PRACTICE QUESTIONS TO PREP FOR EXAM-I

UNITS: **1, 2A, 2B, 3A**

Problem Sets: **PS1, PS2, PS3, PS4**

Scheduled Tutorials: **ST1, ST2**

MATERIALS TESTED ON EXAM-I INCLUDE:

- Assigned pre-class readings
- Supplemental materials assigned as pre-, in- or post-class readings/visualization
- In-class materials: PPTs, worksheets
- Scheduled Tutorial materials: Pre- and In-tutorial worksheets, PPTs
- Homework and practice questions assigned
- Use the Learning Objectives to guide your prep.

EXAM-I INFORMATION:

- Date of Exam: Feb. 14th, 2019 (Thursday)
- Time of Exam: 6:30 to 8:30pm.

The exam is 2h long <u>in total</u>. This includes:

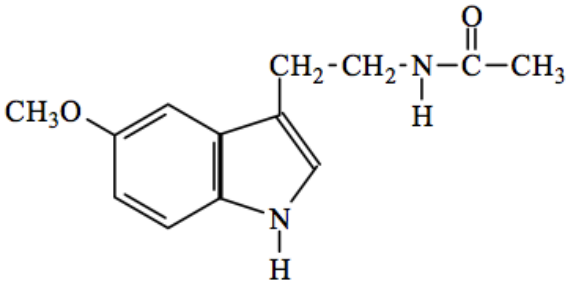
- Individual Component of the exam: ~1h 20m (80 min)
- Transition to Group Component: ~10m
- Group Component of the exam: ~25min.

The questions in this document are similar in style to the BIOL201 exam (you may also notice that they are similar in style to the BIOL201 Problem Sets). They are designed to be used along with the rest of the BIOL201 resources (listed above) to prepare for the exam. This document is **<u>NOT</u>** similar in length to the real exam.

We encourage you to try these at the <u>end</u> of your studying, using only a <u>clean copy</u> of the Exam 1 Reference Material to guide you.

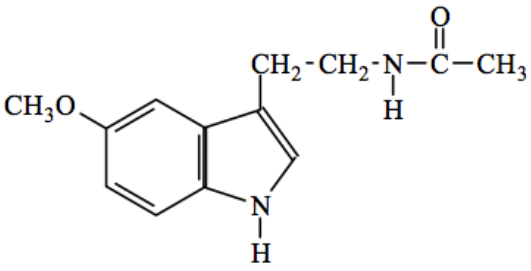
QUESTION 1 (4 points)

The following modified amino acid derivative is called "melatonin." Melatonin is important in the regulation of circadian rhythms.



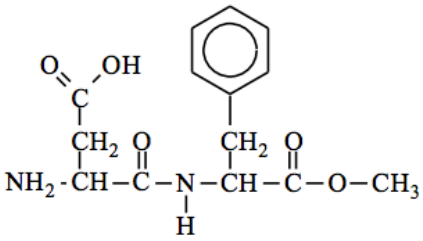
a. In the space below, redraw the structure of melatonin showing the ionic form of any group(s) that ionize in aqueous solution in the pH range of 2 - 10.
Note: this question will be marked "correct minus incorrect".

b. On the illustration below, circle all the atoms that serve as H-bond acceptors at pH 7.
Note: this question will be marked "correct minus incorrect"



QUESTION 2 (12 points)

Aspartame is an artificial sweetener. The neutral, unionized form is shown below.

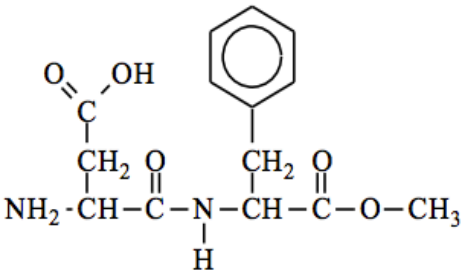


Predominant Species at pH 3.5

a. In the box above, draw the **Predominant Species** (PS) of aspartame expected to exist in Diet Coke (pH 3.5). Choose the most appropriate pKa values from the reference sheet.

b. Estimate to the nearest 1% (e.g. 18%, 76% etc.) the % of aspartame molecules that exist as zwitterions in Diet Coke (pH 3.5).

c. In the box below, draw the structure of the Average Molecule (AM) of aspartame at pH 3.5.



