NAME:_____

Biochemistry I, Spring Term 2005 - Second Exam:

There are a total of 10 pages in this exam, including this one. Please check that you have all the pages and write your name on every page before you begin.

Enzyme Kinetics:

For ([E]+[S]<-->[ES]-->[E]+[P])

$V_{max} = k_2[E_T] = k_{cat}[E_T]$
$K_M = (k_{-1}+k_2)/k_1$

$$v = \frac{V_{MAX}[S]}{K_M + [S]}$$

Double reciprocal plot:

$$\frac{1}{v} = \frac{K_M}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}}$$

$$v = \frac{\frac{V_{MAX}}{\alpha'}[S]}{\frac{\alpha}{\alpha'}K_M + [S]}$$

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

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

α'=1 for competitive inhibition
α'>1 for non-competitive inhibition

$$\alpha = \frac{slope([I]>0)}{slope([I]=0)}$$

$$\alpha' = \frac{y-\text{int}([I]>0)}{y-\text{int}([I]=0)}$$

General Thermodynamics:

R=8.3 J/mol-deg
T=300K, RT=2.5 kJ/mol @ 300K
$\Delta G^O = -RT\ln K_{eq}$
$\Delta G = \Delta H - T\Delta S$
S=RlnW

van't Hoff Plot: $\ln K_{EQ} = \frac{-\Delta H}{R}\frac{1}{T} + \frac{\Delta S}{R}$

Amino Acid Names:

Alanine: Ala
Arginine: Arg
Asparagine: Asn
Aspartic Acid: Asp
Cystine: Cys
Glycine: Gly
Histidine: His
Isoleucine: Ile
Lysine: Lys
Leucine: Leu
Methionine; Met
Phenylalanine: Phe
Proline: Pro
Serine: Ser
Threonine: Thr
Tryptophan: Trp
Tyrosine: Tyr
Valine: Val
Glutamine: Gln
Glutamic Acid: Glu

Ligand Binding:

$$\boldsymbol{Y} = \frac{[\boldsymbol{ML}]}{[\boldsymbol{M}]+[\boldsymbol{ML}]}$$

$$\boldsymbol{Y} = \frac{\boldsymbol{K_A}[\boldsymbol{L}]}{1+\boldsymbol{K_A}[\boldsymbol{L}]}$$

$$\boldsymbol{Y} = \frac{[\boldsymbol{L}]}{\boldsymbol{K_D}+[\boldsymbol{L}]}$$

Scatchard Plot: Y/[L] versus Y
$Y/[L] = -Y/K_D + 1/K_D$
$\nu/[L] = -\nu/K_D + n/K_D$

Hill Plot: *log*(Y/(1-Y)) versus *log*[L]
Hill Equation: $log(Y/(1-Y)) = \log K_\pi + n_h log[L]$

Misc:

A=ℜCl
$pH = pK_A + \log([A^-]/[HA])$
$[HA] = [A_T]/(1+R)$ $[A^-] = [A_T]R/(1+R)$

For the reaction: N <--> U:

$K_{eq} = [U]/[N]$
$f_u = K_{eq}/(1+K_{eq})$ $f_n = 1/(1+K_{eq})$

Beer's law: A=ε[X]l

Logs:

MW (Da)	Log(MW)
10,000	4.00
20,000	4.30
30,000	4.48
40,000	4.60
50,000	4.70
60,000	4.78
70,000	4.85
80,000	4.90
90,000	4.95
100,000	5.00

Part A: Multiple Choice (18 pts, 2 pts each)

1. Once a ligand dissociation constant (K_D) has been determined it is possible to calculate
   a) the ligand binding constant (K_a).
   b) the ΔG^o for the binding interaction.
   c) the concentration of ligand required for half-maximal occupancy.
   d) All of the above are correct.

2. In both hemoglobin and myoglobin the oxygen is bound to.
   a) the nitrogen atoms on the heme.
   b) polar pocket in the protein.
   c) histidine residues in the protein.
   d) the iron atom in the heme group.

3. A protein that binds two ligands in a non-cooperative manner will:
   a) show a hyperbolic binding curve
   b) show a curved Scatchard Plot
   c) show a curved Hill Plot.
   d) show a sigmodial binding curve

4. The active site of an enzyme differs from an antibody-antigen binding site in that the enzyme active site
   a) contains modified amino acids.
   b) catalyzes a chemical reaction.
   c) is complementary to a specific ligand.
   d) contains amino acids without sidechains.

