1. (6 pts, 15 min) You are growing a 0.5 L culture of bacteria in growth media that is buffered with 0.05 M Succinate, at an initial pH = 6.2. After 6 hours of growth, the pH rises to 6.5 due to the release of organic bases by the bacteria. Since the bacteria grow optimally at pH 6.2 you would like to return the pH back to 6.2. How many moles of HCl would you need to add to the 0.5 L culture to restore the pH to 6.2? You can assume that the pK_a values of succinate are 4.2 and 5.6.
   Hint: Try one of the following,
   - Sketch a titration curve to approximate how many equivalents of acid you would need to move from a pH of 6.5 to 6.2.
   - Sketch a curve of fraction protonated versus pH (for the appropriate pKa value) to help you figure out the number of equivalents that would be required to move the pH from 6.5 back to 6.2.

   For questions like this, you should always calculate the fraction protonated (or deprotonated), the only exception should be when pH=pKa. The plots can be used to check to make sure you are on the right track.
   You should do all of your calculations using the pKa that is the buffer region you are working in, in this case pKa=5.6.
   The equivalents of HCl you would add is just the difference in the fraction protonated (or the fraction deprotonated) at the two pH values:

pH=6.5 $R=10^{6.5-5.6} = 10^{0.9} = 7.94$ $f_{HA} = 1/8.94 = 0.112$	pH=6.2 $R=10^{6.2-5.6} = 10^{0.6} = 3.98$ $f_{HA} = 1/4.98 = 0.201$

   The difference is 0.089 equivalents.
   Moles of HCl = eq x $[A_T]$ x V
   = 0.089 x 0.05 M/l x 0.5 L
   = 2.22×10^{-3} moles

2. (5 pts, 10 min) The sequence of three tripeptides are given below. Which of these peptides will bind most tightly a positively charged surface, assuming pH=6.0? Use the pKa values from lecture 5. Briefly justify your answer with reference to the sequence of each peptide. (The different amount of binding to the surface would potentially provide a mechanism to purify the peptides from each other).

   A: Ala-Glu-Leu B: Ala-His-Leu C: Ala-Lys-Leu

   At pH=6, the charge from the mainchain ionizable groups essentially cancel; the amide is fully protonated with a + charge and the carboxy-terminus is fully deprotonated, with a – charge.
   A: The Glu sidechain is fully deprotonated (pH>>pKa), charge = -1, overall charge -1.
   B: The His sidechain is ½ deprotonated, average charge of +1/2, overall charge +1/2.
   C: The Lys sidechain is fully protonated (pH << pKa), charge is +1, overall charge is +1.

   Only peptide A will stick to the positively charged polymer (Thus peptide A could be easily purified from a mixture of all three peptides).

3. (12 pts, 20 min) Digestion of a peptide with Trypsin, Chymotrypsin, and Cyanogen bromide produced the sequence data given below. Note that it was only possible to perform Edman degradation for <u>seven</u> cycles, i.e. it is only possible to determine the sequence of the first seven residues of any peptide, even though the peptide may have been longer.
   i) Determine the sequence of the original peptide. *Show how you arrived at the sequence and demonstrate that your final answer is consistent with the experimental data (10 pts).*
      a) The products from Cyanogen bromide treatment gave peptides with the following sequences:
      Arg-Ser-Cys-Met Gly-Arg-Ser-Thr-Gly Trp-Gly-Ala-Val-Ile-Leu-Met
      b) Trypsin digestion produced one free amino acid (not identified), and the following peptide sequences: Ser-Thr-Gly Ser-Cys-Met-Trp-Gly-Ala-Val
      c) Chymotrypsin digestion produced the sequences of the following peptides:
      Arg-Ser-Cys-Met-Trp Gly-Ala-Val-Ile-Leu-Met-Gly

One possible approach is as follows:
The long fragment from trypsin contains a Met and should overlap with two CNBr fragments. It also contains a Trp, so it should overlap with two Chymotrypsin fragments:

Arg-Ser-Cys-Met Trp-Gly-Ala-Val-Ile-Leu-Met [CNBr fragments]
Ser-Cys-Met-Trp-Gly-Ala-Val [Trypsin fragment]
Arg-Ser-Cys-Met-Trp Gly-Ala-Val-Ile-Leu-Met-Gly [Chymotrypsin fragments]

Giving the following partial sequence.

