01. Amino Acids
Biomolecules

- Protein
- Carbohydrate
- Nucleic acid
- Lipid
peptide $\rightarrow$ polypeptide $\rightarrow$ protein

di-, tri-, oligo-
Table 22.1  Examples of the Many Functions of Proteins in Biological Systems

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural proteins</td>
<td>These proteins impart strength to biological structures or protect organisms from their environment. For example, collagen is the major component of bones, muscles, and tendons; keratin is the major component of hair, hooves, feathers, fur, and the outer layer of skin.</td>
</tr>
<tr>
<td>Protective proteins</td>
<td>Snake venoms and plant toxins protect their owners from predators. Blood-clotting proteins protect the vascular system when it is injured. Antibodies and peptide antibiotics protect us from disease.</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Enzymes are proteins that catalyze the reactions that occur in living systems.</td>
</tr>
<tr>
<td>Hormones</td>
<td>Some of the hormones, such as insulin, that regulate the reactions that occur in living systems are proteins.</td>
</tr>
<tr>
<td>Proteins with physiological functions</td>
<td>These proteins are responsible for physiological functions such as the transport and storage of oxygen in the body, the storage of oxygen in the muscles, and the contraction of muscles.</td>
</tr>
</tbody>
</table>
proteins

fibrous proteins

globular proteins
Figure 4.1 Anatomy of an amino acid. Except for proline and its derivatives, all of the amino acids commonly found in proteins possess this type of structure.
Glycine (Gly, G)  Alanine (Ala, A)  Valine (Val, V)*  
Leucine (Leu, L)*  Isoleucine (Ile, I)*
Serine (Ser, S)  Threonine (Thr, T)*  Cysteine (Cys, C)  cystine  Methionine (Met, M)*
Aspartate (Asp, D)

Glutamate (Glu, E)

Asparagine (Asn, N)

Glutamine (Gln, Q)
Lysine (Lys, K)*

Arginine (Arg, R)*
Phenylalanine (Phe, F)*

Tyrosine (Tyr, Y)

Histidine (His, H)*

Tryptophan (Trp, W)*
Proline (Pro, P)
Hydrophobic (A, G, I, L, F, V, P)

Hydrophilic (D, E, R, S, T, C, N, Q, H)

Amphipathic (K, M, W, Y)
Several Amino Acids Occur Rarely in Proteins

We'll see some of these in later chapters

- **Selenocysteine** in many organisms
- **Pyrrolysine** in several archaeal species
- **Hydroxylysine**, hydroxyproline - collagen
- **Carboxyglutamate** - blood-clotting proteins
- **Pyroglutamate** – in bacteriorhodopsin
- **GABA**, epinephrine, histamine, serotonin act as neurotransmitters and hormones
- **Phosphorylated** amino acids – a signaling device
Several Amino Acids Occur Rarely in Proteins

(a)

\[
\begin{align*}
\text{H}_3\text{N}^+ & - \text{C} - \text{H} \\
\text{CH}_2 & \\
\text{SeH} & \\
\text{COOH} & \\
\end{align*}
\]

\text{Selenocysteine}

\[
\begin{align*}
\text{H}_3\text{N}^+ & - \text{C} - \text{H} \\
\text{CH}_2 & \\
\text{CH}_2 & \\
\text{CH}_2 & \\
\text{HN} & - \text{C} - \text{N} \\
\text{N} & - \text{O} \\
\text{CH}_3 & \\
\end{align*}
\]

\text{Pyrrolysine}
Several Amino Acids Occur Rarely in Proteins

Figure 4.4 (b) Some amino acids are less common, but nevertheless found in certain proteins. Hydroxylysine and hydroxyproline are found in connective-tissue proteins; carboxyglutamate is found in blood-clotting proteins; pyroglutamate is found in bacteriorhodopsin (see Chapter 9).
Several Amino Acids Occur Rarely in Proteins

Figure 4.4 (c) Several amino acids that act as neurotransmitters and hormones.
Stereochemistry of Amino Acids

Perspective drawing

Fischer projections
\[ \text{D-glyceraldehyde} \quad \text{L-glyceraldehyde} \]

\[ \text{D-amino acid} \quad \text{L-amino acid} \]
Why do L-amino acids predominate in biological systems? What process might have selected L-amino acids over their D-counterparts? The meteorite found near Murchison, Australia may provide answers. Certain amino acids found in the meteorite have been found to have L-enantiomeric excesses of 2% to 9%.
Which is \((2S,3R)\)-threonine?
4.2 What Are Acid-Base Properties of Amino Acids?

