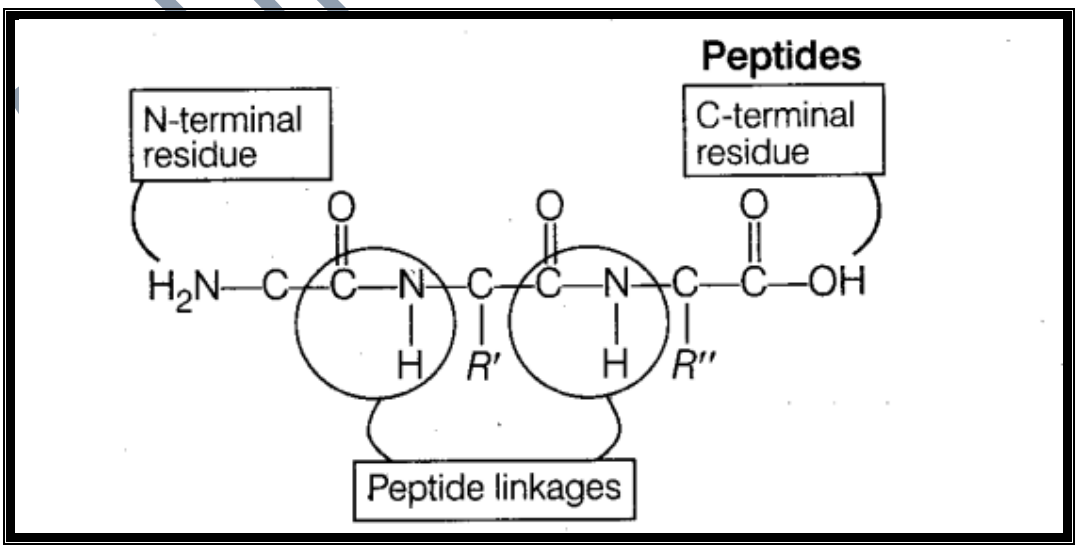
Proteins

- The word protein was coined by **Berzelius in 1838** and was used by **G. J. Mulder first time 1840.**
- **15% of protoplasm** is made up of protein.
- Average proteins contain 16% nitrogen, 50–55% carbon, oxygen 20–24%, hydrogen 7% and sulphur 0.3 – 0.5%. Iron**, phosphorous, copper, calcium, and iodine** are also present in small quantity.
- Proteins are made up of the **20 different amino acids**. These amino acids are joined together by a **covalent linkage** commonly known as a **peptide bond**. The linear sequence of these linked **amino acids is specific for a protein**.

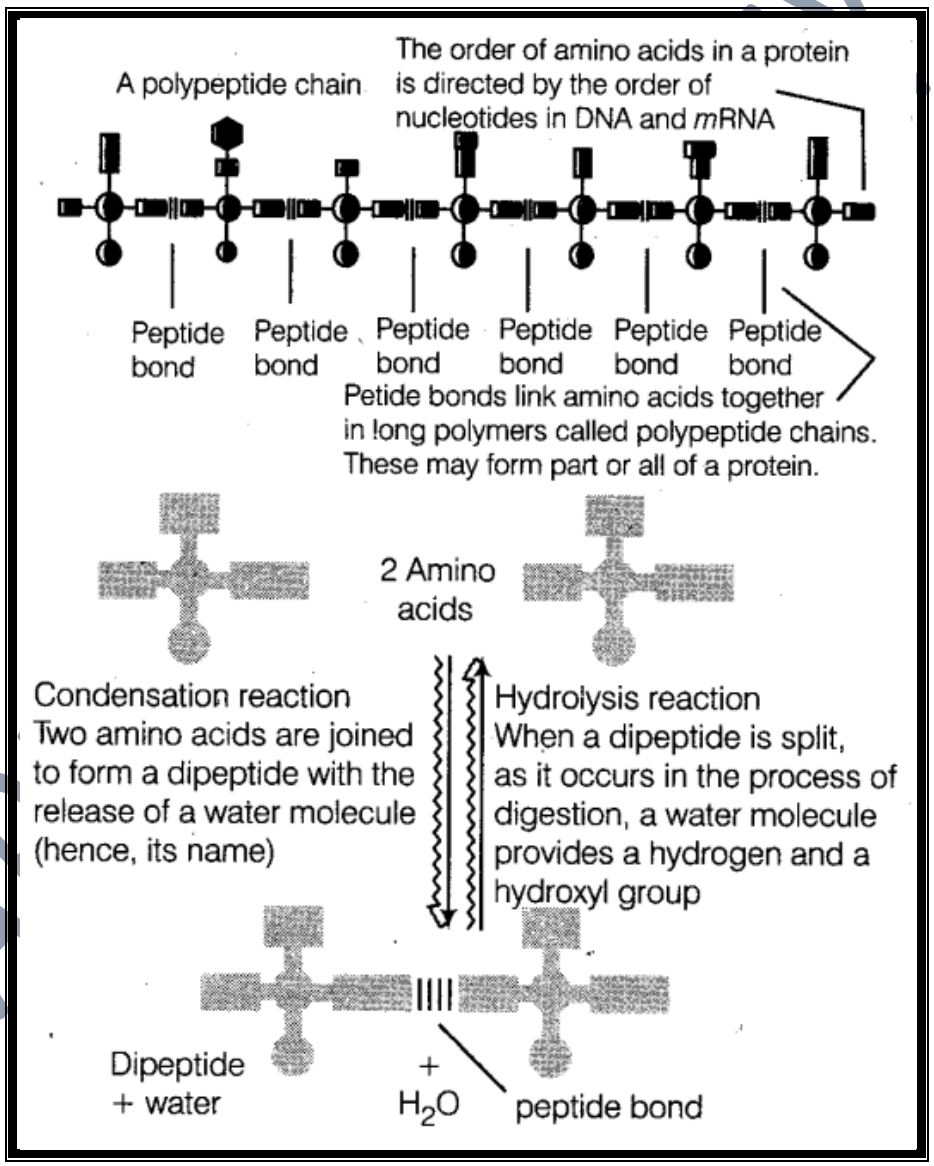
Peptide Bond

Proteins are the linear sequence of amino acids linked together by **peptide bonds**. The peptide bond is chemically, a covalent



There are many peptide bonds in a single protein molecule. Therefore, proteins are also called polypeptide.

- Dipeptide When two amino acids are joined together via a peptide bond, a dipeptide is formed.
- Oligopeptide It is a long, unbranched chain of 2-25 amino acids residues, which are linked by peptide bonds.
- Polypeptide A long chain of many amino acids (>25 amino acid residues) linked end to end by peptide bond.



- Amino acids can combine to form peptide chains by a process called **condensation reaction**. Peptide chains can be broken down by **hydrolysis to simple amino acids.**
- **Insulin (human) has 53 amino acids** arranged in two polypeptide chains of 22 and 31 amino acids. Human **serum albumin has 582 amino acids** in its polypeptide chain.

- Proteins show enormous diversity because of different proportions and sequence of amino acids. The number and **variety of proteins vary from species to species** and within a species from cell to cell.
- A **bacterium Escherichia coli {E. coli)** may have about **3000 types of proteins**. A human liver cell may have millions of proteins. However, all these proteins are synthesized from the **same 20 amino acids**.
- Proteins and amino acids are amphoteric in nature. It means they can react with both acids and bases. Proteins are oxidized by putrefaction process and produce bad smell
- The amino acid sequence contains **necessary information for that protein to fold into a unique three dimensional structure** and correspondingly a unique function. The structure of proteins can be best understood by considering them in four hierarchical levels

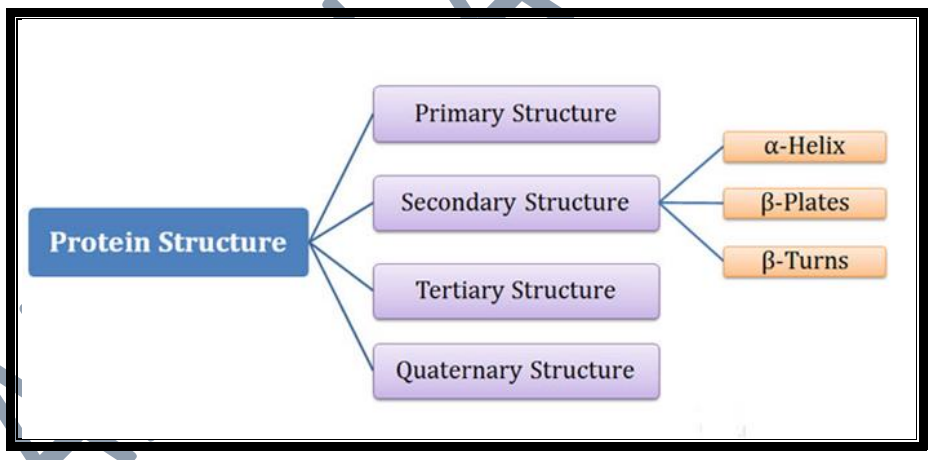
➔ A protein can have **Four** levels of structural organization:

(1). Primary Structure

(2). Secondary Structure

(3). Tertiary Structure

(4). Quaternary Structure



- Secondary, Tertiary and Quaternary structure are together called the **three-dimensional (3D) structure** of the protein.
- All functional proteins will have up to 3 **(tertiary level) of structures.**
- Some proteins will have all the **4 levels of structures (up to quaternary structure)**

