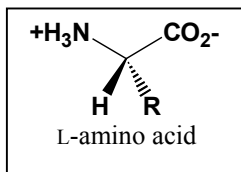
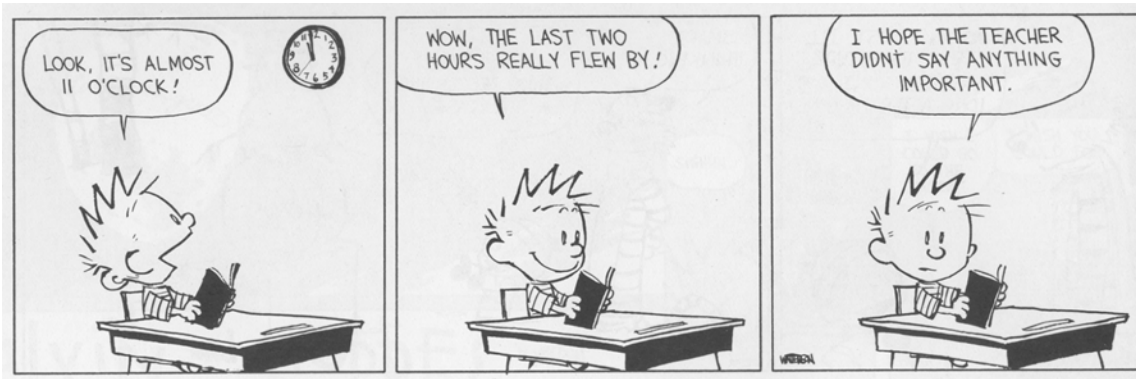


**CHEM 641 Biochemistry First Exam**

Saturday, October 6, 2007, 10am - noon, 100 Kirkbride

Instructor: Bahnson

Please put your name on the front, initial each page, and check to make sure that your exam copy contains all 10 pages. During the exam, take care not to expose your answers to your neighbors. If you have any questions, raise your hand and I will come to you. Remember, no calculators please. Good luck!

Breakdown of Exam

Part I	10 Short Answer	10 pts
Part II	5 multiple choice	10 pts
Part III	2 sections	20 pts
Part IV	5 questions	60 pts
Total		100 pts

Here are some useful constants and equations:

Gas constant -  $R = 8.315 \text{ J mol}^{-1} \text{ K}^{-1}$   
 or  $R = 1.987 \text{ cal mol}^{-1} \text{ K}^{-1}$

$$^{\circ}\text{C} + 273.15 = ^{\circ}\text{K}$$

$$1 \text{ cal} = 4.184 \text{ Joule}$$

$$1 \text{ mM} = 10^{-3} \text{ M}, \quad 1 \text{ \AA} = 10^{-10} \text{ m}$$

Van der Waals  $E = B/r^{12} - A/r^6$

Ionic interaction  $F = e_1 e_2 / D r^2$

<u>amino acid</u>	<u>pKa</u>
Asp	3.9
Glu	4.3
His	6.0
Cys	8.3
Tyr	10.1
Lys	10.5
Arg	12.5
Ser	13.0
Thr	13.0
N-term. amino group	9.5
C-term. carboxy group	2.0

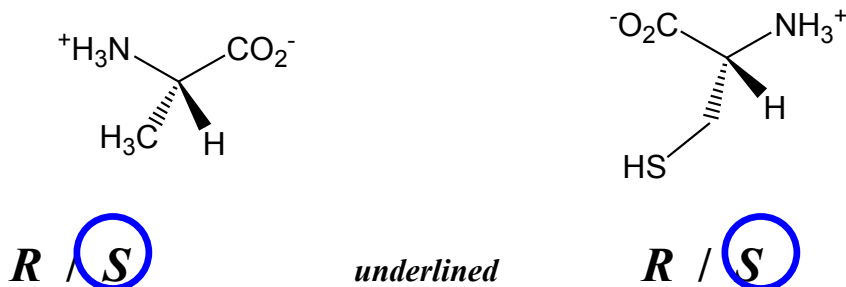
**PART I. Really short Answers (10 pts)**

1. Technique best suited to show polydispersity of a protein sample. \_\_\_\_\_ Native PAGE \_\_\_\_\_
  
2. Method to obtain crystallographic phase from a homologous protein structure. \_\_\_\_\_ Molecular replacement (MR) \_\_\_\_\_
  
