Proteins- lecture part 3 and 4

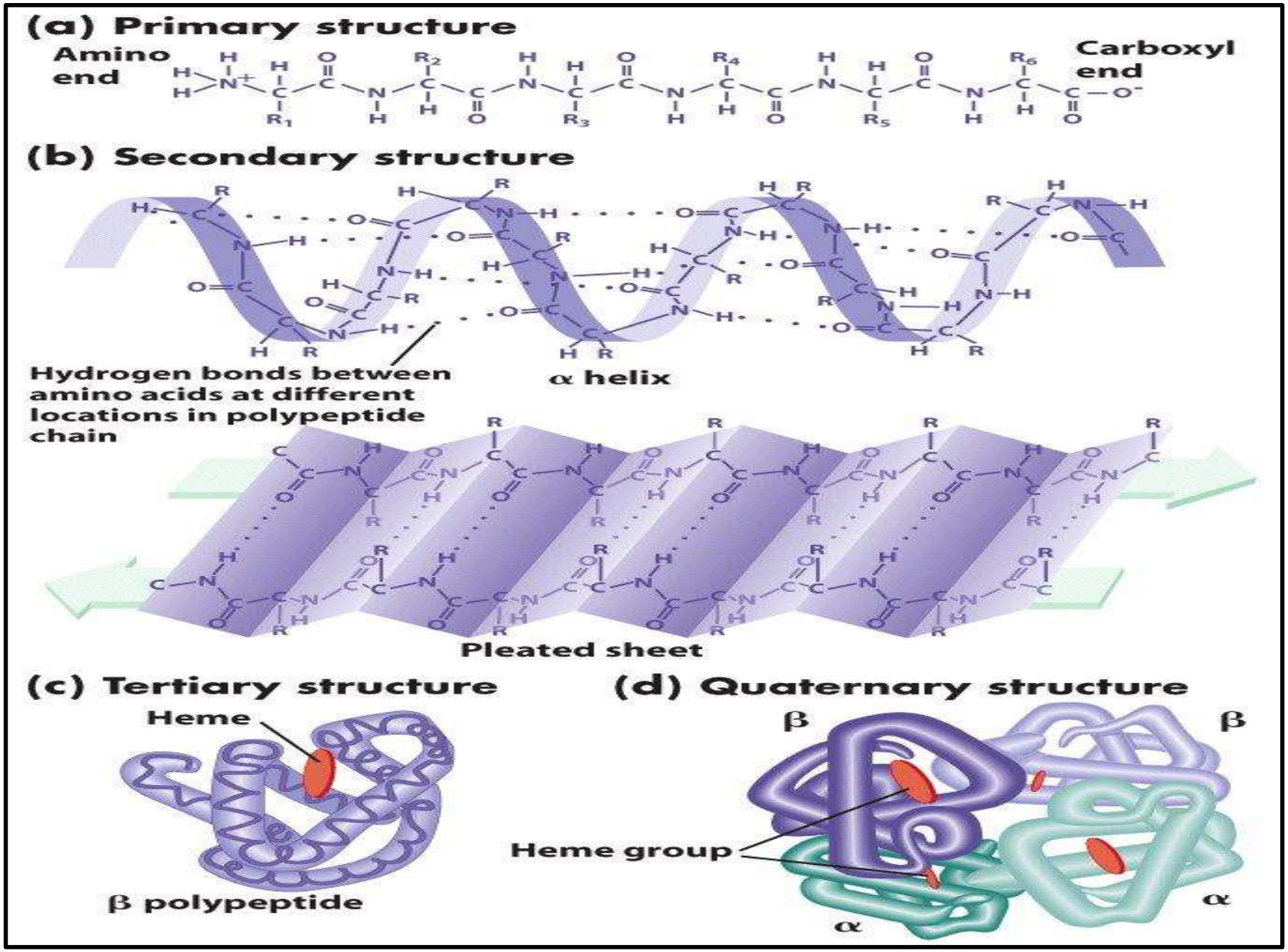
Structural organization of proteins

- Different levels of protein organization

Determination of primary structure of proteins

- Amino acid sequence determination

Levels of structure in proteins



Structural organization of proteins

-Proteins perform a variety of functions
-Functions are closely related to the structures of proteins
-Fundamentally, all proteins are made of amino acids linked to one another by peptide bonds

-A complex three-dimensional structure is formed by:

-Coiling and folding of peptide chains
-Union of several peptide chains with one another

-The three-dimensional structure is also known as **conformation of the protein**

-The conformation is unique to each protein

-The biological functions of a protein depend upon its conformation

-Any change in conformation may lead to loss of function

-The conformation depends upon the sequence of amino acids

Structure of proteins is formed by:

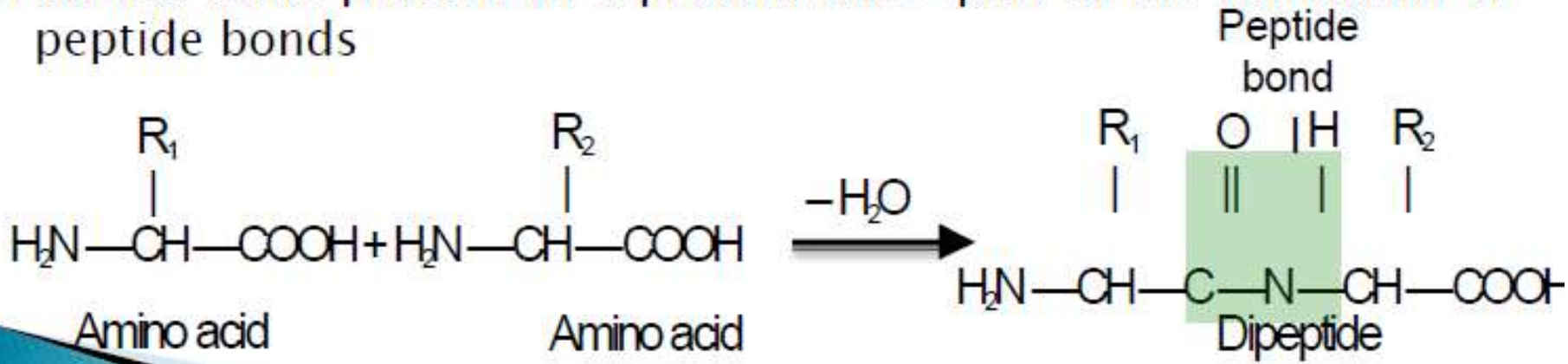
A) Covalent or strong bonds
B) Non-covalent or weak bonds

A) Covalent or strong bonds

These bonds are relatively strong

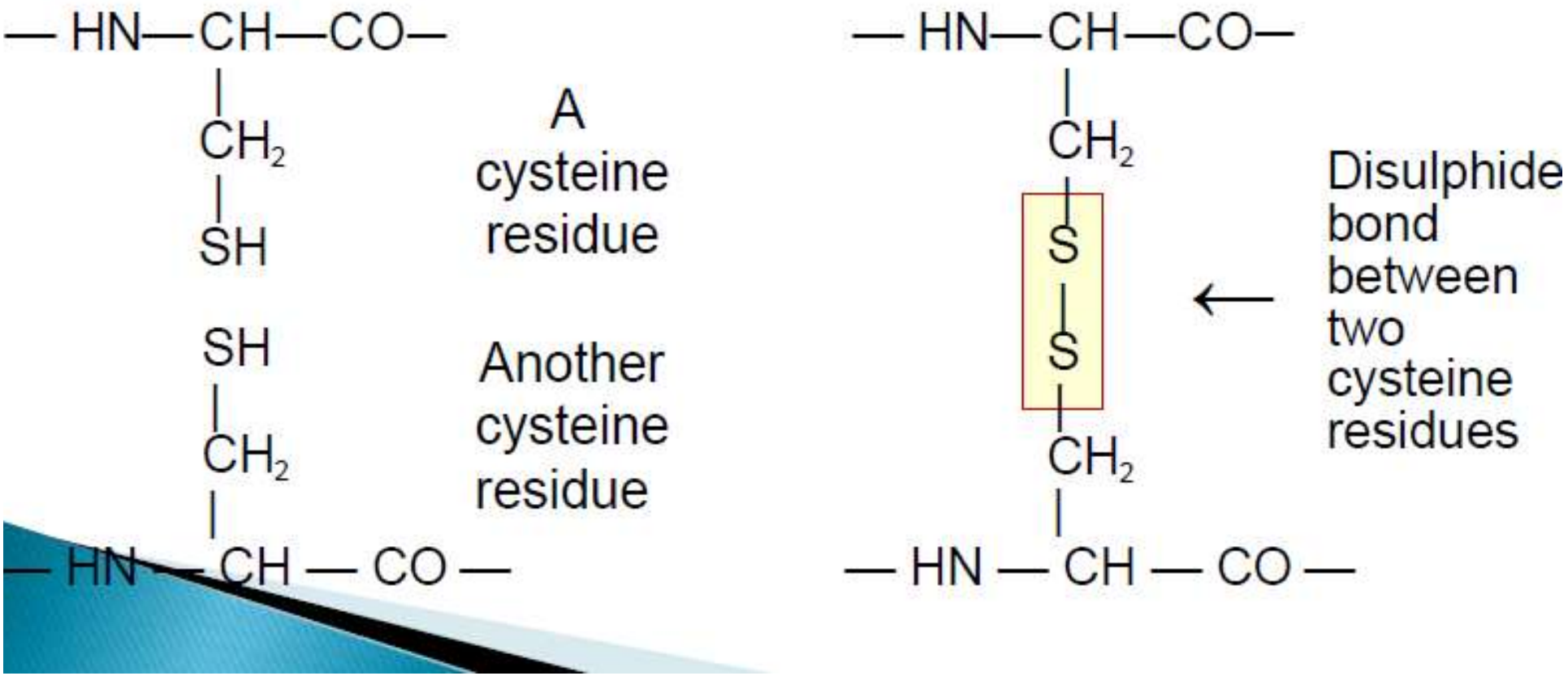
i) Peptide bonds

-These are the basic linkages between two consecutive amino acids
-As they are formed between amino groups and carboxyl groups, they are known as peptide bonds
-All amino acids present in a protein take part in the formation of peptide bonds



