NAME: For GradeScope, please write your FIRST and LAST name in CAPITAL letters WITHIN the box:

Exam I September 25, 2023 Biochemistry I BI/CH 421/621

I.	/48
II.	/12
III.	/30
IV.	/10
TOTAL	/100

I. <u>MULTIPLE CHOICE</u>. (48 points; 3 pts each) Choose the BEST answer to the question and write the letter in the appropriate box to the left.

1. Enzymes are biological catalysts that affect a reaction by:

- A. increasing the amount of free energy released.
- B. decreasing the amount of free energy released.
- C. increasing the change in entropy.
- D. increasing the rate.
- E. decreasing the rate.
- \neg 2. The reaction of two amino acids to form a dipeptide is a(n):
 - A. cleavage.
 - B. condensation.
 - C. group transfer.
 - D. oxidation-reduction.
 - E. rearrangement.
- 3. The enthalpy change ΔH for the formation of ammonia from hydrogen and nitrogen is -46.11 kJ/mol. This reaction is:
 - A. exergonic.
 - B. endergonic.
 - C. exothermic.
 - D. endothermic.
 - E. at equilibrium

4. Water is generally a good solvent for polar molecules and a poor solvent for nonpolar molecules. These solvent properties are best explained by

- A. the high density of liquid water relative to polar solvents
- B. the ability to form intermolecular hydrogen bonds
- C. the density of solid water being less than the density of liquid water
- D. high surface tension
- E. high heat of vaporization
- 5. The properties of water include
 - A. the ability to form hydrophobic interactions with itself
 - B. a disordered structure in the liquid state
 - C. a low dielectric constant
 - D. being a strong dipole, with a partially negative end at the O atom
 - E. a diameter of 5 Å

6. Which of the following pairs of molecules could NOT hydrogen bond with each other?



7. The hydrophobic effect can make important energetic contributions to:

- A. membrane structure.
- B. folded protein structures.
- C. micelles of SDS and protein.
- D. enzyme-substrate interactions.
- E. oil droplets coming together.
- F. all of the above.
- Phosphoric acid is tribasic, with pK_as of 2.14, 6.86, and 12.4. The ionic form that predominates at pH 3.2 is:

A. H_3PO_4 . B. $H_2PO_4^-$. C. HPO_4^{2-} . D. PO_4^{3-} .

9. As the phase changes from water to ice, the decreased density of the water is due to:

- A. the loss of hydrogen bonds
- B. the gain of ionic bonds
- C. the gain of more van der Waals contacts
- D. the spacial arrangement of the assymetrical water molecules as they form the maximum number of hydrogen bonds possible
- E. the hydrophobic effect, which is driven by electrostatic interactions

10. Which of these statements about hydrogen bonds is not true?

- A. Individual hydrogen bonds are much weaker than covalent bonds.
- B. Hydrogen bonds account for the anomalously high boiling point of water.
- C. Individual hydrogen bonds in aqueous water exist for many seconds and sometimes for minutes.
- D. In liquid water, the average water molecule forms hydrogen bonds with 3 to 4 other water molecules.

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11 .	Which of t	the following amino	acids has a sulfur at	om in its side chai	Page 3 n?
	A. Ası D. Met	n B z E	. Glu . Tyr	C. Phe F. Thr	
12.	Of the 20 reason is	standard amino acid that its side chain	s, only i	s not optically act	ive. The
	A. pro B. ala C. gly D. ala E. gly	oline; forms a coval anine; is a simple m ycine; is unbranched anine; contains only ycine; is a hydrogen	ent bond with the ami ethyl group. hydrogen. atom.	no group.	
13.	In a mixtu size-exclu	are of the five prot asion (gel filtratio	eins listed below, wh n) chromatography?	ich should elute se	cond in
	A. cyt B. imr C. rik D. RNA E. set	cochrome c , $M_r = 13$, nunoglobulin G, $M_r =$ ponuclease A, $M_r = 1$ A polymerase, $M_r = 4$ cum albumin, $M_r = 68$	000 145,000 3,700 50,000 ,500		
14.	By adding it is poss	SDS (sodium dodecyl sible to:	sulfate) during the	electrophoresis of	proteins,
	A. det B. pre C. det D. seg E. det	cermine the amino ac eserve a protein's n cermine a protein's parate proteins excl cermine an enzyme's	id composition of the ative structure and b isoelectric point. usively on the basis specific activity.	protein. iological activity. of molecular weight	
15.	The streng the pK _a s c	gth of hydrogen bond of ionizable amino a	s, electrostatic inte cid residues could be	ractions, and the v different in prote	alues of ins than
	A. wat B. pro out C. the D. the	ter forms a clathrat oteins are hydrophil tside e entropy of the wat e microenvironment i	e structure around pr ic on the inside and er increases when pro nside proteins could	oteins hydrophobic on the teins fold up be different than in	n solution
16.	To determine that:	ine the isoelectric	point of a protein, a	gel is first estab	lished
	A. exl an B. con nec C. rei kno	hibits a stable pH of electric field. htains a denaturing gative charges over lates the unknown pr own molecular weight	detergent that can di the protein's surface otein to a series of as, M _r .	es become distribut stribute uniform protein markers wit	ed in h
	D. 1S E. neu bas	wasned with an anti utralizes all ionic ses.	proups on a protein b	protein of interest by titrating them wi	th strong

