Multiple choice (4 points apiece):

1. Hydrophobic interactions make important energetic contributions to:
   A) binding of a hormone to its receptor protein.
   B) enzyme-substrate interactions.
   C) membrane structure.
   D) three-dimensional folding of a polypeptide chain.
   E) all of the above are true.

2. Which of the following is true about the properties of aqueous solutions?
   A) The pH can be calculated by adding 7 to the value of the pOH.
   B) A pH change from 5.0 to 6.0 reflects an increase in the hydroxide ion concentration ([OH\(^-\)]\) of 20%.
   C) A pH change from 8.0 to 6.0 reflects a decrease in the proton concentration ([H\(^+\)]\) by a factor of 100.
   D) Charged molecules are generally insoluble in water.
   E) Hydrogen bonds form readily in aqueous solutions.

3. Which of the following statements about cystine is correct?
   A) Cystine is an example of a nonstandard amino acid, derived by linking two standard amino acids.
   B) Cystine is formed by the oxidation of the carboxylic acid group on cysteine.
   C) Cystine is formed through a peptide linkage between two cysteines.
   D) Two cystines are released when a -CH\(_2\)-S-S-CH\(_2\)- disulfide bridge is reduced to -CH\(_2\)-SH.
   E) Cystine forms when the -CH\(_2\)-SH \(R\) group is oxidized to form a -CH\(_2\)-S-S-CH\(_2\)- disulfide bridge between two cysteines.

4. In a highly basic solution, pH = 13, the dominant form of glycine is:
   A) \(\text{NH}_3^+\text{-CH}_2\text{-COOH}\).
   B) \(\text{NH}_3\text{-CH}_2\text{-COO}^-\).
   C) \(\text{NH}_3\text{\(^+\)}\text{-CH}_2\text{-COOH}\).
   D) \(\text{NH}_2\text{-CH}_3\text{-COO}^-\).
   E) \(\text{NH}_3\text{\(^+\)}\text{-CH}_2\text{-COO}^-\).

5. In a conjugated protein, a prosthetic group is:
   A) a fibrous region of a globular protein.
   B) a nonidentical subunit of a protein with many identical subunits.
   C) a part of the protein that is not composed of amino acids.
   D) a subunit of an oligomeric protein.
   E) synonymous with "protomer."
6. Thr and/or Leu residues tend to disrupt an \(\alpha\)-helix when they occur next to each other in a protein because:
   
   A) an amino acids like Thr is highly hydrophobic.  
   B) covalent interactions may occur between the Thr side chains. 
   C) electrostatic repulsion occurs between the Thr side chains.  
   D) steric hindrance occurs between the bulky Thr side chains. 
   E) the R group of Thr can form a hydrogen bond.

7. The structural classification of proteins (based on motifs) is based primarily on their:
   
   A) amino acid sequence.  
   B) evolutionary relationships. 
   C) function.  
   D) secondary structure content and arrangement. 
   E) subunit content and arrangement.

**Longer questions (70 points total)**

7. (20 points) Fill in the following table:

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<thead>
<tr>
<th>Name</th>
<th>Tryptophan</th>
<th>Glutamic Acid</th>
<th>Lysine</th>
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<tr>
<td>3 letter</td>
<td>Trp</td>
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<tr>
<td>side chain pKₐ</td>
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<td>4.25</td>
<td>10.53</td>
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<tr>
<td>(if ionizable)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>General classification</td>
<td>Aromatic</td>
<td>Negative Charge or Acidic</td>
<td>Positive Charge of Basic</td>
</tr>
<tr>
<td>(nonpolar, polar, etc)</td>
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<td></td>
</tr>
</tbody>
</table>

![Structure](structure.png)
8. (10 points) I am making a buffer with 5 grams of CH₃COONa (Molar mass 82) and 5 grams of CH₃COOH (molar mass 60) in 100 mLs of water. The pKₐ of the acid is 4.75

A. What is the pH of this buffer?

\[ \text{CH₃COONa} = \text{A⁻}, \text{ moles} = \frac{5}{82} = 0.0610 \text{ mol}; \quad \text{CH₃COOH} = \text{HA}, \text{ moles} = \frac{5}{60} = 0.083 \text{ mol} \]

\[ \text{pH} = \text{pKₐ} + \log \left( \frac{\text{A⁻}/\text{HA}}{\text{A⁻}/\text{HA}} \right) = 4.75 + \log(0.0610/0.083) \]

\[ = 4.62 \]

