LAST Name: _____ **FIRST** Name: _____

PRINT **PRINT**

Signature:_____ Student #: _____

THE UNIVERSITY OF BRITISH COLUMBIA
BIOLOGY 201 SAMPLE EXAM-I Answer Key

35% of Final Grade

2 hours

IMPORTANT READ THIS!!

Questions may be answered in pencil

Allowed materials: A scientific calculator

If a pH value differs from a pKa value by +/- 2, a protonation state of 0 or 1 may be assigned

Report all

% rounded with no decimal places (eg. 20%, 31%, 95% etc).
fractional charges rounded to 2 decimal places (eg. -0.67, + 0.99 etc.)
pH values rounded to one decimal place (eg. 6.4)

Question	1	2	3	4	5	6	7	8	9	10	11	12	Total
Student Score													
Maximum Points	5	12	6	6	8	4	4	6	4	8	16	6	85

FACULTY OF SCIENCE RULES GOVERNING FORMAL EXAMINATIONS

(1) Each candidate must be prepared to produce, upon request, a Library/AMS card for identification.

(2) Candidates are not permitted to ask questions of the invigilators, except in cases of supposed errors or ambiguities in examination questions.

(3) No candidate shall be permitted to enter the examination room after the expiration of one-half hour from the scheduled starting time, or to leave during the first half hour of the examination.

(4) Candidates suspected of any of the following, or similar, dishonest practices shall be immediately dismissed from the examination and shall be liable to disciplinary action:
- Having at the place of writing any books, papers or memoranda, calculators, computers, cell phones, audio or video players or other memory aid devices, other than those authorized by the examiners.
- Speaking or communicating with other candidates
- Purposely exposing written papers to the view of other candidates. The plea of accident or forgetfulness shall not be received.

(5) Candidates must not destroy or mutilate any examination material; must hand in all examination papers; and must not take any examination material from the examination room without permission of the invigilator.

EXAM DISRUPTION POLICY FOR EXAM-I

In the event of an exam disruption and students must leave the exam room, the following policies apply:

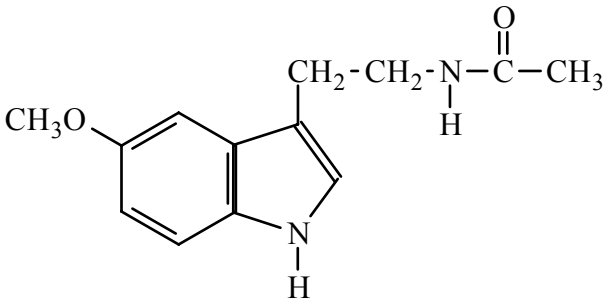
(1) Procedure for exiting the exam room.
- Pick up your question paper.
- Exit the room in an orderly manner through the doors indicated by the invigilators.
- As you exit the room, give your exam paper to an invigilator. Any student failing to produce and exam paper will be asked to wait until all students have exited. Students are **RESPONSIBLE** for the return of their exam paper. Any student failing to do so will receive a grade of "0" for the exam. Students are **STRONGLY CAUTIONED** to guard against theft of theirexam paper by other students. **NO** appeal relating to loss of an exam paper by theft will be considered.

(2) Students **MUST** wait outside the exam room until the disruption has been resolved. During this time, students are **PROHIBITED** from discussing the exam outside the exam room or consulting course materials. Doing so is considered academic misconduct and will result a grade of "0" for the exam.

(3) Once the exam disruption has been resolved, the exam will be continued if at all possible.

Question #1 (5 points)

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



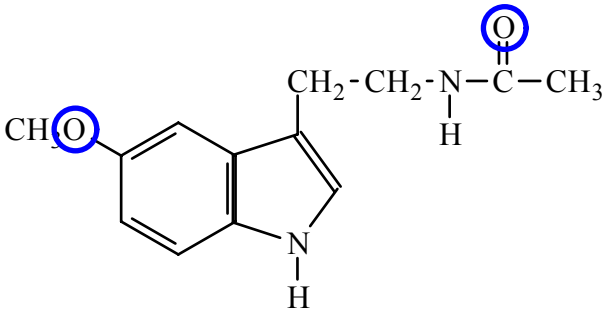
(a) Which **one** of the following groups forms a part of melatonin's structure?

