Biochemistry I, Spring Term 2003 - 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 8 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 $([E]+[S]<-->[ES]-->[E]+[P])$
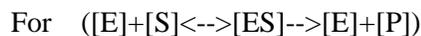
$V_{max} = k_2[E_T] = k_{cat}[E_T]$
$K_M = (k_{-1}+k_2)/k_1$

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

Double reciprocal plot:

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

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

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

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

$\alpha'=1$ for competitive inhibition
$\alpha'>1$ for non-competitive inhibition

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

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

General Thermodynamics:

R=8.3 J/mol-deg
T=300K, RT=2.5 kJ/mol @ 300K

$\Delta G^O = -RTlnK_{eq}$
$\Delta G = \Delta H - T\Delta S$

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

$\mu=\mu^O+RTln[X]$
$S=RlnW$

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
$Y/[L] = -Y/K_D + 1/K_D$
$v/[L] = -v/K_D + n/K_D$

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

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: $A=\varepsilon[X]l$

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

Part A:	_____/24
B1	_____/11
B2	_____/ 5
B3	_____/24
B4	_____/26
B5	_____/10
TOTAL	_____/100

1. Once a ligand dissociation constant (K_D) has been determined for non-cooperative binding it is possible to calculate or determine:

    a) the microscopic ligand binding constant (K_{EQ}).

    b) the ΔG^o for the binding interaction.

    c) the concentration of ligand required for half-maximal occupancy.

    d) all of the above.

2. In both hemoglobin and myoglobin the oxygen is bound to.

    a) the iron atom in the heme group.

    b) the manganese atom in the heme group.

    c) a hydrophobic pocket in the protein.

    d) the same binding site as bis-phosphoglycerate.

3. The conformational changes from the T to the R state

    a) results in a decrease in the binding affinity of oxygen to hemoglobin.

    b) causes loss of heme from hemoglobin.

    c) results in an increase in the binding affinity of oxygen to hemoglobin.

    d) is only found in oxygen binding proteins.

4. Cooperative binding of oxygen that occurs in hemoglobin

    a) minimizes oxygen delivery to the tissues.

    b) maximizes oxygen delivery to the tissues.

    c) can also be observed in myoglobin.

    d) answer b and c.

5. Which of the statements regarding enzymes is false?

    a) Enzymes are usually proteins that function as catalysts.

    b) Enzymes are usually specific.

    c) Enzymes may be used many times for a specific reaction.

    d) The active site of an enzyme remains rigid and does not change shape.

6. The nucleophile that is used in *both* serine proteases and HIV protease is:

    a) Ser.

    b) Asp.

    c) His.

    d) Water.

7. Both competitive and non-competitive inhibitors.

    a) bind to the [ES] complex.

    b) resemble the substrate.

    c) can be used as drugs.

    d) increase the reaction velocity at low concentration.

8. During any successful purification scheme, you would expect

    a) that several dozen steps would be required to purify a protein.

    b) the activity of the enzyme to increase.

    c) the specific activity to increase.

    d) the specific activity to decrease.

B1. (11 pts) The serine protease chymotypsin catalyzes the hydrolyses of the following two substrates, with the indicated kinetic parameters. The structure of these two substrates is shown to the right of the table.

Substrate	k_{cat} (sec^{-1})	K_M (μM)	k_{cat}/K_M (sec^{-1}M^{-1})
Arg-Ala	50.0	100	500,000
Tyr-Ala	8.0	1	8,000,000
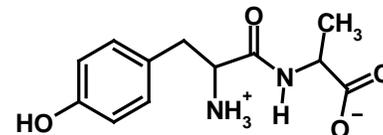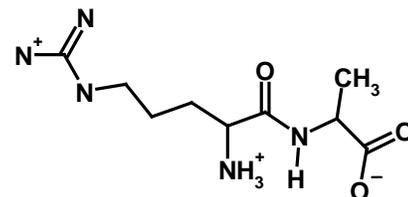
i) Which substrate would produce the most product in a given period of time, assuming that the substrate concentration was 0.01 μM. Justify your answer with either a calculation of the reaction velocities or an approximation of the rates based on limiting conditions (e.g. high or low [S]) Hint $v = \dfrac{E_t k_{CAT}[S]}{K_M + [S]}$. (6 pts)

