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**. (+1)**
   d) Mass spectroscopy of the protein.

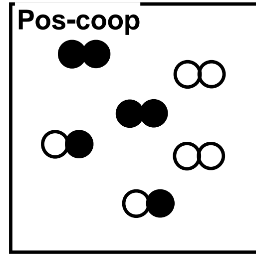
8. In SDS-PAGE Gel Electrophoresis:
   a) Proteins are denatured by the SDS.
   b) Proteins have the same charge-to-mass ratio
   c) Smaller proteins migrate more rapidly through the gel.
   d) All of the above.

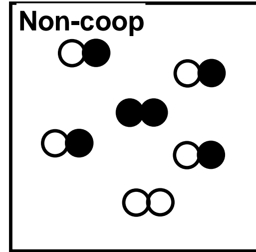
A :	__________/16
B1:	__________/10
B2:	__________/ 4
B3:	__________/ 8
B4:	__________/12
B5:	__________/10
B6:	__________/16
B7:	__________/ 5
B8:	__________/ 9
Tot:	**_________/90**
=	_________ %

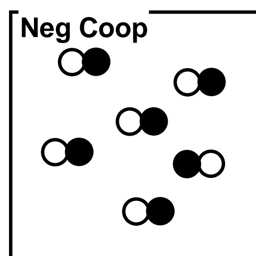
Part B: Please do **all** of the following questions. In some cases you have choices within a question.

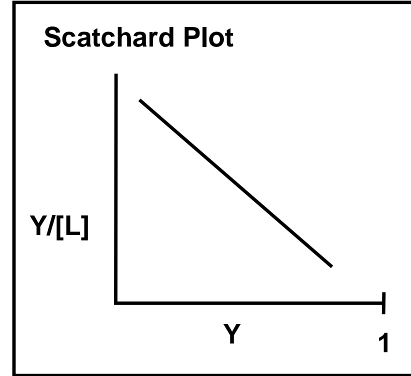
B1 (10 pts): Please do section a), b), c), and ***one*** of section d).

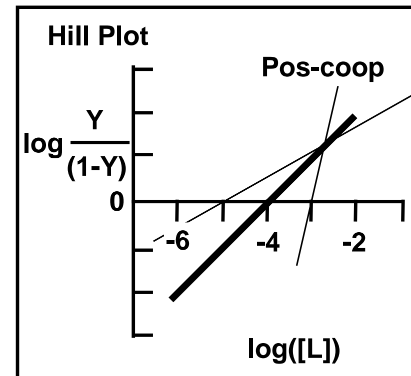
The three boxes to the right represent solutions of dimeric proteins that can bind one ligand/sub-unit, or two ligands per dimer. The top box represents a positive-cooperative system, the middle box a non-cooperative system, and the bottom box a system that shows negative cooperativity. The unfilled 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)

- **6 will be bound, regardless of the degree of cooperativity.**
- **When $[L] = K_D$, one-half of the sites are filled with ligand.**

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).

- **Pos-coop, most will be in the $[ML_2]$ form since the binding of the first ligand enhances the binding of the second.**
- **Non-coop, random distribution since binding to one subunit has no effect on the binding to the other.**
- **Neg-coop, most will be in the [ML] form since once the first one binds, the second will do so with reduced affinity.**

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)

- **The line will be straight since it is non-cooperative binding.**
- **The slope of the line is $-1/K_D$**

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).

- **The slope is greater than one since it is positive cooperativity.**
- **The y-intercept is displaced one unit to the right, reflecting the 10 fold weaker binding or 10 fold higher K_D.**

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).

- **The slope is less than one since it is negative cooperativity.**
- **The y-intercept is displaced one unit to the left, reflecting the 10 fold stronger binding or 10 fold lower K_D.**

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

Yes, provided there is more than one binding site on the protein.

If you also stated that if there was only one binding site, such that the binding of additional ligands cannot occur, therefore the system in non-cooperative. Then you should get 3 ½ pts.

