

Biochemistry I - Exam I - Face Page

The following equations and constants may be useful:

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

R = 8.3 J/mol-K

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

S = R ln W

ln 9 = 2.2

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

ln (a<sup>n</sup>) = n ln a

For the reaction: N  $\rightleftharpoons$  U:

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

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

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

pH = pKa + log [A<sup>-</sup>]/[HA]

[HA] = [A<sup>-</sup>]R/(1+R)

R = [A<sup>-</sup>]/[HA]

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

Amino Acid Names:

Alanine: Ala

Arginine: Arg

Asparagine: Asn

Aspartic Acid: Asp

Cysteine: Cys

Glutamic Acid: Glu

Glutamine: Gln

Glycine: Gly

Histidine: His

Isoleucine: Ile

Leucine: Leu

Lysine: Lys

Methionine: Met

Phenylalanine: Phe

Proline: Pro

Serine: Ser

Threonine: Thr

Tryptophan: Trp

Tyrosine: Tyr

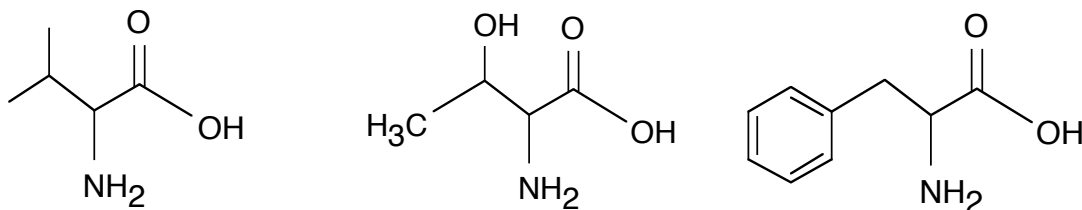
A: _____/14
B1: _____/18
B2: _____/18
B3: _____/6
B4: _____/6
B5: _____/15
B6: _____/8
B7: _____/8
B8: _____/16
Total: _____/100

**This exam consists of 7 pages (including face page). There are a total of 100 points. Allot 1 minute for every 2 points.**

**Part A: Please circle the best answer (2 pts/question, 14 pts total)**

- In the titration of the amino acid glycine, an inflection point occurs:
  - when there is no net charge on the amino acid.
  - when one molar equivalent of base has been added to the fully protonated form.
  - when 1.5 molar equivalent of base has been added to the fully protonated form.
  - at neutral pH.
- Which of the following is *most* correct about the core of a globular, folded protein?
  - There is extensive hydrogen bonding between the side chains of polar residues.
  - Hydrogen bonding between main chain C=O and side chain NH<sub>2</sub> groups is maximized.
  - Extensive van der Waals interactions occur between predominantly non-polar residues.
  - There are nine possible configurations per residue due to rotation about  $\phi$  and  $\psi$  angles.
- Which of the following terms *favours* the *unfolding* of a globular protein?
  - The enthalpy change due to the breaking of noncovalent bonds.
  - The enthalpy change due to the breaking of covalent bonds.
  - The configurational entropy change.
  - The entropy change of the solvent.
- The peptide bond:
  - has partial double bond character and is almost always in the cis configuration.
  - is planar and rigid and almost always in the trans configuration.
  - has three possible configurations due to rotation about the N-C bond.
  - is hydrolyzed spontaneously at neutral pH.
- Which of the following are features shared by both an  $\alpha$ -helix and a  $\beta$ -sheet?
  - Identical  $\phi$  (phi) and  $\psi$  (psi) torsional angles.
  - Hydrogen bonds between electronegative backbone atoms.
  - Hydrogen bonds that are parallel to the main chain.
  - $\beta$ -branched side chains are uncommon.
- Amino acids with  $\beta$ -branched side chains are *best* accommodated in:
  - an  $\alpha$ -helix.
  - a  $\beta$ -sheet.
  - a  $\beta$ -turn.
  - a coiled coil  $\alpha$ -helical structure.
- Which of the following is the “driving force” for protein folding?
  - The restriction of  $\phi$  (phi) and  $\psi$  (psi) torsional angles.
  - The hydrophobic effect.
  - The change in configurational entropy.
  - The formation of  $\alpha$ -helices and  $\beta$ -sheets.

B1. (9 pts) The structure of three amino acids is shown below:

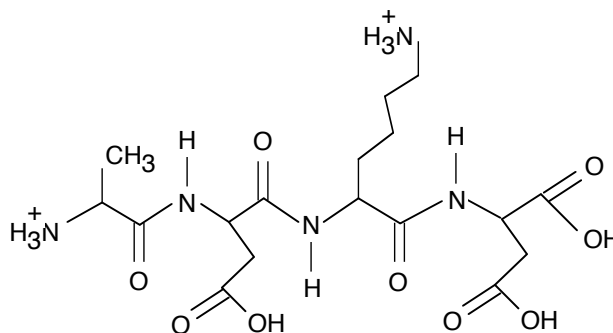


For *each* of these amino acids:

- Identify the amino acid. Then show, by the removal, addition, or replacement of a small group, such as  $\text{CH}_3$ ,  $\text{OH}$ , etc. (not the entire side-chain), how you could convert your chosen amino acid to another amino acid that is chemically most similar to the starting amino acid. For example: Alanine ( $\text{R}=\text{CH}_3$ ) can be converted to Glycine ( $\text{R}=\text{H}$ ) by the replacement of the methyl group with a hydrogen. Include the name of both the original and resulting amino acid. Either redraw the modified amino acid below or indicate your changes on the diagram above. (3 pts)
- Did your change increase, decrease, or not affect the solubility of the amino acid in water? Briefly justify your answer. (3 pts)
- Did your change increase, decrease, or not affect the ability of the amino acid to form a hydrogen bond to water? Briefly justify your answer. (3 pts)

B2. (18 pts) The structure of the fully protonated form of a tetrapeptide is shown:

- Indicate the location of a peptide bond and a freely rotatable bond on the diagram. (4 pts)
- List the sequence of the peptide. (2 pt)
- List each side chain with an ionizable functional group by name and indicate its approximate pKa. (2 pts)



d) *Sketch* the pH titration curve of the tetrapeptide. Be sure to label the axis of your graph, provide the appropriate scale and numbers, and indicate the inflection points. You may assume that the pKa of the amino and carboxy terminus are 9 and 2, respectively. (6 pts)

e) At what pH will there be no net charge on this peptide? (4 pts)

B3. (6 pts)

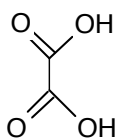
a) *Sketch* an  $\alpha$ -helix. You need not draw the individual atoms. However, indicate the direction of hydrogen bonds as well as the general location of the amino acid side chains in your diagram. Indicate the number of residues per turn of the helix and also state the rule for H-bonding (ie, the \_\_\_ group of residue n donates/accepts an H-bond to/from the \_\_\_ group of residue n+\_\_\_).

B4. (6 pts) Entropy plays an important role in defining the stability of the folded state of globular proteins. List, and then *briefly* discuss, the molecular nature of the entropic terms that affect protein folding. You should clearly state whether the term stabilizes or destabilizes the folded state. You are welcome to use an equation(s) as part of your answer.

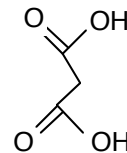
B5. (15 pts)

You would like to make a 0.1 M buffer for an experiment at pH=6.0. You have the following two organic acids to choose from:

Acid	pKa1	pKa2
Oxalic acid	1.23	4.19
Malonic acid	2.83	5.69



Oxalic acid



Malonic acid

- a) Which of these two compounds would you choose and why? (1 pt)
- b) Draw the conjugate acid and base pair in your pH 6.0 buffer, indicating which is the acid and which is the base. (2 pts)
- c) You have only the fully protonated form of the acid in hand and a 1 M solution of NaOH. How would you make a 1 liter solution of your buffer? The amount of acid, as well as the amount of NaOH used should be given in moles. Please show all calculations. (6 pts)
- d) *Briefly* explain why each of the above acids has two different pKa values. (2 pts)
- e) *Briefly* explain why the pH of your buffer will not substantially decrease when protons ( $H^+$ ) are released during a biological experiment carried out in your buffer. (2 pts)
- f) During the course of the biological experiment, protons ( $H^+$ ) are released from one of the substrate organic compounds into the solvent. What would be the consequence of having forgotten to include the buffer if 10  $\mu$ mole ( $1 \times 10^{-5}$  mole) of protons ( $H^+$ ) were released? [Hint: what would the resulting pH be in the absence of buffering?] (2 pts)

B6. (8 pts) The amino acids Proline and Glycine occur frequently in  $\beta$ -turns but only very infrequently in  $\beta$ -helices.

- a) Draw the structure of the tripeptide with the following sequence: Gly-Pro-Gly. (2 pts)
  
  
  
  
  
  
  
  
  
  
- b) State a reason why Pro residues are not commonly found in  $\beta$ -helices. (2 pts)
  
  
  
  
  
  
  
  
  
  
- c) State a reason why Gly residues are not commonly found in  $\beta$ -helices. (2 pts)
  
  
  
  
  
  
  
  
  
  
- d) State one way that  $\beta$ -helices and  $\beta$ -turns differ with respect to hydrogen bonding. (2 pts)

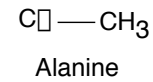
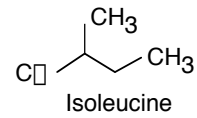
B7. (8 pts) There are four levels of protein structure.

- a) Using the immunoglobulins as an example, briefly discuss the major features of the four levels of protein structure, beginning with the primary structure. (6 pts)

- b) How many antigen binding sites per molecule are there in an intact immunoglobulin? How is this number affected upon cleavage with papain? (2 pts)

B8. (16 pts) An altered version of T4 lysozyme, with a single amino acid substitution of Ile to Ala at position 27 (I27A) has been generated in the lab. In the wild type protein, Ile 27, in the middle of 3 anti-parallel strands, is buried in the hydrophobic core of the protein. The enthalpy and entropy of unfolding (reaction direction  $N \rightarrow U$ ) were measured for both proteins and the values obtained are shown below:

	$\Delta H$	$\Delta S$
Isoleucine side-chain (wt)	116 kJ/mole	360 J/mol-K
Alanine side-chain (I27A)	98 kJ/mol	312 J/mol-K



- Provide an explanation for why the  $\Delta H$  values differ between the two proteins. (4 pts)
- Provide an explanation for why the  $\Delta S$  values differ between the two proteins. (4 pts)
- What is the melting temperature ( $T_m$ ) of each protein? (2 pts)
- What fraction of the mutant protein is unfolded at the  $T_m$  of the wild type protein? (6 pts)