**Problem Set 2: Due Sunday September 8**   *Estimated time: 80 min*

1. (6 pts, 10 min) Imagine a solution of water that is divided into 20 cubes and each cube contains one water molecule. The entropy of this solution is zero because exchanging one water molecule for another does not change anything. In other words, there is only one way to arrange 20 identical molecules and S=RlnW, W=1.

   i) Calculate the entropy associated when one of the water molecules is replaced by a Na ion. Is the entropy change positive or negative, is this favorable or unfavorable?

   There are 20 ways to arrange on Na ion – it can be found in any of the boxes. S=RlnW = 8.3 J/mol-K x ln 20 = + 24.8 J/mol-K. This is positive and favorable.
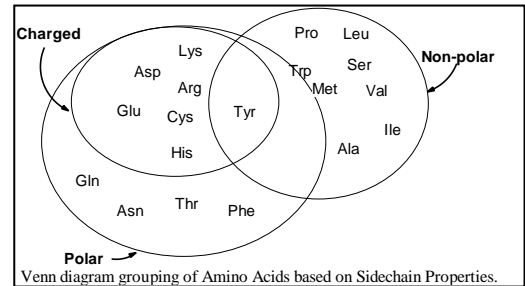
2. (4 pts, 5 min) A Venn diagram that organizes the amino acids by the properties of their sidechains is shown on the right. Glycine is omitted since its sidechain is just a hydrogen atom. As the diagram suggests, a sidechain can be non-polar, charged (in the pH range 0 to 10), or polar. Some amino acids belong to more than one set, e.g. all charged residues are also polar.

Venn diagram grouping of Amino Acids based on Sidechain Properties.

   i) Two amino acids are very clearly in the wrong set. Which are they and where should it be placed? *Briefly justify your answer.*

   Phe – it is completely non-polar

   Ser – it is polar with its OH group.

3. (10 pts, 15 min) An enzyme contains a single histidine (His) that must be **deprotonated** for the enzyme to be active. NMR (nuclear magnetic resonance) was used to measure the fraction protonated for this residue, utilizing the fact that the chemical shift (=absorption wavelength) of the protons on the sidechain change due to protonation/deprotonation.

   i) What is the chemical shift of the fully protonated His? Briefly justify your answer (1 pt).

   120, the value at low pH. 130 is the value for the fully deprotonated.

   ii) Determine the pKa of the histidine from the chemical shift data (2 pts).

   If we have an equal mixture of the two, then the chemical shift will be ½ between the two limits, so a chemical shift of 125 would be observed. This occurs at a pH of 9.

   iii) Plot the curve of activity versus pH, your x-axis should be from pH=4 to pH=10 (submit the plot) (4 pts).
   The activity is zero at low pH, 50% active at pH 9, 90% active at pH 10.

   iv) The pKa for the sidechain on this His is different than free His (pK$_a$ = 6). Based on the shift in pK$_a$, what type of environment surrounds this residue in the protein, i.e. are there positively or negatively charged groups near the His? *Justify your answer* (3 pts).
   This is a weaker acid, so the protonated state must be stabilized by a near-by negative charge.
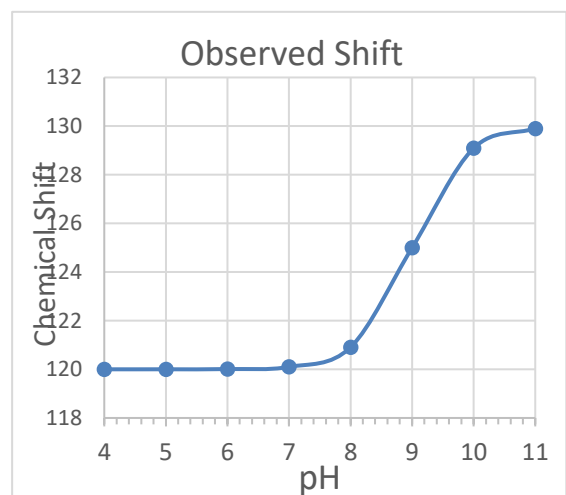
4. (16 points, 25 min) You are required to make a 500 ml solution of 0.2 M adipic acid to use as a buffer to perform a biochemical experiment at pH 6. You only have the fully protonated form of this acid in the lab (shown on the right), plus 1M solutions of HCl and NaOH. Assume that the pK$_a$ values for adipic acid are 4.4 and 6.4.
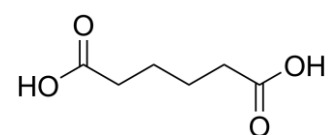
   i) *Sketch* a titration curve for adipic acid (4 pts). The pH should be 4.4 at 0.5 equivalents and 6.4 at 1.5 equivalents. Because the two pKa values are close together, the two buffer regions essentially merge.

   ii) How many *moles* of adipic acid would you need for your buffer solution (2 pts)? The number of moles of weak acid required is just the concentration x volume = 0.2 M x 0.5 L = 0.1 moles.

iii) Why does your answer to part ii) not depend on the pH of the solution (2 pts)? Because the pH will only change the relative concentration of the species in solution, not the total, i.e.:

$[A_T]=[AH_2]+[AH]+[A]$

iv) Calculate the number of equivalents of HCl (or NaOH) that you would need to adjust the pH of the solution. Briefly explain your choice of HCl (or NaOH) (4 pts).

