1. Protein Composition and Structure

- Amino acids and peptides.
- Peptide titrations
- Secondary and tertiary structures.

### Table of pKa Values to know

<table>
<thead>
<tr>
<th>Chemical Group</th>
<th>pKa Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-CO₂ in peptide</td>
<td>3.5</td>
</tr>
<tr>
<td>Sidechain CO₂ (Glu and Asp)</td>
<td>4.5</td>
</tr>
<tr>
<td>Imidazole (Histidine)</td>
<td>6.5 (HIGHLY VARIABLE)</td>
</tr>
<tr>
<td>Sulfhydryl (-SH in Cysteine)</td>
<td>8.5</td>
</tr>
<tr>
<td>Phenol (tyrosine)</td>
<td>10.0</td>
</tr>
<tr>
<td>α-amino group</td>
<td>8.5</td>
</tr>
<tr>
<td>Sidechain amino (lysine)</td>
<td>10.0</td>
</tr>
<tr>
<td>Guanidinyl (arginine)</td>
<td>12</td>
</tr>
</tbody>
</table>
B. amino acid titrations

Can we predict titration curves for amino acids and peptides?

1. Glycine

\[ \text{H}_2\text{N} \text{−} \text{CH} \text{−} \text{CO}_2\text{H} \] at low pH (pH = 1) all glycine have protonation state on far left.

\[ \text{H}_2\text{N} \text{−} \text{CH} \text{−} \text{CO}_2\text{H} \quad \text{pK}_a = 3.5 \]

\[ \text{H}_2\text{N} \text{−} \text{CH} \text{−} \text{CO}_2\text{−} + \text{H}^+ \]

Use H+ eqn to calculate ratio \[ \frac{\text{A}^-}{\text{HA}} \]

\[ \text{pH} = 3 \; \text{or} \; \frac{\text{A}^-}{\text{HA}} = 10^{\frac{3 - 3.5}{10}} = 0.61 \]
\[ pH = 3.5; \quad \frac{A^-}{HA} = 10^{3.5-3.5} = 10^{0} = 1 \]

\[ pH = 4; \quad \frac{A^-}{HA} = 3.2 \]

\[ pH = 5.5; \quad \frac{A^-}{HA} = 100 \]

do the other part.
b. glutamate. Glu E.

Calc pH where net charge is zero. Isoelectric point, pI.

\[ pI = \frac{pK_{a1} + pK_{a2}}{2} = \frac{3.5 + 8.5}{2} = 6 \]

Ionic groups:
- \( \text{R}^+\text{NH}_3^- \): 1
- CO₂⁻: 2
- CO₂H: 1

\[ p\text{I} = \frac{pK_{a1} + pK_{a2}}{2} = \frac{3.5 + 4.5}{2} = 4 \]
2. Peptides (proteins) are polymers of amino acids. Amino acids link together via peptide bonds.

\[
\begin{align*}
\text{N-terminus} & \quad \text{N} - \text{C} - \text{CO} - \text{N} - \text{C} - \text{CO} \\
\text{C-terminus} & \quad \text{R}_1 \quad \text{R}_2 \quad \text{R}_3 \quad \text{R}_4 \\
\end{align*}
\]

Notes:
1. an N-terminus and a C-terminus.
   - N term. amino pK\(_a\) \approx 8.5
   - C-term carboxy pK\(_a\) \approx 3.5

2. Peptide backbone

peptide side chains; R groups
3. Fixed $\alpha$ stereochemistry means that side chains are inverted on adjacent amino acids.

4. Peptide bond has new properties. Of any amide bond:

a. Partial double bond character:

![Partial double bond structure]

Think of as delocalized $\pi$ bond.

Partial double bond character means:
all 6 atoms in peptide bond are planar.

peptide linkages are planar.

partial double bond means hindered rotation.

There is some rotation, but less than if single bond, and more than if double bond.
φ angle involves rotation about N-Cα bond

ψ angle involves rotation about Cα-CO bond.

Partial double bond means cis or trans geometry about peptide bond.

trans conformation
stable, common.

cis conformation
steric hindrance
due to R groups.
unstable, rare.
peptide titrations.

1. Sequence is Ala-Glu-Val-Cys-Gly

\[ \overset{\text{N}}{\text{H}}_{3} \text{AEVCG} - \text{CO}_{2}^{-} \]

draw titration curve for this peptide.

Identify ionizable groups

<table>
<thead>
<tr>
<th></th>
<th>pK_a</th>
<th>pH</th>
<th>( z ) at pH=1</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-term</td>
<td>8.5</td>
<td>8.5</td>
<td>+1</td>
</tr>
<tr>
<td>Glu</td>
<td>4.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cys</td>
<td>8.5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\[ pI = \frac{3.5 + 4.5}{2} = 4 \]

\( pI = 4 \)
Example 2: CHEMISTRY

algorithm.

1. Identify ionizable groups
   list pKa
   charge at pH = 1
   total charge at pH = 1

<table>
<thead>
<tr>
<th>N-term</th>
<th>pKa</th>
<th>charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cys</td>
<td>8.5</td>
<td>+1</td>
</tr>
<tr>
<td>His</td>
<td>6.5</td>
<td>0</td>
</tr>
<tr>
<td>Glu</td>
<td>4.5</td>
<td>-1</td>
</tr>
<tr>
<td>Arg</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Tyr</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>C-term</td>
<td>8.5</td>
<td>-1</td>
</tr>
</tbody>
</table>

if titrating N
   if pH < pKa
     z = +1
     else
     z = 0
else if titrating C-term
   if pH < pKa
     z = 0
     else
     z = -1
2. sketch plot.
   a. how many eq -OH?
   \[ \text{eq. } \text{OH} = \text{water grops.} \]
   \[ pH = 7.5 \]
   b. label axes.
   c. plot pH vs pOH
   d. connect dots
   e. plot changes

3. calc. \( pH = \frac{6.5 + 8.5}{2} = 7.5 \)
- free rotation about \( \psi, \phi \) angles, but some conformations are more stable than others.
- look at Ramachandran Plot.

2 regions of high stability:
- very common in protein structures.
- have specific names.

a. \( \alpha \)-helix: comb. of \( \psi, \phi \) angles makes backbone look like phone cord.
Properties of α-helices:

a. Stabilized by H-bonds.
   - all C=O point in 1 direction
   - all N-H point in opposite

H-bond pattern

C=O on res. i
H bond with N-H on res. i+4

b. R groups are on outside.
   no space on inside of helix
   - look like rollers in car wash.
   - draw helix in helical wheel
   - connect Ca atoms & show sidechains
can have different "faces" of helix
EAARKLAD

polar face

by hydrophobic face

amphipathic helix
The α-helix

Amino terminus

Carboxyl terminus

(b)