C483 Exam 1
Fall 2016

Name Key Seat Number ________

Student ID ____________________ AI ____________________

The last page of this exam contains pKa values and other information you might find useful.
This exam contains 110 points. The highest score you may earn on this exam is 100 points.

1 ________/20pts

2. ________/10pts

3. ________/20pts

4. ________/10pts

5. ________/10pts

6. ________/10pts

7. ________/10pts

8. ________/10pts

9. ________/10pts

Total:

Regrading: All requests for regrades must be submitted in writing within 48 hours of the return of the exam. You must explicitly state what has been misgraded and why it is an error. The entire exam will be regraded, which could result in points being added or deducted overall.

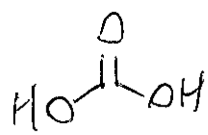
Section 1: Reading guides (50 points)

1. 20 pts. Fill in the blanks (2 points each.)

A. The exclusion of nonpolar substances from an aqueous solution is known as the hydrophobic effect.

B. A barrier such as a lipid bilayer can prevent diffusion, the process of molecules spontaneously moving to equal concentration throughout a solution.

C. Draw the structure of the main acid component of the blood buffer:

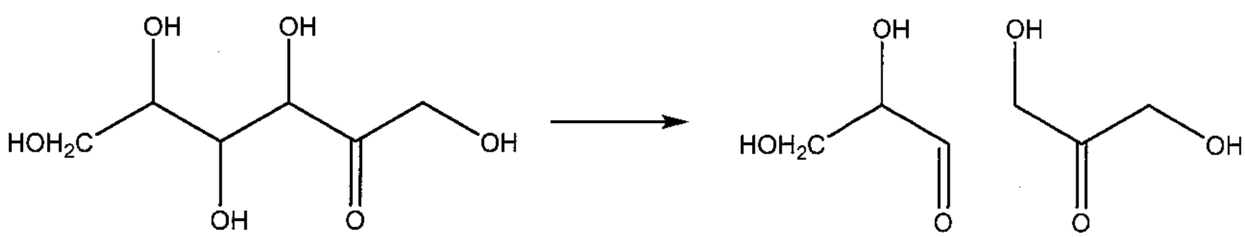


D. A nucleobase attached to ribose is called a nucleoside.

E. A loop or turn is an example of an irregular secondary structure.

F. Peptide chains can be cross-linked through the disulfide bond formed between two cysteine residues.

G. The following reaction would be catalyzed by an enzyme of the lyase class.



H. Keratin is a structural protein that has high tensile strength due to its coiled-coil structure.

I. In chymotrypsin, the Oxyanion hole stabilizes the charge build up on the tetrahedral intermediate of amide hydrolysis.

J. The sigmoidal shape of the oxygen binding curve of hemoglobin suggests that hemoglobin binds oxygen cooperatively.

2. 10 pts. Write True or False (1 points each)

A. True The thermodynamic driving force for assembly of nonpolar groups in water is the accompanying increase in entropy of water molecules that were solvating the nonpolar regions.

B. False Enzymes increase the rate of a reaction by maximization of binding energy to the substrate.

C. False In transcription, the mRNA has the same sequence (except for the substitution of U for T) as the template strand of the DNA.

D. False The nucleic acid backbone is comprised of phosphoanhydride bonds.

E. False Irregular secondary structures are predominantly disordered.

F. True The interior of a globular protein is often regular in its secondary structure in order to allow for hydrogen bonding of its backbone amide groups.

G. True Allosteric binding of 2,3-bisphophoglycerate in the central cavity of hemoglobin shifts the conformational equilibrium toward the tense state.