A:	_____/18
B1:	_____/12
B2:	_____/14
B3:	_____/10
B4:	_____/14
C:	_____/16
D:	_____/16
Tot:	_____/100

5. The steady-state assumption implies
   a) ligands bind to their sites in a continuous fashion.
   b) the concentration of the enzyme-substrate complex [ES] does not change during the reaction.
   c) the concentration of substrate remains constant during the reaction.
   d) Both b and c are correct.

6. A feature in common among *all* serine proteases is:
   a) a hydrophobic specificity pocket.
   b) a pair of reactive aspartic acid residues.
   c) a cluster of reactive serine residues.
   d) a single reactive serine residue.

7. The major problem in the use of drugs to treat HIV infections is:
   a) There are no problems, HIV is completely controlled by existing drugs.
   b) Drugs that are good inhibitors cannot by synthesized.
   c) The drugs are rapidly degraded.
   d) Virus particles with altered (mutant) proteases arise.

8. During any purification scheme, you would expect
   a) the number of different proteins in the sample to decrease.
   b) that the specific activity increases.
   c) that the amount of target protein decreases.
   d) all of the above are correct.

9. An enzyme that produces ethanol from acetaldehyde is being purified from a complex mixture of proteins. What would be the best way to determine the location of this protein *during* the purification scheme?
   a)UV absorption of fractions.
   b)Measure the rate of ethanol synthesis of fractions.
   c)SDS gel electrophoresis of fractions.
   d)Mass spectroscopy of fractions.

Part B: Short Answer.

B1. (12 pts) Please do **one** of the following two choices:

Choice A: Briefly explain the concept of transition state stabilization in enzyme catalysis. Your answer should include a discussion of enthalphic versus entropic effects. Provide an example for one of these effects.

Choice B: Select **either** serine proteases **or** HIV protease and briefly discuss the role of important residues in the cleavage of the peptide bond. If you select serine proteases, you need not give the entire reaction mechanism to complete this question.

B2. (14 pts) Using hemoglobin, or any other suitable protein as an example, provide a *brief* summary of the role of allosteric effects in the control of biological systems. Your answer should include a discussion of relaxed and tense states, and the importance of these terms. Illustrate your answer by selecting **one** of the following three molecules: O_2, H^+, or bisphosphoglycerate, and discuss its role in the regulation of oxygen transport in hemoglobin.

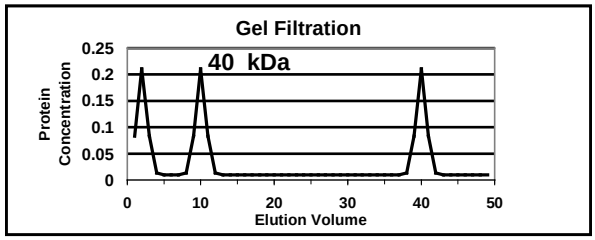
B3. (10 pts) Do **ONE** of the following two choices (The second choice is on the following page.).

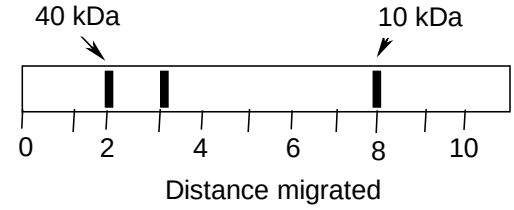
Choice A: You wish to separate the following two proteins from each other, which type of column chromatography would you use? (2 pts) How would you elute the proteins off of the column? (2 pts) Briefly explain why the separation would occur (6 pts).

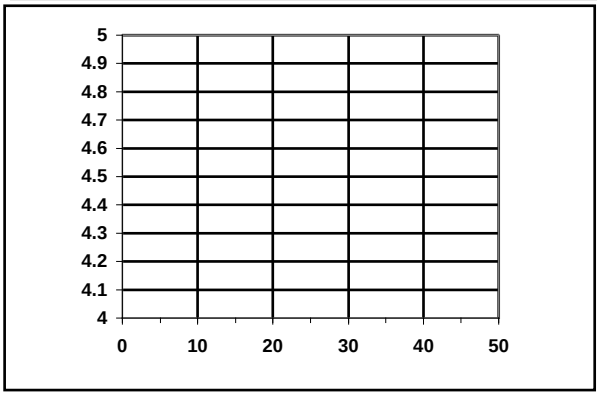
Protein A: isoelectric pH = 7.0. **Protein B**: isoelectric pH = 5.0.

