Fall '10 Hackert CH370 Exam I Name _____UTeID

Given: $c = 3.0 \times 10^{10}$ cm/sec; $k = 1.38 \times 10^{-23}$ J/K; $h = 6.63 \times 10^{-34}$ J-sec; $N_0 = 6.02 \times 10^{23}$ /m $|E(1\%) \times MW| = |10 \times \epsilon|$; $Eff. = 1 / (1 + (R/R_0)^6)$; E = hv

1. Identify each **amino acid** by its **three <u>and</u> one letter codes** (e.g. **Ala / A**, etc.) and each **N base**, **nucleoside** or **nucleotide** by its **full name** in the blanks above or beside the structures (26 pts).

(26)A) _ B) NH_2 H₃N⁺—CH-COO F) H₃N⁺—CH-COO CH_3 K) _ H₃N⁺—CH-COO H₃N⁺—CH-COO ĊН ĊН N) _ O) H_3N^+ — $CH-COO^-$ H₃N⁺—CH-COO CH_2 COO⁻ Q)___ R) _ H₃N⁺—CH-COO H₃N⁺—CH-COO $\dot{C}H_2$ CH₃ U)_ H₃N⁺—CH-COO W) _ `CH₃

2	Consider the following eligenentide:	/airala all raciduae with titratable protone	٠,
∠.	Consider the following offgopeptide.	(circle all residues with titratable protons	,,

L - I - C - K - E - R - D (2)

a) What is the net charge on this oligopeptide at very low pH (pH = 1.0)? _____

(2)

c) What is the approximate pI for this oligopeptide? _____ (show your work)

(4)

3. **Recognition of Terms**: Match each of the first six terms with the phrase that best describes it.

a) arrangement of subunits

b) multiple sequence alignments

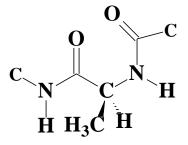
c) independent folding unit with a subunit

Define the last two terms.

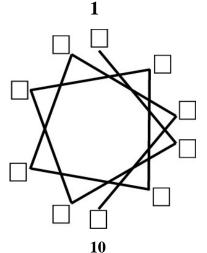
___ domain (6)

- ___ ClustalW
- ___ Homology
- ___ quaternary structure
- ___ BLAST
- d) covalent structure
- Blosum62
- e) sequence comparison algorithm / search sequence databases
- f) substitution matrix
- g) similarity attributed to descent from a common ancestor
- 4. Consider the alanyl residue below. Label the bond rotation angles phi φ and psi ψ , and determine what those values are for this residue: $\varphi =$ ______; $\psi =$ ______ (Watch direction!)

(4)



5. Write an amino acid sequence for a decamer that would fold into an alpha helix with the right side of the helix hydrophobic and the left side hydrophilic in nature. Use the helical wheel to illustrate the validity of your sequence.



(3)

6. For the 5-step enzyme purification shown, **answer** the questions below:

Step	Protein	Volume	Total	Specific Activity
	(mg/mL)	(mL)	Activity	
			(units)	
Crude extract	2.8	235	28,550	43.4
2. Salt ppt	7.2	48	21,750	
3. Ion exhange Chrom	5.8	32	15,250	
4. Affinity Chrom.	2.0	25	12,000	
5. Gel filtration	1.5	30	11,600	

(2) a	a) Which step (#) exploited the differences in charge?
(2) b	b) Which step (#) exploited the differences in size?
(2) c	c) Complete the purification table by filling in the remaining blanks
(3)	d) What is the overall percentage "yield" for this purification scheme?
(2) e	e) Which step (#) of the purification produced the largest % increase in specific activity?
(2) f	Which step (#) of the purification produced the smallest % increase in specific activity?
spheri with t 1.44,	nsider a "gel filtration" column that is 150 cm in length and 2.50 cm in diameter. It is packed with ical beads that are 0.13 mm in diameter with a V _o that is 33% of V _{tot} . The column is calibrated trypsin inhibitor (~21.5 kD) and β-galactosidase (~116 kD) which gave Ve /Vo values of 2.63 and respectively. An unknown protein is then eluted from the column. Calculate the partition coefficients for the two standard proteins (show work). trypsin inhibitor β-galactosidase
b)	If an unknown protein had a partition coefficient that was exactly the average of the two calculated for the two standards , what would be the best estimate for the molecular weight for the unknown protein? (show work)
(4)	

8. Light and Energy: Calculate the frequency and energy in **kJ/mole** of visible light of wavelength =

500 nm (Show work - Draw a Box around your answer).

(5)

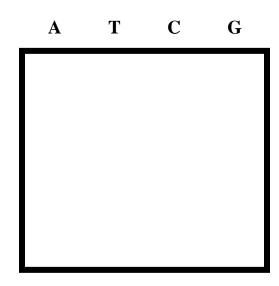
9. A protein has a molar extinction coefficient of 13' 0.50 cm cuvette was found to have a T of 42% at coefficients of tryptophan ($\varepsilon = 5.6 \text{ x } 10^3 \text{ M}^{-1}\text{cm}^{-1}$ as given and the molecular weight of the protein	t a wavelength of 280 nm. The molar extinction) and tyrosine ($\varepsilon = 1.4 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$) at 280 nm are					
a) What is the absorbance for this sample pr	rotein solution?					
(3)						
b) Calculate the $\mathbf{E}(1\%)$ extinction coefficien	b) Calculate the $\mathbf{E}(1\%)$ extinction coefficient for this protein at 280 nm.					
(2)						
c) Calculate the concentration of this protein	solution in mg/mL.					
(3)						
10. In addition to being a more sensitive way to meafluorescence spectroscopy. Identify other types fluorescence properties listed below:	· · · · · · · · · · · · · · · · · · ·					
fluorescence property	type of experiment / measurement					
a. <u>emission wavelength for λmax</u>	measure concentration of emitter					
(6) b. <u>shift in emission wavelength for λmax</u>						
c. <u>change in fluorescence depolarization</u>						
d. <u>resonance energy transfer</u>						
folding. The instrument gives reliable data wher maximum separation distance that these two chro (Show Work for credit. Draw a box around your	for possible use in a FRET experiment on ribosome the efficiency is at least 20%. What is the omophores can be useful with this instrument?					
(5)						

12. Briefly describe the basis of the Maxam-Gilbert method of DNA seque	encing.
(4)	

13. Consider the following nucleic acid sample: 5'-ATGCCTTAGCT-3' used as the template in a dideoxy sequencing experiment by an undergraduate assistant. On the "gel" below, draw the expected gel pattern that *would occur if* the student used aprimer with a 5'- fluorescent label, but forgot to add the dideoxy GTP to that reaction mixture.

(4)

DNA polymerase I + 4 dNTPs + ddATP ddTTP ddCTP ddGTP



(Please sign your name on the back of your exam in a manner that you can recognize it when it is returned.)