NAME KEY

MMBB 380 - Fall 2006
EXAM 2 - Part I

You may use a calculator for this portion of the test.

R = 8.314 J $K^{-1}mol^{-1}$ RT ln (x) = 5700 J/mol log (x) ln (x) = 2.30 log (x)

(16,000 pts)

1. Assume an enzyme catalyzes the reaction, S ⇌ P, and follows Michaelis-Menten kinetics. For a particular enzyme concentration, the K_M = 150 μM & the V_{max} = 230 μmol sec^{-1}. The molecular mass of the enzyme is 35,000 Da.

(8,000 pts)

a) If you begin the assay with [P] = 0.00 μM, what concentration of S will produce an initial rate (v) of 100 μmol min^{-1}?

v = 100 μmol/min (min/60 sec) = 1.67 μmol/sec

$v = \frac{V_{max}[S]}{K_M + [S]}$ $v/V_{max} = [S]/(K_M + [S])$

1.67 μmol/sec/(230 μmol/sec) = 0.00726 = [S]/(K_M + [S])

0.00726 K_M + 0.00726 [S] = [S]

0.00726 (150 μM) = 1.09 μM = (1.00-0.00726) [S] = 0.99 [S]

[S] = 1.09 μM/0.99 = 1.10 μM

If you incorrectly used 100 μmol sec^{-1}: [S] = 115 μM

(8,000 pts)

b) You determine that 1.00 mg of the enzyme was present in the above assays. Determine the k_{cat} of this enzyme.

$\frac{230\ \mu mol/sec}{1.00\ mg}$ = 230 μmol rxn sec^{-1} mg^{-1} = 230 x 10^{-6} mol rxn sec^{-1} mg^{-1}

$\frac{1.00\ mg}{(35{,}000\ g/mol)} \frac{1\ g}{(1{,}000\ mg)}$ = 2.86 x 10^{-8} mol in a mg of enzyme

$k_{cat} = V_{max}/[E_T] = \frac{230 \times 10^{-6}\ mol\ rxn\ sec^{-1}}{2.86 \times 10^{-8}\ mol}$ = 8050 sec^{-1}

(16,000 pts)

2. Creatine phosphate (phosphocreatine) can undergo the following reaction.

$$\text{Creatine phosphate} + H_2O \rightleftharpoons \text{creatine} + P_i \qquad \Delta G^o = -43.1 \text{ kJ/mol}$$

(10,000 pts)

a. You make a pure solution of 10.0 mM creatine phosphate and then add a trace amount of an enzyme that catalyzes the given reaction. Determine the equilibrium concentrations of creatine and creatine phosphate when the solution is maintained at a temperature of 25°C.

Because of the large ΔG, nearly all of the creatine~P will be converted to creatine & P_i

Thus, [creatine] = [P_i] = 10.0 mM = 0.0100 M

$\Delta G = \Delta G^o + 5700$ J/mol log [creatine] [Pi]/[creatine~P]

$0 = -43{,}100$ J/mol + 5700 J/mol log (0.0100 M) (0.0100 M)/[creatine~P]

$+43{,}100$ J/mol = 5700 J/mol log (1×10^{-4})/[creatine~P]

$\frac{43{,}100 \text{ J/mol}}{5700 \text{ J/mol}} = \log 10^{-4}/[\text{creatine~P}] = 7.56$

$10^{7.56} = 3.63 \times 10^7 = 10^{-4}/[\text{creatine~P}]$

[creatine~P] = $10^{-4}/3.63 \times 10^7 = 2.75 \times 10^{-12}$ M

(6,000 pts)

b. The enzyme catalyzing the above reaction would be classified as a (an): (circle one answer)

