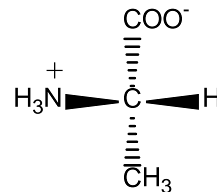
Chemistry 4055 (Spring 2013)
Biochemistry I- Introduction to the Chemistry of the Animal Cell
Chapter 3 HW Assignment

1. Would you expect the following configuration of alanine (Ala, A) to be the dominant form in our body?



2. Write the three letter code and single letter code for glutamine and glutamate and explain how these AA are similar and different?

3. What is the concentration (M) of a protein if the absorbance of the protein @ 280 nm is 0.987, which is the sum of the absorbances of the 10 Trp amino acid (AA) residues that it consists of?

Given Beer's Law A = εbc where the pathlength is b = 1 cm and the extinction coefficient is ε(Trp) = 5,690 $M^{-1}cm^{-1}$.

4. If the physiological pH of intracellular organelles can range from 5.5 to 7.4, explain what role His in a protein could serve in reactions that the protein engages in in these organelles.

5. Sketch the pH titration curve for Glu. Also draw all forms of Glu that are involved in this titration and identify their corresponding charge states and pKa values, and determine the pI of the amino acid.

6. Draw the structure for HAPPY. Note: We have not yet discussed how to draw peptides in 3D so just draw it as a planar, 1D structure.

7. Say you were tired one day and you accidentally extracted proteins from a mouse kidney in the wrong buffer. You are only interested in studying proteins of 10 kDa molecular weight or higher and need to fix your mistake or Professor Tinoco will be very mad. What is the best way to fix this mistake?

8. Draw a general workflow for protein extraction, purification, and identification.

9. Say you have a mixture of four proteins with the following properties.

Protein A: MW of 30 kDa, pI = 5.0, binds to nitrilotriacetic acid
Protein B: MW of 35 kDa, pI = 4.5, low solubility and will precipitate in 5% (w/v) $(NH_4)_2SO_4$
Protein C: MW of 100 kDa, pI = 4.7

Protein D: MW of 30 kDa, pI = 7.4

Identify fractionation strategies to purify each protein. What techniques should you use to verify the success of each fractionation step?

10. You discover and purify a new enzyme, generating the purification table below.

Procedure	Total protein (mg)	Activity (units)
1. Crude Extract	20,000	4,000,000
2. Precipitation (salt)	5,000	3,000,000
3. Precipitation (pH)	4,000	1,000,000
4. Ion exchange chromatography	200	800,000
5. Affinity chromatography	50	750,000
6. Size-exclusion chromatography	45	675,000

a. Calculate the specific activity of the enzyme solution after each purification procedure. (Show your math.)

b. Which of the purification procedures used for this enzyme results in the greatest relative increase in purity?

c. Was there any additional improvement in purification between steps 5 and 6?

11. What protein identification technique is currently the best to use for a protein from an organism with a sequenced genome? Does this protein have to be 100% pure?

12. You have to identify the AA sequence of an unknown peptide using the following information and protocols.

Step 1: The protein is hydrolyzed and an amino acid analysis, which defines the AA composition revealed the total number of AA present and how many of each AA are present.

A 1	C 2	D 1	F 1	G 2
H 2	K 1	L 1	P 3	R 2
S 2	V 1	W 1	Total: 20	

Step 2: Chemical labeling with FDNB revealed H to be the amino terminal residue.

Step 3: The protein is reduced with DTT to break any disulfide bonds.

Step 4: Some of the protein is cleaved with trypsin, which cleaves at the carboxyl side of Lys and Arg. The protein is then sequenced by Edman degradation. The following peptides were obtained following 100% cleavage success with trypsin.

PP
HASPR
HGFLVCR
DGWSCK

Step 5: Some of the protein is cleaved with chymotrypsin, which cleaves at the carboxyl side of Phe, Trp, and Tyr. The protein is then sequenced by Edman degradation. The following peptides were obtained following 100% cleavage success with chymotrypsin.

SCKHGF
HASPRDGW
LVCRPP