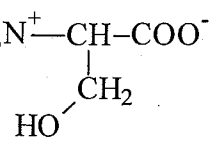
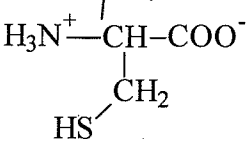
Fall '09 **CH370** Name KEY
Hackert **Exam I** UTeID _____

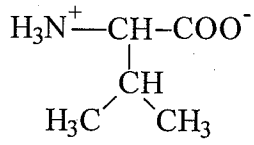
Given: c = 3.0 x 10^{10} cm/sec; k = 1.38x10^{-23}J/K; h = 6.63 x 10^{-34} J-sec; No = 6.02 x 10^{23} /m

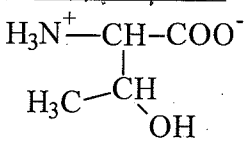
1. Identify each **amino acid** by its **three and one letter codes** (e.g. **Ala / A**, etc.) in the blanks above (16) the structures shown below.

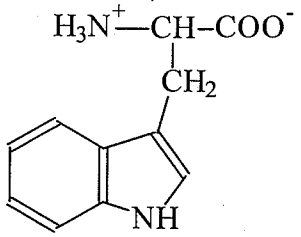
(½/½)

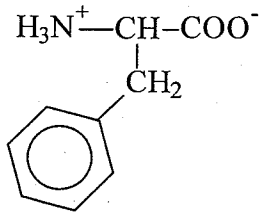
A) Ser / S 

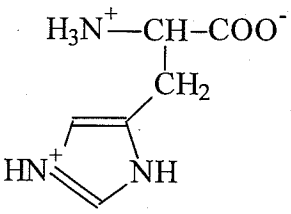
B) Cys / C 

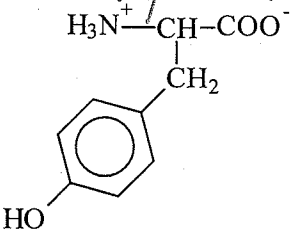
C) Val / V 

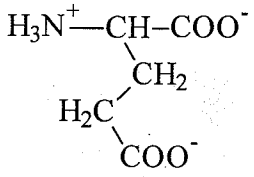
D) Thr / T 

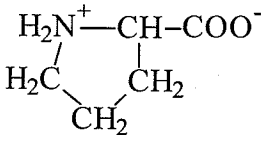
E) Trp / W 

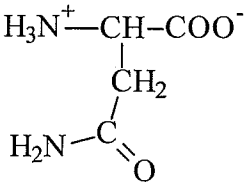
F) Phe / F 

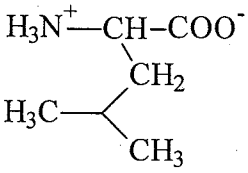
G) His / H 

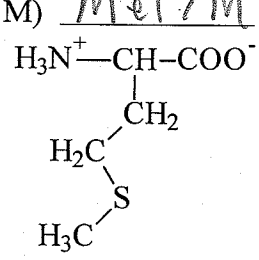
H) Tyr / Y 

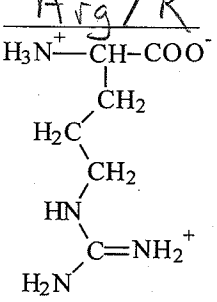
I) Glu / E 

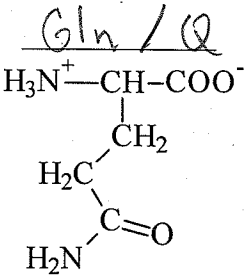
J) Pro / P 

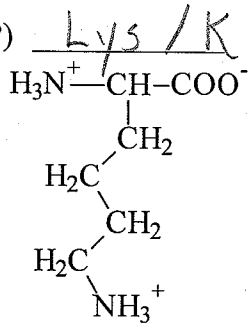
K) Asn / N 

L) Leu / L 

M) Met / M 

N) Arg / R 

O) Gln / Q 

P) Lys / K 

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

2-all 1-most (2) 

a) What is the net charge on this oligopeptide at very low pH (~pH = 1.0)? +4

(2) (2)

(3) c) What is the approximate pI for this oligopeptide? 8

work (3) **show your work**

(2) good work but mistake

3. **Glossary:** Define or use **KEY WORDS** to describe the meaning of each of the following terms used in Bioinformatics (**NO MORE THAN 2 LINES OF TEXT SHOULD BE USED!!**).

