

COURSE
Fundamentals of
Biochemistry

Unit: 2

PROTEINS

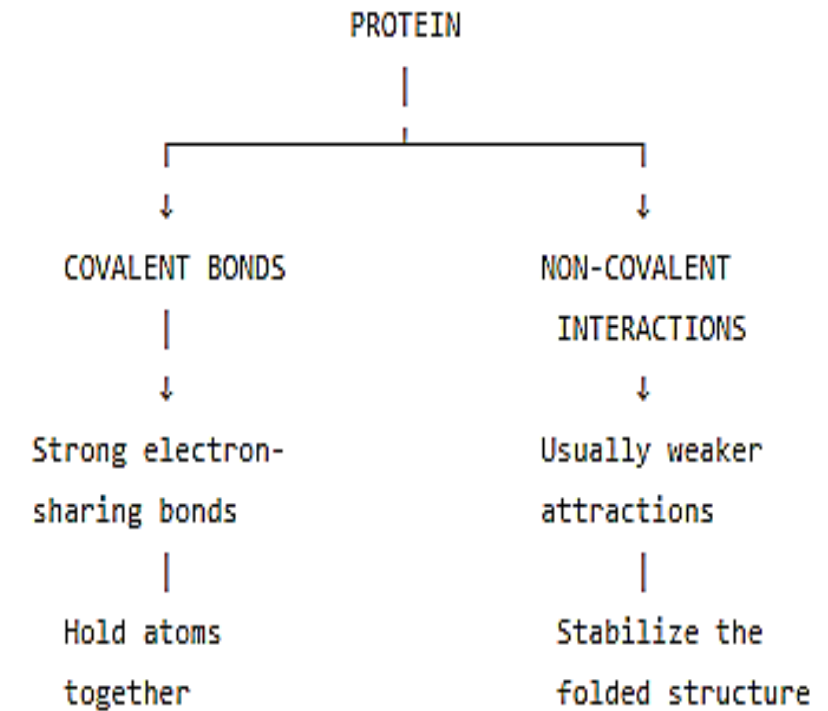
Ms. Sarojmoni Sonowal
Assistant Professor
Department of Zoology
Pub Kamrup College

Proteins are polymers of amino acids in which the amino acid sequence folds into a specific three-dimensional shape.

Proteins have different levels of structural organization:

- **Primary structure**
- **Secondary structure**
- **Tertiary structure**
- **Quaternary structure**

Each level is stabilized by particular types of chemical interactions OR Bonds



Covalent vs Non-covalent Interactions in Protein Structure

Feature	Covalent	Non-covalent
Nature of interaction	Electrons are shared between atoms.	Attraction without conventional electron sharing.
Strength	Generally strong.	Generally weaker individually.
Reversibility	Usually harder to break.	Often readily reversible.
Types / examples	Peptide bonds, disulfide bonds.	Hydrogen bonds, ionic interactions, hydrophobic effect, van der Waals forces.
Role in protein folding	Builds/links the polypeptide chain; can strongly stabilize folds.	Maintains and stabilizes higher-order folding.

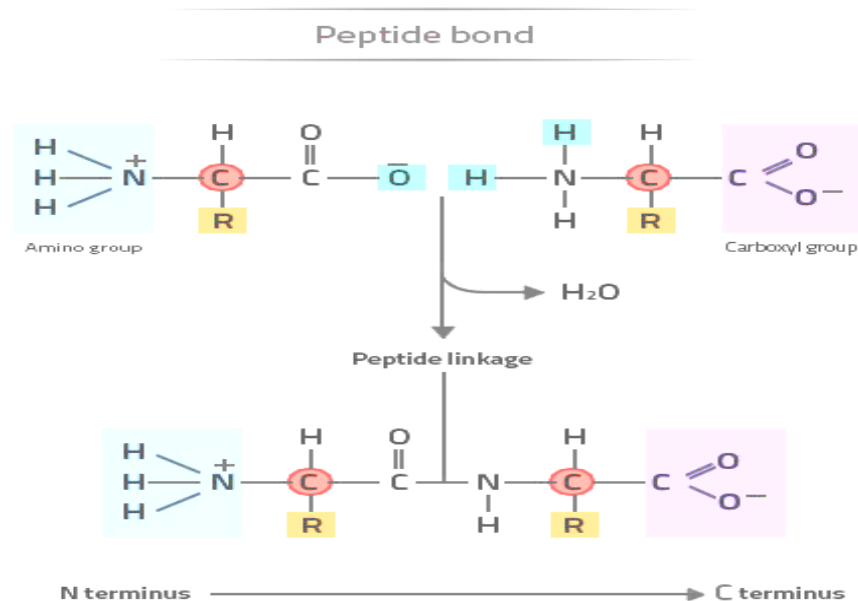
1. Primary Structure – Amino Acid Sequence

The primary structure is the exact linear sequence of amino acids in a polypeptide chain extending from the **N-terminal end** to the **C-terminal end**.

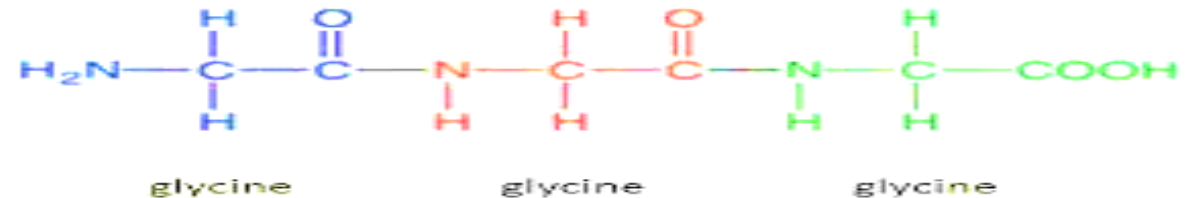
Amino acids are joined together through **peptide bonds**.

Ala — Gly — Val — Leu — Lys — Ser — Phe

PRIMARY STRUCTURE



Tripeptide



**Bond stabilizing primary structure:
Peptide bond**

It is a **covalent bond** between the carboxyl group of one amino acid and the amino group of the next.

The primary structure is extremely important because it determines how the protein can fold into higher levels of structure.

A change in even one amino acid can sometimes alter protein folding and function.

2. Secondary Structure – Local Folding

The polypeptide chain does not remain completely straight.
Parts of the chain fold or coil into regular shapes.

These regular arrangements are called secondary structures.

The polypeptide backbone begins to fold into regular local structures, mainly:

α -helix

β -pleated sheet

**β -turns and
loops**

The two most common types are:

(A) α -helix

(B) β -pleated sheet

The major stabilizing force is hydrogen bonds between peptide backbone groups.

PROTEIN SECONDARY STRUCTURE

Alpha Helix

Right-handed coiled structure.

Hydrogen bond between C=O of residue i and N-H of residue $i+4$.