ii) Disulphide bonds

-A disulphide bond is formed between two cysteine residues
-The sulphydryl groups of residues are linked together



B) Non-covalent or weak bonds

-Non-covalent bonds are much weaker than the covalent bonds
-But they contribute significantly to the stability of protein structure

The main non-covalent bonds in proteins are

(i) Hydrogen bonds
(ii) Electrostatic bonds
(iii) Hydrophobic bonds

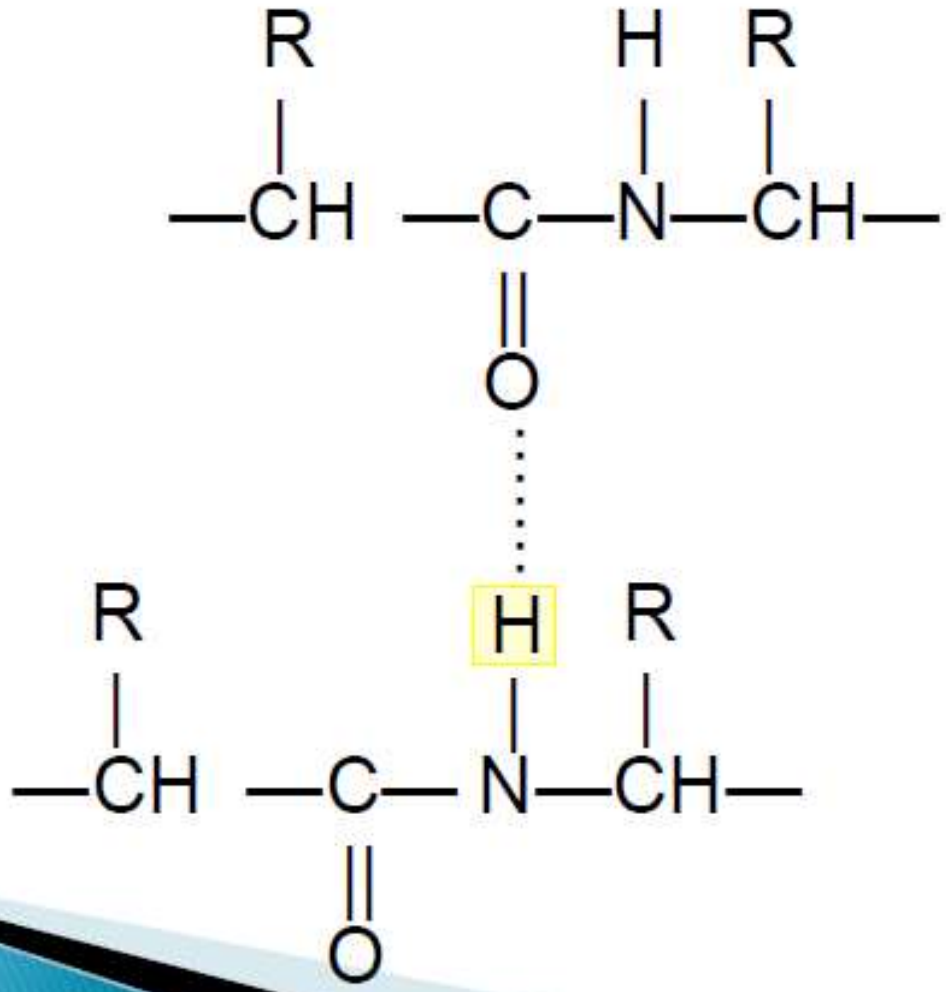
(i) Hydrogen bonds

-Hydrogen bonds are formed between two peptide linkages

-The peptide linkages may be present in the same polypeptide or in different polypeptide chains

-The hydrogen atom of the N–H group participating in a peptide bond is shared between nitrogen and oxygen atoms

-The nitrogen atom involved in sharing belongs to one peptide bond, and the oxygen atom belongs to another peptide bond



(ii) Electrostatic bonds

-Electrostatic bonds or salt bonds are formed between two oppositely charged groups

-Side chains of several amino acids contain ionizable groups e.g. amino groups, carboxyl groups, sulphydryl groups, phenol groups etc

-Such groups may form electrostatic bonds with other groups bearing opposite charges

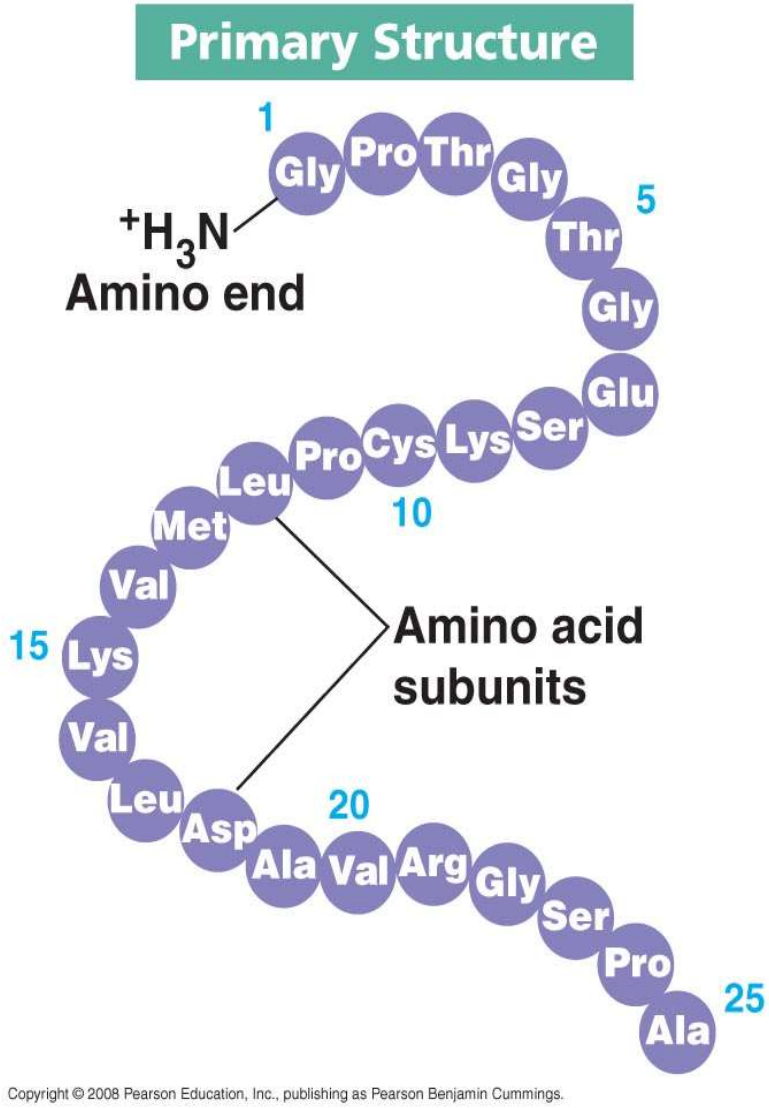
(iii) Hydrophobic bonds

-The side chains of non-polar amino acids attract each other because of their hydrophobic nature

-However, this is only a physical attraction and no chemical bonds are really formed

Primary structure

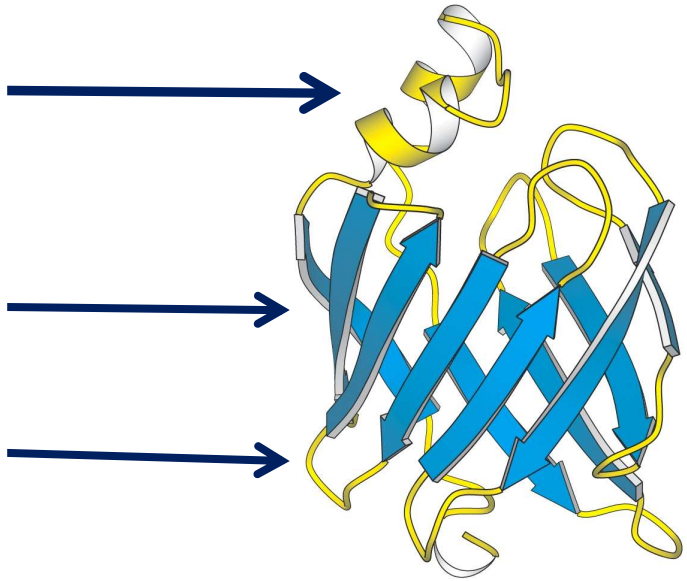
- **Linear sequence of amino acids** joined by peptide bonds in a polypeptide chain refers to the primary structure.
- The **peptide bonds** form the **back bone.**
- Amino end in the primary structure is the beginning residue of a polypeptide and known as **amino terminus.** The other end is a **carboxyl terminus.**
- The free –NH2 group of the terminal amino acid is called as **N-terminal end and the free –COOH end is called C-terminal end.**
- Potential to form hydrogen bonds between oxygen of carboxyl group of one amino acid and hydrogen of amino group of another amino acid.