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

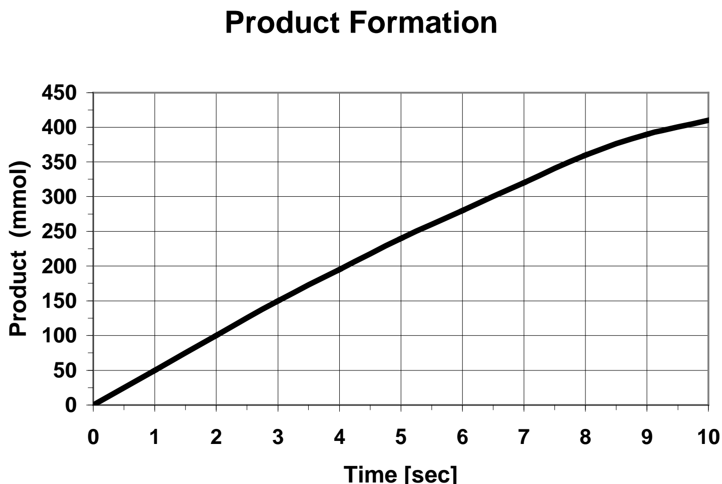
Choice A: A dialysis bag contains 10 μM of a protein the binds one ligand in a non-cooperative manner. Assume that the K_D is 1 μM and the ligand concentration outside the dialysis bag is 1 μM.

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.



Choice A:

When [L] = K_D the protein will be half-saturated with ligand. Therefore the total amount of ligand in the bag will be 1μM (free ligand) plus 5 μM (bound ligand), or a total of 6 μM.

The fractional saturation is: $Y=[ML]/[M_t]$ = 5μM/10μM = 0.5.

Choice B:

The slope at short times (e.g. t<3 secs) is used to define the initial rate (-2 if initial rate is not used).

The rate is dP/dt = 50 mmol/sec (e.g. 250 mmol of product are produced in 5 sec).
Since v=1/2 V_{MAX}, [S] = K_M.

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).

Choice A:

The following points must be stated (2 pts each).

1. **The protein exists in two conformations, which are in equilibrium: Tense (T) and Relaxed (R).**
2. **The tense form has low activity.**
3. **The relaxed form has high activity.**

Any 3 of the following should be stated: (2 pts each)

- **The T and R form differ in conformation.**
- **Allosteric activators increase the R form.**
- **Allosteric inhibitors increase the T form.**
- **Homotropic allosteric compounds affect their own binding.**
- **Heterotropic allosteric compounds affect the binding of a different ligand.**

Choice B:

	O_2	BPG
+4 pts	Binds to Fe^{2+} in heme group.	Binds to + charged pocket formed between two subunits of hemoglobin
+4 pts	Binding to the Heme moves the proximal His residue, which moves Helix F. This changes the interaction between subunits, causing the unbound subunit to go to the R state.	BPG stabilizes the T state by binding to the pocket between the two subunits. When BPG is not present this pocket can close when the Hb goes to the R state.
+4 pts	The increased affinity as O_2 is bound causes a sigmoidal-shaped binding curve. This allows Hb to be fully saturated with O_2 in the lungs and release most of its oxygen in the tissues.	BPG levels are increased at high altitude as an adaptive response to low O_2. The BPG reduces the affinity by stabilizing the T-form, shifting the binding curve to the right – i.e. more O_2 is required for binding. The shape of the binding curve is also changed, such that the same amount of O_2 is delivered.

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.

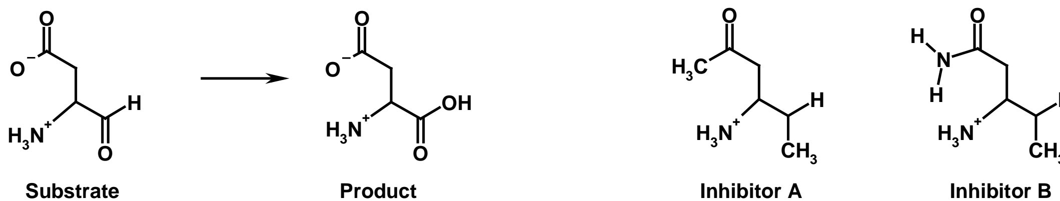
Choice A:

- **Enzymes lower the energy of the transition state of the reaction (+ 4pts).**
- **This increases the amount of the transition state, increasing the rate of the reaction (+2 pts)**
- **The energy of the transition state is decreased by:**
  1. **Entropic terms – the catalytic groups are correctly arranged in the folded enzyme, e.g. the Ser, His , and Asp residues in serine proteases. (+2 pts)**
  2. **Enthalpic terms – there is a direct enthalpic interaction between the transition state and the enzyme. In the case of serine proteases, the oxyanion hole stabilizes the negatively charged transition state by provided two positively charged NH groups.(+2 pts)**

