Some equations and formulae that might or might not be useful:

$\Delta G = \Delta H - T\Delta S$	$K_w = [H^+][OH^-] = 10^{-14}\ M^2$
$\Delta G = \Delta G^0 + RT\ lnQ$	$pH = -\log[H^+]$
$\Delta G = \Delta G^0 + RT\ ln(\{[products]\}/\{[reactants]\})$	$K_a = [H^+][A^-]/[HA]$
$K_{eq} = \{[products]\}_{eq}/\{[reactants]\}_{eq}$	$pH = pK_a + \log([A^-]/[HA])$
$\Delta G^0 = -RT\ ln(K_{eq})$	$[A^-]/[HA] = 10^{pH-pKa}$
$K_{eq} = exp(-\Delta G^0/RT)$	$pH = pK_a + \log[x/(c_0 - x)]$
$R = 8.31\ J/K/mol = 0.00831\ kJ/K/mol$	(c_0 is the initial molar concentration of a weak acid, and x denotes molar equivalents of the conjugate base)
$T(K) = t(^oC) + 273.16^o$	

Table 1: Peptide bond cleavage reagents

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 1. (15 points)
Consider a hypothetical reaction A + B $\rightleftarrows$ C which takes place at 25ºC. The initial molar concentrations of A and B were 30 mM and 50 mM, respectively, and there was no C. When the molar concentration of B dropped to 30 mM, the ΔG for the forward reaction was -2.0 kJ/mol. Determine the value of ΔG^0 and the equilibrium constant for this reaction.

Problem 2. (25 points) This problem deals with arginine.
A. For what pH values can arginine amino acid be used as an effective buffer?

B. What ***fraction*** of arginine has its guanidino group in the deprotonated form at pH=12?

C. You have a 200 mL sample of 50 mM solution of arginine at the pH value that equals the pKa of the arginine's guanidino group. You need to bring the pH of the sample to 12 (see previous question). You have at your disposal 1M HCl and 1M NaOH. Which of the two reagents and in what volume you need to add to your sample in order to achieve your goal? *Explain your reasoning and assumptions.*

Problem 3. (23 points) Consider a penta-peptide with the following amino acid sequence: **NIHRC**
A. Estimate the isoelectric point of this peptide. *Show your calculation and explain your reasoning.*
B. Draw the **complete structure** (including hydrogens) of the penta-peptide. Show the main forms of all ionizable groups at the isoelectric point.
C. You want to use ion-exchange chromatography to purify this peptide. The peptide is in Tris buffer at pH 7.2. What type of column do you need if you want the peptide to be retained on the column and not washed out in the flow-through? *Explain your reasoning.*

Problem 4. (23 points) Suppose you have a mixture of five proteins listed in the table below.

Protein	pI	Number of amino acids
A	10.6	250
B	9.6	700
C	7.5	100
D	4.5	620
E	3.5	80

1. Predict the order in which these proteins will migrate on the PAGE SDS gel, starting with the fastest.

2. Predict the order in which these proteins will elute from a gel-filtration (size-exclusion) column, starting with the fastest.

3. You loaded an aliquot (small portion) of this mixture on a cation column (that bears negatively charged groups). The proteins are in sodium acetate buffer at pH 5.0. List the proteins that will bind to the column.

4. You also loaded another aliquot of the mixture, this time on an anion column (that bears positively charged groups), and the buffer is HEPES (pH 7.5). List the proteins that will bind to the column.

5. Now it's time to do the separation. Devise a scheme that will allow you to unambiguously separate and purify all these proteins using a combination of the preparative chromatographic methods mentioned in the previous questions. Explain your reasoning. Remember, you goal is to separate the proteins unambiguously.

Problem 5. (14 points) In the course of amino-acid sequence determination of an unknown peptide enzymatic cleavage was combined with subsequent sequencing. You obtained the following results.

1. Chymotrypsin (see Table 1 on the equations sheet) cleaved the peptide into the following three fragments:

NLW
IAQF
KGEILRVY

2. Treatment with trypsin produced three penta-peptides.

A. Based on these data determine the correct amino acid sequence of the peptide. *Briefly explain how you arrived at your answer.*

B. Now that you *know* the sequence of the peptide, design/propose a reaction using only one of the cleavage reagents from Table 1 that will allow unambiguous determination of the peptide's sequence in a single step.