Secondary structure

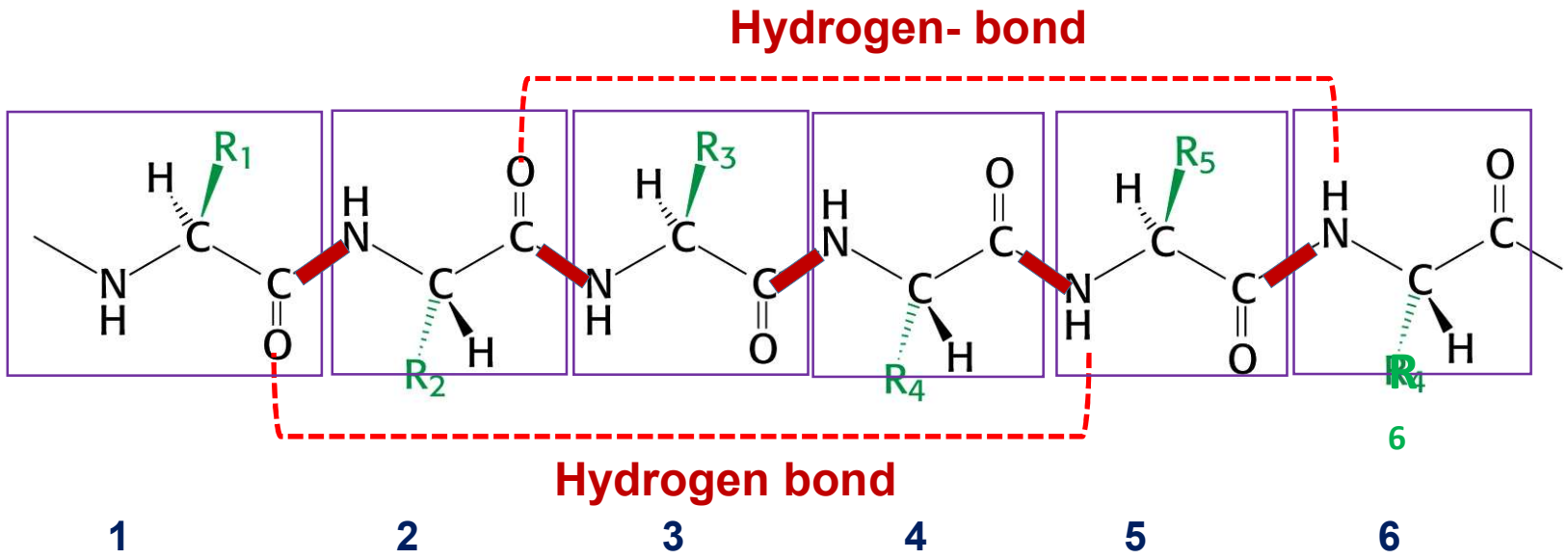
- Amino acids in primary structure interact with one another to form the secondary structure.
- Interaction occurs through **hydrogen bonds**.
- The common secondary structures found in proteins are:

- alpha (α)-helix
- Beta (β)-strand/sheets
- β-turns or reverse turns



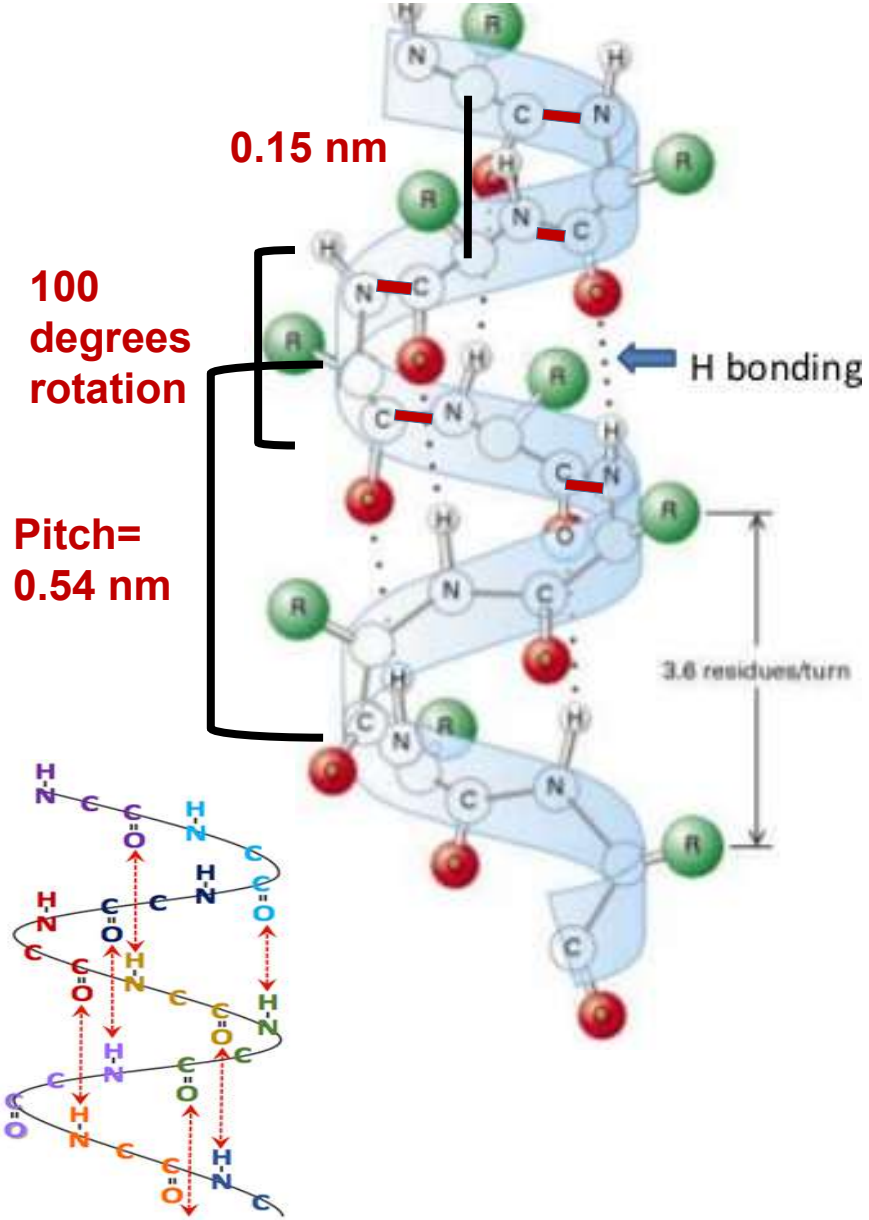
Alpha (α)-helix

- Rod-like structure, the polypeptide chain forms a spiral or a coiling with the backbone forming inner part of the coil and the side chains extend outward in helical array.
- Stabilized by **the intrachain hydrogen bonds** formed between the oxygen atom of carboxyl group of one amino acid and hydrogen atom of amino group of other amino acid.
- Hydrogen bond between every i (Residue 1) and i+4 (Residue 5) residue of polypeptide chain. “i” refers to any number of amino acid. If it “i” is first aa, “i+4” is the fifth amino acid.



