

(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:

- (4) a) Which amino acid's side chain would have an expected pKa around 8? _____
b) Which four atoms define the "phi" angle when specifying the conformation of a polypeptide backbone? _____
c) Humans possess three forms of the protein antizyme – AZ-1, AZ-2 and AZ-3. These proteins have homologous sequences and are more accurately referred to as _____.
d) Where would one expect to find DNA with a syn conformation for the N base orientation? _____

2. Estimate the pI of the oligopeptide: D – E – C – E – M – **D** – E – R (pI ~ _____)
(2)

3. Consider a "gel filtration" column that is 100 cm in length and 2.50 cm in diameter. It is packed with spherical beads that are on average 0.22 mm in diameter with a density of 1.31 g/cm³. Assume that V_o is 36% of V_{tot}. The column is calibrated with trypsin inhibitor (~21.5 kD) and β-galactosidase (~116 kD) which gave V_e/V_o values of 2.67 and 1.42, respectively. Estimate the elution volume of a protein of **molecular mass 75.6 kD**. (V_e ~ _____ mL)
(3)

4. Given the sizes and pI's of the following proteins, predict the order in which these proteins would be eluted off a CMC (carboxymethyl cellulose) column run at pH 7.0 and eluted with a salt gradient from 0.10 M NaCl to 2.0 M NaCl.

	A) Serum albumin	B) Hemoglobin	C) Chymotrypsin
Size (kD):	68.5	64.5	23
pI:	4.9	6.8	9.5

- (2) Order off column: (1st off) _____ (last off)

5. The absorbance of UV light at 280 nm by proteins is mostly due to the aromatic amino acids tyrosine and tryptophan. Lactate DH is a tetramer with each subunit having 332 a.a. (36,507 Da) and containing 6 residues of tryptophan ($\epsilon = 5.6 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$), 5 residues of tyrosine ($\epsilon = 1.4 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$) and 8 residues of phenylalanine ($\epsilon = 0.2 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$).

- a) Estimate the **molar extinction coefficient** for this protein at 280 nm. ($\epsilon =$ _____)
(3)

- b) Estimate the **E^{1%}** for this protein at 280 nm. (**E^{1%}** = _____)
(2)

- c) Calculate the **absorbance** and **percent transmission** for a solution of this protein at a concentration of **0.60 mg/mL** from a cell with a path length of **0.50 cm** measured at **280 nm**.
(2) (**A** = _____ ; **%T** = _____)

6. Consider a FRET experiment where the measured efficiency of energy transfer between two chromophores is 20.0%. If R_o = 40.0 Å for these two chromophores, **estimate the separation** of the two chromophores. (**R** = _____)
(2)

I hereby declare that I did this work independently: _____