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Biochemistry I, Spring Term 2002 - Second Exam:

This exam has a total of 100 points and is divided into two sections. You must do ALL of the questions. There are a total of 8 pages in this exam. 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.

Part A: _____

B1 _____

B2 _____

B3 _____

B4 _____

B5 _____

B6 _____

B7 _____

TOTAL _____

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

$\Delta G^O = -RT\ln K_{eq}$
$\Delta G = \Delta H - T\Delta S$

$\mu = \mu^O + RT\ln[X]$
$S = R\ln W$

Ligand Binding:

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

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

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

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:

$A = \varepsilon Cl$

$pH = pK_a + \log([A^-]/[HA])$

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Section A (24 pts): (3 pts/question). Circle the letter corresponding to the *best* answer.

1. The binding affinity of a ligand to a protein is affected by temperature if:
   a) the enthalpy of binding is zero.
   b) the entropy of binding is zero.
   c) the enthalpy of binding is not zero.
   d) the entropy of binding is not zero.

2. In comparing the binding of two drugs to an enzyme, one would compare
   a) the Hill coefficient, n_h, of each drug.
   b) the K_M of each drug.
   c) the pK_a of each drug.
   d) the K_I values of each drug.

3. Equilibrium dialysis can be used to
   a) measure K_D.
   b) measure K_I.
   c) measure K_I'.
   d) a) and b)

4. Myoglobin _____ while hemoglobin _____. (Make this true using the statements below)
   a) binds oxygen with positive cooperativity, does not
   b) is an enzyme, is not
   c) binds oxygen, binds oxygen with positive cooperativity
   d) binds bisphosphoglycerate (BPG), does not.

5. The active site of an enzyme
   a) binds oxygen with positive cooperativity.
   b) contains residues important for catalysis.
   c) is the binding site for non-competitive inhibitors.
   d) is the binding site for allosteric activators.

6. The analysis of enzyme kinetics using steady-state methods
   a) assumes $d[ES]/dt = 0$.
   b) provides an accurate description of the reactions at all times.
   c) can only be used if the product does not inhibit the enzyme.
   d) cannot be applied when inhibitors are present.

7. The specific activity of an enzyme
   a) the rate of product production over time.
   b) the total mass of the protein in a purification.
   c) should increase during a purification.
   d) is not a useful criteria for monitoring purity.

8. In gel filtration chromatography, proteins are separated on the basis of their:
   a) molecular weight.
   b) positively charged sidechains.
   c) different isoelectric points.
   d) negatively charged sidechains.

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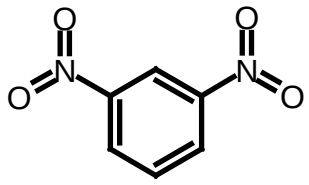
Section B:

B1. (10 pts) Two rival companies make an enzyme that degrades highly toxic chlorinated biphenyls to non-toxic compounds. The steady state kinetic properties of the two enzymes are given below. Given that the concentration of chlorinated biphenyls in landfills are approximately 0.1 μM, which company has the better enzyme? Justify your answer with a quantitative calculation of the efficiency, or velocity, of each enzyme at this substrate concentration ([S]=0.1μM).

	K_M	V_{MAX}
Company A	1 μM	50 μM/sec
Company B	10 μM	500 μM/sec

B2. (8 pts) Dinitrophenyl (DNP) binds to its antibody via non-polar interactions between itself and two Tryptophan (Trp) residues on the antibody. The binding constant (K_{EQ}) of DNP is 10^{6} M^{-1}.



