

C383 Exam 1  
Spring 2017

Name \_\_\_\_\_ Seat Number \_\_\_\_\_

Student ID \_\_\_\_\_ Circle your section: M T W R

**The last page of this exam contains equations, constants, pKa values, and other information you might find useful.**

The exam consists of 34 questions worth 100 points plus 8 bonus points on a total of 11 pages. It will be scored out of 100 points, and the maximum score that can be received is 100 points.

1-15 \_\_\_\_\_/30 multiple choice

16-30 \_\_\_\_\_/30 fill in the blank

31 \_\_\_\_\_/10

32 \_\_\_\_\_/10

33. \_\_\_\_\_/10

34. \_\_\_\_\_/10

Bonus \_\_\_\_\_/8

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: Multiple Choice. 15 questions, 2 points each. Circle the letter of the correct answer.**

1. A succinic acid/succinate buffer with ten times as much of the conjugate acid as the conjugate base would have a pH of

- A. 3.2
- B. 4.2
- C. 4.6
- D. 6.6
- E. None of the above

2. \_\_\_\_\_ has a net charge of zero at pH 7.

- A. histidine
- B. lysine
- C. arginine
- D. glutamate
- E. aspartate

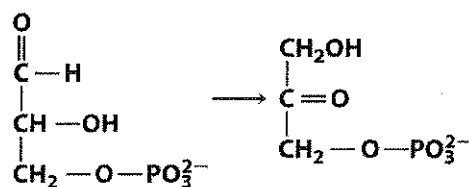
3. Which amino acid sidechain would contribute to protein folding through the hydrophobic effect to the greatest degree?

- A. T
- B. G
- C. K
- D. Q
- E. I

4. Which statement concerning the peptide bond is false?

- A. It is planar
- B. It is polar.
- C. It is predominantly in the trans configuration.
- D. It has two dihedral angles.
- E. It has significant double bond character.

5. The following reaction would be catalyzed by an enzyme of what class?



- A. oxidoreductase
- B. Transferase
- C. hydrolase
- D. isomerase
- E. lyase

6. An amphipathic  $\beta$ -sheet is most likely to be found
- A. on the surface of a fibrous protein
  - B. in the core of a fibrous protein
  - C. on the surface of a globular protein
  - D. in the core of a globular protein
  - E. equally in all the above.
7. Which would be most effective in separating a group of proteins of differing sizes?
- A. homogenization
  - B. salting out
  - C. gel filtration
  - D. enzyme assay
  - E. isoelectric focusing
8. To determine the specific activity of a protein sample,
- A. the sample must be pure.
  - B. the % yield must be known.
  - C. the sample must be denatured.
  - D. the total activity and total protein must be known.
  - E. more than one of the above.
9. If  $\Delta G^\circ$  of a reaction is +17 kJ/mol,
- A.  $[P]/[R] \sim 1000$
  - B.  $K_{eq} \sim 1/1000$
  - C.  $\Delta G' = -17$  kJ/mol
  - D. The reaction is spontaneous at equilibrium.
  - E. More than one of the above.
10. Which of the following terms best describes this statement: The enzyme changes conformation when it binds to substrate.
- A. Lock and key
  - B. transition state stabilization
  - C. Active site
  - D. proximity effect
  - E. Induced fit
11. Which of these terms describes an enzyme bound with its cofactor?
- A. Apoenzyme
  - B. coenzyme
  - C. holoenzyme
  - D. prosthetic group
  - E. cosubstrate