- 17. The pK of the carboxyl group of glycine (pK = 2.3) is less than that for acetic acid (pK=4.8) due to \cdot
 - A. the electronegativity of the carbonyl oxygen
 - B. the electrostatic stabilization by the adjacent amino group
 - C. the electronegativity of the nitrogen in the adjacent amino group
 - D. the electronic nature of amino acids
 - E. the fact that acetic acid can be a zwitterion and glycine cannot8
- 18. A student was given a task of identifying the contents of five bottles of amino acids from which the labels had fallen off. Each of the original bottles contained one of the following: R, H, C, P, and W. Which of the following methods could be most readily employed to identify tryptophan?
 - A. Electrophoresis
 - B. Ultraviolet spectroscopy
 - C. Gel filtration
 - D. Analytical ultracentrifugation
 - E. Optical rotation
- **II. STRUCTURES.** (12 points)
- 19. Draw the structure of Asp-Ala-His (Aspartylalanylhistidine) in the ionic form that predominates at pH 7.5. Put an arrow pointing at the group that would become deprotonated after an increase in the pH.



III. SHORT ANSWER/FILL-IN. (30 points)

Give a **brief** answer or diagram for each problem or question below. Write your answers in the box.

20. You observe an interaction in a protein between two Alanine residues that are 2 Å apart. What non-covalent bond are you most likely observing? (4 pts)

If the strength of this bond were 1 kcal/mol, what would the strength be at 20 Å?

In the same protein, you observe another interaction between an Aspartate and an Arginine that are 3 Å apart. What non-covalent bond are you most likely observing?

- If the strength of this bond were 5 kcal/mol, what would it be at 30 Å?
- 21. A buffer contains 0.1 mol of lactic acid ($pK_a = 4.0$) and 0.1 mol of sodium lactate per liter. What is the pH of the buffer? [NOTE: log 1=0, log 3=0.5, log 0.33=-0.5, log 0.005=-2.3, log 0.05=-1.3, log 0.5=-0.3] (6 pts)



What is the total concentration of the buffer?

Calculate the pH after adding 5.0 mL of 10 M HCl to 1.0 L of the buffer. What is the change in the pH?

рH

Calculate the change in pH after adding 5 mL of 10 M HCl to 1.0 L of pure water, which has a pH of 7.0.





Is there a significant effect in stabilizing the pH given the addition of acide due to the presence of lactic acid? 22. Given the following general reaction: (5 pts) 100 amino acids 1 protein of 100 amino acids in length

What is the ΔS for this reaction as written, positive or negative?

What is the ΔH for this reaction as written, positive of negative?

Which of the two thermodynamic parameters above contributes more to making this reaction favorable? In other words, which provides more to a possible negative free energy change?

Given the information provided, can you say whether the overall reaction is favorable (Y or N)?

Total

protein

In the cell, this is obviously a favorable reaction, what is the cell doing to ensure a negative ΔG ?

Procedure

23. Fill in the blanks on the following purification table. (15 pts)

Fraction

volume

separation (ml)(units) (units/mg) (%) or step (mg) 1. Crude cellular extract 1,400 10,000 100,000 10 100 2. Precipitation 280 3,000 32 96,000 3. Ion-exchange chromatography 400 80,000 200 90 4. Size-exclusion chromatography 80 100 60,000 600 5. Affinity chroma-5 5 50,000 tography

Activity

Specific activity

* All data represent the status of the sample *after* the procedure indicated in the first column has been carried out.

Which numbered step afforded the best purification?

Which numbered step was the least effective in purification?

If your protein was fairly pure after step #5, what is the approximate expression level of your protein in the liver as a percentage? In other words, what percentage of the overall proteins in liver are your protein?

If the column used in step #3 were a cation-exchange column run at pH 8, would the pI of your protein be <8, >8, or equal to 8 (circle your choice)?

The final prep has a protein concentration of:

If you performed an isopycnic centrifugation purification as step #6 using a sucrose gradient, and the final specific activity were 2000 U/mg, what conclusions might you make about this new step?



Yield





Basis of

IV. MATCHING. (10 points)

24. For each of the pairs of amino acids described in the lists on the right, put the number from the left in the appropriate box that best describes the bond between them.



