_M, *y*-intercept.

6. The major problem in the use of drugs to treat HIV infections is:
   a) Drugs that are good inhibitors cannot by synthesized.
   b) The drugs interfere with normal digestion.
   c) The drugs are rapidly degraded.
   d) Virus particles with altered (mutant) proteases arise.

7. A protein that synthesizes tyrosine is being purified. What would be the best way to determine the location of this protein in chromatography fractions?
   a) UV absorption.
   b) Measure the rate of tyrosine synthesis.
   c) SDS gel electrophoresis of the protein.
   d) Mass spectroscopy of the protein.

8. In SDS-PAGE Gel Electrophoresis:
   a) Proteins are denatured by the SDS.
   b) Proteins have the same charge-to-mass ratio
   c) Smaller proteins migrate more rapidly through the gel.
   d) All of the above.

A :_____/16

B1:_____/10

B2:_____/ 4

B3:_____/ 8

B4:_____/12

B5:_____/10

B6:_____/16

B7:_____/ 5

B8:_____/ 9

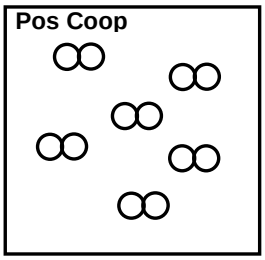
Tot:_____/90

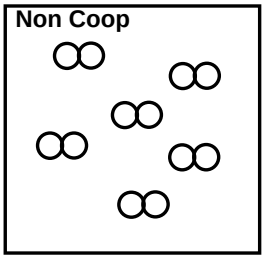
= _____ %

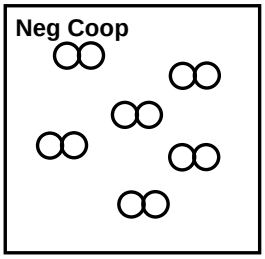
Part B: Please do **all** of the following questions. In some cases you have choices within a question.

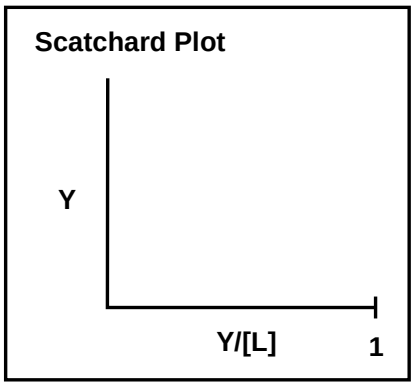
B1 (10 pts): Please do section a), b), c), and ***one*** of section d).

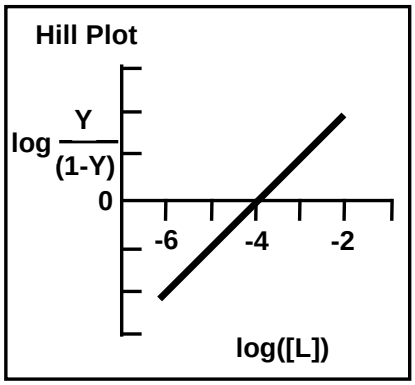
The three boxes to the right represent solutions of dimeric proteins that can bind one ligand/sub-unit, or two ligands per dimer. The top box represents a positive-cooperative system, the middle box a non-cooperative system, and the bottom box a system that shows negative cooperativity. The unfilled circles represent proteins that do not have ligand bound. You are to fill in the circles to represent bound ligands.











a) Each box contains 12 binding sites. How many sites will be occupied when [L] = K_D? Why? (1 pt)

b) Shade the proteins in each box to represent the *approximate* distribution of bound ligands at [L] = K_D. Do not draw anything that represents free ligand. (3 pts).

c) Sketch, in the space to the upper right, the Scatchard plot that you would obtain for the non-cooperative system. Briefly describe in the space below how you would you obtain K_D from such a plot. (4 pts)

d) The lower right part of the diagram shows a Hill Plot. This plot already contains the Hill curve for the non-cooperative system. Do ***either*** of the following two choices. Be sure to clearly indicate your selection and briefly justify your drawing. You need only draw the linear portion of the Hill plot, near *log* [Y/(1-Y)] =0.

Choice i) Sketch the Hill plot that you would expect to observe for the *positive* cooperative system, assume that the binding, on average, was 10 fold *weaker* than the non-cooperative system (3 pts).

OR

Choice ii) Sketch the Hill plot that you would expect to observe for the *negative* cooperative system, assuming that the binding, on average, was 10 fold *stronger* than the non-cooperative system. (3pts).

B2 (4 pts). Can a monomeric protein show cooperative binding? Yes or No? Briefly justify your answer.

B3 (8 pts): Please do one of the following two choices. Be sure to indicate your selection.

