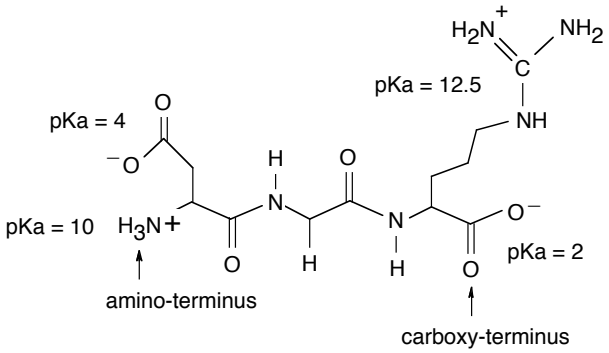
Biochemistry – Problem Set 2

Problem Set 2 solution key **36 points total**

1. **(2 pts)** Fig. 3.3 shows the non-ionized forms of the side chains of Aspartic (pK_a~3.9) and Glutamic Acid (pK_a~4.2). At pH 7, both of these side chains should be
   de-protonated. Only at **pH~3** would a fraction of either of these amino acids be found with their α-COOH (pK_a~2) de-protonated and the side-chain COOH (pK_a~4) protonated. **(2 pts)**
2. **(10 pts)** The structure of Asp-Gly-Arg is drawn below. **(5 pts for structure, 2 pts for correct assignment of amino and carboxy terminus, 2 pts for correct assignment of all charges)**



Group		Protonation state	Contribution to charge (q)
Amino-terminus	pH<pKa by at least 2 pH units	Fully protonated	+1
Carboxy-terminus	pH>pKa by at least 2 pH units	Fully deprotonated	-1
Aspartic acid side chain	pH>pKa by at least 2 pH units	Fully deprotonated	-1
Arginine side chain	pH<pKa by at least 2 pH units	Fully protonated	+1

   The net charge is obtained by summing the contribution to the charge from each group. Thus **q = 0**. **(1 pt)**

3. **(6 pts)** (a) Calculate the fraction of the His side-chains protonated at pH 6.0, 6.5 and 7.0. **(1 pt each)**

   $pH = pKa + \log\frac{[A^-]}{[HA]}$ and $R = \frac{[A^-]}{[HA]}$

   At pH 6.0, $R = 10^{(pH-pKa)} = 10^0 = 1$. Therefore $f_{HA} = 1/(1+R) =$ **0.5**
   At pH 6.5, $R = 10^{0.5} = 3.16$. Therefore $f_{HA} =$ **0.24**
   At pH 7.0, $R = 10^1 = 10$. Therefore $f_{HA} =$ **0.09**
   (b) At pH 7.0, 91% of the His side-chains are deprotonated. **Thus the enzyme is likely to be very active**. **(3 pts)**

4. **(5 pts)** Determine the sequence of the 10 residue peptide. **(2 pts if sequence is correct, full credit for explanation).**
What you want to look for are overlapping sequences in the fragments that were generated by different cleavages.

For example the CNBr cleavage fragment [Gly-Phe-Leu-Lys] overlaps the Chymotrypsin fragment [Leu-Lys-Val-His], suggesting the partial sequence: **[Gly-Phe-Leu-Lys-Val-His]**.

Similarly, the Trypsin fragment [Met-Gly-Phe-Leu] overlaps the CNBr fragment [Gly-Phe-Leu-Lys], suggesting the partial sequence: **[Met-Gly-Phe-Leu-Lys-Val-His]**.

Finally, the Trypsin fragment [Val-His-Met-Cys] overlaps both the Chymotrypsin fragment [Leu-Lys-Val-His] and the CNBr fragment [Cys-Ala], suggesting the complete 10 residue sequence:

Met-Gly-Phe-Leu-Lys-Val-His-Met-Cys-Ala

This sequence is consistent with the original data presented in the problem:

CNBr cleavage:	**Gly-Phe-Leu-Lys**-Val-His-Met **+ Cys-Ala**
Chymotrypsin digestion:	**Met-Gly-Phe + Leu-Lys-Val-His**-Met-Cys-Ala
Trypsin digestion:	**Met-Gly-Phe-Leu**-Lys **+ Val-His-Met-Cys**

5. **(9 pts)** (a) The length of the proteasome is approximately **160 (± 15 A°) long and 150 (± 20 A°). (3 pts if answer is in the ballpark)**
(b) Chain S has **1 Trp and 4 Tyr** residues. **(Give full credit if only 3 Tyr were counted) (3 pts)**
(C) Since the absorbance of the three chromophores is additive, the extinction coefficient is:

$$\varepsilon = 1 \times 5{,}050\, M^{-1}cm^{-1} + 4 \times 1{,}440\, M^{-1}cm^{-1} = 10{,}810\, M^{-1}cm^{-1}$$

(The Phe residues do not contribute significantly to the absorbance because the λ_{MAX} of Phe is significantly lower than 280 nm).
The protein concentration is obtained using Beer's Law

$$[X] = A/\varepsilon = 0.8/10{,}810\, M^{-1}cm^{-1}(1cm) = 7.4 \times 10^{-5}\, M = 74\,\mu M$$

(3 pts) (Again, give full credit if only 3 Tyr were counted and they got 85 μM).

6. **(4 pts)**
   i. The side-chains of an α-helix point **outward** from the main-chain. **(1 pt)**

ii. The hydrophobic face is more likely to face the **interior** of the protein because it would not interact favorably with water. **(1 pt)**
iii. The hydrogen bonds in a β-sheet are **perpendicular** to the β-strands. **(1 pt)**
iv. The side-chains in a β-sheet point **are perpendicular to** the plane of the sheet. **(1 pt)**