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Biochemistry I-First Exam Spring 2001 Solution key:

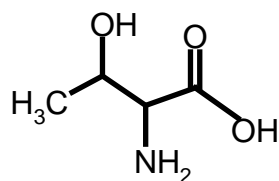
Part A: (3 points each, 24 points total) Circle the best answer. Partial credit is given in some cases.

- Structures of the antibody that binds PCP shows that the tight binding of PCP to the antibody is primarily due to:
 - formation of covalent bonds
 - van der Waals forces.: **Also a good answer, but not the best: 2pts**
 - hydrogen bonds
 - hydrophobic forces': **Best answer 3 pts**
- Unfolding of an single α -helix in a non-polar solvent (i.e. benzene, hexane, etc) would be less favorable than in water because
 - of the formation of ordered benzene molecules around the non-polar sidechains.
 - of the inability to reform hydrogen bonds with the solvent. **Each h-bond broken and not reformed is 25kJ/mol! Very unfavourable.**
 - of the formation of strong van der Waals interactions with the solvent.
 - reduction in the number of conformational states in the unfolded form.
- ΔG^0 is zero for
 - reactions at equilibrium. **This is true for ΔG , but I gave you one point.**
 - reactions that are spontaneous in the forward direction.
 - reactions that are spontaneous in the reverse direction.
 - reactions whose substrates and products have the same energy. **ΔG^0 is defined as the difference in free energy between 1 mol of products and one mole of substrate (reactant)**
- Which of the following are common features between an α -helix and a β -sheet?
 - both have the same phi and psi angle.
 - both have extensive hydrogen bonds. **This is the only common feature**
 - both have hydrogen bonds that are parallel to the direction of the mainchain.
 - answers b and c.
- The peptide bond in proteins is
 - a normal single bond.
 - always in the cis form, except for bonds before proline.
 - always in the trans form, except for bonds before proline. **This is the best answer. 3 pts.**
 - none of the above. **This was ok, because the of the words "always", but not the best - 2pts.**
- In general, a weak acid can act as a buffer
 - at pH values = pK +/- 1.0 **Best answer, 3 pts**
 - at pH values = pK +/- 2.0 **OK answer, 1 pt**
 - at pH values = pK +/- 0.1.
 - at pH values = isoelectric pH.
- Which of the following groups would form the strongest hydrogen bond with a carbonyl group?
 - NH. **NH certainly form strong hydrogen bonds, so 2 pts.**
 - OH. **OH is more electronegative than N, therefore this is the best answer. 3 pts**
 - CH.
 - All would be of equal strength.
- Which of the following statements is *most* correct:
 - Polar residues can be buried in the interior of a protein. **Provided they can find h-bond donor and acceptors, this is perfectly alright.**
 - All hydrophobic amino acids are buried when a protein folds. **No, Only about 50% are buried.**
 - Charged amino acids are never buried in the interior of a protein. **Occasionally, they can be if an opposite charge is nearby.**
 - Tryptophan is only found in the interior of proteins. **Not true, remember lysozyme?**

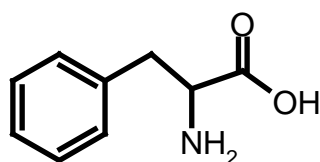
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Part B:

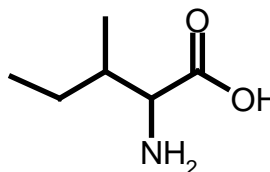
B1. (6 pts) The structure of three amino acids is shown below:



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.

The best choices were:

1. **Thr to Ser (removal of the methyl)**
2. **Phe to Tyr (addition of a OH to the ring)**
3. **Ile to Val (removal of methyl group)**

Other choices were entertained, and some of them were just plain entertaining!

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

1. **Thr to Ser: increased solubility due to loss of a hydrophobic group (methyl)**
2. **Phe to Tyr increased solubility due to gain of polar group (OH)**
3. **Ile to Val increase in solubility due to loss of a hydrophobic group (methyl)**

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.

1. **Thr to Ser: no change**
2. **Phe to Tyr: added both a donor (the H of the OH group) and an acceptor (the O of the OH group)**
3. **Ile to Val no change, couldn't hydrogen bond in the 1st place.**

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

Aspartic Acid: 4
Glutamic Acid: 4
Histidine: 7
Lysine: 9
Arginine: 9

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

Histidine, it's pK_a is closest to 7.0

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

See your textbook. Small errors were accomodated.

ii) 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

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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)

Your plot was scored as the following (this example for Asp):

- x-axis: eq OH added, to 3 equivalents (1 pt)
- y-axis: pH (1pt)
- curve: should have shown three inflection points, at pH 2 (carboxy-terminus), 4 (Asp) and 9(amino terminal) (2 pts)

This is essentially figure 3.7 in the text book.