ii) Which of the two substrates in the above table bind to chymotrypsin with the lowest affinity (i.e. weakest binding)? Justify your answer with reference to the data in the table. Explain this observation based on your knowledge of the active site of chymotrypsin. A simple annotated sketch of the active site will do (5 pts).

B2. (5 pts) What is the *general* mechanism for the acceleration of a chemical reaction by an enzyme? You may use a specific reaction mechanism in your discussion if desired.

Normal Substrate   Drug

B3. (24 pts) The left structure shows the interaction between a normal substrate for HIV protease and the wild-type (naturally occurring) enzyme. The right structure shows the complex formed between a mutant HIV protease (change of the amino acid at position 82) with a drug designed to inhibit the mutant enzyme.

You must do _all four parts_ of this question.

i) What type of inhibitor is this drug, competitive or non-competitive? Your answer should indicate why this particular compound is **not** a substrate _as well as_ discussing why the drug is a competitive or non-competitive inhibitor. It is insufficient to simply state the type of inhibition, you must justify your answer by reference to the structure of the drug (8 pts).
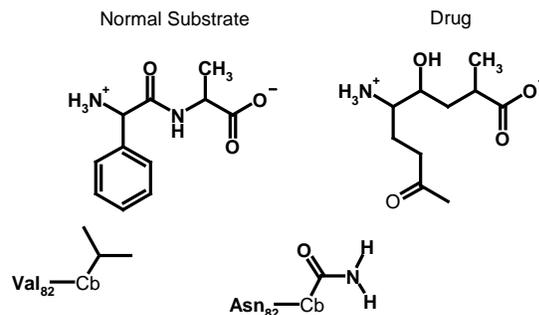
ii) The double reciprocal plot shown in the right margin shows enzyme kinetic data for the _mutant_ enzyme that was obtained in the absence of inhibitor. What is V_{MAX} ? Please show your work (4 pts).
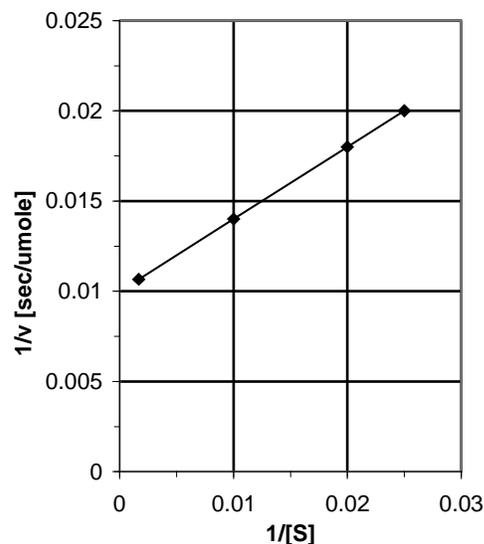
iii) Assume that the drug acts as a competitive inhibitor and that the K_I for the drug is 10 nM. Draw the double reciprocal plot that you would expect to obtain if the inhibitor concentration was equal to **10** nM. Use the graph from part _ii_ for your plot. When you draw your graph you should take care in representing both the slope and the y-intercept as accurately as possible. Use the space below for any calculations (6 pts).
Hint: $\alpha=1+([I]/K_I)$

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B3, Continued:

iv) Do choice A **or** choice B. Answer both parts of A. (6 pts) .