Average molecule at pH = 3.5

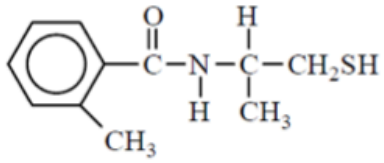
QUESTION 3 (4 points)

Cardophilin is a brand-name drug that is used to treat cardiac arrhythmias. The drug is designed such that it is naturally removed from body via urine after four hours of intake. During initial studies with the drug, it was found that among persons taking the same daily dose of Cardophilin, some possessed slightly higher concentrations of Cardophilin in their blood even after eight hours, if they were also taking another drug that increased the pH of their urine from a normal pH 6 to pH 8.

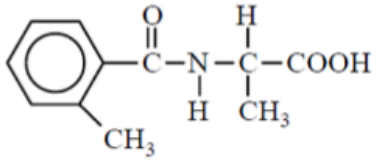
[Urine is produced by the kidney. The kidney works like a molecular filter. It separates mixtures of molecules into fractions containing large and small molecules. Large molecules and cells are generally useful and they are returned to the body. Small molecules can be useful or not useful (waste). Small useful molecules are actively recovered and returned to the body while the waste molecules are eliminated in the urine. Some small molecules can also passively return to the body rather than being excreted in the urine. This can be "good' and "bad" depending on the molecule.]

a. Based on the information provided above, indicate which one of the following illustrations best represents the structure of Cardophilin. Circle your answer. (2 points)

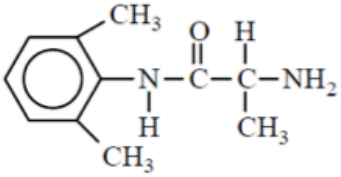
(1)



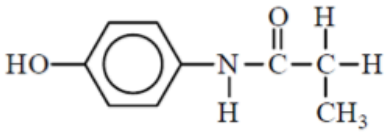
(2)



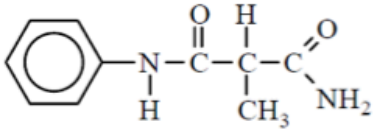
(3)



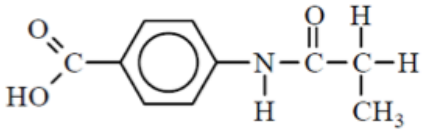
(4)



(5)



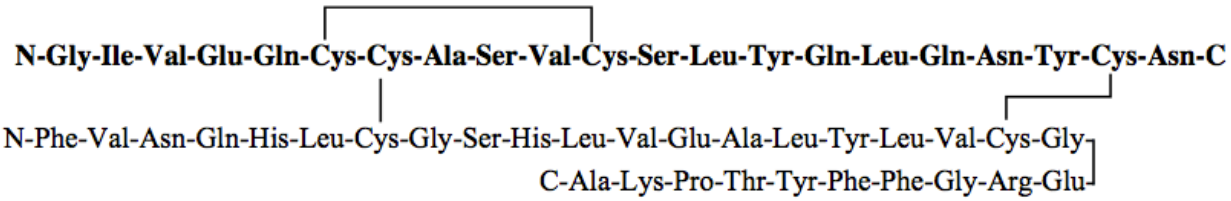
(6)