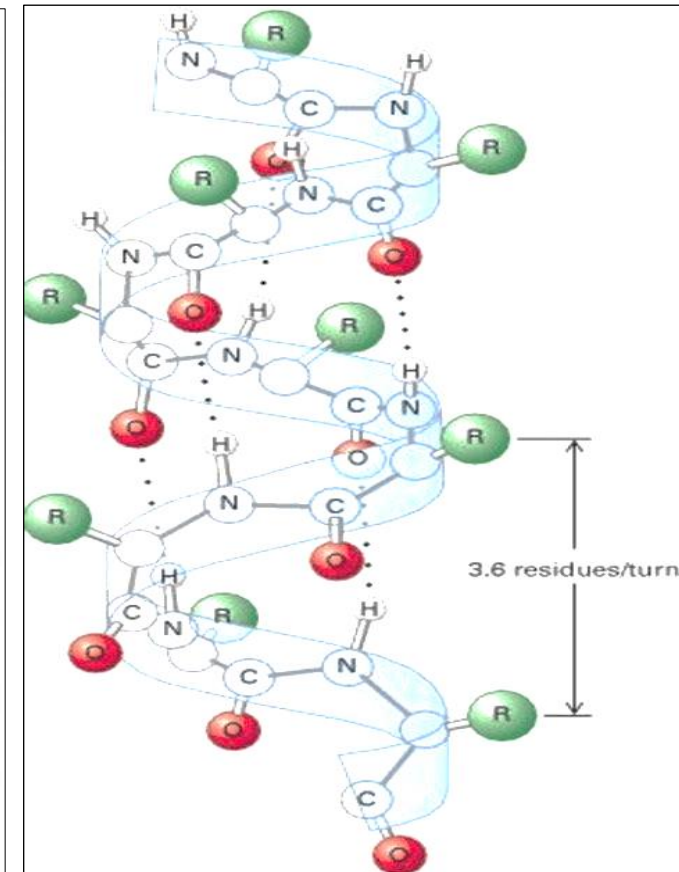
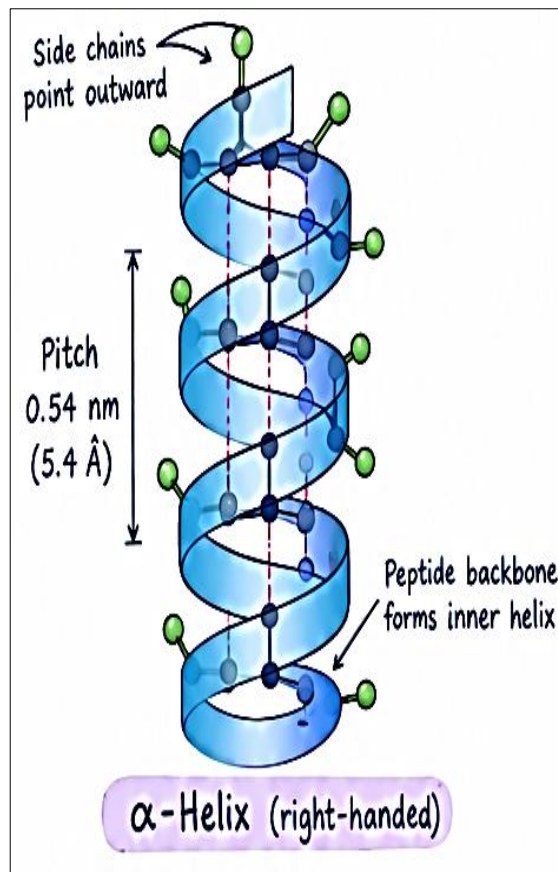
3.6 amino acid residues per turn.

Pitch ≈ 0.54 nm ($5.4 \text{ \AA}$).

Rise ≈ 0.15 nm ($1.5 \text{ \AA}$) per residue.

Side chains point outward.

Peptide backbone forms the inner helix.

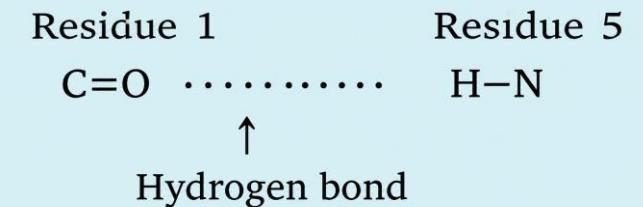


Some amino acids strongly affect α -helix formation.

- **Proline is called a helix breaker**

Its rigid ring structure restricts rotation and can disturb the regular hydrogen-bonding pattern. Therefore: Proline tends to disrupt α -helices.

- Glycine is very flexible because its side chain is only H. Therefore, it can also destabilize regular α -helical structure.



The carbonyl oxygen (C=O) of one peptide bond forms a hydrogen bond with the amide hydrogen (N-H) approximately **four residues earlier** in the chain.

Beta Sheet

Instead of forming a spiral like an α -helix, the polypeptide chain becomes more **extended**.

A β -sheet forms when **two or more stretches of a polypeptide chain lie alongside one another**.

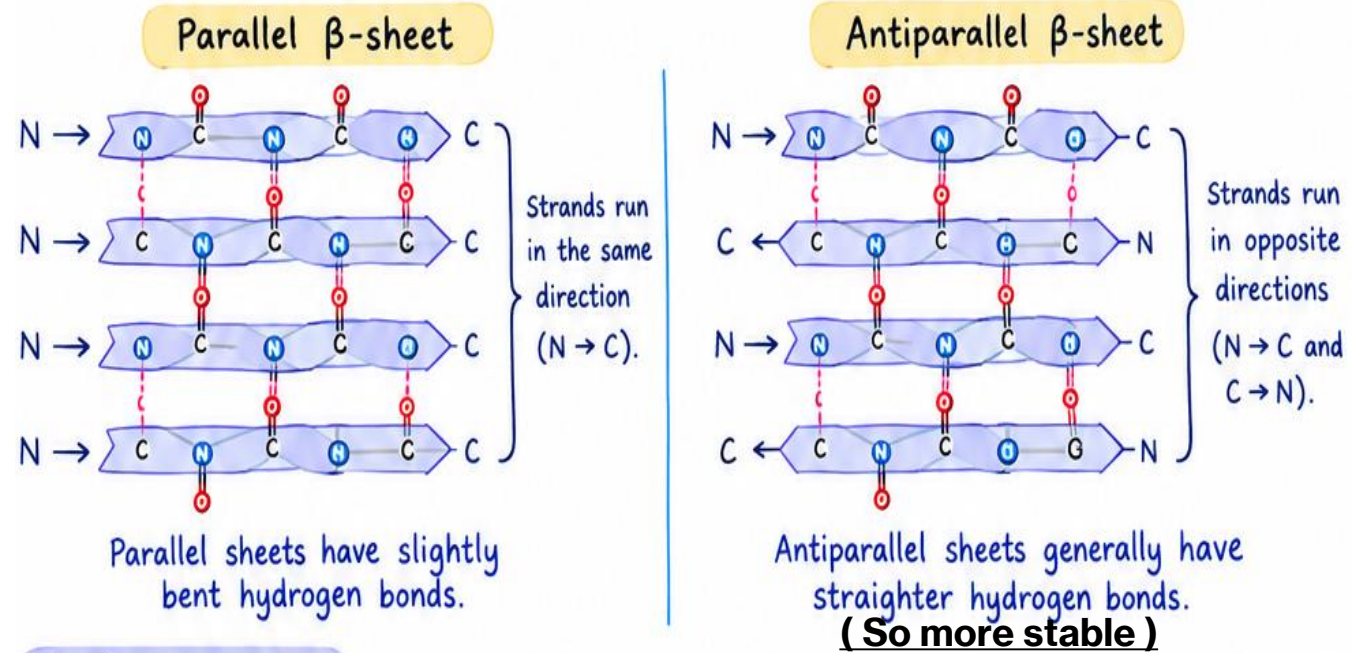
These adjacent chains are held together mainly by: **Hydrogen bonds** between **C=O** and **N-H groups** of neighbouring polypeptide segments.

Each individual extended segment is called a **β -strand**. Several β -strands together form a **β -sheet**

The distance between adjacent amino acids is approx. **3.5 Å**

β -strands + hydrogen bonding --- β -pleated sheet

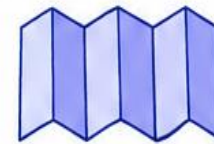
β -sheets can be: **1. Parallel** & **2. Antiparallel**



Quick Comparison



Alpha helix
= Coiled structure



Beta sheet
= Folded / pleated sheet

What stabilizes secondary structure?

