Consider a hexapeptide with the following sequence of amino acids:

The N-terminal residue contains an aromatic ring that can get phosphorylated.
The next residue has the shortest side chain containing 2 methyl groups.
The next residue's side chain is readily oxidized to form covalent bonds.
The next residue has the longest side chain that is negatively charged at neutral pH.
The next residue, when repeated 6 or more times one after another, is used as a tag in affinity chromatography.
Lastly, the C-terminal residue has a nonpolar aliphatic side chain containing only methylene groups.

Answer the following questions. Use the pKa values for side chains (R groups) from Table 3-1 in the Lehninger textbook or from the lecture slides. You can assume the "common" values for the terminal carboxyl and amino groups: pK_1=2.3 and pK_2=9.7, respectively.

(A) Write the amino-acid sequence of this peptide in 3-letter and 1-letter abbreviations.

Tyr-Val- Cys-Glu-His-Pro or YVCEHP

(B) What is the net charge on the peptide at pH = 13?

The pH is above all the relevant pKa values for this peptide, so all ionizable groups are deprotonated. In the deprotonated state only the carboxy-terminus and the side chains of Cys, Tyr, and Asp are charged, which gives the total charge = - 4 (see also the pI calculation table below)

(C) What is the net charge on the peptide at pH = 6?

At pH=6 equals the pKa of His side chain, thus His side chain is half-protonated (i.e. its net charge is 0.5). For all other ionizable groups the pH differs from their pKa values by ~2 units or more, thus these groups are either almost fully protonated (amino-terminus has the charge of +1, Cys and Tyr side chains have zero charge) or almost fully deprotonated (the carboxyl-terminus and the side chain* of Glu have the charge of -1 each). Thus, the total net charge of the peptide is 1.5 – 2 = – 0.5.

Note that the difference between the pH and the pKa of Glu side chain is 1.75, i.e. slightly less than 2, so the Glu side chain is slightly protonated, but the ratio of $[Glu^-]/[Glu] = 10^{1.75} = 56.2$ is much greater than 1, such that the charge of Glu is (– 1)(56.23/57.23) = – 0.98, i.e. almost – 1.

(D) Estimate the isoelectric point of this peptide. *Show the equation you used.*

pI = (pK_{Glu}+pK_{His})/2 = 5.125 (see the pI calculation schematics on the next page).

(E) What fraction of the peptide molecules is in the zwitterionic state at the isoelectric point? *Show the equation you used.*

The fraction is ≈ 0.79 (see my derivation of the equations on the last page).

$$fraction = \frac{[zw]}{[total]} = \frac{1}{1+2/10^{pI-pK_{Glu}}} = \frac{1}{1+2\times 10^{pI-pK_{His}}} = \frac{1}{1+2\times 10^{(pK_{Glu}-pK_{His})/2}} =$$

$$\frac{1}{1+1/10^{pI-pK_{Glu}}+10^{pI-pK_{His}}} \approx 0.7895$$

(F) You want to use ion-exchange chromatography to purify this peptide by retaining it on the column. Do you need a cation or anion column for this if the peptide is in phosphate buffer at pH=6.8? *Explain your reasoning*.

Because the pH > pI, the peptide has a net negative charge. Thus, it will be retained on an **anion** chromatography column.

The charge states of the hexapeptide:

NH_3^+-Tyr-Val-Cys-Glu-His-Pro-COO^-

pK_2=9.7 pK_{Tyr}=10.07 pK_{Cys}=8.18 pK_{Glu}=4.25 pK_{His}=6.0 pK_1=2.3

pK_a	pH	NH_3^+	Tyr	Cys	Glu	His	COO^-	Total Charge	
	1	+	0	0	0	+	0	+2	
2.3	3	+	0	0	0	+	-	+1	
4.25	5	+	0	0	-	+	-	0	← zw
6.0	7	+	0	0	-	0	-	-1	
8.18	9	+	0	-	-	0	-	-2	
9.7	9.9	0	0	-	-	0	-	-3	
10.07	12	0	-	-	-	0	-	-4	

pK_{Glu} pK_{His}

$$pI = (pK_{Glu} + pK_{His})/2 = 5.125$$

[+2] + [+1] + [zw] + [-1] + [-2] + [-3] +[-4] +[0]= [total]

$$\frac{[+1]}{[+2]} = 10^{pI-pK_1} \Longrightarrow [+2] = [+1]/10^{pI-pK_1} = [+1]\times 10^{-2.825} \ll [+1]$$

negligible

$$\frac{[zw]}{[+1]} = 10^{pI-pK_{Glu}} \Longrightarrow [+1] = [zw]/10^{pI-pK_{Glu}}$$

$$\frac{[-1]}{[zw]} = 10^{pI-pK_{His}} \Longrightarrow [-1] = [zw]\times 10^{pI-pK_{His}}$$

$$\frac{[-2]}{[-1]} = 10^{pI-pK_{Cys}} \Longrightarrow [-2] = [-1]\times 10^{pI-pK_{Cys}} = [-1]\times 10^{-3.055} \ll [-1]$$

negligible

$$\frac{[-3]}{[-2]} = 10^{pI-pK_{NH3}} \Longrightarrow [-3] = [-2]\times 10^{pI-pK_{NH3}} = [-2]\times 10^{-4.575} \ll [-2]$$

negligible

$$\frac{[-4]}{[-3]} = 10^{pI-pK_{Tyr}} \Longrightarrow [-4] = [-3]\times 10^{pI-pK_{Tyr}} = [-3]\times 10^{-4.945} \ll [-3]$$

negligible

Hence, we can reduce the number of contributing states: [+1] + [zw] + [-1] ≈ [total]

$$\Longrightarrow [zw](1+1/10^{pI-pK_{Glu}} + 1\times 10^{pI-pK_{His}}) = [total]$$

$$fraction = \frac{[zw]}{[total]} = \frac{1}{1+1/10^{pI-pK_{Glu}} + 1\times 10^{pI-pK_{His}}} = \frac{1}{1+2/10^{pI-pK_{Glu}}} \approx 0.7895 \approx 0.79$$