Arg-Ser-Cys-Met-Trp-Gly-Ala-Val-Ile-Leu-Met-Gly

This sequence contains a Met near the end, indicating that it should overlap with another CNBr fragment:

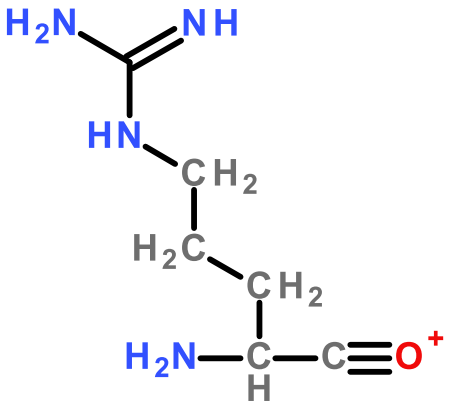
Arg-Ser-Cys-Met-Trp-Gly-Ala-Val-Ile-Leu-Met-Gly
Gly-Arg-Ser-Thr-Gly

Giving the final solution.

Arg-Ser-Cys-Met-Trp-Gly-Ala-Val-Ile-Leu-Met-Gly-Arg-Ser-Thr-Gly

Digestion/cleavage of the above sequence gives the following (detected sequence underlined):
CNBr Digest: Arg-Ser-Cys-Met Trp-Gly-Ala-Val-Ile-Leu-Met Gly-Arg-Ser-Thr-Gly
Trypsin: Arg Ser-Cys-Met-Trp-Gly-Ala-Val-Ile-Leu-Met-Gly-Arg Ser-Thr-Gly
Chymotrypsin: Arg-Ser-Cys-Met-Trp Gly-Ala-Val-Ile-Leu-Met-Gly-Arg-Ser-Thr-Gly

ii) Draw the chemical structure of the b_1 ion that would be measured by mass spectrometry and give its mass (2 pts). Its mass is 156.1 + 1 = 157.1. The extra mass unit comes from the fact that there are two hydrogens on the amino nitrogen, a **residue** in a protein only has one.



4. (5 pts, 5 min) You are sequencing a small protein by mass spectrometry and find the following masses for the b-fragments. What is the sequence of the protein? A table of residue masses is provided on the following page: b_1=58 b_2=129 b_3= 242 b_4= 405 b_5= 518

The mass of each b-fragment is the sum of the residue masses, from the amino terminus, plus one for the fact that the amino terminus is NH_2 (internal mainchain N are NH). It is helpful to take the difference between successive b-fragments to give the residue mass:

Residue 1 = 58-1 = 57 (Glycine)
Residue 2 = 129-58 = 71 (Alanine)
Residue 3 = 113 (Leucine or Isoleucine)
Residue 4 = 163 (Tyrosine)
Residue 5 = 113 (Leucine or Isoleucine)

The sequence is Gly-Ala-(leu or Ile)-Tyr-(leu or ile)
The b-fragments do not permit distinction between leu or ile. However, the sidechains of these two amino acids fragment differently, so it is possible to use mass spec to identify whether it is a leu or ile.

The following questions (6-8) will require you to view JSmol structures.

5. (3 pts, 10 min) Use the protein G structure to learn more about β-stands. Please answer the following questions:
   i) What strand contains residues 42 and 47 (counting strands from the amino terminus).
   The third one (residues 1-9 are the first, 13-21 are the second, 51-56 the fourth).
   ii) What is the distance between the mainchain nitrogen atoms of residue 42 and 47? (spanning 5 residues) 16.7 *Å*

   Can't find the amino terminus? Try the following using the menu: Color:Structures:Cartoon:By scheme:Group. The amino terminus will be blue.

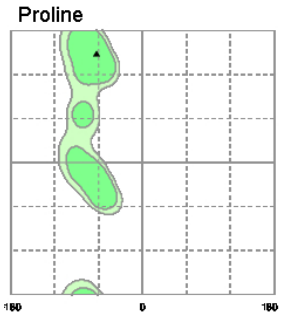
6. (4 pts, 10 min) Use the protein G structure to learn more about α-helices. Please answer the following questions:
   i) What is the distance between the mainchain nitrogen of Ala 26 and Asn 37? 16.77 *Å*
   ii) How many residues would be required to span this distance if these residues were in a β-strand?
   Each residue in a b-strand spans 16.7/5 = 3.34 A, so a b-strand with 5 residues would be long enough.

   Take home message – about twice as many residues are required to make a helix that is the same length as a beta strand.
   iii) Determine the relationship between hydrogen bonded residue in a helix. If the "i^{th}" residue is the C=O acceptor, which residue is providing the donor (e.g i+2).
   The relationship is i, i+4.