Main stabilizing interaction

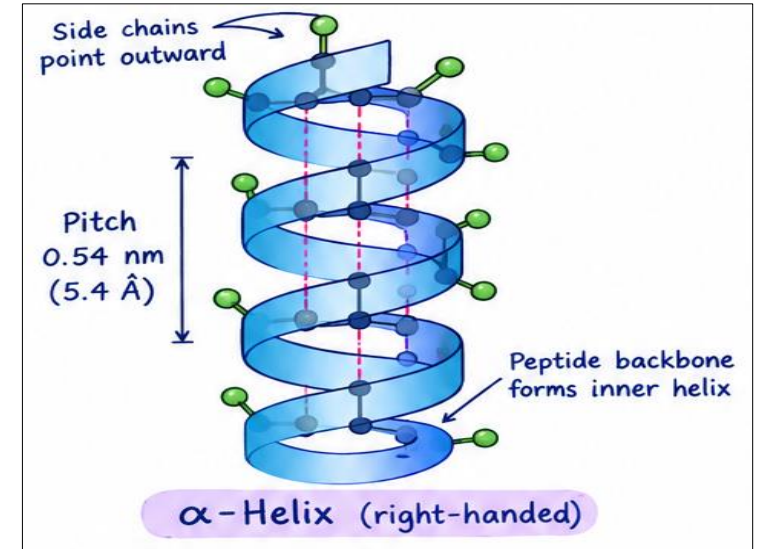
Hydrogen bonds between backbone C=O and N-H groups.

Secondary structure

(Regular folding of the polypeptide backbone)

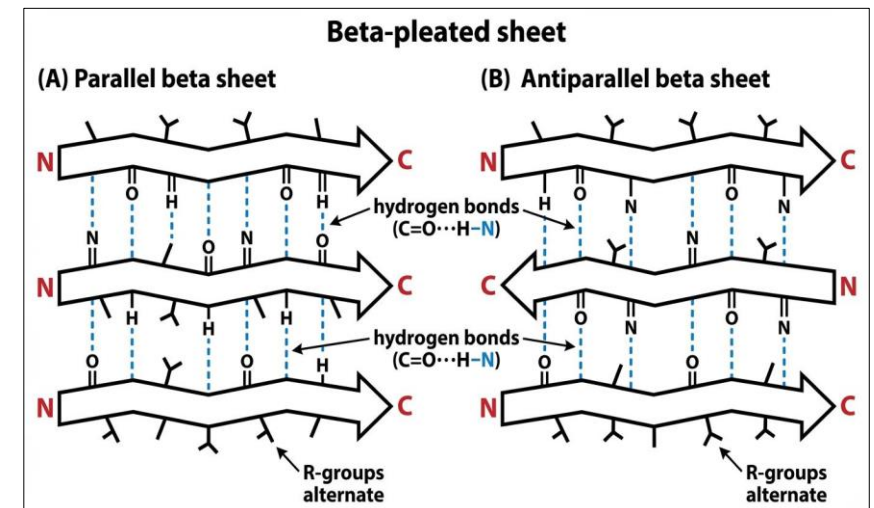
α -helix

- Rigid, rod like coiled structure (helical configuration: right or left handed)
- In almost all proteins: Right-handed (more favourable).
- 3.6 amino acid residues/turn
- 0.54 nm/turn
- 0.15 nm (1.5 Å) distance between adjacent amino acids
- Length of α -helix is usually 10-15 amino acid residues.
- Stabilized by hydrogen bonds
- C=O of one residue bonds with N-H of four residues ahead
- Side chains project outward
- Proline can disrupt the helix



β -pleated sheet

- Extended polypeptide strands
- Adjacent strands are held by hydrogen bonds
- Can be parallel or antiparallel
- Side chains project alternately above and below the sheet
- The distance between adjacent amino acids is approx. 3.5 Å



Bends in protein structure are regions where the polypeptide backbone changes direction

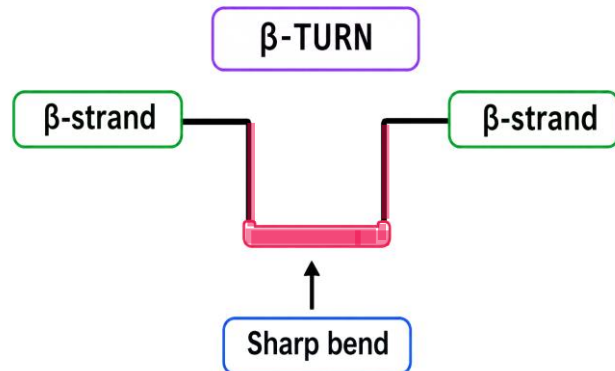
- They are particularly important in **secondary and tertiary structure** because they connect α -helices and β -strands and allow the protein chain to change direction.
- The term “**bend**” can refer to several related structural features, especially **β -turns, loops**

β -TURNS: 3-5 amino acid residues- Third type of secondary structure. A β -turn allows the polypeptide chain to **reverse its direction**.

Turns allow large proteins to fold into highly compact structure

These short U shaped secondary structure are **stabilized by H-Bonds** between their end residues- often contributes to their stability.

Glycine (lacks large R chain) and proline (Build in bend structure) amino acids are commonly present in turns



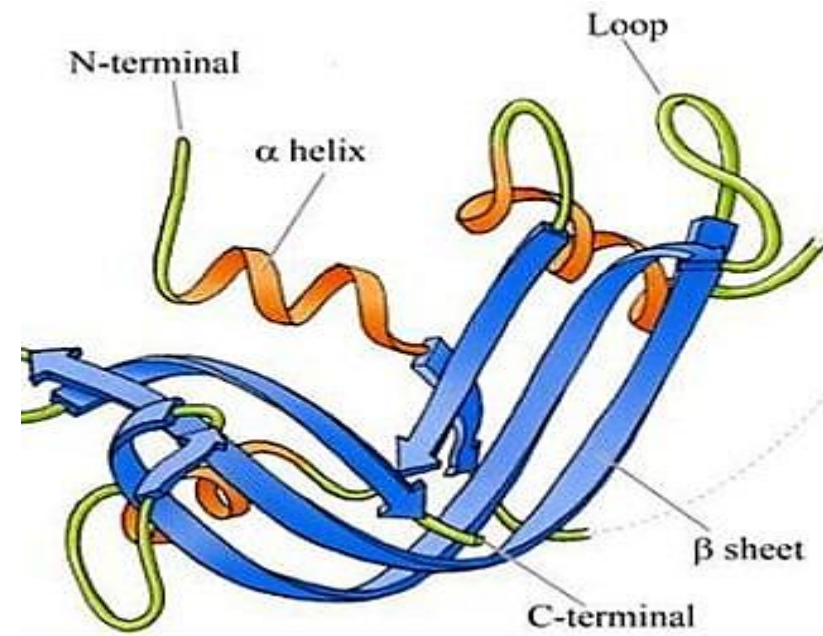
The chain effectively turns back on itself, allowing two regions of the polypeptide to become close together.

