 Name:_____

Biochemistry I - First Exam Face Page

This exam has 8 pages, including this one.

Total Points – 100: Allow 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=2.2 ln1=0

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

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

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

$S = R\ln W$

For the reaction: N <--> U:

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

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

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

$$pH = pK_a + \log\frac{[A^-]}{[HA]}$$

$$R = \frac{[A^-]}{[HA]}$$

$$[HA] = [A_T]\frac{1}{(1+R)}$$

$$[A^-] = [A_T]\frac{R}{(1+R)}$$

A :	_____
B1:	_____
B2:	_____
B3:	_____
B4:	_____
B5:	_____
B6:	_____
B7:	_____
C1:	_____
Total:	_____

Beer's law: $A=\varepsilon[X]l$ ε_{TYR} = 1,400 $M^{-1}cm^{-1}$, ε_{PHE} = 220 $M^{-1}cm^{-1}$, ε_{TRP} = 5,050 $M^{-1}cm^{-1}$

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: (24 points total: 3 points each, Circle the best answer)

1. Most of the important functional groups on biological molecules
   a) contain oxygen and(or) nitrogen and are acidic.
   b) contain oxygen and(or) nitrogen and are basic.
   c) can donate or accept a hydrogen bond.
   d) contain oxygen and(or) nitrogen and are nonpolar.

2. Polyprotic acids such as H_3PO_4, can act as acid-base buffers
   a) at a pH that is the average of all their pK_a values.
   b) at pH values around the first pK_a only.
   c) at pH values halfway between each pKa's.
   d) at pH values around any of their pKa's.

3.Which of the following is **not** a sensible grouping of amino acids based on their polarity properties?
   a) Met and Leu.
   b) Val and Leu.
   c) Glu and Ile.
   d) Asn and Gln.

4. The peptide bond in proteins:
   a) is another name for hydrogen bonds.
   b) is usually cis unless proline is the next amino acid.
   c) is usually trans unless proline is the next amino acid.
   d) can assume 3 different conformations.

5. A β-pleated sheet structure
   a) can occur in both right and left handed forms
   b) has neighboring residues that are hydrogen bonded to each other.
   c) has neighboring chains that are connected by α-helices
   d) has neighboring chains that are hydrogen bonded.

6. The unfolding of a globular protein causes
   a) loss of primary structure.
   b) loss of secondary structure.
   c) both a) and b)
   d) neither a) or b).

7. Below the midpoint of a temperature denaturation curve (T_M) for a protein.
   a) more than half of the protein is denatured.
   b) less than half of the protein is denatured.
   c) the energy of the native state is higher than the denatured.
   d) all of the protein is in the native state.

8. Which of the following causes a change of the entropy of the water during protein folding?
   a) Conformational Entropy.
   b) Hydrophobic Interactions.
   c) Van der Waals Interactions.
   d) Hydrogen Bonds

Part B: Short Answer

B1 (12 pts):

i) In the space below draw the structure of a dipeptide. The first amino acid can be *any* polar, but **not** charged, amino acid and the second amino acid can be *any* amino acid that is predominately or completely non-polar, **except** for Tyrosine. Provide the *name* for each amino acid that you have drawn (6 pts).

ii) Indicate, on the *polar* amino acid, how a single water molecule would hydrogen bond to one of its *sidechain* atoms. Indicate both the donor and acceptor for this interaction. Also indicate the approximate length of the H-bond. (3 pts)

iii) Indicate the following on your diagram.

a) The location of the peptide bond (1pt).

b) The locations of the ϕ (phi) and the Ψ (psi) angles (1pt)

c) The locations of the amino- and carboxy-terminus (1 pt)

B2: (5 pts) **Sketch** either a 12 residue α-helix or a two stranded *parallel* β-sheet (6 residues/strand) (2 pts). If you sketch the β-sheet provide some indication of the directionality of the peptide chain. In either case you **should not** draw all of the atoms, but just indicate the approximate location of residues. In your sketch please indicate the following features:

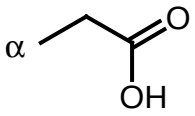
i) Location of hydrogen bonds (1 pt).