Choice A: The diagram from the previous page has been copied
to the right. The dissociation constant (K_D) for the binding of
the normal substrate to the wild-type enzyme and the binding
of the inhibitor to the mutant enzyme were measured using
equilibrium dialysis and were found to be 1 nM and 10 nM,
respectively. A plot of $\ln K_{EQ}$ versus $1/T$ has the same slope
for the normal substrate binding to the wild-type enzyme and
the drug binding to the mutant protein.

Normal Substrate   Drug

Val$_{82}$ K_D=1 nM   Asn$_{82}$ K_D=10 nM
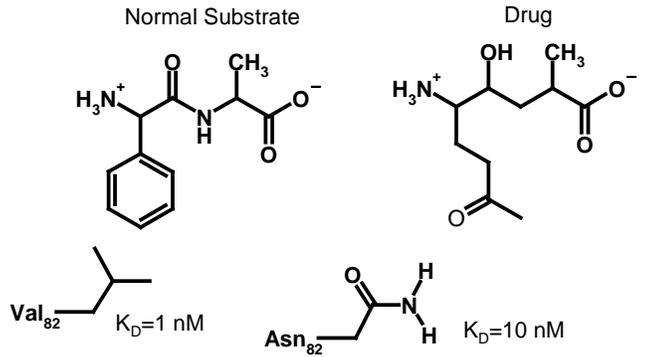
a) What does the fact that the slopes of the $\ln K_{EQ}$ versus $1/T$ are the same for both compounds tell you about the
ΔH^o for binding for both compounds to their respective enzymes? (2 pts)

b) Explain the molecular basis for the differences in the ΔG^o of binding for the normal substrate to the wild-type
enzyme versus the binding of the drug to the mutant enzyme. Your answer should include an explicit
description of the enthalpy and entropy of binding and how these two terms relate to the interaction between
the protein and the ligand. You need *not* calculate ΔG^o, a qualitative discussion will suffice. Hint: $\Delta G^o = \Delta H^o -$
$T\Delta S^o$. (4 pts)

OR

Choice B: If a new mutation arose in which the amino acid at position 82 was replaced
by a lysine residue, how might you alter the structure of the drug to maintain a high
binding affinity? If you do not remember the structure of lysine, indicate what you
think the structure is and then proceed with your answer. You may draw your answer
on the diagram to the right.. Be sure to briefly justify your answer with a discussion
of the relevant intermolecular forces between the altered drug and the Lysine
sidechain on the mutant protein. You should also indicate whether the increased
interaction would be due to changes in enthalphy (ΔH), entropy (ΔS), or both (6 pts).
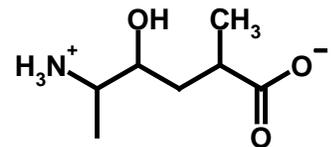
Lys$_{82}$

Hill Plot

B4. (26 pts) A pentameric protein (protein X) has a single binding site for a ligand on each of its subunits and therefore can bind a total of 5 ligands/pentamer. A Hill plot for the binding of the ligand to protein X is shown to the right.

You must attempt *all five* parts of this question.

i) What are the K_D and Hill coefficient (n_h) for binding of the ligand to this protein? Briefly explain how you arrived at your answer. (**Caution**: The scale on the y- and x-axis are not equal, calculate slopes carefully.) (6 pts)
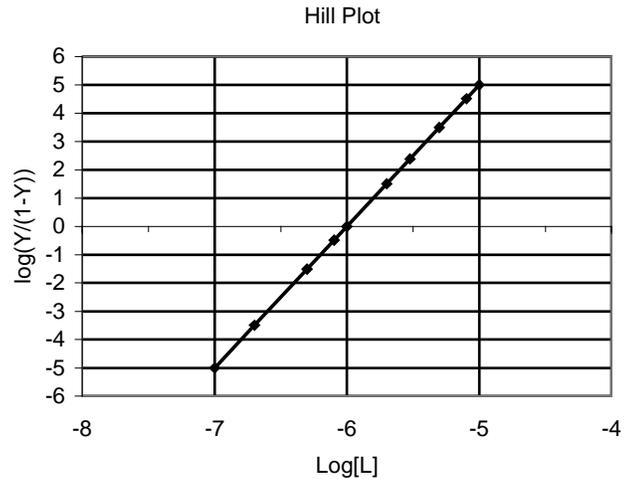
ii) A 4 uM solution of protein-X is placed in a dialysis bag. This concentration refers to the concentration of monomeric units, not pentamers. The free ligand concentration outside of the dialysis bag is adjusted such that it is equal to K_D. What is the *total* ligand concentration inside the bag? Please show your work.(4 pts)

