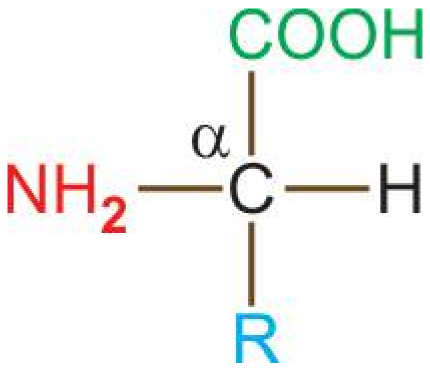
AMINO ACIDS PEPTIDES AND PROTEINS

Learning objectives

- Describe general structure, classification and importance of amino acid with examples
- Describe and discuss definition, various classifications with examples and functions of proteins.
- Describe and discuss primary, secondary, tertiary and quaternary structure of proteins with examples and clinical significance.
- Describe structure - function relationship of normal & abnormal hemoglobin

General Structural characteristic of Amino Acids

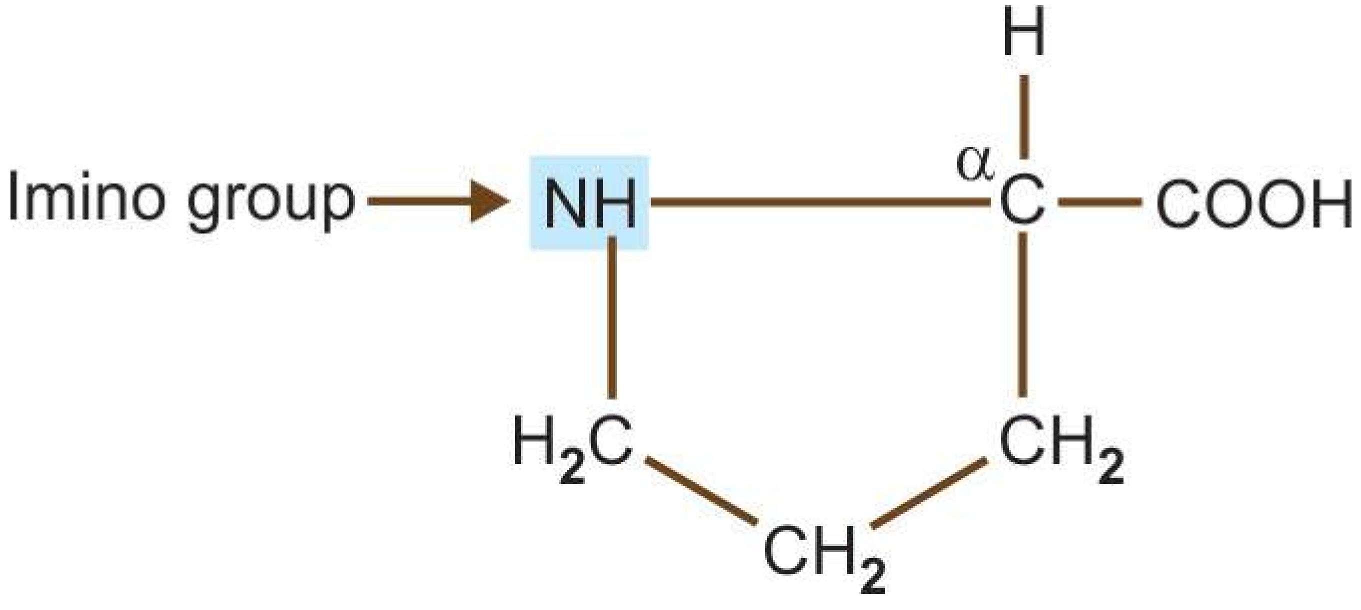
- All the 20 amino acids found in proteins have a carboxyl (COOH) group and an amino ($-NH_2$) group bound to the same carbon atom called the α carbon



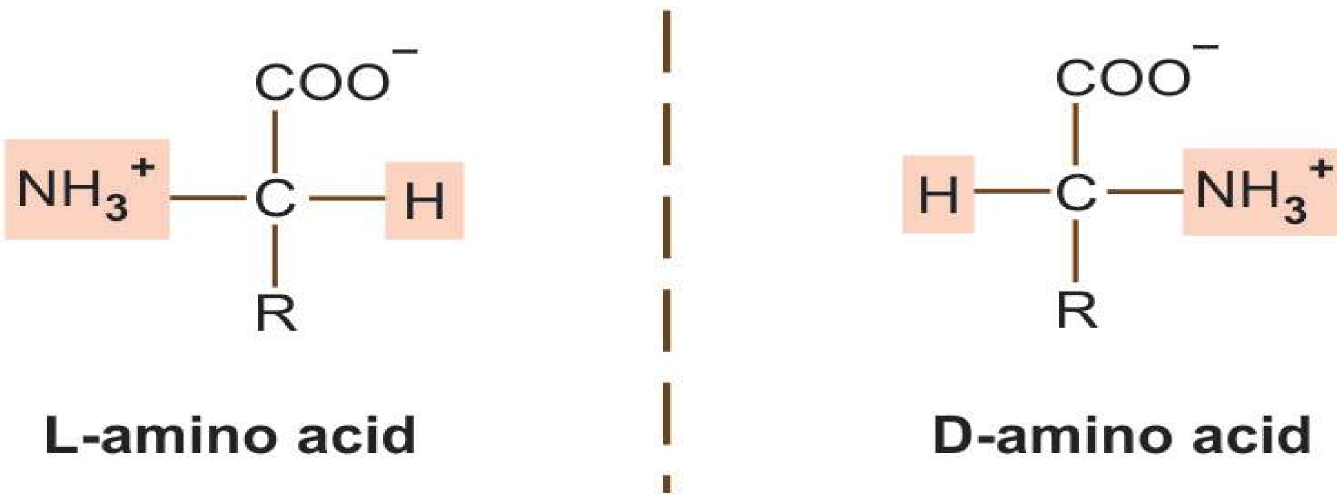
- Amino acids differ from each other in their side chains or R-groups, attached to the α-carbon

- The 20 amino acids of proteins are often referred to as the standard or primary or normal amino acids.

- One of the 20 amino acids, proline is an imino (-NH) (secondary amino group) acid not an amino ($-NH_2$) acid as are other 19.


Imino group
NH
H
α
C
COOH
H2C
CH2
CH2

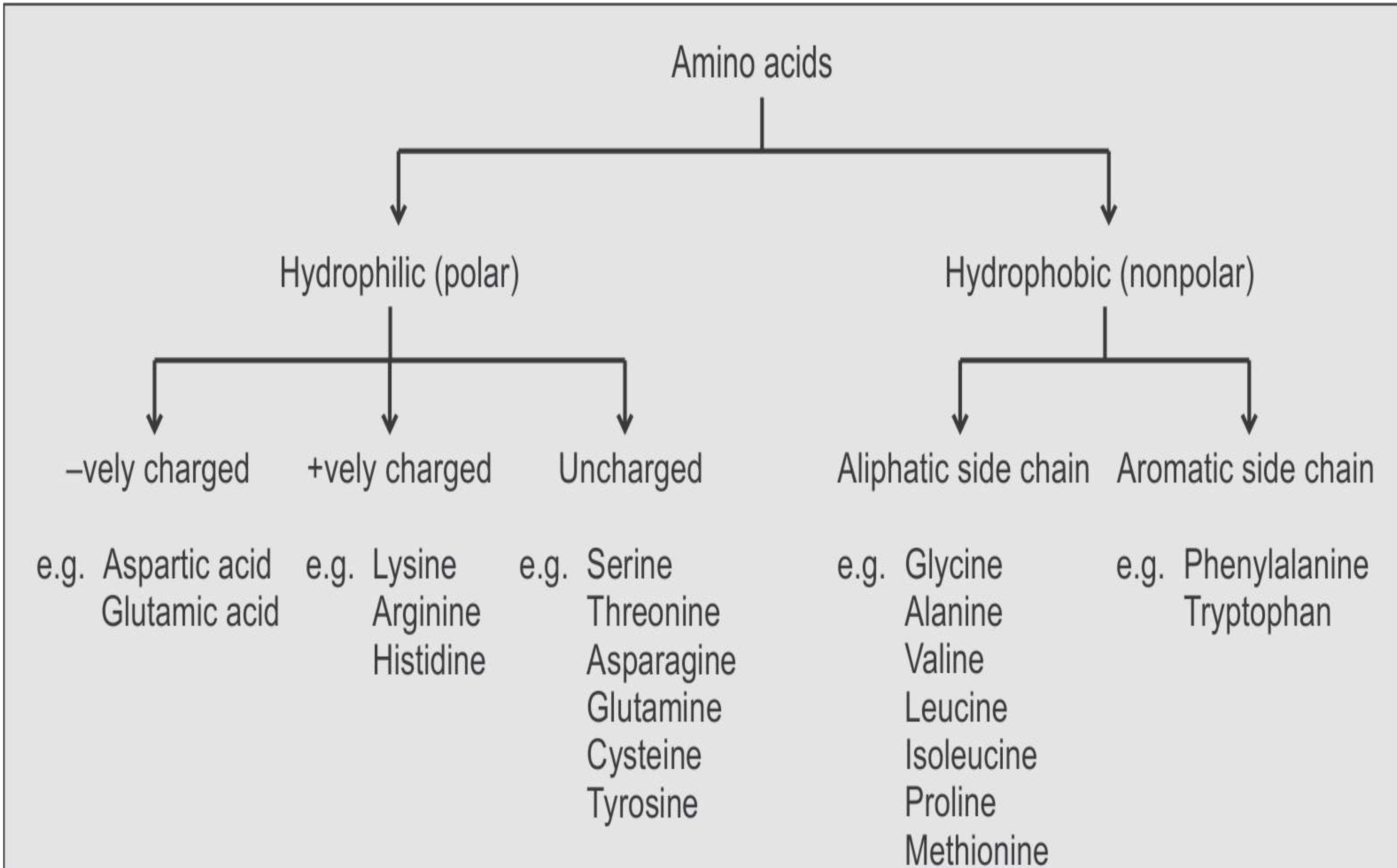
- All naturally occurring amino acids are optically active except glycine (no asymmetric carbon) which is optically inactive
- All the amino acids found in proteins are exclusively of the L-configuration.



