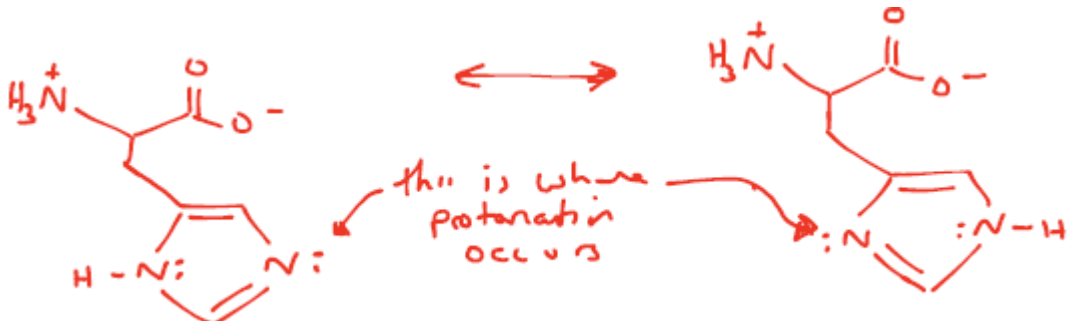
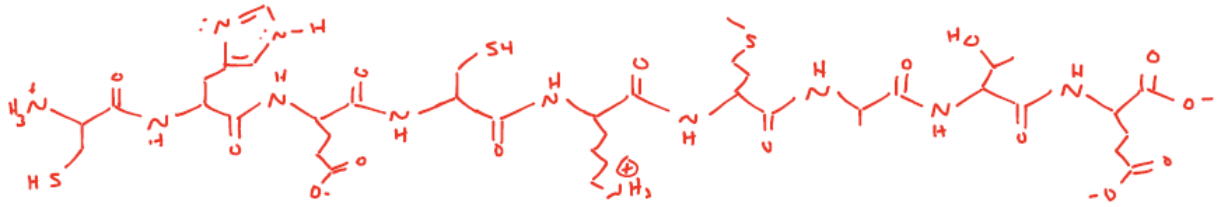
Problem Set 3 (**Due Sept 16th**)

1. Consider histidine. Draw as many resonance structures as you can. Is the ionizable proton (the proton that reacts with water) shared between the two nitrogens? You can draw a lot of different resonance structures with a carbanion formed, but these will not contribute to the average structure very much because they are not particularly stable. The two really important ones are shown below. Each of these would contribute ~50%, so the ionizable proton is shared between the two nitrogen atoms.



2. Consider the peptide CHECKMATE.
   a. Name this peptide. Cystyl-histidyl-glutamatyl-cystyl-lysyl-methionyl-alanyl-threonyl glutamic acid
   b. Write the peptide using three letter nomenclature. CysHisGluCysLysMetAlaThrGlu
   c. Draw the peptide.



   d. Using the standard side chain, N-terminus and C-terminus pKa values, predict the charge of the peptide at pH 6.0, 7.0 and 8.0

pKa 9.69 8.37 6.07 4.07 8.37 10.54 4.07 2.34

d. H3N- C - H - E - C - K - M - A - T - E - COO-

pH 6 +1 0 * -1 0 +1 +0.54 -1 -1

1 + 0.54 - 1 + 1 - 1 - 1 = -0.46 → So pI should be below pH 6!

pH 7 +1 0 +0.11 -1 0 +1 -1 -1

1 + 0.11 - 1 + 1 - 1 - 1 = -0.89

pH 8 +1 * 0 -1 * +1 -1 -1

as done for His → -0.3 each

1 - 0.3 - 1 - 0.3 + 1 - 1 - 1 = -1.6

* for histidine, we are at a pH in the buffer region

$pH = pK_a + \log \frac{A}{HA}$ solve for HA because protonated this is (+1)

