NAME:_____

Biochemistry I, Spring Term 2004 - Second Exam:

This exam has a total of 100 points and is divided into two sections. You must do ALL of the questions, but in many cases you have choices. There are a total of 9 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. Use the space provided to answer the questions.

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
30,000	4.48
50,000	4.70
70,000	4.85
90,000	4.95
100,000	5.00

NAME:_____

Section A (21 pts): (3 pts/question). Circle the letter corresponding to the *best* answer.

Part A:	_____/21
B1	_____/15
B2	_____/ 8
B3	_____/12
B4	_____/14
B5	_____/20
B6	_____/10
TOTAL	_____/100

1. One ligand binds more tightly to a protein than another ligand if:
   a) its K_A (K_{EQ}) is lower than the other ligand.
   b) its K_D is lower than the other ligand.
   c) its Hill coefficient is >1.
   d) its Hill coefficient is <1.

2. A ligand that binds more tightly to a protein than another ligand probably has a
   a) a slower kinetic on-rate
   b) a faster kinetic on-rate.
   c) a faster kinetic off-rate.
   d) a slower kinetic-off rate.

3. If protein binds two ligands in a *non-cooperative* manner, then:
   a) the dissociation constant is [L] when Y=0.5/2
   b) the dissociation constant is [L] when Y=0.5
   c) the dissociation constant is [L] when Y=0.5*2
   d) the dissociation constant cannot be determined from a binding curve in this case.

4. The oxygen bound to hemoglobin or myoglobin is directly attached to the
   a) helix-F in the protein.
   b) the proximal histidine.
   c) the iron atom.
   d) the heme group.

5. Enzymes increase the rate of chemical reactions by
   a) providing suitable catalytic groups.
   b) increasing the population of the transition state.
   c) decreasing the free energy difference ΔG^o of the transition state.
   d) all of the above.

6. HIV protease and Chymotrypsin are similar in that:
   a) both use Serine as the nucleophile.
   b) both are monomeric proteins.
   c) both cleave hydrophobic containing peptides.
   d) both use Aspartate to activate the nucleophile.

7. SDS gel electrophoresis gives the _____ molecular weight and Gel filtration provides the _____ molecular weight.
   a) native, denatured.
   b) denatured, native.
   c) native, native.
   d) denatured, denatured.

NAME:_____

B1. Please answer **three** of the following **six** questions (15 pts total)

1. BPG (bisphosphoglycerate) is involved in the adjustment of Oxygen delivery at high altitude. *Briefly* explain how this works.

2. What is the purpose of a Scatchard plot? When is it appropriate to use it and when is it not?

3. What is the main purpose of a Hill Plot? What two parameters can be obtained from a Hill Plot?

4. What is the difference between microscopic and macrosopic binding constants. Use the example of a protein that binds two ligands in your answer.

5. If the ΔH^o for the binding of a ligand is negative, will the binding constant ($K_A = K_{EQ}$) increase or decrease as the temperature is raised? Briefly justify your answer.

6. How does an antibody differ from an enzyme? How are they similar?

NAME:_____

B2. Answer **one** of the following **two** questions (8 pts)

Choice A:

i) Calculate the fractional saturation given the following equilibrium dialysis data (show your work) (4 pts)

1. Total concentration of protein (macromolecule) inside the dialysis bag: 1 μM
2. Ligand concentration outside the bag: 5 μM
3. Total ligand concentration inside the bag: 6 μM

ii) Is this information sufficient to obtain K_D? Briefly Justify your answer.(4 pts)

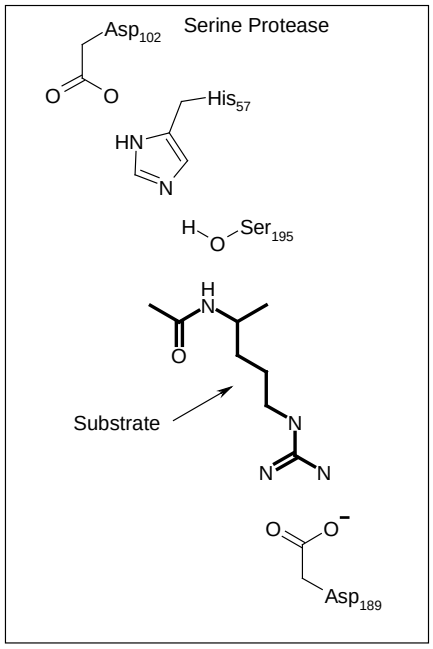
Choice B:

What is the *highest* possible value of the Hill coefficient for hemoglobin? Why? (4 pts) If hemoglobin actually had such a Hill coefficient, do you expect to find any partially liganded species when oxygen is present (e.g. [Hb-O_2], [Hb-$(O_2)_2$], [Hb-$(O_2)_3$])? Justify your answer.(4 pts)

B3. Briefly describe how allosteric effects can be used to control biological processes. Your answer should include a discussion of tense (T) and relaxed (R) states. Give either a *specific* or a *general* example of how allosteric effects can modify the biological function of a protein (12 pts)

NAME:_____

B4: The figure to the right shows the active site region of the serine protease trypsin.(14 pts).