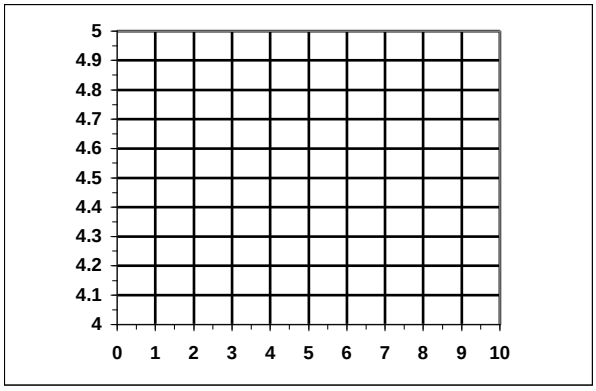
Choice B: You are trying to determine the quaternary structure of a protein. You mix the unknown protein with two molecular weight standards. The size of the standards are 10 and 40 kDa. The elution profile from the gel filtration column is shown to the left. An image of the SDS gel is shown to the right.

i) Determine the quanternary structure of the protein using these data. Please justify your answer. (See face page for log values) (8 pts).









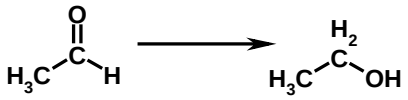
ii) Do the above experiments give you any information regarding disulfide bonds in this protein? Why? (2pts)

B4. (14 pts) Enzyme Inhibitors

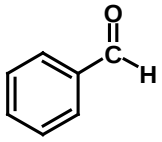
i) Compare and contrast a competitive inhibitor to a non-competitive inhibitor. You answer should include:

- A discussion of the location of the inhibitor binding site. (4 pts)
- How and why the inhibitor affects the enzyme kinetics (i.e. changes K_M or k_{CAT}, or both.). (4 pts)
- How to distinguish each type of inhibitor using a double reciprocal plot. (2 pts)

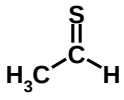
ii) An enzyme catalyzes the following reaction:



Which of the following compounds, A or B, would likely be a competitive inhibitor and which would be a non-competitive inhibitor of the enzyme? Briefly justify your answer (4 pts)



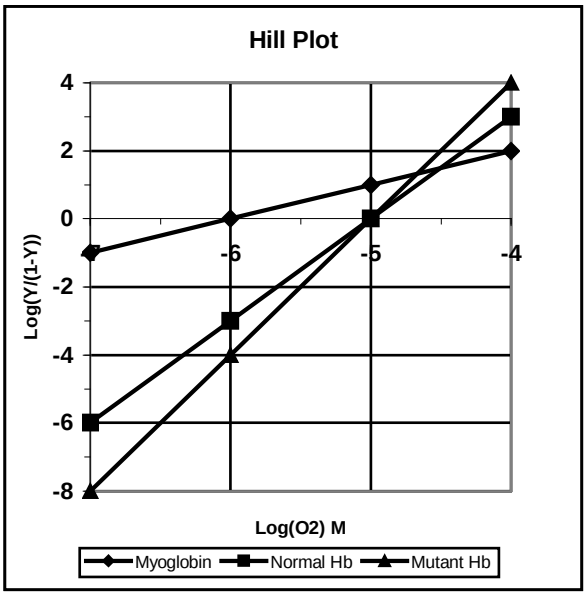
A



B

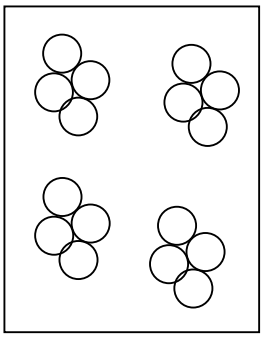
Part C: Oxygen Transport

C1. (16 pts) There are a large number of genetic alterations in the human hemoglobin gene. These often result in a change in the oxygen binding properties. The Hill plot for myoglobin, normal hemoglobin, and a mutant hemoglobin are shown in the diagram to the right.



i) Determine the Hill coefficient and K_D for the mutant hemoglobin. Please describe your approach (4 pts).