α-helical structure

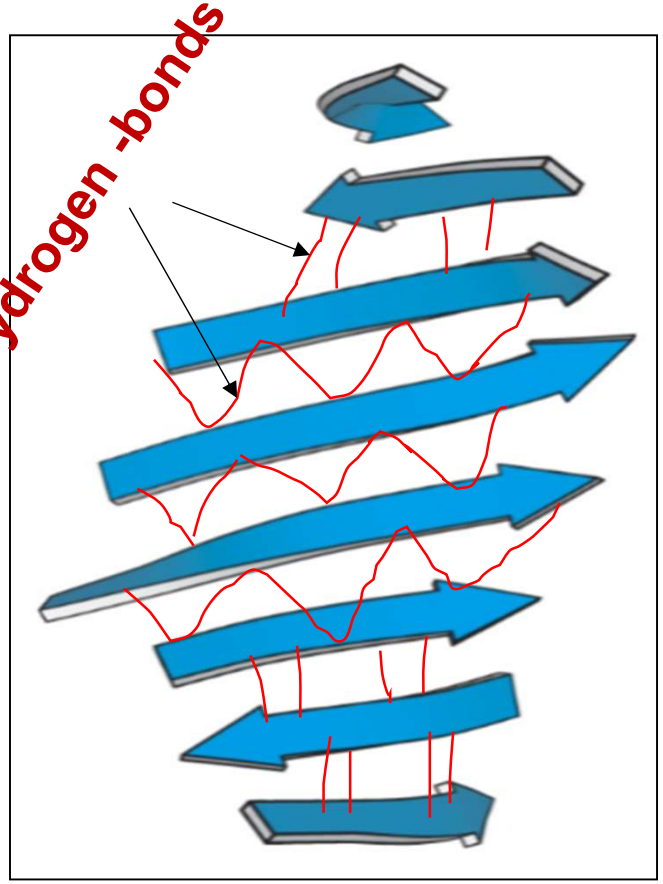
X-ray crystallography (XRC)- determines molecular and atomic positions

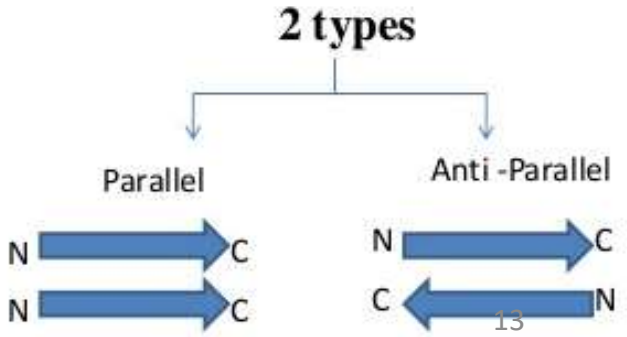


- Amino acids per turn=3.6 residues
- Amino acid distance from one another along the helix axis= 0.15 nm
- Pitch of the helix = **0.54 nm**
- **(Vertical distance between consecutive turns)**
- Screw sense of the helix- describes the direction in which a helical structure rotates with respect to its axis.
- Right handed helix: chain turns in a clockwise direction
- Left handed helix: chain turns in an anticlockwise direction

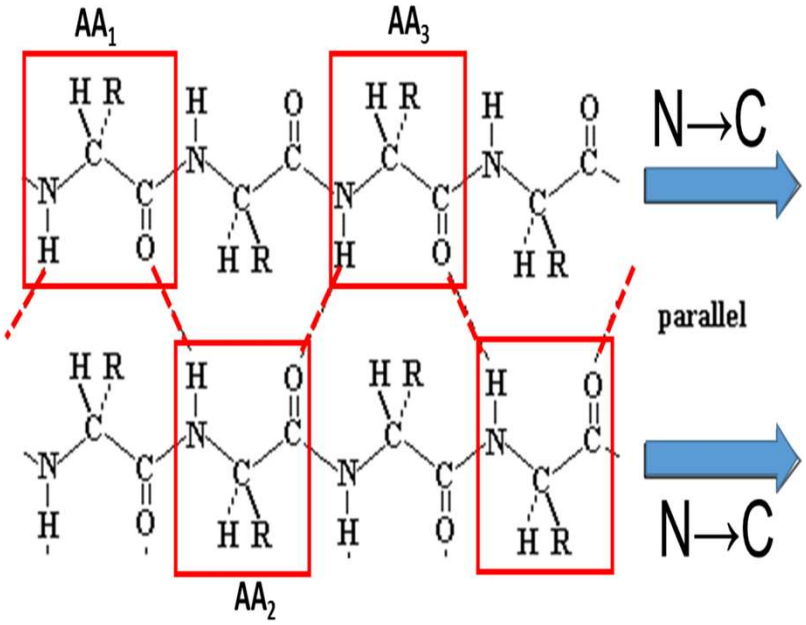
Beta (β)-strand/beta pleated sheet

- β-strand is a linear strand of amino acid.
- β-strands line up as side by side and form hydrogen bonds with one another. This structure is **called β-pleated sheet**.
- The β-pleated sheet is stabilized by **Hydrogen bonds** between -NH and -CO groups of adjacent chains.
- The amino acids in one β-strand can also form hydrogen bonds with **the amino acid in α-helix**.
- Adjacent beta strands can run either in opposite directions **(antiparallel)** or in the same directions **(parallel)** giving rise to **antiparallel beta sheet** and **parallel beta sheet,** respectively.



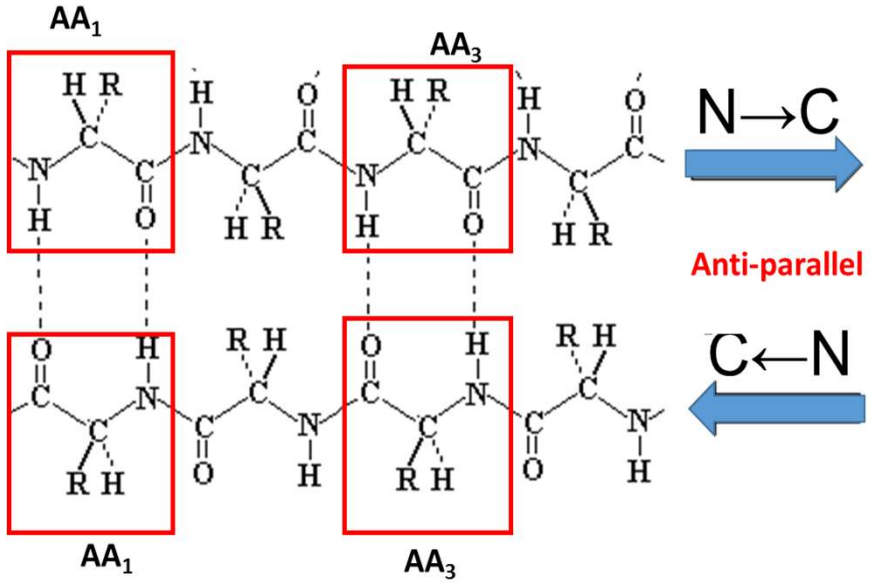


Parallel beta sheet



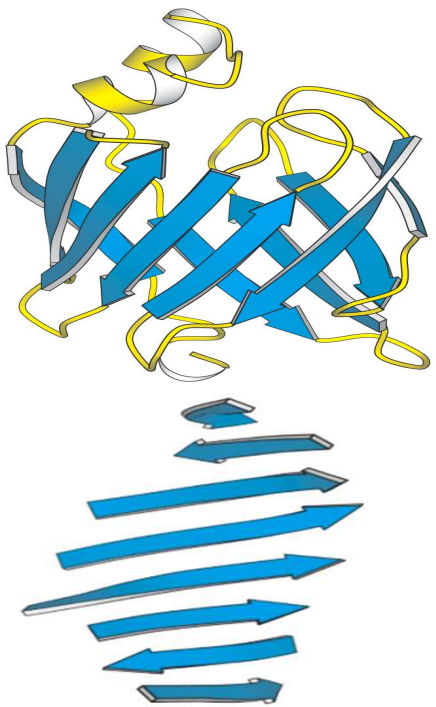
- **Parallel arrangement**: two beta strands run in same directions.
- **Hydrogen bonds**: H-bonds connect each amino acid on one strand with two different amino acids on the adjacent strand, in a zig zag manner, stabilizing the structure. **Amino acid 1 and 3** of one strand **interact with the amino acid 2 on the adjacent strand**..