- Two free D-amino acids D-serine and D-aspartic acid have been found to be present in the brain tissues. D-Alanine and D- glutamate are found in some bacterial cell walls.

Classification Of Amino Acids

- Chemical nature of the amino acid in the solution.
- Structure of the side chain of the amino acids.
- Nature or polarity of the side chain of the amino acids.
- Nutritional requirement of amino acids.
- Metabolic product of amino acids.


Amino acids
Hydrophilic (polar)
Hydrophobic (nonpolar)
–vely charged
+vely charged
Uncharged
Aliphatic side chain
Aromatic side chain
e.g. Aspartic acid
Glutamic acid
e.g. Lysine
Arginine
Histidine
e.g. Serine
Threonine
Asparagine
Glutamine
Cysteine
Tyrosine
e.g. Glycine
Alanine
Valine
Leucine
Isoleucine
Proline
Methionine
e.g. Phenylalanine
Tryptophan

Nutritionally Essential Amino Acids

Phenylalanine	Tryptophan	Histidine
Valine	Isoleucine	Arginine
Threonine	Methionine	Lysine
		Leucine

- The mnemonics : PVT. TIM. HALL
- Arginine and Histidine nutritionally semi essential , synthesized at rates inadequate to support growth of children.

Essential Amino Acids	*Semi-Essential Amino Acids*	*Non-essential Amino Acids*
Phenylalanine **Valine** **Threonine** **Tryptophan** **Isoleucine** **Methionine** **Lysine** **Leucine**	**Histidine** **Arginine**	All the other amino acids

TABLE 5.2: Metabolic classification of amino acid.

Exclusive glucogenic	*Exclusive ketogenic*	*Both ketogenic and glucogenic*
❑ Glycine, alanine, serine ❑ Cysteine, aspartic acid ❑ Asparagine, glutamic acid ❑ Glutamine, proline, histidine, arginine, methionine, valine	❑ Leucine ❑ Lysine	❑ Tryptophan ❑ Phenylalanine ❑ Tyrosine ❑ Threonine ❑ Isoleucine

TABLE 5.3: Biologically important compounds formed by amino acids.

Amino acid	*Formation of biologically important compound*
Tyrosine	Hormone, e.g. thyroxine, skin-pigment, e.g. melanin
Tyrosine and phenylalanine	Hormone, e.g. epinephrine, norepinephrine, and dihydroxyphenylalanine (DOPA)
Glutamic acid	Gamma-aminobutyric acid (GABA) neurotransmitter
Tryptophan	Vitamin niacin
Glycine, arginine and methionine	Creatine
Glycine and cysteine	Bile salts
Glycine	Heme
Aspartic acid and glutamic acid	Pyrimidine bases
Glycine, aspartic acid and glutamine	Purine bases
Beta-alanine	Coenzyme-A
Histidine	Histamine
Tryptophan	Serotonin

Amino acids coded by stop codons

- There are actually 22 rather than 20 amino acids specified by stop codons.
- The two extra ones are selenocysteine and Pyrrolysine

Selenocysteine : 21st Amino acid

- Selenocysteine contains selenium rather than sulfur in cysteine
- Precursor amino acid for selenocysteine is serine. It provides the carbon skeleton of selenocysteine.
- Biosynthesis of selenocysteine requires cysteine, selenite (SeO_4), ATP, a specific $tRNA^{Sec}$, and several enzymes.
- It is encoded by UGA codon, which is normally a stop codon.

- Unlike hydroxyproline and hydroxylysine, selenocysteine is incorporation in peptides during protein synthesis rather than created through a post translational modification.
- Selenocysteine is a constituent of several human enzymes that catalyze redox reactions.

    Glutathione peroxidase,

    Thioredoxin reductase and

    Deiodinase

22nd Amino acid: Pyrrolysine

- Pyrrolysine (Pyl or O) encoded by UAG (normally a stop codon)
- It is similar to lysine, but with an added pyrroline ring linked to the end of the lysine side chain.
- Pyrrolysine was discovered in 2002 at the active site of methyl-transferase enzyme from a methane-producing bacteria

Absorption of ultraviolet light by aromatic amino acids

- Tryptophan, tyrosine and to a much lesser extent phenylalanine, absorb ultraviolet light at a wavelength of 280 nm

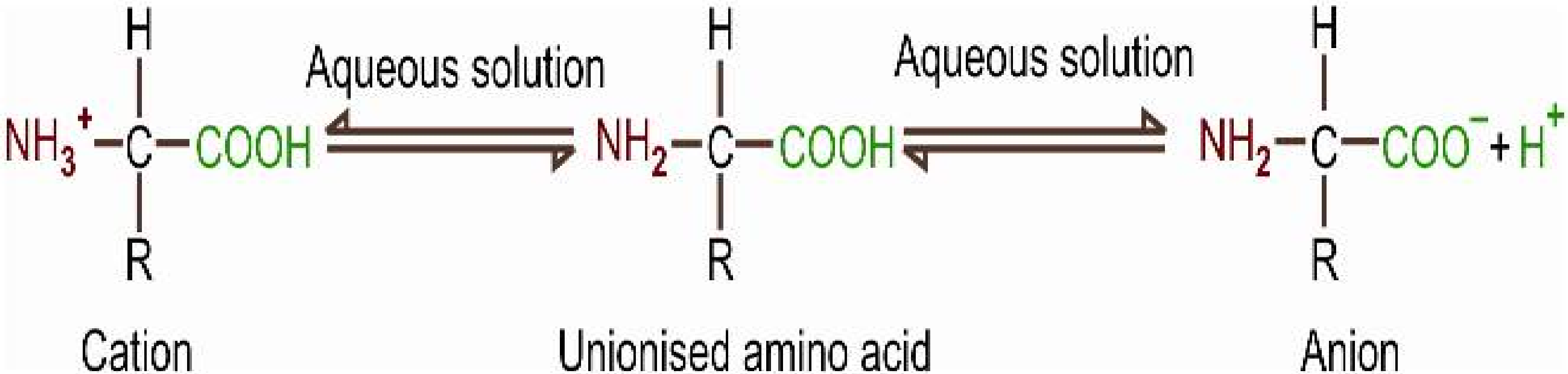
- Measurement of light absorption by spectrophotometer is used to detect and identify molecules and to measure their concentration in solution. Light absorption by phenylalanine generally contributes little to the spectroscopic properties of proteins.

- The absorbance of tryptophan is maximum (four times that of tyrosine) at a wavelength of 280 nm
- Amino acids are colorless because they do not absorb visible light.

