PROTEIN STRUCTURE I

Each protein has a characteristic shape, size and function:

Classification of Proteins on the Basis of Biological Role:

1. **Structural Proteins**
   a. Provide mechanical support to cells and organisms
   b. Give strength to bones, skin and tendons: collagen, elastin

2. **Enzymes**
   a. Proteins that serve as *biological catalysts* for chemical reactions in cells

3. **Transport and Storage**
   a. Carriers for small biomolecules to cellular destinations for use in metabolism or in construction of cell components
   b. Examples: oxygen, ferritin (iron in liver), lipoproteins that transport cholesterol

4. **Muscle Contraction and Mobility**
   a. Actin and myosin are components of skeletal muscle

5. **Immune Proteins and other Protective Proteins**
   a. Proteins used for defensive purposes
      i. Example: Antibodies are proteins that bind and destroy foreign substances like viruses and bacteria

6. **Regulatory and Receptor Proteins**
   a. Proteins that regulate cellular and physiological activity
      i. Hormones
      ii. DNA Binding Proteins – assist in regulation of protein synthesis
   b. Receptors
      i. Proteins that mediate hormone signals and transmit the signal to the inside of the cell
         1. e.g. G-proteins and brain receptors
         2. Aspartame with taste receptor

![Schematic representation of a taste receptor model with three sites that require associations before a sweeter stimulus interacts with a taste bud. The taste bud has a hydrophobic domain, a hydrogen bond donor (A-H) and a hydrogen bond acceptor (E) to complementarily interact with the hydrophobic, hydrogen bond acceptor, and hydrogen bond donor sites of the peptide.](image-url)
PPA – Phenylpropanolamine:
- Nasal decongestant and appetite suppressant
- Old formulations of Alka-Seltzer Cold, Tavist-D and Dexatrim
- PPA is a pressor amine – substance that is capable of raising blood pressure
- Similar to amphetamines (“systemic upper”)
- Interact with α-adrenergic receptors to elicit stimulatory effects in the brain and other tissues
- Produces vasoconstriction
- Raises blood pressure
- Found to increase the risk of hemorrhagic strokes due to increase in blood pressure – blood vessels rupture
- Banned by FDA
- Increased risk more prevalent in women – women are more likely to take diet pills/appetite suppressants
- Higher concentration in diet pills than cold medicine
COMMON THEME: RECOGNITION!!

**All dependent on the fact that proteins have three-dimensional shapes!!

- Proteins interact **selectively** with other proteins or molecules through NON-covalent interactions in order to function.
- Reactant with enzyme
- Transported molecule with transporter
- Protein-protein interactions
- Protein-DNA interactions

- **Non-covalent interactions:**
  - Electrostatic interactions
  - Sterics
  - Van der Waals interactions
  - Hydrogen bonds

PROTEIN STRUCTURE:

- Can be **globular**
  - Spherical or near-spherical
  - Soluble in water – lots of charged R groups
  - Dynamic and flexible
- Can be **fibrous**
  - Elongated and threadlike
  - Not soluble in water – lots of hydrophobic, non-polar R groups
  - Tough
  - Examples: hair, nails, skin
- Can be **membrane proteins**
  - Can be monomers – single polypeptide chain
  - Can be oligomers – multiple polypeptide chains
  - Held together by non-covalent interactions
  - Each polypeptide chain = **subunit** if part of a complex

**Table 3.6** Examples of globular and fibrous proteins

<table>
<thead>
<tr>
<th>Type of Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Globular Proteins</strong></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Transport (oxygen transport)</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>Storage (oxygen storage)</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>Enzyme (RNA hydrolysis)</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Enzyme (bacterial wall hydrolysis)</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>Electron transport</td>
</tr>
<tr>
<td>Immunoglobulin</td>
<td>Defense (antibody)</td>
</tr>
<tr>
<td>Actin</td>
<td>Movement (muscle protein)</td>
</tr>
<tr>
<td><strong>Fibrous Proteins</strong></td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>Structural protein</td>
</tr>
<tr>
<td>Keratin</td>
<td>Structural protein</td>
</tr>
<tr>
<td>Myosin</td>
<td>Movement (muscle protein)</td>
</tr>
<tr>
<td>Elastin</td>
<td>Elasticity</td>
</tr>
</tbody>
</table>

*Table 3.6* Concepts in Biochemistry, 3/e
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- Size expressed in terms of mass: units of Daltons or kilodaltons
- One Dalton = one atomic mass unit
  - Estimate size = # amino acids x average molecular weight aa
  - 110 g/mole per amino acid

