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

$\Delta G = \Delta H - T\Delta S$

$\Delta G = \Delta G'^{0} + RT\,lnQ$

$\Delta G = \Delta G'^{0} + RT\,ln\{[products]/[reactants]\}$

$K_{eq} = [products]_{eq} / [reactants]_{eq}$

$\Delta G'^{0} = - RT\,ln(K_{eq})$

$K_{eq} = exp(-\Delta G'^{0}/RT)$

$R = 8.31\ J/K/mol = 0.00831\ kJ/K/mol$

$T(K) = t(^{o}C) + 273.16^{o}$

$K_w = [H^+][OH^-] = 10^{-14}\ M^2$

$pH = -\log[H^+]$

$K_a = [H^+][A^-]/[HA]$

$pH = pK_a + \log([A^-]/[HA])$

$[A^-]/[HA] = 10^{pH-pKa}$

$pH = pK_a + \log[x/(c_0 - x)]$

(c_0 is the initial molar concentration of a weak acid, and x denotes molar equivalents of a base)

$M = [(m/z)_2 - 1]\,[(m/z)_1 - 1]/[(m/z)_2 - (m/z)_1]$

$n_2 = [(m/z)_1 - 1]/[(m/z)_2 - (m/z)_1]$; $n_1 = n_2 + 1$

← here $(m/z)_1$ and $(m/z)_2$ correspond to n_1 and n_2 protons absorbed on the molecule in ESI-MS; these equations are valid for $n_1 = n_2 + 1$, i.e. $(m/z)_1 < (m/z)_2$

A math refresher: solutions to quadratic equation $a x^2 + b x + c = 0$ are

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

Peptide 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 hypothetic reaction A $\rightleftarrows$ B that takes place at 25ºC. At the start of the reaction, the molar concentration of A was 120 mM. When the molar concentration of A dropped to 80 mM the ΔG for the forward reaction at this time point was -3 kJ/mol. Answer the following questions.

(A) Determine the equilibrium constant for the forward reaction.

(B) Calculate the final molar concentrations of A and B at equilibrium.

Problem 2. (20 points)

This problem deals with glutamate.

A. What ***fraction*** of glutamate has its side chain in the deprotonated form at pH=5? *Explain your reasoning and assumptions.*

B. You have a 250 mL sample of 20 mM solution of glutamate at the pH value that equals the pKa of the glutamate's side chain. You need to bring the pH of the sample to 5 (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. (20 points)

(A) Draw the **complete structure** of the following pentapeptide. Show the main forms of the ionizable groups as they would be at pH=5.

HKEWR

(B) Estimate the isoelectric point of this peptide. *Show your calculation and explain your reasoning.*

Problem 4. (25 points) Suppose you have a mixture of five proteins listed in the table below.

Protein	pI	Number of amino acids
A	6.6	77
B	4.3	120
C	10.1	200
D	7.8	500
E	4.8	600

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

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

(C) You loaded an aliquot (small portion) of this mixture on a cation column (i.e. column that bears negatively charged groups). The buffer you used for this was sodium acetate buffer at pH 4.8. List the proteins that came out in the flow-through, i.e. did not bind to the column.

(D) In order to elute the proteins immobilized on the cation column (in question C) you apply a salt gradient, with NaCl concentration gradually increasing from 0 to 1M. Predict the order in which the proteins will elute as the salt concentration increases.

(E) You also loaded another aliquot of the mixture, this time on an anion column (i.e. column that bears positively charged groups), and the buffer is TRIS (pH 7.0). List the proteins that will bind to the column.

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

Problem 5. (10 points)

In the course of amino-acid sequence determination of a peptide enzymatic cleavage was combined with subsequent sequencing. You obtained the following results. Trypsin cleaved the peptide into the following three fragments:

ENLGF
DAWQR
QIACNK

Asp-N protease cleaved the peptide into the same three fragments.
From these data determine the correct sequence of the peptide. *Briefly explain how you arrived at your answer.*

Problem 6. (10 points)

You need to determine how many subunits are in a 40-kDa homo-multimeric protein (consisting of several identical subunits). For this you decided to use Fred Sanger's method for identification of the N-terminal amino acid. A 200 mg sample of the protein was treated with an excess of 1-fluoro-2,4-dinitrobenzene (Sanger's reagent) under slightly basic conditions until the chemical reaction was complete, and the peptide bonds were then completely hydrolyzed by boiling with concentrated HCl. The resulting hydrolysate contained 8.91 mg of the Leucine derivative of 2,4-dinitrobenzene (Leu-DNP; MW = 297). No 2,4-dinitrophenyl derivatives of α–amino groups of other amino-acids could be found.

Based on these data, how many subunits are there in the structure of this protein? *Show your calculations and explain how you arrived at your answer.*