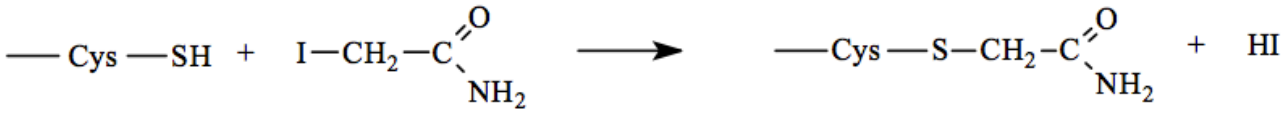
b. Briefly explain the reasoning for your choice in part **a** in the space provided below. In your written explanation, do not refer to any of the structures you did not choose in part **a**. (2 points)

QUESTION 4 (6 points)

Consider the protein insulin:



In order to separate and purify the 21-residue subunit A (sequence **shown in bold above**) from the subunit B (the non-bolded sequence), a biochemist disrupts all the S-S-linkages in the protein, then she treats the subunit mixture with iodoacetamide which reacts with thiol groups, **AMIDATING** them, thereby making them unreactive. The reaction is shown below for your information:



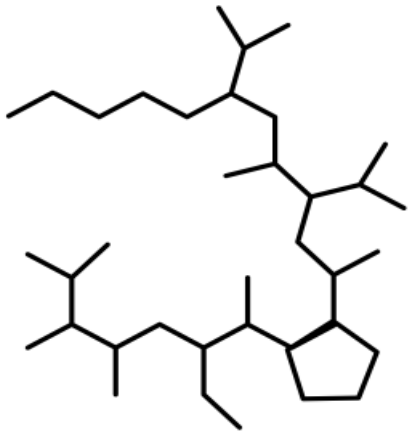
When the mixture of amidated subunits is applied to an anion-exchange column at pH 7, subunit A binds to the column but subunit B does not. The biochemist decides to elute the subunit A from the column using a buffer with the same pH as the pI of the amidated subunit A.

Using the "Two-pH method", estimate the pI of the **AMIDATED** subunit A. For full credit, you will need to show your work in the table set-up for the "Two-pH method"

pKa ↓ pH →	
Net charge	

QUESTION 5 (8 points)

As a summer intern with a pharmaceutical company, you are joining a team of X-ray crystallographers, organic chemists, and protein chemists who are working on a highly confidential drug design project. The team is designing an inhibitor to a key protein in the life cycle of an extremely lethal virus that has suddenly emerged and is spreading rapidly, and the pressure is on. You are filling in for a team member who has been tragically struck by the virus, and who is lying comatose in the hospital, quarantined and near death. Just before falling victim to the virus, this team member claimed to have come up with **THE** inhibitor, a pentapeptide, that he was sure would be a potent drug against the viral target. However, he collapsed before he was able to present the proposed peptide to the group. You are given his lab notebook and access to his computer account, and are asked to figure out what compound he had in mind. In his computer you find a coordinate file that gives you the relative locations of the heavy atoms of the pentapeptide (no hydrogen atoms are included, single and double bonds are shown equally as solid lines, and the type of atom is not identified). This yields the following molecular scaffold/skeleton.



A. given the scaffold structure above, indicate **ALL** the possible residues that could occur at each position of this peptide. Write **ALL** the possibilities in the boxes below from N to C terminus.
Answer using the three-letter residue abbreviations.

Next, you search his notebook and find the following relevant information:

- All residues **except one** have the potential to form PDPD **or** IPD bonds with water.
- The pI of the peptide appears to be somewhere between 8 and 9

B. Based on this information write the linear sequence of the pentapeptide. **Again, answer using the three-letter residue abbreviations**

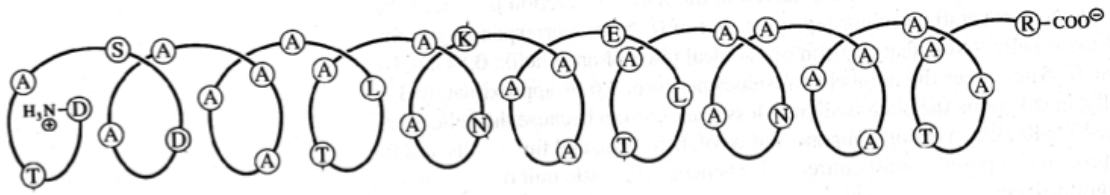
N-_____-_____-_____-_____-_____-C

QUESTION 6 (6 points)