Dinitrophenyl

i) Calculate the free energy (ΔG^{o}) associated with the binding of DNP to the antibody at T=300K.(5 pts)

ii) Assume that one of the Trp residues in the binding site is replaced by an Alanine, calculate the free energy for the binding of DNP to this *altered* antibody. (Assume that the free energy to transfer one Trp from a non-polar solvent to water is +10 kJ/mol and that for Alanine is +2 kJ/mol). State any assumptions you have made in your calculation. (3 pts)

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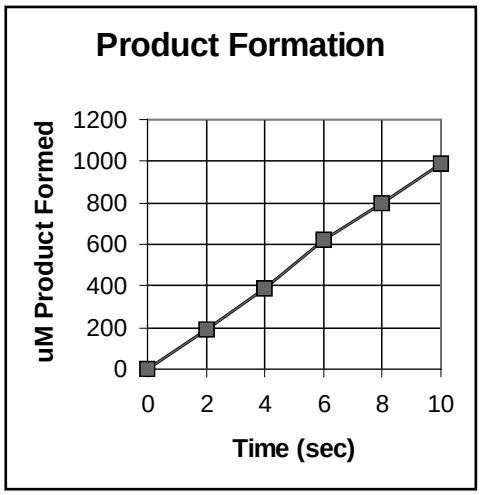
B3: (10 pts) Do **EITHER** Part A or Part B.

Part A) *Enzyme kinetics*: A plot of product produced as a function of time is shown to the right. You can assume that the reaction is in steady-state over the entire time period.



i) Calculate the velocity of the enzyme reaction, including units.(3 pts)

ii) Given that V_{MAX} is 100 μM/sec, what was the substrate concentration in this reaction? If you feel that you cannot obtain [S], state why (3 pts).

iii) Sketch, in the above graph, the curve you would expect to observe if a <u>non</u>-competitive inhibitor was added at a concentration *equal* to its K_I'. Briefly justify your logic below.(4 pts)

OR

Part B) *Ligand binding*: The total concentration of a protein within a dialysis bag is 10μM ([M]+[ML] = 10μM). This dialysis bag is placed in a beaker containing buffer and ligand is added to the buffer outside the dialysis membrane. After equilibrium is reached the concentration of the ligand outside the dialysis bag is 30 μM while the concentration of the ligand inside the dialysis bag is 40μM. Assume that the protein contains one binding site for the ligand.

i) What is the free ligand concentration, [L], inside the dialysis bag? (2 pts)

ii) What is the concentration of bound ligand, [ML], inside the dialysis bag.(3 pts)

iii) Calculate the fractional saturation for this ligand concentration.(1 pt)

iv) What is K_D for this binding reaction? If you feel that you cannot obtain K_D, please state why.(3 pts)

v) How would your answer to iii) change if this protein had <u>two</u> binding sites for this ligand?(1 pt)

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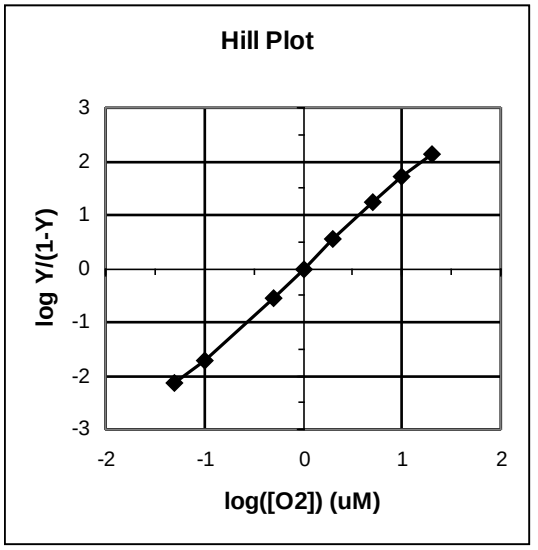
B4:(12 pts) A Hill plot for the binding of oxygen to Hemoglobin from clams is shown to the right. Clam hemoglobin is dimeric, with N=2 binding sites. The binding data that generated this plot is shown below:



$[O_2]$ μM	Y
0.05	0.007
0.10	0.019
0.50	0.223
1.00	0.500
2.00	0.778
5.00	0.945
10.00	0.980
20.00	0.992

i) What is the average K_D for oxygen binding to this hemoglobin? Explain how you arrived at your answer. (6 pts).

ii) Does the binding of O_2 to clam hemoglobin show negative cooperativity or positive cooperativity? Justify your answer. How does its cooperativity compare to that of human hemoglobin (n_h=3). (4 pts)

iii) Myoglobin from clams is similar to myoglobin from humans. It binds oxygen with a 10 fold *higher* affinity than hemoglobin. *Sketch* the Hill plot for the binding of O_2 to clam myoglobin on the above plot (2 pts).