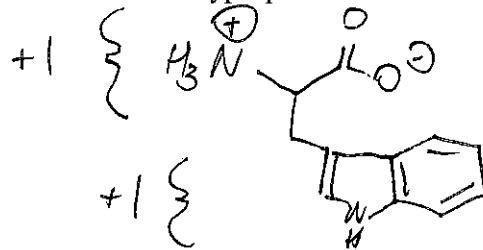
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12. The DNA double helix is stabilized relative to its single strand form primarily by
- A. hydrogen bonding of base pairs
  - B. backbone phosphate interactions
  - C. hydrogen bonding and base stacking
  - D. hydrophobic effect and van der Waals forces
  - E. none of the above
13. A nucleobase attached to ribose:
- A. B-DNA
  - B. pyrimidine
  - C. nucleotide
  - D. deoxyribose
  - E. none of the above
14. The histidine sidechain in an enzyme active site has a pKa of 6.8. About what percent of the histidine sidechains are ionized at pH 7.4?
- A. 20%
  - B. 40%
  - C. 50%
  - D. 60%
  - E. 80%
15. Anfinsen treated Ribonuclease A with urea and mercaptoethanol then allowed it to renature to demonstrate:
- A. the lock and key model of enzyme binding
  - B. semiconservative replication
  - C. protein folding is governed by the primary sequence
  - D. the central dogma.
  - E. the relationship of melting point to GC-content of the double helix.

**Section 2: Fill in the blank. 15 questions 2 points each**

16. Intrinsically unstructured proteins do not have discrete three-dimensional structure under physiological conditions.
17. Enzymes of the transferase class that transfer a phosphate from ATP to another molecule are called Kinases.
18. SDS-PAGE is a technique that uses electrophoresis to separate proteins based on their size.
19. Enzymes bind better to the Transition State than they do to the substrate.
20. Proteins with cysteine residues are capable of forming covalent bonds called disulfide bonds that stabilize protein tertiary structure.
21. The residues of a polynucleotide are linked by phosphodiester bonds.
22. A recurring pattern of super secondary structure is known as a motif.
23. Collagen is a fibrous protein containing hydroxyproline that breaks down in the case of a Vitamin C deficiency.
24. The amino acid proline is not commonly found in  $\alpha$ -helices because its peptide bond is not capable of being a hydrogen bond donor.
25. Valine is a hydrophobic amino acid with three carbon atoms in its sidechain.
26. A parallel  $\beta$ -sheet has strands running  $N \rightarrow C$  hydrogen bonded to strands running  $N \rightarrow C$ .
27. RNA is less stable than DNA due to the presence of the 2' -OH.
28. The sigmoidal shape of the DNA melting curve suggests that denaturation is behaving cooperatively.

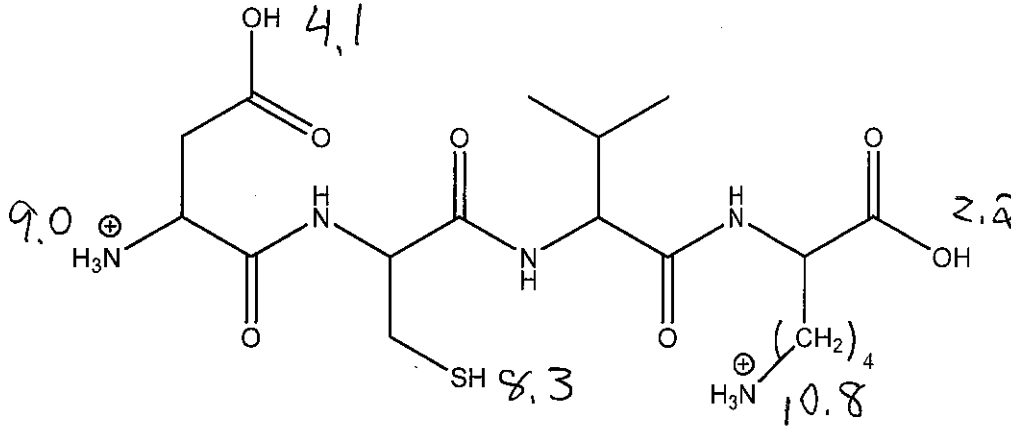
29. Draw the zwitterion form of tryptophan:



30. Amino acids that we must eat because we cannot synthesize them are called essential amino acids.

**Section 3. Problems. 4 questions 10 points each.**

31. Beverly Biochemist has synthesized the tetrapeptide below, and wants to purify it from other peptides in the mixture by isoelectric focusing. It is drawn in its major ionization state at pH 1.