(1) indole
(2) imidazole
(3) pyrrolidine
(4) pyrimidine
(5) purine

(b) In the space below, redraw the structure of melatonin showing any groups that ionize in aqueous solution in their ionic form. **Note**: this question will be graded "right minus wrong."

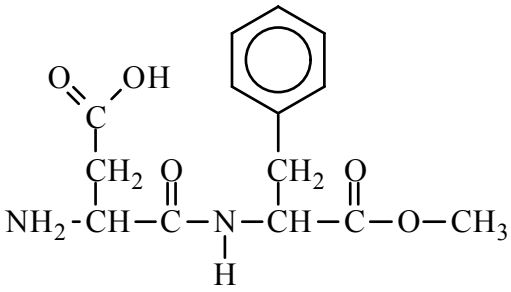
There are no chemical groups which ionize in aqueous solution

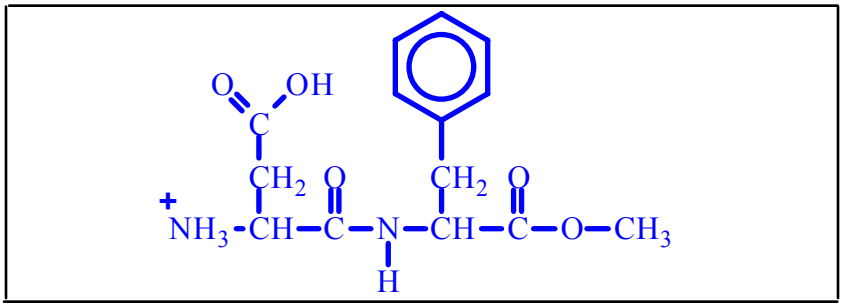
(c) Circle any **single** atoms that serve as H-bond acceptors at pH = 7. **Note**: this question will be graded "right minus wrong."



Question #2 (12 points)

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





PMS at pH = 3.5

(a) Draw the **principle molecular species** of aspartame expected to exist in Diet Coke (pH 3.5). Choose the most appropriate pKa values from the reference sheet.

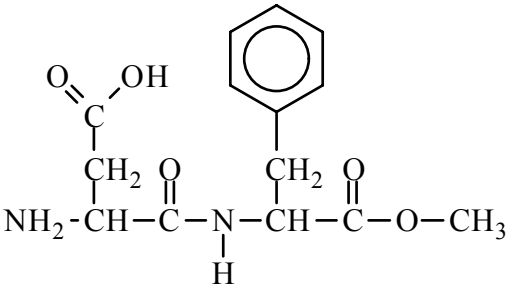
(c) Estimate to the **nearest 1%** (eg. 18%, 76% etc) the % of aspartame molecules that exist as isoelectric zwitterions in Diet Coke (pH 3.5).

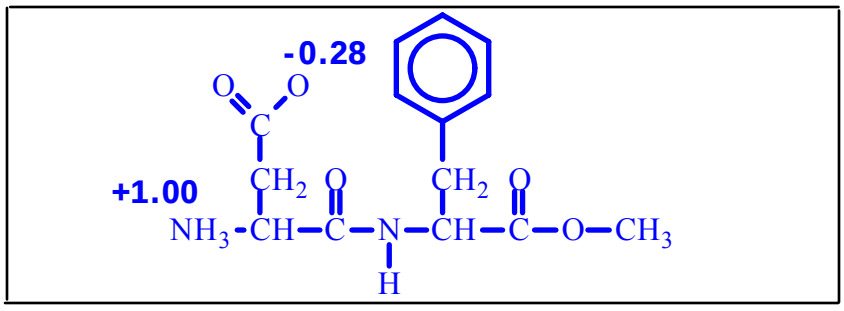
NH_3^+/NH_2 group: no calculation required: 100% NH_3^+

COOH/COO^- group: pKa = 3.9
3.5-3.9 = log COO^-/COOH
COO^-/COOH = 0.398/1 = 28% COO^-

Final answer = (0.28) (1) = 28% or 29%

(d) Draw the structure of the **average** aspartame molecule at pH = 3.5. Give fractional charges to 2 decimal places.