1. Oxidoreductase

2. Ligase

3. Hydrolase

4. Lyase

5. Transferase -3 k pts

6. Isomerase

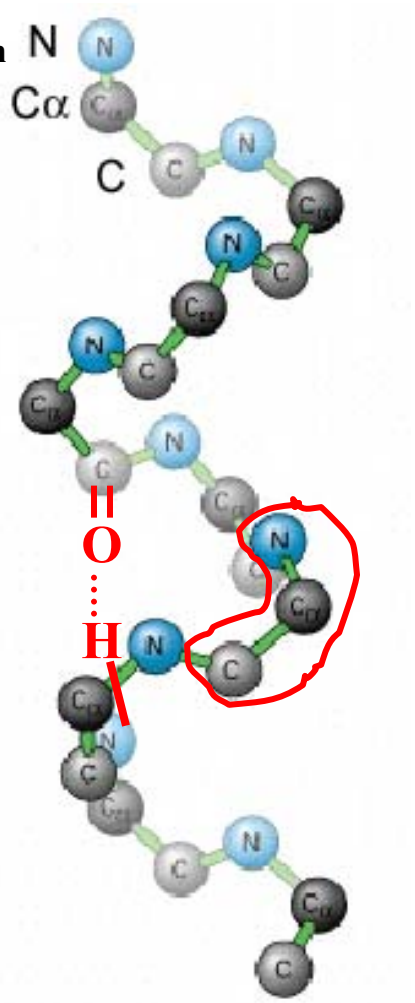
NAME KEY

MMBB 380 - FALL 2006
EXAM 2 - Part II

You may not use a calculator for this portion of the exam.
Please read and answer each question carefully. Good Luck!

(10,000 pts)

1) **Shown at the right is the polypeptide backbone structure of an alpha helix. The first amino acid backbone atoms are labeled where N is the amide nitrogen and Cα is the alpha carbon.**

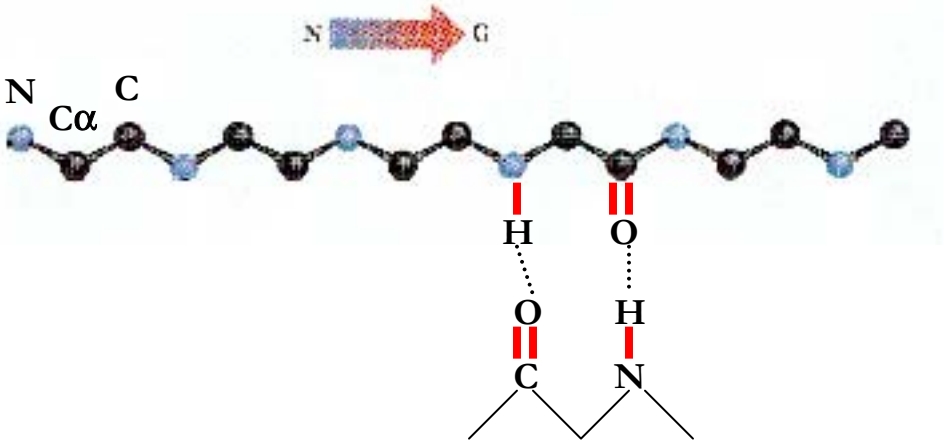


   a. **Identify amino acid #6 in this diagram by circling all backbone atoms attributed solely to residue #6.**

   b. **Using this diagram, show all hydrogen bonds that would be expected to involve amino acid # 4.**

(8,000 pts)

2) **The following represents a single β-strand. The polypeptide backbone atoms of the first amino acid are identified where N is the amide nitrogen and C is the carbonyl carbon. Use this diagram to show all β-strand hydrogen bonding that would be expected of amino acid #4. Show additional strands as needed.**



(6,000 pts)

3) What is the minimum number of amino acids that can participate in the β-turn of a protein? (Hint: think about how the β-turns use hydrogen bonding for stabilization)

A. Three -3 k pts

B. Four H-bonding occurs between amino acid 'n' & 'n+3' – requires 4 aa.

C. Five -4 k pts

D. Six

E. Seven

(6,000 pts)

4) Describe Levinthal's Paradox regarding protein folding.

Proteins can not fold randomly because it would take far too long (>10^{77} years for a 100 aa protein). Since proteins can obviously fold much faster than that, there must be other factors operating.

(8,000 pts)

5) In an Anfinsen-style experiment, fully active ribonuclease (RNAase) is treated, in order, with (1) 8 M urea followed by (2) introduction of β-mercaptoethanol (β-ME) in 8 M urea followed by (3) removal of β-ME with exposure to O_2 followed by (4) removal of 8 M urea. You would expect the resulting ribonuclease activity to be closest to:

A. 200%

B. 100% - 5 k pts

C. 50%

D. 25%

E. 0%

(8,000 pts)

6) What metal is found at the center of the myoglobin prosthetic group? What is the biologically active oxidation state of the metal?