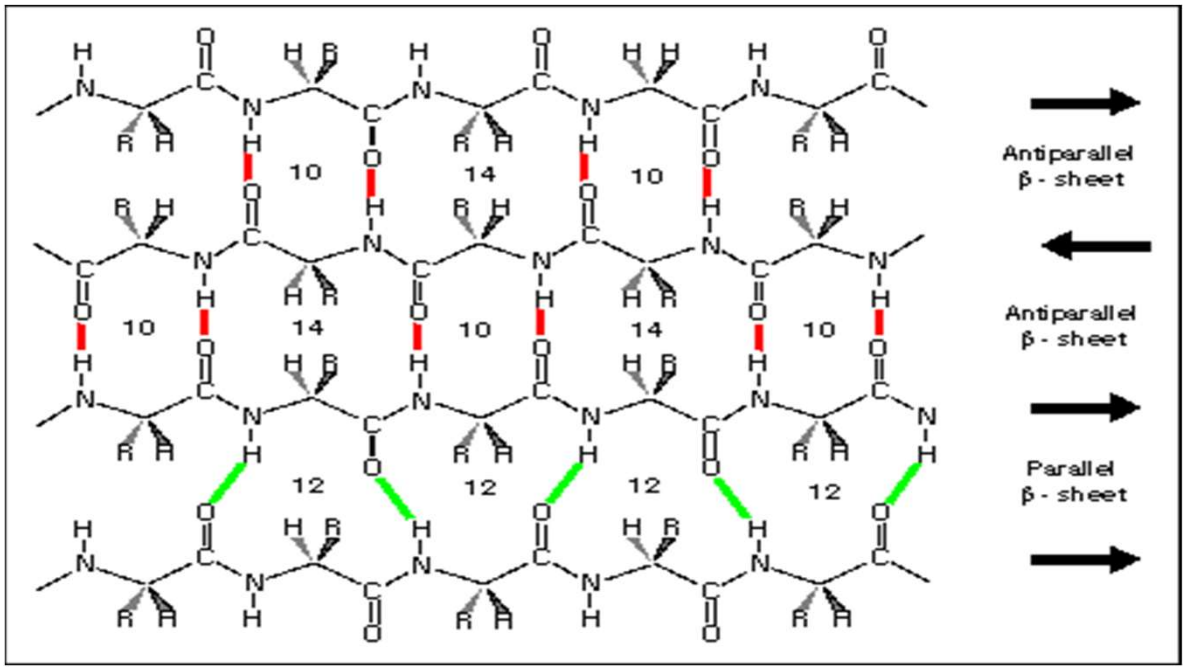
Antiparallel beta sheet



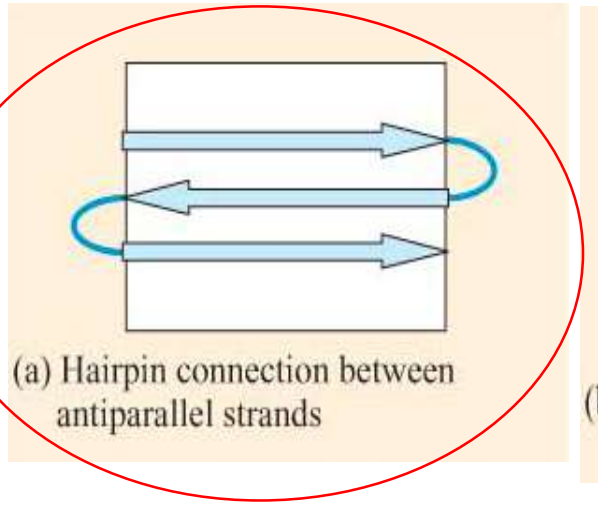
- **Antiparallel arrangement**: two beta strands run in opposite directions.
- **Hydrogen bonds**: The -NH group and the –CO group of each amino acid are hydrogen bonded to the –CO group and the –NH group of a partner on the adjacent beta strand. The hydrogen bonding pattern in **Antiparallel beta sheet** is **" i of one strand and i of adjacent strand, i+2 of one strand and i+2 of adjacent strand, i+4 of one strand and i+4 of adjacent strand.**

