1. (2 pts) Fig. 3.3 shows the non-ionized forms of the side chains of Aspartic (pKa≈3.9) and Glutamic Acid (pKa≈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 \(-\text{COOH}\) (pKa≈2) de-protonated and the side-chain COOH (pKa≈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)

<table>
<thead>
<tr>
<th>Group</th>
<th>Protonation state</th>
<th>Contribution to charge (q)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino-terminus</td>
<td>pH&lt;pKa by at least 2 pH units</td>
<td>Fully protonated +1</td>
</tr>
<tr>
<td>Carboxy-terminus</td>
<td>pH&gt;pKa by at least 2 pH units</td>
<td>Fully deprotonated -1</td>
</tr>
<tr>
<td>Aspartic acid side</td>
<td>pH&gt;pKa by at least 2 pH units</td>
<td>Fully deprotonated -1</td>
</tr>
<tr>
<td>Arginine side chain</td>
<td>pH&lt;pKa by at least 2 pH units</td>
<td>Fully protonated +1</td>
</tr>
</tbody>
</table>

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)

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

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 Å°) long and 150 (± 20 Å°). (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:

   \[
   \varepsilon = 1 \times 5.050 \text{M}^{-1} \text{cm}^{-1} + 4 \times 1.440 \text{M}^{-1} \text{cm}^{-1} = 10.810 \text{M}^{-1} \text{cm}^{-1}
   \]

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

   \[
   [X] = \frac{A}{\varepsilon} = \frac{0.8}{10.810 \text{M}^{-1} \text{cm}^{-1}} \text{ (1cm)} = 7.4 \times 10^{-5} \text{ M} = 74 \mu\text{M}
   \]

   (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 \( \alpha \)-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 $\beta$-sheet are **perpendicular** to the $\beta$-strands. (1 pt)

iv. The side-chains in a $\beta$-sheet point **perpendicular** to the plane of the sheet. (1 pt)