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Biochemistry I-First Exam Spring 2001

There are a total of 80 points on this Exam. Plan your time accordingly. This is about 2 points/minute of time. Some questions of low point value may take you longer to do and perhaps should be left to the end of the exam. Unless otherwise stated, each section of a question has equal weight.

Before beginning please write your name on every page. Use the space provided to answer the question.

Calculate: means to determine a number with units.

Sketch means to draw an approximate curve that illustrates the most important features of the curve.

Grade:

Part A: _____

B1: _____

B2: _____

B3: _____

B4: _____

B5: _____

B6: _____

The following equations and constants may be useful:

$T=300\text{K}$ and $\text{pH}=7.0$ unless otherwise stated.

$R=8.3 \text{ J/mol-K}$ $RT=2.5 \text{ kJ/mol @ } 300\text{K}$

$\log 2=0.3$ $\ln 10=2.3$

$$\Delta G^0 = -RT \ln K_{eq}$$

$$\Delta G = \Delta H - T\Delta S$$

$$S = R \ln W$$

For the reaction: $N \rightleftharpoons U$:

$$K_{eq} = [U]/[N]$$

$$f_u = K_{eq}/(1+K_{eq})$$

$$f_n = 1/(1+K_{eq})$$

$$\text{pH} = \text{pK}_A + \log([A^-]/[HA])$$

$$[HA] = [A_T]/(1+R)$$

$$[A^-] = [A_T]R/(1+R)$$

$$R = [A^-]/[HA]$$

Beer's law: $A = \epsilon[X]l$

Amino Acid Names:

Alanine: Ala

Arginine: Arg

Asparagine: Asn

Aspartic Acid: Asp

Cystine: Cys

Glycine: Gly

Histidine: His

Glutamine: Gln

Glutamic Acid: Glu

Isoleucine: Ile

Lysine: Lys

Leucine: Leu

Methionine: Met

Phenylalanine: Phe

Proline: Pro

Serine: Ser

Threonine: Thr

Tryptophan: Trp

Tyrosine: Tyr

Valine: Val

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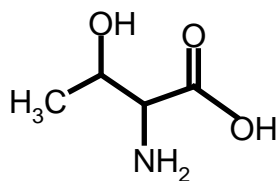
Part A: (3 points each, 24 points total) Circle the best answer. Partial credit is given in some cases.

1. Structures of the antibody that binds PCP shows that the tight binding of PCP to the antibody is primarily due to:
 - a) formation of covalent bonds.
 - b) van der Waals forces.
 - c) hydrogen bonds.
 - d) hydrophobic 'forces'.
2. Unfolding of an single α -helix in a non-polar solvent (i.e. benzene, hexane, etc) would be less favorable than in water because
 - a) of the formation of ordered benzene molecules around the non-polar sidechains.
 - b) of the inability to reform hydrogen bonds with the solvent.
 - c) of the formation of strong van der Waals interactions with the solvent.
 - d) reduction in the number of conformational states in the unfolded form.
3. ΔG^0 is zero for
 - a) reactions at equilibrium.
 - b) reactions that are spontaneous in the forward direction.
 - c) reactions that are spontaneous in the reverse direction.
 - d) reactions whose substrates and products have the same energy.
4. Which of the following are common features between an α -helix and a β -sheet?
 - a) both have the same phi and psi angle.
 - b) both have extensive hydrogen bonds.
 - c) both have hydrogen bonds that are parallel to the direction of the mainchain.
 - d) answers b and c.
5. The peptide bond in proteins is
 - a) a normal single bond.
 - b) always in the cis form, except for bonds before proline.
 - c) always in the trans form, except for bonds before proline.
 - d) none of the above.
6. In general, a weak acid can act as a buffer
 - a) at pH values = $pK \pm 1.0$
 - b) at pH values = $pK \pm 2.0$
 - c) at pH values = $pK \pm 0.1$.
 - d) at pH values = isoelectric pH.
7. Which of the following groups would form the strongest hydrogen bond with a carbonyl group?
 - a)NH.
 - b)OH.
 - c)CH.
 - d)All would be of equal strength.
8. Which of the following statements is *most* correct:
 - a) Polar residues can be buried in the interior of a protein.
 - b) All hydrophobic amino acids are buried when a protein folds.
 - c) Charged amino acids are never buried in the interior of a protein.
 - d) Tryptophan is only found in the interior of proteins.