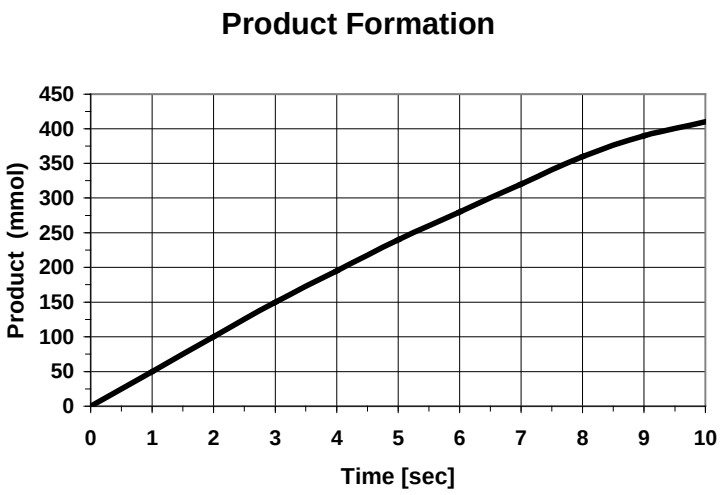
Choice A: A dialysis bag contains 10 μM of a protein the binds one ligand in a non-cooperative manner. Assume that the K_D is 1 μM and the ligand concentration outside the dialysis bag is 1 μM.

i) At equilibrium, what is the *total* concentration of ligand inside the bag? Briefly indicate your approach (5 pts).

ii) What is the fractional saturation of the protein under these conditions? Please show any calculations (3 pts).

OR

Choice B: An enzyme has a K_M of 1μM and a V_{MAX} of 100 mmoles product formed/sec. The product formation as a function of time is shown to the right for an unknown substrate concentration. What substrate concentration was used in this reaction? Please show your work/justify your answer.



B4 (12 pts): Please do one of the following two choices. Please indicate the choice that you are answering.

Choice A: Briefly describe the *conceptual* formalism that is used to describe cooperative behavior of ligand binding. Your answer should include a definition/discussion of tense and relaxed states as well as the role of homotropic and heterotropic allosteric in cooperativity.

OR

Choice B: Hemoglobin binds both O_2 and bisphosphoglycerate as ligands. Both ligands affect the cooperativity of the system. Select **one** of these ligands and answer the following questions.

i) Briefly describe in molecular terms the "binding site" for the ligand, i.e. why does the ligand bind where it does? (4 pts)

ii) Briefly describe, in *molecular* terms, how the binding event is related to the cooperativity of the system, i.e. how is the conformation of hemoglobin affected by the binding of ligand? (4 pts)

iii) Briefly describe the *functional* importance of the binding of your choice of ligand on oxygen transport/delivery (4 pts).

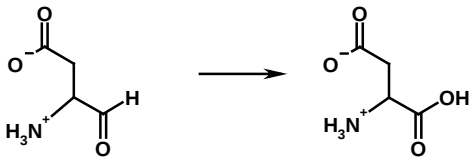
B5 (10 pts): A solution of free Histidine, Aspartic acid, and Serine will cause peptide bond hydrolysis at a somewhat faster rate than pure water. However, the same three residues in a serine protease accelerate the reaction rate several thousand fold. Similarly, a solution of Aspartic acid is much less efficient at peptide bond hydrolysis than the HIV protease. Please answer one of the following two choices:

Choice A: Explain why enzymes in general are capable of enhancing reaction rates. You answer should discuss both enthalpic and entropic terms. Provide an example.

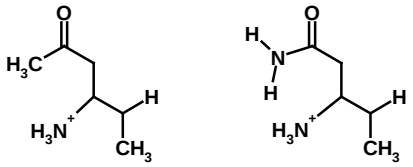
OR

Choice B: Select *either* the HIV protease, *or* one of the serine proteases (trypsin, chymotrypsin, elastase) and briefly discuss the reaction mechanism. Your answer should mention the role that residues in the active site play in *both* catalysis and substrate specificity. Feel free to answer this question by means of a drawing.

B6 (16 pts): The diagram below shows the substrate and product of an enzyme. The reaction that is catalyzed is an oxidation of an aldehyde to carboxylic acid. The K_M for this substrate is 1 μM. The structures of two inhibitors of this enzyme are also shown. Enzyme kinetics were measured in the absence of inhibitor, and in the presence of 10 μM of both inhibitors. The resultant data has been plotted on a double-reciprocal plot. The slopes and intercepts of the lines on this plot are also given.

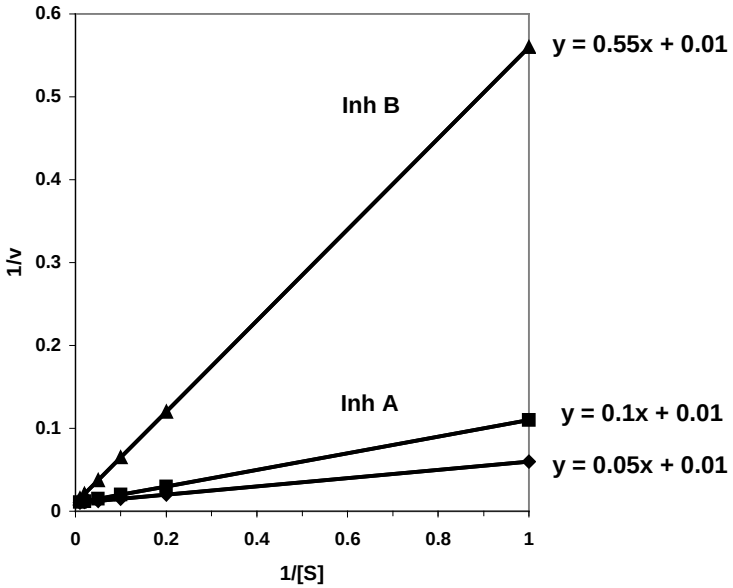


Substrate **Product**



Inhibitor A **Inhibitor B**

a) Explain why the inhibitors are not substrates for this enzyme (2 pts).