Properties Of Amino Acids

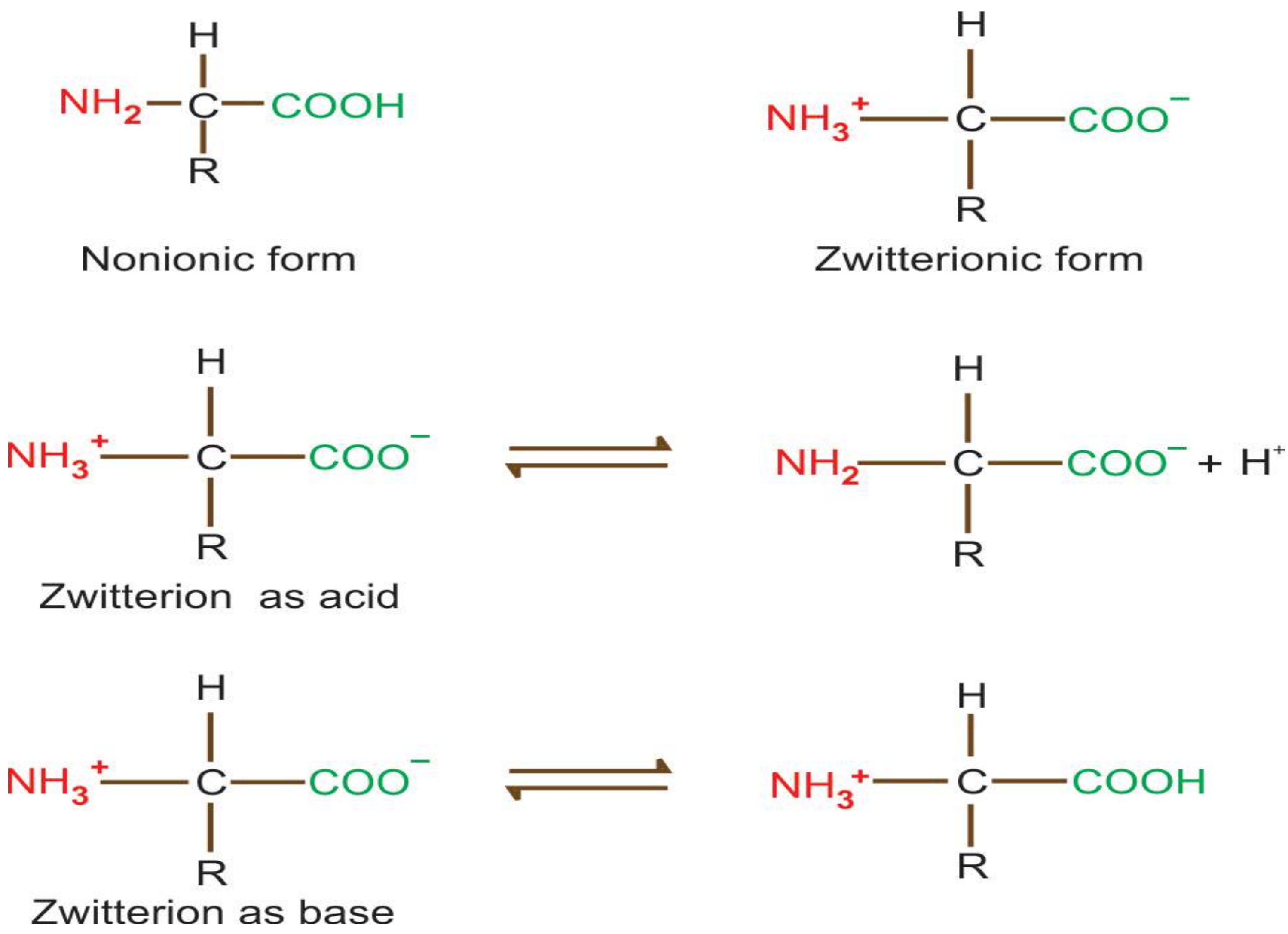
- Genetic code specifies an amino acid.
- Amino acid not coded by genetic code are called derived amino acid
- All naturally occurring amino acids are optically active except glycine (no asymmetric carbon) which is optically inactive
- Amino acid coded by stop codon are selenocysteine and Pyrrolysine.

- Amino acid exist in three charged state, positive negative or neutral depending on the two factors:
  - Isoelectric pH of the amino acid
  - pH of the surrounding media



Ionisation of Amino Acid

Nonionic and zwitterionic forms of amino acid. A zwitterion can act as either an acid (proton donor) or base (proton acceptor).



Iionization of Amino Acids

Due to ionizing property of amino acids, amino acids exert:

- Acid base behavior.
- Amphoteric properties (zwitter ion formation)
- Buffering activity.

- Molecules which carry equal number of ionizable groups of opposite charge and as a result bear no net charge are called zwitterions or ampholytes.

- Zwitter is a German word which means hermaphrodite.

- The net charge of an amino acid depends upon the pH of the medium.

- At pH less than Isoelectric pH (pI) amino acid is positively charged because ionized COO^- group accepts a proton and becomes uncharged (COOH).

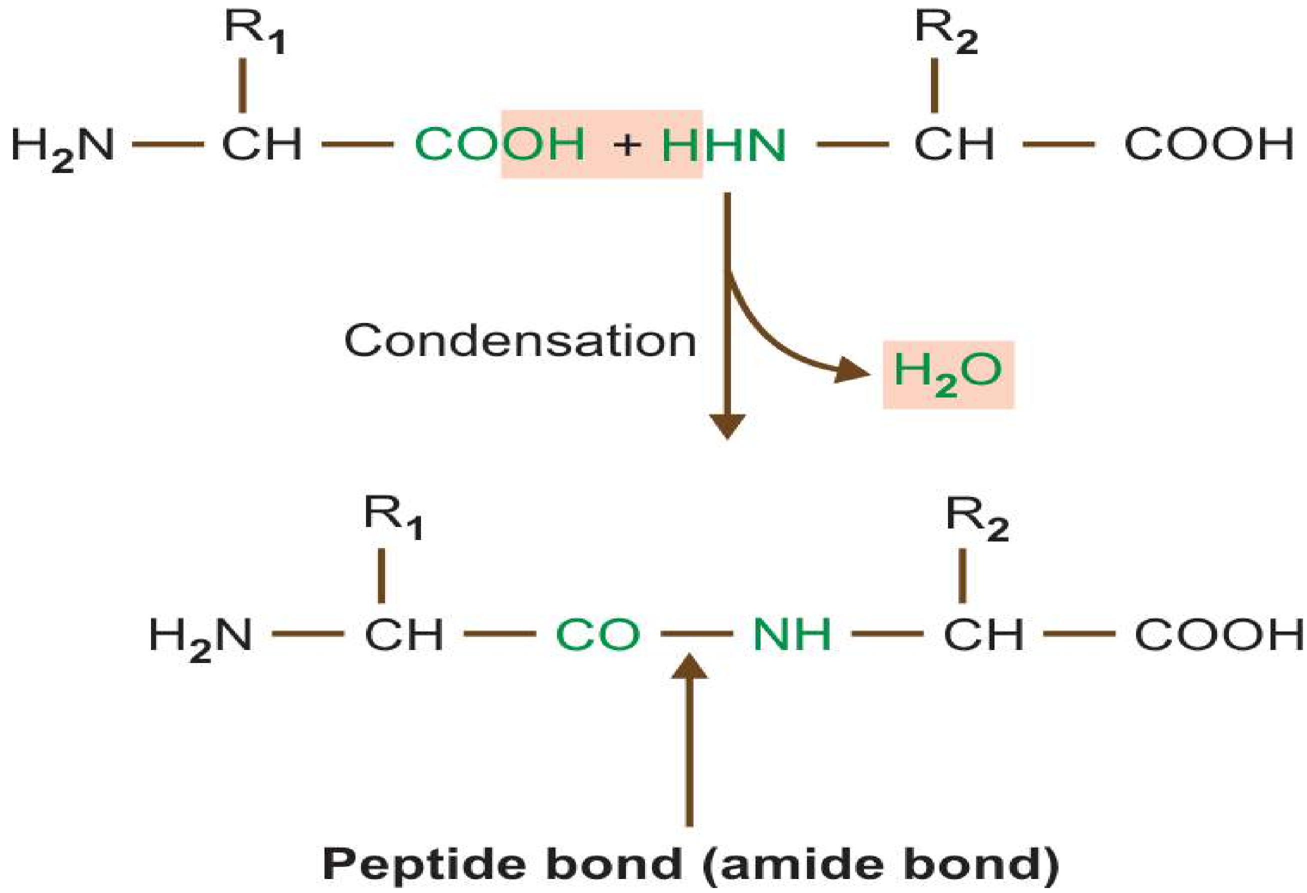
- While at pH greater than Isoelectric pH (pI) it is negatively charged as the NH_3^+ group loses its proton and becomes uncharged.

- At physiological pH (7.4) carboxyl group is negatively charged and amino group is positively charged.

PROTEIN

Definition

- Proteins are macromolecules composed of one or more polypeptide chains, each with a characteristic sequence of amino acids linked by **peptide bonds**.
- Each protein has specific and unique sequence of amino acids that defines both its three dimensional structure and its biologic function.



Formation of peptide bond.

TABLE 5.8: Biologically important peptides.

