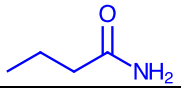
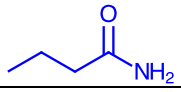
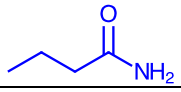
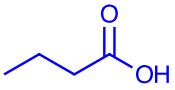
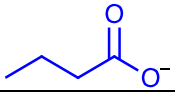
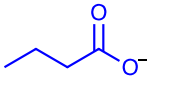
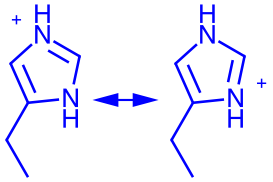
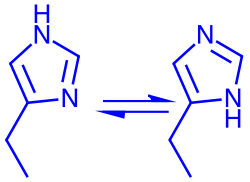
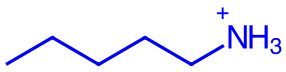
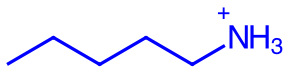
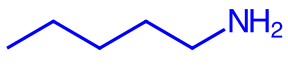
Problem Set #2 Name Solutions

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.

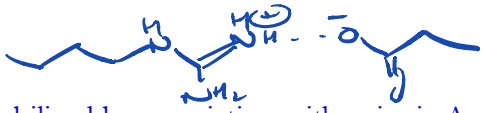
Amino acid	**pH 3**	**pH 7**	**pH 11**
Gln			
Glu			
His*		50:50 mixture of the stuff at pH 3 and the stuff at pH 11.	
Lys			

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

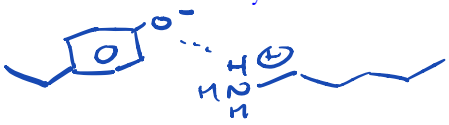
2. The pK_a of an acid may be altered by local conditions. For example the pK_a of acetic acid is lower in water than it is in hexane, because water stabilizes the conjugate base better, so acetic acid is a stronger acid (more ready to react to form the base) in water. Draw the following interactions 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).

pK_a will increase because anionic conjugate base becomes destabilized in non-polar environment.

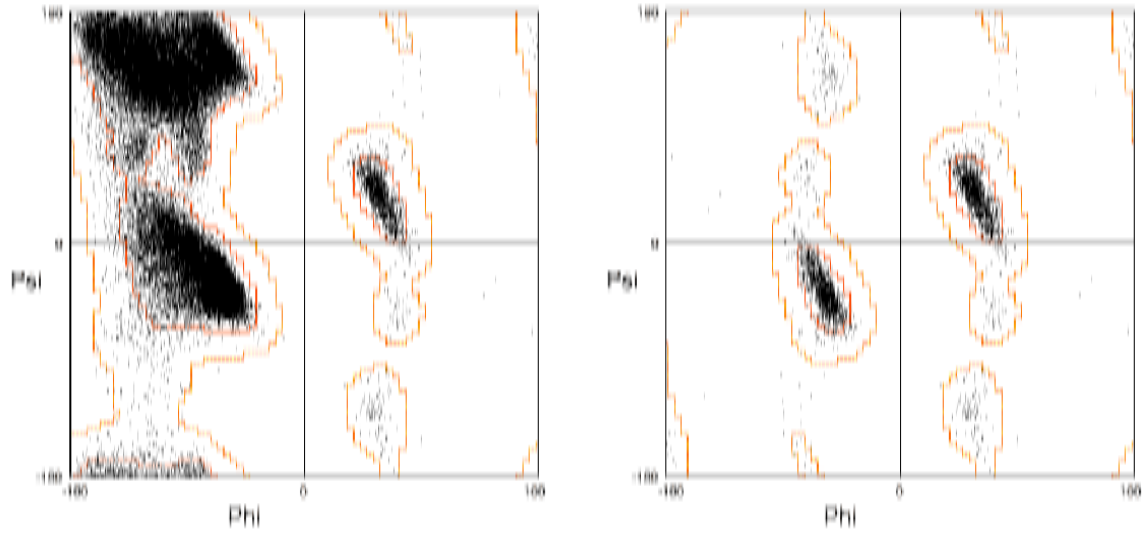
b. Arginine adjacent to an aspartate side chain 

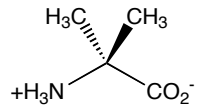
pK_a will increase because cationic conjugate acid is stabilized by association with anionic Asp.

c. Tyrosine H-bonded to lysine. 

pK_a will decrease because ammonium ion stabilizes anionic conjugate base of tyrosine.

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.

You betcha – one of its two islands of stabilitiy is smack at -60°, -60°.

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

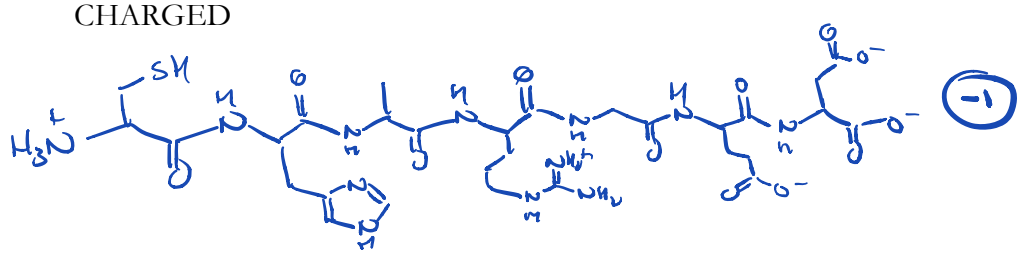
Less flexibility – it has fewer conformations available to it, so Aib has a rigidifying effect on the peptide.