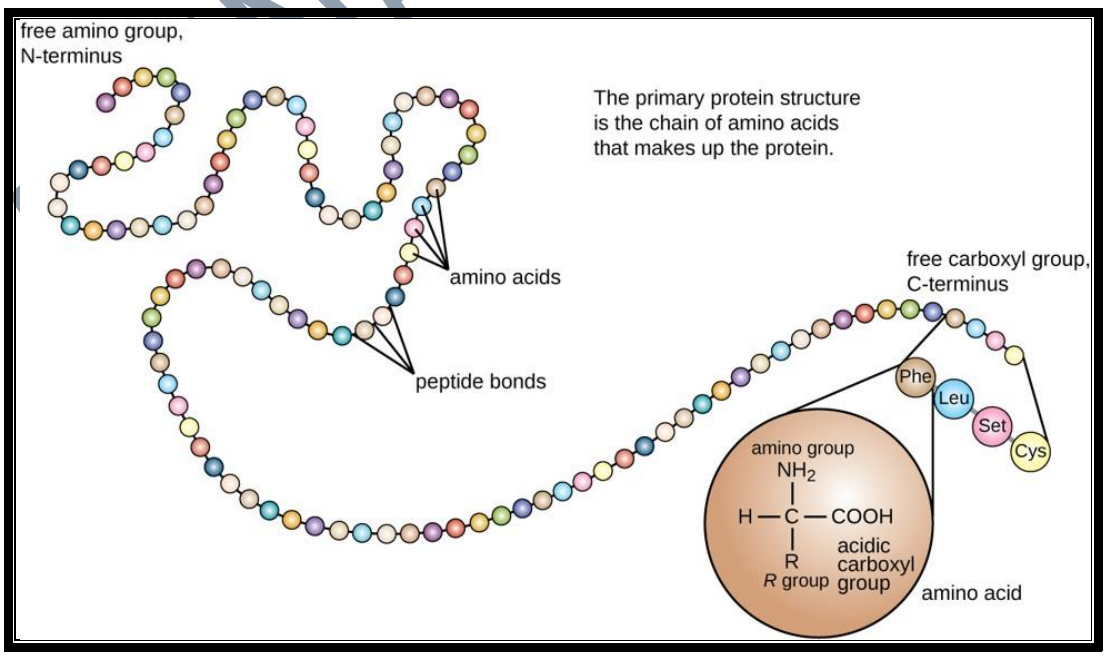
The primary structure of proteins

- The **amino acid sequence of a protein** is known as its **primary structure**.

- Knowing the primary structure for a protein is important because even small changes (due to mutations) in the primary structure can lead to **improper folding** and hence impairment or complete loss of function.
- The primary structure will tell you two main things: (i) The **number of amino acid residues** in the protein and (ii) the **sequence of amino acids**.
- The 'sequence' information contains the correct order of amino acids in the protein starting **from N-terminal to C-terminal.**
- The primary structure of a protein will determine all other levels of structural organization of a protein (**secondary, tertiary and quaternary).**
- The primary structure is stabilized by **Peptide Bonds** (**Covalent Bond**).
- The first study about an unknown protein will be its sequence determination **(determination of primary structure).**
- First sequenced protein: **Insulin** by **Frederick Sanger**.

Importance of Primary Structure:

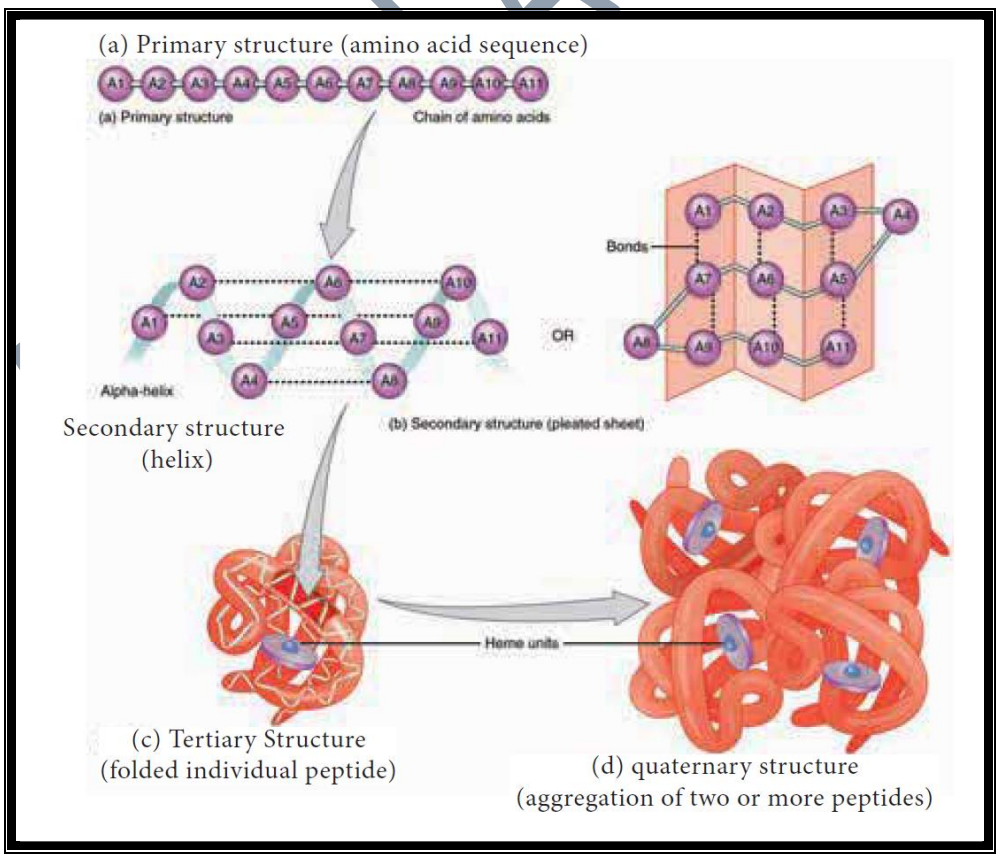
- Three dimensional **(3D) structure**
- **Function** of the protein
- Cellular **location**
- **Evolution** of the protein
- Primary structure data can be used for the sequence searching from the **protein databases**



Three Dimensional Structures of Proteins

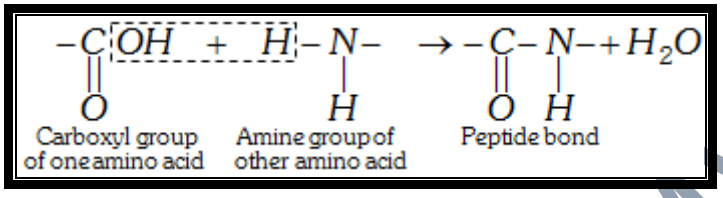
- ➔ The backbone of a protein contains hundreds of individual **bonds**.
- ➔ Free rotation is **possible around many** of these bonds.
- ➔ The free rotation allows an unlimited number of conformations around these bonds.
- ➔ However, **each protein has a specific** (unique) **structural conformation**.
- ➔ This unique structural formation of a protein is called its **3D structure**.
- ➔ The spatial arrangement of atoms in a protein is called its '**Conformation**'.
- ➔ Proteins in their functional, folded conformations are called **native proteins**.
- ➔ The conformations of a protein are mainly stabilized by weak interactions such as **hydrogen bonds**, hydrophilic interactions, hydrophobic interactions etc..
- ➔ These weak interactions can be easy distorted with less expenditure of energy.
- ➔ Proteins may have **three levels of Three Dimensional (3D)** Organizations. They are:
  - ✚ **Secondary structure**
  - ✚ **Tertiary structure**
  - ✚ **Quaternary structure**

Structural organization of proteins



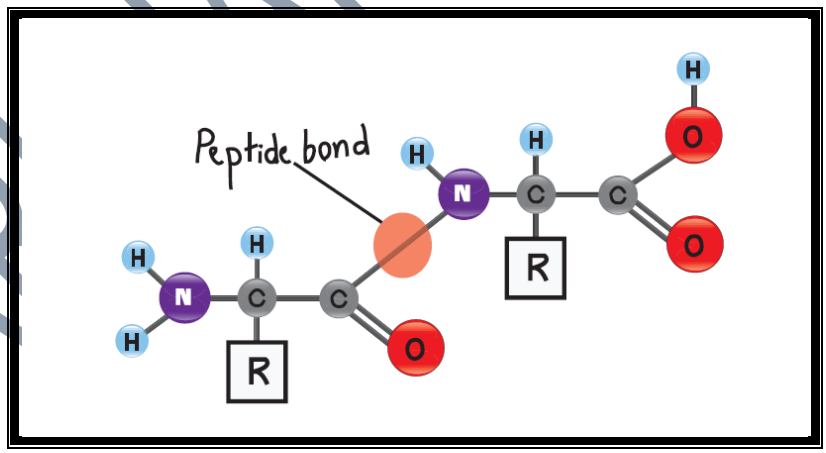
Peptide bonds

- **Formation of proteins-peptide bond:** Proteins are formed by joining the carboxyl group of one amino acid to the [alpha]amino group of another amino acid. The bond formed between two amino acids by the elimination of a water molecule is **called a peptide linkage or bond. The peptide bond is simply another name for amide bond.**



- The **amino acids in protein** are covalently linked together to form **peptide bonds**.
- Peptide bonds are amide linkages between **the α carboxyl group of one amino acid** and the **α amino group of another amino acid**. For example, serine and alanine can form a peptide called s**erylalanine**
- Since **two amino acids are joined together**, this molecule is known as a **dipeptide.** If many **amino acids are joined together** in the **same way to form a single chain**, such a chain is known as a **polypeptide**. The atoms excluding the side chains of amino acids in a polypeptide are known together as the back bone or **main chain of the polypeptide**

Chemical structure of a dipeptide and a peptide bond



Peptide bonds have some important properties

- Peptide bonds are generally in **trans conformation**. However in rare conditions peptide bonds formed by **proline can adopt a cis conformation**.
- Peptide bonds have a partial **double bond character**, which gives them a planar nature and hence **cannot be rotated**.

➔ Since peptide bonds are amide **linkages the –C=O and –NH** groups cannot donate or accept **protons and are uncharged.** The net charge of a polypeptide can come only from the **N terminus amino** group, **C terminus carboxyl group** and the side chains of the amino acids.