Fe^{+2}

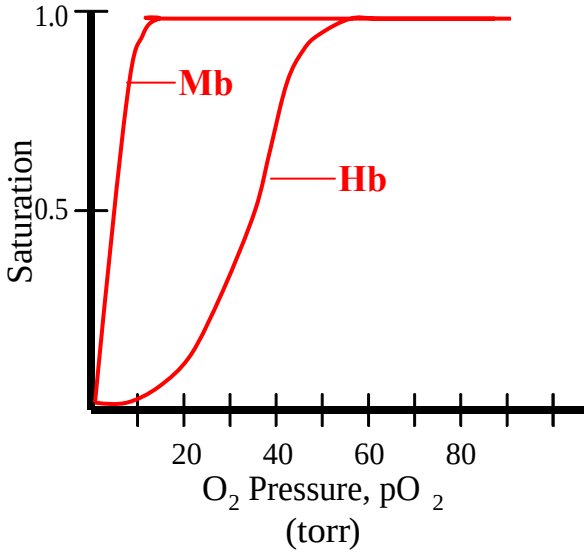
In reference to proteins, what is the general definition of a prosthetic group?

Nonpolypeptide unit or molecule that must bind the protein to confer biological activity.

(6,000 pts)

7) Using this diagram, carefully illustrate how O_2 binding differs between myoglobin and hemoglobin

Label any lines drawn.



(6,000 pts)

8) Which of the following molecules compete with O_2 for the *same* binding site in hemoglobin? Circle all that apply.

A. CO All the others exert allosteric control over O_2 binding, not direct competition

B. CO_2

C. H^+

D. 2,3-bisphosphoglycerate (2,3 BPG)

E. none of the above

(7,000 pts)

9) Describe the role of the proximal histidine in the biological function of hemoglobin. What role, if any, does the proximal histidine play in the cooperative binding of O_2?

Proximal His: Binds directly with the Fe^{2+} in the heme. The binding of O_2 to Fe^{2+} flattens the plane of the heme and pulls the proximal His and its helix. This results in a conformational change which increases O_2 binding in the other three subunits.

Distal His: forces bent geometry of CO & O_2 binding; this weakens CO binding but does not weaken O_2 binding

(5,000 pts)

10) The alpha (α) and beta (β) subunits of adult human hemoglobin:

A. have nearly identical primary and tertiary structures -3 k pts

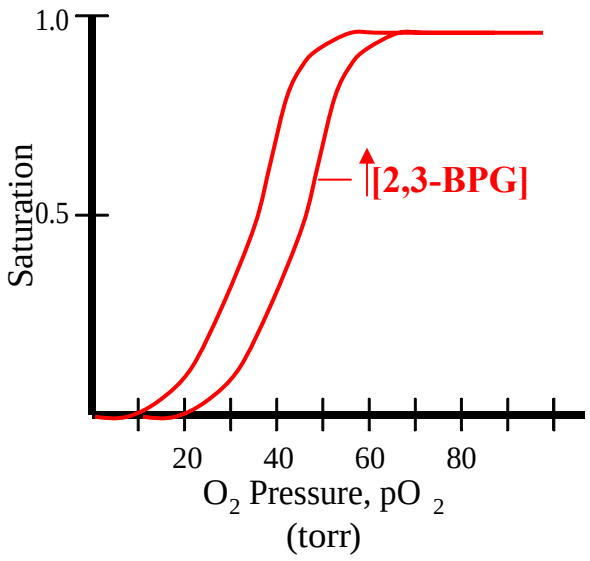
B. have nearly identical primary structures, but very different tertiary structures

C. have very different primary structures, but very similar tertiary structures

D. have very different primary and tertiary structures

(6,000 pts)

11) Using this diagram, carefully illustrate how an increase of 2,3 bisphosphoglycerate will affect the binding of O_2 to hemoglobin. Identify any lines drawn.



With increased 2,3-BPG, the sigmoidal curve shifts to the right

(6,000 pts)

12) According to the Bohr Effect, increased partial pressure of CO_2 has what affect on hemoglobin? Circle all that apply.

A. favors the T state of hemoglobin
B. favors the R state of hemoglobin
C. enhances binding of O_2 to hemoglobin
D. enhances release of O_2 from hemoglobin
E. none of the above

(8,000 pts)

13) Sickle cell anemia is considered a molecular disease.