First calculate the fraction protonated and deprotonated at the desired pH, using the closest pKa:

$R=10^{pH-pKa} = 10^{6.0-6.4}=10^{-0.4} = 0.4$

$f_{HA} = 1/ (1+R) = 0.714$

$f_A = R/(1+R) = 0.286$

Since the pH < pKa, we expect to find more protonated species.

Since we are starting with AH_2, we need to add NaOH. It is necessary to add 1 full eq to fully deprotonate the first proton ($AH_2 \rightarrow AH$) and then 0.286 equivalents to generate the correct ratio of AH and A.

Equivalents of NaOH = 1.286

v) Convert the equivalents of HCl (or NaOH) to number of moles (2 pts).

Equivalents is defined as the number of moles of titrant to moles of weak acid, therefore the moles of NaOH is:

moles NaOH = eq NaOH x V x A_T = (moles NaOH/moles weak acid) x V (L) x (moles weak acid/L)

moles NaOH = 1.286 x V x A_T = 0.1286 moles of NaOH

vi) Explain why the two pKa values are different, even though the same chemistry occurs for each ionization (4 pts).

Deprotonation of the first -COOH generates a negative charge. This makes it more difficult to deprotonate the second since its deprotonation places two negative charges on the molecule. The energy of the A^{2-} state is raised by the unfavorable electrostatic interaction.

5. (15 points, 25 min) View the JSmol structure associated with your last name (see webpage) and answer the following questions. **Please indicate your "section" on your homework (A, B, or C)**

   i) Draw the chemical structure of this three-amino acid peptide, assuming pH = 7.0. Omit hydrogens attached to carbon (5 pts).
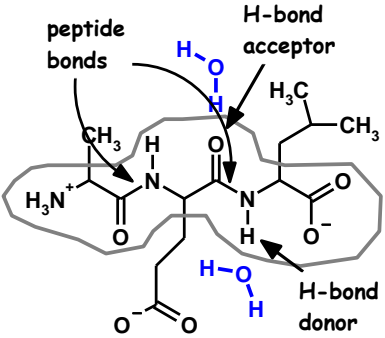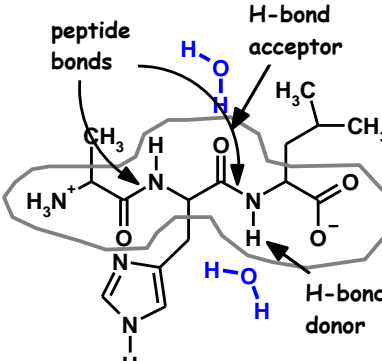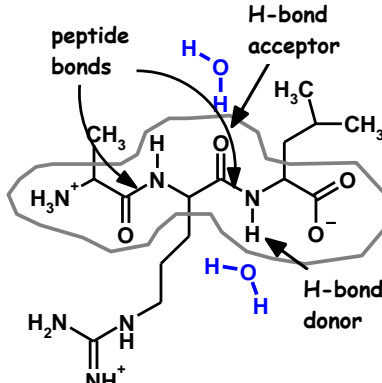
   ii) Write the correct sequence of your peptide. You will need to look at the sidechain groups to determine the identity of each amino acid residue; the labels provided by JSmol are erroneous (2 pt).

   iii) Draw a dotted line on your diagram that completely encloses all the mainchain atoms of this peptide (2 pt).

   iv) Which pair of labeled atoms (A-F) on the Jmol structure represent the peptide bond, indicate this bond on your sketch (2 pts).

   v) Which labeled atom can act as a hydrogen bond acceptor? Indicate this atom on your sketch and draw a water molecule forming a hydrogen bond with this atom (2 pts).

   vi) Which labeled atom (or pairs of atoms) on the Jmol structure can act as a hydrogen bond donor? Indicate this atom on your sketch and draw a water molecule forming a hydrogen bond with this atom (2 pts).

	Section A	Section B	Section C
i	Ala-Glu-Leu	Ala-His-Leu	Ala-Arg-Leu
ii	Note: The amino term is 100% protonated at pH 7 (pH << pKa). Both carboxylates are fully deprotonated (pH >> pKas).	Note: The amino term is 100% protonated at pH 7. The carboxylate is fully deprotonated. The His sidechain is 10% protonated.	Note: The amino term is 100% protonated at pH 7. The arg sidechain is fully protonated, and the carboxyl is fully deprotonated.
iii	See diagram.	See diagram	See diagram
iv	Peptide bond: B-D	Peptide bond: B-D	Peptide bond: B-D
v	H-bond acceptor: C	H-bond acceptor: C	H-bond acceptor: C
vi	H-bond donor: D & E	H-bond donor: D & E	H-bond donor: D & E