Part A: Select **three** of the following **four** residues and briefly discuss its role in the overall catalytic mechanism of the enzyme (12 pts).

i) Asp102

ii) His57

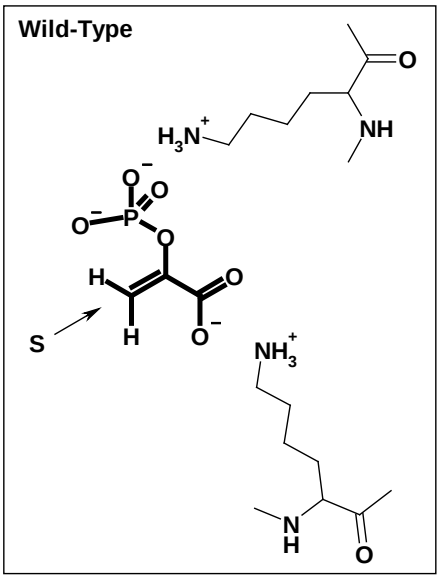
iii) Ser195

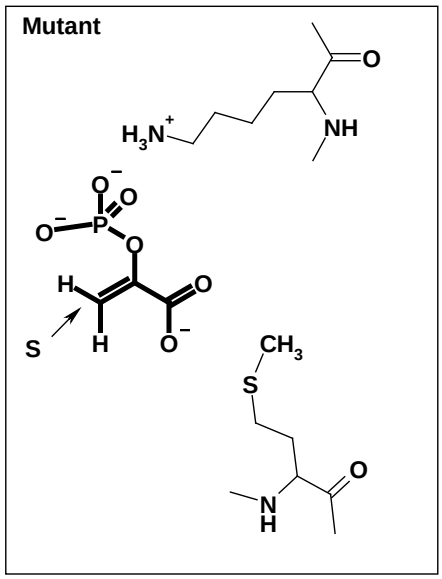
iv) Asp189

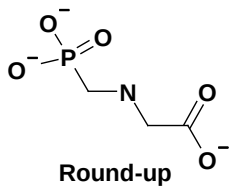
Part B: Which one of the above four residues performs the same role as the deprotonated Aspartate 25 in HIV protease? (2 pts).

NAME:_____

B5: (20 pts) The left panel shows a substrate bound in the active site of the enzyme that is responsible for the synthesis of phenylalanine in plants. This enzyme is readily inhibited by 'Roundup', a widely used herbicide. The bound substrate is shown in thick lines and labeled with 'S' and residues that belong to the enzyme are shown in thin lines. The right panel shows a mutant enzyme that has been isolated from plants that are resistant to 'Roundup'. The structure of Roundup is shown to the far right.



