

(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 8? C (Cys)

b) Name two differences in conformational characteristics between B-DNA and Z-DNA:

B-DNA - right handed

B-DNA

ribose C2' endo; bases anti

Z-DNA - left handed

Z-DNA

deoxyribose C3' endo; base syn at dG

2. Estimate the pI of the oligopeptide: N-P-R-Y-I-C-A-T-E-COO⁻ (pI ~ 6)

~pKa

10

12

16

8

4

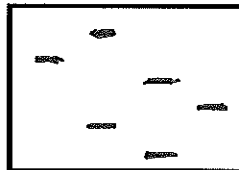
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(2) +2 → +1 → 0 → -1 → -2 → -3 → -4

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

Original 5'-ATGCAG-3'

New 3'-TACGTC-5'



CTGCAT
5' → 3'
new strand

Template? (2) 5'-ATGCAG-3'

4. What is the role of luciferase and name of the sequencing technique that utilizes this protein?

(2) Action of luciferase converts ATP → light / pyrosequencing

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

Property: Size Charge Polarity Affinity
(4) Technique: gel filtration ion exchange reverse phase affinity chrom.
size exclusion cation/anion HIC (IMAC/His tag)

6. What is the percentage yield for a purification step that started with 47 mL of solution at 1.1 mg/mL with a specific activity of 356 and ended with 14 mL of solution at 2.4 mg/mL with a specific activity of 511?

(2) 93%

	Vol (mL)	[P] $\frac{\text{mg}}{\text{mL}}$	Total Prot (mg)	Spec. Act.	Total Activity	% Yield
1)	47	1.1	51.7	356	18,405	
2)	14	2.4	33.6	511	17,170	93%

7. 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 1.33 g/cm³. Assume that V_0 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_0 values of 2.50 and 1.50, respectively. What is the best estimate of molecular mass (kD) for an unknown protein with $V_e/V_0 = 2.15$?

(3) $(M \sim \underline{38.5} \text{ kD})$

$V_T = 4 \cdot \pi \cdot r^2 = 491 \text{ cm}^3$

$V_0 = .36 V_T = 177 \text{ cm}^3$

$V_T - V_0 = 314 \text{ cm}^3$

$V_{e, \text{inhib.}} = 2.50 \times V_0 = 443 \text{ mL}$

$V_{e, \text{unk.}} = 381 \text{ mL}$

$V_{e, \beta\text{-gal}} = 266 \text{ mL}$

$K = (V_e - V_0) / (V_T - V_0)$

	K	log M
t.inh.	0.847	4.33
unk.	0.650	<u>4.58</u>
β -gal	0.283	5.06

$x = 4.33 + \frac{.197}{.564} (.73)$

8. Proteins A, B, C, and D with MWs corresponding to 25 kDa, 185 kDa, 67 kDa, and 85 kDa and pIs

(1) corresponding to 5.3, 7.6, 4.4, 9.6, respectively, are added onto a CMC (carboxy methyl cellulose) 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 (neg. charged matrix; A and C do not stick; D probably more + at pH 6.9 than B.)

9. (1) Which of the above proteins would come off first when all are loaded on a G-200 column? B

10. (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.

I hereby declare that I did this work independently:

Matt