➔ Despite not being ionisable, **the –C=O and –NH** groups of peptide **bonds are polar** and can involve in the formation of **hydrogen bonds**. This property is important for the formation of **secondary structures of proteins**.

Secondary structure of proteins

❋ The back bone of a **polypeptide forms regular structural arrangements** by making hydrogen bonds with its neighbouring amino acids. As a rule, these hydrogen bonds are always between the main chain **–NH group and –C=O** group.

❋ Secondary structure is the special local conformation of some part of a **polypeptide chain.**

❋ It is the folding pattern of the regular polypeptide backbone.

❋ Different types of secondary structures occur in nature.

❋ The secondary structures are stabilized mainly by **Hydrogen Bonds.**

❋ Hydrogen bonds are weak electrostatic interactions between an electro negative atom and a hydrogen which is covalently linked to another electro negative atom.

❋ Three most important secondary structure in protein are:

- **α-Helix**
- **β-Conformations (β-plates)**
- **β-Turns**

α Helix

❋ **The α-helix is the** most common **secondary structure.**

❋ They are regular structures that **repeat every 5.4 Å.**

❋ It is the simplest arrangement of a **polypeptide chain.**

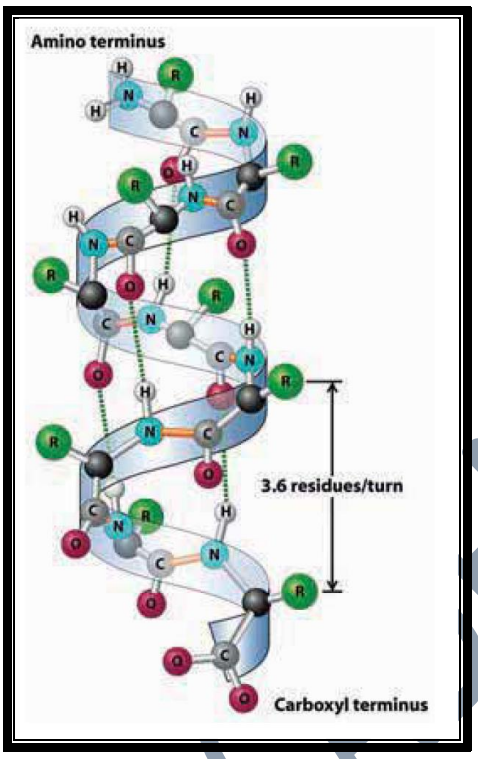
❋ The α-helical structure of the protein was proposed by **Pauling and Corey in 1951.**

❋ The polypeptide backbone is tightly wound around an imaginary axis drawn longitudinally through the middle of the **helix, and the R groups** of the amino acid residues protrude **outward from the helical backbone.**

❋ Pitch of helix: **The repeating unit of the helix.**

- The pitch is a **single turn of the helix** which extends **about 5.4 Å.**
- Each helical turn in **α-helix contains 3.6 amino acids**.
- The **helical twist of the α-helix** in all protein is **right-handed.**
- The α- helix is **stabilized by hydrogen bonds**.
- The α-helix is so common in protein because it makes optimal use of **internal hydrogen bonds.**
- Hydrogen bonds are formed between the **hydrogen attached to the electronegative nitrogen atom of the peptide** linkage and the electronegative carbonyl oxygen atom of the fourth amino acid on **the amino-terminal** side of **the peptide bond.**
- Within the α-helix, every peptide bond participates in hydrogen bonding.
- All hydrogen bonds **together provide considerable** stability to the α-helix.
- All polypeptide **cannot form a sable α-helix.**
- The interactions between **the amino acid side** chains can stabilize or destabilize the **α-helix.**
- For example, if a polypeptide chain has a long stretch of **Glutamic Acid residues**, this segment the chain **will not form an α-helix.**
- The negatively charged carboxyl group of the adjacent Glu residues repels each other strongly so that they prevent the formation of the α-helix.
- Similarly, a **polypeptide rich in Proline** will not form **an α-helix.**
- In proline, **the nitrogen atom is part** of a rigid ring and rotation about the **N – Cα bond is not possible.**
- Thus **proline** introduces a destabilizing kink in the **polypeptide and hence proline** is very rarely found **in α-helix**.
- It is a **spiral (helical) structure** of a **tightly packed** and coiled main chain of a **polypeptide** with the side chain **groups of amino acids** protruding **outside**.
- The **helical structure** is achieved by the formation of hydrogen bonds between the –C=O of an nth amino acid with the –NH group of n+4th amino acid. Each turn of an α helix contains **3.6 amino acids**.
- The helices are mostly right handed but there are rare instances where **left handed α** helices are also **present in proteins.** The amino acid **Proline** can produce a kink in an α helix as its **secondary amino group** is not geometrically compatible inside an α helix.

Structure of an α helix



β pleated sheets (β-Conformations)

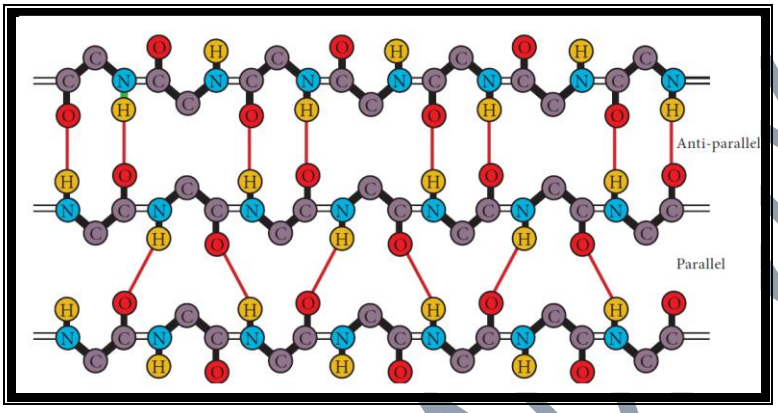
- In a **β pleated sheet**, two or more **segments of a polypeptide chain** line up next to each other forming a sheet like structure held together by **hydrogen bonds**.
- The strands of a **β pleated sheet may be parallel where the N- and C- termini** of the strands match up or **antiparallel where the N-terminus** of one strand is positioned next to the **C-terminus of the other.**
- The β-conformation is an extended form of a **polypeptide chain**.
- Here the polypeptide backbone is extended into a **zigzag structure**.
- The zigzag polypeptide chains can be arranged side-by-side to form a structure resembling a series of pleats called **β-sheets**.
- Here also the structure is stabilized by **hydrogen bonds**.
- However, unlike α-helix, the hydrogen bonds are formed between adjacent segments of the chain.
- The R-groups of adjacent amino acids protrude from the **zigzag structure** in opposite direction creating the alternating pattern.
- The polypeptide chains in the **β-sheets may be arranged either in parallel** (the same direction) **or anti-parallel** (opposite direction).

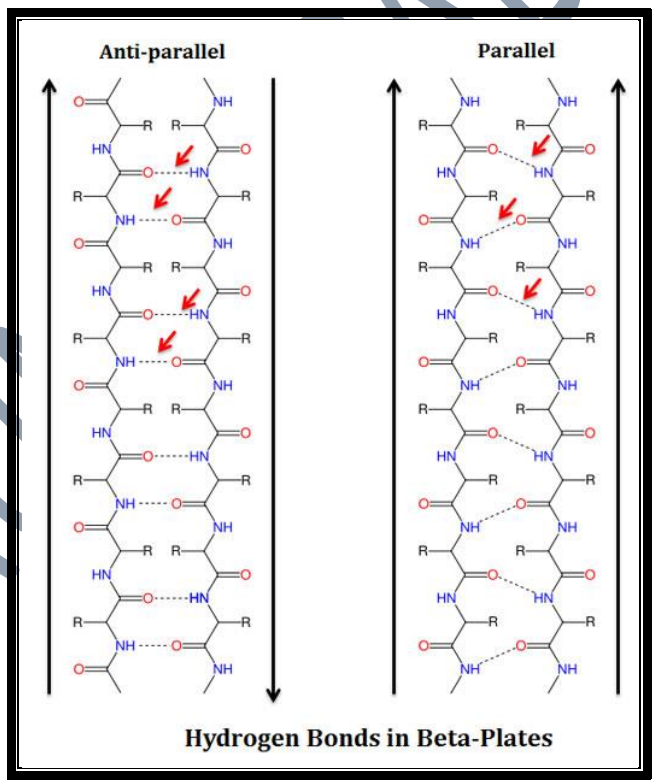
Based on this the β-Plates are classified into two types:

(a) Anti-parallel β-Plates

(b) Parallel β-Plates

Structures of parallel and anti parallel β sheets

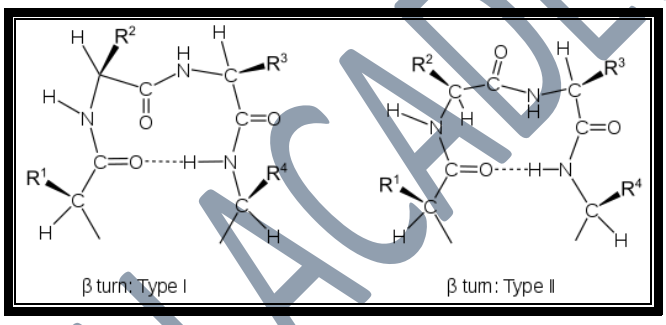




β-Turns:

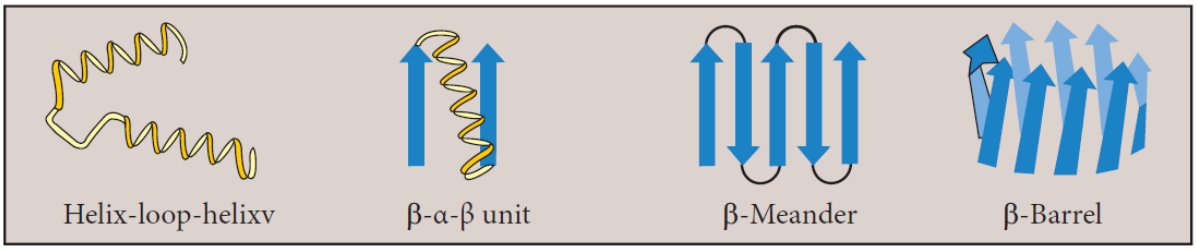
✱ The β-turns are very common in proteins, where the peptide make a turn or loop (peptide make a reverse direction).