Peptide	*Example*
Tripeptide	Glutathione Thyrotropin-releasing hormone (TRH)
Pentapeptide	Enkephalins
Octapeptide	Angiotensin II
Nonapeptide	Oxytocin Vasopressin [antidiuretic hormone (ADH)] Bradykinin
Decapeptide	Angiotensin I

Classification of Proteins

They are most conveniently classified on the basis of

1. Function
2. Molecular shape
3. Composition
4. Nutritional quality.

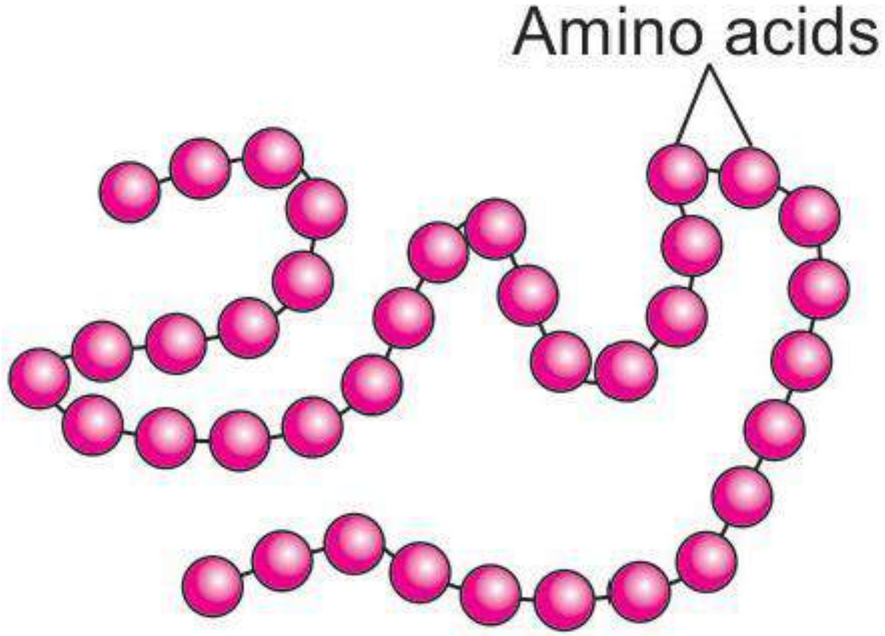
Structure of Proteins

Four levels of organization Protein structure

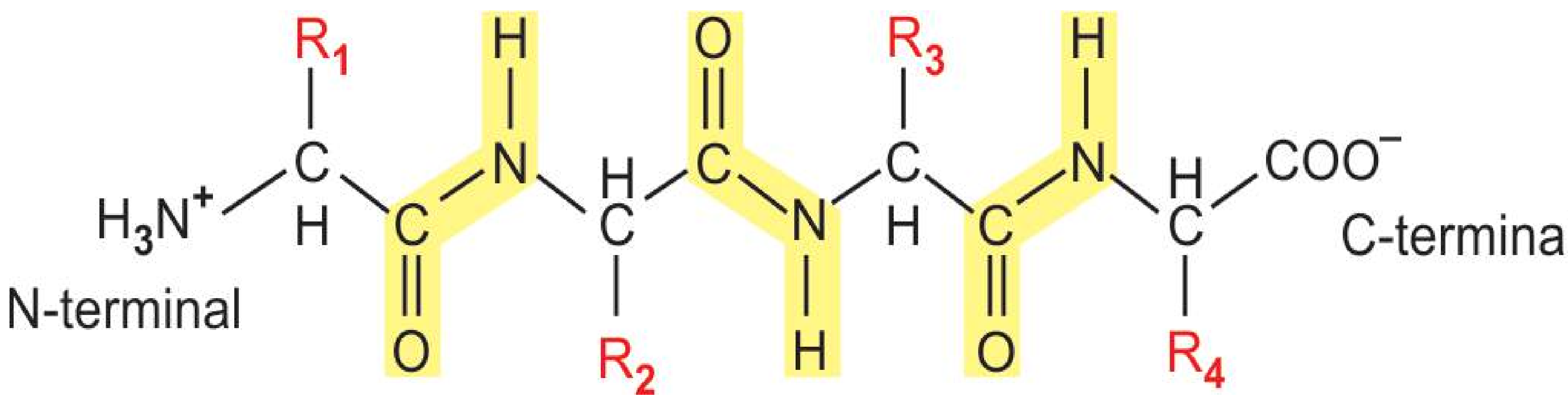
1. Primary structure
2. Secondary structure
3. Tertiary structure and
4. Quaternary structure.

PRIMARY STRUCTURE OF PROTEINS

Sequence of amino acids linked together by covalent peptide linkage is called primary structure of the protein



Backbone of a polypeptide chain showing N-terminal, C-terminal and variable side (R).



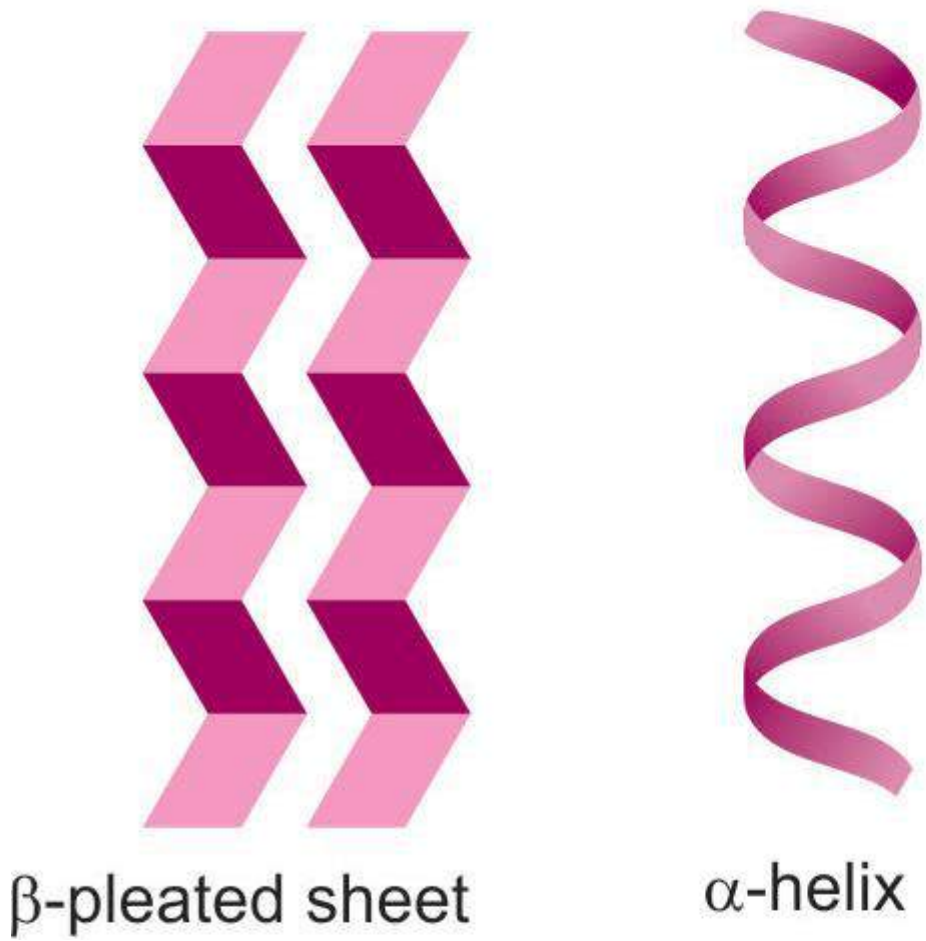
Secondary Structure Of Proteins

- For stability of primary structure hydrogen bonding between the hydrogen of NH and oxygen of C=0 group within the polypeptide chain occur which gives rise folding and twisting of primary structure.
- Regular folding and twisting of the polypeptide chain brought about by hydrogen bonding is called secondary structure of protein.

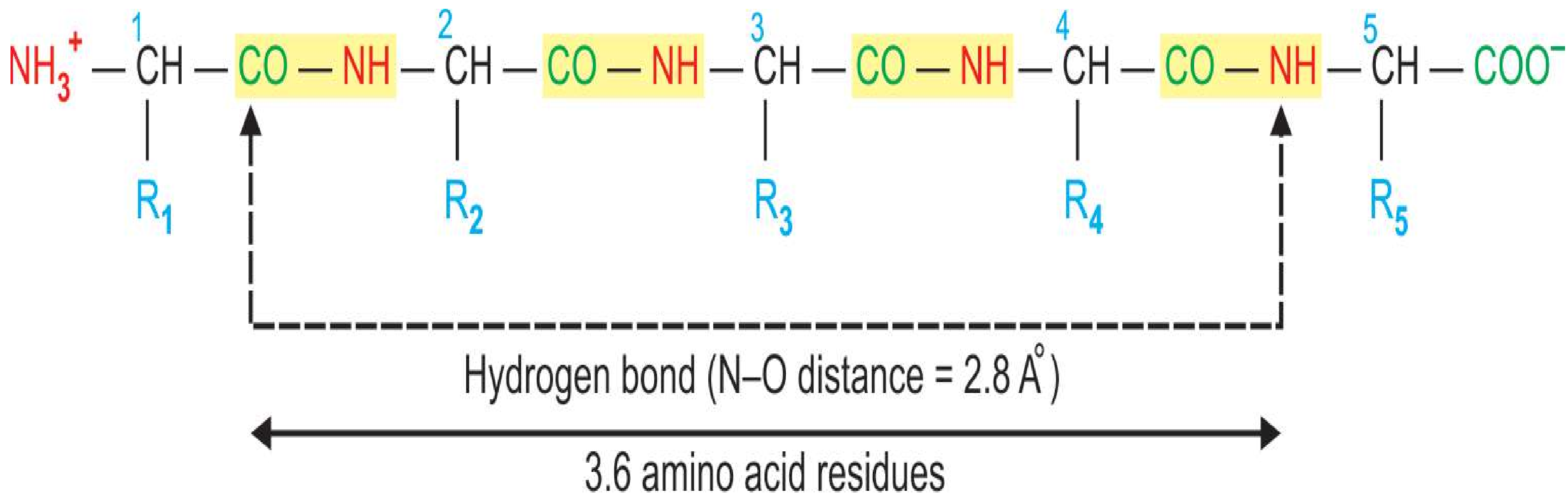
The different kinds of secondary structure are

- α-Helix (helicoidal state)
- β-Pleated sheet (stretched state)
- β-turn and loops.