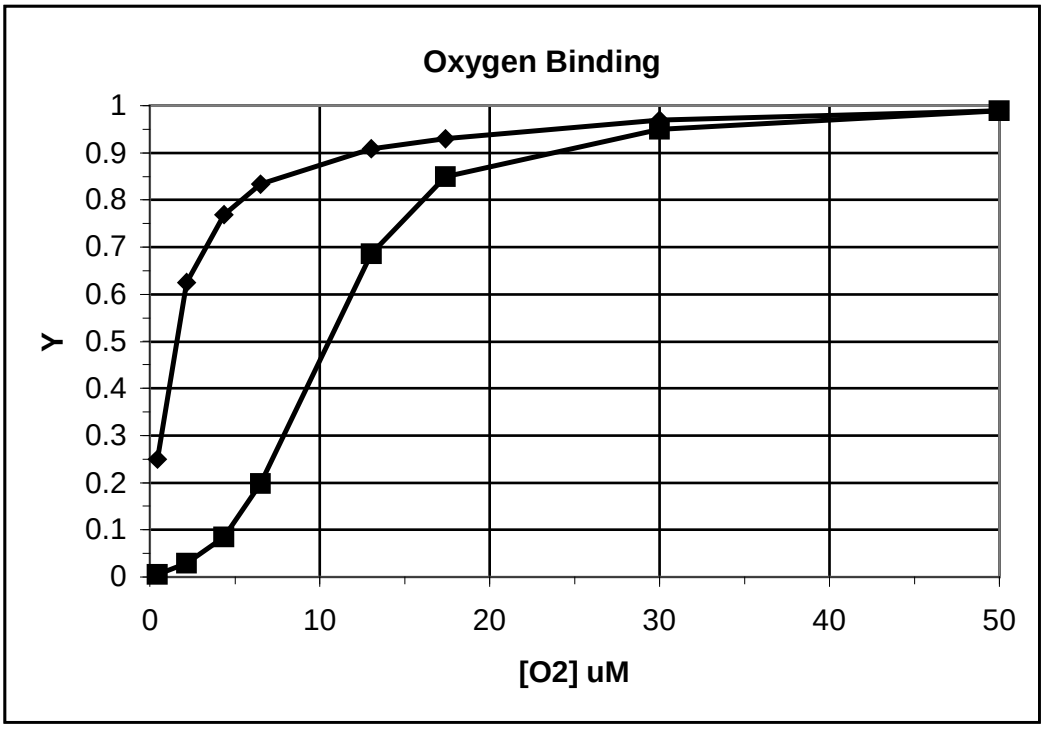
ii) If a solution of the mutant hemoglobin was at an oxygen concentration to give Y=0.5, describe, or draw, the distribution of oxygen molecules on the tetramer. (Feel free to use the diagram to the right). Justify your answer with clear reference to the Hill coefficient for the mutant hemoglobin (4 pts).



continued on next page.

Question C1, continued....

iii) On the basis of this Hill plot, sketch the oxygen binding curves for the mutant hemoglobin. The oxygen binding curves for myoglobin and hemoglobin are shown on the graph below. Draw the curve as accurately as possible, reflecting the K_D value as well as the degree of cooperativity for the mutant hemoglobin. (4 pts).

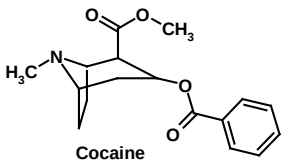


iv) Using your sketch from *part iii*, briefly discuss whether this mutant hemoglobin would be more or less efficient at delivering oxygen to the tissues. You can assume that the concentration of oxygen in the lungs is 50 uM and that in the tissues it is 10 uM (4 pts).

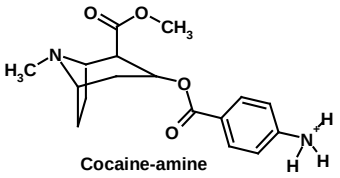
Part D: (16 pts) Quantitative analysis of enzyme kinetics or ligand binding.

Please attempt **ONE** of the following two questions.