3. Write the equation relating Gibbs free energy and entropy. \_\_\_\_\_  $\Delta G = \Delta H - T\Delta S$  \_\_\_\_\_
  
4. Most significant weak force interaction of the dimerization of a protein. \_\_\_\_\_ hydrophobic interactions \_\_\_\_\_
  
5. Protein technique used to analyze purity based on antibody recognition. \_\_\_\_\_ Western or Immunoblot \_\_\_\_\_
  
6. Common motif of anti-parallel  $\alpha$ -helix proteins. \_\_\_\_\_ helix-turn-helix, leucine zipper \_\_\_\_\_
  
7. and 8. Name one Nobel prize winner mentioned in class [others were accepted also](#) and what they did. \_\_\_\_\_ Perutz and Kendrew \_\_\_\_\_ Mb and Hb structure \_\_\_\_\_
  
9. The torsion angle that rotates the  $C\alpha$ -C bond of a protein backbone. \_\_\_\_\_  $\Psi$  \_\_\_\_\_
  
10. Name of through space interactions used to provide NMR distance restraints for a protein structure. \_\_\_\_\_ NOE \_\_\_\_\_

## PART II. Select the one best answer [10 pts]

- E** 1. The force or forces critical for a hydrophobic core of a protein to fold starting from a primary sequence:
- Van der Waals
  - Hydrophobic interactions
  - Hydrogen bonding
  - Induced-dipole induced-dipole
  - All of the above
- C** 2. An  $\alpha,\beta$ -barrel protein fold always has:
- an extensive anti-parallel  $\beta$ -sheet forming an interior barrel
  - amphiphilic  $\beta$ -sheets
  - non-polar side chains on each side of its  $\beta$ -sheet
  - a well defined quaternary structure
  - non-polar side chains on the surface of the protein
- C** 3. Two globular protein's that have the same protein fold would have
- sequence homology
  - similar functions
  - a hydrophobic core
  - all of the above (A, B and C)
  - none of the above
- E** 4. Which statement is **TRUE** about a protein's backbone structure?
- The phi and psi angles define the relative orientation of peptide planes
  - It is possible to rotate the peptide bond despite its double bond character
  - The primary sequence determines the fold of a protein
  - A protein's backbone is polar
  - All of the above are true