LOOPS

Loops are irregular regions that connect

α -helix — loop — β -sheet

- They are often found on the surface of proteins.
- Loops are longer than turns



3. Tertiary structure

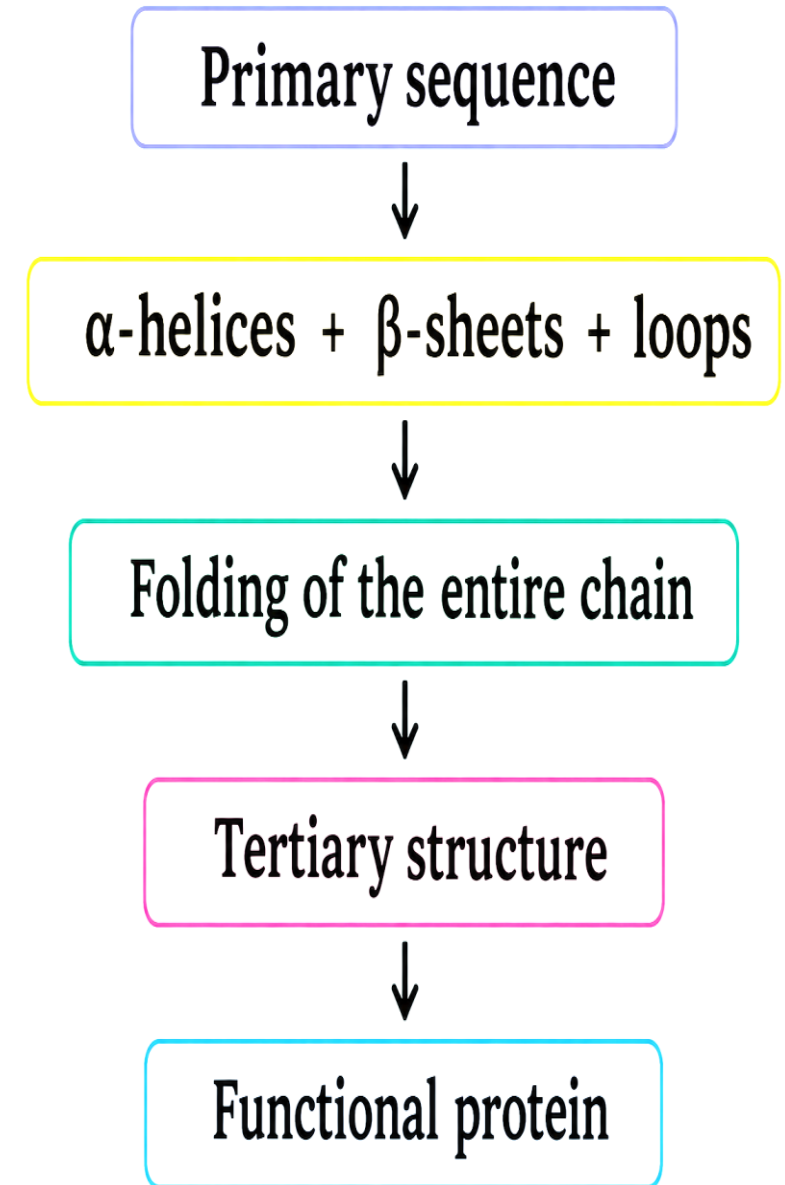
The **tertiary structure** is the overall three-dimensional arrangement of a **single polypeptide chain**. It describes how all the secondary-structure elements are arranged relative to one another.

The α -helices, β -sheets, turns and loops fold together to form domains and a compact functional protein.

Forces stabilizing tertiary structure

Tertiary structure is stabilized by several interactions. The major ones are:

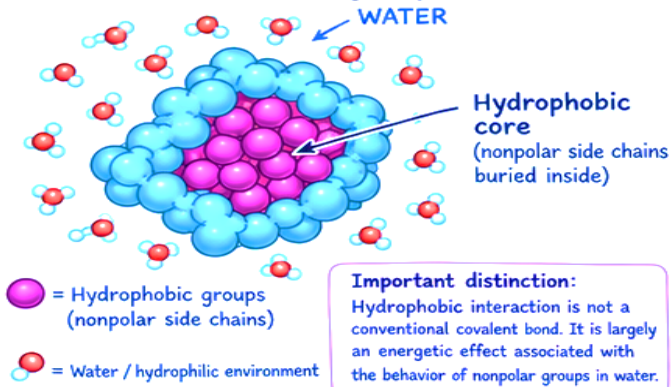
1. **Hydrophobic interactions**
2. **Hydrogen bonds**
3. **Ionic interactions / salt bridges**
4. **Disulfide bonds**
5. **Van der Waals forces**



Interactions or bonds stabilizing tertiary protein structure

Hydrophobic interactions

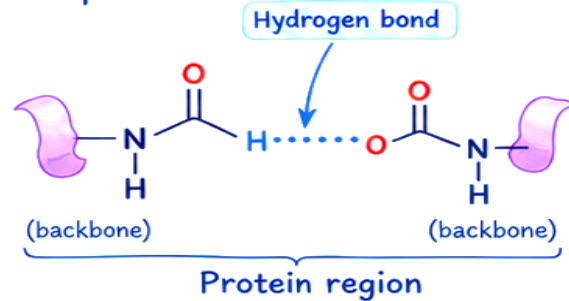
- These are extremely important in the folding of proteins in aqueous environments.
- Nonpolar amino acid side chains tend to avoid contact with water.
- Examples of predominantly hydrophobic side chains:
Valine, Leucine, Isoleucine, Methionine, Phenylalanine
- During folding, these residues become buried inside the protein, forming a hydrophobic core.



Hydrogen bonds

- Hydrogen bonds can form between:
 - polar side chains
 - backbone groups
 - side chain and backbone groups.

Example:



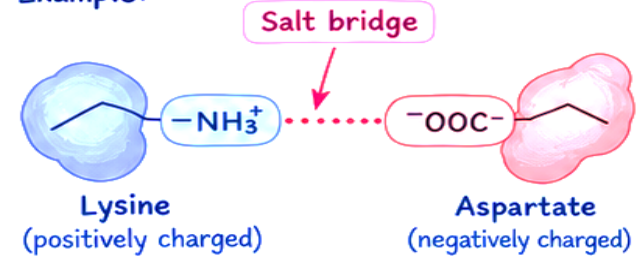
- Hydrogen bonds are individually weak compared with covalent bonds, but many hydrogen bonds together can make a substantial contribution to protein stability.

Ionic interactions / Salt bridges

- Charged amino acid side chains can attract one another.
- Common examples include:



Example:



- These interactions can contribute to the tertiary structure of proteins.

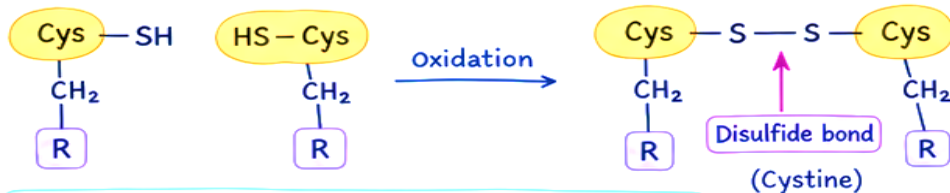
Peptide bonds hold the amino-acid sequence together, while

- hydrophobic interactions,
- hydrogen bonds,
- ionic interactions,
- van der Waals forces and
- disulfide bonds

stabilize higher-order folding to varying degrees.

Disulfide bonds

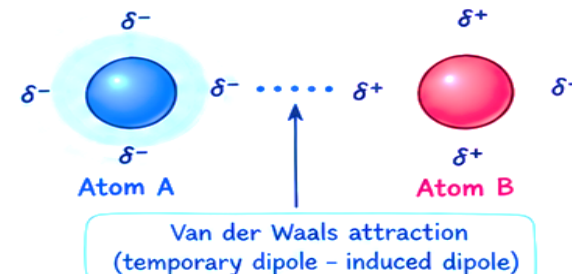
- Disulfide bonds are strong covalent bonds formed between the sulfur atoms of two cysteine residues.
- Two cysteine side chains:



- Disulfide bonds can connect:
 - two regions of the same polypeptide chain, or
 - two different polypeptide chains.
- They are especially important for many proteins functioning outside cells.

Van der Waals interactions

- When atoms are very close together, temporary fluctuations in electron distribution can produce weak attractive forces called van der Waals interactions.
- Individually they are weak, but a large number can contribute significantly to the stability of a tightly packed protein.



Although individually weak, many van der Waals interactions between atoms in a tightly packed protein structure add up to give significant stability.

