**Bonus Problem.** (total 10 points). The amino acid sequence of a hypothetic protein contains 10 histidines and 10 aspartic acids, the rest of amino acids have either nonpolar or polar uncharged R-groups. Assuming that all $pK_a$ values are the same as for free amino acids, calculate the average charge of the protein molecule at the pH values indicated below. Explain your reasoning.

(a) at pH = 6.0 .

This pH corresponds to His pKa; therefore, 50% of His R groups are protonated. Their total charge is then +5. All Asp's are deprotonated: their total charge is −10. The total charge of a protein is then:

$$\underbrace{(+5)}_{\text{His's}} + \underbrace{(-10)}_{\text{Asp's}} + \underbrace{(+1)}_{\text{N-term}} + \underbrace{(-1)}_{\text{C-term}} = -5.$$

(b) at pH = 4.85 From Hend.-Hasselb. equation: $\frac{[A^-]}{[HA]} = 10^{pH-pKa}$.

For His: the ratio of deprotonated to protonated R groups is: $\frac{[His]}{[His^+]} = 10^{4.85-6.0} = 10^{-1.15}$. The total charge of all His's is then

$$Z_{His} = (+10) \times \frac{[His^+]}{[His] + [His^+]} = 10 \times \frac{1}{10^{-1.15}+1}$$

For Asp: $\frac{[Asp^-]}{[Asp]} = 10^{4.85-3.7} = 10^{1.15}$, and the total charge

$$Z_{Asp} = (-10) \times \frac{[Asp^-]}{[Asp^-]+[Asp]} = (-10)\left(1 - \frac{[Asp]}{[Asp^-]+[Asp]}\right) = (-10)\left(1 - \frac{1}{1+10^{1.15}}\right)$$

$$= (-10)\frac{1}{10^{-1.15}+1}.$$

So, $Z_{His} = -Z_{Asp}$, and since the N- and C-termini have charges +1 and −1, the total charge = 0.

Another consideration: this $pH = pI = \frac{pK_R(Asp) + pK_R(His)}{2}$.

Therefore, the number of positively charged His's = number of negatively charged Asp's;