Statements	True (T) or False (F)?
The hydrophobic effect refers to the stabilization of protein structure due to H-bonds between the hydrophobic side chains of amino acyl residues.	
During polypeptide folding, the number of H-bonds formed is much larger than the number broken.	
As compared to the unfolded state, the folded state of a polypeptide is a highly stable one.	
Motionally restricted water molecules surrounding oligomeric proteins stabilize their oligomeric structure.	
When a polypeptide folds, most polar groups of the peptide linkage maintain H-bonds with water.	
A particular polypeptide folds spontaneously with a $\lvert\Delta H_{FOLD}\rvert = 0$. For this polypeptide $\lvert T\Delta S_{WATER}\rvert$ must be > $\lvert T\Delta S_{POLY}\rvert$	
A protein with a low % content of α-helix tends to possess a high % content of β-sheet.	

QUESTION 7 (8 points)

The diagram below is a 37-amino acid alpha-helical blood protein synthesized by polar fish, known as the winter flounder. The protein is a biological "antifreeze". It inhibits ice crystal formation and helps to keep the fish's serum liquid below 0°C.



A. Estimate $\Delta H_{H\text{-}BOND}$ in kJ for the folding of 1 mol of this protein in water. Consider only the H-bonds involving the peptide linkages of the polypeptide backbone. That is, ignore H-bonds by/with R-groups and the N and C termini.

Make the following assumptions:

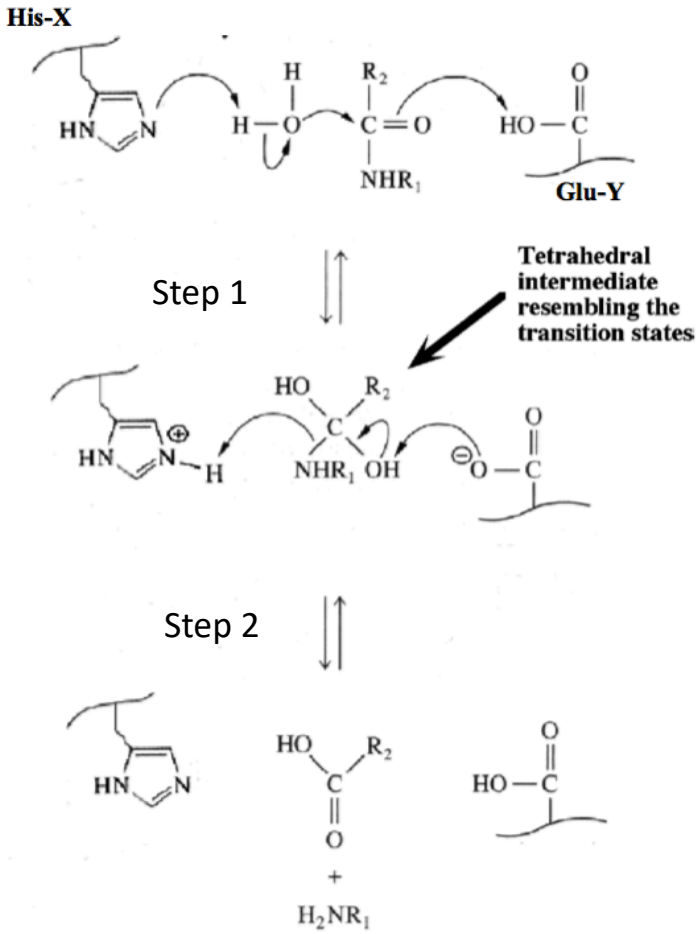
- The heat energy (ΔH) released when forming an H-bond between C=O and H-N = -15 kJ/mol (at 25°C, 1 atm).
- The heat energy (ΔH) released when forming an H-bond involving H_2O = -12 kJ/mol (at 25°C, 1 atm)
- All H-bond acceptor atoms accept **only one** H-bond.
- There are 3.6 residues/turn of an α-helix and each turn involves a 0.54 nm translation with respect to the long axis of the helix.
- The **four "unsatisfied"** backbone N-H and C=O groups at the amino and carboxy- termini, respectively, maintain their H-bonds with water in the folded state. This is equivalent to peptide bond groups between four residues, that continue to maintain their H-bonds with water at the N- and C- termini.