3. Tertiary structure cont.....

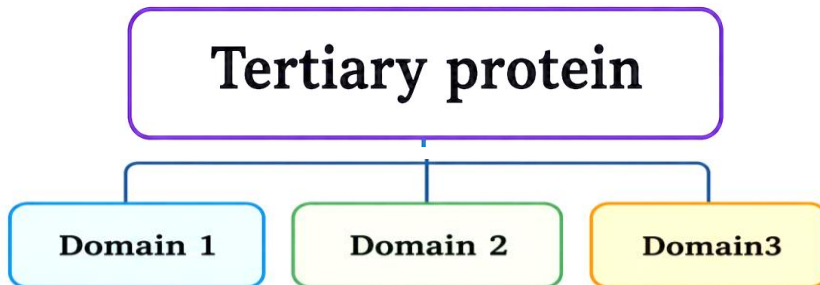
The fundamental unit of tertiary structure is **the Domain**

It is a polypeptide chain or a part of a polypeptide chain that can **fold independently** into a **stable 3-D tertiary structure**

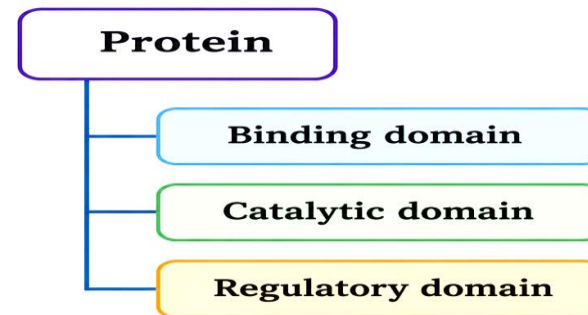
A **protein domain** is a region of a protein that can often fold into a relatively stable structural and functional unit.

A protein may contain a single domain or more than one domain. A small protein often consists of only one Domain.

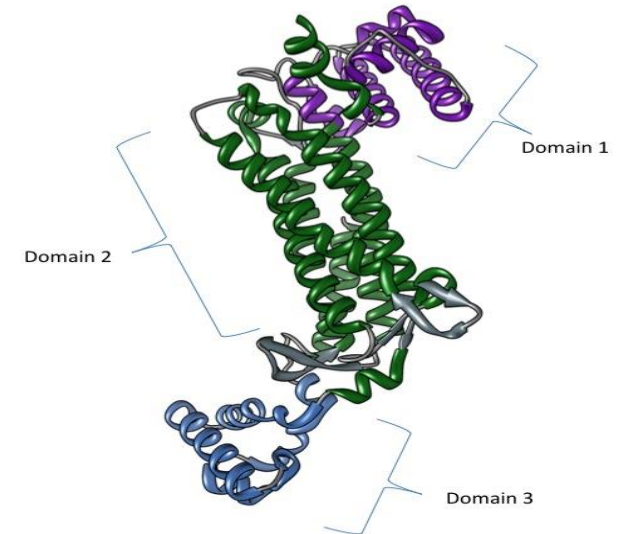
For example:



Different domains can have different functions.



This is particularly important in large enzymes and regulatory proteins.



Tertiary structure

1. Myoglobin

- Found in muscles
- Binds the oxygen required for aerobic metabolism;
- Associated with a heme group, which is actually responsible for binding oxygen;

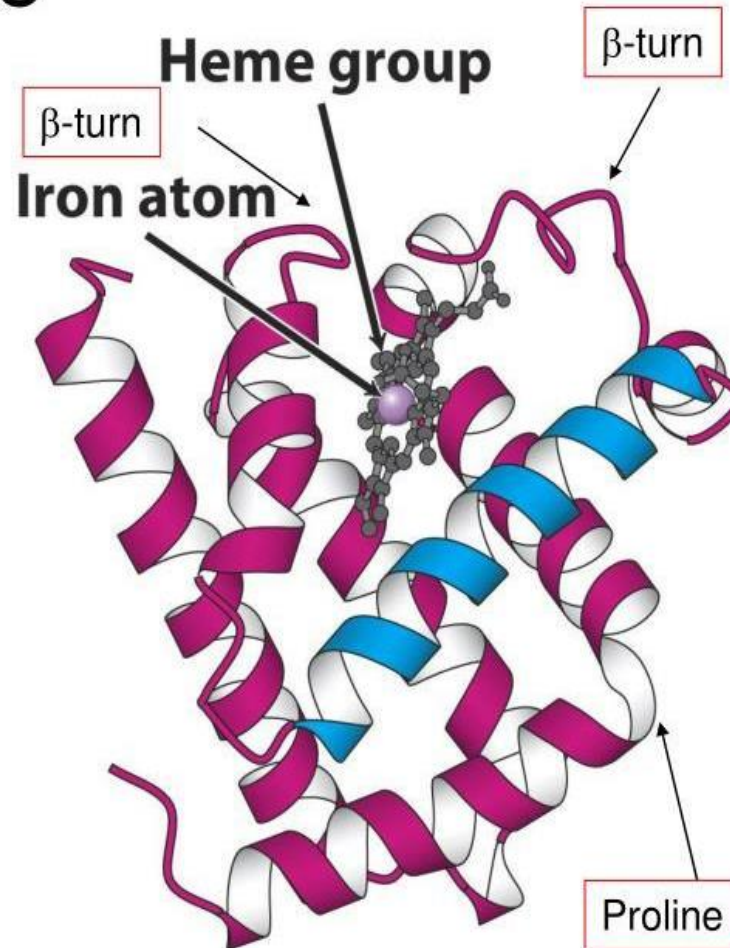


Figure 2-48a
Biochemistry, Sixth Edition
© 2007 W.H. Freeman and Company

**Other examples
of proteins**
with a well-defined tertiary
structure

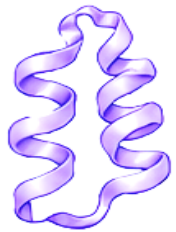
- Lysozyme
- Ribonuclease A
- Chymotrypsin
- Trypsin
- Cytochrome c

QUATERNARY STRUCTURE OF PROTEINS

Definition

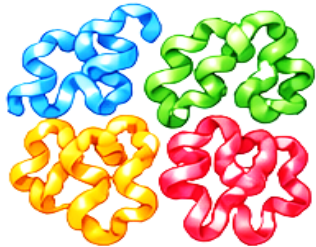
The **quaternary structure** describes the arrangement and interaction of two or more polypeptide subunits within a functional protein complex.

Not every protein has quaternary structure.



Single polypeptide

Tertiary structure

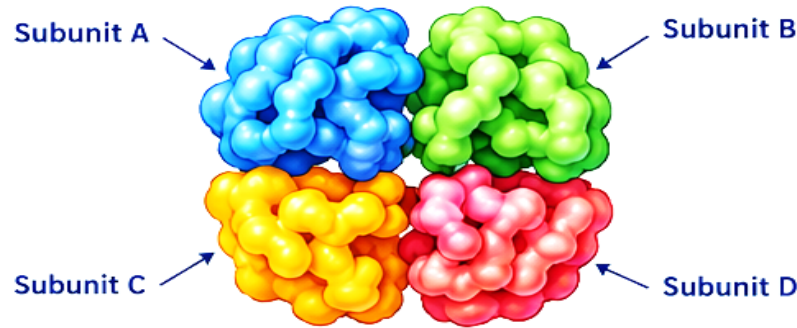


Multiple polypeptides

Quaternary structure

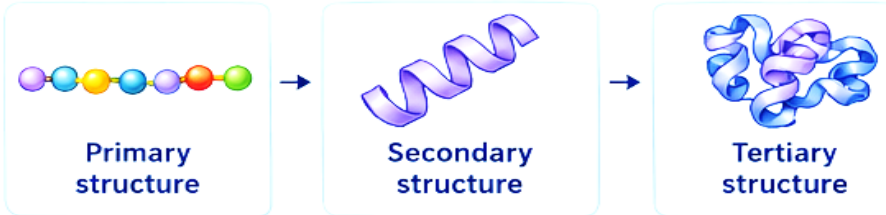
Subunits

Each individual polypeptide is called a **subunit**.