- Amino Acids are Weak Polyprotic Acids
- The degree of dissociation depends on the pH of the medium

\[ \text{H}_2\text{A}^+ + \text{H}_2\text{O} \rightarrow \text{HA}^0 + \text{H}_3\text{O}^+ \]

\[ \frac{[\text{HA}^0][\text{H}_3\text{O}^+]}{[\text{H}_2\text{A}^+]} = K_{a1} \]
The second dissociation (the amino group in the case of glycine):

- \( \text{HA}^0 + \text{H}_2\text{O} \rightarrow \text{A}^- + \text{H}_3\text{O}^+ \)

\[
K_{a2} = \frac{[\text{A}^-][\text{H}_3\text{O}^+]}{[\text{HA}^0]}
\]
4.2 What Are Acid-Base Properties of Amino Acids?

Figure 4.5 The ionic forms of the amino acids, shown without consideration of any ionizations on the side chain.
The adjacent $\alpha$-amino group makes the $\alpha$-COOH group more acidic.
4.2 What Are Acid-Base Properties of Amino Acids?
You should know these numbers and know what they mean

- Alpha carboxyl group - $\text{pK}_a = 2$
- Alpha amino group - $\text{pK}_a = 9$
- These numbers are approximate, but entirely suitable for our purposes.
<table>
<thead>
<tr>
<th>Amino acid</th>
<th>$pK_a$ α-COOH</th>
<th>$pK_a$ α-NH$_3^+$</th>
<th>$pK_a$ side chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>2.34</td>
<td>9.69</td>
<td>—</td>
</tr>
<tr>
<td>Arginine</td>
<td>2.17</td>
<td>9.04</td>
<td>12.48</td>
</tr>
<tr>
<td>Asparagine</td>
<td>2.02</td>
<td>8.84</td>
<td>—</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>2.09</td>
<td>9.82</td>
<td>3.86</td>
</tr>
<tr>
<td>Cysteine</td>
<td>1.92</td>
<td>10.46</td>
<td>8.35</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>2.19</td>
<td>9.67</td>
<td>4.25</td>
</tr>
<tr>
<td>Glutamine</td>
<td>2.17</td>
<td>9.13</td>
<td>—</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.34</td>
<td>9.60</td>
<td>—</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.82</td>
<td>9.17</td>
<td>6.04</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2.36</td>
<td>9.68</td>
<td>—</td>
</tr>
<tr>
<td>Leucine</td>
<td>2.36</td>
<td>9.60</td>
<td>—</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.18</td>
<td>8.95</td>
<td>10.79</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.28</td>
<td>9.21</td>
<td>—</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.16</td>
<td>9.18</td>
<td>—</td>
</tr>
<tr>
<td>Proline</td>
<td>1.99</td>
<td>10.60</td>
<td>—</td>
</tr>
<tr>
<td>Serine</td>
<td>2.21</td>
<td>9.15</td>
<td>—</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.63</td>
<td>9.10</td>
<td>—</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>2.38</td>
<td>9.39</td>
<td>—</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.20</td>
<td>9.11</td>
<td>10.07</td>
</tr>
<tr>
<td>Valine</td>
<td>2.32</td>
<td>9.62</td>
<td>—</td>
</tr>
</tbody>
</table>
Protonated at physiological pH
pKa = 6.0

protonated imidazole $\rightleftharpoons$ imidazole

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histidine

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You should know these numbers and know what they mean

- Arginine, Arg, R: $pK_a$ (guanidino group) = 12.5
- Aspartic Acid, Asp, D: $pK_a = 3.9$
- Cysteine, Cys, C: $pK_a = 8.3$
- Glutamic Acid, Glu, E: $pK_a = 4.3$
- Histidine, His, H: $pK_a = 6.0$
pK\textsubscript{a} Values of the Amino Acids

You should know these numbers and know what they mean

- Lysine, Lys, K: pK\textsubscript{a} = 10.5
- Serine, Ser, S: pK\textsubscript{a} = 13
- Threonine, Thr, T: pK\textsubscript{a} = 13
- Tyrosine, Tyr, Y: pK\textsubscript{a} = 10.1
What is the pH of a lysine solution if the side chain amino group is 3/4 dissociated?

\[ pH = 10.5 + \log_{10} \frac{[3]}{[1]} \]

- pH = 10.5 + (0.477)
- pH = 10.977 = 11.0

- Note that, when the group is ¾ dissociated, ¾ is dissociated and ¼ is not; thus the ratio in the log term is ¾ over ¼ or 3/1.

