Biochemistry I - First Exam Face Page

This exam has 9 pages, including this one.

Total Points – 84: Allot 2 points/minute

The following equations and constants may be useful:

T=300K and pH=7.0 unless otherwise stated.

R=8.3 j/mol-K RT=2.5 kJ/mol @ 300K

Log2=0.3 ln10=2.3

ln9=2.20 ln2=0.69

$\Delta G^0 = -RT\ln K_{eq}$

$\Delta G = \Delta H - T\Delta S$

$S = R\ln W$

$\ln(a^n) = n \ln a$

For the reaction: N ⇔ U:

$K_{eq} = [U]/[N]$

$f_u = K_{eq}/(1+K_{eq})$

$f_n = 1/(1+K_{eq})$

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

$[HA]=[A_T]/(1+R)$

$[A^-]=[A_T]R/(1+R)$

$R=[A^-]/[HA]$

Beer's law: $A=\varepsilon[X]l$

A :_____/14	
B1:_____/ 6	
B2:_____/ 6	
B3:_____/12	
B4:_____/ 8	
B5:_____/ 6	
B6:_____/ 8	
C1:_____/ 6	
C2:_____/ 8	
C3:_____/10	
Tot:_____/84	

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

Part A: (14 points total: 2 points each, Circle the *best* answer)

1. Which of the following group can serve as an *effective* donor of hydrogen bonds.
   a) N-H
   b) C-H
   c) S-H
   d) C=O

2. A tri-protic weak acid can act as an *effective* buffer
   a) at all pH values.
   b) when the pH is approximately equal to any of its pK_a values
   c) half-way between pK_{a1} and pK_{a2}.
   d) at the middle pK_a only.

3. Which of the following is most correct:
   a) Polar amino acids are never buried in the interior of a protein.
   b) Polar amino acids are seldom buried in the interior of a protein.
   c) All hydrophobic amino acids are buried when a protein folds.
   d) Tyrosine is only found in the interior of proteins.

4. Formation of a hexa (6)-peptide from individual amino acids would release how many water molecules?
   a) Three.
   b) Four.
   c) Five.
   d) Six.

5. Which of the following has *no* effect on the energetics of protein folding?
   a) Conformational Entropy.
   b) Hydrophobic Interactions.
   c) Covalent bonds.
   d) Hydrogen Bonds.

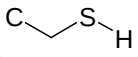
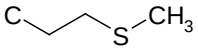
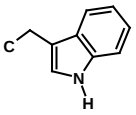
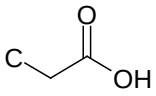
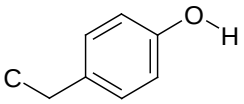
6. The free energy change due to *unfolding* of a protein is positive, therefore
   a) the temperature is equal to T_M.
   b) the enthlapy of the reaction is zero.
   c) the temperature is above T_M.
   d) the temperature is below T_M.

7. Which of the following regions of antibodies bind antigens?
   a) Disulfide bonds.
   b) Constant regions.
   c) F_v fragments.
   d) F_c fragments.

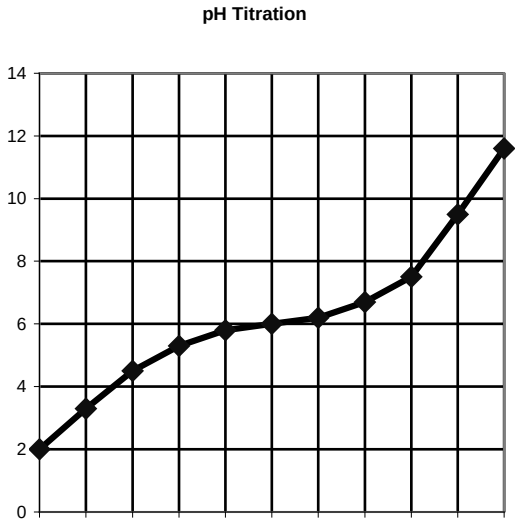
Part B: Short Answer

B1: (6 pts)

Correctly match **three** of the six descriptions to the amino acid side-chain (only the side chain of the amino acid is shown). Place the correct letter next to the description. In cases where there is more than one correct choice, only one is required for full credit. Item 4 has been done for you as an example.

