Proteins

Part 2

C 2112

Coverage

1.Functions and importance of proteins in the living system= Where are they found?

2.Types of proteins= How can we classify proteins?

3.Forces stabilize the protein = What are the possible interactions between A.As?

4.Structures of the protein = Why 3D structure?

Protein functions

* Catalytic function: digestions, hydrolysis, biosynthesis, oxidation and reduction, muscle contraction and so on.

* Structural proteins: rigidity and stiffness for cells, such as keratin (hair and nails), collagen (bones, ligaments and skin), and elastin (more flexible than collagen).

* Transport and store: some proteins carry substances, nutrition and drugs throughout the blood stream into cells or out of cells.
Sugars, cholesterol or oxygen, such as hemoglobin which carries oxygen from lungs to body tissues.
The lipoproteins LDH and HDL transport the insoluble form of cholesterol form liver to the tissues.

* Defense proteins: some proteins help to protect the body against virus or fight infections. immoglobulines or antibodies.

* Regulating proteins: some proteins act to maintain the acid-base balance of fluids, such as Albumin and Globulin. Proteins can bind hormones for regulating purposes, such as the peptide hormones insulin and glucagon, which regulate blood sugar.

Types of proteins

Based on the composition

Simple	Conjugated
Albumin	Nucleoprotein (Ribosome, virus)
Globulin	Lipoprotein (Chylomicrone)
Glutens	phosphoprotein (Casein)
Protamine	Metalloprotein (Ferritin and hemoglobin)
Histones	Glycoprotein (Muncie)
Scleroproteins	Flavoprotein (FAD (Flavin adenine di nucleotide)
	Hemoprotein (hemoglobin)

Based on the shape

Fibrous (water insoluble)	Globular (water soluble)
α-keratin	Myoglobin
	Hemoglobin
collagen	Ribonuclease
	Chymotrypsin
	Lactate dehydrogenase

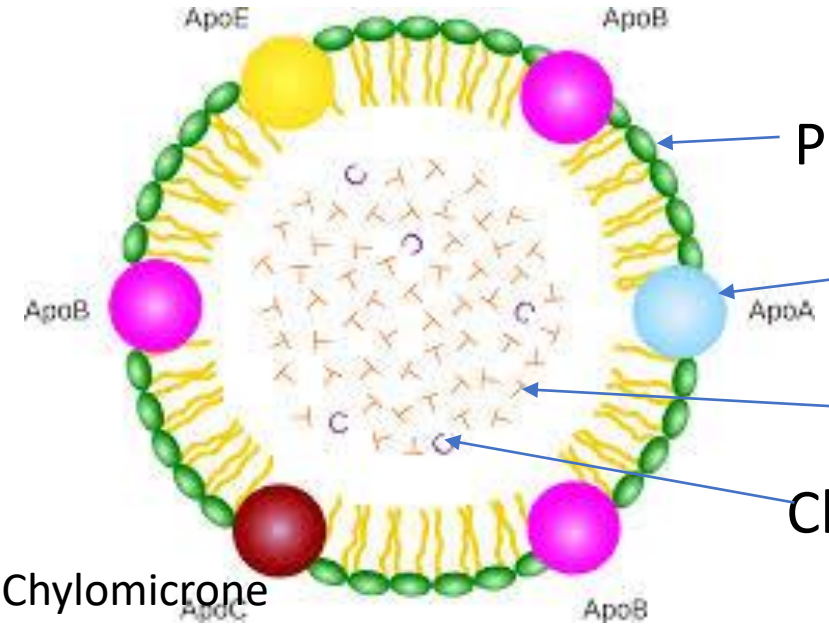
How Can we classify proteins?