- These are secondary structural elements with four amino acids that can reverse (turn) the direction of a polypeptide and thus help the polypeptide to form a globular shape.
- They are mostly found on the surface of proteins. The amino acids **proline and glycine** are more frequently found in β turns.
- They are also mostly found to connect **two different α helices or β strands** to form super secondary structure motifs such as helixturn helix, beta meander, beta barrel etc.
- In globular proteins, nearly one-third of **the amino acid residues are in β turns.**
- The β-turns are the connecting elements that **link successive runs polypeptide chain.**
- The β-turn connects the ends of two adjacent segments of anti-parallel β-sheets.
- The β-turn structure is an **180º turn** involving four amino acid residues
- The **carbonyl oxygen** of the first residue forms a hydrogen bond with the amino group hydrogen of the fourth amino acid in the turn.



- **Glycine (Gly) and Proline (Pro**) resides frequently occurs in **β-turns**.
- **Glycine due t**o its very small size **(the R group is – H) allows β turns**.
- **Proline** is an **imino acid** with its side chain covalently linked with the **amino group.**
- **Proline** residue in the peptide bond assumes the **'cis'** configuration.
- The 'cis' conformation is very amenable to **tight turns**.
- There are several **types of β turns of with type I and Type II** are most common.
- **The type I β-turn** is found more than twice the **frequency of type II.**
- In type II, the third residue will always be a **Glycine residue.**

The secondary structure elements connected through β turns



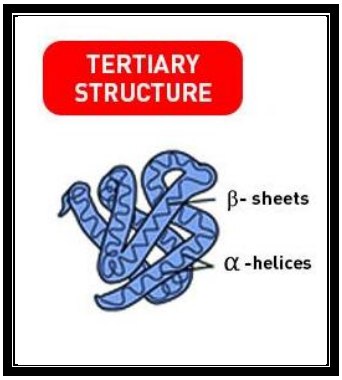
Comparison between α helix and β pleated sheet

α Helix	β Sheet
1. The interacting residues are always from a continuous stretch of polypeptide chain.	1. The interacting residues are not from a continuous stretch of a polypeptide chain.
2. Forms a tightly coiled structure	2. Forms a fully extended structure
3. The hydrogen bonds are parallel to direction of the polypeptide backbone	3. The hydrogen bonds are perpendicular to the direction of the polypeptide back bone
4. Can exist as a right handed or left handed helix	4. Can exist as parallel or anti parallel sheets
5. The amino acids Methionine, alanine, leucine, glutamic acid and lysine have higher probabilities of occurrence in α helix.	5. The amino acids isoleucine,valine,threonine, phenylalanine and tyrosine have higher probabilities of occurrence in a β sheet.

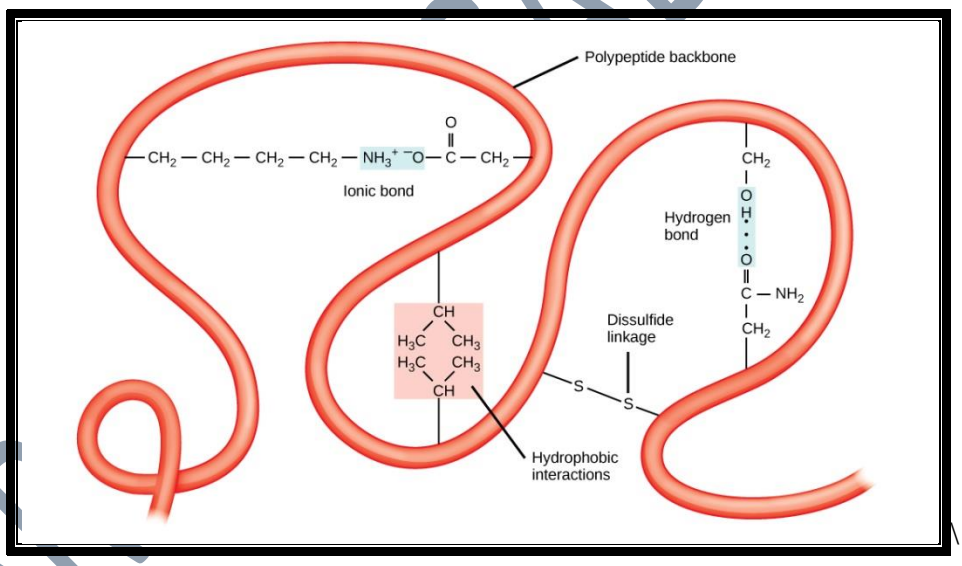
Tertiary structure

- The polypeptide folds in such a way that the **secondary structure elements** are packed compactly to form an overall **three-dimensional** structure called its **tertiary structure**.
- The **tertiary structure** will have a single **polypeptide "backbone**" with one or more secondary structures.
- Tertiary structure is defined by the **atomic coordinates**.
- Tertiary structures in a protein are stabilized by both **covalent and non-covalent bonds.**
- Covalent bond: **Disulfide bonds (between two Cys residues**)
- **Non-covalent interactions**: **Ionic interactions (electrostatic attractions**), hydrophilic **interactions**, **van der Waals interactions.**
- The term '**Domain**' is used to denote a single functional unit of a protein.
- A protein may have many domains with specific functions.
- A protein with a single subunit only has up to the tertiary structure.
- The tertiary structure is stabilized mainly by the interactions between the R groups (side chains) of the amino acids.
- The interactions that contribute to tertiary structure are **hydrogen bonds, ionic interactions, dipole-dipole interactions and Vander Waals Forces**.
- The side chains with like charges such as **Lys and Arg repel** one another, while those with opposite charges such as **Lys and Asp** can form an ionic interaction. **Similarly, polar R groups can form hydrogen** bonds and other **dipole-dipole interactions**.
- The **amino acids with non polar, hydrophobic R** groups cluster together on the inside of the protein through hydrophobic interactions. This cluster is also **known as the hydrophobic core** and it is an important **feature of globular proteins**.

- Similarly, the **hydrophilic amino acids,** the **amino acids with side chains containing charged groups are present on the surface of globular** proteins to interact with surrounding water molecules.



- The **sulphur containing side** chains of **two cysteine** residues can form a covalent bond known as a disulfide bond. The disulfide bonds help to bring together two different parts of the same polypeptide or two different polypeptides together and are the **only covalent interactions** involved in the formation of tertiary structure.