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.

Using Asp as an example:[list group and charge]

pH=1: NH (+), Sidechain(0), COOH(0)	Net=+1
pH=2: NH (+), Sidechain(0), COOH(-1/2)	Net=+1/2
pH=3: NH (+), Sidechain(-0.1),COOH(-0.9)	Net=0
pH=4: NH (+), Sidechain(-0.5),COOH(-1.0)	Net=-1/2

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.

Use: $\text{pH}=\text{pKa}+\log[\text{A}^-]/[\text{HA}]$. Again for Asp:

$$\begin{aligned}8 &= 4 + \log R \\4 &= \log R \\R &= 10^4 \\[\text{HA}]/[\text{A}^-] &= 10^{-4}\end{aligned}$$

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?

Hydrophobic effect: ordering of water molecules around exposed non-polar groups. This leads to a large favourable *entropy* gain when the non-polar groups are removed from water as they fold into the interior of the protein.

Van der Waals force: general attractive force between any two atoms. Breakage of these leads to a positive *enthalpy* change.

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?

$$\Delta G = \Delta G^\circ + RT \ln K_{eq}$$

ΔG - direction of the reaction. If $\Delta G < 0$ the reaction is spontaneous as written.

ΔG° - position of the reaction. The energy difference between the reactants and products gives the equilibrium constant. From which, the position of the reaction can be calculated.

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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.

See Figure 4.11 in Campbell for the sketch. As for forces:

- $\alpha\alpha$ -unit: hydrophobic and van der Waals
- β -meander: Hydrogen bonds between the strands and van der Waals and 'hydrophobic' forces between the strands.

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!)

Since both of these structures exist as a single unique conformation, they both have an entropy, $S=0$ ($W=1$). Therefore the entropy change is zero.

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)

Anti-parallel, because the strands run in opposite direction (N terminal to C-terminal)

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

- a) psi (Ψ) angle **This defines the torsional, or dihedral, angle for the bond between the $C\alpha$ atom and the CO.**
- b) phi (Φ) angle **This defines the torsional angle for the bond joining the N and the $C\alpha$ atoms.**
- c) amino-terminal end of one of the strands **"Blue end" of any strand.**
- d) peptide bond : **Bond between each amino acid residue, between the CO and the N atoms on adjacent residues.**

You should have only lost one point if you simply interchanged the phi and psi angles.

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)

The best answer would be a main-chain hydrogen bond between the NH of one residue and the CO of another, between the β strands (See Figure 4.1 in Campbell). The length of this bond is 3.0\AA from the nitrogen to the oxygen, or 2.0\AA from the amide proton to the oxygen.

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)

$\epsilon = 2 \times 4,500 + 1,000 = 10,000$. Note that this protein contains two Trp residues.

$A = \epsilon c l = (.001 \text{ moles/l}(10,000) \text{ l/mole-cm}(1) \text{ cm} = 10$

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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)

Since ϵ increases by 10% for all residues, you simply multiply the above answers by 1.1, giving:

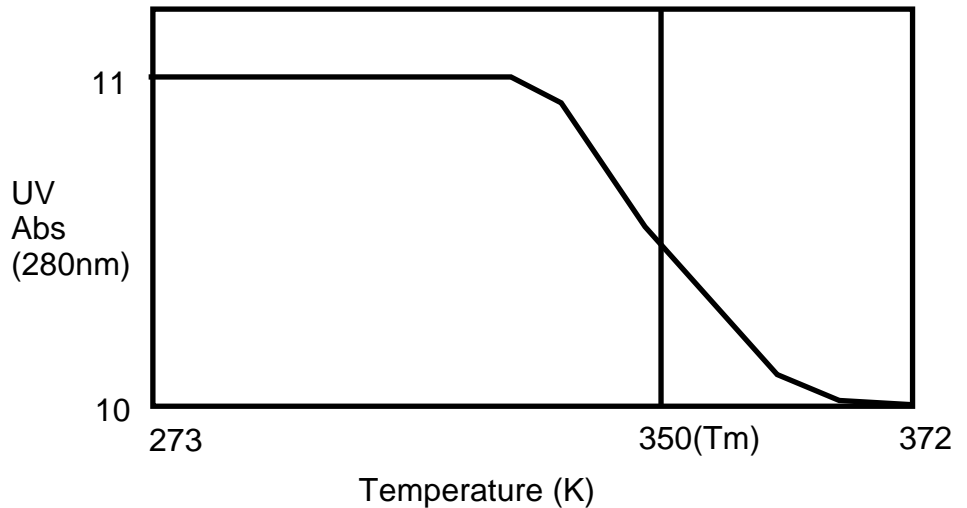
$$\epsilon=11,000$$

$$A=11$$

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.