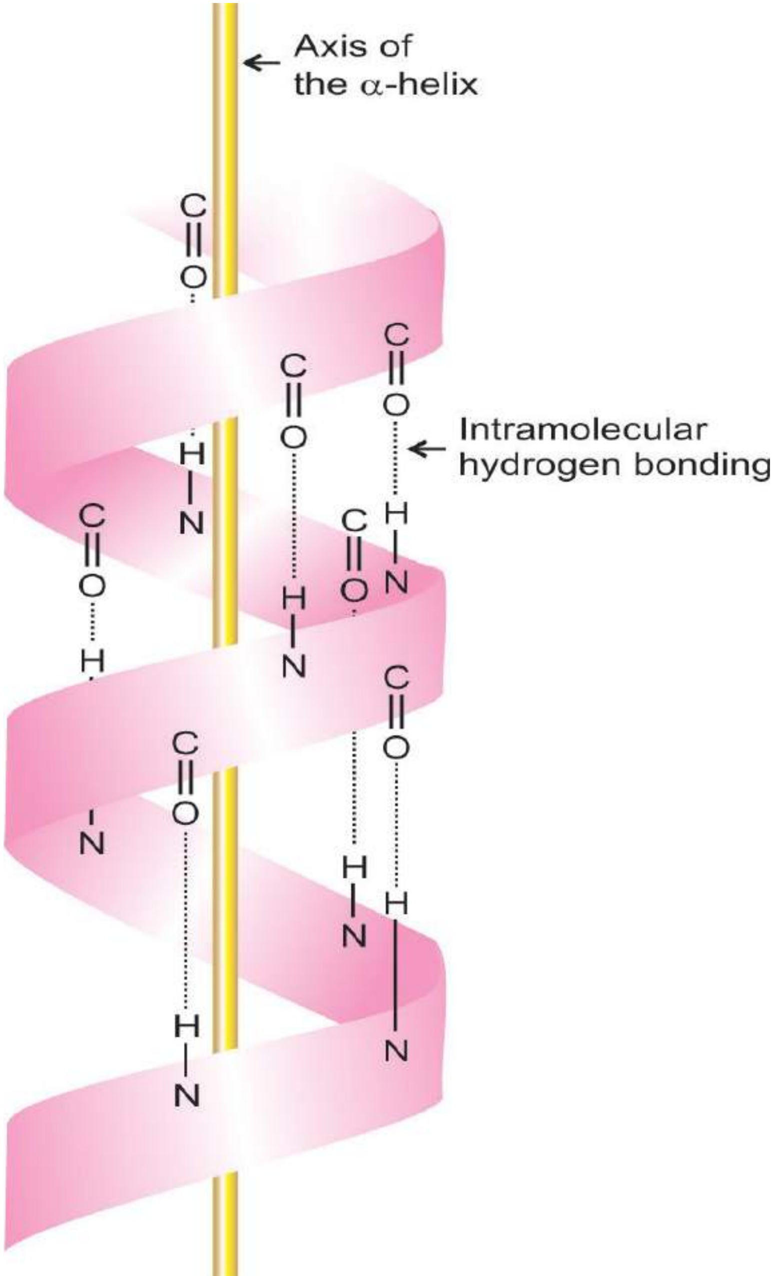
Secondary protein structure
Folding of the polypeptide chain into helices or sheets



Formation of hydrogen bond in α-helix. In α-helix, the CO group of residue 1 forms hydrogen bond with the NH group of residue 4.



Schematic diagram of α-helical structure of protein



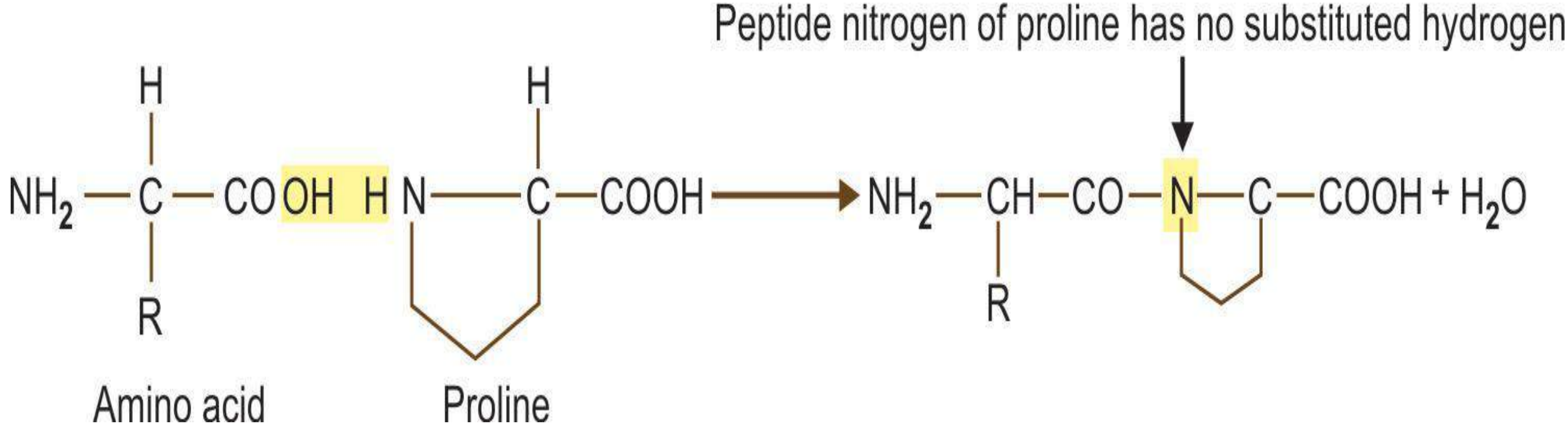
α –HELIX

- First structure elucidated by Pauling and Corey.
- Due to intramolecular hydrogen bonding backbone of polypeptide chain is twisted by an equal amounts around each α-carbon & forms a coil or helix.
- C=O group of each amino acid is bonded to the -NH of the amino acid that is situated four residues ahead in the linear sequence

- Hydrogen bonds have an essentially optimal nitrogen to oxygen (N-0) distance of 2.8 Å.
- A complete turn of helix contains an average of 3.6 amino acid residues and the distance it rises per turn (its pitch) is 0.54 nm
- The axial distance between adjacent amino acids is 1.5 Å
- Proteins contains only L-amino acids, for which a right handed α- helix is more stable and only right handed α-helices are present in protein.

- Proline will not allow the formation of α- helix. It disrupts the conformation of the helix, producing a bend.
- Glycine because of its small size induces bends in the helix.
- Alanine favors α- helix.
- Examples of proteins whose major secondary structure is α- helix.
    - Hemoglobin
    - Myoglobin
-

Peptide bond formation with proline



β-Pleated sheet (stretched state)

- β because it was the second structure elucidated by Pauling & Corey.
- Polypeptide chains are fully extended . β -sheet appear "pleated" or zigzag and these structures are therefore often called "β -pleated sheet'.
- Unlike α-helix, β-pleated sheets are composed of two or more polypeptide chains.

- β-pleated sheet is stabilized by interchain hydrogen bonds

- The axial distance between adjacent amino acids in β-pleated sheet is 3.5Å in contrast with 1.5Å for the α-helix.

- The arrangement of polypeptide chains in β -pleated sheet conformation is either Parallel or Anti-parallel pleated sheet.