Based on the composition

Albumin

Simple

Conjugated



Phospholipids

Protein

Triglyceride

Cholesterol

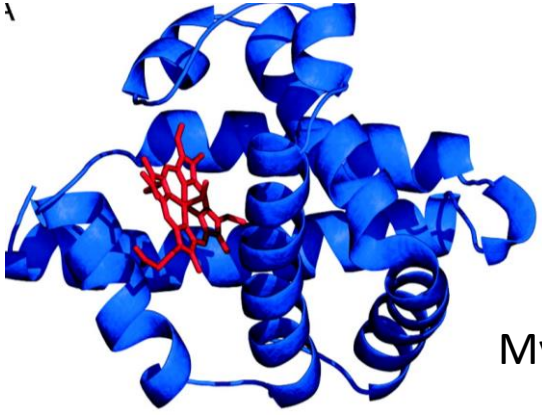
Chylomicrone

Based on the shape

Fibrous

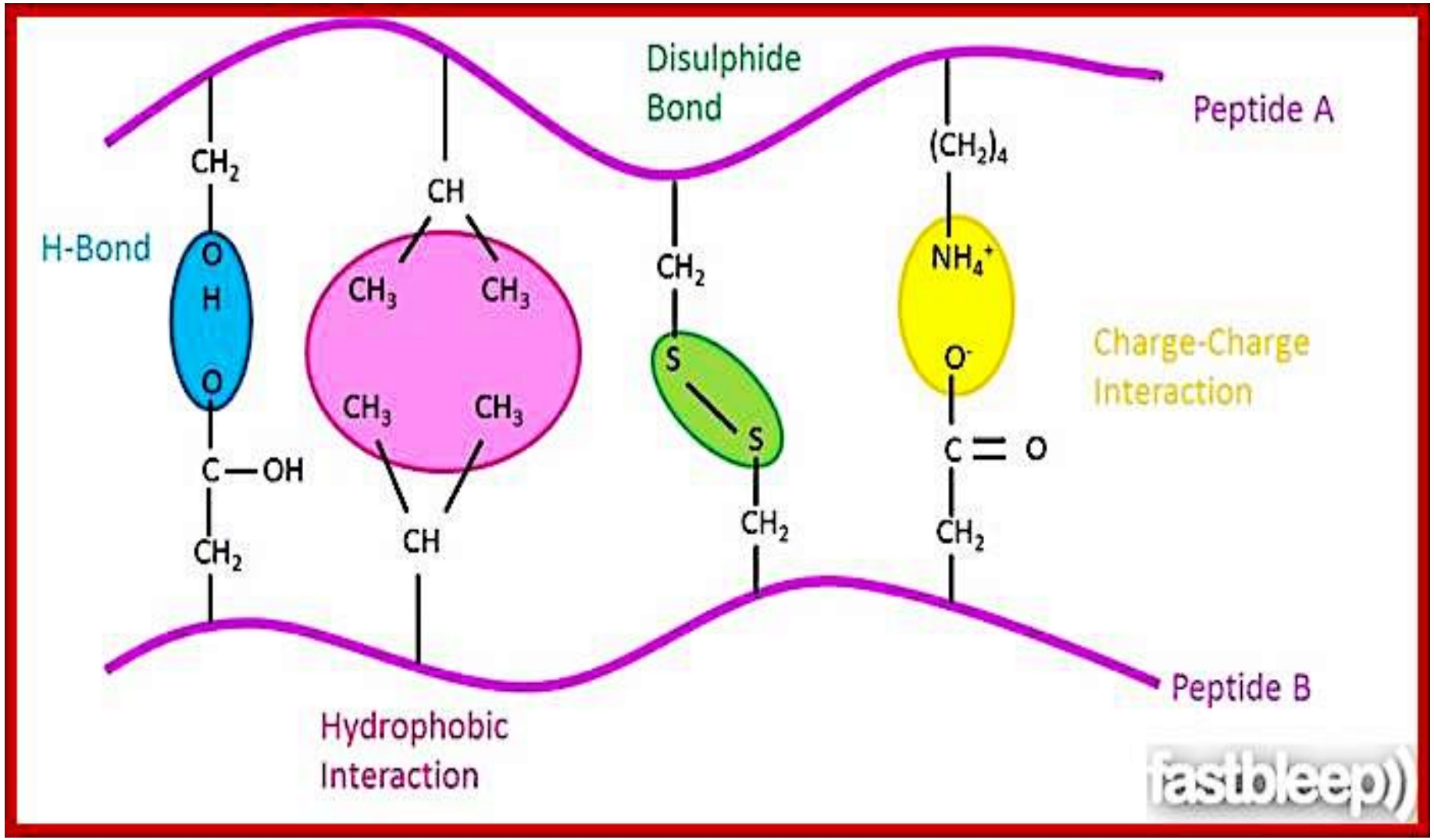