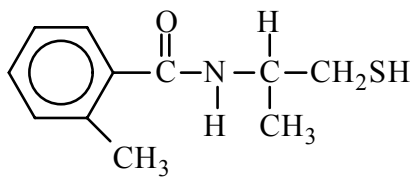




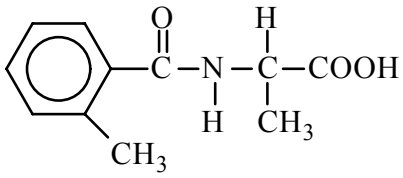
Average molecule at pH = 3.5

Question #3 (a) First indicate which **one** of the following structures is the structure of Cardophilin. **Circle** your answer.

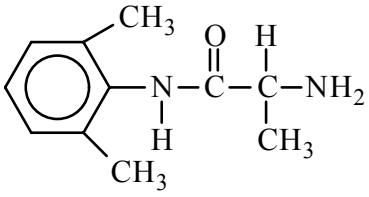
(1)



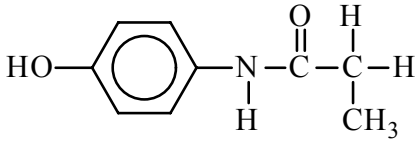
(2)



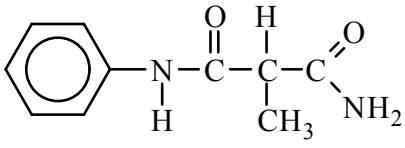
(3)



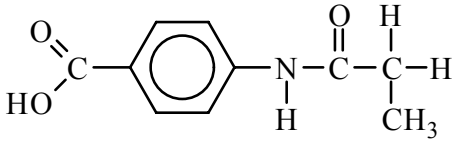
(4)



(5)



(6)



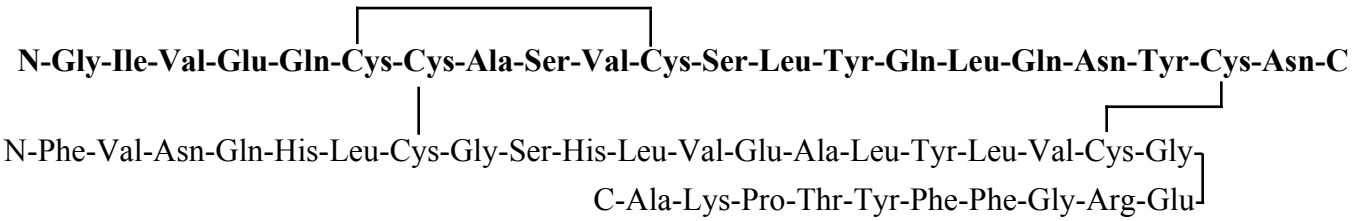
(b) Now briefly explain the reasoning for your choice **(a)** in the space provided below. In your written explanation, do **not** refer to any of the structures you did not choose in **(a)**. You must explain your choice **(a)** based solely on the merits of your choice alone. Use **either** "average molecule language" or "PMS language" in your explanation.

Average molecule language: Passive rebasorption of a molecule from the urine into the blood occurs more readily if the molecule is uncharged. The net charge on a molecule is influenced by the pH. If the pH of the urine is raised, structure 3 becomes less positively charged thus promoting its diffusion across membrane lipid bilayers from the urine into the blood.