5. Circle **R or S** for the chiral atom of each molecule below (1 pt each).



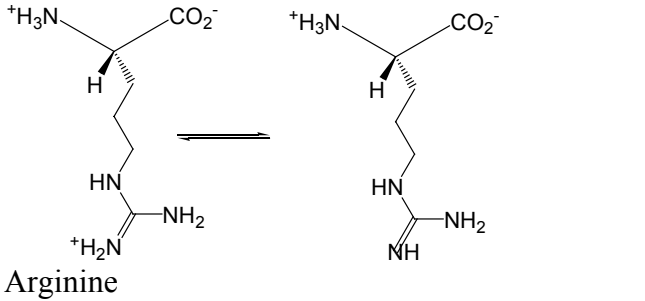
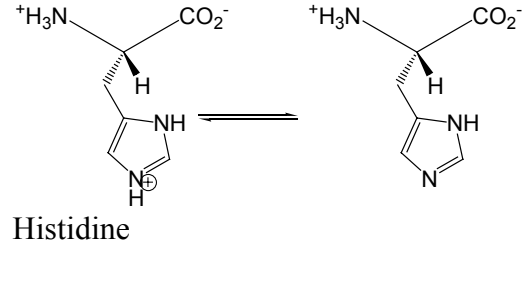
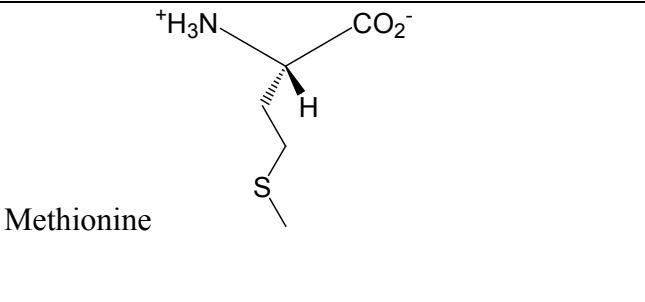
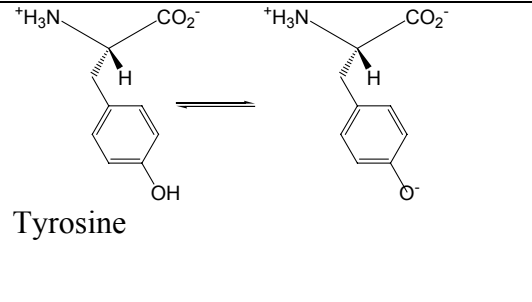
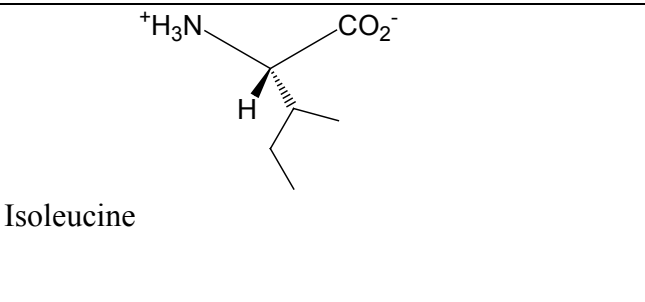
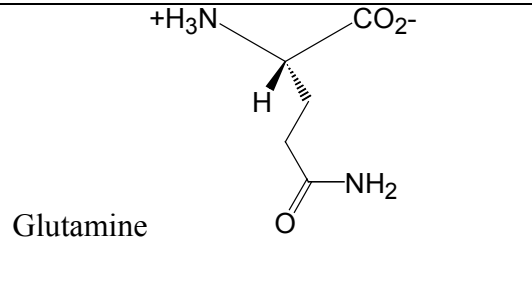
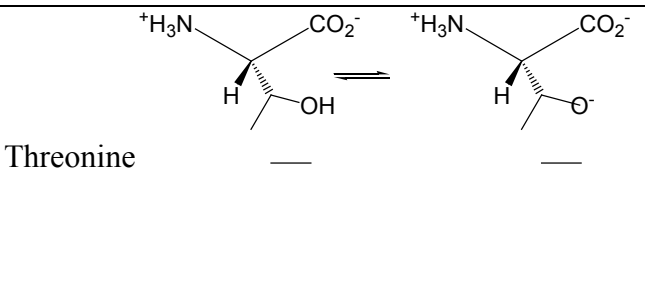
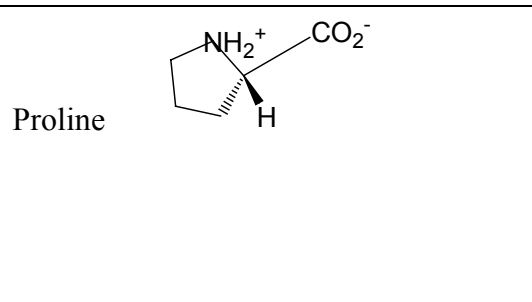
*underlined*

**PART III. Amino Acids**

**1. Write the SINGLE LETTER CODE of the following 8 amino acids [4 pts]**

Glutamine <u>  Q  </u>	Leucine <u>  L  </u>	Phenylalanine <u>  F  </u>	Aspartate <u>  D  </u>
Tryptophan <u>  W  </u>	Valine <u>  V  </u>	Asparagine <u>  N  </u>	Tyrosine <u>  Y  </u>

**2. Amino Acid Structures [16 pts]** Draw the entire structures in each box of the following 8 naturally occurring amino acids. For any side chains, which can exist in a protonated and unprotonated form, draw each form.

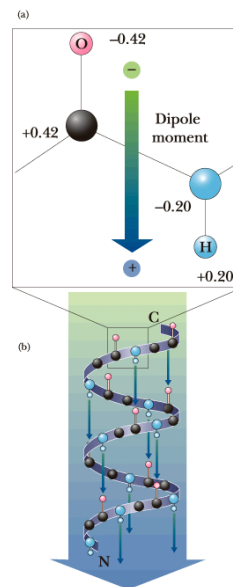
 <p>Arginine</p>	 <p>Histidine</p>
 <p>Methionine</p>	 <p>Tyrosine</p>
 <p>Isoleucine</p>	 <p>Glutamine</p>
 <p>Threonine</p>	 <p>Proline</p>

**PART IV. - Slightly Longer Answer Problems****1.  $\alpha$ -helices**

a) [5 pts] Draw how two peptide planes align in an  $\alpha$ -helix showing the H-bond formed. Based on your drawing, predict the overall dipole of this  $\alpha$ -helix. Explain briefly.