- Examples of proteins whose major secondary structure is

  β -pleated sheet

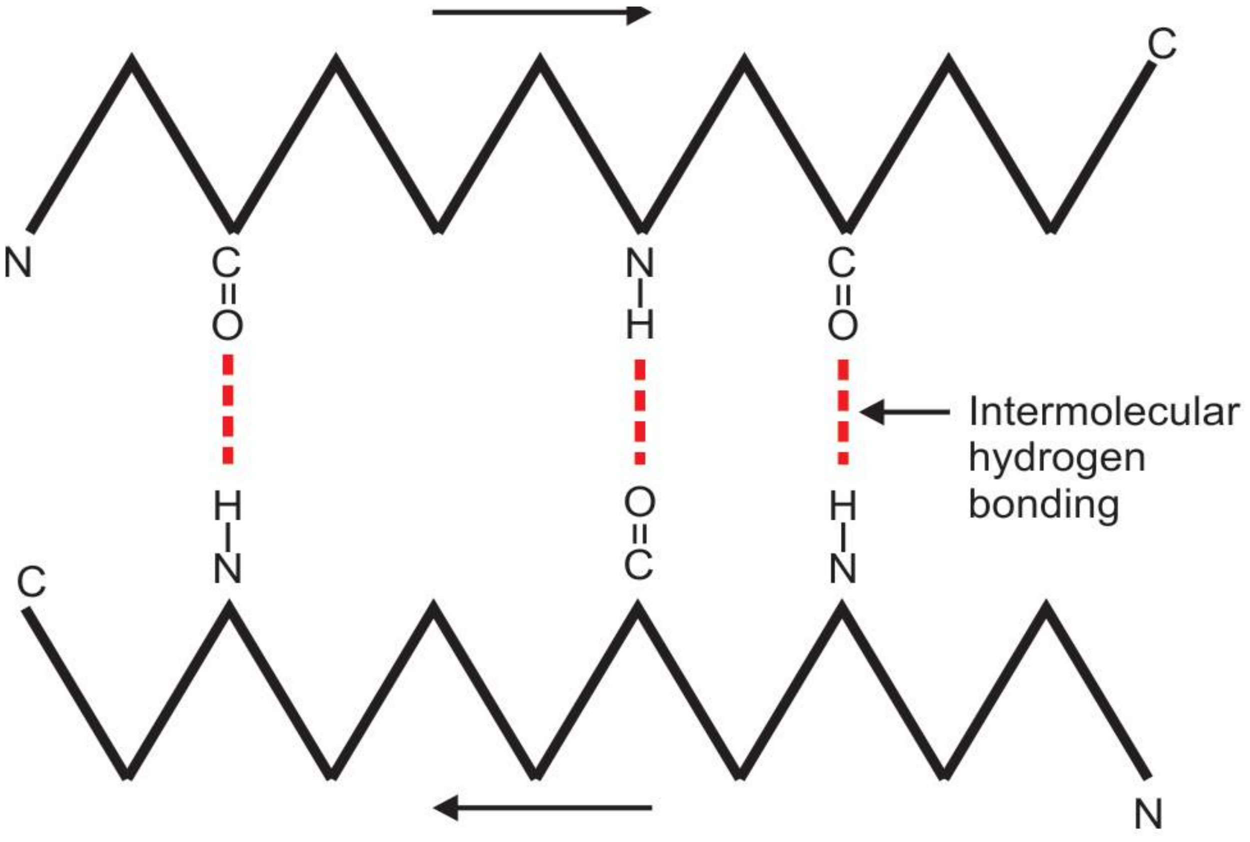
  - Parallel β -pleated sheet : Flavodoxin

  - Anti-parallel β -pleated sheet: Silk fibrion

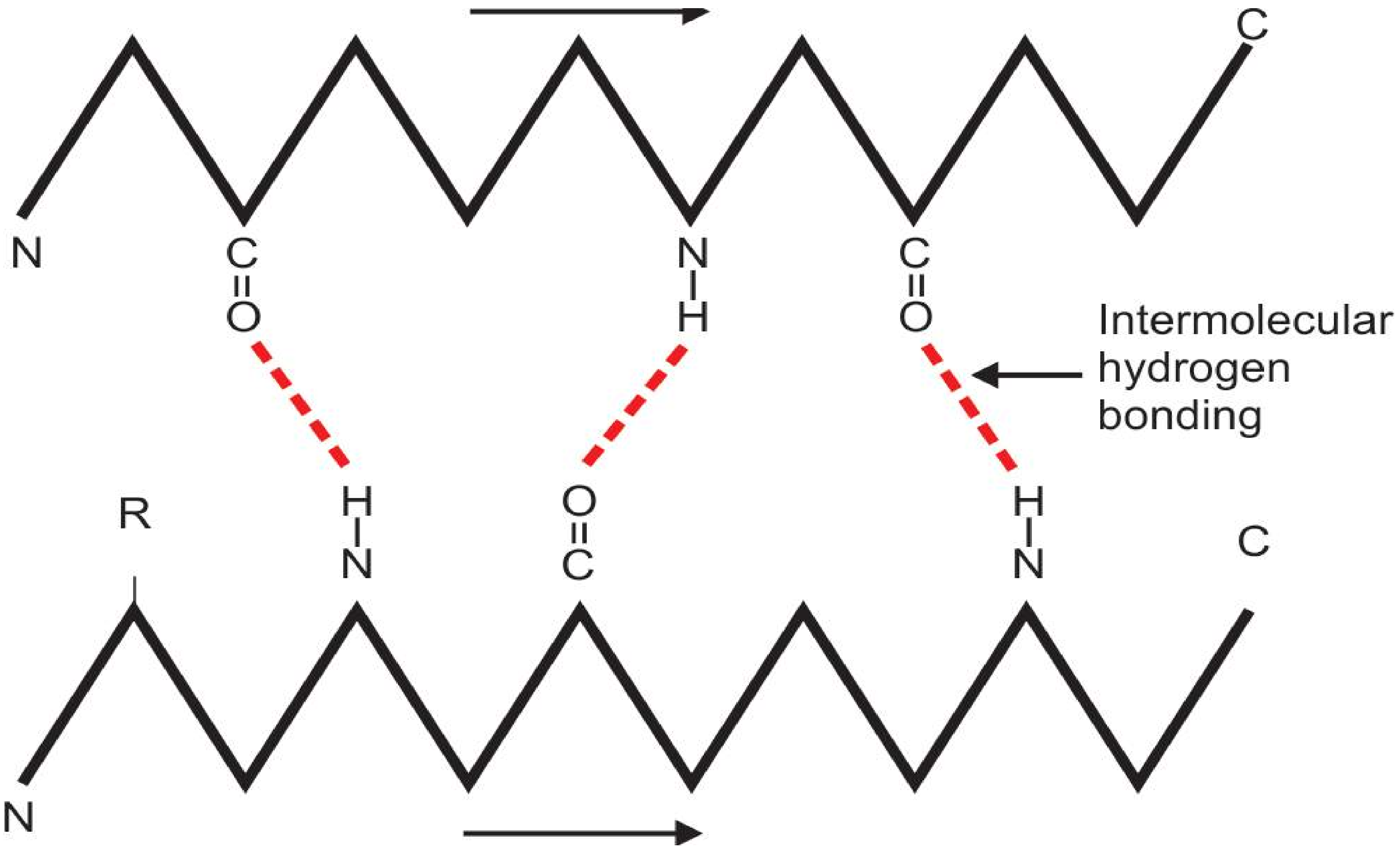
  - Both Parallel and Anti-parallel β sheet:

    Carbonic anhydrase

Antiparallel



Parallel



- β-pleated sheet occurs as a principal secondary structure in amyloid proteins found in people with amyloidosis e.g., Alzheimer disease

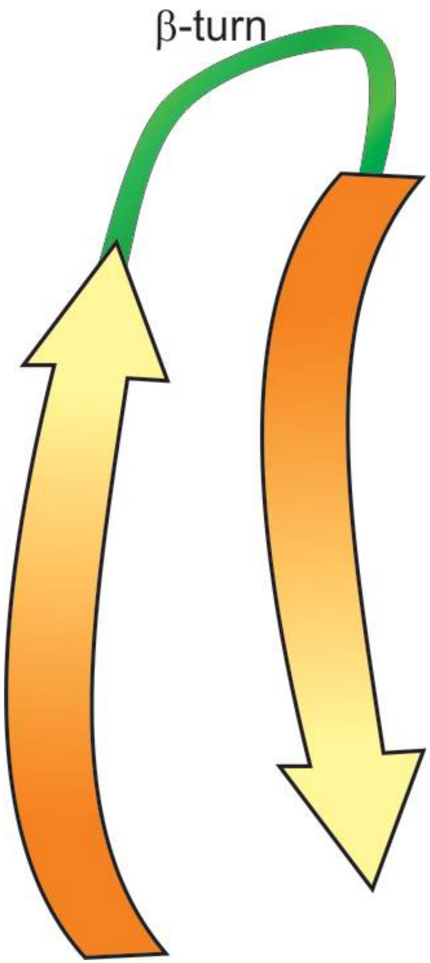
- Amyloid proteins that accumulate are misfolded proteins derived from immunoglobulins.

- Amyloid proteins are insoluble and their accumulation in tissues and organs disrupt normal physiological process.

Turns and bends

- Turns and bends: short segments of amino acids that join two units of a secondary structure
- A β turn involves four amino acid residues, in which the first residue is hydrogen bonded to the fourth, resulting in a tight 180^0 turn.
- The peptide groups of the central two residues do not participate in any inter-residue hydrogen bonding.

Figure 5.22: Two antiparallel beta strands connected by a bend, i.e. β-turn.



- β-turn is a space saving method of turning a corner and cause polypeptide chains to be compact molecule
- Glycine and proline residues often occur in b-turns, the former because it is small and flexible; the latter because peptide bonds involving imino nitrogen of proline assume the cis configuration, a form that is particularly responsible to a tight turn.
- β-turns are often found near the surface of a protein, and they constitute readily accessible sites, or epitopes, for recognition and binding of antibodies.