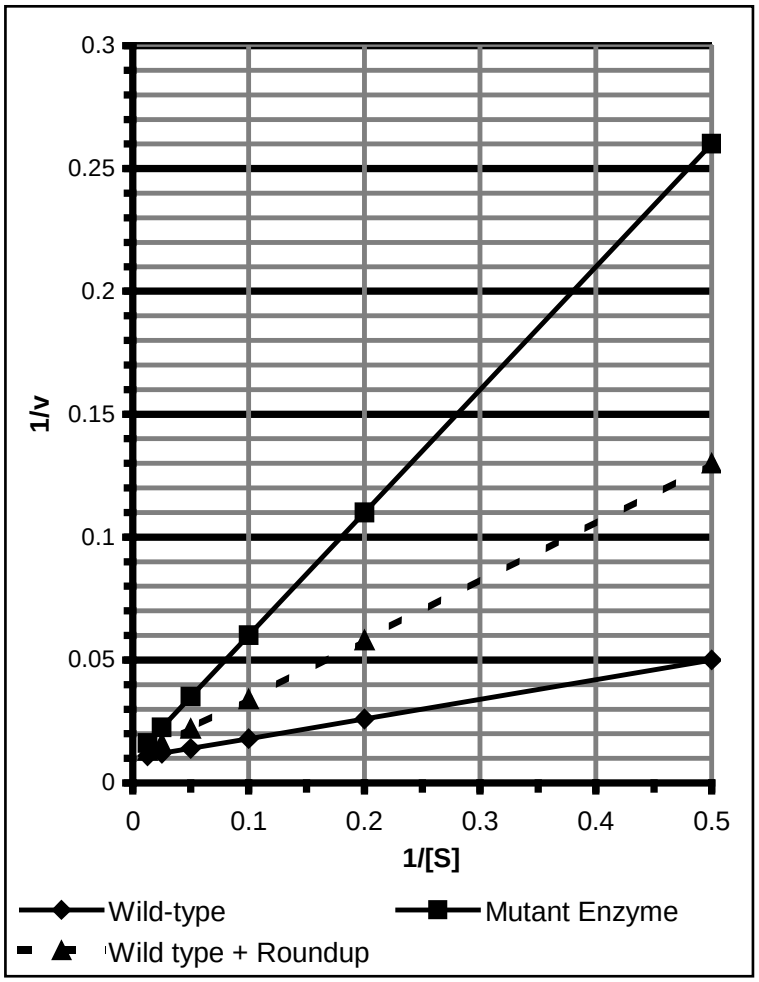




i) How does the mutant enzyme differ from the wild-type enzyme? (2 pt).

ii) The enzyme velocity data of the wild-type and mutant enzyme have been plotted on a double reciprocal plot (**solid lines**) shown to the right. Both of these plots are in the ***absence*** of the inhibitor. (The dotted line is the wild-type enzyme in the presence of the inhibitor.)

Has the mutation affected K_M or V_{MAX} of the enzyme? (It is not necessary to obtain numerical values, but explain how you arrive at your answer.(4 pts)



iii) Based on your answer to *part ii)* does the mutation affect the ability of the enzyme to bind the substrate or the enzyme's ability to catalyze the chemical reaction? Justify your answer (3 pts).

iv) What type of inhibitior is Roundup - a competitive one or a non-competitive inhibitor? Justify your answer with reference to the *chemical structure* of Roundup *as well* as the curve plotted on the figure on the previous page. (4 pts)

v) Determine either K_I or K_I and K_I' (depending on the type of inhibitor) from the data presented in the graph on the previous page. The concentration of inhibitor was 10 μM in the experiment (3 pts)

vi) Roundup binds poorly to the mutant enzyme, How might you modify Roundup such that it will become a more effective inhibitor of the mutant enzyme and therefore be able to kill the plant? If you are successful in the redesign of Roundup, how will the K_I values change? Will they increase or decrease? Why? (4 pts)

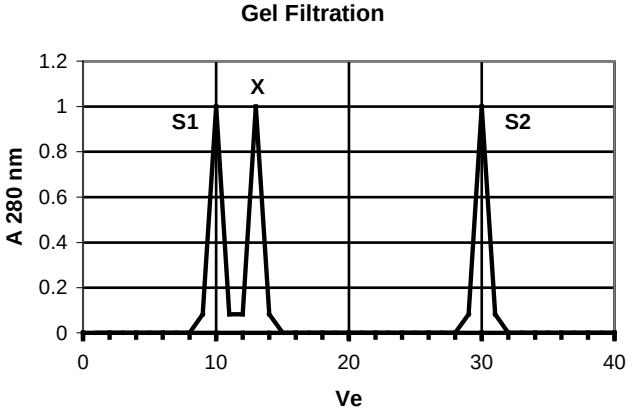
NAME:_____

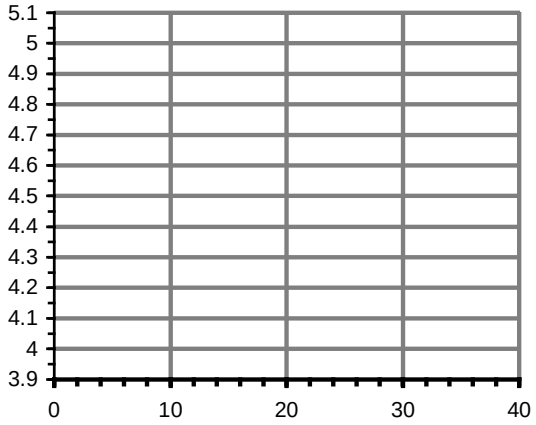
B6 (10 pts)**:** Do **one** of the following **three** choices:

Choice A: Biochem Bob is trying to purify a single protein from a complex mixture of proteins. He knows the protein that he is trying to purify has a large number of Aspartic and Glutamic acid residues, and no Lysine, Arginine, or Histidine residues. He loads the mixture onto a _____ exchange column at pH 7.0. Much to his dismay, his protein cannot be washed off (eluted) from the column. He asks you what to do, and you say add _____ or _____ and your protein will come off the column..

