Biochemistry – Problem set 2 *Due Mon Sept 19* Total Time required: ~ 80 min

A: The following problems from Campbell are recommended. Please do not submit them for grading; the answers are in the back of the text.

Chapter 3: 1-5, 7-9, 11, 15, 22 Chapter 4: 1, 19, 21

B: Please submit answers to the following problems for grading:

1. (2 points, 5 min) The structures of Aspartic acid and Glutamic Acid, shown in Fig. 3.3 of the Campbell textbook, are incorrect at pH 7. Draw the amino acids in their correct ionization states at this pH. (1 pt) At *approximately* what pH might the structures in Fig. 3.3 actually represent the ionization states of the two amino acids? (1 pt)

2. (10 points, 15 min) Sketch the structure of the following tri-peptide in an extended chain conformation at pH 7.0. (5 pts) Clearly label the amino- and carboxy-terminus of the peptide. (2 pts) Also indicate the location of all formal charges. (2 pts)

   Aspartic acid – Glycine - Arginine

   Determine the overall net charge on this molecule at this pH. (1 pt)

3. (6 points, 10 min) The enzyme Nucleoside Diphosphate Kinase has a critical catalytic Histidine residue with a pKa of 6.0.
   (a) Calculate the fraction of the His side chain protonated at pH 6.0, 6.5 and 7.0. (4 pts)
   (b) The deprotonated form of the critical His residue is required for its activity. Given the result of your calculation in part (a), is Nucleoside Diphosphate Kinase likely to be highly active at pH 7.0? (2 pts)

4. (5 points, 20 min) Digestion of a 10 residue peptide with Cyanogen bromide, Chymotrypsin and Trypsin produced the sequence data given below. Note that it was only possible to perform Edman degradation for <u>*four*</u> cycles, i.e. it is only possible to determine the sequence of the first four residues of any peptide (*the actual peptide may have been longer*). Determine the sequence of the original peptide. *Show your work and demonstrate that your final answer is consistent with the experimental data.*

   a. Cyanogen bromide cleavage produced two peptides, each of which gave the following sequences from Edman degradation:

      Gly-Phe-Leu-Lys and Cys-Ala

   b. Chymotrypsin digestion produced two peptides:

Leu-Lys-Val-His and Met-Gly-Phe

c. Trypsin digestion produced the following peptide sequences:

Val-His-Met-Cys and Met-Gly-Phe-Leu

5. (9 points, 20 min) The following questions will require you to view the structure of the 20S proteasome using Chime. Remember that you need to have the Chime plug-in installed. The link to the chime page is on the problem set page (the link to the problem set page is found on the home page).

   (a) The 20S proteasome is a large cylindrical macromolecular protein complex that is responsible for protein degradation in prokaryotes and eukaryotes. It is a hollow chamber made up of 28 subunit polypeptide chains that assemble into 4 stacked rings. Unfolded proteins are injected through the hollow core where they undergo proteolysis. View the structure and use the relevant commands in Chime to determine the *approximate* (to 10-20 angstroms) overall dimensions of the 20S proteasome (length & diameter). (3 pts)
   (b) Use the commands in Chime to determine the number of Tryptophan and Tyrosine residues in the S chain (one of the 28 subunits). (3 pts)
   (c) If a solution of pure chain S has an absorbance of 0.8 at 280 nm, what is the concentration of protein in this solution? Assume that λ_{MAX} is at 280 nm for both residues and use the extinction coefficients given in lecture 5. You can assume that the path length is 1 cm. (3 pts)

6. (4 points, 10 min) Answer the following questions about protein secondary structure:
   (a) Do the amino acid sidechains in an α-helix point inward or outward (or both) from the main chain? (1 pt)
   (b) An amphipathic α-helix has hydrophilic residues on one side of the helix and hydrophobic residues on the other. Which face is likely to face the interior of the protein and why? (1 pt)
   (c) Are the hydrogen bonds in a β-sheet parallel or perpendicular to the β-strand? (1 pt)
   (d) Do the amino acid sidechains in a β-sheet point parallel or perpendicular to the plane of the sheet? (1pt)