(6) BLAST - Basic Local Alignment Search Tool (BLAST is a fast sequence comparison algorithm) (2)

PAM - "Percent Accepted Mutation" - A unit to measure evolutionary distance, 1 PAM unit ≈ 1% change. (2) OK - if describe matrix

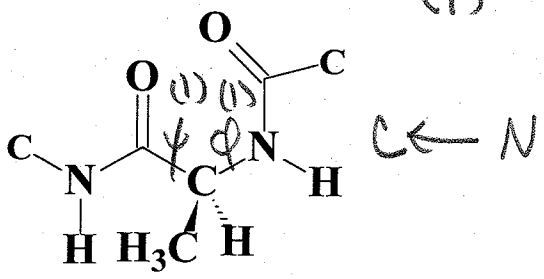
Homology - Similarity attributed to decent from a common ancestor. (2)

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 = 0° (1); ψ = 180° (1)

(4)

1pt each

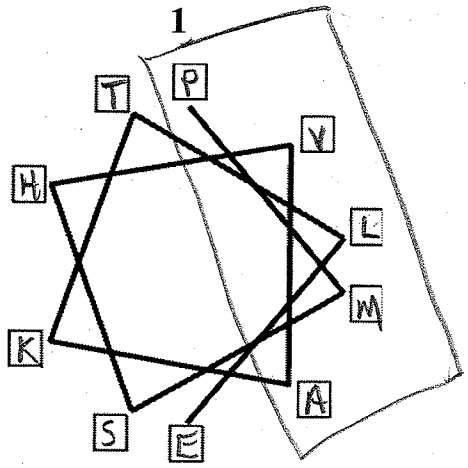
-2 if done correctly for N→C



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

Grade the helical wheel. -2 hydrophilic boxed in (3)

1 P-M-S-H-V-A-K-T-L-E 10



6. Which **general property** (we discussed 4) of proteins is exploited for each of the following methods to separate and purify proteins. Fill in the blanks appropriately.

(3)

	(method)	**(property)**
A)	Dialysis	size
B)	CM chromatography	charge
C)	"Nickel" column	specificity (affinity using a His_6-tag)

1pt each

7. **Complete the table below** by calculating the specific activities for the following steps used to purify an enzyme, and **then answer** the questions below: **(Show your work)**

Step	[Protein] (mg/mL)	Volume (mL)	Total Activity (units)	Specific Activity
1. Crude extract	5.2	285	56,550	38
2. Salt ppt	10.4	38	35,750	90
3. Ion exhange Chrom	7.5	26	31,250	160
4. Affinity Chrom.	2.6	4.2	23,400	2143
5. Gel filtration	1.9	4.8	21,600	2368

4 pts

(4) Fill in the table with the specific activity values.

(2) a) What is the overall percentage "yield" for this purification scheme? 38% (2)

(2) b) What is the number fold improvement for this purification scheme? 62X (2)

8. Consider a "gel filtration" column that is 100 cm in length and 2.0 cm in diameter. It is packed with spherical beads that are 0.14 mm in diameter with a V_o that is 34% of V_{tot}. The column is calibrated with two standards, "Std A" (~26 kD) and "Std B" (~145 kD) which gave Ve /Vo values of 2.42 and 1.26, respectively. An unknown protein is run on the same column and gave a Ve /Vo value of 1.86. (Show work for credit and draw a Box around your answer).

a) Calculate the elution volumes for the two standards and the unknown.

1pt each (3) Ve. 1pt if right method but wrong V_{Tot} (3)

$V_{Tot} = h \cdot \pi \cdot r^2$

$= 314.2\ cm^3$

$V_o = 0.34 \cdot V_{Tot} = 106.8\ cm^3$

	$V_e\ (cm^3)$	
"A"	256	(1)
Unk	199	(1)
"B"	135	(1)

b) Calculate the **partition coefficients** for the two standards and the unknown.

1pt each

$K_{av} = \frac{V_e - V_o}{V_{tot} - V_o} = \frac{V_e - V_o}{207.4\ cm^3}$

	K_{av}	log M
"A"	0.719 (1)	4.41
Unk	0.445 (1)	
"B"	0.136 (1)	5.16

c) Estimate the molecular weight for the unknown protein. 57.9 kD

(3) -2 for not using K and log MW.

$\log M^{Unk} = \log M^A + \left(\frac{K^A - K^{Unk}}{K^A - K^B}\right)(\log M^B - \log M^A)$

$= 4.41 + \left(\frac{.719 - .445}{.719 - .136}\right)(5.16 - 4.41)$

$= 4.41 + \left(\frac{.274}{.583}\right)(0.75) = 4.76 = \log M^{Unk}$

9. Briefly (**IN NO MORE THAN 4 LINES**) using **KEY WORDS** or TEXT describe how his-tags enable a biochemist to more efficiently purify proteins.