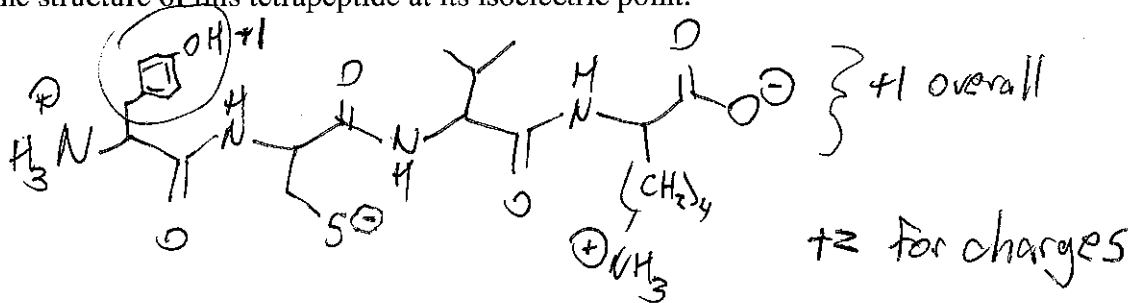


A. At what pH in the isoelectric focusing gel should she look for this purified peptide? Explain how you determined your answer.

The pH has to be above 4.1 so that both carboxylates are deprotonated to give a net zero charge, but +2 below 8.3 so that cysteine is not deprotonated

$$pI = \frac{4.1 + 8.3}{2} = 6.2 \quad (+2)$$

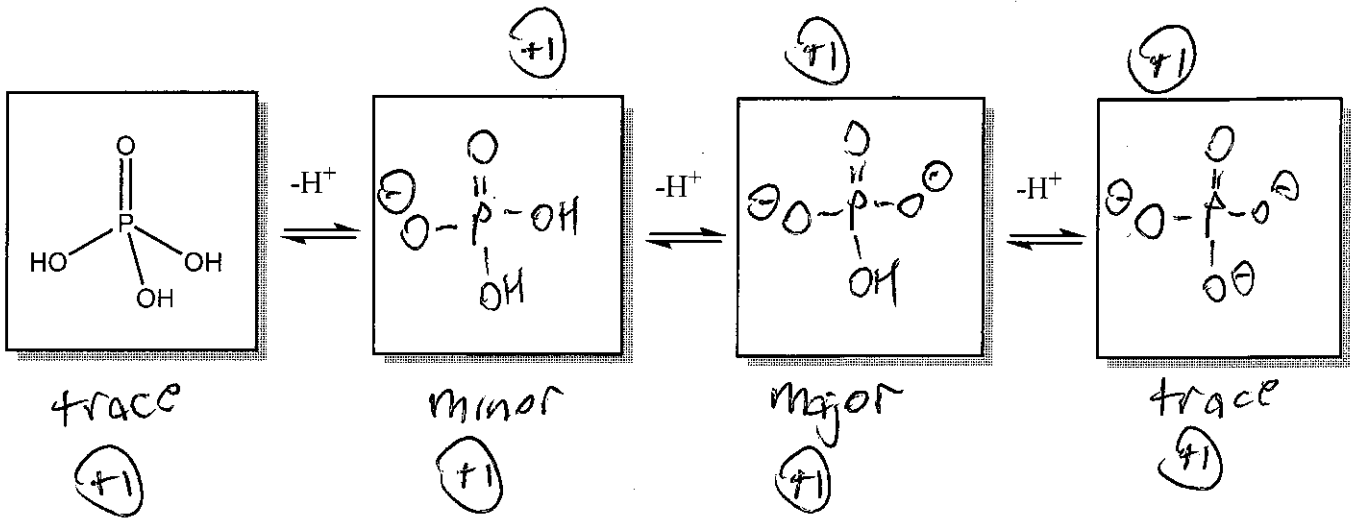
B. Beverly also made a tetrapeptide based on the one above in which she mutated D → Y. Draw the structure of this tetrapeptide at its isoelectric point.