Loops

Unlike bends and turns, loops are long segments of amino acid that joins two secondary structures.

TERTIARY STRUCTURE

- The peptide chain, with its secondary structure, may be further folded and twisted about itself forming three dimensional arrangement of the polypeptide chain
- The three dimensional folded compact and biologically active conformation of a protein is referred to as its tertiary structure, e.g. myoglobin
- It indicates, in 3-dimentional space, how secondary features –helices, sheets, bends, turns, and loops assemble to form domains.

Figure 5.23: Schematic tertiary structure of protein.

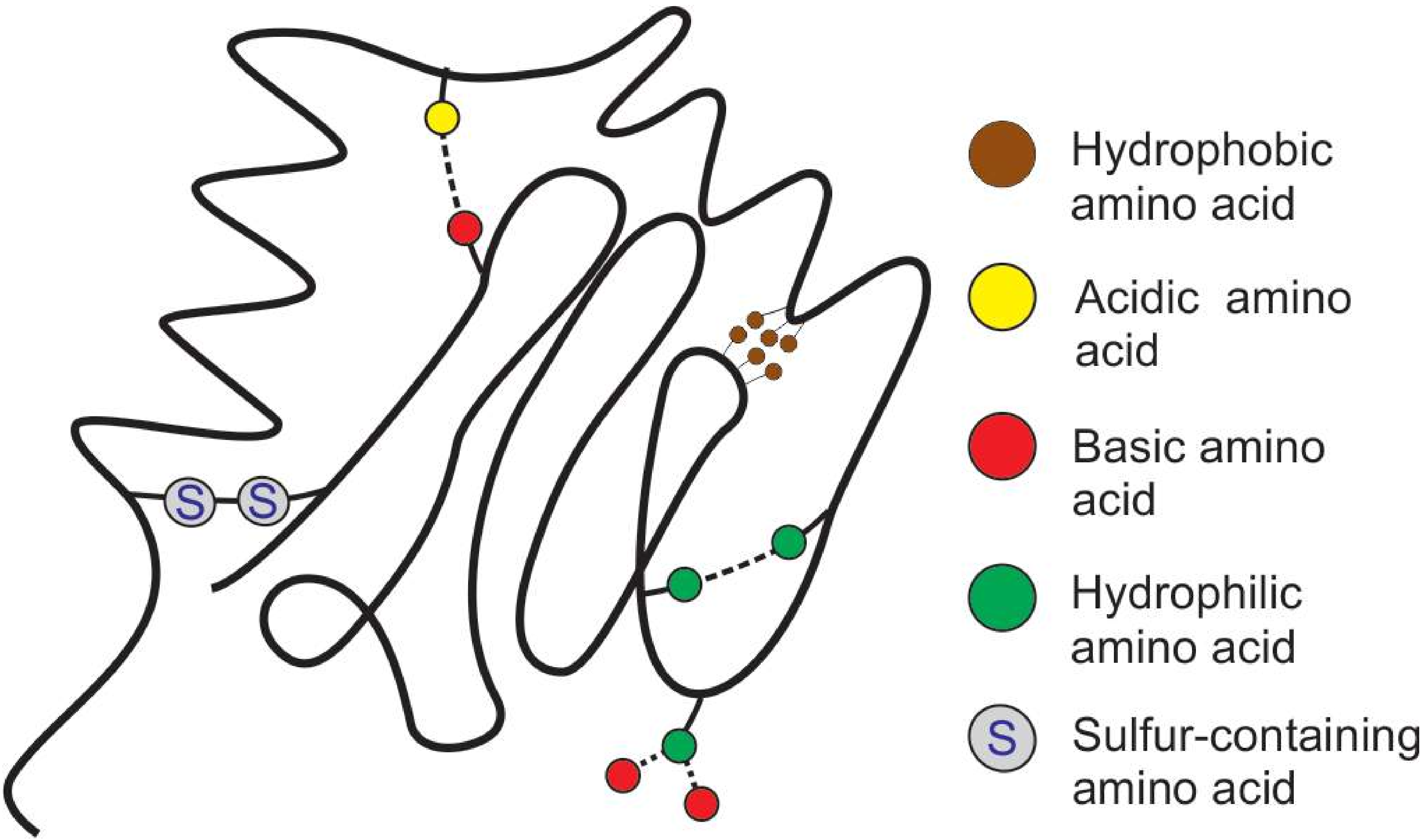
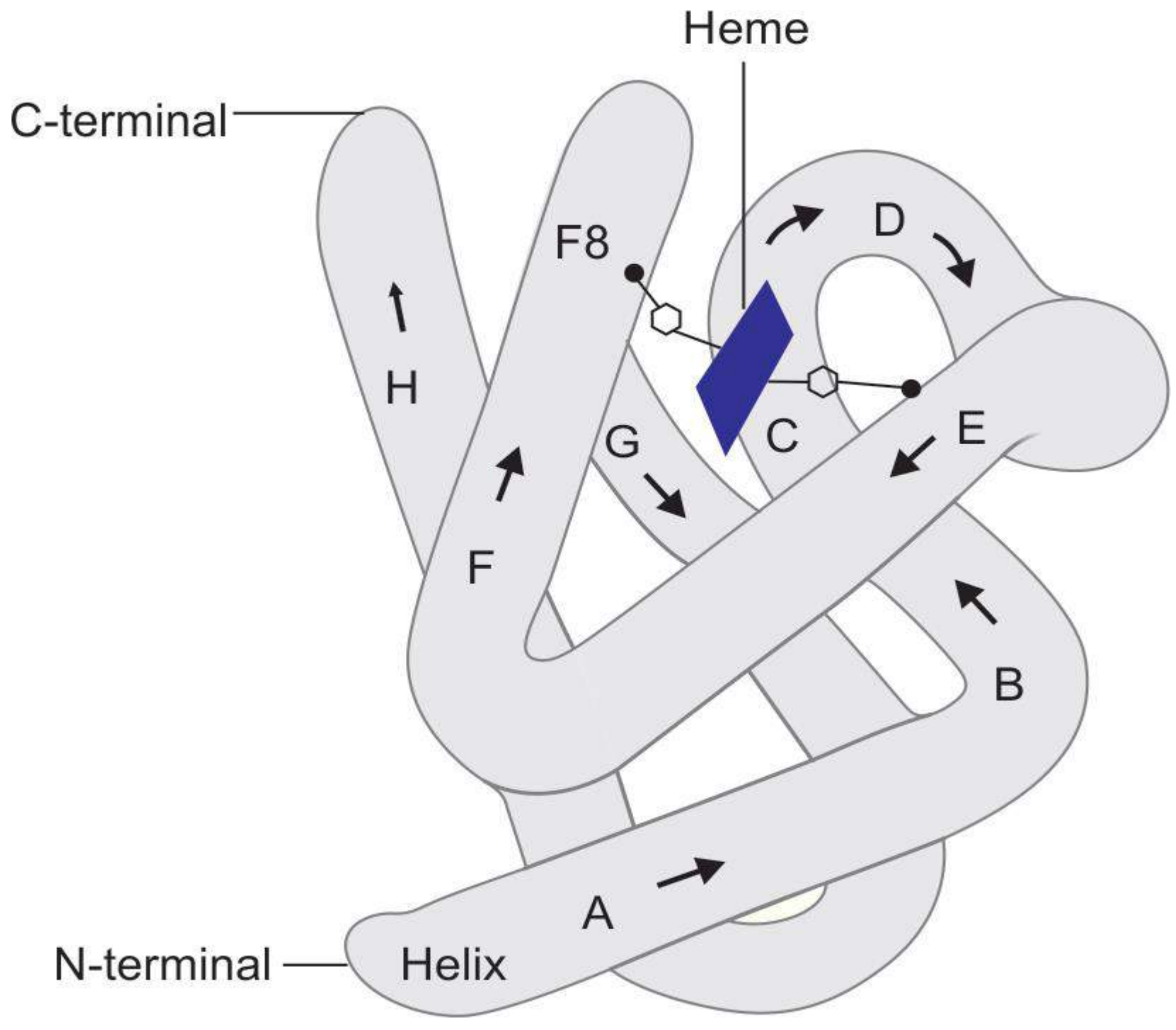


Figure 5.24: Tertiary structure of myoglobin.



