

There are seven questions on five pages. There is a total of 105 points, hence there are 5 bonus points.

1. (15 points) Use the above graph (see graphs in chapter 5 for examples) to answer the following questions. Three different variants (A, B, and C) of the same heme protein were analyzed for their ability to bind oxygen. The results of these experiments are plotted in the above graph.
  1. Which of the heme proteins has the highest affinity for oxygen?
  2. What are the  $P_{50}$  values for A, B and C ?
  3. Which of the variants displays cooperative binding? Explain your rationale.
  
2. (15 points) Draw the structure for the following peptide at pH 7. Then, calculate the pI for this peptide. Use  $pK_a=8$  for the amino terminus group and  $pK_a =3.4$  for the carboxy terminus group. You know all of the other  $pK_a$  values for the R groups.

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3. (10 points) The following peptide was cleaved with chymotrypsin and the resulting peptides were separated by cation exchange chromatography. What are the peptide fragments? What is their order of elution for these peptides at pH 5 from an cation exchange column. For all of the peptides, the N terminus amino group  $pK_a$  is 8 and the C terminus carboxyl  $pK_a$  is 3.4.

## PIGNWQRGVYAKILHFMEADV

4. (20 points) A peptide was hydrolyzed with 6M HCl at 100°C overnight, and the following amino acids were detected:

H 2	E 3	I 1	W 1
S 2	G 1	A 1	T 3
Q 1	R 1	C 2	

Another sample of the intact peptide was digested with an enzyme and then the sample was separated by anion exchange chromatography at pH 6. Only one fraction was obtained the first time the researcher did the anion exchange column. Then, the researcher realized that a step had been omitted in this process. A new sample of peptide was used for the appropriate reaction(s), and then the product was digested with the same enzyme. This time, there were two fractions when the mixture was separated by anion exchange chromatography. These two fractions were then subjected to Edman degradation.

These are the two sequences of the peptide fragments:

HITESQCS                    CTHEGREATW

- a. What enzyme was used to cleave the peptide?
  
  
  
  
  
  
  
  
  
  
- b. Why was there only one fraction from the first anion exchange column?
  
  
  
  
  
  
  
  
  
  
- c. What step did the researcher forget the first time? What reaction(s) were needed?
  
  
  
  
  
  
  
  
  
  
- d. What is the sequence of the peptide?
  
  
  
  
  
  
  
  
  
  
- e. One more reaction could be done to determine the N-terminus of the peptide. Do you need to do this reaction to determine the sequence of this peptide? What is the reaction?
  
  
  
  
  
  
  
  
  
  
- f. Which of the fragments eluted first from the anion exchange column?
  
  
  
  
  
  
  
  
  
  
- g. What is Edman degradation? How does it work?
  
  
  
  
  
  
  
  
  
  
5. (15 points) What are the main driving forces for the folding of globular proteins?
  
  
  
  
  
  
  
  
  
  
6. (10 points) Why is vitamin C an essential nutrient for humans? Describe the disease that results from a vitamin C deficiency. Describe the reaction(s) that require(s) vitamin C.
  
  
  
  
  
  
  
  
  
  
7. (20 points) Four diseases were presented in class. These diseases all involved the same protein, but because there are different types of the protein present in different tissues, different symptoms occur with each disease. Describe the disease that your group presented. Fully describe the protein that is involved and the cause, treatment and symptoms of the disease.