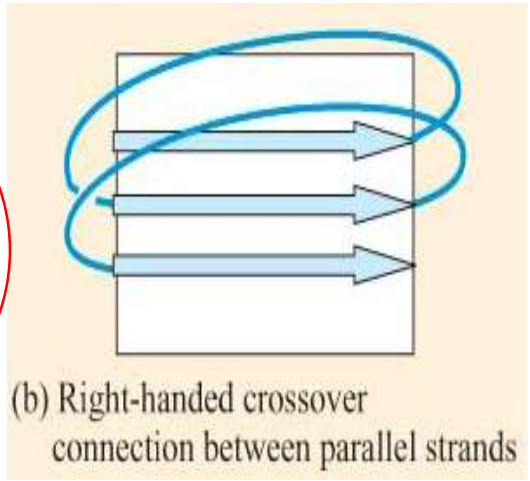
Mixed beta sheet

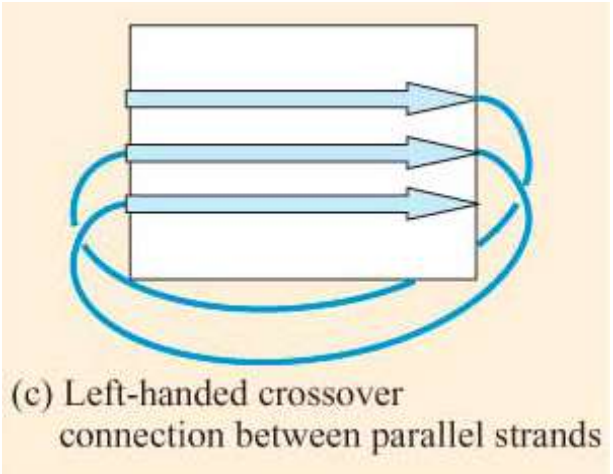




How beta strands are joined to one another in mixed beta sheet

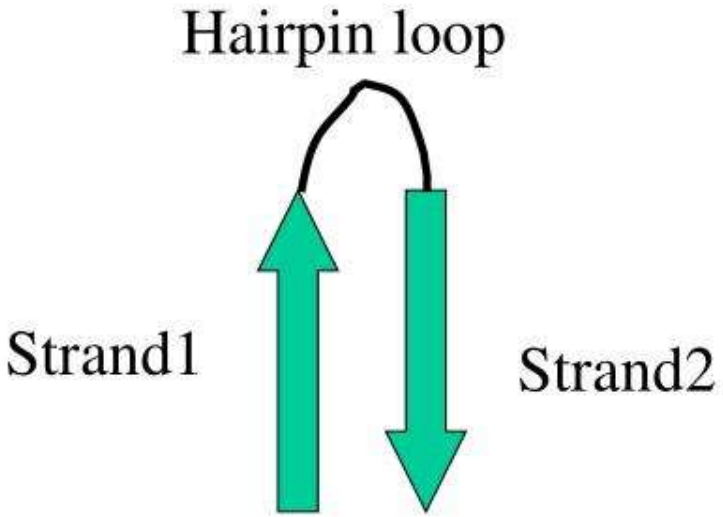

(a) Hairpin connection between antiparallel strands

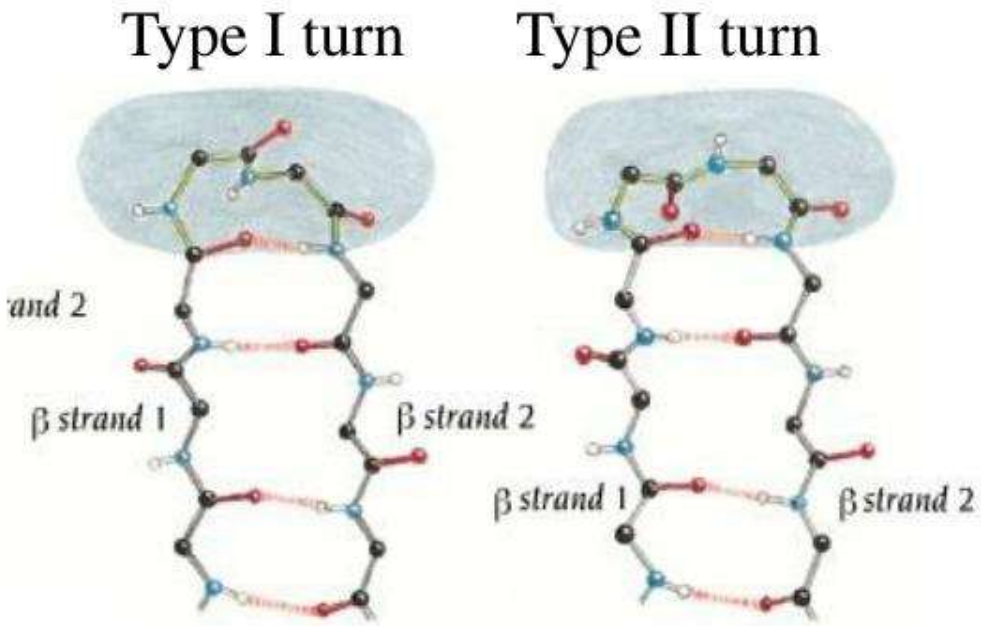

(b) Right-handed crossover connection between parallel strands


(c) Left-handed crossover connection between parallel strands

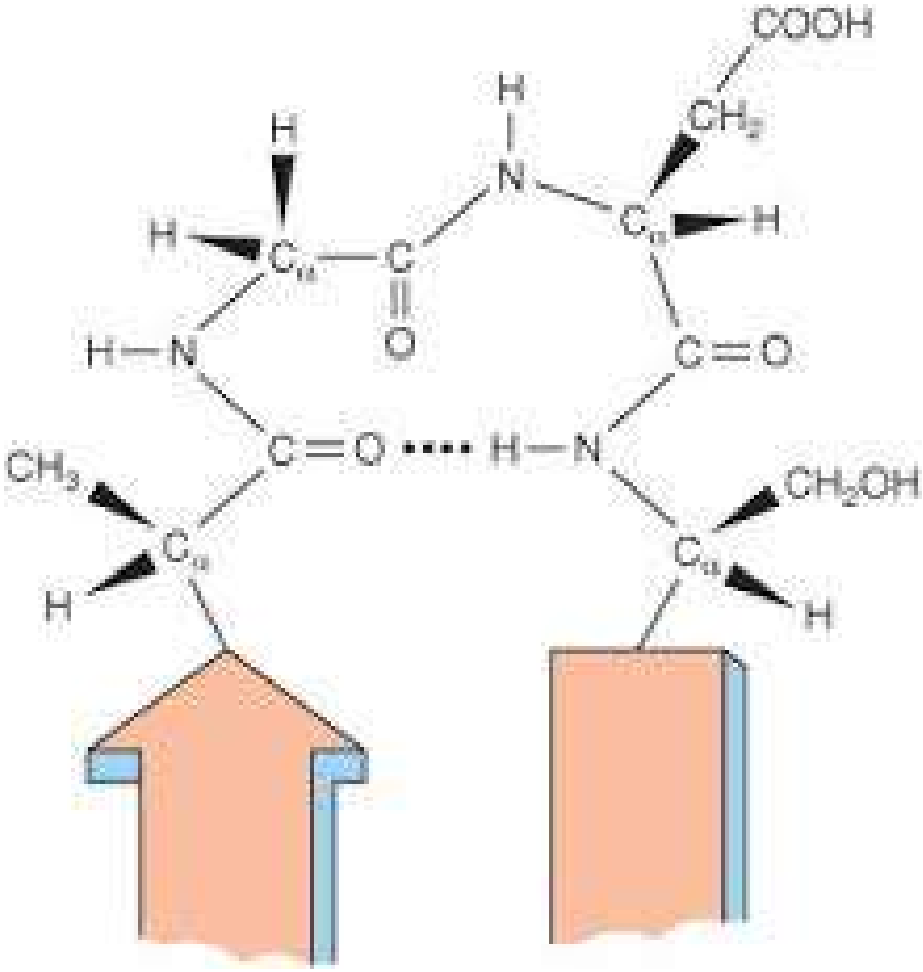
Hairpin loops and reverse turns

- Loops, which connect two adjacent antiparallel beta strands are called hairpin loops
- 2 residues long hairpin loops are often called reverse turns, beta turns or simply turns



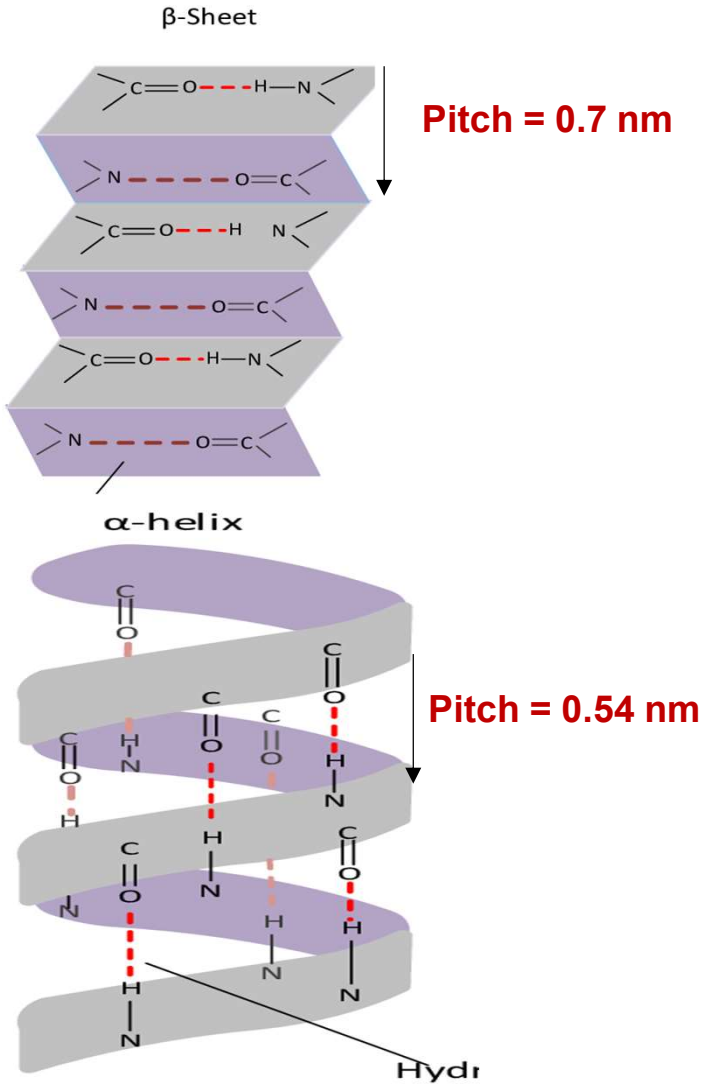


- Permits the change of direction of the peptide chain to get a folded structure.
- It gives a protein globularity rather than linearity.
- H bond stabilizes the beta bend structure.
- Proline and Glycine are frequently found in beta turns.
- Beta turns often promote the formation of antiparallel beta sheets.
- Occur at protein surfaces.
- Involve four successive aminoacid residues



Comparative measurements of alpha helix and beta strands

	Alpha helix	Beta strands
The distance between two adjacent amino acids / axial distance	0.15 nm	0.35 nm
Amino acids covering one turn	3.6 amino acids	2 amino acids
Pitch (extended length along the helix axis)	**0.54 nm (5.4 Å)** which is 0.15 nm x 3.6 amino acids	**0.7 nm,** which is 0.35 x 2



- Polypeptides in the **beta-conformation** are **far more extended** than those **in the alpha-helical conformation.**

Tertiary structure

-The polypeptide chain is folded in complex ways

-Folding produces different types of secondary structures in different regions of the chain

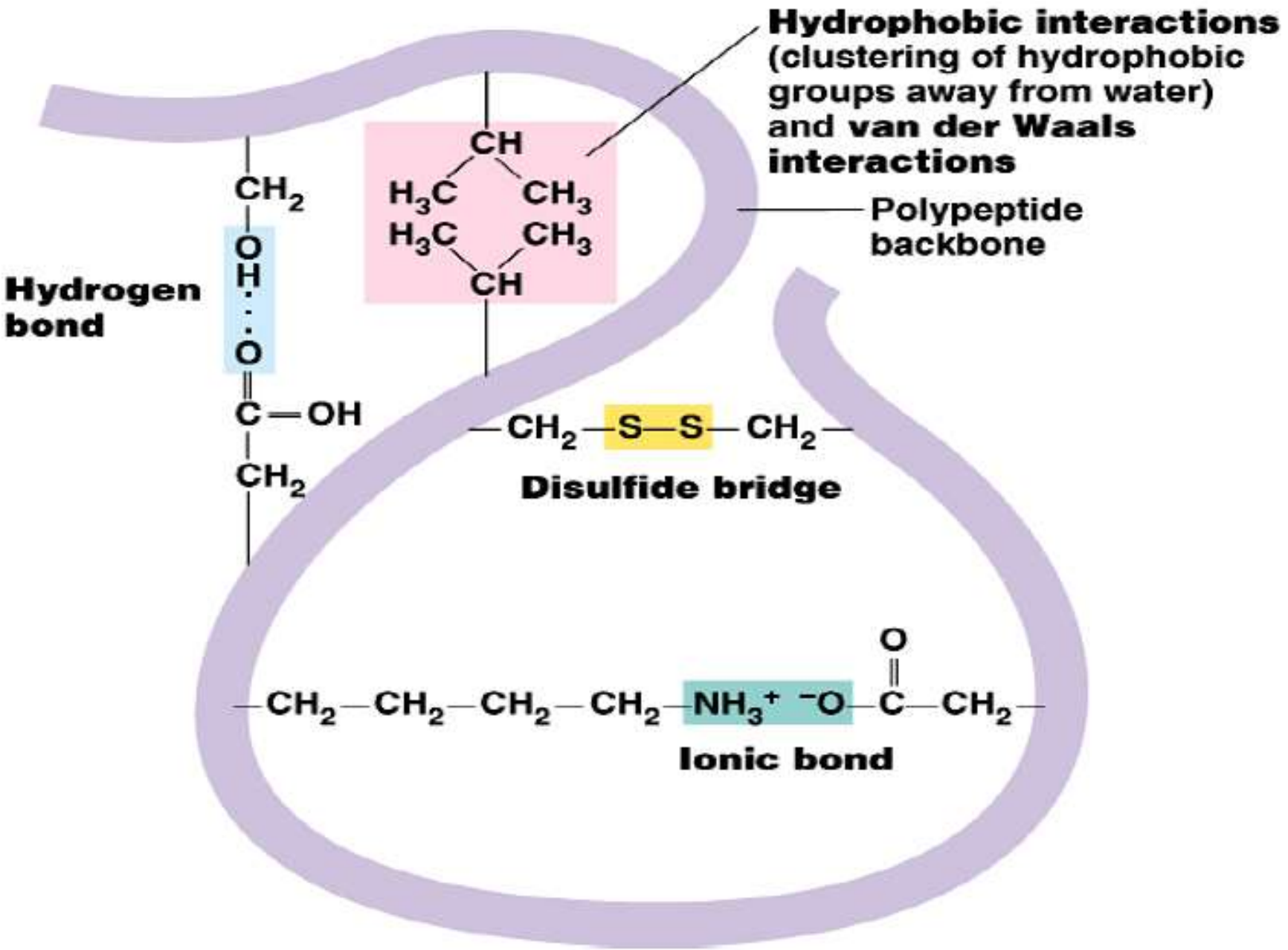
-Some supersecondary motifs are also formed