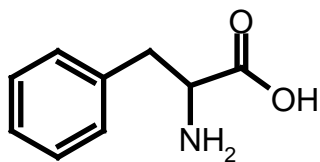
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Part B:

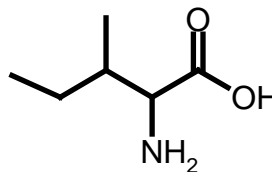
B1. (6 pts) The structure of three amino acids is shown below:



Threonine



Phenylalanine



Isoleucine

Select **one** of these amino acids and:

i) Show, by the removal, addition, or replacement of a small group, such as CH₃, OH, etc., (i.e. not the entire sidechain) how you could convert your chosen amino acids to another amino acid that is chemically most similar to the starting amino acid. For example: Alanine (R=CH₃) can be converted to Gly (R=H) by replacement of the methyl group with a proton. Either redraw the modified amino acid below or indicate your changes on the diagram above.

ii) Did your change increase or decrease the solubility of the amino acid in water? Briefly justify your answer.

iii) Did the change increase, decrease, or not affect, the hydrogen bonding capability of the amino acid? Justify your answer with the explicit mention of the atoms that are hydrogen bond donors or acceptors.

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B2. (14 pts) Indicate the approximate pKa of the side chain of **each** of the following amino acids (4 points).

Aspartic Acid:

Glutamic Acid:

Histidine:

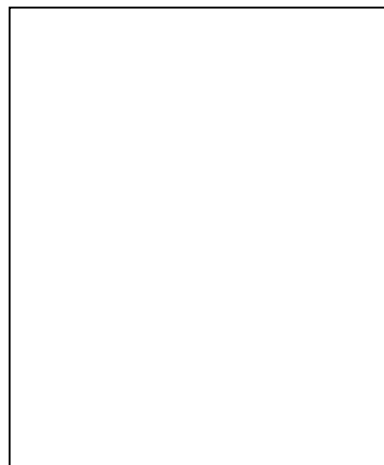
Lysine:

Arginine:

i) Based on your pKa values, which of the above would be suitable buffers at pH=7.0. Why? (2 pts)

ii) Pick **one** of these amino acids and draw its structure and identify it by name.(2 pts)

iii) Now sketch the pH titration curve that you would obtain if the amino acid you picked in part *ii* were contained in the peptide of the following sequence: Gly-X-Gly. Be sure to label the axis of your graph, provide an appropriate scale, and indicate the inflection points(s). Use the box to contain your graph. You may assume that the pKa of the amino terminus and the carboxy terminus are 9.0 and 2.0, respectively.(4 pts)



Do either part iv or part v (2pts):

iv) Indicate the isoelectric pH of this peptide on your graph. Briefly describe how you arrived at your answer.

v) What is the ratio of protonated to unprotonated **sidechain** at pH 8.0 for your choice? Please show your calculations. If you don't have a calculator, work the problem as far as you can without it.

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B3: (8 pts) Do **two** of the following **four** questions:

B3a: Briefly distinguish between the hydrophobic effect and van der Waals forces as they apply to protein folding. Which is energetically more important for stabilizing the folded state of proteins?

B3b. Briefly discuss the difference between ΔG and ΔG° . Which of these provides information on the direction of the reaction? Which of these provides information on the position (e.g. fraction of unfolded protein, amount of ligand bound) of the reaction?

B3c: Pick **one** of the two super-secondary structures.

- i) α - α unit (two helix bundle)
- ii) β -meander

Sketch its structure and briefly discuss the forces that would stabilize its structure.

B3d: What is the change in entropy when a 20 residue α -helix adopts a β -strand configuration? Justify your answer (**Hint:** *Think* before you calculate!)

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B4. (8 pts) The handout contains a chime image of a portion of a β -sheet from lysozyme (oxygen is colored red, nitrogen pale blue, carbon grey. Note that none of the sidechains are shown and the amide protons are also not shown.)

i) Is this sheet parallel or anti-parallel? Why?(2 pts)

ii) Place the following labels on the image, in the correct location (4 pts)

- a) psi (Ψ) angle
- b) phi (Φ) angle
- c) amino-terminal end of one of the strands
- d) peptide bond

(if you feel that you cannot interpret the chime image for labeling purposes, then draw a short peptide in the space below and label it accordingly.)

iii) Draw one hydrogen bond that you would expect to find in this structure. Label the donor and acceptor atoms and indicate the typical length for a hydrogen bond.(2 pts)

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B5. (13 Pts) A protein has two Trp residues and one Tyr residue. You can assume that the extinction coefficient for Trp and Tyr are 4,500 and 1,000 L/M-cm, respectively at 280 nm.