$HA + A = 1$

$A = 1 - HA$

$6 = 6.07 + \log \frac{A}{HA}$

$-0.07 = \log \frac{1 - HA}{HA}$

$10^{-0.07} = \frac{1 - HA}{HA}$

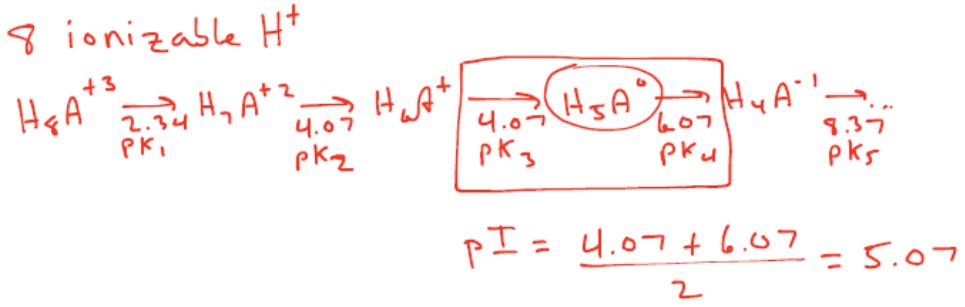
$10^{-0.07} HA = 1 - HA$

$(1 + 10^{-0.07}) HA = 1$

$HA = \frac{1}{1 + 10^{-0.07}} = 0.54$

→ this is the total positive charge from Histidine @ pH 6

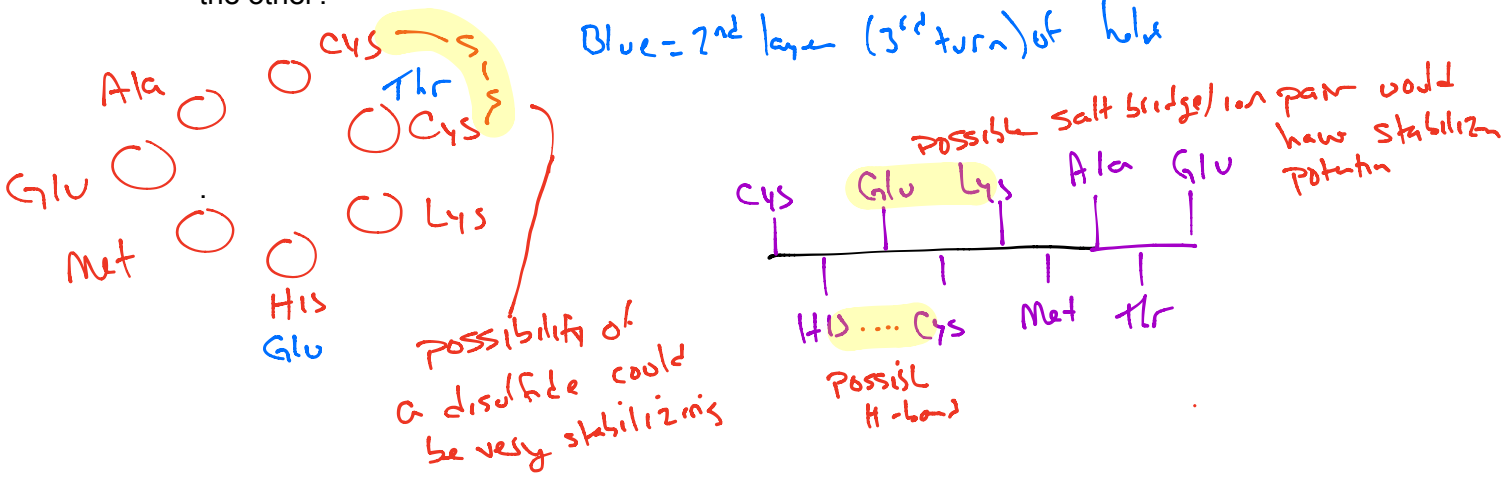
e. Determine the pI of CHECKMATE.