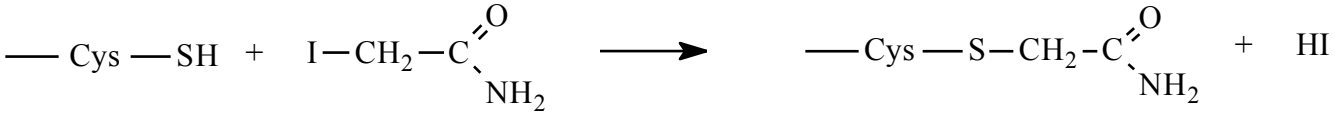
PMS language:: Passive rebasorption of a molecule from the urine into the blood is promoted if the molecule is uncharged. The proportion of a molecule with a single ionizing group that exists in uncharged form is influenced by the pH. If the pH of the urine is raised, the proportion of structure 3 in solution that exists in neutral form increases thus promoting its diffusion across membrane lipid bilayers from the urine into the blood.

Question #4 **(6 points)**

Consider the protein insulin:



In order to purify the 21 residue **A** subunit (**bolded above**) away from the B subunit, a biochemist disrupts all the protein's -S-S-linkages then she treats the subunit mixture with iodoacetamide which reacts with thiol groups **AMIDATING** them.



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

Using the tabular "2 pH method", estimate the pH_I of the **AMIDATED**, wild type **A** subunit.

Pka ↓ / pH →		2.1	4.1	9.8	10.5	13.5
Asn α-COOH	**2.1**	-0.5	-1	-1	-1	-1
Glu R-COOH	**4.1**	0	-0.5	-1	-1	-1
Gly α-NH_2	**9.8**	+1	+1	+0.5	?	?
Tyr (R-OH)x2	**10.5**	0	0	?	-0.5	-1
Ser (R-OH)x2	**13.5**	0	0	?	?	-0.5
Net Charge		**+0.5**	**-0.5**	**~-1.66**	**~-1.34**	**?**

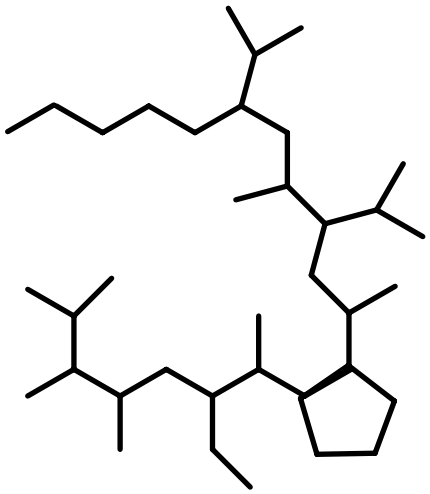
$pH_I = (2.1+4.1)/2 = 3.1$

Notes:

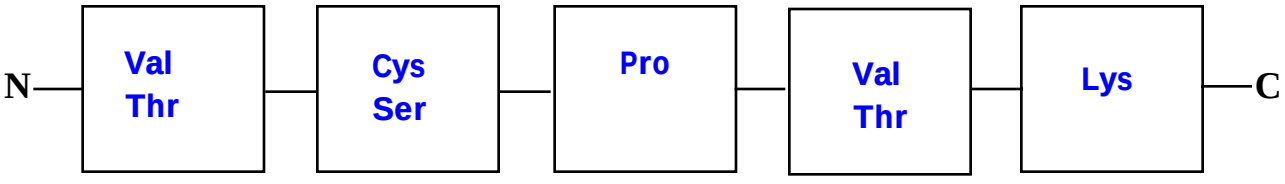
- ✓ **The Cys-SH groups do not ionize because they have been blocked by the non-ionizing amide group.**
- ✓ **"?" – in the pH_I table are left for you to calculate and add to it – good practice.**
- ✓ **Once calculations for pHs 2.1 and 4.1 are done, the pH_I can be estimated.**
- ✓ **Calculations for pHs 9.8 and 10.5 and 13.5 are not really required.**