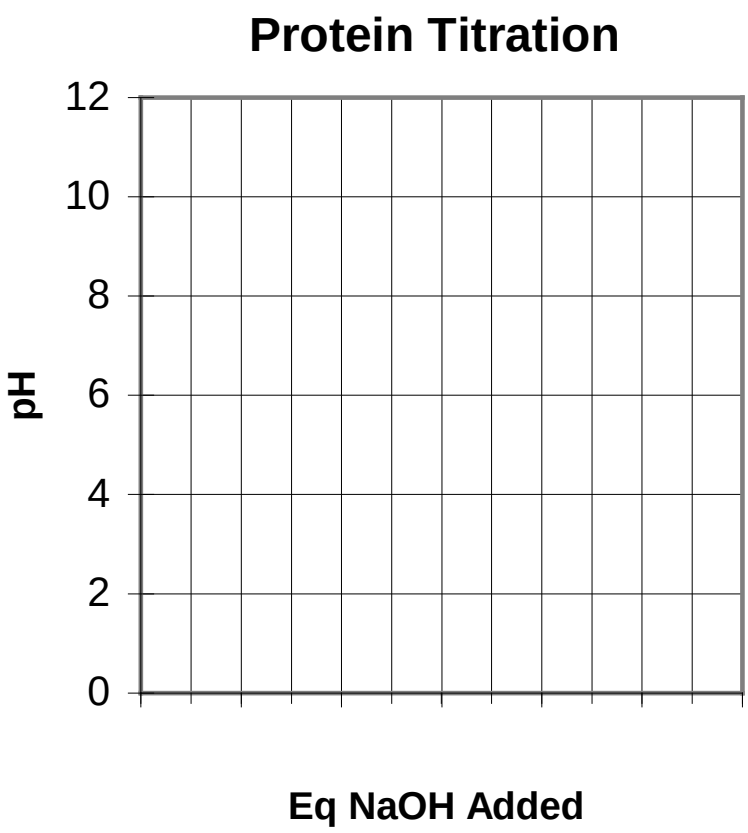
ii) Location of side-chain groups (1 pt).

iii) The approximate length of your structure (1pt).

B3: (17 pts) A protein solution has an optical absorbance of 1.0 . The sequence of this protein is unknown, but it contains the following amino acids (plus others of no consequence to this problem).

- Four (4) Tryptophan residues.
- Four (4) Tyrosine residues.
- Two (2) Aspartic acid resides. The side-chain for this residue is:





i) What is the most likely wavelength that was used for the optical absorbance measurement? (1 pt)

ii) What is the pK_a of the side chain of Aspartic acid? (1 pt)

iii) Calculate the extinction coefficient, ε, for this protein.(3 pts)

iv) What is the concentration of this protein in solution.(3 pts)

v) Sketch the complete pH titration of this protein, on the graph given above. If you are unsure of any pK_a values, simple state what you think their values are and draw the titration curve accordingly. The x-axis should be in *equivalents* of base added. Be sure to place the appropriate numerical values on the x-axis. (5 pts)

vi) How many moles of NaOH, per liter of solution, would you have added by the end of the titration? Justify your answer based on the amino acid composition of the peptide and your calculated concentration. (4 pts)

B4: (8 pts) Using the chart below, briefly describe **either** the role of van der Waals forces **or** the hydrophobic effect in protein folding. Please indicate which effect you are discussing. A sample answer for hydrogen bonds has been provided for you.

Interaction	**Effect on characteristics of Folded state (3 pts)** (Give one characteristic and why the interaction causes that characteristic)	**Enthalpy or Entropy (1 pt)**	**Molecular nature of the interaction (4 pts)** (one or two sentences)
Hydrogen bonds	*Almost all hydrogen bonds are formed when proteins are folded since the energetic cost of breaking, but not reforming a hydrogen bond is high.*	*Enthalpic*	*The hydrogen in the N-H group is electropositive, allowing it to form a favorable interaction with the electronegative oxygen on mainchain C=O groups.*

B5: (6 pts) [Choose only **one** of the following **three** questions]

Choice a) Briefly describe the structure of a β-barrel and explain why it is intrinsically more stable than a β-sheet composed on the same number of strands.

OR

Choice b) Sketch, and name, any super-secondary structure you like, briefly describe the fundamental forces that hold it together.

OR

Choice c) Briefly discuss how the pK_a of an amino acid sidechain could be affected by its environment in the folded form of the protein. Provide an illustrative example in your discussion.