A. Which molecule is defective in this disease? The β subunit of adult hemoglobin

B. How does the defective molecule differ from the normal (wild-type) molecule?
Amino acid # 6 is altered; Glu_6 becomes Val_6

C. How does this defect give rise to sickled or abnormally shaped cells?

When the pO_2 is low enough so that the hemoglobin is completely deoxygenated, a second hydrophobic domain is revealed. The hydrophobic Val-6 interacts with the 2nd hydrophobic domain from another Hb. If the second Hb is also HbS, it too can bind another Hb. This continues until large aggregates form to the point of distorting the cell into elongated or tortured shapes.

(6,000 pts)

14) A deficiency in dietary Vitamin C results in which human disease condition?

Scurvy

Which post-translational protein modification is specifically disrupted by lack of Vitamin C?

hydroxylation

(6,000 pts)

15) Examination of a human patient who died prematurely due to Crueztfeld-Jacob Disease (CJD) reveals structural information regarding both the infectious and noninfectious forms PrP protein. These two proteins would be expected to:

A. have nearly identical primary and tertiary structures – 3 k pts
B. have nearly identical primary structures, but very different tertiary structures
C. have very different primary structures, but very similar tertiary structures
D. have very different primary and tertiary structures

(6,000 pts)

16) An enzyme, E, catalyzes the reaction: A + B ⇌ C + D
Identify how addition of E would affect the free energy associated with this reaction. Mark one category each for A, B & C.

	Decrease	No change	Increase
A. Gibb's Free Energy of reaction (ΔG)		X	
B. standard Gibb's Free Energy of reaction (ΔG°)		X	
C. Gibb's Free Energy of activation ($\Delta G^{\ddagger}$)	X		

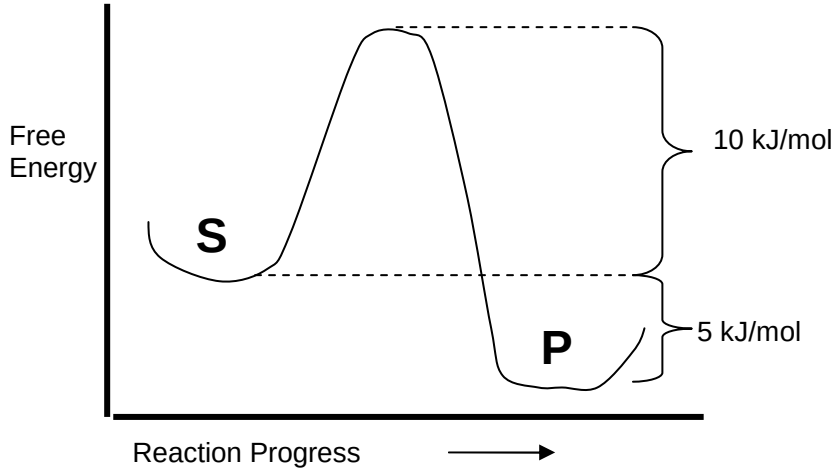
(6,000 pts)

17) Briefly describe Koshland's Induced Fit Model of enzymes.

The structure of free substrate and empty active site are not a perfect complementary match for one another. Instead, substrate binding induces a conformational change at the binding site of the enzyme; the enzyme can also cause conformational changes within the substrate.

(8,000 pts)

17) The following is a free energy diagram illustrating the chemical reaction: S ⇌ P.



The change in free energy of activation of S being converted to P would be:

A. -15 kJ/mol
B. -10 kJ/mol
C. -5 kJ/mol
D. 0 kJ/mol
E. +5 kJ/mol
F. +10 kJ/mol
G. +15 kJ/mol

The change in free energy of activation of P being converted to S would be:

A. -15 kJ/mol
B. -10 kJ/mol
C. -5 kJ/mol
D. 0 kJ/mol
E. +5 kJ/mol
F. +10 kJ/mol
G. +15 kJ/mol

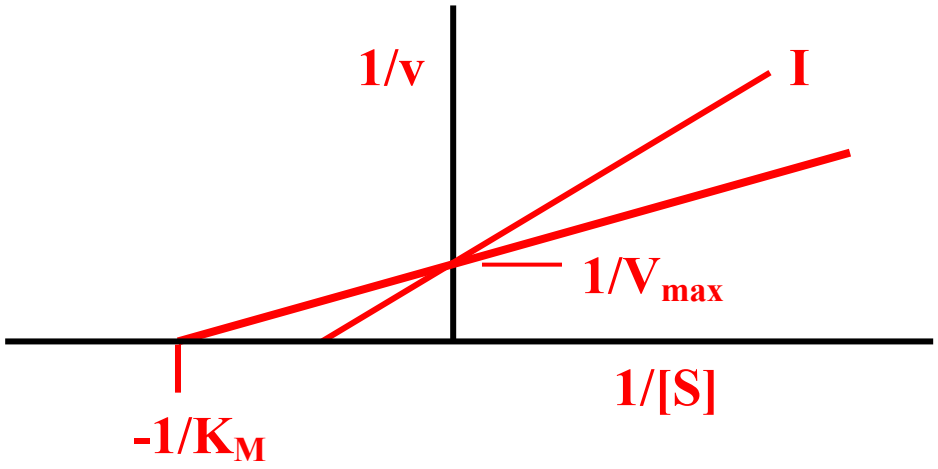
(5,000 pts)

18) *Briefly* describe how a MALDI mass spectrometer (MS) ionizes and transfers peptides to a gas phase.

MALDI = Matrix-assisted LASER desorption ionization. MALDI uses a LASER to simultaneously ionize and vaporize the peptides.

(12,000 pts)

19) Use the following axes to diagram a double reciprocal (Lineweaver-Burk) plot for an enzyme catalyzed reaction, S ⇌ P. Label both axes and show how this diagram can be used to determine V_{max} and K_M. Show how a competitive inhibitor would affect this plot.



(9,000 pts)

20) You identify a non-competitive inhibitor for an enzyme that follows Michaelis-Menten kinetics. You would expect this inhibitor to alter K_M and/or V_{max} as follows. Circle all that apply.

(5,000 pts)

A. increase K_M

B. decrease K_M

C. increase V_{max} **-3 k pts**

D. decrease V_{max}

E. both K_M and V_{max} would be unaltered

(4,000 pts)

If K_M and/or V_{max} is altered in the presence of the noncompetitive inhibitor, provide a mathematical equation showing how they have changed.

$V_{max}^{app} = V_{max} /(1 + [I]/K_i)$

(5,000 pts)

21) The fastest known enzyme can turn over how many reactions per second?

A. 4,000

B. 40,000

C. 400,000

D. 4,000,000 **-2.5 k pts**

E. 40,000,000

(5,000 pts)

22) Under standard conditions, the change in Gibb's free energy of reaction for hydrolysis of the gamma phosphate of ATP (producing ADP) is:

-30.5 kJ/mol

(5,000 pts)

23) The ratio of k_{cat}/K_M for an enzyme is considered to be:

A. a direct measure of affinity between substrate and enzyme
B. the catalytic efficiency when substrate is much less than K_M
C. inversely proportional to V_{max}
D. the fraction of sites filled with substrate
E. linearly dependent on enzyme concentration

(5,000 pts)

24) A suicide inhibitor like penicillin is best characterized as a:

A. competitive inhibitor – 3 k pts
B. noncompetitive inhibitor
C. uncompetitive inhibitor
D. mixed mode inhibitor
E. irreversible inhibition

Bonus Question (8,000 pts)

A common type of osteogenesis imperfecta (brittle bone disease) results from a specific mutation of one collagen gene that results in a single amino acid substitution that weakens the collagen fibers found in bone. Identify which of the 20 common amino acids is most likely altered in the mutated state and explain the rationale behind your decision.

Based on lecture, one could make an argument for glycine, proline or lysine. Glycine is, in fact, the culprit because the triple helix requires every third amino acid be Gly because the side chain sticks directly into the middle of the triple helix. Since there isn't any room inside the triple helix, even a substitution of Ala for Gly disrupts the structure and can significantly weaken the collagen fibers.

Page #	Points Possible	Points
Part I	32,000	
3	18,000	
4	28,000	
5	24,000	
6	20,000	
7	24,000	
8	13,000	
9	26,000	
10	15,000	
Bonus	8,000	
Total	200,000	