C. Would you expect to be able to separate the tetrapeptide from its mutant more effectively using size exclusion chromatography or ion exchange chromatography?

ion exchange (+2)

32. A. Phosphate is found in significant concentration in blood. In the boxes below is phosphoric acid, a polyprotic acid with  $pK_{a1} = 1.8$ ,  $pK_{a2} = 6.8$ , and  $pK_{a3} = 12.1$ . Draw the three other ionization states of phosphate. Indicate which of these ionization states is(are) the major species found in the blood (pH 7.4), which is(are) the minor species, and which is(are) found only at trace amounts.



B. A new deep-sea invertebrate was found that has some tissues with pH of about 9.5. Bob Biochemist has proposed that phosphate might serve as a pH buffer in these tissues. Is he right or wrong? Explain.

(+1) wrong

Buffer is only effective  $\pm 1$  from  $pK_a$ .

(+2) Phosphate buffer works in the pH ranges of 5.8-7.8 and 11.1-13.1, but not 9.5.

33. The isomerization of glucose-6-phosphate (G-6-P) to fructose-6-phosphate (F-6-P) has an equilibrium constant of 0.50 at 298 K and pH 7. In a certain liver cell, the concentration of F-6-P was determined to be 4.2 mM, and the concentration of G-6-P was determined to be 6.2 mM.

A. What is the free energy of this reaction at equilibrium?

(+2)  $\Delta G' = \text{zero}$

B. What is the free energy of this reaction when  $[F-6-P] = [G-6-P] = 1.0 \text{ M}$ ?

when  $[P] = [R] = 1 \text{ M}$ ,  $\Delta G' = \Delta G^{\circ'}$

(+3) 
$$\Delta G^{\circ'} = -RT \ln K_{eq}$$

$$= -8.314 \frac{\text{J}}{\text{mol K}} (298 \text{ K}) \ln(0.5) = +1.7 \frac{\text{KJ}}{\text{mol}}$$

C. What is the free energy of this reaction in this liver cell?

$$\Delta G'' = \Delta G^{\circ'} + RT \ln \frac{P}{R}$$

(+3) 
$$= 1700 \frac{\text{J}}{\text{mol}} + 8.314 \frac{\text{J}}{\text{mol K}} (298) \ln \frac{4.2 \times 10^{-3}}{6.2 \times 10^{-3}}$$

$$= 740 \frac{\text{J}}{\text{mol}}$$

D. Under which of the conditions above, if any, is the reaction spontaneous as written? Explain.

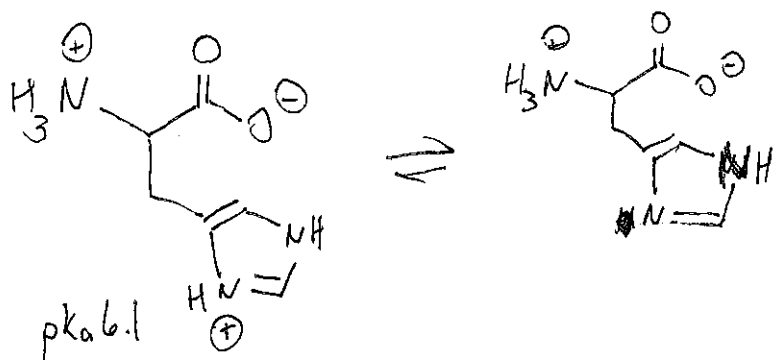
It is not spontaneous under any of these conditions -  $\Delta G'$  must be negative to be spontaneous

(+2)





**Bonus:** As an estimate, we say that Histidine is primarily in an ionization state with a net charge of zero at pH 6.5. But what is the actual net charge on histidine at pH 6.5? Show your work.