OC p60 Henderson-Hasselbalch eq
If \( \text{pH} < \text{p}K_a \), acidic form
If \( \text{pH} > \text{p}K_a \), basic form
Isoelectric point (IP): no net charge

![Alanine structure](image)

$\text{pK}_a = 2.34$

$\text{pK}_a = 9.69$

$$pI = \frac{2.34 + 9.69}{2} = \frac{12.03}{2} = 6.02$$
\[ \text{pI} = \frac{8.95 + 10.79}{2} = 9.87 \]

\[ \text{pI} = \frac{2.19 + 4.25}{2} = 3.22 \]
Reactions of Amino Acids

• Carboxyl groups form amides & esters
• Amino groups form Schiff bases and amides
• Edman reagent (phenylisothiocyanate) reacts with the $\alpha$-amino group of an amino acid or peptide to produce a phenylthiohydantoin (PTH) derivative.
• Side chains show unique reactivities
  • Cys residues can form disulfides and can be easily alkylated
  • Few reactions are specific to a single kind of side chain
A jellyfish (Aequorea victoria) native to the northwest Pacific Ocean contains a **green fluorescent protein**. GFP is a naturally fluorescent protein. Genetic engineering techniques can be used to “tag” virtually any protein, structure, or organelle in a cell. The GFP chromophore lies in the center of a $\beta$-barrel protein structure.
The prosthetic group of GFP is an oxidative product of the sequence –FSYGVQ–.
Amino acid substitutions in GFP can tune the color of emitted light. Shown here is an image of African green monkey kidney cells expressing yellow fluorescent protein (YFP) fused to $\alpha$-tubulin, a cytoskeletal protein.
• All amino acids absorb at infrared wavelengths
• Only Phe, Tyr, and Trp absorb UV
• Absorbance at 280 nm is a good diagnostic device for amino acids
• NMR spectra are characteristic of each residue in a protein, and high resolution NMR measurements can be used to elucidate three-dimensional structures of proteins
Figure 4.10 The UV spectra of the aromatic amino acids at pH 6.
Figure 4.11 Proton NMR spectra of several amino acids.
Electrophoresis (based on charge)

**Separation of AA**
- Analytical separation
- Preparative separation

**Arginine**
- \[ \text{H}_2\text{NCONHCH}_2\text{CH}_2\text{CH}_2\text{CHCO}^- \]
- \[ \text{pl} = 10.76 \]

**Alanine**
- \[ \text{CH}_3\text{CHCO}^- \]
- \[ \text{pl} = 6.02 \]

**Aspartate**
- \[ \text{OCCH}_2\text{CHCO}^- \]
- \[ \text{pl} = 2.98 \]
Ninhydrin test (AA is purple)
Paper Chromatography and Thin-Layer Chromatography
(based on polarity)

Solvent: H$_2$O/AcOH/BuOH

MIT 5.301 Chemistry Laboratory Techniques
http://www.youtube.com/watch?v=EUn2skAAjHk
Ion-Exchange Chromatography

Nonpolar nature of the column caused it to retain nonpolar amino acids longer than polar amino acids.

Water softener OC p1032
Fractions sequentially collected

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CHIRALITY
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LEVADRO ANTIDEPRESSANT ANIMATION
After addition of ninhydrin

At 570 nm
Synthesis of Amino acids

Hell-Volhard-Zelinski reaction

\[
\begin{align*}
\text{RCH}_2\text{C} \text{OH} & \quad \text{a carboxylic acid} \\
\xrightarrow{1. \text{ Br}_2, \text{ PBr}_3} & \quad \text{RCH}_2\text{C} \text{OH} \\
\xrightarrow{2. \text{ H}_2\text{O}} & \quad \text{RCH} \text{Br} \\
\xrightarrow{\text{excess NH}_3} & \quad \text{RCHC} \text{O}^- \\
+ \text{NH}_4\text{Br}^- & \quad \text{an amino acid}
\end{align*}
\]
The reaction shown in the diagram is the reduction of a carboxylic acid (RCO\textsubscript{2}H) using excess ammonia and sodium borohydride (NaBH\textsubscript{3}(OCCH\textsubscript{3})\textsubscript{3}). The reaction converts the carboxylic acid into an amide (RCONH\textsubscript{2}) and ammonia (NH\textsubscript{3}).
Higher yield
\[ \text{acetamidomalonic ester} \]