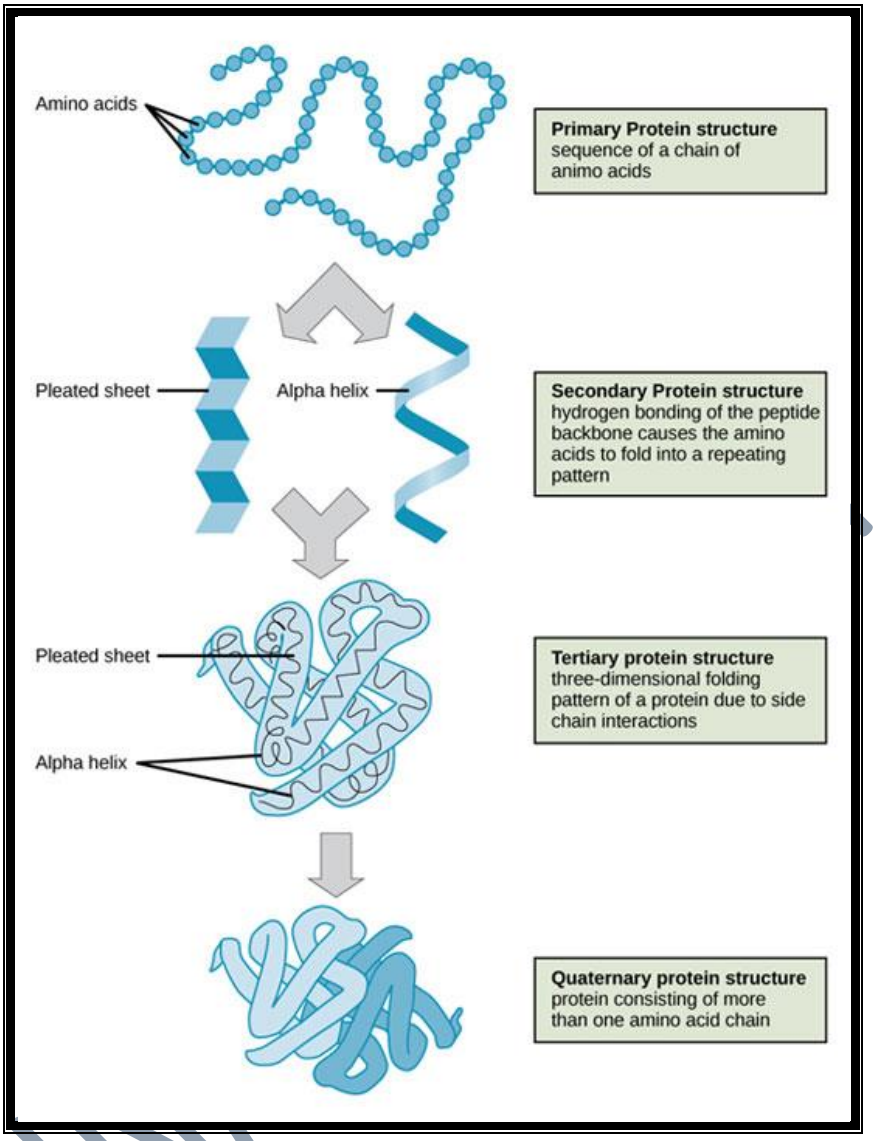


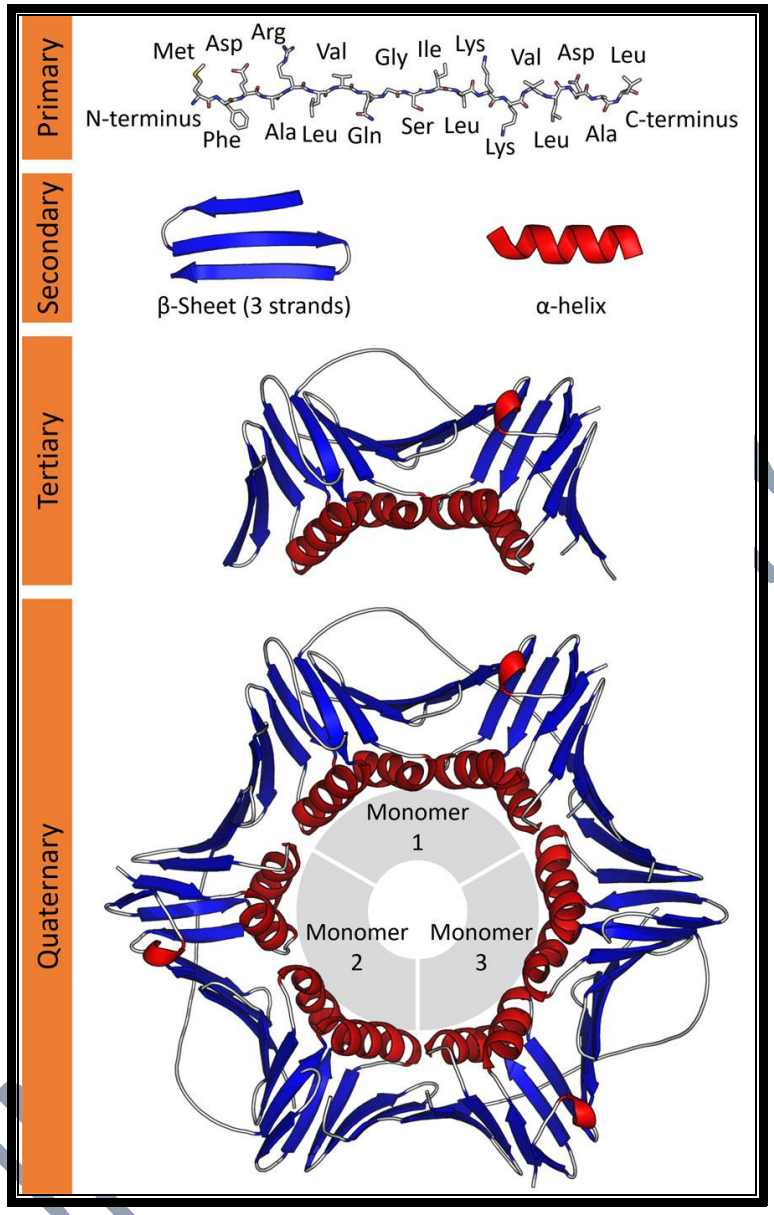
The tertiary structure of proteins is determined by a variety of chemical interactions. These include hydrophobic interactions, ionic bonding, hydrogen bonding and disulfide linkages.

Quaternary structure of proteins

- Proteins that are made up of a single polypeptide chain have only three levels of structure. Some proteins are made up of more than one polypeptide chain. In such cases the tertiary structures formed by each of those polypeptide chains come together to **form a quaternary structure.**

- These individual polypeptide chains are also known as subunits. Hemoglobin, a protein which carries oxygen in **blood** is made up of four subunits. Similarly, **DNA polymerase,** an **enzyme which synthesizes** new strands of **DNA is composed of ten subunits**. The same types of interactions that contribute to **tertiary structure** are also involved in stabilization of the **quaternary structure**.
- Majority of functional protein contains more than **one polypeptide** chains and such a protein is said to be **oligomeric**.
- Each peptide forms a **sub-unit** or **monomer** or **protomer**.
- The arrangement of **protein monomers in three-dimensional** complexes in a multi-subunit protein is called **quaternary structure**.
- For a protein to have a quaternary structure, it should fulfil two conditions:
- It should have more than **one polypeptide subunits**
- There should not have **permanent (covalent**) interaction between the subunits (like **disulfide bond).**
- **Insulin** does not have the quaternary structure even if it **contains two subunits.**
- The **two polypeptides** in insulin are covalently connected **with two disulfide bonds**.
- Thus, insulin can have up **to tertiary structure (not quaternary structure).**
- Bonds stabilizing quaternary structure: **hydrogen bonds, hydrophilic interactions**, **hydrophobic interactions, van der Waals interactions.**


Amino acids
Primary Protein structure
sequence of a chain of animo acids
Pleated sheet
Alpha helix
Secondary Protein structure
hydrogen bonding of the peptide backbone causes the amino acids to fold into a repeating pattern
Pleated sheet
Alpha helix
Tertiary protein structure
three-dimensional folding pattern of a protein due to side chain interactions
Quaternary protein structure
protein consisting of more than one amino acid chain



Chemical Bonds Involved in Protein Structure and Conformation

Proteins are the polymers of amino acids. Amino acids are **joined together by a special type of covalent bond (peptide bond) to form linear structures** called polypeptides. The polypeptides are then folded into specific structures to form the functional conformation of the protein. The folding of proteins into specific shapes and conformations are assisted and stabilized by many types of bonds in them. Some of these bonds are strong bonds whereas others are **weak interactions**. Important types of bonds involved in protein structure and conformation are **Peptide bonds**, **Ionic bonds, Disulfide bonds, Hydrogen bonds and Hydrophobic Interactions.** The current post describes the importance of each of these bonds and their role in the functional conformation of the protein.

Different types of bonds present in a protein

(1). Peptide bond

(2). Ionic bond

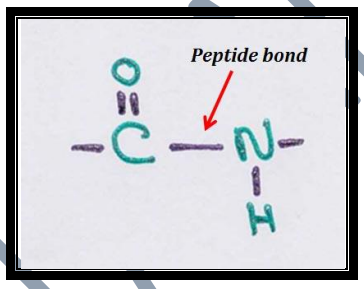
(3). Disulfide bond

(4). Hydrogen bond

(5). Hydrophobic Interactions

(1). Peptide Bond

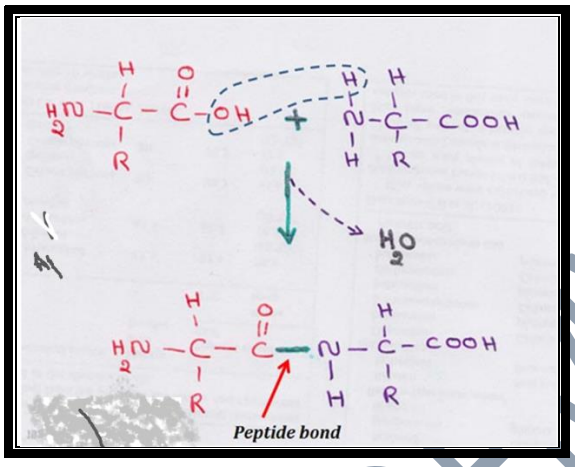
- a covalent bond formed between the carboxylic group of one amino acid and the amino group of another amino acid.
- Peptide bond is a strong covalent bond with high bond dissociation energy.
- It is formed by the joining of two amino acid residues during protein synthesis.
- The carboxylic group (- COOH) of one amino acid combine with the amino group ($-NH_2$) of another amino acid to form the peptide bond.



The peptide bond is represented as follows:

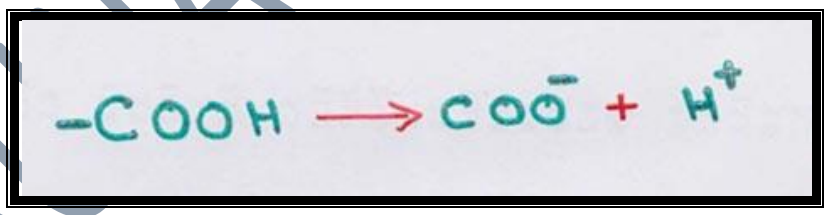
- Peptide bond formation is an example for a **condensation or elimination reaction.**
- One molecule of water is eliminated during the formation of peptide bond by the condensation reaction **of two amino acids**.
- The resulting compound after the peptide bond formation is called **a dipeptide.**
- A dipeptide has a free amino group at **one end and a carboxylic group** at the other end.
- The free amino **group or carboxyl group of a dipeptide** can form another peptide bond with a third amino acid and so on.
- Many amino acids join together in this manner to form a polypeptide.
- Peptide bond formation is facilitated by the enzyme **Peptidyl transferase** during the **translation process of protein synthesis**.
- **Peptidyl transferase** enzyme is a **ribozyme; it is a part of the ribosomal RNA (rRNA) of large subunit of ribosome.**

- In prokaryotes the **23S rRNA and in eukaryotes the 28S rRNA** acts as the Peptidyl transferase enzyme.
- The primary structure of the **protein is stabilized by peptide bonds**.

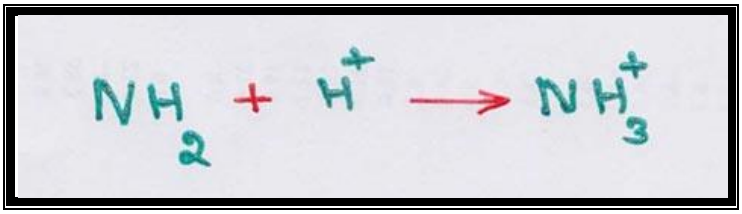


(2). Ionic bond:

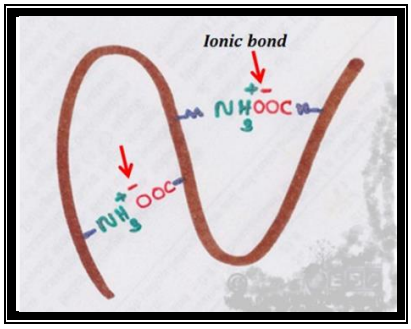
- Ionic bond definition: a chemical bond formed between two ions of opposite charges.
- In proteins, the ionic bonds are formed between the ionized acidic or basic groups of amino acids.
- The R groups (side chain) of certain amino acids contain additional acidic ($-COO^-$) or basic ($-NH_3^+$) groups.
- These R groups can ionize to produce charged groups at certain pH.
- Acidic R groups will be negatively charged since they release the H^+ ions.