1. Absorbs UV light	____	a. 
2. Forms disulfide bonds in proteins.	____	b. 
3. Side chain pK_a =4.0. Residue also found in the active site of HIV protease.	____	c. 
4. Amino acid that is not chiral.	__C__	d. 
5. Has both polar and non-polar character.	____	e. 
6. CNBr cleaves after this residue.	____	f. 

B2: (6 pts) A titration curve of a mono-protic acid is provided on the right.



i) Label the x-axis and y-axis, including the units (2 pts.)

iii) Briefly explain how the pK_a of the acid is obtained from this experiment. What is the pK_a for this particular acid (3 pts).

iv) Which amino acid has a side chain with the same pK_a (1 pt)?

B3: (12 pts) The partial structure of a dipeptide is shown below.

i) Convert this to a tripeptide by adding a glycine residue to either end. [The sidechain of Gly is a proton](3 pts).

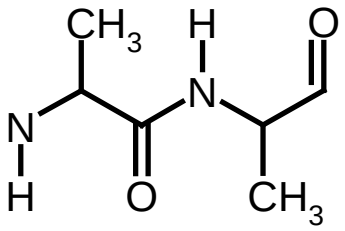
ii) Give the sequence of the peptide (e.g. Tyr-Trp-Phe) (1 pt).

iii) Indicate the location of:

- The *first* peptide bond (1 pt).
- The *first* ϕ torsional angle or bond (1 pt).
- The *first* ψ. torsional angle or bond (1 pt).
- The amino terminus (1 pt).

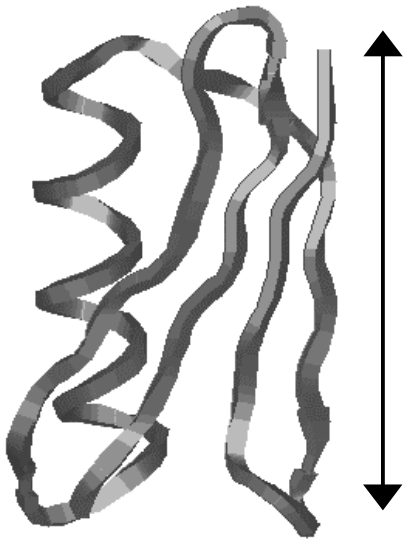
iv) Which of the above three bonds is always considered to be planer? Why is it planer? (2 pts)

v) Draw a water molecule forming a hydrogen bond to one of the amide groups in this peptide. Label the donor and acceptor in the diagram (2 pts)



B4: (8 pts) An image of protein G is shown to the right.

i) An arrow has been drawn to the right of the figure. What is the *approximate* length of this arrow in angstroms. Briefly justify your answer (1 pt).



ii) Circle, or otherwise clearly indicate, on the structure to the right, **two** of the following three structural features (2 pts).

- α-helix
- β-strand
- β-hairpin

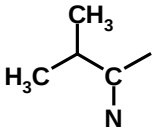
Be sure to indicate which of the three you have selected.

B4: - Continued:

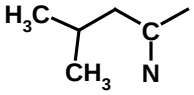
iii) Discuss the major energetic term that *stabilizes* any one of the above structural features. A simple chemical diagram of the interaction can be used in your answer. (5 pts)

B5: (6 pts) Explain why the core of a folded protein consists mainly of non-polar residues. Your answer should include a discussion about changes in thermodynamic parameters as well as provide some information at the molecular scale, i.e. simply stating "It is lowest in free energy" is not sufficient.