H. False Alanine, which has pKa values of 9 and 2, would serve as an effective buffer at pH 5.5.

I. True Myosin and actin are both NTP binding proteins.

J. True Ligases catalyze bond-forming reactions concomitant with ATP hydrolysis.

3. 20 pts. Short answer (5 points each)

A. Hydrophobic residues usually appear at the first and fourth positions in the seven residue repeats of polypeptides that form coiled-coils. Why do polar or charge amino acids usually appear in the remaining five positions? Which of the following sequences is more likely to appear in a coiled-coil? Ile-Gln-Glu-Val-Glu-Arg-Asp or Trp-Gln-Glu-Tyr-Glu-Arg-Asp?

question 5.49

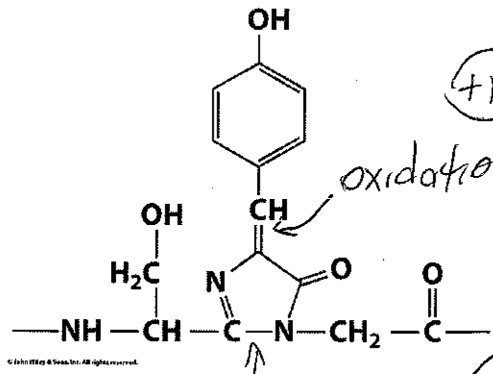
- Polar/charged groups are in the other positions because they point out toward water (+3)

- Ile Gln Glu Val Glu Arg Asp (+2)

B. The structure below is part of the green fluorescent protein. Identify the three amino acids that make up this segment. Indicate the bond that arises from a cyclization reaction. Identify the bond that results from an oxidation reaction.

ser - tyr - gly

(+1) (+1) (+1)



oxidation (+1)

cyclization (+1)

C. Assuming a pKa of 6.1, what percent of histidine sidechains are ionized at a pH of 7.4?

$$pH = pK_a + \log \frac{A^-}{HA}$$

(+2)

$$7.4 = 6.1 + \log \frac{A^-}{HA}$$

$$20 = \frac{A^-}{HA}$$

(+3)

$$\% HA = \frac{1}{1+20} = 4.8\%$$

D. Explain why the fact that GC base pairs have more hydrogen bonds than AT base pairs does not contribute to the increased stability of GC-rich nucleic acids.

(+5)

The relative strength of H-bonds is not much different in the double helix than single stranded DNA because both maximize H-bonding. The single stranded structure has H-bonds between the bases and water.

Section 2: Problems (10 points each)

4. How much of a 5 M HCl solution can be added to one liter of a 0.10 M HEPES buffer (pH = 7.25) so that it does not drop below a pH of 7.15?

Start:

$$pH = pK_a + \log \frac{A^-}{HA}$$

$$7.25 = 7.55 + \log \frac{A^-}{HA}$$

$$0.50 = \frac{A^-}{HA}$$

$$\% A^- = \frac{.5}{1.5} = 33\% \text{ of } 1\,L\left(0.10\,\frac{mol}{L}\right) = 0.033\,mol$$

End:

$$pH = pK_a + \log \frac{A^-}{HA}$$

$$7.15 = 7.55 + \log \frac{A^-}{HA}$$

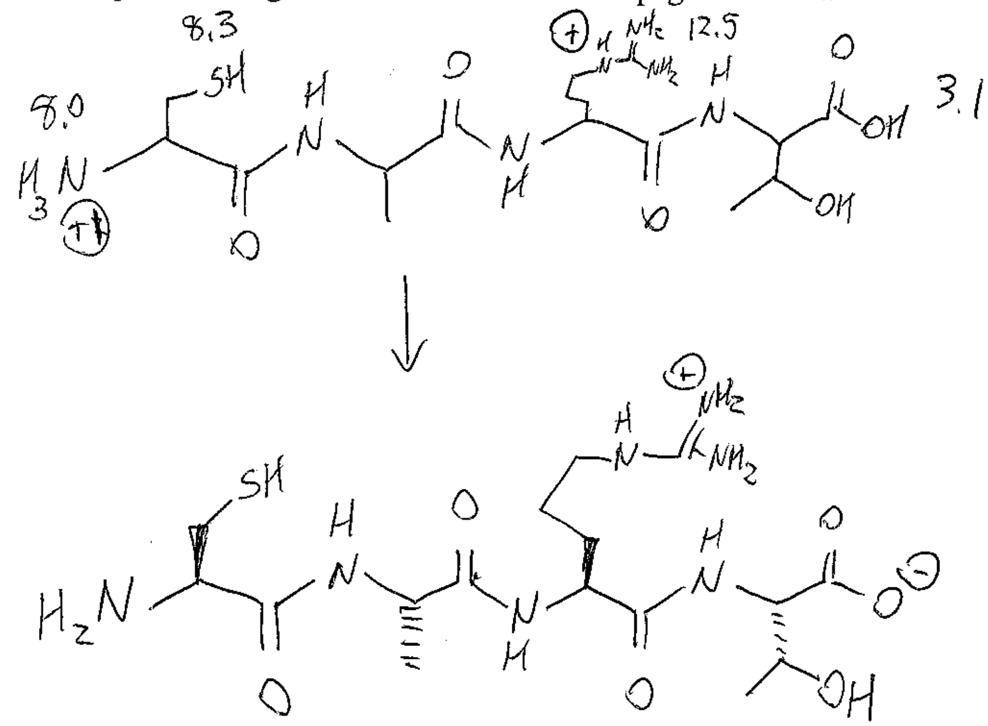
$$0.40 = \frac{A^-}{HA} \qquad \% A^- = \frac{.4}{1.4} = 29\% \text{ or } 0.029\,mol$$

