Answer key

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 phosphate, 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. Calculate how many moles of HCl you would need to add to the 0.5 L culture to restore the pH to 6.2. You can assume that the second pKa value for phosphate is 7.2. [Hint: 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. Alternatively, sketch a curve of fraction protonated versus pH to help you calculate the number of equivalents that would be required to move the pH from 6.5 back to 6.2].

You need to calculate the fraction protonated (or deprotonated) at the two pH values, the difference gives you the number of equivalents are required to go from one pH to the other. Since we are using the middle buffer region, the pKa to use is 7.2

\[ R_{pH=6.2} = \frac{10^{6.2-7.2}}{10^{-1}} = 0.1 \], therefore \( f_A = 0.91 \) at pH=6.2.

\[ R_{pH=6.5} = \frac{10^{6.5-7.2}}{10^{-0.7}} = 0.2 \], therefore \( f_A = 0.83 \) at pH 6.5.

Quick check: Both of these numbers should be more than 0.5, since both pHs are below the pKa. More should be protonated at pH=6.2, so the \( f_A \) values look reasonable.

The difference is 0.08 equivalents. Therefore the number of moles of HCl to add is:

\[ \text{moles HCl} = \frac{\text{eq HCl} \times [A_T] \times V}{0.08 \times 0.05 \text{ moles/L} \times 0.5 \text{ L}} = 0.002 \text{ moles} \]

2. (8 pts, 10 min)

i) Estimate the pl of the peptide that you viewed for problem 5 on Pset 2 (sequence is Ala-Glu-Leu). You can do this by calculating the charge at a number of different pH values, or use excel to do these calculations for you (5 pts)

This would occur around a pH of 3.0. At this pH, the charge on the amino group is +1, COOH mainchain is -0.9, charge on the COOH sidechain is -0.1.

You can easily program this calculation in Excel. The formula for the average charge over all of the molecules is:

\[ q = \sum \left( f_{A_{\text{Amino}}} q_{A_{\text{Amino}}} + f_{A_{\text{COOH sidechain}}} q_{A_{\text{COOH sidechain}}} + f_{A_{\text{COOH mainchain}}} q_{A_{\text{COOH mainchain}}} \right) \]

In this particular case it simplifies as:

\[ q = f_{A_{\text{Amino}}} ( +1 ) + f_{A_{\text{COOH sidechain}}} (-1) + f_{A_{\text{COOH mainchain}}} (-1) \]

\[ = \frac{1}{(1 + R^{\text{Amino}})} + (-1)(R^{\text{Side}}/(1+R^{\text{Side}})) + (-1)(R^{\text{Main}}/(1+R^{\text{Main}})) \]

where R is calculated using pKa values of 9, 4, and 2, respectively. The plot of average charge versus pH is shown on the right, it crosses the axis at pH=3.0

ii) Assume you want to purify this peptide for a mixture of other peptides. One method is ion exchange chromatography, where small beads with a surface charge are used to capture charged molecules from solution by electrostatic forces between the bead and the charged molecule. In this case you have anion exchange beads which have a positive charge (so anions stick). What should the pH of your solution be to capture your peptide? How would you release your peptide from the bead after washing away other peptides that did not bind to the bead? (3 pts)

You want to do the chromatography above a pH of 3, so that the peptide has a net negative charge. Lowering the pH below the pl should release the peptide.

3. (8 pts, 15 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 seven cycles, i.e. it is only possible to determine the sequence of the first seven residues of any peptide. Keep in mind that the peptide may have been longer. 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.

i) The products from Cyanogen bromide treatment gave peptides with the following sequences:

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

ii) Trypsin digestion produced one free amino acid (not identified), and the following peptide sequences:

- Ser-Thr-Gly
- Ser-Cys-Met-Trp-Gly-Ala-Val

iii) Chymotrypsin digestion produced the sequences of the following peptides:

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

Biochemistry I

Problem Set 3

September 6, 2016
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

4. (5 pts, 5 min) A famous scientist has determined the structure of an important new protein by a technique called X-ray crystallography. They proudly show you the Ramachandran plot for their new protein structure (on right). On the basis of the Ramachandran plot, why is unlikely that they have determined the structure correctly? Please justify your answer.

This structure is likely wrong because most of the phi/psi angles are in the high energy region of the plot. When proteins fold, most residues will assume phi/psi angles that are in low energy regions.

Use the Jmol page to answer the following questions.
5. (5 pts, 10 min) Pick the first (closest to the amino terminus) β-stand in your protein and give the following:
i) Starting residue (n_s)  
ii) Ending residue (n_e)
iii) Length (l), in Å, of the beta strand (measured from the same atom in the start and ending residue), then calculate the length of a single residue in a beta strand = l/(n_e-n_s+1)

Can’t find the amino terminus? Try the following using the menu: Color:Structures:Cartoon:By scheme:Group. The amino terminus will be blue. See the Jmol tutorial for how to measure distances.

<table>
<thead>
<tr>
<th>Protein G (Å)</th>
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<tbody>
<tr>
<td>Start residue</td>
</tr>
<tr>
<td>End residue</td>
</tr>
<tr>
<td>Length (Å)</td>
</tr>
<tr>
<td>Length/residue</td>
</tr>
</tbody>
</table>

Take home message: If you looked at multiple β-strands you would see that they vary in the number of residues, but are fairly uniform in the distance/residues, which is about 3 Å.

6. (5 pts, 10 min) Complete the same analysis as Q5, but for the α-helix in the protein.
i) What is the relative distance spanned by a single residue in an alpha helix versus a beta strand?
ii) What is the linear distance spanned by one turn of a helix?

\( n_s = 23, n_e = 37 \), Length = 21 Å, \( \text{length/residue} \approx 1.5 \text{ Å} \), i.e. about \( \frac{1}{3} \) that of a residue in a beta strand

ii) Since there are 3.6 residues/turn, one turn spans \( 3.6 \times 1.5 \text{ Å} = 5.4 \text{ Å} \).
7. (5 pts, 10 min) Use this helix (from Q6) to determine the rule, or relationship, that describes the relative position in the polypeptide chain of mainchain hydrogen bond acceptors and donors in an α-helix, i.e. if you are given the residue number of an acceptor (e.g. residue # 15), your rule should predict the residue number of the hydrogen bond donor (Sample incorrect answer: The hydrogen bond donor for a residue in a helix is found at i+1, i.e. the residue immediately following donates the hydrogen bond. The general rule is that the NH group of one residue donates a hydrogen bond to the residue that is 4 away, i.e. the NH group from residue i, donates to the C=O of residue i-4.

8. (5 pts, 10 min) The absorbance (A) of a solution of your protein was measured at λ=280 nm and found to be 1.5. The path length is 1cm. What is the concentration of the protein in this solution? Please show your work. Instructions on determining the composition of a protein are given on the Jmol page.

<table>
<thead>
<tr>
<th>Protein G (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#Trp/#Tyr</td>
</tr>
<tr>
<td>ε</td>
</tr>
<tr>
<td>[X]=A/εl</td>
</tr>
</tbody>
</table>