7. (5 pts, 10 min) The absorbance (A) of a solution of protein G was measured at λ=280 nm and found to be 0.75. The path length is 1cm. Using the composition from the Jmol page (see instructions on the page), and the extinction coefficients given in lecture, determine the concentration of the protein in solution. Please show your work. Protein G contains 1 Trp and 3 Tyr residues, so its molar extinction coefficient is 9,970 (5,500 + 3 x 1490). The concentration is 0.75/9970 = 7.52×10^{-5} M.

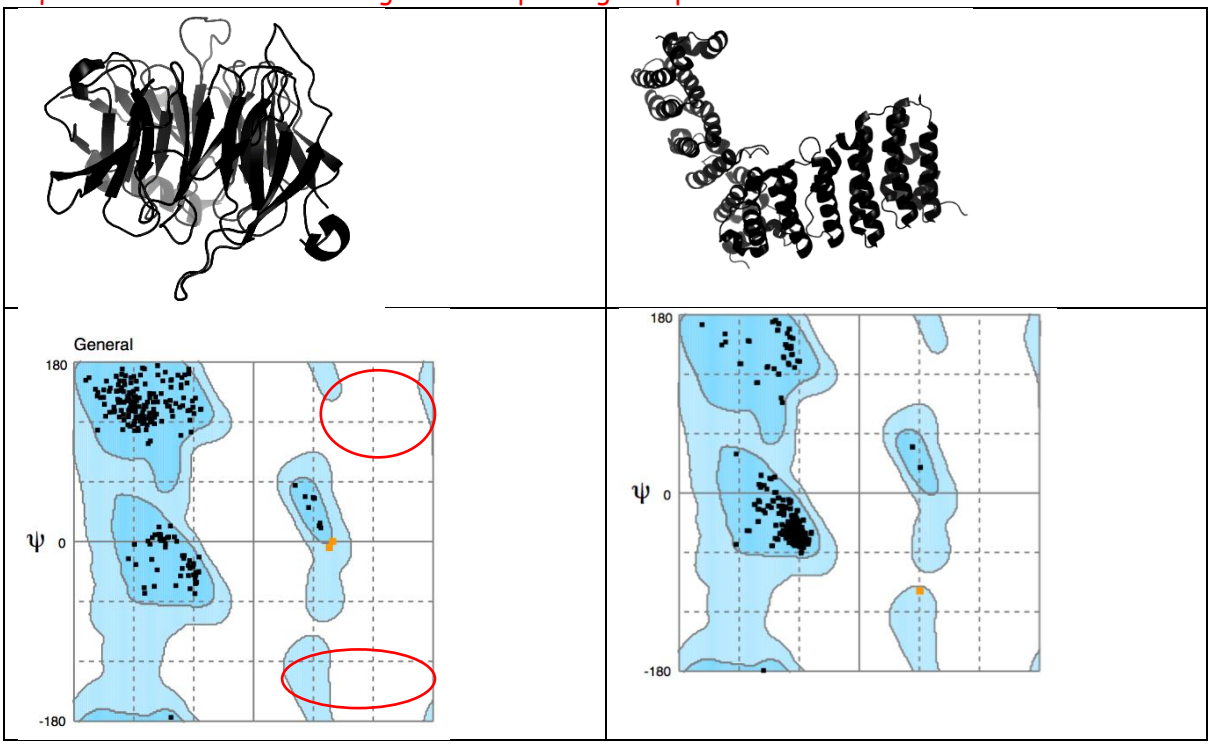
8. (5 pts, 10 min). The Ramachandran plot for proline is shown on the right. Explain why the plot for proline is much more restrictive than the other 18 amino acids.

   Note that the only permissible angles are on the right. The x-axis refers to possible angles for rotation about the N-C-alpha bond. In a proline, because of the ring, this angle is fixed to about -90, i.e. on the left side of the Ramachandran plot.



9. (5 pts, 5 min) Below are the Ramachandran plot for two proteins.
   i) Match the protein (A or B) to its corresponding Ramachandran plot. Please justify your answer.
   ii) Glycine appears to be very rare in both of these proteins. How could you conclude that from the Ramachandran plots alone?

i) Beta-sheets and alpha-helices are the predominant secondary structure elements in protein A and B, respectively. In plot 1 most of the residues are in the upper left quadrant where the bond angles characteristic of beta-sheets are located. In plot 2, most of the residues are in the lower left quadrant where the bond angles corresponding to alpha helices are concentrated.



ii) Glycine is probably rare because there are no residues (dots) in the region of the plot that glycine can occupy because of its smaller sidechain (H). These regions are circled in the above left plot.