

(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:

- (5) a) Which amino acid's side chain would have an expected pKa around 6? _____
 b) Which four atoms define the "psi" angle when specifying the conformation of a polypeptide backbone? _____
 c) Name the nucleoside containing a deoxyribose sugar and the N-base cytosine. _____
 d) T/F: The amino acid sequences of two proteins are aligned and found to be 78% identical and cleared related. Thus you can correctly state that the two proteins are 78% homologous? _____
 e) The "E value" found in a BLAST search refers to _____

2. Estimate the pI of the oligopeptide: R – I – C – H – A – R – D (pI ~ _____)

(2)

3. Consider a "gel filtration" column that is 100 cm in length and 3.00 cm in diameter. It is packed with spherical beads that are on average 0.27 mm in diameter with a density of 1.31 g/cm^3 , with a V_o determined to be 37% 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 2.74 and 1.47, respectively. Estimate the **molecular mass (kD)** of an **unknown** protein that has a $V_e/V_o = 1.84$. (M ~ _____ kD)

(3)

4. Given the sizes and pI's of the following proteins, predict the order in which these proteins would be eluted off a DEAD column run at pH 7.0 and eluted with a salt gradient from 0.10 M to 4.0 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 5 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 10 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. ($E^{1\%} =$ _____)

(1)

c) Calculate the **absorbance** and **percent transmission** for a solution of this protein at a concentration of **0.70 mg/mL** from a cell with a path length of **1.00 cm** measured at **280 nm**.

(2) ($A =$ _____ ; $\%T =$ _____)

6. Consider a FRET experiment where the measured efficiency of energy transfer between two chromophores is 40.0%. If $R_0 = 40.0 \text{ \AA}$ for these two chromophores, **estimate the separation** of the two chromophores. ($R =$ _____)

(2)

I hereby declare that I did this work independently: _____