- Basic R groups will be positively charged since they accept the H^+ ions from the medium.



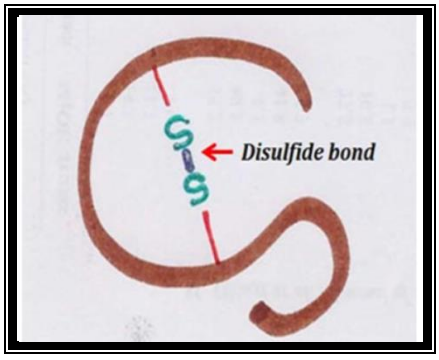
- After the ionization of side chain as mentioned above, the amino acids in the protein chain can attract or repel each other based on their charges.
- The attraction of oppositely charged **R groups** results in the **formation of ionic bonds**.



- **Ionic bonds** are weak bonds and they are very **fragile in an aqueous medium**.
- Even a change in the **pH may** breakdown the ionic bonds.
- This is the reason for the **denaturation of proteins** in the **acidic or basic medium**.
- Tertiary and quaternary structures of proteins are stabilized by **ionic bonds**.

(3). Disulfide bond

- Disulfide bond: a covalent bond formed from two thiol groups of two cysteine residues in a protein.
- The cysteine (Cys or C, a sulfur containing amino acid) contain a highly reactive sulfhydryl group (-SH) in its side chain (R group).
- The sulfhydryl is highly polar and highly reactive.
- If two molecules of a cysteine line up alongside each other, the neighboring sulfhydryl groups can be oxidized.
- This reaction results in the formation of a permanent covalent connection between two cysteine residues called disulfide bond.
- Disulfide bond in protein chemistry is better known as the disulfide bridge or S-S bond.



- Disulfide bond is a **covalent bond**.
- They are very strong bonds and are **not easy to break.**
- A disulfide bond may be formed between the **cysteine residues of same polypeptide** chain or different polypeptide chain of a functional protein.

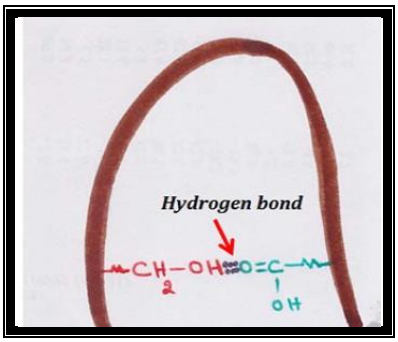
- Disulfide bonds stabilize the **tertiary structures of the protein.**

(4). Hydrogen bond:

- Hydrogen bond is an electrostatic attraction between a hydrogen atom, which is covalently bound to a high electronegative atom (such as **Oxygen and Nitrogen**), to another electronegative atom of same or different molecules of their close vicinity.
- Hydrogen present in **the –OH group or –NH_2 of amino acids** become slightly electropositive.
- This is due to the high electronegativity of O and N when compared to hydrogen.
- Due to the high **electronegativity, Oxygen and Nitrogen** attract the shared electron of hydrogen more towards them.
- Thus hydrogen attached to these high electronegative atoms will get a partial positive charge called **δ positive** whereas the electronegative atoms will get a partial negative charge called **δ negative**.
- Consequently, the slightly positive H is then attracted towards the neighboring electronegative **oxygen of -C=O or nitrogen atom of -NH2 group**.
- These **-C=O and NH_2** groups occur along the length of the polypeptide chain in regular sequence.
- Thus the formation of hydrogen bonds gives a regular shape to the polypeptide chain such as **alpha helix and beta plates.**
- Hydrogen bonds are **very weak bonds**.
- Occurrence of hydrogen bonds in high frequency makes a considerable contribution towards the **molecular stability of proteins.**
- Hydrogen bonds are involved in stabilizing the **secondary, tertiary and quaternary structures of proteins.**

(5). Hydrophobic Interactions

- Some R groups in the amino acids are non-polar.
- Example: alanine, valine, isoleucine, leucine and methionine
- The non-polar R groups are hydrophobic and they try to stay away from water.
- In a long polypeptide chain, there may be many such non-polar amino acids which may be adjacent to each other or separated by polar R groups.
- In an aqueous environment (inside the cell) the linear polypeptide will fold into such a shape that the hydrophobic amino acids come in close contact with each other and they try to exclude the water due to its hydrophobicity.



- By this method, the peptide chain of a globular protein will fold into a spherical shape in the **aqueous environment.**
- In a folded protein the hydrophobic groups tend to **orient towards the inner side** of the protein.
- The hydrophilic residues will form a shell over the **hydrophobic moieties**.
- The hydrophilic shell makes the protein **soluble in the aqueous environment**.
- Similarly, in the unit membrane, the orientations of membrane proteins are also affected by **the hydrophilic and hydrophobic interactions.**
- The hydrophilic domain of membrane protein orient towards the exterior of the membrane whereas the **hydrophobic domain will orient towards** the interior (to the lipid portion)

- **Peptide bonds :** strongest bond in proteins.
- **Hydrogen bonds** : These occur between hydrogen and oxygen atoms of various groups.
- **Disulphide bond** : These bonds form between - SH group of amino acids (e.g., methionine, cysteine). These bonds are second strongest bond and stabilise the tertiary structure of protein.
- **Hydrophobic bonds** : Present between amino acids which have hydrophobic side chains, e.g. aromatic amino acids.
- **Ionic bonds** : Formation of ionic bond occurs between two opposite ends of a protein molecule due to electrostatic attraction. Majority of proteins and enzymes in protoplasm exhibit tertiary structure.

Classification of Proteins Based on Structure and Function

Proteins are important macromolecules of the cells, formed by the polymerization of amino acids according to the sequence of genetic code in the **mRNA.** Proteins are the mode of expression of the genetic information. They perform a variety of duties in the cells such as they act as the structural **components of cells, enzymes,** hormones, pigments, storage proteins and some toxins in the cells. The proteins are classified into many categories based on **different criterions.**

I). Classification of Proteins based on the Structure of Proteins

Based on the structure, proteins are classified into 3 groups.

(A). Fibrous Proteins

(B). Globular Proteins

(C). Intermediate Proteins

(A). Fibrous Proteins

- They are **linear (long fibrous) in shape**.
- Secondary structure is the most important functional structure of fibrous proteins.
- Usually, these proteins **do not have tertiary structures.**
- Physically fibrous proteins are very tough and strong.
- They are insoluble in the water.
- Long parallel polypeptide chains cross linked at regular intervals.
- Fibrous proteins form long fibres or sheaths.
- Functions of fibrous proteins: perform the structural functions in the cells.

  Examples of fibrous proteins: **Collagen, Myosin, Silk and Keratin.**

(B). Globular Proteins

- Globular proteins are **spherical or globular in shape.**
- The polypeptide chain is **tightly folded into spherical shapes.**
- Tertiary structure is the most important functional structure in **globular proteins**.
- Physically they are soft **than fibrous proteins.**
- They are readily **soluble in water.**
- Most of the proteins in the cells belong to the **category of globular proteins.**
- Functions: Form enzymes, antibodies and some hormones.

  Example: **Insulin, Haemoglobin, DNA Polymerase and RNA Polymerase**

(C). Intermediate Proteins

- Their structure is intermediate to linear and globular structures.
- They are short and more or less linear shaped proteins
- Unlike fibrous proteins, they are soluble in water.
- Function: blood clotting proteins

  Example: **Fibrinogen**

(II). Classification of Proteins based on Composition:

Two broad categories of proteins according to its composition, they are:

(A). Simple Proteins

(B). Conjugated Proteins

(A). Simple Proteins

- Simple proteins composed of ONLY amino acids.
- Proteins may be fibrous or globular.
- They possess relatively simple structural organization.

Example: Collagen, Myosin, Insulin, Keratin

- **Albumins** -Serum albumin, egg albumin
- **Globulins** -Serum globulin, tissue globulin
- **Glutelins**-Glutenin, oryzenin
- **Prolamins**-Zein, hordein
- **Albuminoids**-Keratin, collagen, fibroin
- **Histones**- Nucleic acid
- **Protamins**-Salmine, sturine

Is DNA a protein?

DNA is often associated with proteins in the nucleus called histones, but DNA itself is not a protein. No. DNA is a nucleic acid consisting of phosphate and sugar groups based on purine and pyrimidine, while proteins are large molecules made up of one or more long amino acid chains.

(B). Conjugated Proteins

- Conjugated proteins are complex proteins.
- They contain one or more non-amino acid components.
- Here the protein part is tightly or loosely bound to one or more non-protein part(s).

- The non-protein parts of these proteins are called prosthetic groups.
- The prosthetic group may be metal ions, carbohydrates, lipids, phosphoric acids, nucleic acids and FAD.
- The prosthetic group is essential for the biological functions of these proteins.
- Conjugated proteins are usually globular in shape and are soluble in water.
- Most of the enzymes are conjugated proteins.