collagen

Globular



Myoglobin

Forces stabilize the protein structure

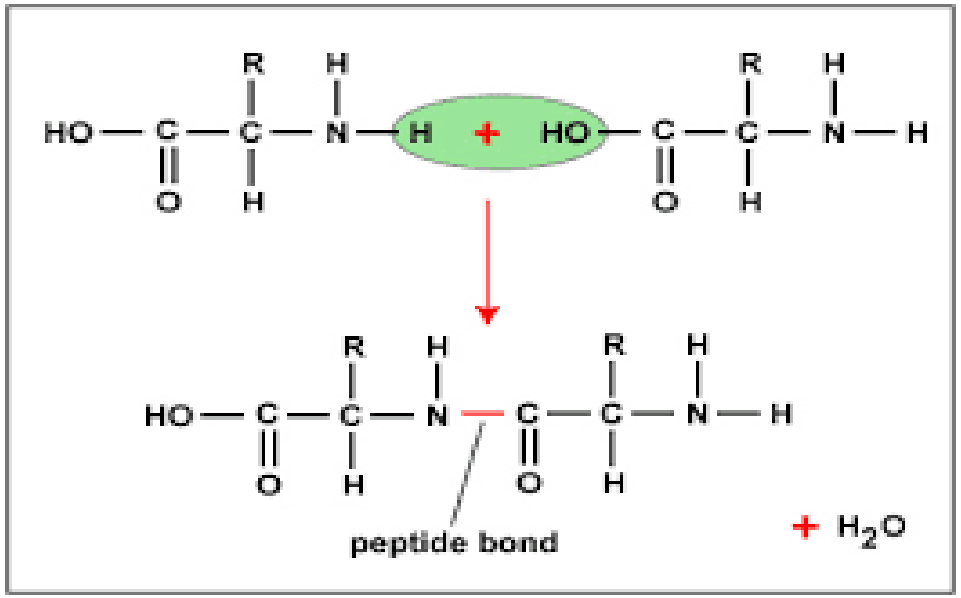


Type of Forces in Proteins

Covalent bonds

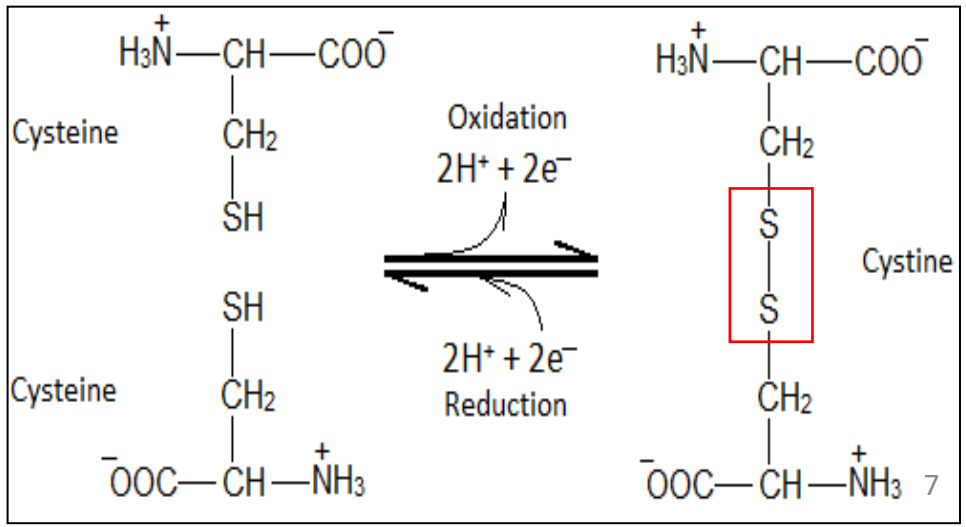
1.Peptide bonds:

Between C=O and NH of the amid group of 2 A.As

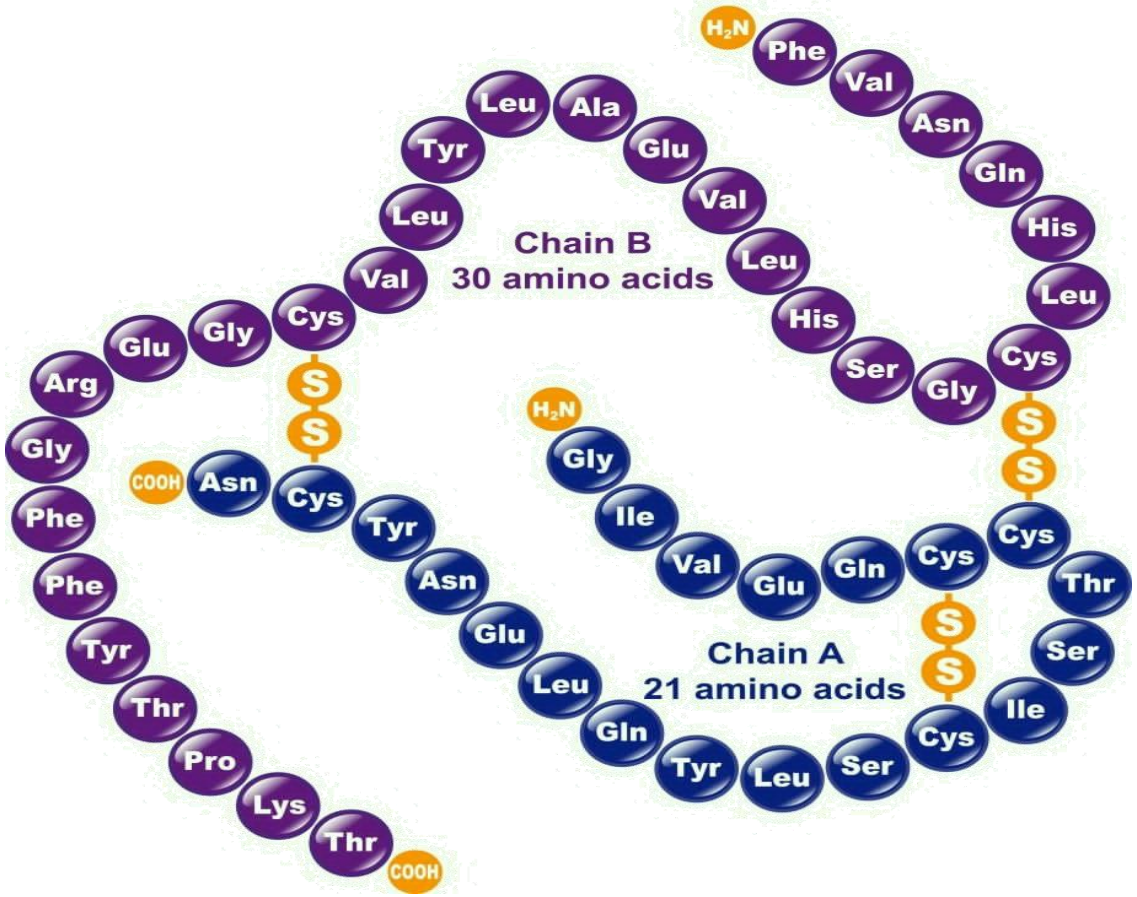


2. **Disulfide bonds:**

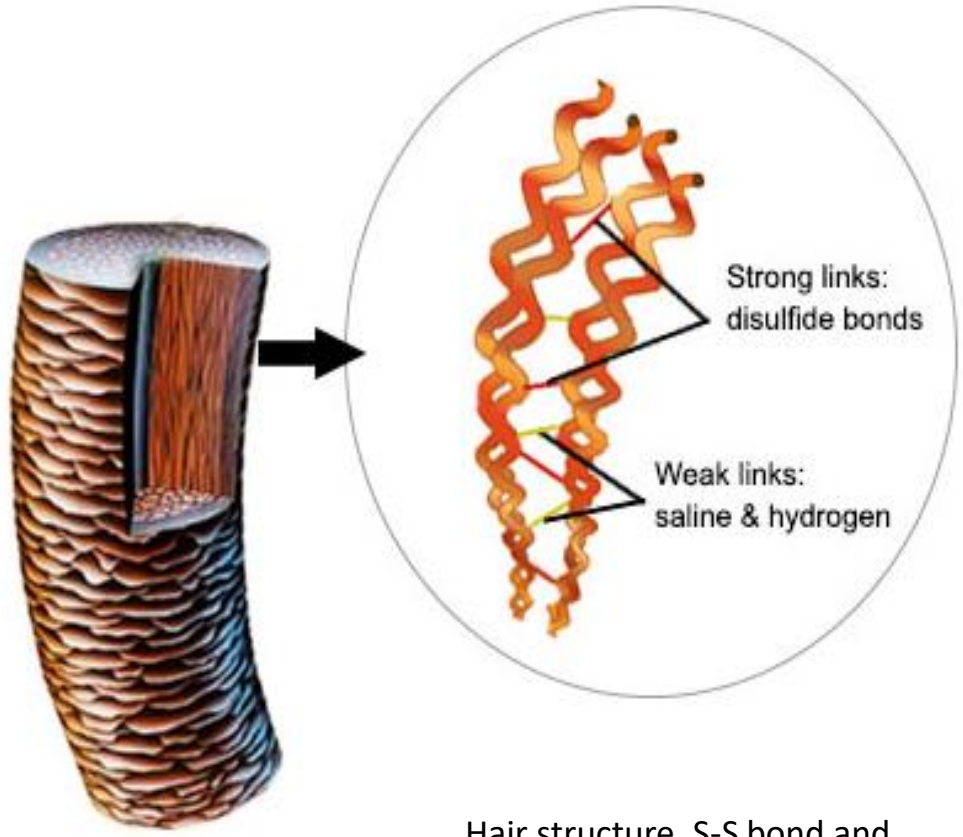
between any cysteine residues present. Cysteine residues can form disulfide bridges (also called disulfide linkages)



