My answers to the exam problems are on the next pages.

I strongly recommend that you try doing these problems yourself BEFORE looking at my solutions.

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.

We don't know yet the concentrations at equilibrium, but we can determine the equilibrium constant through ΔG^0. Concentrations at that time point: [A] = 0.08 M, [B] = 0.12M - 0.08M = 0.04 M. Since we know ΔG at this point, we can calculate $\Delta G^0 = \Delta G - RT \times \ln([B]/[A])$ = - 1.283 kJ/mol; and from here we get $K_{eq} = \exp(-\Delta G^0/RT) = 1.6787$

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

The equilibrium concentrations of A and B obey the mass conservation law $[A]_{eq} + [B]_{eq} = 120$ mM = 0.12 M (the initial concentration of A); therefore if we can determine (say) $[A]_{eq}$, we automatically know $[B]_{eq} = 0.12M - [A]_{eq}$. Using the equation that relates the equilibrium constant to the ratio of concentrations at equilibrium, $K_{eq} = (0.12M - [A]_{eq})/[A]_{eq} = 1.6787$; and solving it for $[A]_{eq}$ we get $[A]_{eq} \times (1+1.6787) = 0.12M \rightarrow [A]_{eq} = 0.0448$ M = 44.8 mM; and $[B]_{eq} = 75.2$ mM.

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.*

Using the Henderson-Hasselbalch equation, we get $[E^-]/[E] = 10^{pH-pKa}$, where E designates Glu with the protonated side chain carboxylic group and E^- designates Glu with deprotonated side chain, and pKa = 4.25. At pH=5, this ratio (let's call it *r*) equals $r = [E^-]/[E] = 10^{-0.75} = 5.6234$. The fraction of E molecules with deprotonated side chain is $[E^-]/([E]+[E^-]) = r/(1+r) = 5.6234/(1+5.6234) = 0.849 \approx 0.85$.

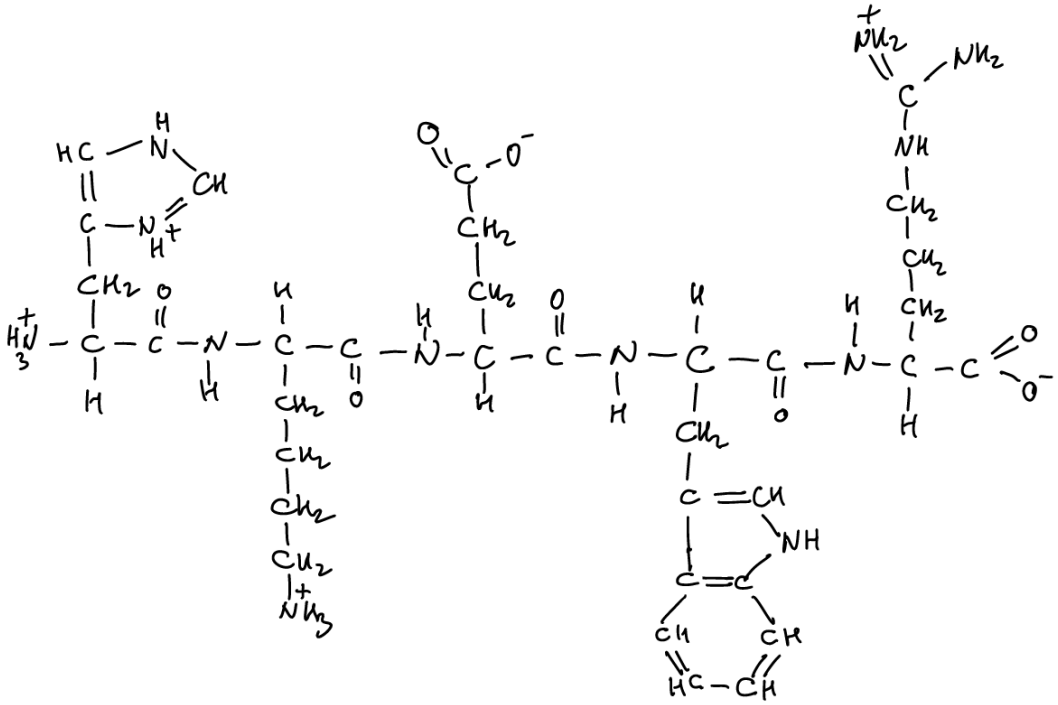
B. You have a 250 mL sample of 20 mM solution of glutamate at the pH value that equals the pKa of the glutamate's side chain. You need to bring the pH of the sample to 5 (see previous question). You have at your disposal 1M HCl and 1M NaOH. Which of the two reagents and in what volume you need to add to your sample in order to achieve your goal? *Explain your reasoning and assumptions.*

250 mL of 20 mM solution of glutamate contain 0.25 L × 20 mM = 5 mmol of Glu. At the pH = pKa = 4.25, this amount is split equally into 2.5 mmol of base (deprotonated Glu, E^-) and 2.5 mmol of acid (protonated Glu). To increase the pH from 4.25 to 5.0 we need to add a base, hence NaOH.
Based on the answer to the previous question, at pH 5 we will have 0.15 × 5 mmol = 0.75 mmol of protonated Glu (acid, E) and 4.25 mmol of deprotonated Glu (base, E^-). Thus to go from 2.5 mmol of E^- to 4.25 mmol we need to add 4.25 - 2.5 = 1.75 mmol of NaOH, which corresponds to 1.75 mL of 1M NaOH.
Note that because this added volume is much smaller than 250 mL, this calculation ignores an increase in the total volume (from 200 mL to 201.75 mL).