Protein complex

Each subunit can possess its own:

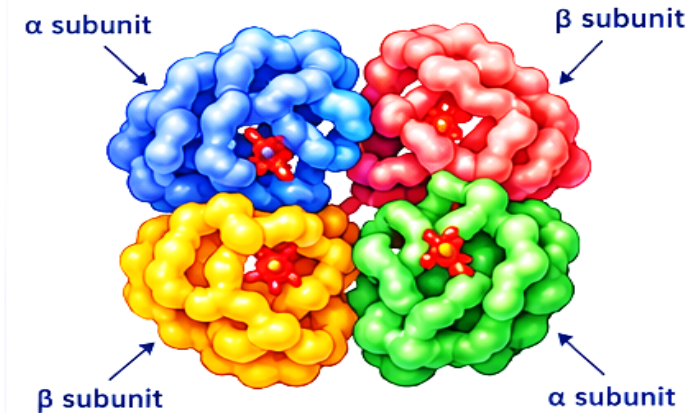


The subunits then associate to form the quaternary structure.

Example: Hemoglobin

Hemoglobin is a classic example of a protein with quaternary structure. It contains four polypeptide subunits:

- 2 α subunits
- 2 β subunits



Hemoglobin ($\alpha_2\beta_2$)

- = α subunit
- = β subunit
- ★ = Heme group (binds oxygen)

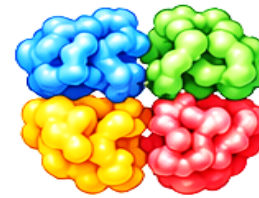
Summary of Quaternary Structure



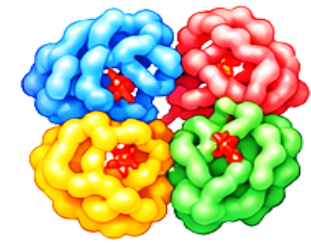
Single polypeptide chain
(subunit)



Individual subunits
(each with its own 1°, 2° and 3° structure)



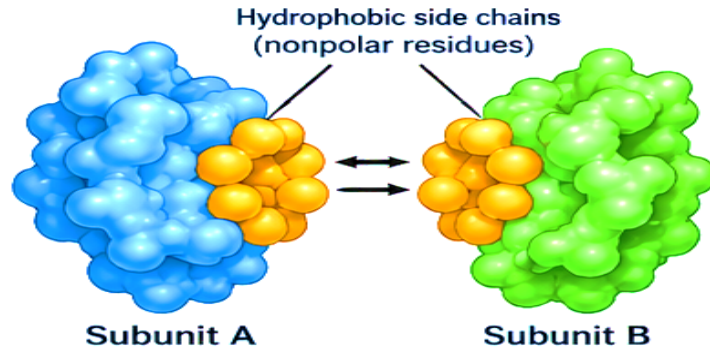
Association of subunits
(Quaternary structure)



Functional protein complex
(e.g., Hemoglobin)

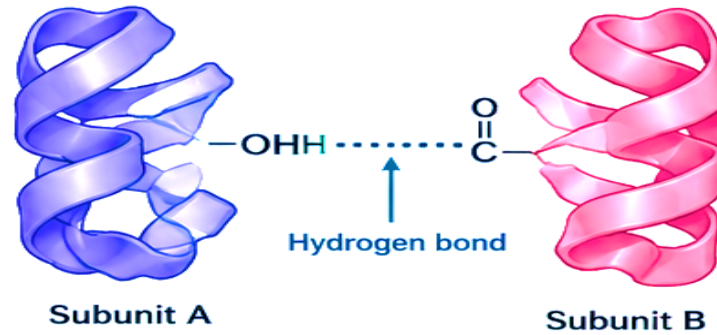
Stabilization of Quaternary Structure

Hydrophobic interactions



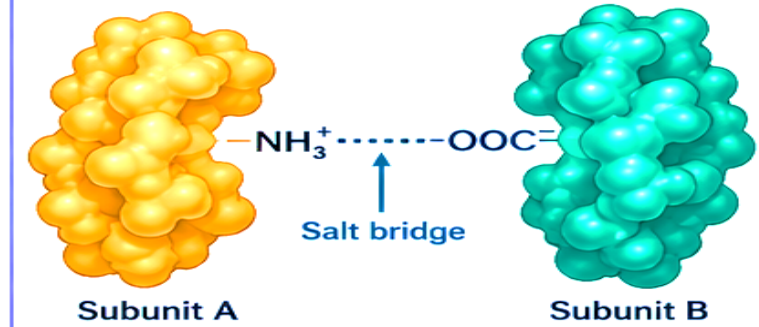
- Nonpolar (hydrophobic) surfaces of different subunits associate in an aqueous environment, avoiding water.
- This helps bury hydrophobic residues and stabilizes the quaternary structure.

Hydrogen bonds



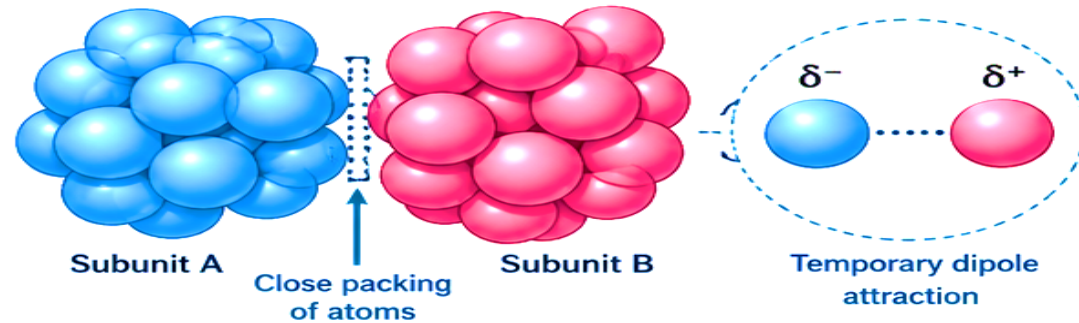
- Hydrogen bonds can form between polar side chains or backbone groups of different subunits.
- They contribute to the stability and specificity of subunit association.

Ionic interactions



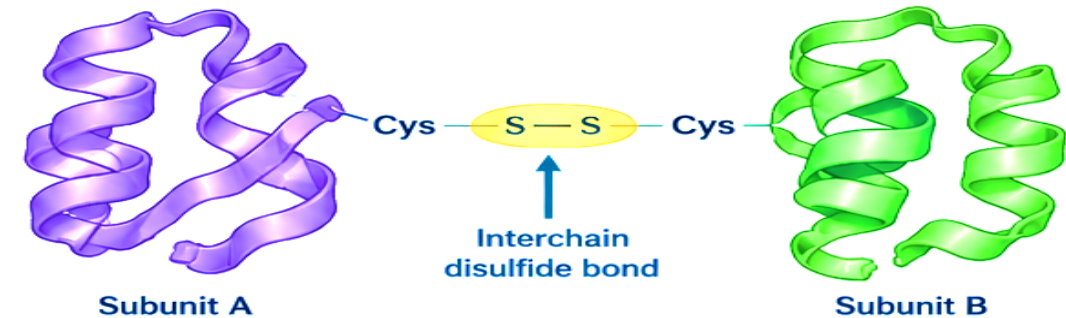
- Oppositely charged amino acid side chains (e.g., Lys^+ and Asp^- , Lys^+ and Glu^- , Arg^+ and Asp^- , Arg^+ and Glu^-) attract each other.
- These ionic interactions help stabilize the quaternary structure.