Based on the nature of prosthetic groups, the conjugated proteins are further classified as follows:

- **Phosphoprotein:** Prosthetic group is phosphoric acid, Example- **Casein of milk, Vitellin of egg yolk.**
- **Glycoproteins:** Prosthetic group is carbohydrates, Example – **Most of the membrane proteins, Mucin (component of saliva).**
- **Nucleoprotein:** Prosthetic group is nucleic acid, Example – proteins in chromosomes, structural proteins of ribosome.
- **Chromoproteins:** Prosthetic group is pigment or chrome, Example: **Haemoglobin, Phytochrome and Cytochrome.**
- **Lipoproteins:** Prosthetic group is Lipids, Example: **Membrane proteins**
- **Flavoproteins:** Prosthetic group is FAD (Flavin Adenine Dinucleotide), Example: **Proteins of Electron Transport System (ETS).**
- **Metalloproteins:** Prosthetic group is Metal ions, Example: **Nitrate Reductase.**

Sl. No.	Inorganic Ions as Cofactors	Name of Enzyme	Function of Enzyme
1	Cu^{2+}	Cytochrome c oxidase	Complex IV in the ETS
2	Fe^{2+} Fe^{3+}	Cytochrome c oxidase	Complex IV in the ETS
		Catalase	Catalyzes the decomposition of hydrogen peroxide to water and oxygen
		Peroxidase	Peroxidation reaction
3	K^{+}	Pyruvate kinase	Catalyzes the final step of glycolysis to yield pyruvate
4	Mg^{2+}	Arginase	Final enzyme of the urea cycle, combine arginine + water to produce ornithine and urea
		Ribonucleotide reductase	Catalyzes the formation of deoxyribonucleotides from ribonucleotides
5	Mo	Dinitrogenase	Reduction of atmospheric nitrogen to Ammonia (nitrogen fixation)
6	Ni^{2+}	Urease	Catalyzes the hydrolysis of urea into carbon dioxide and ammonia
7	Se	Glutathione peroxidase	Reduce lipid hydroperoxides to their corresponding alcohols; reduce free hydrogen peroxide to water.
8	Zn^{2+}	Carbonic anhydrase	Catalyze the rapid interconversion of carbon dioxide and water to bicarbonate and protons and vice versa
		Alcohol dehydrogenase	Fermentation of sugar
		Carboxypeptidase A & B	Protease enzyme, cleave a peptide bond at the carboxy-terminal (C-terminal)

Protein Prosthetic group Examples

- **Glycoproteins**- Carbohydrate-Mucin, serum albumin
- **Phosphoproteins**- Phosphoric acid -Caesin, vitellin
- **Lipoproteins** -Lipid -Lipoproteins of blood serum
- Nucleoproteins- Nucleic acid-Nuclein
- **Chromoproteins**-Carotenoid pigment- Rhodopsin
- **Metalloproteins**- Metal Chlorophyll,- haemoglobin

(III). Classification of Protein based on Functions:

(A). Structural Proteins:

✚ Form the component of the connective tissue, bone, tendons, cartilage, skin, feathers, nail, hairs and horn.

✚ Most of them are fibrous proteins and are insoluble in water.

Example: **Collagen, Keratin and Elastin.**

(B). Enzymes:

- They are the biological catalysts.
- Enzymes reduce the activation energy of reactants and speed-up the metabolic reactions in the cells.
- Most of them are globular conjugated proteins

  Example: **DNA Polymerase, Nitrogenase, Lipase**

(c). Hormones:

- They include the proteinaceous hormones in the cells.

  Example: Insulin, Glucagon, ACH

(D). Respiratory Pigments

- They are coloured proteins
- All of them are conjugated proteins and they contain pigments (chrome) as their prosthetic group.

  Example: **Haemoglobin, Myoglobin**

(E). Transport Proteins

- They transport the materials in the cells
- They form channels in the plasma membrane
- They also form one of the components of blood and lymph in animals.

  Example: Serum albumin

(F). Contractile proteins

- They are the force generators of muscles
- They can contract with the expense of energy from ATP molecules.

  Example: Actin, Myosin

(G). Storage Proteins

- They act as the store of metal ions and amino acids in the cells.
- Found in seeds, egg and milk
- Abundantly seen in pulses (legume seeds).

  Example: **Ferritin which stores iron, Casein, Ovalbumin, Gluten of Wheat**

(F). Toxins

✚ They are toxic proteins

Example: **Snake venom**

Properties of proteins

➔ Generally proteins are colourless and tasteless. However, there are exceptions for example Hemoglobin is red in colour.

➔ The solubility of proteins is influenced by pH. Proteins are least soluble at their iso electric point.

➔ All protein solutions are optically active. The magnitude of optical activity depends on temperature, the wave length of light used and the **concentration of protein**.

➔ The amino acid sequence of a protein is determined by the information found in the cellular genetic code.

➔ The genetic code is the sequence of nucleotide bases in nucleic acids **(DNA and RNA) that code for amino acids.**

➔ These gene codes not only determine the order of amino acids in a protein, but they also determine a protein's structure and function.

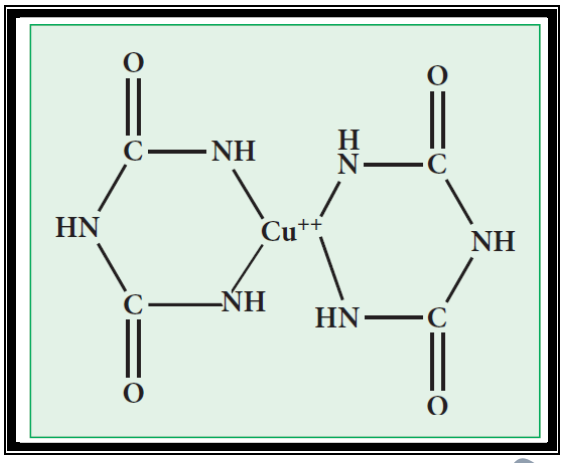
➔ Since proteins are macro molecules, their sizes are quantitatively expressed in terms of their molecular weights with kilo **Daltons as their unit (kDa**). For example the human serum albumin has a molecular weight of 66 kDa.

➔ Because of their giant size, proteins exhibit colloidal properties such as low diffusion and Tyndall effect.

➔ Proteins undergo hydrolysis upon treatment with concentrated mineral acids like HCl and yield constituent amino acids as their hydrochlorides. Similarly, proteolytic enzymes like trypsin and chymotrypsin **hydrolyse proteins**.

➔ When proteins are treated with alkaline copper sulphate solution (Biuret reagent) they form a violet coloured complex called **Biuret complex**. This reaction can be used as a qualitative and quantitative test for proteins.

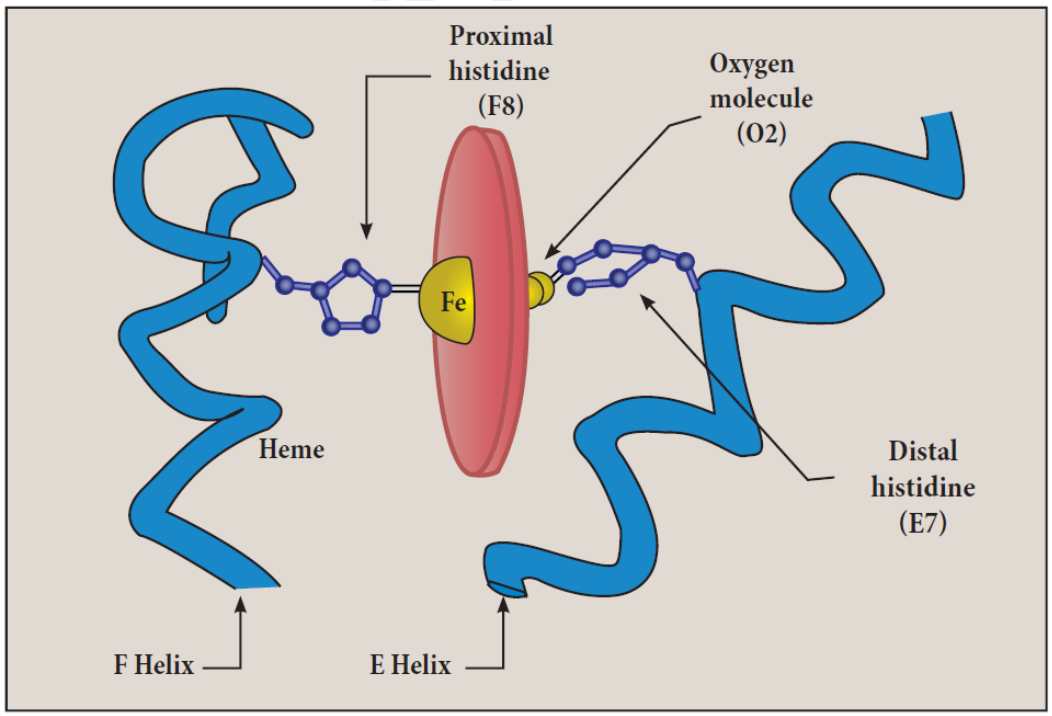
Biuret complex



Haemoglobin – an example for globular protein

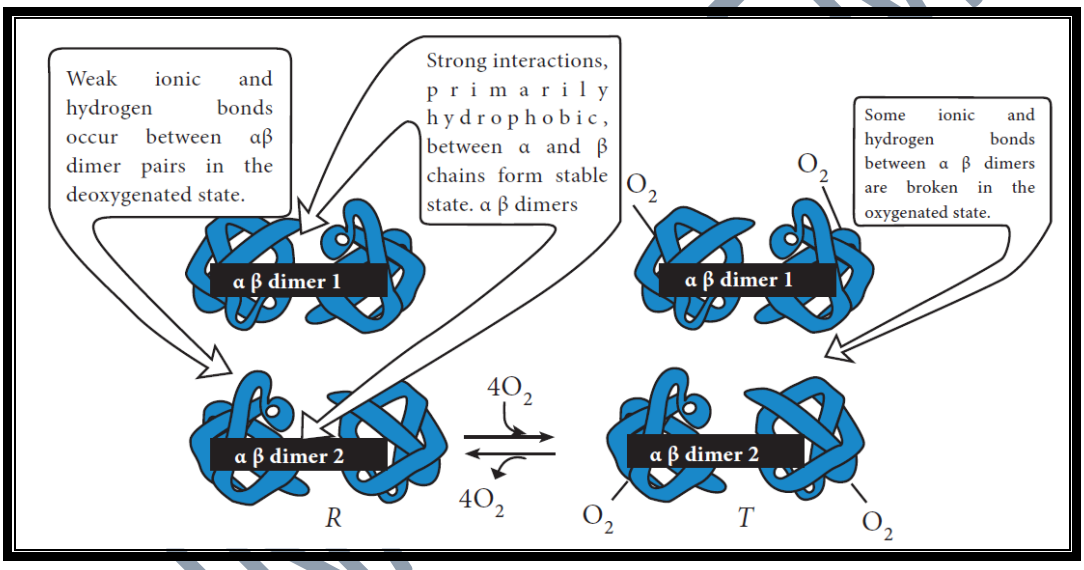
- Haemoglobin is found in red blood cells and is involved in the transport of oxygen from lungs to tissues. It is a tetramer containing four polypeptide chains – 2α chains and 2 β chains. Each of these chains contains a prosthetic group called heme. Heme is a protoporphyrin ring complexed with Fe2+. This Fe2+ ion can form six bonds, four with the nitrogen atoms of the porphyrin ring, one with a histidine of hemoglobin and the other with oxygen. Thus every haemoglobin can carry four O2 molecules.