B. Assuming ΔG_{FOLD} is solely dependent on $\Delta H_{H\text{-}BOND}$ alone, is there the potential for the protein to fold in water?

Yes **No** (Circle)

QUESTION 8 (16 points**)**

The following diagram shows a mechanism for the enzyme-catalyzed hydrolysis of an amide (linkage) in the dipeptide substrate R_1-R_2. "His-X" and "Glu-Y" are two residues in the active site of the enzyme. The thick arrow indicates a tetrahedral intermediate resembling the two transition states for the reaction:



Based on the illustrations for the enzyme mechanism, answer the following questions (next page) about how the enzyme increases the rate at which the peptide linkages are hydrolyzed in aqueous solution. For full credit, it WILL BE necessary to draw some relevant molecular structures to illustrate your answers.

a. For Step #1 of the reaction ONLY, explain how His-X contributes to an increase in the reaction rate brought about by the enzyme. You will need to draw two structures that depict the intermediate. Show one structure where His-X is not involved and one structure where His-X is involved.

(8 points: 4 points for explanation, 2 points each for 2 structures)

Features of the Intermediate (and TSs)	**Features of the intermediate (and TSs)**
NO His-X involved	**With His-X** involved
NO Glu-Y involved	**NO** Glu-Y involved

Use the two structures you have drawn above to guide your explanation of how His-X contributes to an increase in the reaction rate brought about by the enzyme.

b. For Step #1 of the reaction only, explain how Glu-Y contributes to an increase in the reaction rate brought about by the enzyme. (8 points: 4 points for explanation, 2 points each for 2 structures). You will need to draw two structures that depict the intermediate. Show one structure where His-X is involved but not Glu-Y, and one structure where both His-X and Glu-Y are involved.

Features of the Intermediate (and TSs)

With His-X involved

NO Glu-Y involved

Features of the intermediate (and TSs)

Both His-X and Glu-Y involved

Use the two structures you have drawn above to guide your explanation of how Glu-Y contributes to an increase in the reaction rate brought about by the enzyme.

QUESTION 9 (4 points)

In an effort to purify histone proteins from nuclear extracts, a biochemist applies a sample of nuclear proteins to a column containing a matrix composed of cellulose derivatized with DNA. Following the application of a sample of the nuclear proteins in buffer at pH 7, the column is washed with an NaCl gradient in buffer at pH 7.

What kind(s) of column chromatography is the biochemist using?

	Yes / No
Cation-exchange chromatography	_____
Anion-exchange chromatography	_____
Affinity chromatography	_____
Size exclusion chromatography	_____

QUESTION 10 (4 points)

In an attempt to separate two monomeric proteins, a biochemist conducts some exploratory experiments, but he turns out to be a very unlucky biochemist.

a. He finds that neither protein binds to a cation-exchange column or an anion-exchange column at pH 7. What could be a plausible reason for this? (1 point)

b. He finds that both proteins elute immediately in the void volume when run on a size exclusion column. Explain what this means. (1 point)

c. Despite these initially disappointing results, how should the biochemist proceed further to separate and purify the two proteins? He only has access to size exclusion chromatography and ion-exchange chromatography. (2 points)

QUESTION 11 (4 points)

You want to achieve the best separation of the following four proteins:

Protein A: 30 kDa, pI 8.0

Protein B: 32 kDa, pI 7.0

Protein C: 90 kDa, pI 7.9

Protein D: 91 kDa, pI 6.5

Which of the following separation method(s) would you choose? Explain the anticipated results of each method with appropriate diagram(s).

i. SDS-PAGE

ii. IEF

iii. 2D-PAGE

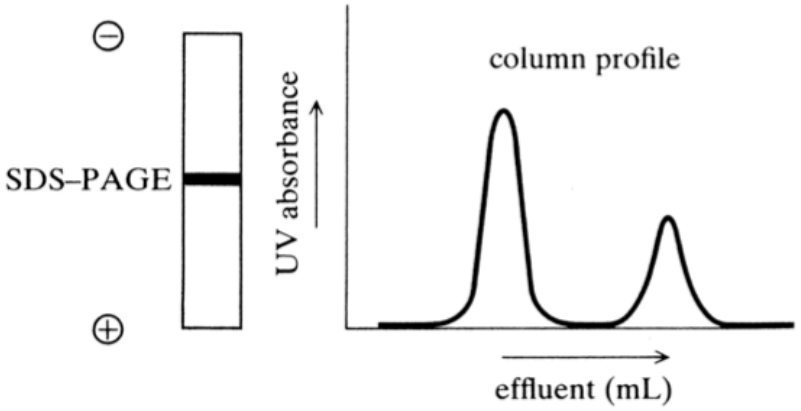
iv. ion-exchange chromatography

v. size-exclusion chromatography

vi. Affinity chromatography

QUESTION 12 (6 points**)**

Two identical samples of a **protein mixture** (containing many different proteins) were analyzed separately, one by standard SDS-PAGE, and the other by ion-exchange chromatography. The results are shown below. Assume all proteins in the mixture are monomeric.

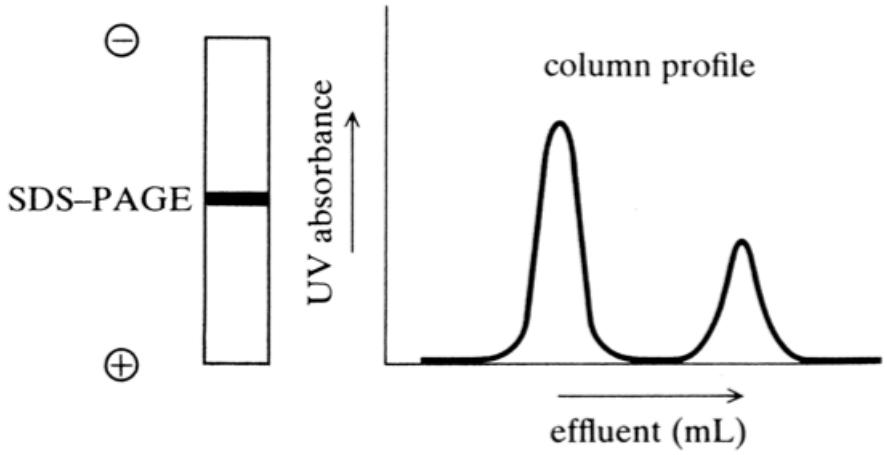


a. Describe the data from the SDS-PAGE and the column chromatography experiments (1 point), and explain what it means without drawing any conclusions (1 point)

b. What can be concluded about the composition of the protein mixture in the sample, based on the data shown? (2 points)

c. Can you conclude there are only two proteins in the mixture? Explain. (2 points)

QUESTION 13 (5 points)
Two identical samples of a **pure protein** (purified protein, performing a known single function) were analyzed separately, one by standard SDS-PAGE, and the other by ion-exchange column chromatography in the presence of 8 M urea (urea disrupts hydrogen bonding). The results from the two experiments are shown below.



a. Describe the data from the SDS-PAGE and the column chromatography experiments (1 point), and explain what it means without drawing any conclusions (1 point)

b. State four conclusions that can be made about the structure of the protein (4 points)