Van der Waals interactions



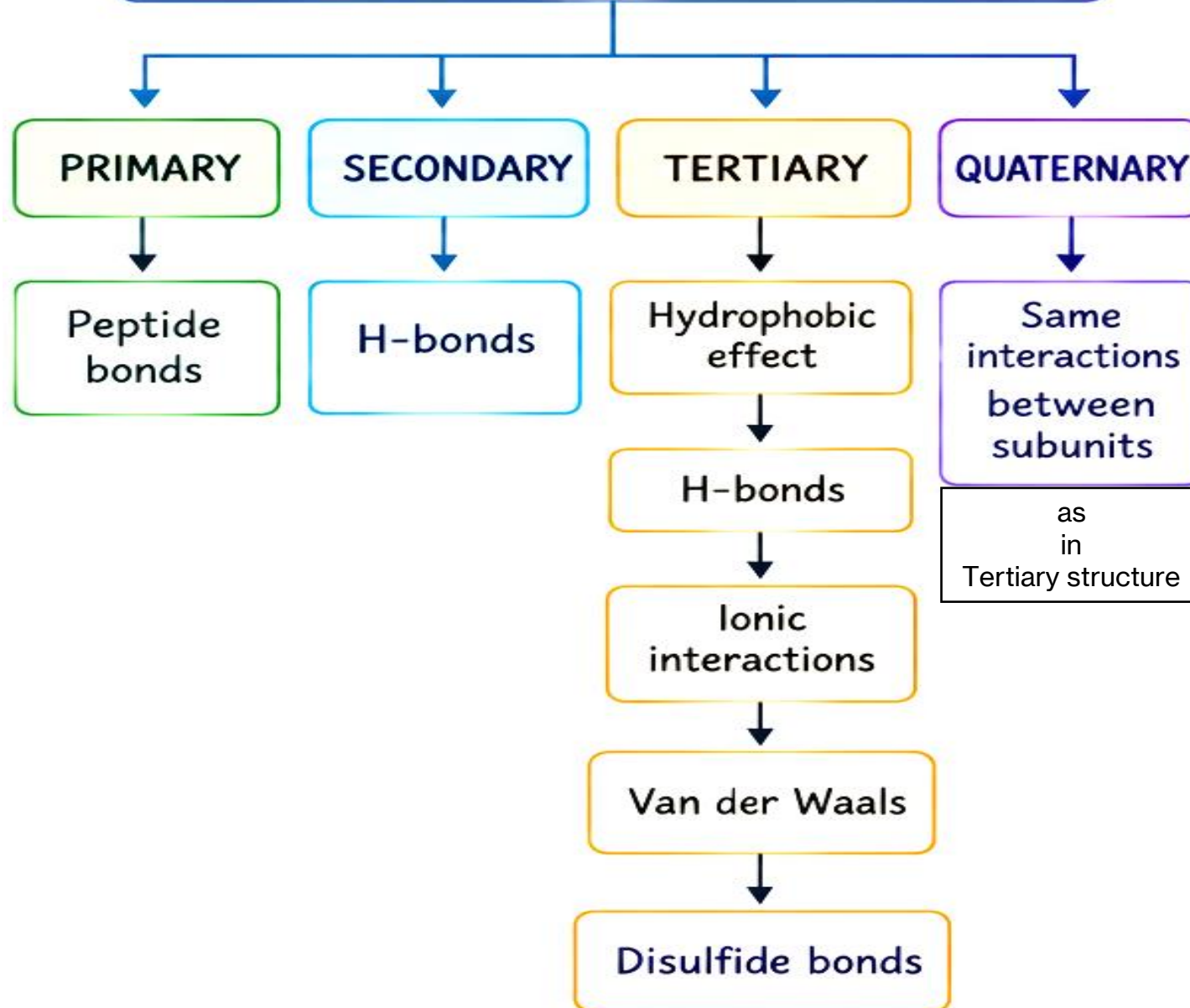
- Atoms of neighboring subunits come into close contact, causing temporary fluctuations in electron distribution.
- These weak attractions (van der Waals forces) occur in large numbers and collectively contribute to the stability of the quaternary structure.

Disulfide bonds



- Formed between the sulfur atoms of cysteine residues from different subunits.
- These strong covalent bonds help link subunits together and provide additional stability, especially in many extracellular proteins.

PROTEIN STRUCTURAL LEVELS



DENATURATION OF PROTEINS

Denaturation of Proteins

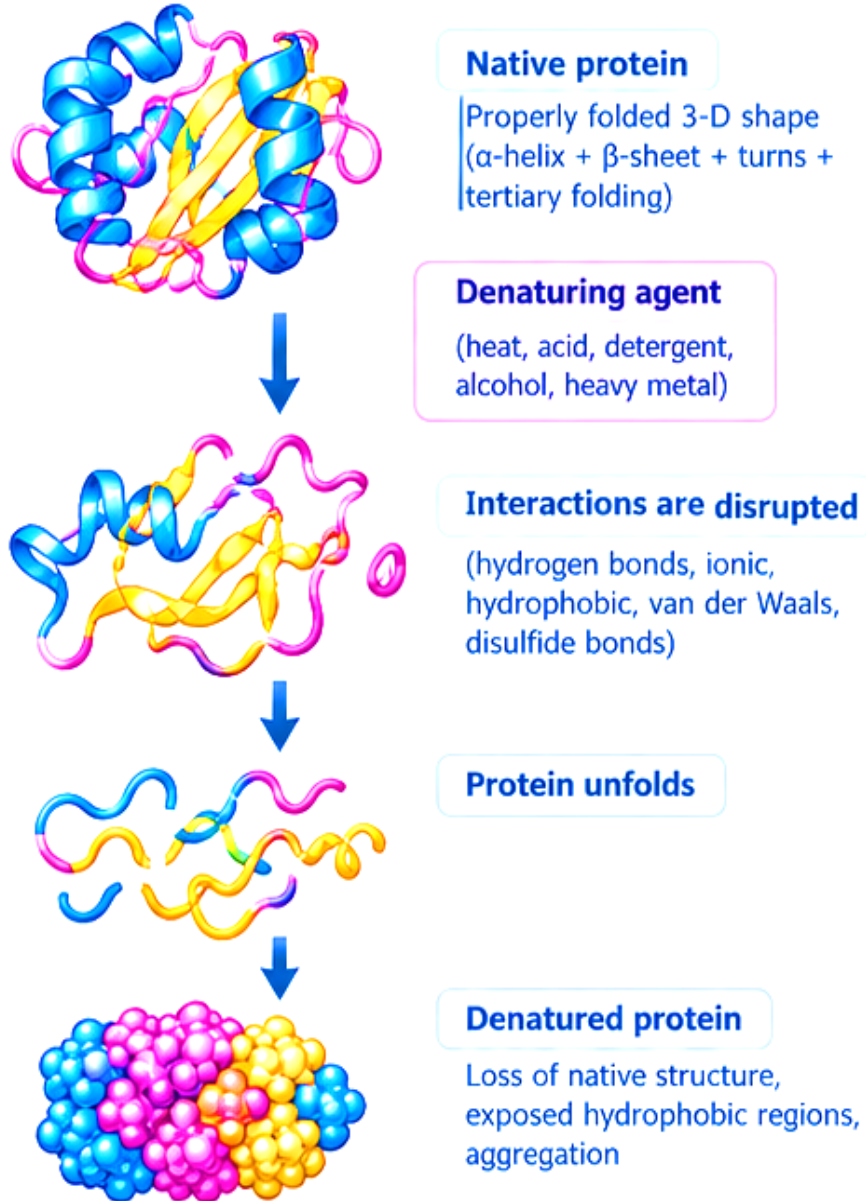
Protein denaturation is the process in which a **protein** loses its normal or **native three-dimensional conformation** due to changes in the physical or chemical environment.

The **primary structure** of a protein is determined by its **amino-acid sequence** and is held together by **peptide bonds**, whereas the **secondary and tertiary structures** depend mainly on **non-covalent interactions** such as **hydrogen bonds**, **ionic interactions**, **hydrophobic interactions** and **van der Waals forces**, as well as **covalent disulfide bonds**.

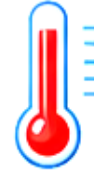
During denaturation, these interactions are disrupted, causing the protein to unfold or lose its normal shape.

Denaturation usually **does not involve breaking the peptide bonds**. Therefore, the amino-acid sequence or primary structure generally remains intact, while the higher levels of organization, particularly **secondary and tertiary structure**, are disturbed.

Process of Denaturation



Example of a Denaturant



Heat

- Increases molecular motion and disrupts weak interactions.