(6)

2pts His-tag
2pts - Ni col.
1pt - clarity
1pt - elution

- commercially available vectors for expressing protein with added His_6-tag at either end (N-term or C-ter
- His_6-tagged protein confers specificity to bind to Ni column
- All other proteins pass thru column, elute with imidazole gra

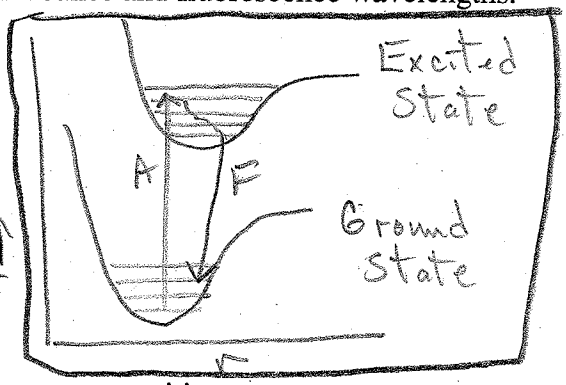
10. Fluorescence: Draw a "Frank-Codon" **DIAGRAM** that illustrates why there is always a "red shift" when comparing absorbance and fluorescence wavelengths.

(6)

$E^A > E^F$

$\Rightarrow \lambda^F > \lambda^A$

longer wavelength ↑ E
$\Rightarrow$ red shift



Since the energy difference in "Abs" is always greater than that released during "F",
$\rightarrow \lambda^F > \lambda^A$

11. 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:

(6) (2pts each)

fluorescence property	type of experiment
a. emission wavelength for λmax	measure concentration of emitter
b. shift in emission wavelength for λmax	folding
c. change in fluorescence depolarization	binding
d. resonance energy transfer	(FRET) separation distance

12. Light and Energy: Calculate the frequency and energy in **kJ/mole** of visible light of wavelength = 600 nm and X-rays of wavelength = 0.10 nm (1.0 Å). (Recall that E = hν ; Also note that for reference, the –C-C- single bond energy is about 350 kJ/mol) (Draw a Box around your answers)

(6)

$E = h\nu = h \cdot \frac{c}{\lambda} = 6.63 \cdot 10^{-34}\ J \cdot s \left(\frac{3.0 \times 10^{17}\ nm/sec}{\lambda}\right)$

-1 each if J/photon vs kJ/mole

i) 600nm: $E = 3.32 \cdot 10^{-19}\ J/phot$ or $\boxed{200\ kJ/mol}$

ii) 0.1nm: $E = 1.99 \cdot 10^{-15}\ J/phot$

$E = \left(1.99 \cdot 10^{-15}\ \frac{J}{phot.}\right)\left(\frac{6.02 \cdot 10^{23}}{m}\right) = \boxed{1.2 \cdot 10^{6}\ kJ/mol}$

13. You have to design an experiment to measure the absorbance of a protein solution that is 1.5 mg/mL. The protein is a monomer of 715 amino acids (MW ~ 78,458). The protein contains 10 W, 12 Y and 16 F. Given the following 280 nm molar extinction coefficients - tryptophan ($\varepsilon = 5.6 \times 10^3\ M^{-1}cm^{-1}$), tyrosine ($\varepsilon = 1.4 \times 10^3\ M^{-1}cm^{-1}$) and phenylalanine ($\varepsilon = 0.2 \times 10^3\ M^{-1}cm^{-1}$) - answer the following questions (Show work for credit. Draw a Box around your answers.):

a) Estimate the molar extinction coefficient for the protein at 280 nm.

(4) Assume all A_{280} is due to W, Y, and F $\Rightarrow |A| = |\varepsilon|$ for 1M

(2 pts OK-setup) 4 pt.-correct

$A = 5600(10)(1) + 1400(12)(1) + 200(16)(1)$

$A = 56{,}000 + 16{,}800 + 3200 = 76{,}000$

$\Rightarrow \boxed{\varepsilon = 76{,}000\ M^{-1}\ cm^{-1}}$

b) Estimate the E1%

(4) -1 no units

$|E^{1\%}| \cdot M = |\varepsilon| \cdot 10 \Rightarrow E^{1\%} = \frac{10 \cdot \varepsilon}{MW} = \boxed{9.68 \frac{dL}{g \cdot cm}}$ -1 no units

c) You have three cuvettes available with path lengths of 0.50 cm, 1.0 cm and 2.0 cm. Which cuvette would you choose to measure A_{280}? 0.50, and explain your answer in 1 or 2 sentences.

(3) -3 for 2.0 -1 for 1.0 with 0 explanation

A would be > 1 for the 1.0 cm or 2 cm cuv.

d) Calculate the A for this solution for the cuvette you have selected. $1.5\ mg/mL = 0.15\ g/dL$

(3) Note: OK, if consistent A = 1.45 for 1cm cuvette

$A_{280} = E^{1\%} \cdot c \cdot l = \left(9.68 \frac{dL}{g \cdot cm}\right)\left(0.15 \frac{g}{dL}\right)(0.50\ cm)$

$\boxed{A = 0.726}$

14. 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.)

(6) 2 pts

$\boxed{Eff = \frac{1}{1 + (r/r_o)^6}}$; $0.20 = \frac{1}{1 + x^6} \Rightarrow 0.20 + 0.20x^6 = 1$

$x^6 = 4$

$x = 1.26$

6 pts for correct answer

$\boxed{r = 1.26 \cdot r_o = 46.6\ Å}$

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