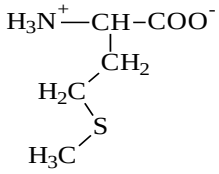
Fall '10 **CH370** Name _____
Hackert **Exam I** UTeID _____

Given: c = 3.0 x 10^{10} cm/sec; k = 1.38x10^{-23}J/K; h = 6.63 x 10^{-34} J-sec; No = 6.02 x 10^{23} /m
|E(1%) x MW| = |10 x ε|; Eff. = 1 / (1 + (R/Ro)^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) _____



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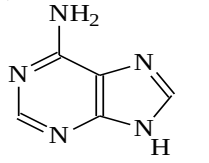

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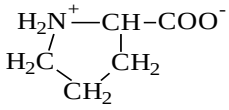

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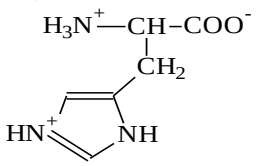

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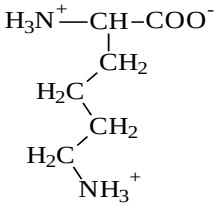

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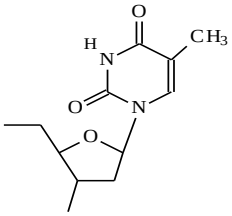

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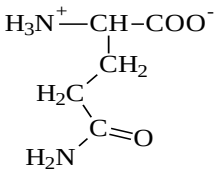

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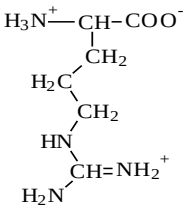

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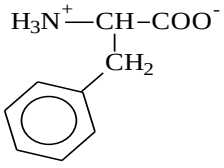

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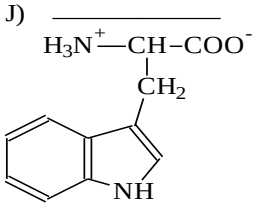

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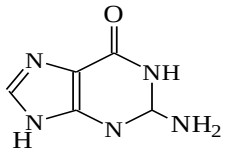

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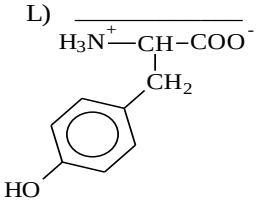

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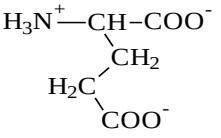

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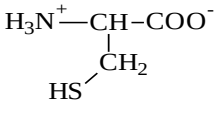

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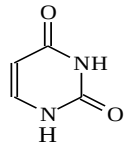

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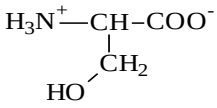

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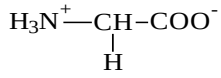

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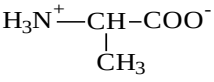

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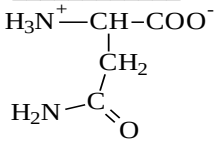

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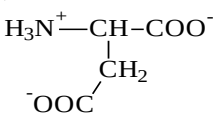

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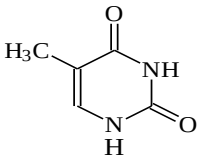

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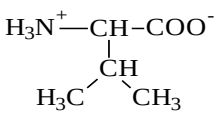

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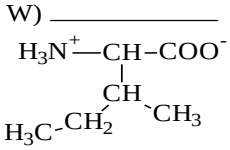

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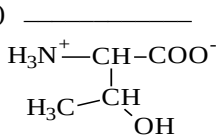

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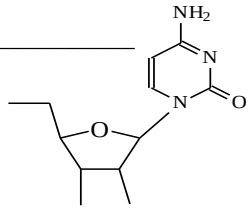

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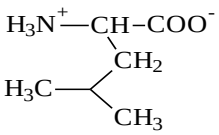


2. Consider the following oligopeptide: **(circle all residues with titratable protons)**

(2) **L – I – C – K – E – R – D**

a) What is the net charge on this oligopeptide at very low pH (pH = 1.0)? _____
(2)

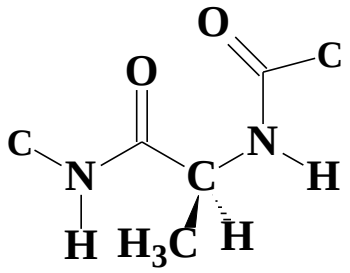
c) What is the approximate pI for this oligopeptide? _____ (**show your work)**
(4)