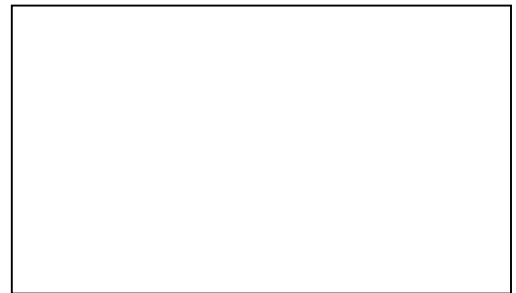
i) Calculate the extinction coefficient for the denatured form of this protein. You should assume that the residues in the denatured form absorb light in a manner that is identical to the amino acids in solution. Now calculate the absorbance, at 280 nm, of a 1 mM solution of unfolded protein.(2pts)

ii) Assume that protein folding **increases** the extinction coefficients for both Trp and Tyr by 10%, calculate the extinction coefficient for the folded form of this protein. Calculate the absorbance of a 1 mM solution of the folded protein.(2pts)

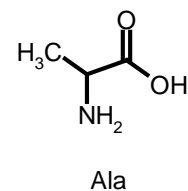
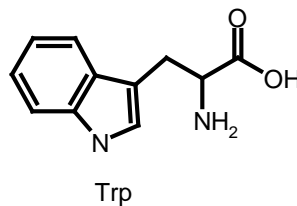
iii) A 1 mM (0.001 M) solution of this protein is heated from 273K to 373K and the absorbance of the solution is measured at different temperatures.

Sketch, in the box to the right, the curve of absorbance of ultraviolet light (280 nm) versus temperature from 273K to 373K. Be sure to label the x and y axis of your graph.

You can assume that this protein has a T_m of 350K, a ΔH° of 200 kJ/mol, and a ΔS° of 571 J/mol-deg (i.e. Very similar to the thermodynamics of ProteinG).(4 pts)



iv) A mutation was made in the protein, such that a completely buried Trptophan residue was replaced by an Alanine residue. This change does not affect the overall structure of the protein. The measured ΔH° of unfolding decreased by 5 kJ/mol in the mutant protein. Give two possible reasons for this decrease in enthalphy. Briefly justify your answer.(4 pts)



v) Calculate the fraction of the *folded* form of this mutant protein at 350K, assuming that the contribution of ΔS° is the same for both proteins.(1 pt)

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B6. (7 pts) Do **either** part a **or** part b.

B6a: The following peptide forms the central strand of an external β -sheet in a protein.

Val-Asp-Asp-Asp-Met-Arg-Val-Lys-Ala-Glu-Trp-Ser-Cys-Gly

i) Give the sequences of peptides produced by (3 pts):

Cyanogen bromide (CNBr) treatment.

Trypsin treatment.

Chymotrypsin treatment.

ii) Assuming that it is only possible to perform 5 cycles of Edman degradation on each isolated peptide, can you obtain the sequence of this peptide with the above fragments? Briefly justify your answer.(2 pts)

iii) Could this region of the protein participate in disulfide bonds? Why?(1 pt)

iv) This sequence forms an amphipathic sheet in the protein. However, the amino acid sequence presented above is not 'ideal' for a amphipathic sheet. How might you modify (i.e. change to another amino acid) a residue to increase the stability of the protein? Briefly justify your choice.(1 pt)

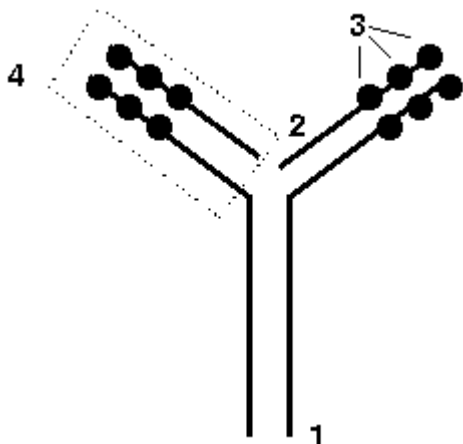
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B6b:

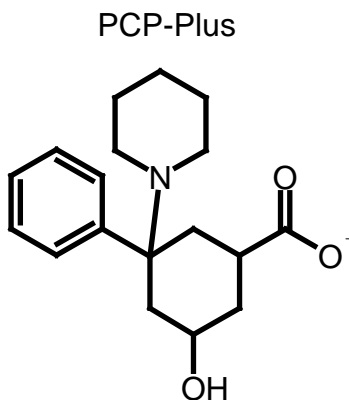
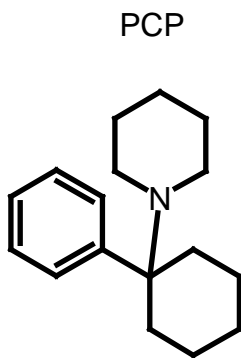
i) The following is a sketch of an IgG class immunoglobulin. (Item 4 refers to the dotted box and is a proteolytic fragment of the antibody).(2 pts)

a) Please provide names for the numbered segments.

b) Which of these is most important for the binding of the antibody to its antigen?



ii) A new drug has hit the street. This drug is a variant of PCP, called PCP-Plus. The structures of both of these are shown below. You work for a company that produces antibodies for the treatment of overdose of PCP. How might you modify your antibody such that it would be effective against this new form of PCP? In your discussion state the amino acids you would incorporate into the modified antibody and how they would interact with the new form of PCP. You can simply draw the amino acids on the figure below.(3 pts)



iii) Briefly explain why type O blood can be given to an individual of any other blood type.(2 pts)