NO. 011		
Test	Correct	Answer
1	D	
2	В	
3	С	
4	В	
5	D	
6	D	
7	F	
8	В	
9	D	
10	С	
11	D	
12	Е	
13	В	
14	D	
15	D	
16	А	
17	В	19
18	В	

Answer Key for Exam 1 09/25/23



The peptide, with two peptide bonds (+1 point) must have an amino-terminal Aspartate residue, an Alanine residue, and a carboxyl-terminal His residue (+1 point each). The imidazole side chain is mostly deprotonated because the pH is well above the pK_a of ~6. The carboxylates are deprotonated as well, but the amino-terminal amine is still protonated (+1 point each for charges). Arrow pointeing to charged amino-terminus (1 point).

pI: the fully protonated peptide has a net charge of +2, and the fully deprotonated peptide has a net charge of -2. So, the four deprotonation reactions in order are: $+2 \rightarrow +1 \rightarrow 0 \rightarrow -1 \rightarrow -2$, with the pKa values going from 3 (Cterm), to 4(Asp), to 6(His), to 9(N-term). The zwitterion would be governed by the values of 4 and 6, with a pI values of 5 ((4+6)/2=5). But if you used the values more precisely from the table of 4.4 and 6.5 you get 5.5. If you use the AA values, which is incorrect, you would get 4.9. Correct values would be between 5-6.

No. on

No. on Test Correct Answer

20 20.1: Van der Waals. The Ala-Ala is so close they have induced dipole-induced dipole interactions

20.2: 1x10-6 kcal/mol. Since from Coulomb's Law, the strength varies by 1/r6 for VdW, and it's a 10-fold change in r, its 10-6 of 1 kcal/mole

20.3: Salt Bridge. The Asp(-) and Arg(+) are fully charged and interact easily.

20.4: 0.5 kcal/mol. The 10-fold change in distance (r) is a 10-fold change in energy of the bond according to Coulomb's law.

21 21.1: pH = 4 Since the $[A_-] = [HA]$, both 0.1 M, the Henderson-Hasselbalch (HH) equation says the pH = pK the because $log([A_-]/[HA])=log 1=0$

21.2: 0.2 M Since both are 0.1 M and both are part of the buffer reaction (HA \rightarrow A– + H+), you add them.

21.3: pH = 3.5 Since you are adding 0.05 moles of a strong acid (10 M/L x 0.005L = 0.05 moles), this protonates half of the 0.10 mol of lactate. You are left with 1.5 mol of lactic acid and 0.5 mol lactate. The ratio is 0.05/0.15 = 0.33. The log of this is given in the NOTEs as -0.5. So, according to the HH equation the pH = 4-0.5 = 3.5.

21.4: change in pH is -0.5 The pH of 4 at the start is reduced to 3.5, which is a pH change of -0.5.

21.5: change in pH is -5.7 Since you add the same number of mole strong acid as before, this is 5x10-2 M, and the log of 0.05, which is given in the NOTEs, is -1.3. From pH = $-\log[H+]$, the pH = 1.3. Starting from pH=7, it has dropped 5.7 pH units.

21.6: Yes

22 22.1: Since there is more entropy of reactants, the Δ S=Products–Reactants; less–more is negative

22.2: Since there is lower heat energy in the bonded protein, the Δ H=Products-Reactants; less-more is negative

22.3: ΔH Since favorability is a negative $\Delta G = \Delta H - T \Delta S$, the negative ΔS gives a + value to the overall free energy change, whereas the ΔH gives a negative value and helps the overall favorability be more negative.

22.4: N You don't know just how positive the entropy term is, so the overall change in free energy could be negative or positive.

22.5: Coupling. You need to describe some way cells incorporate a more exergonic reaction(s) with the unfavorable reactions to make this favorable.

Procedure or step	Fraction volume (ml)	Total protein (mg)	Activity (units)	Specific activity (units/mg)	Overall Yield (%)	Basis of separation
1. Crude cellular extract	1,400	10,000	100,000	10	100	
2. Precipitation	280	3,000	96,000	32	96	Solubility
 Ion-exchange chromatography 	90	400	80,000	200	80	, Charge
 Size-exclusion chromatography 	80	100	60,000	600	60	Size (MW)
5. Affinity chroma- tography	5	5	50,000	10,000	50	Binding

23 23.1 - 23.6 filled in on Table.

* All data represent the status of the sample *after* the procedure indicated in the first column has been carried out.

23.7: The best purification is Step #5

23.8: The least effective purification is Step #4

23.9: 0.1%, or 1/1000, which is the fold purification from a specific activity of 10 to one of 10,000

23.10: Circle >8 because it has a positive charge at pH 8, so it have not been titrated through a net charge of 0 (pl) and there are still excess protons.

23.11: 1 mg/mL, from the Table

23.12: Since the specific activity went DOWN, the activity must have been lost. Your protein was inactivated somehow.

24

1.	4
2.	1
3.	2
4.	2
5.	1
6.	3
7.	3
8.	4
9.	2
10.	5