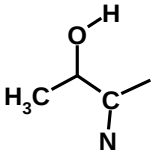
B6: (8 pts) The core of a protein contains a Valine residue in the wild-type enzyme. This is replaced by Leucine in one mutant and Threonine in another. The thermodynamic parameter associated with unfolding of each of these proteins is provided below the amino acid side-chain. The direction of the reaction is considered to be from the Native state to the Unfolded state (N→U).



Valine



Leucine



Threonine

ΔH^o	+200 kJ/mol	+190 kJ/mol	+205 kJ/mol-deg
ΔS^o	+600 J/mol-deg	+595 J/mol-K	+605 J/mol-deg

i) (5 pts) Select **ONE** of the mutants, either the Leucine or Threonine substitution, and provide an explanation for the change in enthalpy (ΔH^o). Your answer should include a description of the molecular interactions in the folded form of the protein. You can supplement your answer with a simple sketch.

ii) Only do **one** of the following **three** parts (3 pts)

a) What is the T_M for the wild-type protein? Briefly justify your approach.

OR

b) Briefly explain how the enthalphy would be obtained from a protein melting (denaturation) curve.

OR

c) Briefly explain how you would obtain the fraction of wild-type molecules that are unfolded at any temperature.

Part C: Detailed Calculations - You must attempt all three questions. However, you have two choices within each question.

C1: Do one of the following two questions (6 pts).

i) A protein that is 20 amino acid residues in length folds into a stable structure. Assume that the protein forms **all but one** hydrogen bond when it folds and that the unsatisfied H-bond is not accessible to water. Calculate the enthalpy of unfolding. State whatever assumptions you make regarding the energetics of hydrogen bond formation.

OR

ii) A 20 residue protein can fold into either an α-helix or a β-strand (i.e. both species can be found in a solution of the folded protein at the same time). Calculate the entropy for the transition from the unfolded to the folded states. If you do not have a calculator, simply write out the relevant equations.

C2: (8 pts) Do one of the following two questions:

i) The primary sequence of a 10 residue peptide is being determined using Edman degradation and proteolytic cleavage. Only the sequence of the first four residues of a peptide are obtainable, regardless of its length. The following data were obtained:

a). Sequencing of the intact peptide gave the first four amino acids: Ala-Cys-Met-Val

b) Sequencing of each peptide produced from Trypsin cleavage gave:

Ala-Cys-Met-Val Phe-Thr-Ser-Gly

c) Sequencing of each peptide produced from Chymotrypsin cleavage gave:

Ala-Cys-Met-Val Thr-Ser-Gly-Met

Determine as much of the peptide sequence as possible and give the most probable sequence for the missing residue(s).

OR

ii) A protein contains one Trp (ε_{280} = 5000 $M^{-1}cm^{-1}$) residue and five Tyrosine residues (ε_{280} = 1000 $M^{-1}cm^{-1}$). A solution of this protein has an absorbance of 0.5 for a 1cm path length. What is the concentration of the protein in solution?

C3: (10 pts) Do one of the following two questions:

i) You want to make 1 L of a 0.5 M buffer solution with a pH = 5.0. The reaction that you are trying to control the pH of generates protons.

Your choices of acids are acetic acid (pK_a = 4.0) or imidazole (pK_a = 6.0).

a) Explain which buffer compound you would use and why. If you are uncertain of what to choose, just pick one and move on, either choice will be graded in the following sections (2 pts).

b) Determine the correct ratio of the acidic and conjugate base form of the buffer.(5 pts)

c) Explain how you would make the buffer, assuming you only have the fully protonated form of the acid in your laboratory.(3 pts)

OR

ii) An enzyme has a single lysine residue in its active site. In order for the enzyme to be fully active, this lysine side-chain must be positively charged (protonated). You can assume that the pK_a of the lysine side chain is 7.0.

a) Draw, using the graph to the right, the pH dependence of the enzyme activity. Justify your graph, preferably with a sample calculation. (6 pts)

b) This pK_a is significantly lower than the normal pK_a for a lysine residue. Suggest how this might occur. Illustrate your answer with an appropriate diagram of the lysine residue in the active site of this enzyme (4 pts)