Question #5 (8 points)

As a summer intern with a pharmaceutical company, you are joining a team of NMR spectroscopists, 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 this 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. You start at the computer. You find a coordinate file that gives you the relative locations of the heavy atoms of the pentapeptide (no hydrogen atoms are included, and the type of atom is not identified). This yields the following molecular scaffold



(a) Given the scaffold structure above, indicate **ALL** the possible residues at each position of this peptide, that is, write **ALL** the possibilities in the boxes below. **Answer using the three letter residue abbreviations**. Be careful about assigning the N and C termini.



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

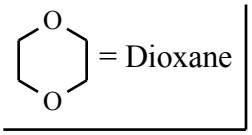
- All residues **except one** have the potential to form PDPD **or** IPD bonds with water.
- The pH_I of the peptide appears to be somewhere between 8 and 9.

(b) Based on this information write the linear sequence of the pentapeptide. **Again answer using the three letter residue abbreviations**.

N - Thr - Cys - Pro - Thr - Lys - C

Question #6 (4 points)

In the organic solvent dioxane, an amino acid chain composed entirely of **leucine** adopts an α-helical conformation however one composed entirely of **isoleucine** adopts a "random coil conformation." Explain the reason for this. The term "random coil" is used by protein biochemists to refer to a conformation lacking any recognizable repetitive structure.



= Dioxane

Leucine and isoleucine side chains are chemically identical except they have different branching patterns. Ile branches closer to the α-carbon, that is closer to the polypeptide backbone, while Leu branches "farther out." Because α-helices are tightly packed structures with little empty space, branching close to the backbone must sterically interfere with the backbone being able adopt an α-helical conformation. Note: Because of the chemical similarities between Leu and Ile the solvent is not an issue

Question #7 (4 points)

The C-terminal region of a monomeric protein possesses the following sequence:

-Val-Asp-Asp-Val-Phe-Ser-Gln-Val-Cys-Thr-His-Leu-Asp-Thr-Leu-Lys-

Is there any pattern in the sequence that suggests that it is a part of an α-helix? Explain.

Yes. The hydrophobic residues are separated by 3-4 residues. In between are hydrophilic residues. If this linear sequence was folded into an a-helix it would be amphipathic. Likely the hydrophobic side would face the interior of the folded protein while the hydrophilic side would face water.

Question #8 (6 points) **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. **F**

During polypeptide folding, the number of H-bonds formed is much larger than than the number broken. **F**

As compared to the unfolded state, the folded state of a polypeptide is highly stable one. Motionally restricted water molecules surrounding oligomeric proteins stabilizes their oligomeric structure **F**

.

When a polypeptide folds, most polar groups of the peptide linkage maintain H-bonds with water. **F**

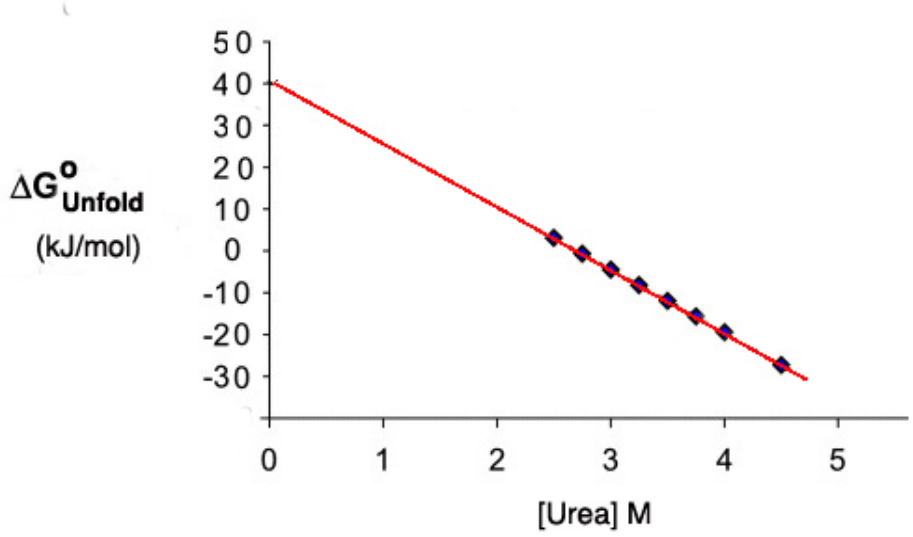
A polypeptide folds spontaneously with a $|\Delta H_{FOLD}| = 0$ **T**