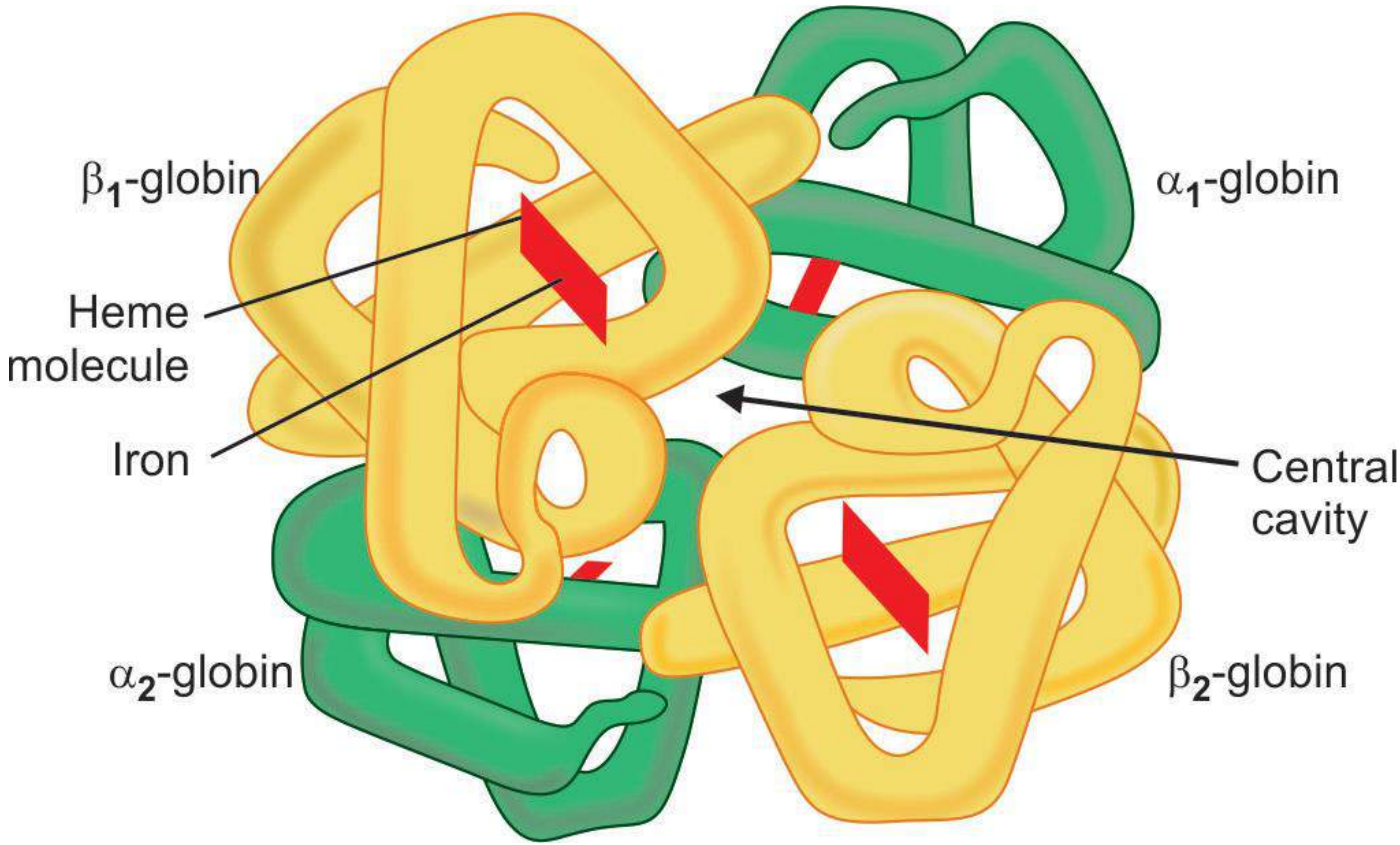
Tertiary Structure Stabilizing Forces

- Hydrogen bonds
- Hydrophobic interactions
- Van der Waals forces
- Disulfide bond
- Ionic (electrostatic) bonds or salt bridges

Quaternary Structure Of Protein

The arrangement of polypeptide subunits in three dimensional complexes is called the quaternary structure of the protein

Figure 5.25: Quaternary structure of polymeric protein hemoglobin.



Quaternary Structure Stabilizing Forces

- Hydrophobic interactions
- Hydrogen bond
- Ionic bonds.
- Vander Waal's forces

Examples of proteins having quaternary structure

- lactate dehydrogenase
- Hemoglobin
- Creatine kinase

- Insulin has two polypeptide chains which are connected by disulphide bond.
- It does not have quaternary structure
- Insulin is the first protein in which complete sequencing of amino acids was done by Sanger

Bonds Responsible for Protein Structure

1. Covalent bond
   - Peptide bonds
   - Disulphide bond
2. Noncovalent bond
   - Hydrogen bond
   - Hydrophobic bond or interaction
   - Electrostatic or ionic bond
   - Van der Waals interactions.

Covalent Bond

- Peptide bonds (–CO-NH–) : bond formed by the condensation of the amino group of one amino acid with the carboxyl group of another amino acid with a removal of a water molecule

- Disulfide bond (-S-S-) : bond formed between the sulfhydryl group (-SH) of side chain of cysteine residues.

Noncovalent Bonds

- Hydrogen bond : formed between -NH and -CO groups of peptide bond by sharing single hydrogen. Side chains of some amino acids can also form hydrogen bond.
- Hydrophobic bond or interaction : formed by interaction between nonpolar hydrophobic R groups (side chain) of amino acids like alanine, valine, leucine, isoleucine, methionine, phenylalanine and tryptophan.

- Electrostatic or ionic bond or salt bond : formed between oppositely charged groups such as amino ($NH3^{+}$) terminal and carboxyl (COO^{-}) terminal groups of the peptide and oppositely charged R-groups of polar amino acid residues.

- Van der Waals interactions : include both an attractive and a repulsive forces (between both polar and nonpolar side chain of amino acid residues).

Properties of proteins

- Colloidal nature

Colloidal protein molecules exert osmotic pressure.

Colloidal osmotic pressure or oncotic pressure

exerted by protein maintain blood volume.

- Molecular weight

Albumin = 6,9000

γ-Globulin = 1,60,000.

- Solubility

globular proteins, such as, albumin have higher solubility than elongated fibrous proteins. Moreover, smaller molecules are more soluble than larger molecules.

- Shape of the protein

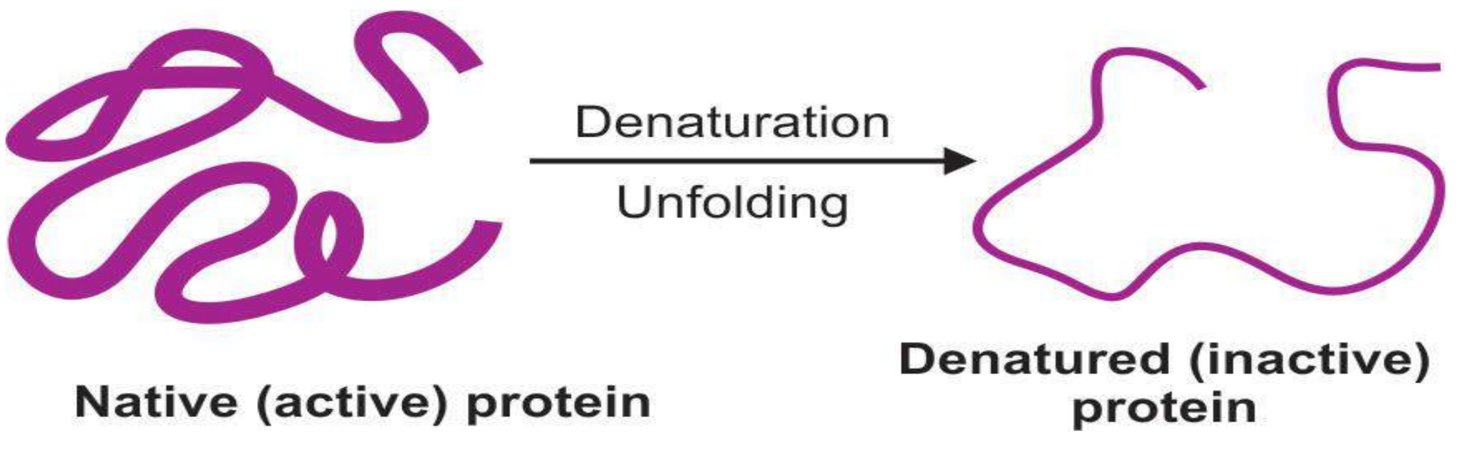
Scleroproteins like keratin, collagen are in the form of fibers. While soluble proteins tend to be of rounded shape and are called globular proteins.

- Amphoteric nature
- Isoelectric pH of the protein
- Hydration of proteins

Shell-like layer of water, called the *“solvation layer”* or *water envelope* is held around each protein particle in an aqueous medium.

Denaturation of protein

Disorganization of primary, secondary, tertiary and quaternary structure by breaking of Hydrogen bond, ionic bond and hydrophobic bond without breakage of any peptide linkage.



Denaturation of proteins leads to:

- Unfolding of natural coils of native protein
- Decrease in solubility and increase in perceptibility
- Loss of biological activities
- Increased digestibility

Denaturing agents

- Physical agents
- Chemical agents
- Mechanical means.

Significance of denaturation

- Digestibility of native protein is increased
- in blood analysis to eliminate the proteins of the blood

Coagulation

Irreversible denaturation. For example boiled egg.