Peptide planes are all lined up with their carbon oxygens pointing to the C-terminus of the helix.

This leads to a dipole with a positively charged N-term of +0.5 and a C-term of -0.5.

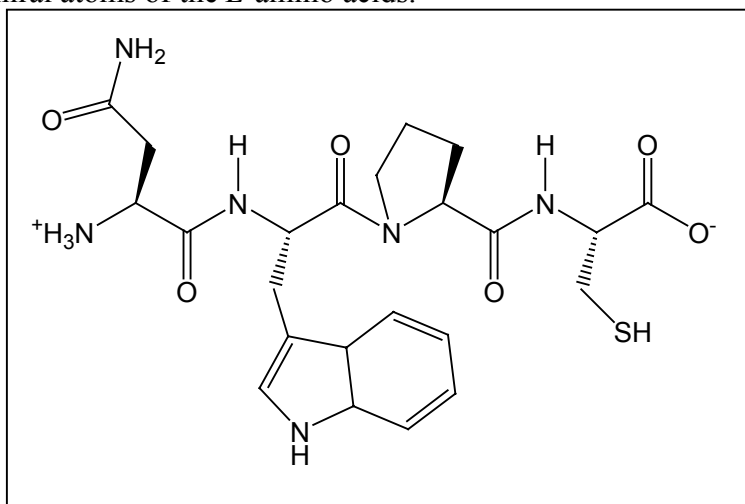


**Part b [5 pts]** Describe two separate reasons why the amino acid proline is not typically found in the middle of an  $\alpha$ -helix.

- 1) The backbone N lacks an amide hydrogen, so it can not make a H-bond in middle of helix.
- 2) Due to covalent attachment of side chain to N, the Phi torsion angle is restricted to a non-helical angle, thereby giving the helix a kink if Pro is in it.

**2. Peptides**

a. [7 pts] Draw the structure of the tetra-peptide Asn-Trp-Pro-Cys as it would appear at pH 7.0 including the full structures of the side-chains. Also show proper stereochemistry around any chiral atoms of the *L*-amino acids.



b. [3 pts] Estimate the net charge on the peptide at pH 4.3, 8.3 and 10.9.

pH 4.3 0

pH 8.3 -0.5

pH 10.9 -2

c. [5 pts] Suppose this peptide is biologically active only when proline's peptide bond is a *cis*-peptide bond. Furthermore, suppose this biologically active peptide is a pharmaceutical target (in other words a money making drug). In addition to standard peptide synthesis, which can be done with a peptide synthesizer, what would be needed in production in order to make the biologically active form?

**One possible answer:**

**After synthesis, the *trans*- and *cis*-form, which exist at a 4:1 ratio of the peptide, would need to be isolated by chromatography. The *cis*-form would then be ready for use. The *trans*-form could then be treated with a *cis-trans* proline isomerase to bring the solution  $K_{eq}$  to 4:1. This could be purified to get more of the *cis*-proline form. This process could be repeated several times.**

**3. Protein Heterogeneity [10 pts]** List and briefly describe 5 different ways proteins can display heterogeneity. With each of the 5 ways you chose, briefly state how the protein could be purified of the heterogeneity, or describe how each type of heterogeneity could be avoided.