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.*

This peptide contains 6 titratable groups: the N-terminal amine (pK_2=9.7), the imidazole group of histidine (pK_H=6), the ε-amine (side chain) of lysine (pK_K=10.53), the side chain carboxyl group of glutamate (pK_E = 4.25), the guanidine group of arginine (pK_R=12.48), and the C-terminal carboxyl (pK_1=2.3). The table below considers charge states of each of these groups at different pH in order to determine the pKa's responsible for the formation of the zwitterionic state.

pH	NH_2	H	K	E	R	COOH	Net charge of the predominant species
1	+	+	+	0	+	0	+4
3	+	+	+	0	+	-	+3
5	+	+	+	-	+	-	+2
9	+	0	+	-	+	-	+1
10	0	0	+	-	+	-	0 (zw)
11	0	0	0	-	+	-	-1

The isoelectric point can then be estimated as $pI = (pK_2+pK_K)/2 = (9.7+10.53)/2 = 10.115$.

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.

Smaller in length proteins usually migrate faster. The order will be: **A, B, C, D, E.**

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

The bigger the protein size is, the faster it will move through the size-exclusion column, hence the earlier it will elute. The order will be opposite to that for SDS PAGE: **E, D, C, B, A.**

(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.

At this pH proteins A, C, and D will be positively charged, and B will be negatively charged, and E will be neutral. The former three proteins will bind to the column, so the answer is: **B, E.**

(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.

The smaller the charge of a protein at that pH the weaker is its interaction/attraction to the oppositely charged beads. Thus proteins having their pI closer to the pH will elute earlier. The order is expected to be: **A, D,** and then **C.**

(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.

At this pH proteins **A**, **B**, and **E** will be negatively charged, and therefore 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.

There are several scenarios. Most important: take advantage of the differences in the size and the pI values. What you want to avoid is trying to separate proteins that are close in size (e.g. 77 a.a from 120 a.a, 120 a.a from 200 a.a., or 500 a.a from 600 a.a.) using size exclusion chromatography or those that are close in their pI values (e.g. 4.3 from 4.8) by ion-exchange chromatography.
For example, using your answers to the above questions you can

(1) first separate A, C, and D from the rest of the proteins using a cation-exchange column and a buffer with pH 5 or slightly higher;
(2) then take the flow-through (which contains B and E) and separate these proteins from each other using size-exclusion chromatography: you should be able to easily separate 120 a.a. protein from 600 a.a. protein;
(3) then elute proteins A, C and D from the cation-exchange column by adding high-salt buffer and separate them using size-exclusion chromatography (77 a.a. from 200 a.a. from 500 a.a.), or even separate them by the time when they elute from the cation-exchange column when using a shallow salt gradient.

Or in the first step you can use an anion-exchange column with pH 6 or a bit lower to separate A, C, and D (now in the flow-through) from B and E (which will stick to the column), and then follow steps 2 and 3.

Or you can first use size-exclusion chromatography to separate D+E from A+B+C, and then use the cation or anion exchange column to separate D from E. Separating A from B from C could require more than one ion-exchange column run or a shallow salt gradient.

Or some other scenario… as long as it makes sense.

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.*
The sequence of the peptide is **QIACNK DAWQR ENLGF**

You realize that because trypsin cleaves after R and K, the peptide ending with F must be the C-terminal fragment. Also, because Asp-N cleaves before D and E, the peptide that starts with a Q must be the N-terminal fragment. This leaves the peptide that starts with D and ends with R to be the middle peptide.

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 (DNP-Leu; MW = 297 Da). 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.*

Solving this problem requires counting the number of N-termini per one molecule of the protein.
The number of moles of the protein in this reaction is N_P= 200 mg/40000 g = 5×10^{-6} moles.
The number of moles of DNP-Leu obtained is $N_{DNP\text{-}Leu}$ = 8.91 mg/297 g = 3×10^{-5} moles.
The ratio of the two gives: $N_{DNP\text{-}Leu}/N_P = 6$.
Thus 6 molecules of DNP-Leu were found per molecule of the protein – this indicates that there are 6 N-termini, i.e. 6 polypeptide chains, hence the protein consists of 6 subunits. It must be a hexamer.