Problem Set #2 Name_____

Chem 391 – Due in class on 9/13/18

1. I have created a link to a computer modeling exercise using PyMOL software on the assignments webpage. Please complete the exercise and submit the final image to me via email (≤4 people can collaborate on one image). You can use computers in room 203, or download the software yourself.

1. Draw the **side chains** of the following amino acids with the appropriate protonation states for the pH's identified below.

Amino acid	**pH 3**	**pH 7**	**pH 11**
Gln			
Glu			
His*			
Lys			

*His has two resonance forms at pH 3 and two tautomers at pH 11. Please draw both at each pH.

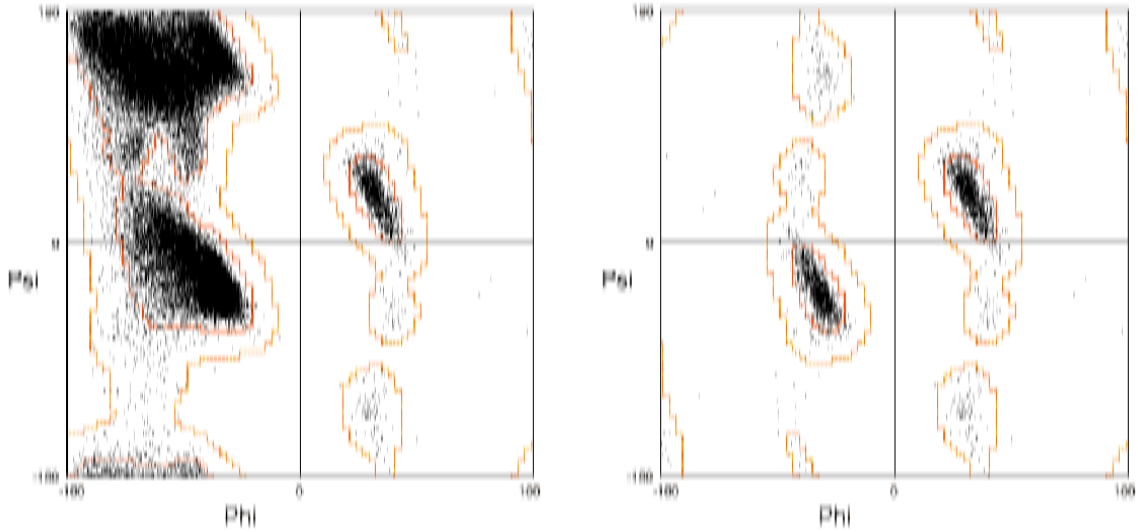
2. The pK_a of an acid may be altered by local conditions. For example the pK_a of acetic acid is lower in water than it is in hexane, because water stabilizes the conjugate base better, so acetic acid is a stronger acid (more ready to react to form the base) in water. Draw the following interactions and suggest how each might affect the pK_a of the underlined amino acid **side chain**.

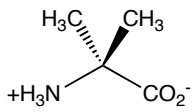
a. Cysteine in a 50:50 mixture of water and ethanol (a reduced polarity environment).

b. Arginine H-binding to an aspartate side chain.

c. Tyrosine H-bonded to a lysine side chain.

3. The following are the Ramachandran plots for alanine (left) and aminoisobutyric acid (Aib; right), an α-amino acid with two methyl groups on the α carbon atom.





Consider a helix-forming peptide that substitutes an alanine residue with Aib.

a. Is Aib capable of adopting α-helical conformation? Explain briefly.

b. Is a peptide containing Aib capable of greater conformational flexility than one with Ala, or less? Explain briefly, referring to the above plots.

c. Would Aib shift the equilibrium between random coil and helix towards helix or towards coil? Suggest enthalpic and/or entropic rationales as appropriate.

4. Draw the following peptides and indicate their total charge at pH 7.

a. CHARGED

b. ACIDIFIER

5. An MS/MS sequencing project is being conducted. A tryptic digest of the protein is performed, and from the primary MS spectrum of these peptides a peak with m/z ratio of 240.8 is selected. Following CID fragmentation, the peaks (corresponding to B & Y fragments) are observed at the following m/z ratios:

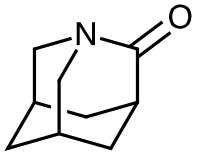
132.2, 147.1, 231.3, 250.3, 334.5, 349.5

What is the sequence of the peptide with m/z ratio of 240.8? Show your reasoning.

(See: http://db.systemsbiology.net:8080/proteomicsToolkit/FragIonServlet.html for a helpful fact checker)

6. When proteins are embedded in a biological membrane, and that portion of the peptide chain that crosses the membrane is always found in either alpha-helical or beta sheet conformation. Provide a thermodynamic explanation for that observation (use words like entropy and enthalpy to make me smile).

7. For every rule I give you, there's an exception. The following amide is protonated on the nitrogen, not the oxygen. Furthermore it hydrolyzes rapidly with a half-life in minutes instead of years as for the typical peptide bond. *Provide an explanation for this odd behavior.* Feel free to check out Komarav et al. (2015) *JACS* **137**, 926. They solved the crystal structure of the little beasty.



Questions related to Haney et al. (2016) "Thermodynamic origin of α-helix stabilization by side-chain cross-links in a small protein" *Org. Biomol. Chem.* **14**, 5768.

1. What is the goal of this research - what hypothesis is being tested? Why is this hypothesis of general interest? See if you can find an instance of a "stapled helix" being considered as a therapeutic compound.

2. Describe any limitations to previous approaches to addressing this problem and how this paper provides a superior approach. Focus also on the pairs of peptides shown in Figure 1 and how they contribute to an effective experimental design.

3. Methionine is replaced with norleucine in the peptides studied here. Draw both amino acids. There is concern regarding methionine oxidation. How is methionine modified by oxidation?

4. The authors describe purification by reverse phase HPLC.

a. Define the solid phase (solid support) and contrast that to silica gel.

b. How does the elution gradient compare to elution gradients used in silica gel, and why is that difference appropriate?

c. Elution times are given for peptides 2a and 2b in Supplementary Figure 1. Explain why the relative times are what they are.

6. CD spectra and temperature dependent "melting curves) are provided for the three sets of stapled helices in supplementary figure 2.

a. What information do the CD spectra provide in each instance?

b. How is a melting temperature obtained from the plots of ellipticity vs. temperature? A sketch may help.

c. Are the T_m results what you would expect from the CD spectra? Rationalize any similarities/differences.

7. We will discuss the technique used to obtain thermodynamic data at a later date. In the meantime, summarize the results.

a. What is the general observation of the effect of stapling on $\Delta H_{folding}$? Why or why not? Make explicit comparisons within Table 1. Is that what you/the authors expected?

b. What is the general observation of the effect of stapling on $\Delta S_{folding}$? Is that what you/the authors expected? Why or why not? Make explicit comparisons within Table 1.