**Listed below are the 5 most common answers:**

**1. Polydispersity or oligomerization**

**purify by size exclusion chromatography**

**or solve the problem by adding detergents to coat hydrophobic surface**

**2. disordered domain**

**reconstruct the expression construct to express individual domains**

**or**

**other reasonable approaches to purify based on a difference**

**3. Cys oxidation**

**solution, keep in reducing conditions with DTT or beta-mercapto-EtOH**

**4. ASN/GLN hydrolysis**

**solution: keep at pH 7.0, use immediately and/or store protein at -80 C**

**5. proteolysis**

**solution:**

**use protease inhibitors during prep**

**or**

**purify by size exclusion chromatography**

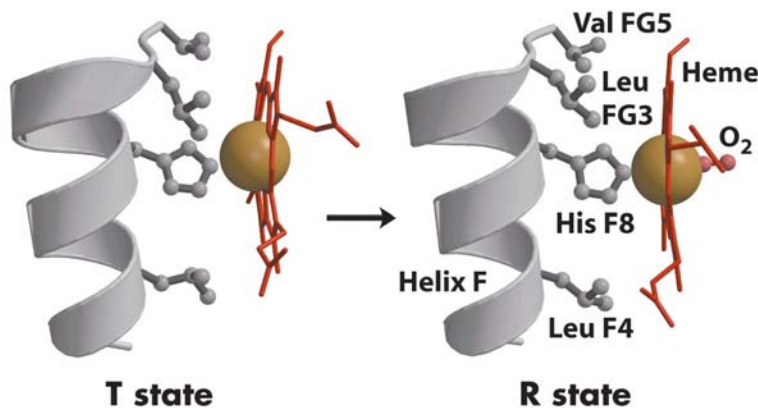
#### 4. Hemoglobin

a. [5 pts] Hemoglobin (Hb) is homologous to myoglobin (Mb) and shares a similar function, which is to bind  $O_2$ . Additionally Hb has cooperative properties suited for its physiological function to transport  $O_2$  in the blood and hand it over to Mb in metabolizing cells. In order to fine tune its cooperative function, Hb has interactions that stabilize either the R-state or the T-state. Describe 2 interactions for each state (4 total) that are present physiologically, and explain how the interactions are controlled to suit the overall function.

**R-State: Oxygen and higher pH stabilize the R-state.** As mentioned in class, oxygen binding pushes all four subunits into R-state as a homotropic positive effector. An increase of pH drives Hb into the R-state as well. This is due to the protonation state of histidine side chains in the intersubunit regions of the T-state. At higher pH (7.6 in lungs), these histidines lose their charge, and therefore lose the favorable salt bridge of the T-state of Hb.

**T-state: 2,3-BPG and  $CO_2$  stabilize the T-state.** As mentioned in class, 2,3-BPG has a specific binding site in the T-state of Hb for the sole purpose to stabilize the T-state. The T-state of Hb is also stabilized by N-terminal carbamylation by  $CO_2$ . This makes sense, that near metabolizing cells, Hb would bind  $CO_2$  in order to switch it the T-state (unload oxygen), as well as bring this  $CO_2$  back to the lungs for expiration.

a. [5 pts] Describe the conformational change that is induced by  $O_2$  binding to the active sites of Hb. To assist your explanation, use a drawing with at least helix-F, the Fe-coordinated histidine of helix-F, the Fe atom, a simplified heme plane, and the  $O_2$  molecule that binds.