This means that $|T\Delta S_{WATER}|$ must be > $|T\Delta S_{POLY}|$.

A protein with a low % content of α-helix tends to possess a high % content of β-sheet **T**

Question #9 (4 points)

Below are some data from a partial denaturation experiment conducted at 25°C.



(a) Estimate Keq_{FOLD} from these data.

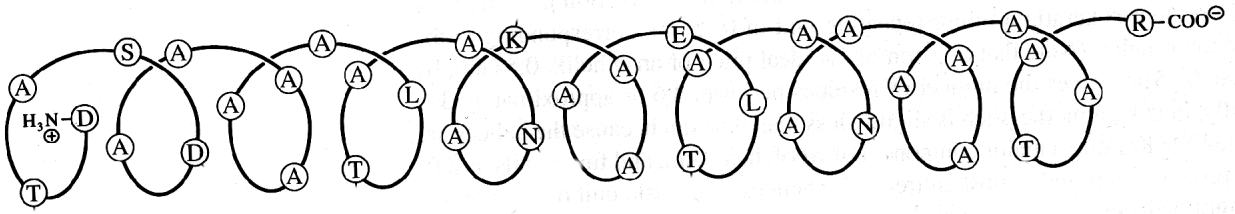
$-40{,}000 = -2476 \ln Keq_{FOLD}/1$ $\qquad Keq_{FOLD} = 1.03 \times 10^{7}$

(b) Can ΔH_{FOLD} and $T\Delta S_{FOLD}$ be estimated from a partial denaturation experiment?

Yes **No** **(Circle)**

Question #10 (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 H-bonds involving the peptide linkages of the polypeptide backbone. That is, ignore anything to do with R-groups (amino acid side chains) and the N and C termini.

Make the following assumptions:

(1) The energy (at 25°C/1 atm) required to break a H-bond between C=O and H-N in an α-helix = **15 kJ/mol**.
(2) The energy (at 25°C/1 atm) required to break a H-bond involving H_2O = **12 kJ/mol**.
(3) All H-bond acceptor atoms accept only one H-bond.
(4) The 4 "unsatisfied" backbone C=O and N-H groups maintain their H-bonds with water in the folded state.

UNFOLDED STATE

 # H-bonds between H_2O and peptide linkages

= 33 x 2 = 66

↑

Ignores groups at the ends of the a-helix which are assumed to maintain their H-bonds with water in the folded state