Fill in the three blanks (3 pts), explain why Bob's protein remains bound to the column at pH 7.0 (3 pts), and discuss how *each* of your suggestions will release his protein from the column (4 pts).

Choice B: You are trying to determine the quaternary structure of protein X. You run the protein on a gel filtration column and SDS-page Gel electrophoresis. The SDS-page gel electrophoresis shows a *single* band from your protein, giving a molecular weight of 10 KDa (10,000 Da). The elution profile of the gel filtration column is shown below. The peaks labeled S1 and S2 correspond to 100 KDa and 10 KDa standards, respectively. A table of logs can be found on the face page. What is the quaternary structure of this protein? Briefly explain your approach (6 pts) and label the axis of any plots that you use (2 pts).

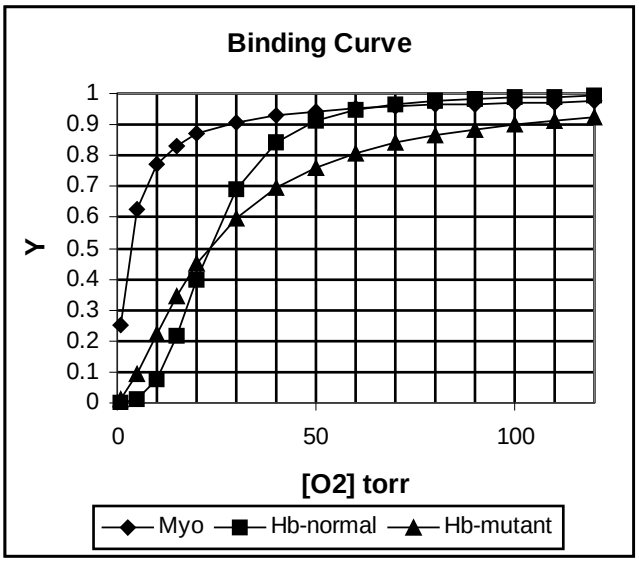




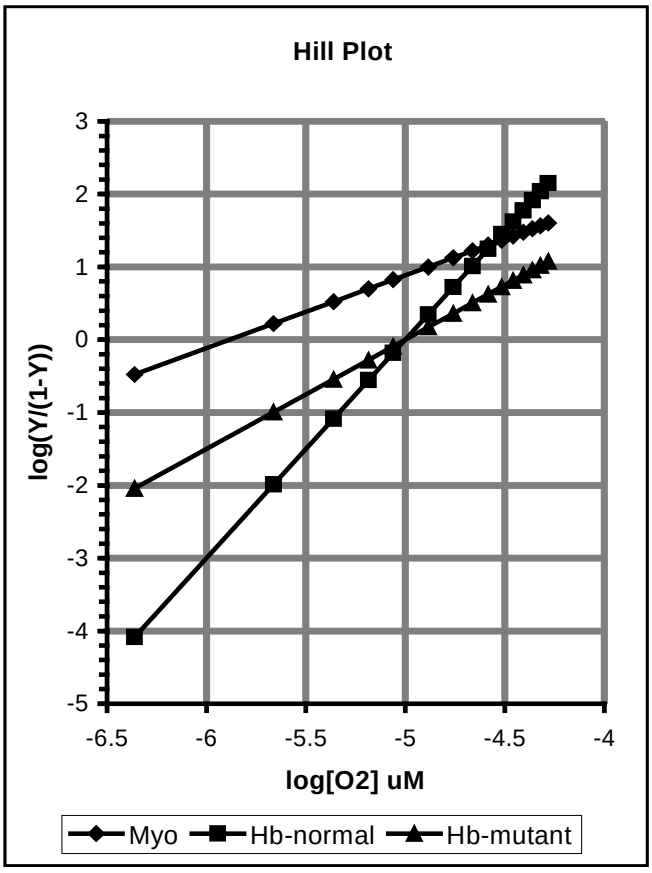
Can you say anything about the presence or absence of disulfide binds in protein X (2 pts)? Why?

NAME:_____

Choice C: The binding curves and corresponding Hill plot shows data for the binding of oxygen to Myoglobin, normal Hemoglobin, and a mutant Hemoglobin.



i) Obtain the Hill coefficient, n_h, for the mutant hemoglobin. Please show your work (2 pts).



ii) Is the mutant hemoglobin more or less cooperative than normal hemoglobin? (Normal hemoglobin has a Hill coefficient of 3.0.) Justify your answer, with reference to either the Hill coefficients or the binding curves.(3 pts)

iii) Discuss how this change in cooperativity would effect oxygen delivery to the tissues by the mutant enzyme. You may find it useful to refer to the binding curves. Oxygen levels in the lungs are 100 torr, while those in the tissue are about 20 torr. (5 pts)