\[ \text{CH}_3\text{CH}_2\text{O}^- \rightarrow \text{CH}_3\text{CH}_2\text{OH} \]

\[ \text{Br}^- \]

\[ \text{HCl, H}_2\text{O} \Delta \]

\[ \text{CH}_3\text{CO}_2\text{H} + \text{CO}_2 + \text{H}_3\text{N}^+\text{CH}_2\text{CO}_2\text{H} \]

\[ \text{acetic acid} \]

\[ \text{an amino acid} \]
Strecker synthesis

an aldehyde $\xrightarrow{\text{NH}_3}$ an imine $\xrightarrow{-\text{C}≡\text{N}}$ \(\text{RCHC}≡\text{N}+\text{NH}_3\) \(\xrightarrow{\text{HCl, H}_2\text{O}}\) \(\text{RC}≡\text{C}−\text{OOH}+\text{NH}_3\)

an amino acid

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Kinetic resolution

In kinetic resolution, two enantiomers show different reaction rates in a chemical reaction, thereby creating an excess of the less reactive enantiomer. This excess goes through a maximum and disappears on full completion of the reaction. Kinetic resolution is a very old concept in organic chemistry and can be used in the organic synthesis of chiral molecules. It has been surpassed by other methods.

\[
\begin{align*}
\text{d-amino acid} + \text{l-amino acid} & \rightarrow \text{N-acetyl-d-amino acid} + \text{N-acetyl-l-amino acid} \\
& \quad + \text{N-acetyl-d-amino acid}
\end{align*}
\]
4.7 What is the Fundamental Structural Pattern in Proteins?

- Proteins are unbranched polymers of amino acids.
- Amino acids join head-to-tail through formation of covalent peptide bonds.
- Peptide bond formation results in release of water.
- The peptide backbone of a protein consists of the repeated sequence \(-N-C_\alpha -C_\text{o}\)-.
- "N" is the amide nitrogen of the amino acid.
- "C_\alpha " is the alpha-C of the amino acid.
- "C_\text{o}" is the carbonyl carbon of the amino acid.
peptide bond (amide bond)

\[
\begin{align*}
\text{H}_3\text{NCH}^+ \text{C}_\text{O}^- & \quad \text{H}_3\text{NCH}^+ \text{C}_\text{O}^- \\
\text{R} & \quad \text{R}' \\
\text{H}_3\text{NCH}^+ \text{C}_\text{O}^- & \quad \text{H}_3\text{NCH}^+ \text{C}_\text{O}^- \\
\text{R} & \quad \text{R}''
\end{align*}
\]

\[\downarrow\]

\[
\begin{align*}
\text{H}_3\text{NCH}^+ \text{C} \quad \text{NHCH} \quad \text{NHCH} \quad \text{C}_\text{O}^- \\
\text{R} & \quad \text{R}' & \quad \text{R}'' \\
\quad & \quad & \quad
\end{align*}
\]

\[2 \text{ H}_2\text{O}\]

the N-terminal amino acid

peptide bonds

the C-terminal amino acid

a tripeptide

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Glu, Cys, His, Val, Ala

the pentapeptide contains the indicated amino acids, but their sequence is not known

Val-Cys-Ala-Glu-His

the amino acids in the pentapeptide have the indicated sequence

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The Peptide Bond

- Is usually found in the *trans* conformation
- Has partial (40%) double bond character
- Is about 0.133 nm long - shorter than a typical single bond but longer than a double bond
- Due to the double bond character, the six atoms of the peptide bond group are always planar
- N partially positive; O partially negative
40% double-bond character
4.7 What is the Fundamental Structural Pattern in Proteins?

