

(Complete this graded homework independently, place all answers on this page, show work below, on back, or on attached pages. No credit for late work)

1. Terminology:

- (2) a) Which amino acid's side chain would have an expected pKa around 12? Arg (R)  
 b) What is the approximate distance between adjacent alpha Cs in proteins, and the approximate diameter of an alpha helix?

0.38 nm (3.8 Å) / ~ 1.0 nm (10 Å)

2. Estimate the pI of the oligopeptide:  $\overset{+}{H}N-\overset{+}{R}-I-D-D-L-E-\overset{0}{C}H_2$  (pI ~ 4)  
 (2)  $\begin{matrix} 10 & 12 & 4 & 4 & 4 & 2 \\ +2 & +1 & 0 & -1 & -2 & -3 \end{matrix}$

3. Size, charge, polarity and affinity are all characteristics of proteins that can be exploited to purify a protein, name one separation technique that capitalizes on each of these characteristics:

Property:	Size	Charge	Polarity	Affinity
(4) Technique:	<u>Size Exclusion Gel Filtration</u>	<u>Ion Exchange</u>	<u>HIC (Reverse Phase)</u>	<u>Affinity Chrom.</u>

4. What is the percentage yield for a purification step that started with 68 mL of solution at 1.1 mg/mL with a specific activity of 26 and ended with 8.5 mL of solution at 2.2 mg/mL with a specific activity of 100? 96%  
 (2) Start:  $68 \text{ mL} \times 1.1 \text{ mg/mL} \times 26 \text{ EU/mg} = 1945 \text{ EU}$   
 End:  $8.5 \text{ mL} \times 2.2 \text{ mg/mL} \times 100 \text{ EU/mg} = 1870 \text{ EU}$

5. Consider a "gel filtration" column that is 100 cm in length and 2.25 cm in diameter. It is packed with spherical beads that are on average 0.22 mm in diameter with a density of 1.33 g/cm<sup>3</sup>. Assume that V<sub>0</sub> is 33% of V<sub>tot</sub>. The column is calibrated with trypsin inhibitor (~21.5 kD) and β-galactosidase (~116 kD) which gave V<sub>e</sub>/V<sub>0</sub> values of 2.50 and 1.50, respectively. What is the best estimate of molecular mass (kD) for an unknown protein with V<sub>e</sub>/V<sub>0</sub> = 2.00.

(M ~ 47.9 kD)

	V <sub>e</sub>	K	log M
tryp.	327	.73	4.33
unk	262	.49	<u>4.68</u>
β-gal	196	.24	5.06



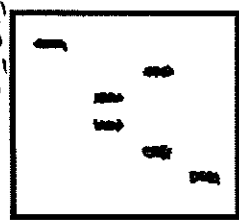
6. Proteins A, B, C, and D with MWs corresponding to 20kDa, 225kDa, 50kDa, and 85kDa and pIs (1) corresponding to 5.3, 6.9, 4.4, 9.6, respectively, are added onto a CMC (carboxy methyl cellulose) <sup>⊖</sup> charge ion exchange column at pH 6.9 and then eluted with an increasing salt gradient. Which protein would you predict to be off the column last? D  
 at pH 6.9 = ~0 -- ++

7. (1) Which protein above would come off last when all are loaded on a G-200 (GF) column? A  
 (= smallest)

8. (1) Two common affinity tags used to purify a protein are the His6 tag and the Maltose Binding Protein tag, once bound, the protein is eluted with imidazole and maltose, respectively for these two tags.

9. Given the following standard, dideoxy sequencing gel result, what is the sequence (5' → 3') of the

Newly synth. strand: 3'-A-C-T-T-C-G-5'  
 Template strand: 5'-T-G-A-A-G-C-3'



5' GCTTCA 3'  
 new strand

- (2) original template DNA? 5'-TGAAGC-3'

10. What is the role of luciferase and name of the modern sequencing technique that utilizes this protein? (2) Luciferase converts ATP → light / Pyrosequencing

I hereby declare that I did this work independently: \_\_\_\_\_