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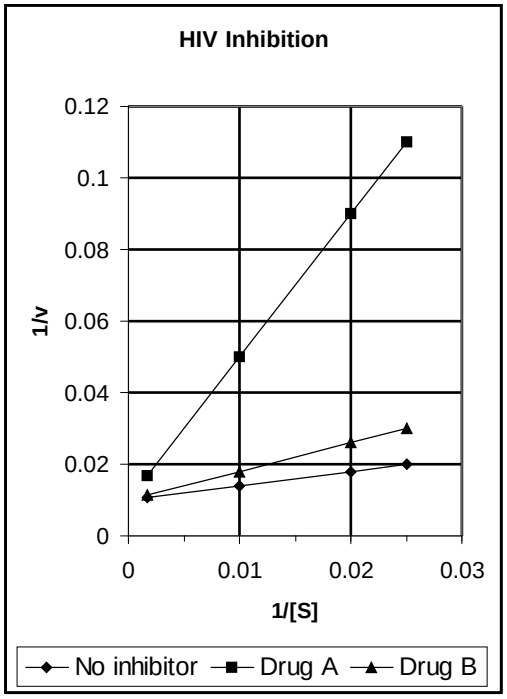
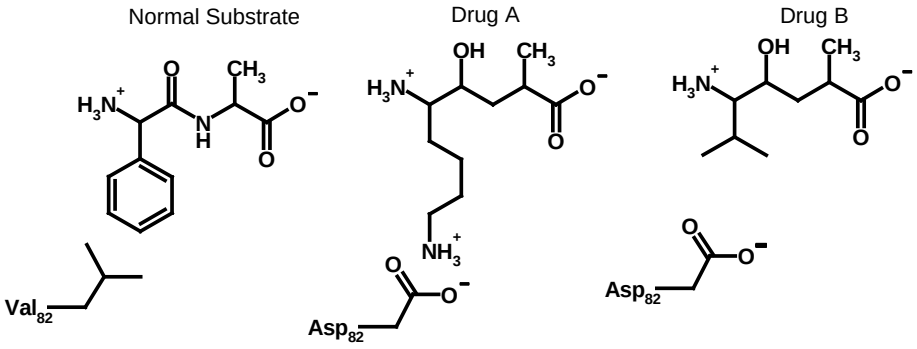
B5:(16 pts) A mutant of the HIV protease that arises after treatment of patients with HIV protease inhibitors has the amino acid Valine82 replaced by Aspartic Acid. A double reciprocal plot of the inhibition of the mutant HIV protease with two protease inhibitors (A and B) is shown to the right. The concentration of both inhibitors is 1 nM.



The interaction of the normal substrate with the non-mutant HIV protease (Val82) is shown below (left diagram). The structures of Drug A and Drug B are shown (middle and right) interacting with the mutant HIV protease (Valine 82 replaced by Aspartic acid).



i) In the left-hand diagram of the normal substrate, place an 'X' through the bond that would normally be cleaved by the HIV protease (2 pts)

ii) Are these drugs competitive or non-competitive inhibitors? Justify your answer.(6 pts)

iii) On the basis of the data presented in the double reciprocal plot, which drug is the better inhibitor? (Although you may find it useful to calculate K_I values, you may answer the question by a comparison of relative values).(4 pts)

iv) Is your answer to part iii consistent with the interaction between the drugs and the mutant enzyme? Briefly justify your answer with reference to inter-molecular forces commonly found in biochemistry.
If you could not do section iii, infer what would be the better inhibitor based on the structures shown above.(4 pts)