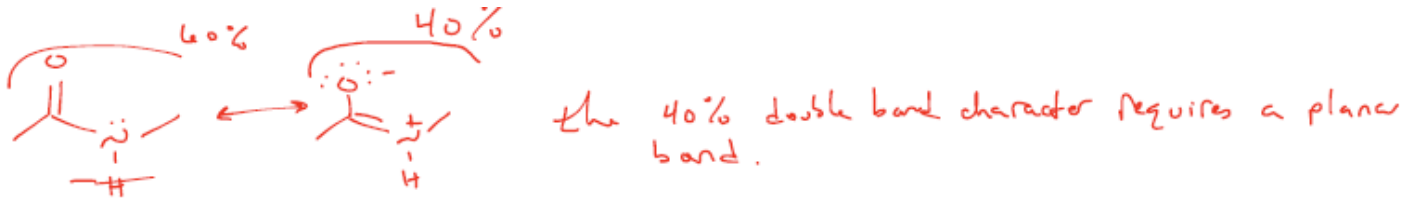
f. Can the side chains of this peptide be modified by any of the chemical modifications discussed in class (acetylation, phosphorylation, or disulfide formation)? E, H, and T can all be phosphorylated. The two cysteines can be oxidized to disulfides. In theory, E, H, and T can all be acetylated, but it's definitely more common to find primary amines getting acetylated. Lysine can be acetylated.

g. Based on your chemical intuition, predict if any of the side chain pKas will be modified based on the physical proximity to other ionizable groups. Any side chain in close proximity to another charged group will potentially be modified. For example, the HEC interaction might produce a modified pKa.

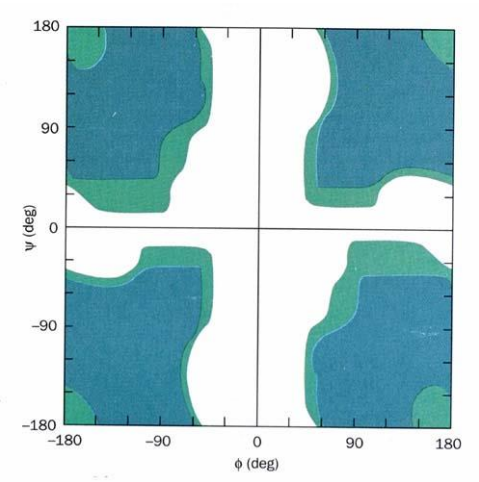
h. Consider this peptide forming into an α-helix vs. a β sheet. Is there any preference for one over the other?



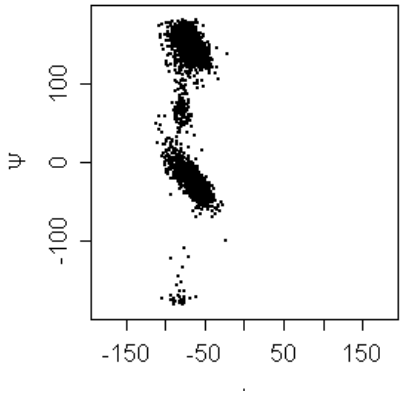
3. Please sketch a peptide bond and justify why it is planar.



Consider the Ramachandran Plot for polyglycine. Why is the $\Phi = 0$ and $\Psi = 0$ region not populated? Why are positive phi values allowed when they are not observed in other amino acids? A complete answer will include a couple sketches. Glycine's R group is the smallest of all amino acids, so it is not as conformationally restricted as other amino acids. The 0 regions are not populated because that is where the carbonyl oxygen or N-H will maximally interfere with neighboring atoms.