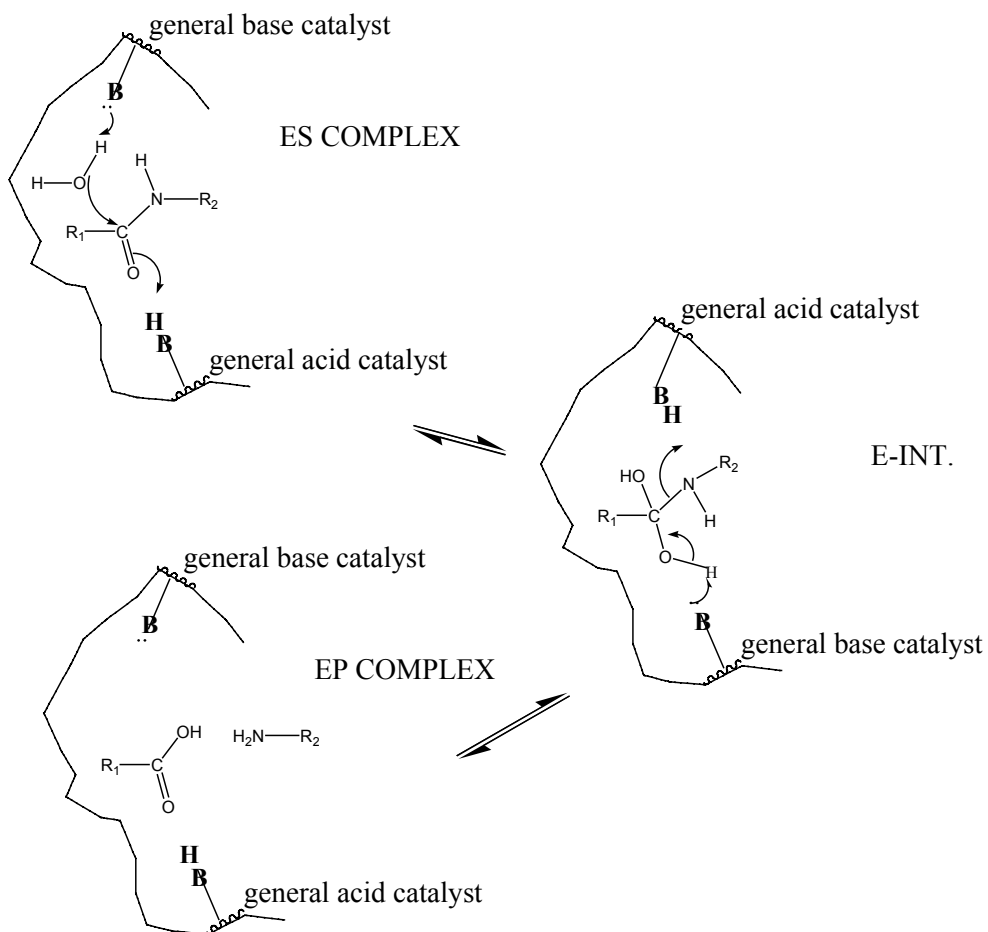
**$O_2$  binding shifts Helix F by 0.4 Å to right**

**This shift breaks salt bridges in the same subunit, as well as between neighboring subunits.**

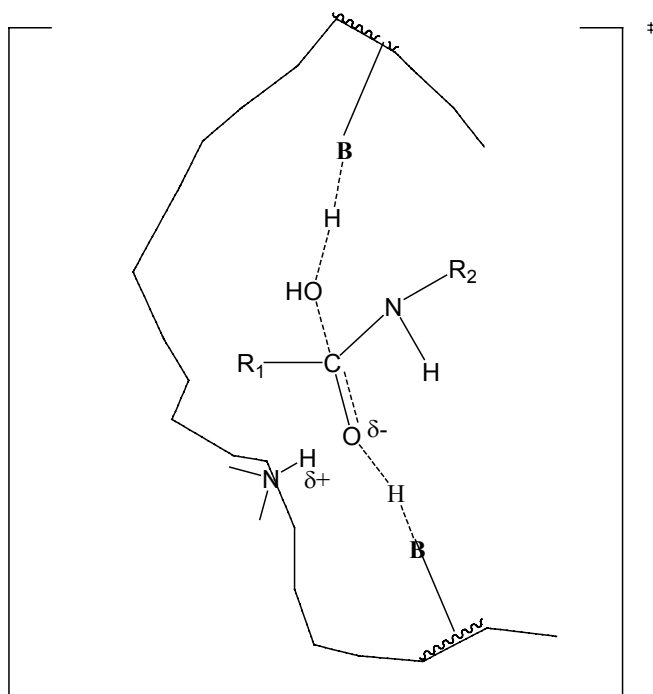
**$T_4 \rightarrow R_4$**

### 5. Protease Mechanism

**Part a. [10 pts]** Draw the complete mechanism (with arrows coming from the electrons) for a simple hydrolysis reaction of a peptide bond (protease). For maximum points show a two-step mechanism with a single tetrahedral intermediate. Also show the side chain of two separate amino acid residues simultaneously involved in the mechanism as general acid/base catalysts. If you want you can depict the general acid/base side chains as  $-B-H$  and  $-B$ :



**Part b. [5 pts]** For the first step of the mechanism drawn above, draw a reasonable transition-state. In your drawing include the involvement of general acid/base catalysts. Also depict an additional interaction the enzyme might have with the transition-state, in order to catalyze the reaction.



The additional interaction depicted above is a partial positive charge from a neighboring enzyme active site backbone amide, binding to the partial negative charge of oxygen in transition state. Any reasonable interaction like this was accepted here.