Figure 4.16 (c) The peptide bond is best described as a resonance hybrid of the forms shown on the two previous slides.
The Peptide Bond

Figure 4.15 The *trans* conformation of the peptide bond.
The coplanar relationship of the atoms in the amide group is highlighted here by an imaginary shaded plane lying between adjacent $\alpha$-carbons.
2 R—SH \[\xrightarrow{\text{mild oxidation}}\] Br\(_2\) (or I\(_2\)) \[\rightarrow\] RS—SR

a thiol

a disulfide

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mechanism for oxidation of a thiol to a disulfide

\[
\begin{align*}
\text{R--S}^- & \rightleftharpoons \text{HO}^- \quad \text{H}_2\text{O} \\
\text{R--S}^- & \xrightarrow{\text{Br--Br}} \text{R--S--Br} \\
\text{R--S}^- & \xrightarrow{\text{R--S}^-} \text{R--S--S--R} + \text{Br}^- \\
& + \text{Br}^-
\end{align*}
\]
RS—SR  \[\xrightarrow{\text{reduction}}\]  \[2 \text{ R—SH}\]

a disulfide  \[\rightarrow\]  a thiol
2HSCH₂CHCO⁻ + NH₃ → mild oxidation → O⁻OCCHCH₂S=SCH₂CHCO⁻ + NH₃

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polypeptide

oxidation

reduction

disulfide bridges cross-linking portions of a polypeptide
“Peptides”

- Short polymers of amino acids
- Each unit is called a residue
- 2 residues - dipeptide
- 3 residues - tripeptide
- 12-20 residues - oligopeptide
- many - polypeptide
One or more polypeptide chains

- One polypeptide chain - a monomeric protein
- More than one - multimeric protein
- Homomultimer - one kind of chain
- Heteromultimer - two or more different chains
- Hemoglobin, for example, is a heterotetramer
- It has two alpha chains and two beta chains
A-chain
Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn

B-chain
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Ala

insulin

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Enkephalins synthesized by the body to control pain

Tyr-Gly-Gly-Phe-Leu leucine enkephalin

Tyr-Gly-Gly-Phe-Met methionine enkephalin

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morphine
Peptide hormones

**bradykinin**  \[\text{Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg}\]

**vasopressin**  \[\text{Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH}_2\]

**oxytocin**  \[\text{Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH}_2\]

*Bradykinin* inhibits the inflammation of tissues

*Vasopressin* controls blood pressure

*Oxytocin* induces labor in pregnant women by stimulating the uterine muscle
200 times sweeter than sucrose
D-amino acid is bitter.
Destroy harmful oxidizing agents in the body

Glutathione

Reducing agent ↔ Oxidizing agent

Oxidized glutathione
The Sequence of Amino Acids in a Protein

- Is a unique characteristic of every protein
- Is encoded by the nucleotide sequence of DNA
- Is thus a form of genetic information
- Is read from the amino terminus to the carboxyl terminus
Chemical Synthesis of peptide
Solid Phase Synthesis of Peptides

- R. Bruce Merrifield and his collaborators pioneered the solid-phase synthesis of polypeptides in the laboratory
- Carboxy terminus of a nascent peptide is covalently anchored to an insoluble resin
- After each addition of a residue, the resin particles are collected by filtration
- Automation and computer control now permit synthesis of peptides of 30 residues or more
glycine

H₂NCH₂CO⁻

alanine

H₂NCHCO⁻

O

CH₃

peptide bond is formed between these groups

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di-tert-butyl dicarbonate + H₂NCH₂CO⁻ → N-protected glycine

Boc

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N-protected Gly + dicyclohexylcarbodiimide (DCC) → activated group

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\[
\text{CH}_3\text{C}-\text{OCN}\text{HCH}_2\text{C}-\text{O}-\text{C} \rightarrow \text{CH}_3\text{C}-\text{OCN}\text{HCH}_2\text{C}-\text{O}-\text{C} + \text{H}^+
\]

**tetrahedral intermediate**

**new peptide bond**

**Gly-Ala**

**dicyclohexylurea**

*a diamide*
N-protected Gly-Ala → N-protected Gly-Ala-Val

1. DCC
2. H₂NCHCO⁻
Merrifield automated solid-phase synthesis of a tripeptide

\[
\text{N-protected amino acid}
\]

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N-protected amino acid

N-protected and C-activated amino acid
**N-protected amino acid**

CH₃\(\text{O} \text{C-} \text{NHCHCOH}\)

+ DCC →

CH₃\(\text{O} \text{C-} \text{NHCHCOO} \\text{DCC}\)

**N-protected and C-activated amino acid**

CH₃\(\text{O} \text{C-} \text{NHCHC} \text{C-} \text{NHCHC} \text{C-} \text{NHCHCO-CH}_2\)

+ CF₃COOH

CH₂Cl₂

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Automated solid-phase peptide synthesis

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