4. Consider the Ramachandran Plot (right). Predict what amino acid is represented here and discuss why you came to this conclusion. This is proline. Due to the cyclical side chain, proline is restricted to ϕ angles of ~60°



5. Amphipathic proteins are peptide chains that have folded into a conformation that contains a hydrophobic region and a hydrophilic region on the **surface**. How could this be accomplished in a β-sheet structure? How about an α-helical? Since β sheets alternate R-group orientation after every peptide bond, an amino acid sequence that alternates hydrophobic and hydrophilic side chains would result in an amphipathic β sheet. Helices can end up amphipathic if the A and D positions are hydrophobic, the B, C, and F positions are hydrophyllic, and E and G are typically charged. This is according to the heptad repeat.

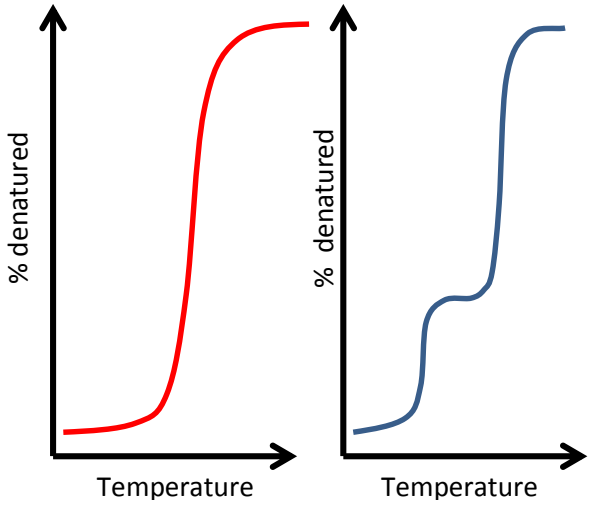
6. What are the common ϕ and ψ angles in alpha helices. What H-bonding pattern stabilizes this structure? A helices tend to be centered around (-75, -30). The backbone carbonyl of the n^{th} amino acid will H-bond with the backbone N-H of the $(n+4)^{th}$ amino acid.

7. Please describe the difference between parallel and antiparallel β-sheets. Anti-parallel sheets have H-bonding patterns with C=O lining up end on to an N-H in an adjacent strand. Parallel

8. Consider the hydrophobic effect.
    a. What role does the hydrophobic effect play in protein folding? Packing of the hydrophobic core of globular proteins is the major driving force for protein folding. The hydrophobic effect describes how this process occurs.
    b. Is this an enthalpically or entropically driven phenomenon? Why? Entropically driven. When hydrophobic molecules are forced to interact with water, very strong H-bonds are formed between water molecules to 'cage' the hydrophobic molecule. When the hydrophobe is transferred to a non-polar environment, the cage is broken ($\Delta H > 0$) but a lot of disorder in water molecules is created ($\Delta S > 0$)

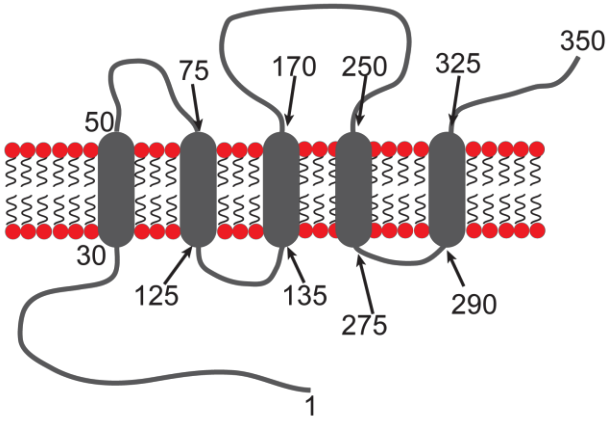
9. As we discussed in class, protein unfolding can be monitored using a variety of spectrophotometric techniques.