The folding occurs due to formation of: Disulphide bonds, Hydrogen bonds, Electrostatic bonds, Hydrophobic bonds

-Some amino acid residues which are distant from each other in the polypeptide chain are brought closer

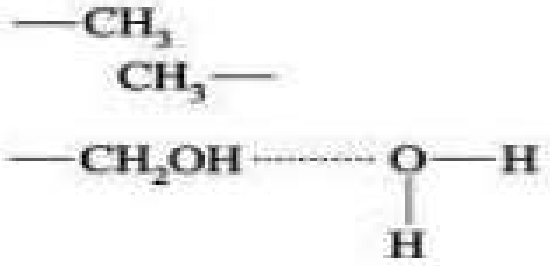
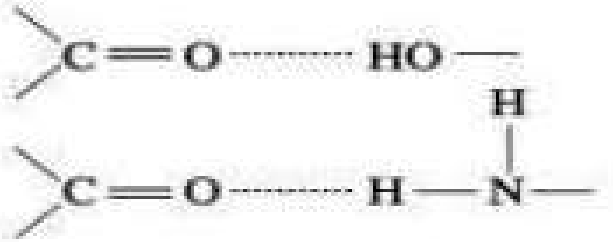
-Some residues are buried into the interior of the molecule

-Some are exposed on the surface of the molecule


Hydrophobic interactions
(clustering of hydrophobic groups away from water)
and van der Waals interactions
Polypeptide backbone
Hydrogen bond
CH
H3C
CH3
H3C
CH3
CH
CH2
OH
O
C
OH
CH2
CH2
S
S
CH2
Disulfide bridge
O
CH2
CH2
CH2
CH2
NH3+
−O
C
CH2
Ionic bond

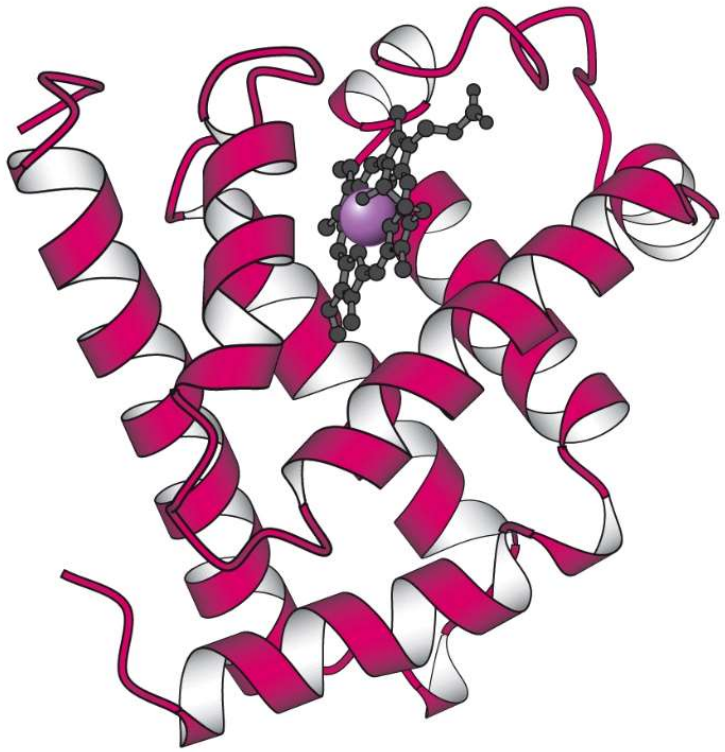
Stabilizing Interactions of Tertiary Structures

Table 20.5 Some Cross-Links in Tertiary Structures

	Nature of Bonding	Example
Hydrophobic interactions	Attractions between nonpolar alkyl and aromatic groups form a nonpolar center that is repelled by water	
Hydrophilic Interactions	Attractions between polar or ionized R groups and water on the surface of the tertiary structure	
Salt bridges	Ionic interactions between ionized R groups of acidic and basic amino acids	
Hydrogen bonds	Occur between polar side groups of amino acids	
Disulfide bonds	Strong covalent links between sulfur atoms of two cysteine amino acids	

Myoglobin -an example of a Globular protein

- Myoglobin (oxygen carrier protein in muscle) in its tertiary structure



- **Globular proteins** fold up into compact, spherical shapes.
- Their functions include biosynthesis, transport and metabolism.
- For example, myoglobin is a globular protein that stores oxygen in the muscles.
  - myoglobin is a single peptide chain that is mostly α-helix
  - the O_2 binding pocket is formed by a heme group and specific amino acid side-chains that are brought into position by the tertiary structure

Fibrous Proteins

- **Fibrous proteins** consist of long fibers and are mainly structural proteins.

- For example,

  α-keratins are fibrous proteins that make hair, fur, nails and skin.

  - hair is made of twined fibrils, which are braids of three α-helices (similar to the triple helix structure of collagen)
  - the α-helices are held together by disulfide bonds

  β-keratins are fibrous proteins found in feathers and scales that are made up mostly of β-pleated sheets

DETERMINATION OF TERTIARY STRUCTURE

- The known protein structures have come to light through:
- **X-ray crystallographic studies**
- **Nuclear Magnetic Resonance studies**
- The atomic coordinates of most of these structures are deposited in a database known as the Protein Data Bank (PDB).
- It allows the tertiary structures of a variety of proteins to be analyzed and compared.

Quaternary structure

-Many proteins are made up of two or more polypeptide chains

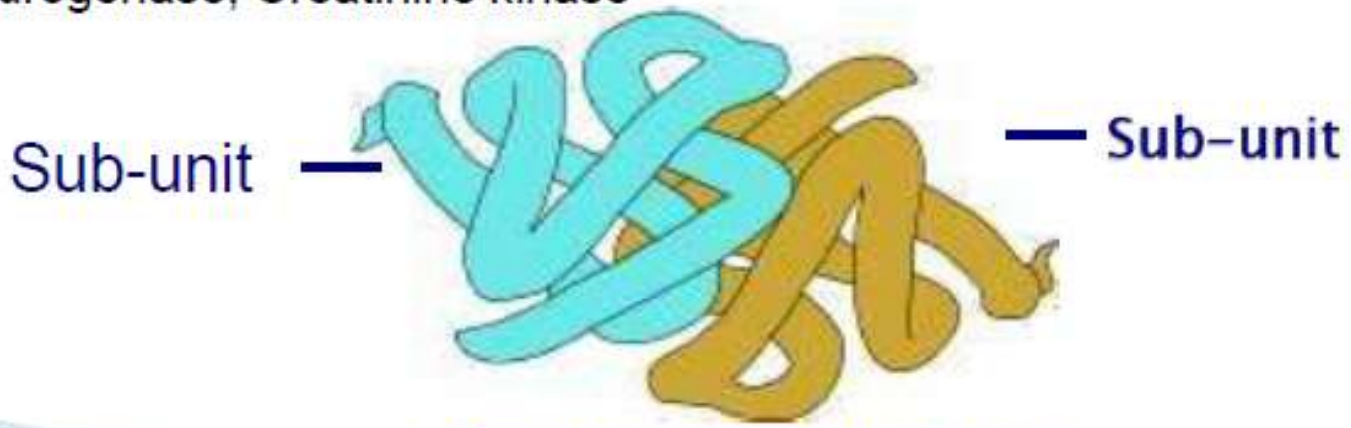
-Each chain is known as a protomer or a sub-unit