Choice A:



Cocaine



Cocaine-amine

An antibody that is used clinically to treat cocaine overdose **binds cocaine with a K_D of 1 μM**. The binding of a modified cocaine (cocaine-amide, right structure) to this antibody were tested using equilibrium dialysis. To do this experiment the F_{ab} fragment at a **concentration of 1μM** was placed in the dialysis bag and different amounts of cocaine-amine were placed in the solution outside of the dialysis bag. After equilibrium was reached, the concentration of cocaine-amine inside and outside the dialysis bag was measured, giving the following values:

Experiment Number	[Cocaine-amine] Outside	[Cocaine-amine] Inside
1	1.0 μM	1.10 μM
2	10 μM	10.5 μM

i) Calculate the fractional saturation for *each* of the above data points (2 pts)

ii) (4 pts)Determine the K_D for the binding of cocaine-amine to the antibody.
You answer should consist of:

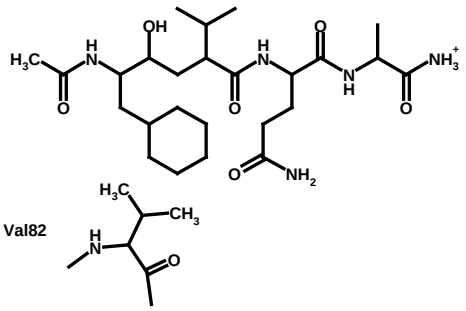
- The approach you took to obtain K_D.
- A list of alternative approaches that you could have taken to obtain the K_D

iii) Based on your answer to *part ii*, does cocaine-amine bind more tightly, or weaker, to the antibody? Justify your answer. (2 pts)

iv) Regardless of your answer to *part iii*, how might you modify the structure of the antibody to *enhance* the binding to cocaine-amine and *reduce* the binding to cocaine? Be explicit and draw a diagram showing the potential interaction between the antibody and the cocaine-amine. *Use the back of the previous page to answer this question.* (8 pts)

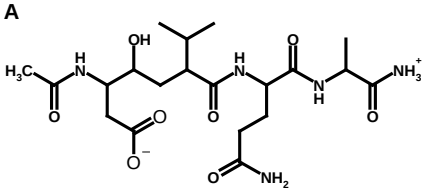
Choice B:

The drawing to the right shows an potent HIV protease inhibitor interacting with Val82 from the enzyme. The K_I for this inhibitor is 1 nM. A mutant HIV protease has arisen in an infected individual and drug A and drug B were tested to see which one would be the better drug to treat this patient. Initial velocity data was acquired as a function of substrate in the absence of inhibitor, or in the presence of 5 nM of drug A, or in the presence of 5 nM of drug B. These data were plotted on the double reciprocal plot shown to the right. The equations for each line are given on the chart. Please answer the following questions.

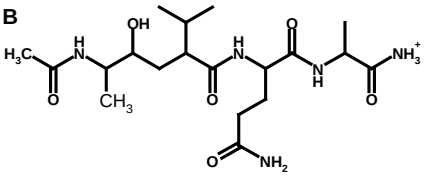


Val82

Drug A

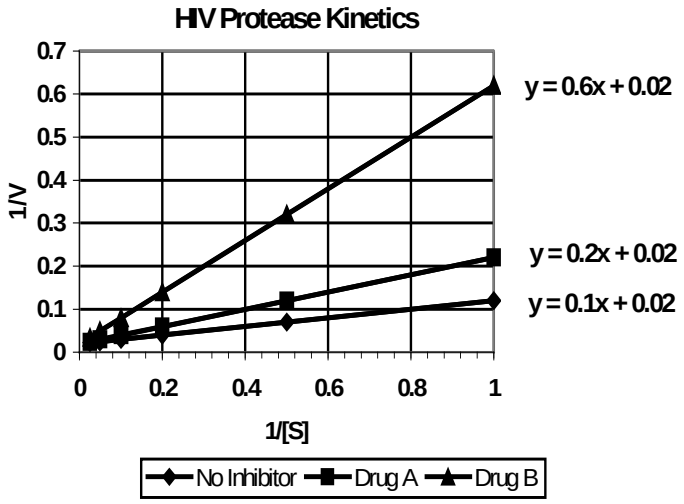


Drug B



i) Obtain the K_I values for each drug. Please show your work. (4 pts).

ii) Why are the K_I' values meaningless for these drugs? (2 pts).



iii) Which drug binds to HIV protease more tightly? Justify your answer using your K_I values (2 pts).

iv) On the basis of your K_I data, and the structures of the two drugs, provide a plausible suggestion for the amino acid side chain that has replaced Val82 in the mutant enzyme. Be explicit and draw a diagram showing the potential interaction between the enzyme and the drug. *Use the back of the previous page to answer this question.* (8 pts).