Binding of oxygen to heme in hemoglobin



❋ Haemoglobin is an alpha helical protein, meaning it does not contain β sheets as its secondary structural elements. The haemoglobin tetramer structure could be considered as **a dimerized dimer of (αβ)1 and (αβ)2**. The α and β chain in each dimer are held together strongly by **hydrophobic interactions**. The interactions **between (αβ)1 and (αβ)2** are comparatively weaker hydrogen bonds and ionic interactions. This allows the dimers to move with respect to each other forming two different conformational states: a relaxed **'R' conformation and a taut 'T' conformation**. The binding and release of oxygen switches the hemoglobin between these two states.

R and T conformations of haemoglobin



❋ Haemoglobinopathies are a set of diseases caused by synthesis of structurally abnormal haemoglobins, insufficient amount of haemoglobins or both. Sickle cell anemia, thalasemia, porphyria etc are examples of haemoglobinopathies.

Collagen - an example for fibrous protein

❋ Collagen is the most abundant protein found in humans. Unlike globular structures discussed above, collagen forms a long coiled fibrous structure. Each collagen molecule consists of three polypeptide chains which forms an elongated triple helical structure

❋ The **amino acid sequence of these polypeptide** chains are always repeating units of Gly-**X-Y**. Where **X is often proline and Y** is either hydroxyproline or hydroxylysine. The hydroxyl group of hydroxylysine can also be glycosylated with glucose or galactose.

There are various types of collagen which can be broadly categorized into three groups.

- The fibril forming collagens present in **skin, bone, cartilages, tendons** and blood vessels etc provide tensile strength to the corresponding tissues.
- The network forming collagens form network like structures beneath the membranes providing **them mechanical strength.**
- The fibril associated collagens connect two fibril forming collagens or a fibril forming collagen with other **components of extra cellular** matrix.

Denaturation and Protein folding

- Each protein has a unique three dimensional structure. Upon changes in various factors like **temperature, pH, ionic strength** or exposure to certain chemicals like urea lead to disruption of its **three-dimensional** structure and turn back into an unstructured string of amino acids. When a protein loses its higher-order structure, but not its primary structure, it is said to be denatured. Denatured **proteins are not functional**.
- For some **proteins, denaturation** can be reversed. Since the primary structure of the polypeptide is still intact it may be able to re-fold into its original structure, if it is returned to its **normal environment**. Many proteins do not fold by themselves, but instead get assistance from other **proteins like chaperons**.
- The process by which a **polypeptide chain acquires its 3-dimensional** structure is known as **protein folding**. It is a complex process and the exact mechanism by which a protein folds to its three dimensional structure has not been understood so far. Quite often many proteins **misfold.** Some proteins when misfolded form fibrillar structure of β pleated sheets. This **misfolding** can be spontaneous or could be because of mutations. These misfolded proteins aggregate in neurons and **can lead to amyloid disease** such as Alzheimer's disease, which is a neuro degenerative disease.
- Strong acids and alkalis, heavy metals, heat, UV radiations, and detergents can denature aprotein.
- Collagen is the most abundant protein in animal world and **ribulose biphosphate carboxylase oxygenase (Rubisco)** is the most abundant protein in the whole of the biosphere.
- **P-proteins** are involved in the transport of organic compounds **through phloem**.
- **Snake venom, ricin of castor, and bacterial toxins** are **proteinaceous** in nature. **Protamines** are basic proteins associated with **DNA of chromosomes**, these are rich in

lysine and arginine. Monellin, a protein is the sweetest chemical obtained from an **African berry.**

Chemical properties

☎ **Salt formation** : Due to presence of both [-N{{H}_{2}}] and [COOH] groups in proteins, they form salts with acids and bases. Casein is present in milk as calcium salt.

☎ **Hydrolysis** : The simple proteins are **hydrolysed by acids, alkalies or enzymes** to produce amino acids. Following steps are involved in the hydrolysis and the final product is a mixture of amino acids.

☎ **Oxidation** : Proteins are oxidised on burning and putrefaction. The products include amines, nitrogen, carbon dioxide and water. The bad smell from decaying dead animals is largely due to the formation of amines by **bacterial oxidation of body proteins**.

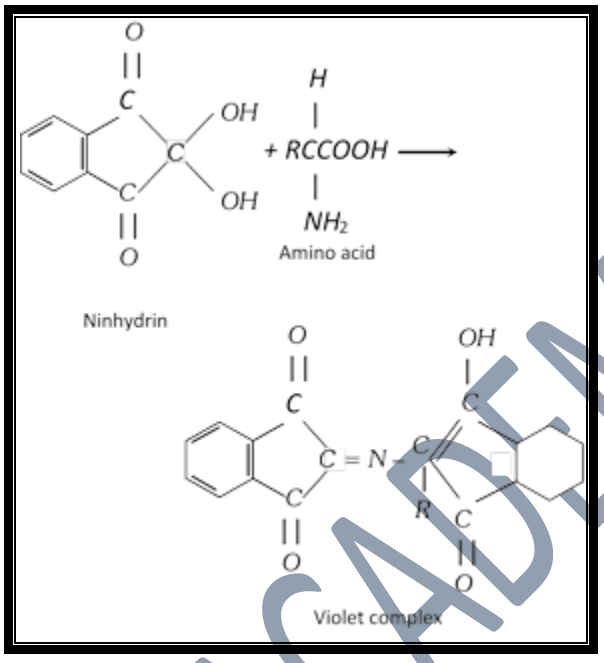
☎ **Biuret test :** On adding a **dilute solution of copper sulphate** to alkaline solution of protein, a **violet colour is developed.** This test is due to the presence of peptide [(CONH)] linkage.

☎ **Xanthoproteic test :** Some proteins give yellow colour with concentrated nitric acid (formation of yellow stains on fingers while working with nitric acid in laboratory). The formation of **yellow colour** is due to reaction of nitric acid with benzenoid structures. Thus, when a protein solution is warmed with nitric acid a **yellow colour** may be developed which turns orange on addition of **[N{{H}_{4}}OH\] solution**.

☎ **Millon's test :** When millon's reagent (mercurous and mercuric nitrate in nitric acid) is added to a protein solution, a white precipitate which turns **brick red on heating,** may be formed. This test is given by proteins which yield tyrosine on hydrolysis. This is due to presence of **phenolic group.**

☎ **Ninhydrin test :** This test is given by all proteins. When a protein is boiled with a dilute solution of ninhydrin, a violet colour is produced.

☎ **Nitroprusside test :** Proteins containing [-SH] group give this test. When sodium nitroprusside solution is added to proteins having[-SH] group, a **violet colour is developed.**



☎ **Molisch's test :** This test is given by those proteins which contain carbohydrate residue. On adding a few drops of alcoholic solution of [alpha -]naphthol and concentrated sulphuric acid to the protein solution, a violet ring is formed.

☎ **Hopkins-Cole test :** On adding concentrated sulphuric acid down the side containing a solution of protein and glyoxalic acid, a violet colour is developed.

☎ **In textile :** Casein (a milk protein) is used in the manufacture of artificial wool and silk.

☎ **In the manufacture of amino acids :** Amino acids, needed for medicinal use and feeding experiments, are prepared by hydrolysis of proteins.

☎ **In industry :** Gelatin (protein) is used in food products, capsules and photographic plates. Glue (protein) is used as adhesive and in sizing paper. Leather is obtained by tanning the proteins of animal hides.

☎ **In controlling body processes :** Haemoglobin present in blood is responsible for carrying oxygen and carbon dioxide. Harmones (proteins) control various body processes.

****

TABLE 4.1. Classification of amino acids and their abbreviations

Group	Amino acid and its abbreviation
I. containing one NH_2 and one C-OH group	1. Glycine (gly), 2. Alanine (ala), 3. Leucine (leu), 4. Isoleucine (ile), 5. Serine (ser), 6. Threonine (thr)
II. containing one NH_2 and two C-OH groups	1. Glutamic acid (glu), 2. Aspartic acid (asp)
III. amides of dicarboxyl	1. Glutamine (gln) 2. Asparagine (asn)
IV. containing additional NH group (basic amino acids)	1. Lysine (lys), 2. Arginine (arg), 3. Histidine (his)
V. cyclic amino acids (imino)	1. Proline (pro), 2. Hydroxyproline (hypro)
VI. containing benzyle group (aromatic amino acids)	1 Phenylalanine (phe), 2. Tryptophane (trp), 3. Tyrosine (tyr)
VII. containing sulphur	1. Methionine (met), 2. Cysteine (cys)