Here is a sample of problems from previous exams.

Problem 1. Consider a short peptide with the amino acid sequence GQEMHR.

(1) Write the amino acid sequence of the peptide in three-letter code for amino acids
(2) Draw the structure of the side chain (the R group) of the C-terminal residue of this peptide at neutral pH.
(3) Indicate the total charge (in the units of electron charge) of the predominant species in solution with the following pH values: 5.0, 7.0, 11.0, 14.0
(4) Determine the isoelectric point for this peptide.

Problem 2. Consider a hypothetic reaction A ←→ B + C.

Molar concentrations of the compounds at the beginning of the reaction that takes place at 25oC were [A]=0.1 M, [B]=0, [C]=0. Assume we can monitor the course of the reaction by measuring the concentration of B.

(1) Molar concentration of B at the equilibrium was $[B]_{eq}$= 40mM. From these data you have to calculate the standard change in free energy for the forward reaction.
(2) Ten minutes after the reaction started, the concentration of B was 20 mM. What is the ΔG of the reaction at this point? Is the forward reaction still favorable?

Problem 3. Cacodylic acid is a weak acid with Ka = 5.37 10^{-7} M. You prepared 500 mL of 0.2 M stock solution of this acid.

(1) Determine the pH of this solution. Show your assumptions and calculations.
(2) What is the pH range in which cacodylic acid can act as a good buffer?
(3) You want to adjust the pH of the stock solution prepared in question (1) so that it corresponds to the ideal buffering conditions for cacodylic acid. You have at your disposal only two reagents: 1M NaOH and 1M HCl. Which of the reagents (if any) could serve your purpose? Explain your reasoning.
(4) After you decided which reagent to use (see previous question), you start adjusting the pH. Calculate the volume of the reagent that you have to add to the stock solution (prepared in question (1)) in order to bring the pH to the optimal buffering conditions for cacodylic acid.

Problem 4. The molecular weight of an unspecified protein, at physiological conditions, is 70,000 Dalton, as determined by sedimentation equilibrium measurements and by gel filtration chromatography. The SDS-polyacrylamide gel electrophoresis (SDS PAGE) of the protein yields a single band corresponding to molecular weight of 70,000 Dalton. However, in the presence of

the reducing agent, β-mercaptoethanol, the SDS PAGE shows two bands, corresponding to molecular weights of 30,000 and 20,000 Dalton.

(a) From these data, describe the native protein in terms of the number of subunits present, their molecular weight, stoichiometry of subunits, and the kinds of bonding (covalent, noncovalent) existing between the subunits.

(b) You treated your protein with protease (e.g. trypsin), ran the product of the proteolytic cleavage on the SDS PAGE, and discovered that the SDS gel in the absence of β-mercaptoethanol still shows one band, but shows 4 bands in the presence of β-mercaptoethanol. How would you explain this result?
(c) How would your answers to problem (a) change if the molecular weight of the protein was 100,000 Dalton?

Problem 5. You have to determine the amino acid sequence of a peptide. You perform the following steps using enzyme cleavage of your peptide (see table below):

Step 1. Treatment with trypsin yields three fragments with the following sequences (in the order of their length): **WGA, AGTK, YLDR**
Step 2. Treatment with chymotrypsin gave the following three peptide fragments: **GA, LDRW, AGTKY**.

(a) What is the sequence of your peptide?
(b) Explain why step 1 alone was not sufficient to unambiguously determine the sequence of your peptide.

Assume you cannot perform step 2 as described above because your lab ran out of chymotrypsin supply. You are desperate to have the answer and you are looking for an alternative method to use in step 2. Consider the following possibilities:
(c) Can you use any of the other three cleavage agents listed in the table in order to unambiguously determine the sequence? Explain your answer.
(d) Would peptide hydrolysis with 6M HCl help you? Explain your answer.
(e) Would Sanger's reagent (1-fluoro-2,4-dinitrobenzene) or dansyl chloride help you? Explain your reasoning.

Treatment	Cleavage points	Cleavage location at
Trypsin	Lys, Arg	carboxyl side of these residues
Chymotrypsin	Phe, Tyr, Trp	carboxyl side of these residues
Asp-N protease	Asp, Glu	amino side of these residues
Pepsin	Phe, Tyr, Trp	amino side of these residues
Cyanogen bromide	Met	carboxyl side of this residue

Problem 6. Suppose you have a mixture of five proteins listed in the table below.

#	Protein	pI	Mol.Weight, kDa
A	Ovalbumin	4.6	45
B	Myoglobin	7	16.7
C	Serum albumin	4.9	68.5
D	Ubiquitin	6.4	8.5
E	Cytochrome c	10.6	13

(a) Indicate the order in which these proteins will elute from a gel-filtration column (starting with the one that elutes first). You can use letters A-E (see table) for simplicity.

(b) You load this mixture on a cation exchange column (i.e. column that bears negatively charged groups). The buffer you use for this column is acetate buffer, pH 4.76.
-- List proteins that will appear in the flow-through (i.e. will not bind to the column).
-- In order to elute those proteins that are immobilized on the column, you then apply a linear salt gradient, with NaCl concentration gradually increasing from 0 to 1 M. Indicate the order in which the proteins bound to the column will elute as the salt concentration increases.

(c) You repeat the same procedure as in (b) but now you use anion exchange column (that bears positively charged groups), and the buffer is TRIS (pH 8.0). Answer the same questions as in (b).

(d) You load this protein mixture and run it on the SDS PAGE. Predict the order they will migrate on the SDS gel, starting with the fastest.

(e) You are interested in further studies of cytochrome c. Based on the results of your experiments above, devise a reliable procedure for its purification from this mixture of proteins.