-The sub-units may be similar or dissimilar

-The sub-units are joined to each other by non-covalent bonds

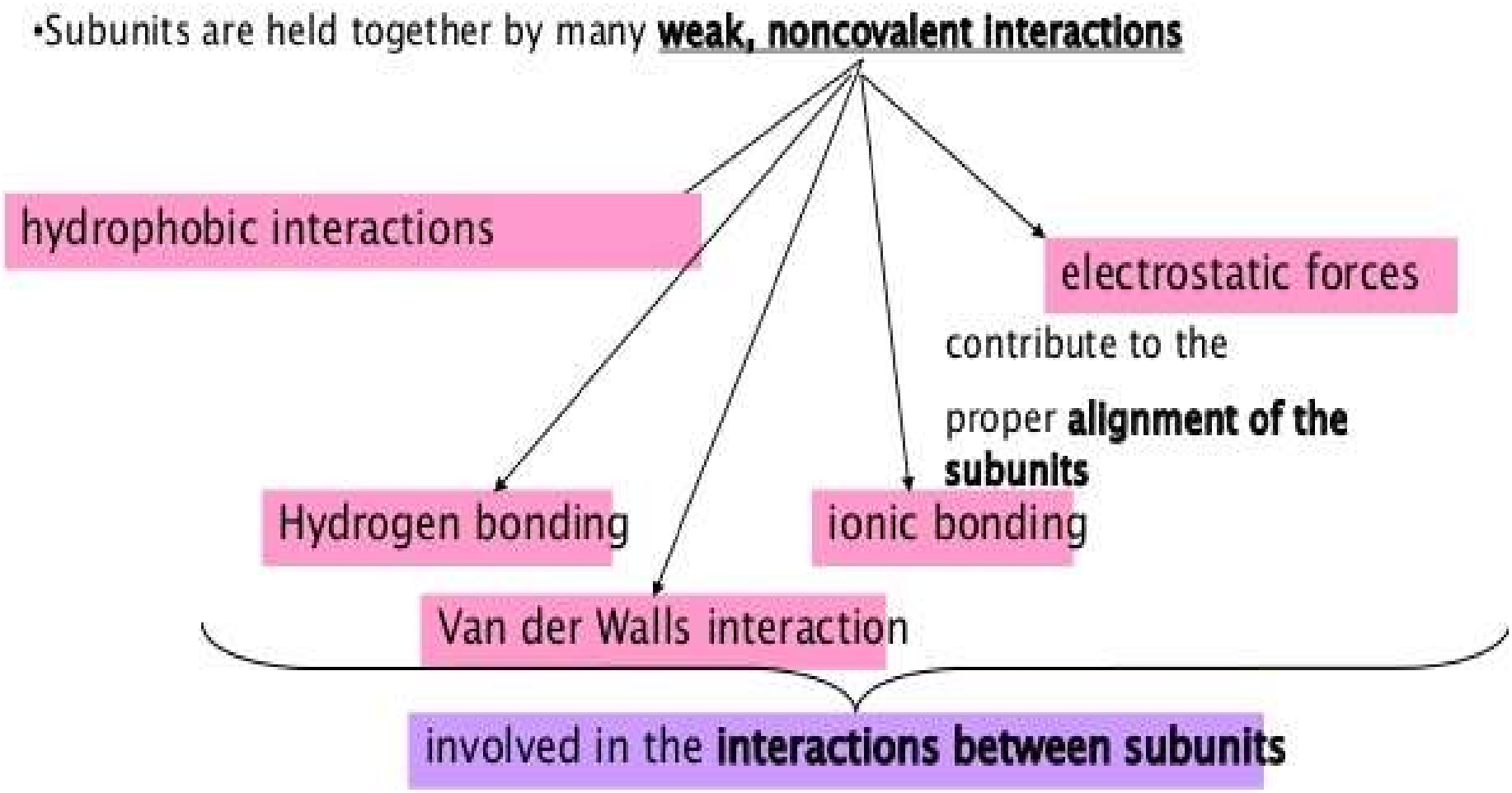
-Joining of sub-units produces the quaternary structure of the protein

Examples of proteins having quaternary structure are: Haemoglobin, Lactate dehydrogenase, Creatinine kinase

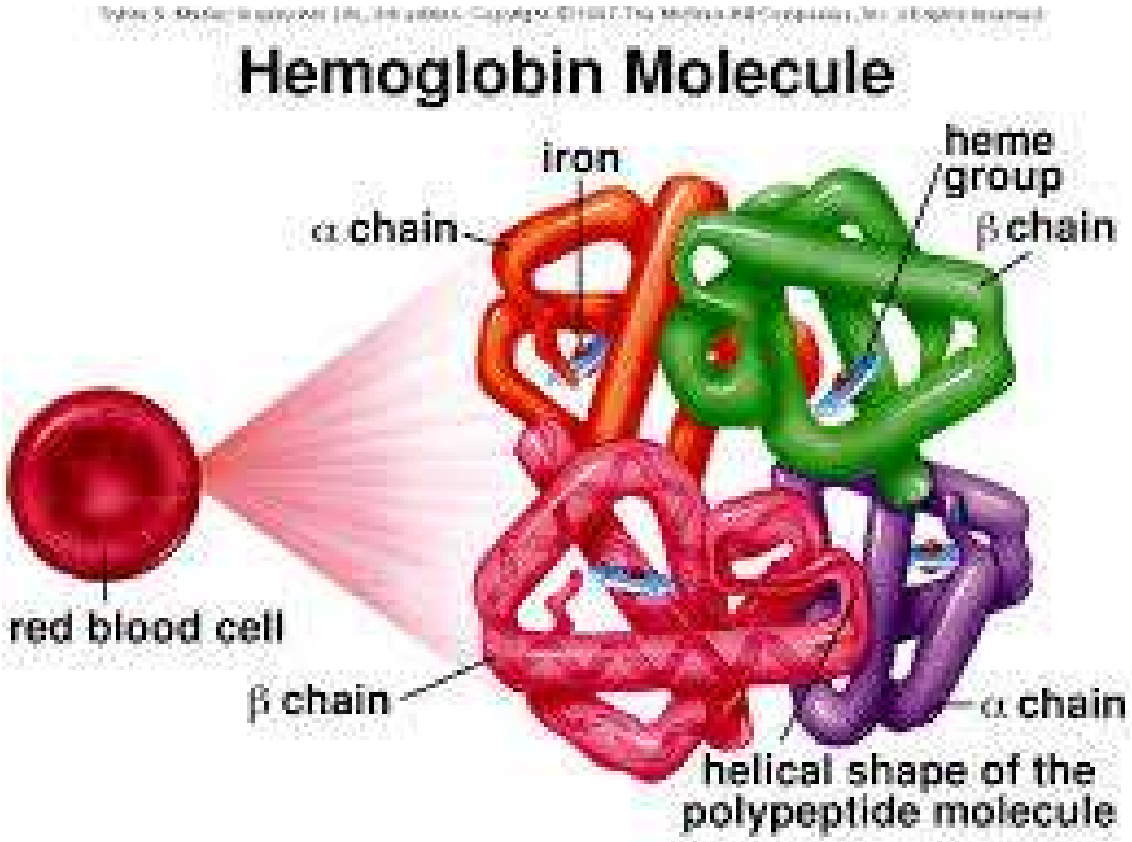


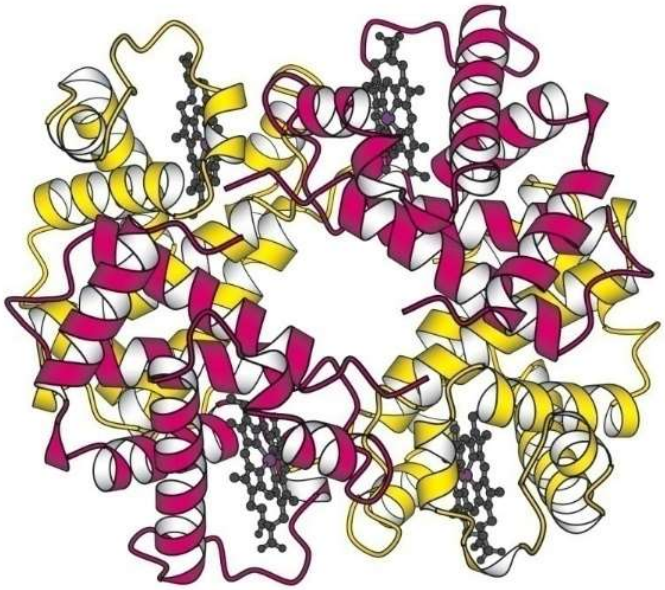
Quaternary structure

Quaternary Structure



Quaternary Structure





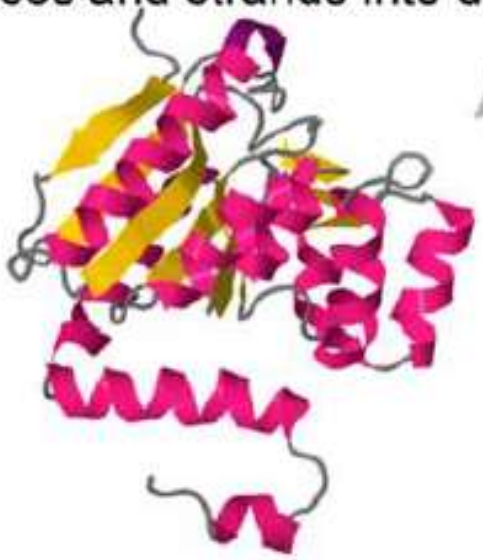
Primary Structure = sequence of amino acids

3-letter code
Lys-Thr-Tyr-Phe-Pro-His-Phe-Asp-Leu-Ser-His-**Gly** ...

1-letter code
KTYFPHFDLSH**G**

Secondary Structure = **alpha helices**, **beta strands**

Tertiary Structure = fold helices and strands into domains



Quaternary Structure (Biological Units) = functional assemblies of chains (subunits)