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B6: Do **EITHER** Part A, Part B, **or** Part C in the space below. (8 pts)

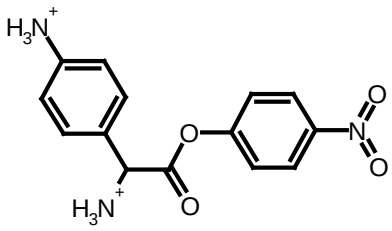
Part A. The concentration of bis-phosphoglycerate (BPG) increases in your blood at high altitude, leading to a change in the binding of oxygen to hemoglobin. Briefly explain how the binding of BPG to hemoglobin changes the oxygen binding properties of hemoglobin.

OR

Part B. Discuss the role of proton ($[H^+]$) binding to hemoglobin in enhancing the delivery of oxygen to exercising muscle.

OR

Part C: Consider the small organic molecule shown to the right:



Which enzyme, Trypsin or Chymotrysin, would show a *lower* K_M with this substrate? Justify your answer based on your knowledge of the active site of these two enzymes.

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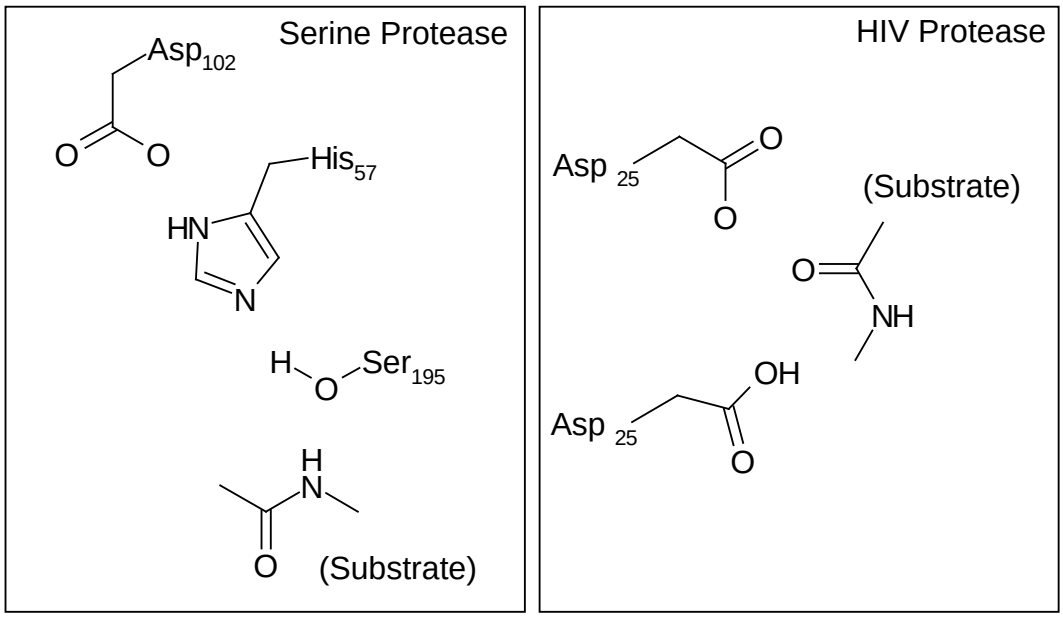
B7: (12 pts) The following diagram shows the active site region of Serine proteases (left) and the HIV protease (right).



Please answer **ONE** of the following three questions. Be sure to discuss the role of transition state stabilization in your answer, regardless of the question you choose.

Part A: Compare and contrast the mechanism of these two enzymes.

OR

Part B: Give a detailed description of the mechanism of Serine Protease.

OR

Part C: Give a detailed description of the mechanism of HIV Protease.