Therefore 0.004 mol of HCl can be added

$$5\,\frac{mol}{L}(x\,L) = 0.004\,mol$$

$$= 0.0008\,L \text{ or } \boxed{0.8\,mL}$$

5. Draw the structure of the polypeptide CART in its predominant form at its isoelectric point. Use the pKa values given in the table on the last page of the exam.



+2 Backbone

+1 Stereochem

+1 C

+1 A

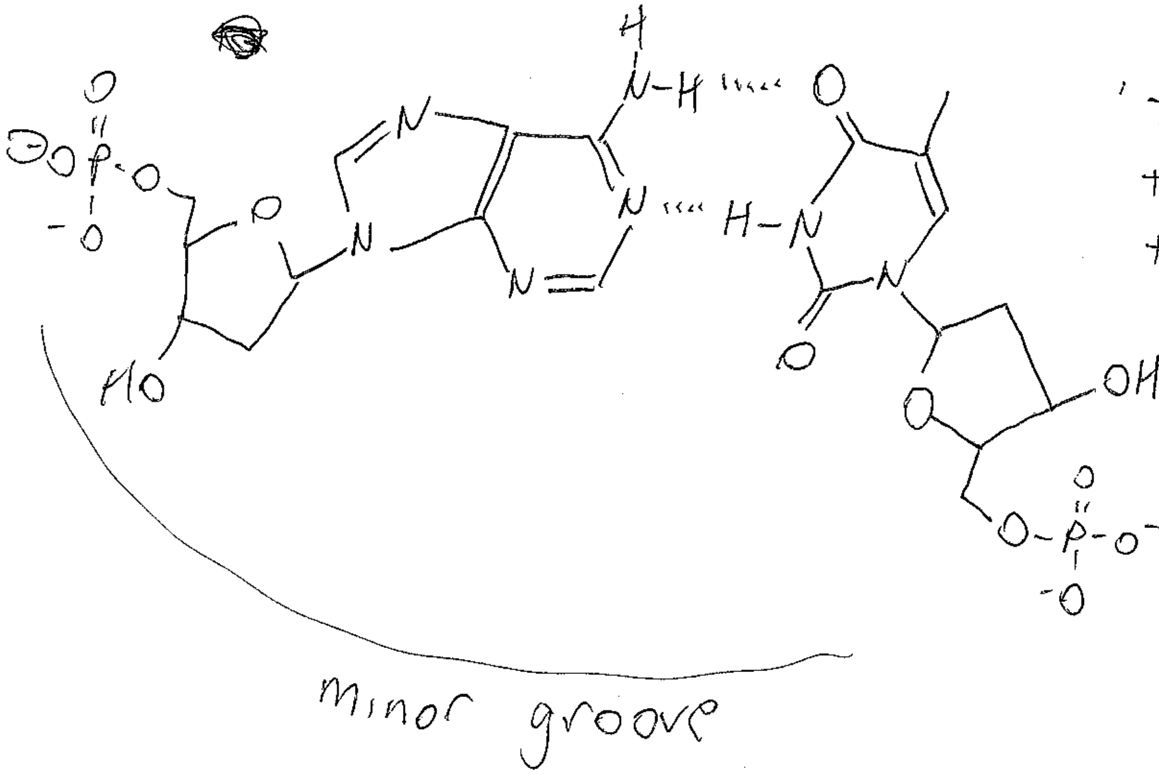
+1 R

+1 T

+1 net zero charge

+2 For ionization (-1 for each ionization mistake, maximum -2)

6. Draw full structures of dAMP base paired to dTMP. Indicate the hydrogen bond donors and acceptors.



+1 deoxyribose

+1 monophosphate

+2 A

+2 T

Using this figure, explain why the DNA helix has a major and minor groove.