Type of Forces in Proteins



Human insulin



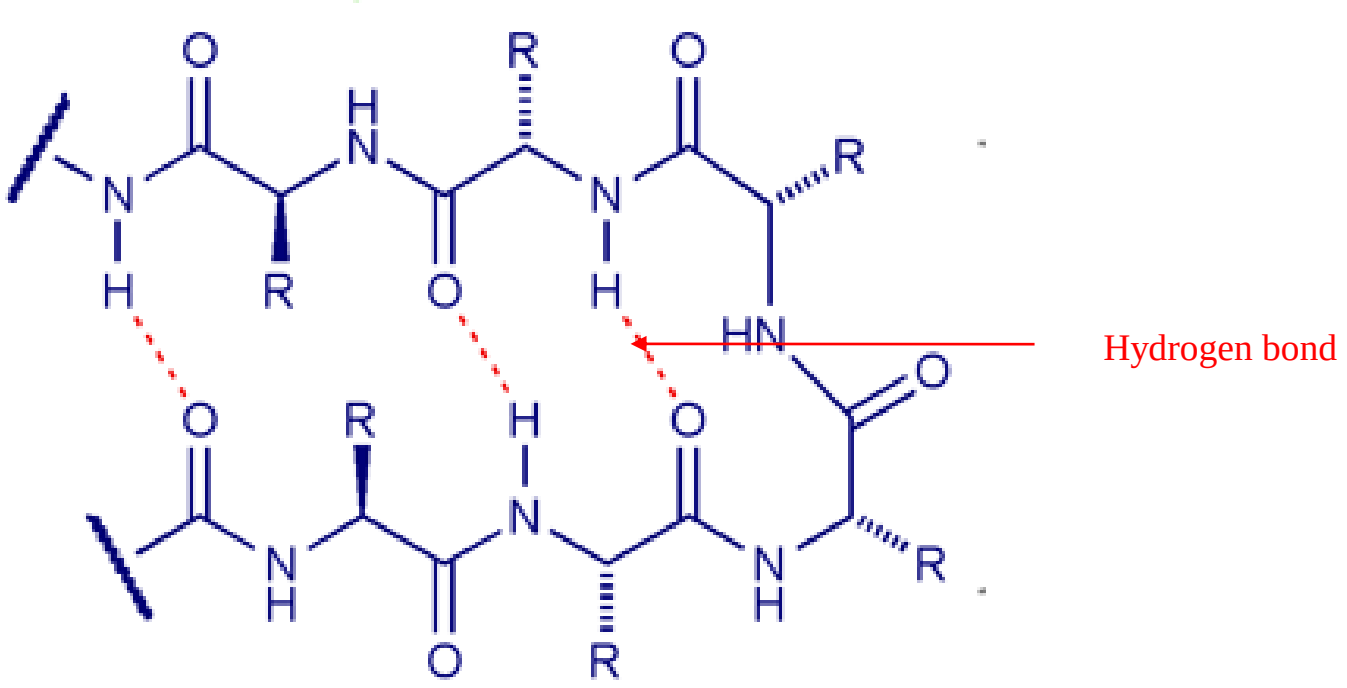
Hair structure, S-S bond and hydrogen bond

Type of Forces in Proteins

Non covalent bonds

3. **Hydrogen bonds:** between water and the protein and within the protein itself= folding and stability of the protein

Hydrogen bonds C=O of each peptide bond in the strand and the hydrogen of the N-H group of the peptide bond.