Strong acids or bases

- Alter ionization of side chains and disrupt salt bridges and hydrogen bonds.



Organic solvents

- Interfere with hydrogen bonds, hydrophobic interactions and solvent environment.



Detergents

- Disrupt hydrophobic interactions.



Reducing agents

- Break disulfide bonds ($-S-S-$) to $-SH$ groups.

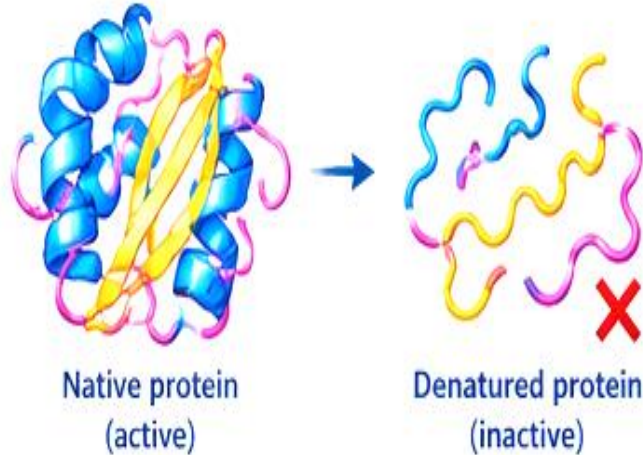


Heavy metal ions

- Bind to charged groups and sulfhydryl groups.

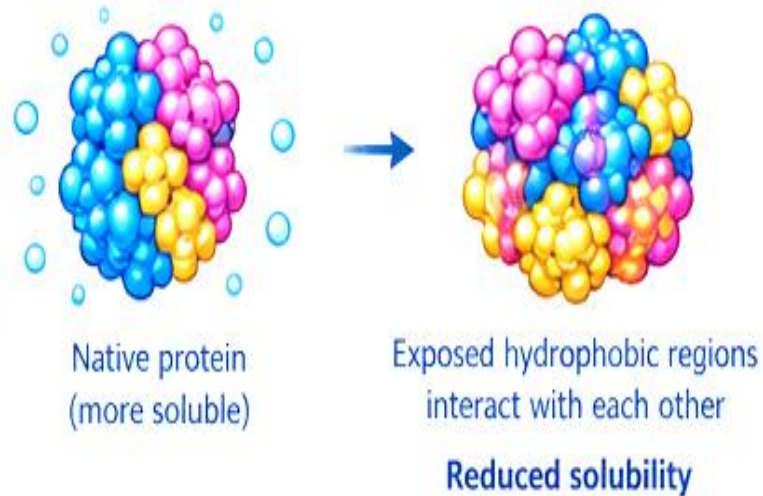
Consequences of Protein Denaturation

1. Loss of Biological Activity



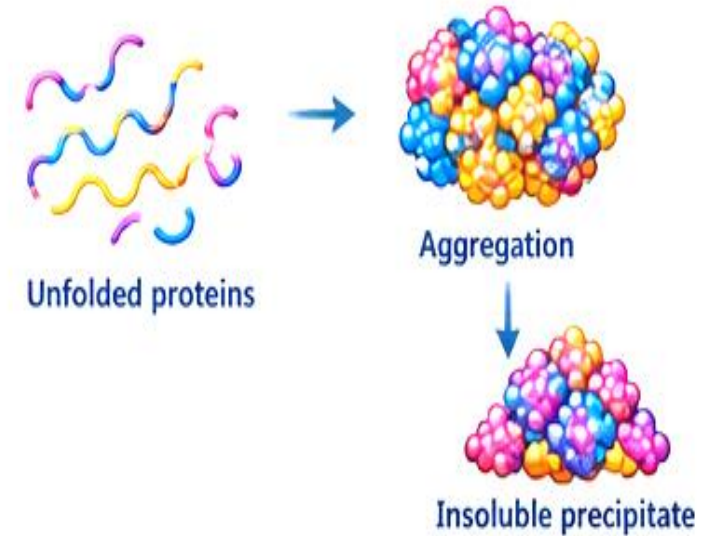
- The active or binding site may be altered, resulting in reduced or complete loss of activity.

2. Loss of Solubility



- Hydrophobic regions become exposed and interact, making the protein less soluble.

3. Aggregation and Precipitation



- Unfolded proteins can interact with each other through exposed hydrophobic regions and other groups.

The extent of denaturation determines the extent of functional loss. A protein may undergo **partial denaturation**, retaining some activity, or **complete denaturation**, resulting in almost complete loss of biological activity.

SIMPLE & CONJUGATE PROTEINS

Simple and Conjugated Proteins

Basic Concept

- Proteins are classified into two main types: **Simple proteins** and **conjugated proteins**.
- Simple proteins are made up of only **amino acids**, while conjugated proteins consist of a protein part (**apoprotein**) combined with a non-protein component (**prosthetic group**).
- The non-protein component is called a **prosthetic group**. A conjugated protein without its prosthetic group is called an **apoprotein**.
- Conjugated proteins are further classified based on the nature of their prosthetic groups, e.g. **carbohydrates**, **lipids**, **metal ions** etc.

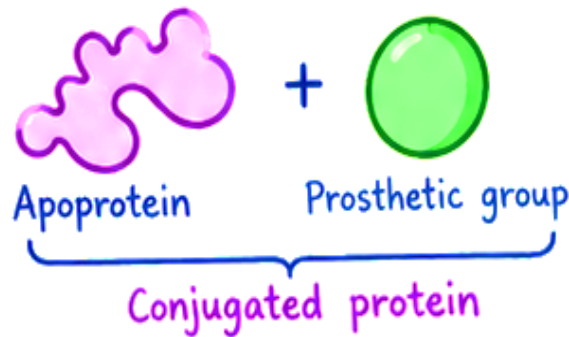
Types of Proteins

Simple Proteins

- Only amino acids
- No non-protein component
- Examples: Albumin, Globulin, Prolamin, Glutelin

Conjugated Proteins

- Protein + non-protein component
- Non-protein part = Prosthetic group
- Example: Glycoproteins, Lipoproteins, Phosphoproteins, Metalloproteins, Hemoproteins



Examples of Conjugated Proteins

Class	Examples	Prosthetic group / Non-protein component
Glycoproteins	- Fibronectin - Cadherin	Carbohydrate component (oligosaccharides).
Lipoproteins	Chylomicron	Lipid component (lipids + protein).
Metalloproteins	- Ferritin (Iron) - Alcohol dehydrogenase (Zinc) - Cytochrome oxidase (Copper and Iron) - Nitrogenase (Molybdenum and Iron)	Metal ions (e.g., Fe, Zn, Cu, Mo)
Hemoprotein	- Hemoglobin (Transport of oxygen) - Myoglobin (Storage of oxygen in muscle) - Cytochrome c (Electron transport chain) - Cytochrome P450 (Hydroxylation of xenobiotics) - Catalase (Degradation of hydrogen peroxide)	Heme group (iron-containing porphyrin).

Some examples with functions



Hemoglobin
(transports oxygen)



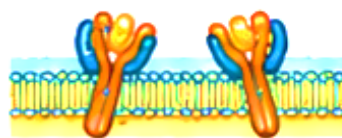
Immunoglobulin (Glycoprotein)
(immune function)



Chylomicron (Lipoprotein)
(transport of lipids)



Ferritin (Metalloprotein)
(stores iron)



Transferrin (Glycoprotein)
(transports iron)



Cytochrome c (Heme protein)
(electron transport)

- Simple proteins = only amino acids (no non-protein part).
- Conjugated proteins = protein + prosthetic group (carbohydrate, lipid, metal ion or heme).

References:

Kumar, P. & Mina, U. (2024). *Life Sciences: Fundamentals and Practice—Part I*. 9th ed. Pathfinder Publication, New Delhi.

Nelson, D. L. & Cox, M. M. *Lehninger Principles of Biochemistry*. W. H. Freeman.