B6:(9 pts)

i) Sketch the structure of an antibody in the box to the right. On your sketch identify the following (5 pts)

- light chain
- heavy chain
- approximate location of inter-chain disulfide bonds
- region of the protein that binds antigen.

ii) What amino acid is used to form disulfide bonds? (1 pt)

iii) Briefly discuss how antibodies can be used to remove toxic substances from an individual. (3 pts)

B7: (7 pts) [Answer **one** of the following **two** questions]

Choice a) A small, 12 residue protein was digested with Chymotrypsin.. These fragments were sequenced by Edman degradation. Due to low amounts of the peptide, it was only possible to obtain the first four amino acids for the intact peptide.

Chymotrypsin Fragments: Ala-Gly-Leu-Phe Lys-Cys-Ser Arg-Cys-Glu-Met-Tyr

Sequencing of the intact peptide gave the following: Ala-Gly-Leu-Phe

i) There is insufficient information to obtain the sequence of the peptide. Why? (2 pts)

ii) What other cleavage reagent would you use to solve this problem? Why? Briefly explain how the new information may allow you to complete the sequence. (4 pts)

iii) Which *three* amino acid residues does chymotrysin recognize and cleave after? (1 pt)

OR

Choice b) A biochemical reaction that you are studying consumes protons. You wish to run this reaction at a pH of 8.3, using Tris buffer, which has a pK_a of 8.3. *Tris can only be purchased in the basic form.* How much HCl would you need to add to a liter of a 0.05 M solution of Tris base to make this buffer. Please justify your answer, *preferably* with a numerical calculation.

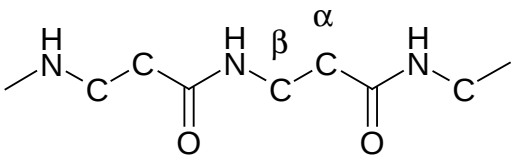
Part C: Detailed Calculations. Do only **one** of the following **two** questions. Please indicate the question that your are attempting.

C1. (12 pts) A 60 residue polypeptide that is composed of α-**amino acids** folds into a stable tertiary structure. At 300K, the native form is 10 kJ/mol more stable than the unfolded form.

i) Calculate the change in the entropy of the backbone when the protein denatures. Briefly explain the underlying assumptions that you are making in your calculation (4 pts)

ii) Given that the native form of the protein is more stable than the unfolded form by 10 kJ/mol at 300K, what is the overall contribution of the hydrophobic effect, van der Waals, and hydrogen bonds to the stability, or Gibbs energy, of the folded form of the protein at that temperature. You only need to calculate the sum of all of these, *not* each individual effect. (5 pts)

iii) If the α-amino acids in this protein are replaced by β-amino acids, the protein unfolds. Assuming that the hydrophobic effect, van der Waals, and hydrogen bonding were unaffected by this change, why did the protein unfold?



A **β-amino acid** has the amino group placed at the β-position of the carboxylic acid instead of the normal α-position. The structure of a peptide containing β-amino acids is shown on the right. The α- and β-carbons are labeled. As a consequence, there are three freely rotatable single bonds associated with each residue. (3 pts)

OR

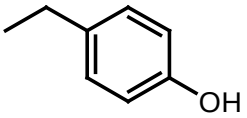
C2. (12 pts) A protein has the following enthalpy and entropy for denaturation (N→U):

$\Delta H^0 = +300$ kJ/mol $\Delta S^o = +1000$ J/mol-deg

i) What is the T_M, or melting temperature, of this protein? Show your work.(2 pts)

ii) Replacement of a buried Tyrosine residue by a glycine residue lowers the melting temperature to 292 K. Assuming that there is no effect on the entropy change during unfolding, what is the change in enthalpy, ΔH^o? The structure of the sidechains of Gly and Tyr are shown to the right. (3 pts)

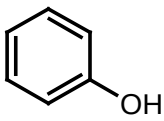
Glycine
(Mutant)



Tyrosine
(Wild-type)

iii) What fraction of the mutant protein is unfolded at the T_M of the wild-type protein? Please show your work (4 pts)

iv) If phenol is included with the mutant protein during the temperature denaturation experiment, the T_M is returned to the value found for the wild-type protein. At this concentration, phenol has no effect on the melting temperature of the wild-type protein. Explain this observation with reference to the forces that stabilize the folded structures of proteins. The structure of phenol is shown on the right (3 pts).



Phenol