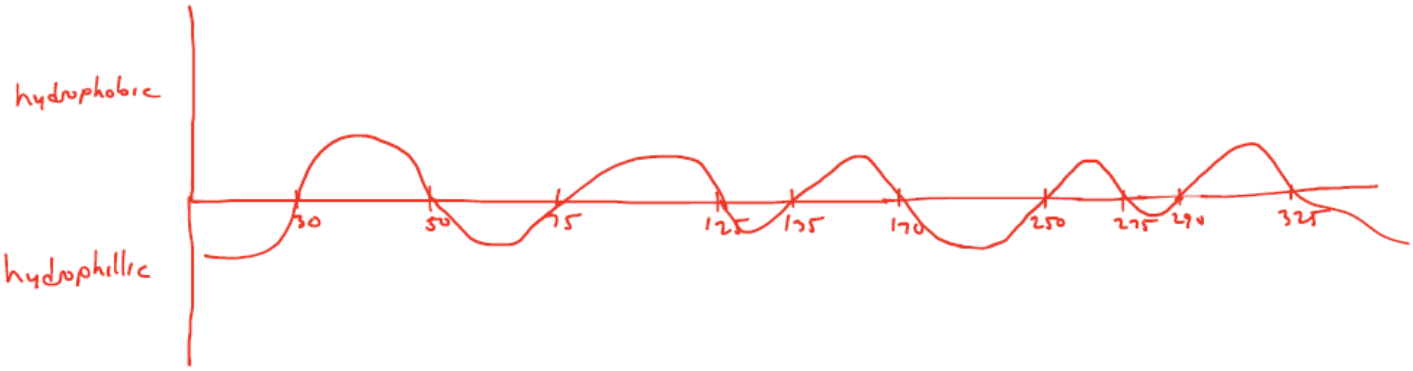
10. Describe how you can use circular dichroism spectroscopy to determine the melting temperature of a protein.



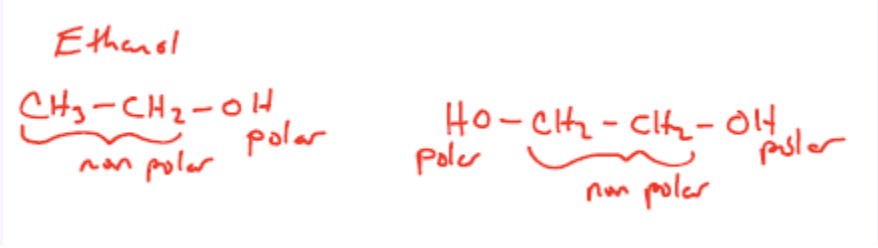
    a. What does the red melting curve tell us about the cooperativity of protein unfolding? Do you expect folding to be the same? It shows that unfolding is cooperative. Yes, folding will be the same.
    b. Think carefully about what you see in the blue curve. What does this unfolding profile suggest? This suggests that there are two independent domains. This could be due to quaternary structure (two proteins interacting to form an active enzyme), or it could be a multidomain protein.
    c. Suggest a sequence of events that could lead to a polypeptide chain folding into it's native conformation. The protein is synthesized at the ribosome. A molecular chaperone (GroEL/ES or HSP family member) binds to the protein, encapsulates it, and allows the folding to occur. Without the chaperone, the odds of the protein folding properly is very low. Other factors may be important as well – for example an enzyme may be necessary to help the protein ensure the proper disulfide bonds.

11. Using the schematic below for a membrane spanning protein (for clarification, the solid ellipses are embedded in the biological membrane and the loops are not), predict the hydrophobicity vs. amino acid number plot.





12. Ethylene glycol (2-hydroxyethanol) is a less effective protein denaturant than ethanol. Justify this observation. Protein denaturation will occur when hydrophobic regions of the protein core is disturbed. Ethanol, with its hydrophobic tail will be better at penetrating into the core than ethylene glycol because the latter has polar groups on both sides of the molecule.



13. Using the pdbID that I sent to you on Tuesday evening, determine:
    a. The name of your protein.
    b. The chemical reaction that it catalyzes.
    c. Using the tools that you learned last week, determine if homologues exist in other organisms. List the % Identity and % Similarity (% Positives) for at least two.