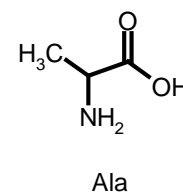
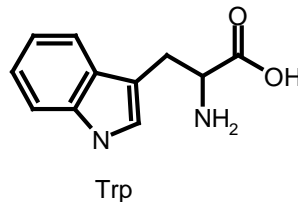
You can assume that this protein has a T_m of 350K, a ΔH° of 200 kJ/mol, and a ΔS° of 571 J/mol-deg (i.e. Very similar to the thermodynamics of ProteinG).(4 pts)



There are two features of this curve that are of note:

- 1. The absorbance decreases as the protein unfolds because the unfolded form absorbs less light.**
- 2. At T_m , the absorbance is exactly half-way between that for the fully folded protein (11 units) and the unfolded protein (10 units)**

iv) A mutation was made in the protein, such that a completely buried Trp 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 enthalpy. Briefly justify your answer.(4 pts)



Since ΔH is decreased there are weaker interactions in the protein that contains Ala. This may be due to either (or both).

- 1. loss of an H-bond from the indole**
- 2. loss of van der Waals contacts, since the Ala, being smaller, would not pack as well inside the protein.**

Some people said the hydrophobic effect would be smaller. This is true in fact, but since the ΔS didn't change, this was not the best answer.

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)

Since you are at the T_m , you should note that ΔG° is zero for the wild-type protein. Therefore ΔG° for the mutant is -5000 J/mol.:

$$K_{eq} = -5000/RT$$

$$K_{eq} = -5000/(8.3)(350)$$

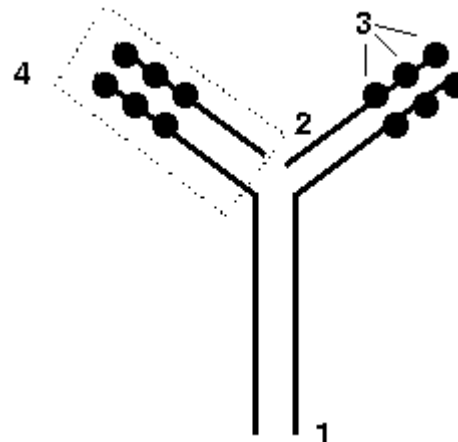
$$K_{eq} = 5.50$$

Therefore, the fraction folded (or native) is: $f_n = 1/(1+K_{eq}) = 1/6.5 = 0.15$.

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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)



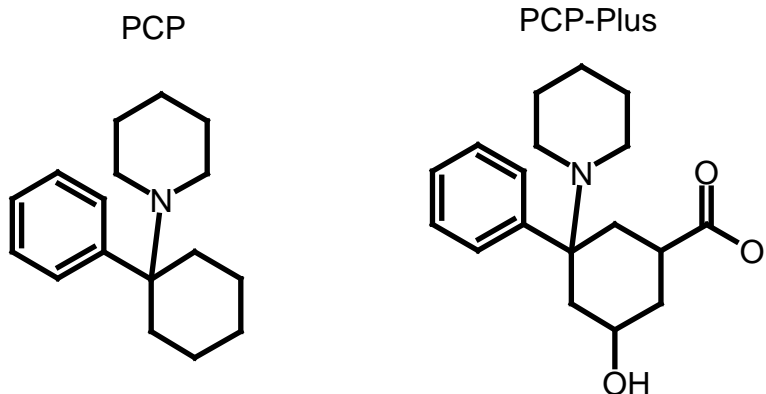
a) Please provide names for the numbered segments.

1. Heavy Chain
2. Light Chain
3. Hypervariable Regions
4. Fab fragment

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

3. Hypervariable Regions

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)



There are two differences between PCP and PCP-plus. The addition of a hydrogen bonding group (OH) and an ionizable group (COOH). You could modify the antibody such that a hydrogen bond donor/acceptor was placed near the OH group, allowing the formation of several hbonds. You could also place a positively charged residue near the COOH group. Lys or Arg would be best because they are positively charged over a wide pH range. His would also work for pH values less than approximately 7.

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

Blood cells from type O individuals have no antigens on their surface. Therefore, they can be given to anyone since the recipients antibodies can't bind to the incoming red cells.