Energy required to break H-bonds in the unfolded state

= 66 x 12 = 792 kJ

FOLDED STATE

# of H-bonds between H_2O molecules = 66/2 = 33

# of H-bonds in the α-helix

33

↑

a-helices have 4 less H-bonds than the number of residues

Energy required to break H-bonds in the folded state

= (33 x 12) + (33 x 15) = 891 kJ

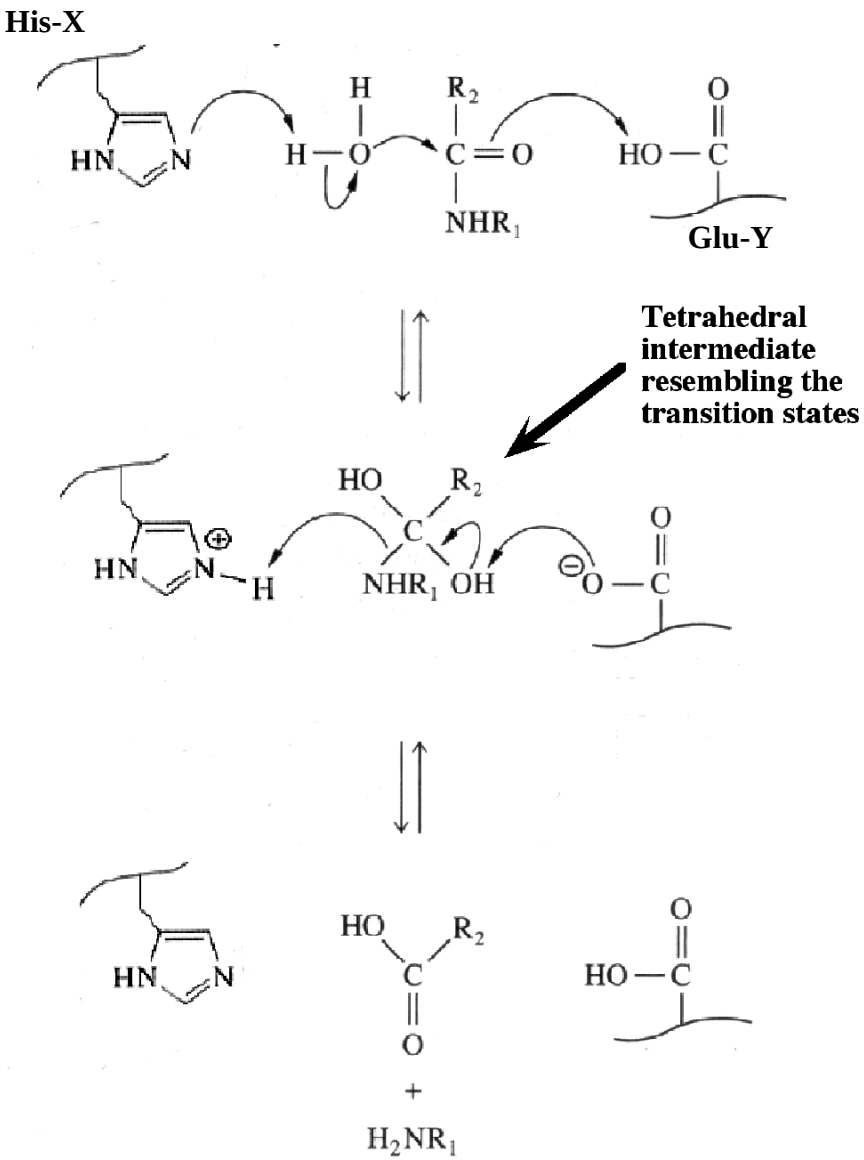
Because it requires more energy to break the H-bonds in the final state, the final state is more strongly bonded thus $\Delta H_{H\text{-}BOND}$ ~ -99 kJ

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

YES **No** **(Circle)**

Question #11 (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.



Answer the **two** questions on p.13 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 relevent molecular structures to illustrate your answers. See next page**.

Name: _____ **Student #** _____

Question #11 (16 points)

Answer the questions below about how this enzyme increases the rate at which the peptide linkages are hydrolyzed in aqueous solution.
For **FULL CREDIT**, it **WILL BE** necessary to draw some relevent molecular structures illustrating features of the transition state.