iii) The diagram to the right represents a solution of protein-X (only four pentamers are shown). Illustrate the distribution of bound ligand when Y=0.5 by shading the protein molecules that have ligand bound (1 pt). Sample answers for Y=0.0 and Y=1.0 are provided at the bottom of the figure. Justify your answer (3pts) (Hint: What is n_h ?)
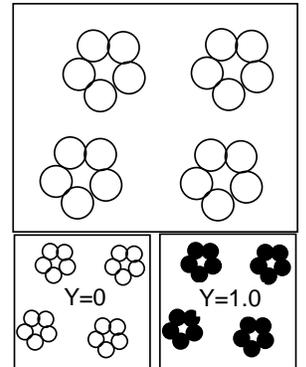
iv) A heterotropic allosteric compound *increases* the affinity of the ligand to protein X by 10 fold, but makes the binding *completely non-cooperative*. Answer *either* choice A *or* choice B, but not both.

    Choice A: Draw, on the plot above, the Hill curve you would expect to observe for the binding of the ligand to the pentamer in the presence of this allosteric compound. Justify your answer in the space below or on the back of page 5.(4 pts).

<div align="center">

OR

</div>

    Choice B: Describe, or sketch, the Scatchard plot for binding of the ligand to the pentamer that would be observed in the presence of this allosteric compound. What is the shape of the plot (e.g. linear, curved) and what is its slope? Use the back of page 5 if you need more room. (4 pts)

Problem B4, part v, is found on the next page.

Problem 4, Continued:

v) (8 pts) An SDS gel is run on protein X to determine its molecule weight. Two proteins of known molecular weights were included for calibration purposes. Standard 'A' had a molecular weight of 1,000 Da and standard 'B' had a molecule weight of 100,000 Da. The plot of log(MW) versus distance migrated is shown to the right. Please answer the following two questions.

a) If protein X migrated 6.0 cm from the top of the gel, what is its molecular weight? Explain how you arrived at your answer or illustrate your approach using the graph to the right. (4 pts)

SDS-Gel

Log(MW) versus Distance Migrated (cm)
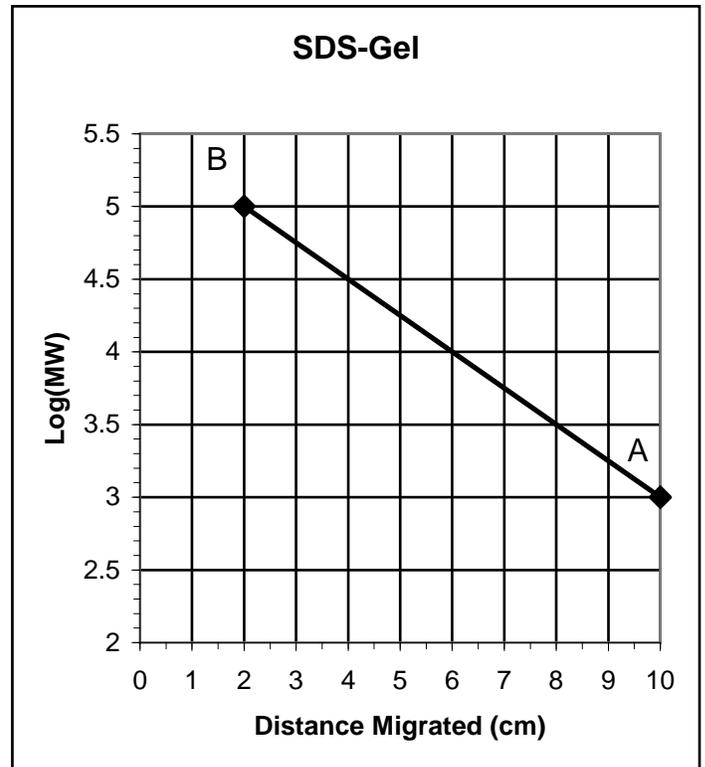
b) Is the above molecular weight equal to the molecular weight of the entire pentamer or individual subunits? Why?(4 pts)