Choice B:

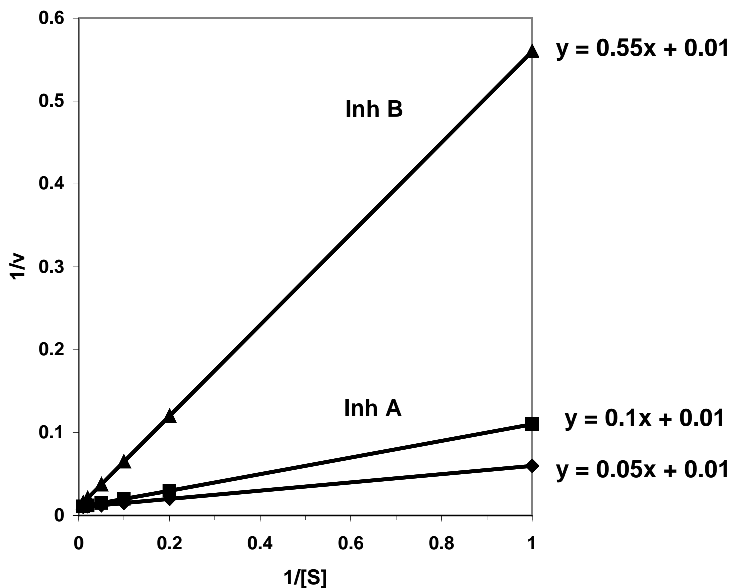
HIV Protease	Trypsin
• The two catalytic residues, Asp25 and Asp25' activate water as the nucleophil by transfer of a proton from water to the deprotonated Asp. • Nucleophilic attack of the water causes peptide bond cleavage and release of both products. • There is no acyl-intermediate. (+ 5pts)	• This is a two step reaction. In both steps the His-Asp pair activate a nucleophil by transfer of the proton from Ser or water. • In the first step, the deprotonated Ser attacks the carbonyl oxygen, breaking the peptide bond. • The first product leaves, the second product has formed an ester with Ser (Acyl enzyme) • Activation of water leads to cleavage of the acyl enzyme by the same mechanism as with the Ser, causing release of the 2nd product.
• HIV protease binds substrates with large-nonpolar groups due to the presence of Val82 in the enzyme's active site. (+5 pts)	• Trypsin binds substrates with pos. charged sidechains due to the presence of an negatively charged Asp residue in the enzyme's active site (note that this Asp is different than the one discussed above. (+5 pts)

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.



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

The aldehyde group has been replaced by an methyl group. This must affect the oxidation reaction in some way (you need not state more than this)



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

The K_I is found from α, as follows:

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

α is the ratio of the slopes for line obtained with inhibitor divided by the slope in the absence of inhibitor:

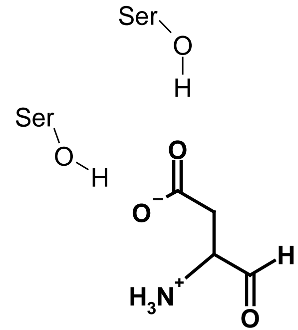
Inhibitor A	**Inhibitor B**
α = 0.1/0.05 = 2.0	α = 0.55 /.05 = 11
K_I = 10 μM / (2 –1) = 10 μM	K_I = 10 μM / (11 –1) = 1 μM

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

Inhibitor B, it has the lower K_I

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)

- **Inhibitor B binds as well to the enzyme as the original substrate since the K_I is equal to K_M. Therefore the carbonyl oxygen on the substrate that was replaced by a methyl group on the inhibitor must not affect substrate binding.**
- **The high K_I for inhibitor A indicates that replacement of the carboxylic acid group by a ketone has reduced affinity. Therefore there must be an interaction between both oxygens in the substrate. This cannot be a charge interaction, since inhibitor B has replaced the carboxylic acid group with an amide. Therefore two hydrogen bonds could be formed between the substrate and the enzyme, as indicated above; two serine residues in the enzyme are interacting with the substrate.**
  Most of the points were awarded for recognizing that the interaction is with the carboxylic acid group (+2 pt) and that this was a complementary interaction between the substrate and the enzyme (+ 2 pts). e.g showing lysine or arg interacting with the substrate should be +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)

- **ΔH^o These are polar interactions. ΔS^o would not be involved since changes in entropy are associated with non-polar (hydrophobic) interactions.**

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.