+4 The phosphate groups are not 180° across which means than one side of the base pair is more accessible, etc.

7. The octapeptide AVGWRVKS was digested with the enzyme trypsin. Would ion exchange or size exclusion (gel filtration) chromatography be most appropriate for separating the products, or would both work well? Explain.

Digest: AVGWR VK S (+1)

Size exclusion - all different sizes, but will have similar charge (+2) (+2)

Suppose that the peptide had, instead, been digested with chymotrypsin. Would the optimal separation technique change or not? Explain.

(+1) AVGW RVKS

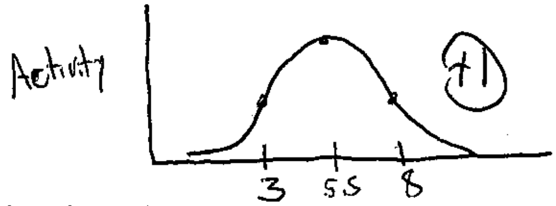
0 +2

(+2) Same size

(+2) Different charge

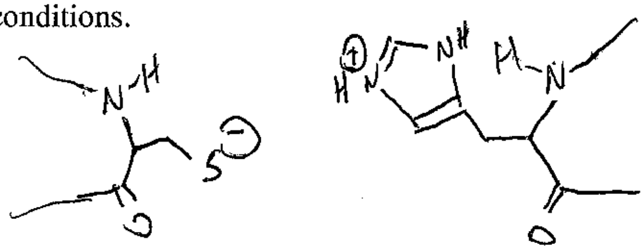
8. The enzyme bromelain (found in pineapple) is a cysteine protease, analogous to a serine protease such as chymotrypsin. The active site contains an important histidine residue, but unlike chymotrypsin, does not contain aspartate. The pKa values of cysteine and histidine in this active site are 3 and 8 respectively.

A. Draw a curve of enzyme activity as a function of pH for this enzyme. What is its approximate pH optimum?



(+1) pH ~ 5.5 (+1)