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

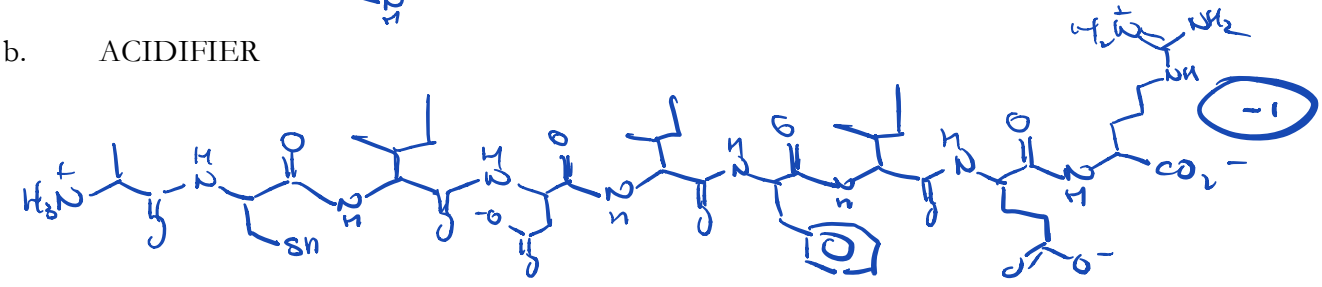
To helix – because the Aib is constrained and more likely to adopt helical conformation than Ala, it will lose relatively less entropy upon forming a helix than alanine.

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.

a. There are 6 peaks total, that indicates a tetrapeptide (three b fragments and three y fragments).

b. We know that y_1 will be either Lys or Arg + mass 19 (for two extra protons + OH). Lys would give 147.1 and Arg would give 175.2 We see a peak at 147.1, thus the C-terminal residue is **Lys**.

c. The next lightest peak is at 132.2. This must be the N-terminal residue of the peptide (+ mass of 1 H). That must be **Met**.

d. Now to explain 231.3 or 250.3? One of them is Met + residue 2, the other is Lys + res 3.

i. 231.3 – 132.2 = 99.1
250.3 – 132.2 = 118.1
Ha! 99.1 is **Val**, and there ain't no residue that is 118.1! Res #2 is **Val**

ii. To verify,
250.3-147.1 = 103.2, that's **Cys** as residue 3!
231.3 -147.1 = 84.2, and there ain't no 84.2.

So, we now have enough to claim a sequence of MetValCysLys

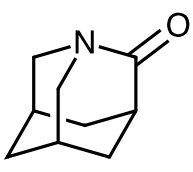
e. To confirm further:
334.5 -231.3= 103.2, so we see Cys attached to Met and Val
349.5 – 250.3 = 99.2 , and we get **Val** attached to Cys and Lys.

So, again: **MetValCysLys**! Plug it into the website above and we see that the $M+2H^+$ is 240.8.

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).

When a peptide chain enters the membrane, the backbone amides lose H-bonds they make with water. Only by forming secondary structure (H-bonding with themselves) can those backbone amides retain favorable enthalpic interactions.

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.



Note that this structure enforces sp^3 hybridization on the nitrogen and makes e^- pair sharing with the carbonyl impossible. As a result, the nitrogen behaves as a normal amine and is readily protonated – which makes hydrolysis relatively easy.

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.

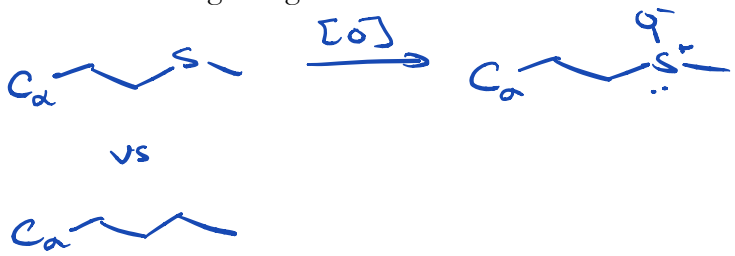
1. What is the goal of this research - what hypothesis is being tested? Why is this hypothesis of general interest? See if you can find an instance of a "stapled helix" being considered as a therapeutic compound.

They hypothesize that stapled helices achieve thermodynamic stability through pre-organization of the peptide. As it turns out, alpha helices are often important in protein-protein interactions related to disease states.

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.

Other attempts look at stapled helix stability indirectly through their ability to interact with target proteins, which confuses 2° structure stability with peptide-protein interactions.

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.

RP HPLC uses a hydrocarbon modified solid support so that it is non-polar. In this case C18 groups provide a non-polar surface.

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

Here they eluted with a gradient of decreasing polarity with water being replaced by acetonitrile. The decreased polarity will gradually elute more and more non-polar solutes.

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

2b comes off slower than 2a because the staple renders the groups less polar and 2b will stick to the non-polar surface more avidly.

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?

Generally speaking it informs us of the similarity in 2° structure between the derivatives and WT. The more or less negatve ellipticity at 222 nm gives evidence of greater or less helicity.

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

T_m is the point at which the 2° structure is 50% lost, the inflection point in the plot.

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

Interestingly, T_m increases for stapled helices relative to the wild type regardless of whether they make the helix "more or less helical" based on CD spectra. But 3b – the stapled helix that has greater inherent helicity than the wild type does have the greatest helicity.

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 $\Delta H_{folding}$? Make explicit comparisons within Table 1. Is that what you/the authors expected? Why or why not?

Intriguingly, all stapled helices fold with a less negative ΔH°, indicating that either (a) the unfolded stapled helix has some inherent greater enthalpic stability than the WT, or (b) the folded stapled helix is in some way enthalpically strained relative to the WT.

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.

In all cases the ΔS of folding is less negative (more favorable) for stapled helix. That is consistent with the hypothesis that pre-organization of the stapled peptide reduces the entropic cost of forming the helix.