- **Specific activity is the ratio of the activity or amount of the protein that is being purified, divided by the total amount of protein. This should increase during a purification scheme since the amount of contaminating proteins decreases, reducing the amount of total protein.**

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 discuss how tightly bound proteins can be eluted from the column.

All of these methods involve small beads which are packed into a column. A mixture of protein flows over the beads.

a) Anion Exchange	**Beads have + charges on them, neg charged proteins bind. (3 pts)**
b) Cation Exchange	**Beads have - charges on them, pos charged proteins bind. (3 pts)**
c) Affinity	**Beads have ligands on them, proteins that bind the ligand stick.(3pts)**

The strength of the interaction between the protein and the bead will affect how much liquid must be flow down the column before the protein elutes. Proteins that bind weakly elute first. Some bind so tightly that the solvent conditions have to be changed in order for the protein to elute. Usually an increase in salt will disrupt the interaction. Alternatively, the pH can be changed, which will change the charge on the protein (+2 pts)

d) gel filtration chromatography

In this case this case the beads are porous, consequently small proteins enter the beads, and because the spend some time inside the bead, they elute later than larger proteins. Since the proteins do not interact with the beads per se, there is no need to change solution conditions to release the protein.

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

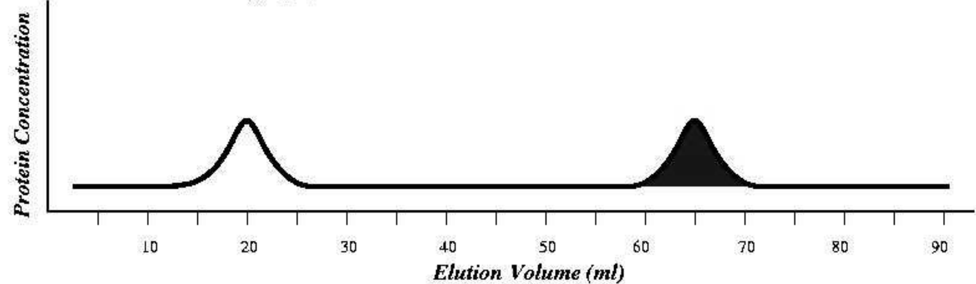
Protein	MW (Daltons)	# aspartic & glutamic acid residues ($pK_a = 4$)	# of lysine residues ($pK_a = 9$)
A	12,000	5	10
B	12,000	0	3
C	20,000	2	1

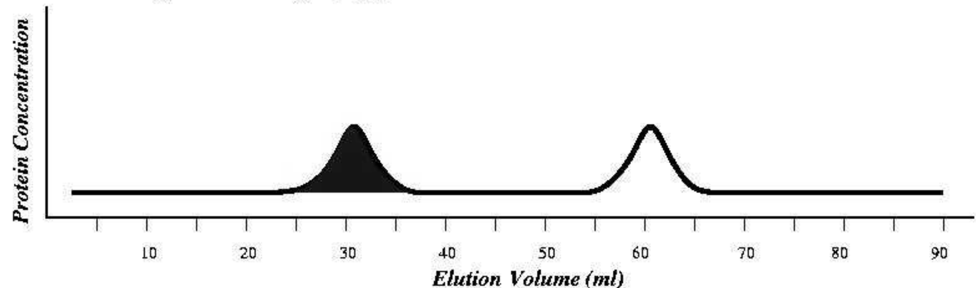
A mixture of these three proteins was subjected to the following two step purification scheme:

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).

This peak elutes last, so it should contain the smaller proteins, A and B.

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).

A mixture of A and B was loaded on the column. The protein with the smallest + charge will elute first.

At pH=7.0 the Asp/Glu residues will be fully deprotonated and have a charge of –1. The Lysine residues will have a charge of +1. So the net charge on each protein is:

q_A = -5 + 10 = +5

q_B = -0 + 3 = +3

Therefore the first peak is protein B.

If you thought that all three proteins were present in this separation by cation exchange, then protein C, with a charge of –1 would be the first one off since it won't bind at all. If your reasoning was correct you should get 4/5 points. –1 for not realizing that this column was the second step in the purification scheme.