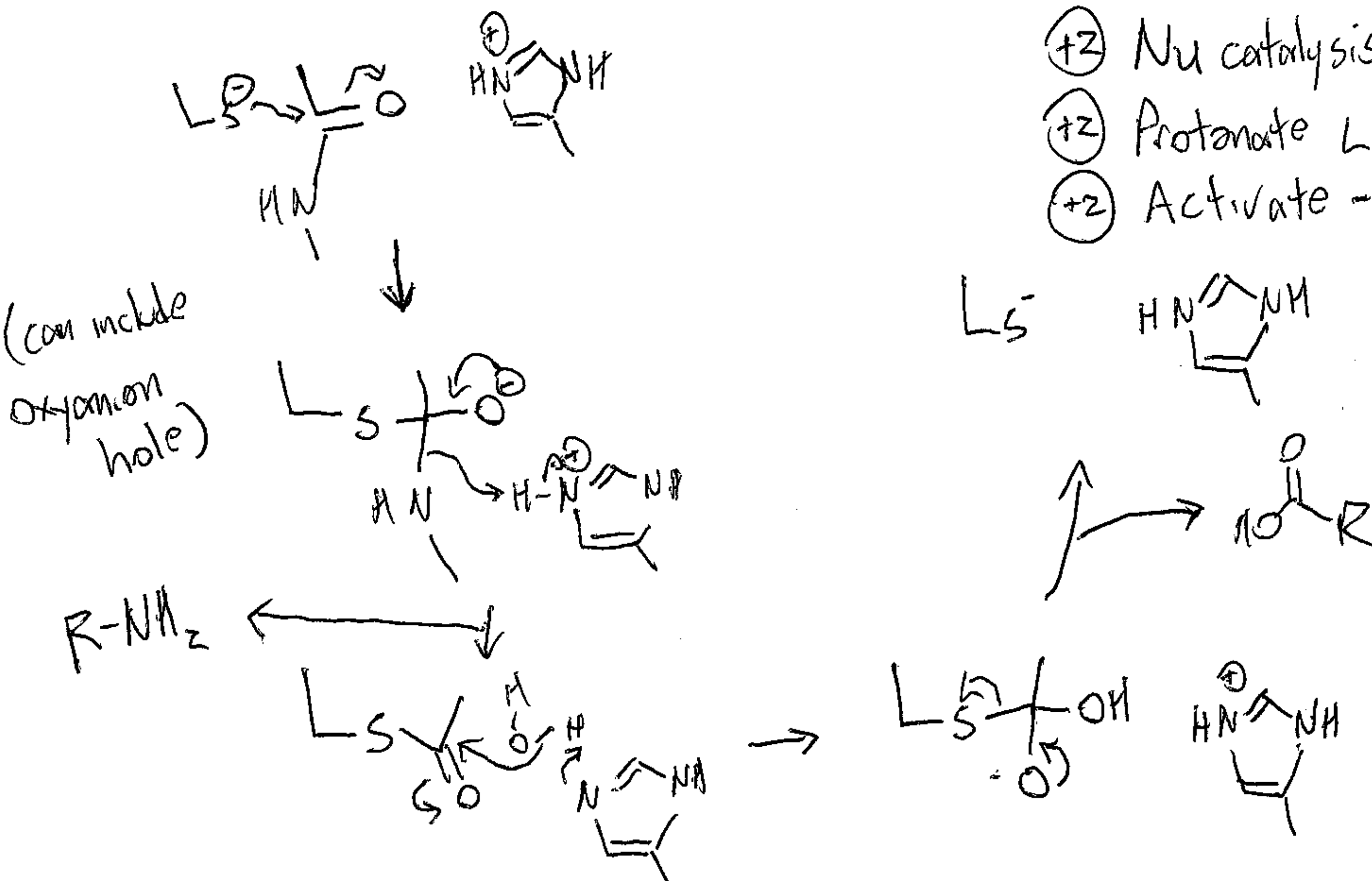
B. Draw the active site residues in their predominant ionization states under optimum pH conditions conditions.



(+1)
(+1)

C. Based on your understanding of chymotrypsin, draw an arrow mechanism for peptide hydrolysis catalyzed by bromelain. Include the roles of both the cysteine and histidine residues.

(+2) Nu catalysis
(+2) Protonate LG
(+2) Activate -OH



Section 3: Case study (10pts)

9. A series of synthetic collagen peptides, each 30 residues long, were heated to determine their melting points. Use the data in the table to answer the questions below. (Hyp = hydroxyproline; Flp = fluoroproline. Peptide X = Gly-Lys-Hyp-Gly-Glu-Hyp-Gly-Pro-Lys-Gly-Asp-Ala-(Gly-Ala-Hyp)$_2$-(Gly-Pro-Hyp)$_4$.)

Experiment	Sequence	pH	T_m (°C)
1	(Pro-Hyp-Gly)$_{10}$	1	61
2	(Pro-Hyp-Gly)$_{10}$	7	58
3	(Pro-Hyp-Gly)$_{10}$	11	60
4	Peptide X	1	18
5	Peptide X	7	27
6	Peptide X	13	19
7	(Pro-Pro-Gly)$_{10}$	7	41
8	(Pro-Flp-Gly)$_{10}$	7	91

A. The (Pro-Hyp-Gly)$_{10}$ peptide is about equally stable over all pH ranges. Explain why the pH stability of peptide X is different.

(+3) Peptide X has ionizable sidechains. At pH 7, it is both ⊕ and ⊖, so it can form triple helix.

(+2) At low pH, it is net ⊕, so the strands repel.
At high pH, it is net ⊖, so the strands repel.

B. Propose a theory for the stabilizing effect of Hyp on collagen. Refer to particular data to support your theory.

+2 for H-bonding (exp 2+7)

(+2) Exp 2, 7, and 8

(+3) Binding stability increases with substituents on proline from -H to -OH to -F.

This does not support H-bonds, but suggests e⁻ withdrawing causes increased stability - maybe through conformational or electrostatic changes.