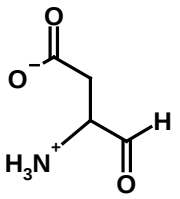
b) What is the K_I value for both inhibitors ([I]=10μM)? Please show your work. (4 pts)

c) Based on the K_I values, which inhibitor binds to the enzyme with higher affinity? (4 pts)

This question continues on the next page…

Q6 – Continues…

d) Sketch the region of the active site that is involved in substrate specificity, i.e. what amino acid sidechains from this enzyme are likely to be involved in recognition of this substrate. To aid your drawing, the structure of the substrate has been repeated here. Briefly justify your answer with reference to the K_I values, the K_M for the substrate (1 μM), and the structure of the inhibitors and substrate. (4 pts)



e) If you measured the ΔH^o and the ΔS^o for the binding of the above substrate to this enzyme, which would provide the larger contribution to binding? Why ?(2 pts)

B7 (5 pts): Please do ***one*** of the following two questions. Please indicate your selection.

Choice A: Provide a definition of specific activity and describe its usefulness in a protein purification scheme.

OR

Choice B: Select **one** of the following purification methods and briefly describe the *principle* by which the method can be used to separate a mixture of proteins. If appropriate, your answer should briefly how tightly bound proteins can be eluted from the column.

a) anion exchange chromatography

b) cation exchange chromatography

c) affinity chromatography

d) gel filtration chromatography

B8 (9 pts): The table below gives the properties of three proteins:

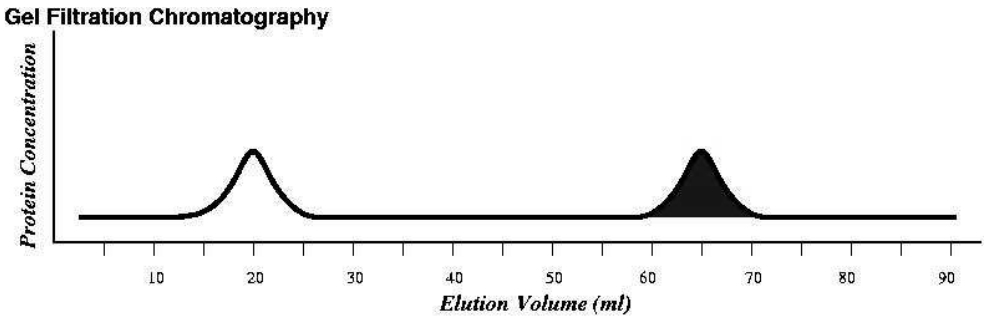
Protein	MW (Daltons)	# aspartic & glutamic acid residues ($pK_a = 4$)	# of lysine residues ($pK_a = 9$)
A	12,000	5	10
B	12,000	0	3
C	20,000	2	1

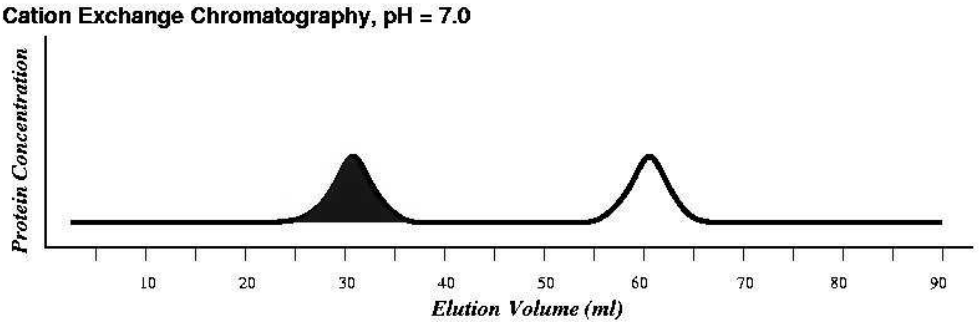
A mixture of these three proteins was subjected to the following two step purification scheme:

i) Gel filtration,

ii) Cation exchange chromatography at pH=7.0.

The elution profiles (UV absorption versus elution volume) for each of these columns is shown to the right. The shaded peak in the gel filtration profile was used to load the cation exchange column.





Please answer the following questions. You should ignore the heights of the elution peaks.

a) What protein(s) are contained in the shaded peak from the gel filtration column? Please justify your answer. (4 pts).

b) What protein is contained in the shaded peak shown in the elution profile of the cation exchange column? Please justify your answer. (5 pts).