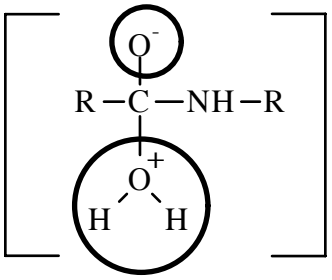
(a) For **Step #1 ONLY,** explain how **His-X** contributes to an increase in the reaction rate brought about by the enzyme. **(8 points: 4 points for explanation, 2 points each for 2 structures)**

First draw the two structures below. Do NOT show any active site residues. (4 points)

0.5 points

4 bonds to C

0.5 points



1 point

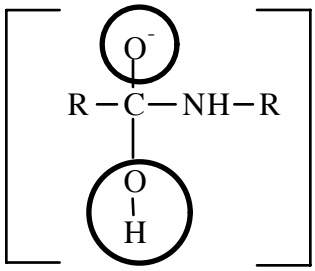
Features of the transition state
No His-X involved
No Glu-Y involved

1 point

0.5 points

4 bonds to C

0.5 points



Features of the transition state
With His-X involved
No Glu-Y involved

Now use the two structures above to guide your explanation. (4 points)

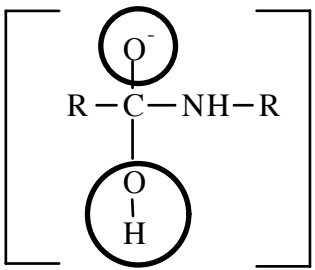
His-X deprotonates H_2O a weak nucleophile, forming ^-OH a strong nucleophile. This avoids the creation of a +ve charge and charge separation on the TS. This increases the stability of the TS relative to the use of water as the attacking nucleophile. It is through this impact on transition state stability that the enzyme increases the reaction rate.

(b) For **Step #1** <u>ONLY,</u> 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)**

First draw the two structures below. Do <u>NOT</u> show any active site residues. (<u>4 points</u>)

<u>0.5 points</u> **4 bonds to C**

<u>0.5 points</u>



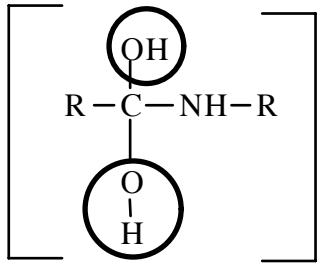
<u>1 point</u>

Features of the transition state
<u>With</u> His-X involved
<u>No</u> Glu-Y involved

<u>0.5 points</u>

<u>1 point</u> **4 bonds to C**

<u>0.5 points</u>



Features of the transition state
<u>Both</u> His-X and Glu-Y involved

Now use the two structures above to guide your explanation. (<u>4 points</u>)

Glu-Y acts an acid donating a H^+ to the TS <u>during</u> -ve charge development on the O-atom as it acquires the electrons of the broken bond. Thus Glu-Y <u>prevents</u> the development of the -ve charge on the TS increasing the stability of the TS. It is through this impact on transition state stability that the enzyme increases the reaction rate.

Question #12 **(6 points)**

(a) 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 application of the sample 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 or No
Cation exchange chromatography	**YES Recall that histones are +vely charged at pH =7 (BIOL 200).**
Anion exchange chromatography	**NO**
Affinity chromatography	**YES Histones bind to DNA (BIOL 200).**
Size exclusion chromatography	**NO**

(b) In attempt to separate two monomeric proteins, a biochemist conducts some exploratory experiments and turns out to be a very unlucky biochemist.

She finds that neither protein binds to a CM cellulose column or a DEAE cellulose column at pH = 7.

She finds that both proteins elute in the void volume when chromatographed on a size exclusion column.

Despite these initially disappointing results, which method should she investigate further to separate and purify the two proteins: size exclusion chromatography or ion-exchange chromatography? Explain.

Because both proteins fail to bind to either a +vely charged chromatographic matrix (DEAE-cellulose) or a -vely charged matrix (CM-cellulose) at pH = 7 both proteins must have a net charge = 0 at this pH, that is, the pH_I of both proteins = 7. Because they have the same pH_I the proteins cannot be separated by ion-exchange chromatography.

It is still a possibility that the proteins might be separated by size exclusion chromatography. Because the both proteins eluted in the void volume both proteins were going only around the beads. The choice of other beads with a different pore size (allowing one protein to enter the beads with the other going around the beads) could yield a separation. This would only work if the two proteins are different sizes (= different molecular masses).