B. If I add 3 mLs of 5.0 M NaOH to the buffer, what is its pH now?

\[ \text{3 mls of 5.0M NaOH} = 0.003 \text{L} \times 5 \text{ mole/L} = 0.015 \text{ mole} \]

\[ \text{HA} + \text{OH}^- \rightarrow \text{A}^- + \text{H₂O} \]

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<th>INITIAL</th>
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<th>FINAL</th>
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<tbody>
<tr>
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<td>0.068</td>
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<tr>
<td>OH⁻</td>
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</tr>
<tr>
<td>A⁻</td>
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<td>+0.15</td>
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</table>

\[ \text{pH} = \text{pKₐ} + \log \left( \frac{\text{A⁻}/\text{HA}}{\text{A⁻}/\text{HA}} \right) = 4.75 + \log(0.068/0.0625) \]

\[ = 4.79 \]

9. (10 points)

A. Draw the structure of the peptide Glu-Lys-Tyr at pH 1 including all the appropriate charges on all ionizable groups.

\[ \text{B. Roughly sketch the titration curve of this peptide with base.} \]

It is hard to sketch this on my word processor. But you should have buffer regions at:

2-3 for the terminal COOH, 4.25 for the Glu, 9-10 for the terminal NH₂, 10.07 for the tyrosine and 10.53 for the lysine

B. What is the pI of this peptide?

The peptide has a +2 charge at the lowest pH, +1 after the first buffer region, and 0 after the second buffer, so the pI is the average of the 2nd and 3rd ionizations = (4.25 + Terminal NH₂)/2

call it (4.25 + 9.5)/2 = 6.875 and this value depends on what you used for your amino terminal pKₐ

10. (10 points) In class I discussed two types of electrophoresis. Describe how these methods are similar and different. Make sure you identify the different physical properties of the protein that are exploited in each kind of electrophoresis to separate different proteins from each other.

In both techniques the protein is placed in an electric field and the charge of the protein is used to make it move in the electric field.

In SDS gel electrophoresis the protein is coated with Sodium Duodecyl sulfate, a detergent that destroys the protein’s structure and coat the protein with negative charges. This makes the protein act like a uniformly charge rod, and the protein’s movement in the get depends only on the proteins molar mass. This technique is used both to determine the purity of the protein and to determine its molar mass.

In Isoelectric focusing electrophoresis the medium that the protein moves in has a pH gradient. Because the protein’s net charge depends on pH, when the protein moves to a region in the medium where the pH=pI, the protein stops moving. This technique is used primarily to determine the pI of a protein.
11. (10 points) We have two major techniques to determine the three-dimensional structure of a protein. Describe these techniques and identify their strengths and weaknesses.

The two main techniques used to determine a protein’s three-dimensional structure are X-ray crystallography and NMR. In X-ray crystallography a single crystal of the protein is placed in an X-ray beam and the diffraction pattern of the X-ray is recorded as the crystal is rotated in the beam. The diffraction pattern is then mathematically transformed with a Fourier transform, and this is used to determine the electron density within the protein. The structure is then determined by fitting models to the electron density pattern. The strength of this technique is that it can be used with proteins of any size. The weakness is that the protein must be in a crystal. Not all proteins make good crystals, and the crystal structure represents a solid state structure which may not reflect the actual solution structure.

In NMR a COSY experiment, which tells which protons are within three bonds of each other, is first used to determine the exact resonance of each proton in the protein. A NOESY experiment, which tells which protons are with a few angstroms of each other is next used to determine what parts of the protein are close to each other. A model is then moved around until all the distance between all the protons match with the NMR information. The strength of this technique is that the structure is determined in solution, so it more accurately reflects the true structure of the protein. Its weakness is that it is limited to smaller proteins, roughly those less than 300 amino acids.

12. (10 points) Compare and contrast the structure of the fibrous protein \( \alpha \)-keratin with the structure of collagen.

Both of these proteins are fibrous proteins, and both are composed of a helical structure. But the structure of the helices are very different.

\( \alpha \)-keratin - the base structure in \( \alpha \)-keratin in the standard right-handed \( \alpha \)-helix. Two of these helices are then twisted around each other in a left-handed coiled-coil, much like a rope. The interface between the two helices is rich in hydrophobic residues.

Collagen - The base structure here is a triple helix of three extended strands to create a left-handed triple helix. The space between the three strands is very limited, so only glycine is small enough to fit, thus 35% of collagen is glycine. The structure also works better if the glycine is followed with a proline or a hydroxyproline, so these residues compose 21% of the amino acids. The small amino acid alanine make up about 11% of the residues. There are also some crosslinks between the collagen fibrils involving lysine, hydroxylysine and histidine.