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

(6)

___ domain	a) arrangement of subunits
___ ClustalW	b) multiple sequence alignments
___ Homology	c) independent folding unit with a subunit
___ quaternary structure	d) covalent structure
___ BLAST	e) sequence comparison algorithm / search sequence databases
___ Blosum62	f) substitution matrix
	g) similarity attributed to descent from a common ancestor

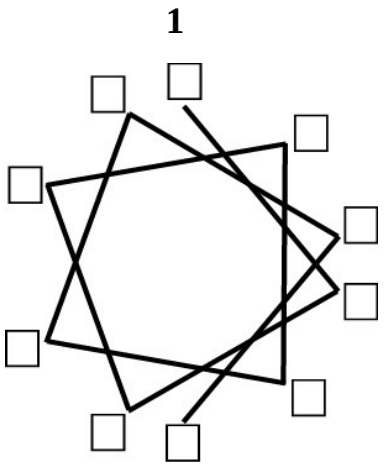
4. Consider the alanyl residue below. Label the bond rotation angles phi φ and psi ψ , and determine what those values are for this residue: φ = _____; ψ = _____ (Watch direction!)
(4)



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)

1 _____ 10



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

Step	Protein (mg/mL)	Volume (mL)	Total Activity (units)	Specific Activity
1. 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) Which step (#) exploited the differences in charge? _____

(2) b) Which step (#) exploited the differences in size? _____

(2) 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) 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?_____

7. Consider a "gel filtration" column that is 150 cm in length and 2.50 cm in diameter. It is packed with spherical beads that are 0.13 mm in diameter with a V_o that is 33% of V_{tot}. The column is calibrated with trypsin inhibitor (~21.5 kD) and β-galactosidase (~116 kD) which gave Ve /Vo values of 2.63 and 1.44, respectively. An unknown protein is then eluted from the column.

a) Calculate the **partition coefficients** for the **two standard** proteins (show work).
trypsin inhibitor _____ β-galactosidase _____

(4)

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 137,450 $M^{-1}cm^{-1}$ at 280 nm. A sample in a standard 0.50 cm cuvette was found to have a T of 42% at a wavelength of 280 nm. The molar extinction coefficients of tryptophan ($\varepsilon = 5.6 \times 10^3\ M^{-1}cm^{-1}$) and tyrosine ($\varepsilon = 1.4 \times 10^3\ M^{-1}cm^{-1}$) at 280 nm are as given and the molecular weight of the protein is 149,000.

a) What is the **absorbance** for this sample protein solution?

(3)

b) Calculate the **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 measure concentration, we discussed many uses for fluorescence spectroscopy. Identify other types of experiments that can make use of each of the fluorescence properties listed below:

	fluorescence property	type of experiment / measurement
a.	emission wavelength for λmax	measure concentration of emitter
(6) b.	shift in emission wavelength for λmax	
c.	change in fluorescence depolarization	
d.	resonance energy transfer	

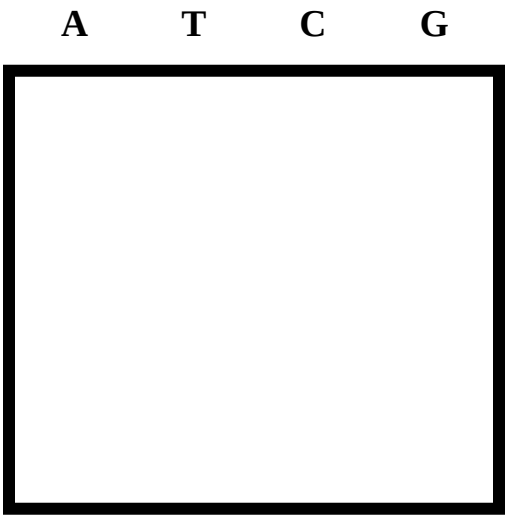
11. You have available a pair of chromophores (Baby Blue-2 and Ruby Red-7) used in food dyes that have a R_o = 37.0 Å (3.70 nm) for consideration for possible use in a FRET experiment on ribosome folding. The instrument gives reliable data when the efficiency is at least 20%. What is the maximum separation distance that these two chromophores can be useful with this instrument? (Show Work for credit. Draw a box around your answer.)

(5)

12. Briefly describe the basis of the Maxam-Gilbert method of DNA sequencing.
(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.)