### Table 3.5

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular Mass (Daltons)</th>
<th>Number of Amino Acid Residues</th>
<th>Number of Subunits a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (bovine)</td>
<td>5,733</td>
<td>51</td>
<td>2</td>
</tr>
<tr>
<td>Cytochrome c (human)</td>
<td>13,000</td>
<td>104</td>
<td>1</td>
</tr>
<tr>
<td>Ribonuclease A (bovine pancreas)</td>
<td>13,700</td>
<td>124</td>
<td>1</td>
</tr>
<tr>
<td>Lysozyme (egg white)</td>
<td>13,930</td>
<td>129</td>
<td>1</td>
</tr>
<tr>
<td>Myoglobin (equine heart)</td>
<td>16,890</td>
<td>153</td>
<td>1</td>
</tr>
<tr>
<td>Chymotrypsin (bovine pancreas)</td>
<td>26,500</td>
<td>241</td>
<td>3</td>
</tr>
<tr>
<td>Hemoglobin (human)</td>
<td>64,500</td>
<td>574</td>
<td>4</td>
</tr>
<tr>
<td>Serum albumin (human)</td>
<td>68,500</td>
<td>550</td>
<td>1</td>
</tr>
<tr>
<td>Immunoglobulin G (human)</td>
<td>145,000</td>
<td>1320</td>
<td>4</td>
</tr>
<tr>
<td>RNA polymerase (E. coli)</td>
<td>450,000</td>
<td>4100</td>
<td>5</td>
</tr>
<tr>
<td>Ferritin (equine spleen)</td>
<td>450,000</td>
<td>4100</td>
<td>24</td>
</tr>
<tr>
<td>Glutamate dehydrogenase (bovine liver)</td>
<td>1,000,000</td>
<td>8300</td>
<td>40</td>
</tr>
</tbody>
</table>

*The number of subunits refers to the quaternary structure.
Table 3.5 Concepts in Biochemistry, 3/e
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### FOUR LEVELS OF PROTEIN STRUCTURE:

- **Primary (1°)**
  - Linear sequence of amino acids in a protein

- **Secondary (2°)**
  - Local 3-dimensional structure of the **PEPTIDE BACKBONE**
    - Ignores the conformation of the side chains

- **Tertiary (3°)**
  - Global arrangement of secondary structure, side chains (R groups), and other prosthetic groups (e.g. metals)

- **Quaternary (4°)**
  - Arrangement of multiple proteins into **complexes**
The four levels of protein structures

(a) Primary structure

(b) Secondary structure

(c) Tertiary structure

(d) Quaternary structure

Primary structure

Secondary structure

Tertiary structure
Look at each level individually:

**PRIMARY STRUCTURE (1°)**

- Defined, non-random **sequence of amino acids** along the peptide backbone
  - Described in two ways:
    - Amino acid **composition**
    - Amino acid **sequence**
    - M-L-D-G-C-G Peptide A
    - M-L-C-D-G-G Peptide B
  - Composition is **IDENTICAL**; Sequence is **DIFFERENT**

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Campbell, Biochemistry, 3e
Text Figure 04.01

- How to determine the **COMPOSITION**
  - Purify the protein of interest – separate away from all other types of proteins and biomolecules
  - Estimate the molecular weight of the protein
  - Establish the composition by complete hydrolysis of the protein under acidic conditions
    - Treat with 6M HCl at 110°C; 12-36 hours
    - Each peptide bond is broken and products are all of the free amino acids
    - Each amino acid is separated, identified and quantified
    - Final result: Know **HOW MANY** of each amino acid present in the original
How to determine the ORDER

- **Determine the C-terminal amino acid**
  - Use carboxypeptidase – enzyme that removes the last (C-terminal) amino acid in a free form by breaking the peptide bond
    - Hydrolyzes the peptide bond nearest the C-terminus
- **Identify the N-terminal amino acids in order**
  - Process called **SEQUENCING**
  - Often difficult to characterize an intact protein
  - Instead, employ a “divide and conquer” approach to analyze peptide fragments of the intact protein
  - Cut large proteins into smaller parts
  - Use enzymes called **PROTEASES**
    - Cleave peptide bond in a specific way
    - TWO Examples:
      - **Trypsin** – Cleave on the C-terminal side of Lys and Arg residues
      - **Chymotrypsin** – Cleave on the C-terminal side of Tyr, Phe, and Trp

- Chemical proteases also can cleave proteins
- **Cyanogen Bromide (CNBr)** – cleave on the C-terminal side of Met
- Agents used to generate an overlapping set of peptides

- If the sequence of each peptide is determined, the entire protein sequence can be reassembled from the fragments
Sequencing of the peptides generated by proteases

- Procedure called **Edman Degradation**
- React the N-terminal amino acid with phenylisothiocyanate
- Derivatized amino acid released as PTH – phenylthiohydantoin
- Each PTH amino acid derivative is identified by chromatography
- Newly exposed N-terminal residue can be derivatized, removed and identified sequentially
- Useful up to 25-50 amino acids