B5: (10 pts) Do only **ONE** of the following five.

Oxygen Binding

Choice A: Sketch, on the graph to the right, the approximate oxygen binding curve for hemoglobin in presence of high levels of BPG (bis phosphoglycerate)(4 pts). The graph already contains the binding curve for hemoglobin under normal conditions of low BPG. Using these curves, explain how elevated levels of BPG are used to enhance oxygen delivery at high altitudes (6 pts)

OR

Choice B: Sketch, on the graph to the right, the approximate oxygen binding curve for myoglobin. (4 pts) The graph already contains the normal binding curve for hemoglobin. Using these curves, briefly explain how oxygen delivery to the tissues is accomplished by these two proteins. (6 pts)
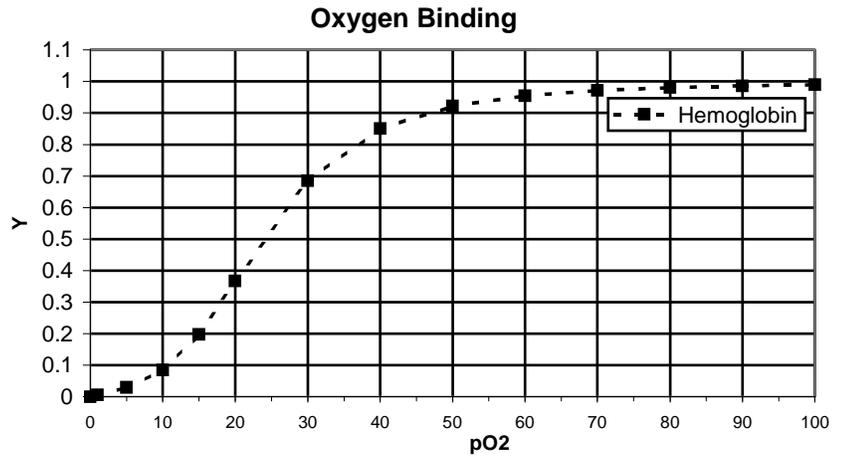
OR

Choice C: Sketch on the graph in the above right the approximate oxygen binding curve for *fetal* hemoglobin. (4 pts) The graph already contains the normal binding curve for adult hemoglobin. Using these curves, briefly discuss how the binding properties of fetal hemoglobin enhance the delivery of oxygen to the fetus.(6 pts)

OR

Choice D: The diagram to the right shows the active site region of a Serine protease and the HIV protease. Answer the following two questions.

  i) In what *two* ways are the reaction mechanisms of these two enzymes similar? (6 pts)

  ii) How do the reaction mechanisms of these two enzymes differ? (4 pts)

OR

Choice E: Select any one of the following four methods of column chromatograpy: gel filtration, cation exchange, anion exchange, or affinity chromatography, and provide a brief answer to *both* of the following questions. A simple annotated diagram is an acceptable answer.

  i) What property of the proteins are utilized in the separation by this form of chromatography? (5 pts)

  ii) How is the protein eluted, or washed, from the column?(5 pts)

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Use the back of pg 7 if you need additional space.