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

$$6.5 = 6.1 + \log \frac{A^-}{HA}$$

$$2.5 = \frac{A^-}{HA}$$

$$\% A^- = \frac{2.5}{3.5} = 71\% \text{ with a net zero charge}$$

$$29\% \text{ with a net 1 charge}$$

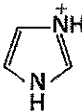
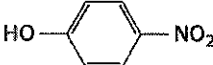
Overall  $\sim +.29$  net charge

Useful Information:

$$\Delta G^{\circ} = -RT \ln K_{eq} \quad R = 8.314 \text{ J/mol.K}$$

$$\Delta G_{\text{reaction}} = \Delta G^{\circ}{}_{\text{reaction}} + RT \ln \frac{[\text{products}]}{[\text{reactants}]}$$

**TABLE 2.4** pK Values of Some Acids

Name	Formula <sup>a</sup>	pK
Trifluoroacetic acid	CF <sub>3</sub> COOH	0.18
Phosphoric acid	H <sub>3</sub> PO <sub>4</sub>	2.15 <sup>b</sup>
Formic acid	HCOOH	3.75
Succinic acid	HOOCCH <sub>2</sub> CH <sub>2</sub> COOH	4.21 <sup>b</sup>
Acetic acid	CH <sub>3</sub> COOH	4.76
Succinate	HOOCCH <sub>2</sub> CH <sub>2</sub> COO <sup>-</sup>	5.64 <sup>c</sup>
Thiophenol	C <sub>6</sub> H <sub>5</sub> SH	6.60
Phosphate	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	6.82 <sup>c</sup>
N-(2-acetamido)-2-aminoethanesulfonic acid (ACES)	H <sub>2</sub> NCOCH <sub>2</sub> NH <sub>3</sub> <sup>+</sup> CH <sub>2</sub> CH <sub>2</sub> SO <sub>3</sub> <sup>-</sup>	6.90
Imidazole		7.00
p-Nitrophenol		7.24
N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)	HOCH <sub>2</sub> CH <sub>2</sub> NH <sup>+</sup> (C <sub>4</sub> H <sub>8</sub> )NCH <sub>2</sub> CH <sub>2</sub> SO <sub>3</sub> <sup>-</sup>	7.55
Glycinamide	<sup>+</sup> H <sub>3</sub> NCH <sub>2</sub> CONH <sub>2</sub>	8.20
Tris(hydroxymethyl)aminomethane (Tris)	(HOCH <sub>2</sub> ) <sub>3</sub> CNH <sub>2</sub> <sup>+</sup>	8.30
Boric acid	H <sub>3</sub> BO <sub>3</sub>	9.24
Ammonium ion	NH <sub>4</sub> <sup>+</sup>	9.25
Phenol	C <sub>6</sub> H <sub>5</sub> OH	9.90
Methylammonium ion	CH <sub>3</sub> NH <sub>3</sub> <sup>+</sup>	10.60
Phosphate	HPO <sub>4</sub> <sup>2-</sup>	12.38 <sup>d</sup>

<sup>a</sup>The acidic hydrogen is highlighted in red; <sup>b</sup>pK<sub>1</sub>; <sup>c</sup>pK<sub>2</sub>; <sup>d</sup>pK<sub>3</sub>.

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### Amino acid pKa values

C-terminal	2.2
N-terminal	9.0
Aspartate, glutamate	4.1
Histidine	6.1
Cysteine	8.3
Tyrosine	10.9
Lysine	10.8
Arginine	12.5

**Table 5.3** Specific cleavage of polypeptides

Reagent	Cleavage site
<b>Chemical cleavage</b>	
Cyanogen bromide	Carboxyl side of methionine residues
O-Iodosobenzoate	Carboxyl side of tryptophan residues
Hydroxylamine	Asparagine-glycine bonds
2-Nitro-5-thiocyanobenzoate	Amino side of cysteine residues
<b>Enzymatic cleavage</b>	
Trypsin	Carboxyl side of lysine and arginine residues
Clostripain	Carboxyl side of arginine residues
Staphylococcal protease	Carboxyl side of aspartate and glutamate residues (glutamate only under certain conditions)
Thrombin	Carboxyl side of arginine
Chymotrypsin	Carboxyl side of tyrosine, tryptophan, phenylalanine, leucine, and methionine
Carboxypeptidase A	Amino side of carboxyl-terminal amino acid (not arginine, lysine, or proline)

Table 5.3  
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