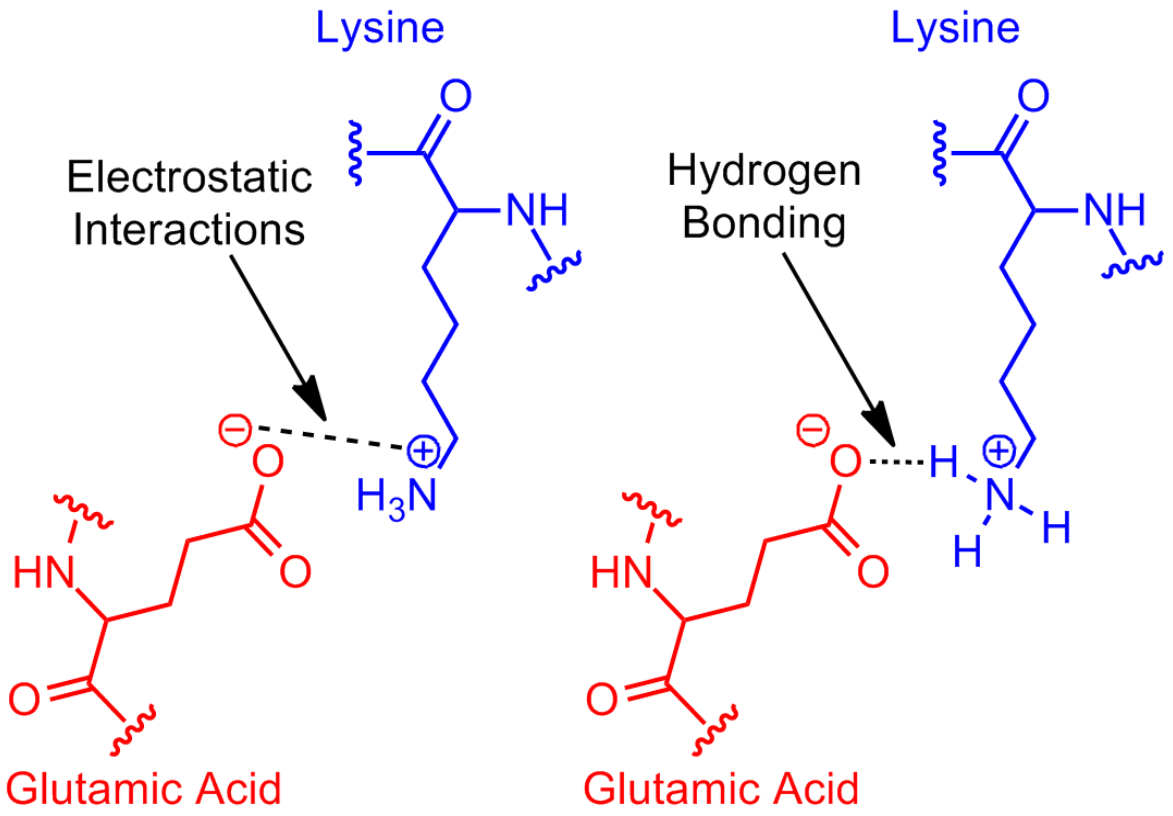


Type of Forces in Proteins

4. **Electrostatic Forces:** Electrostatic forces are mainly between oppositely charged R-groups

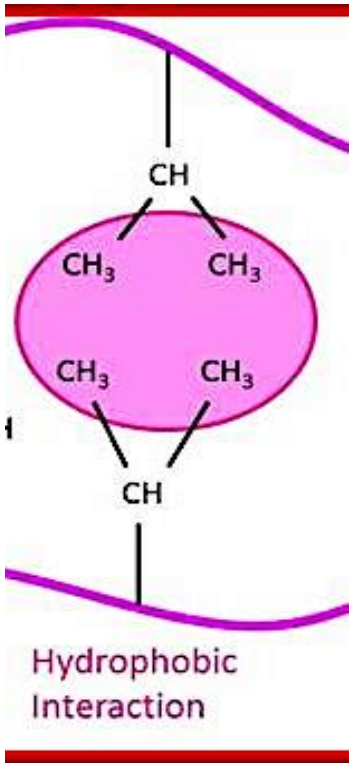
such as

Lys – Glu,
Arg - Asp.

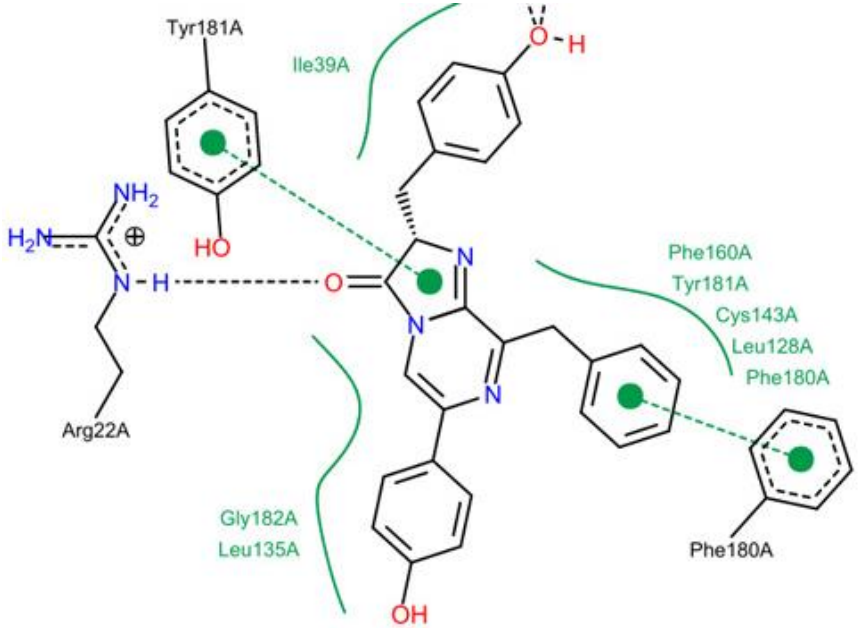


Type of Forces in Proteins

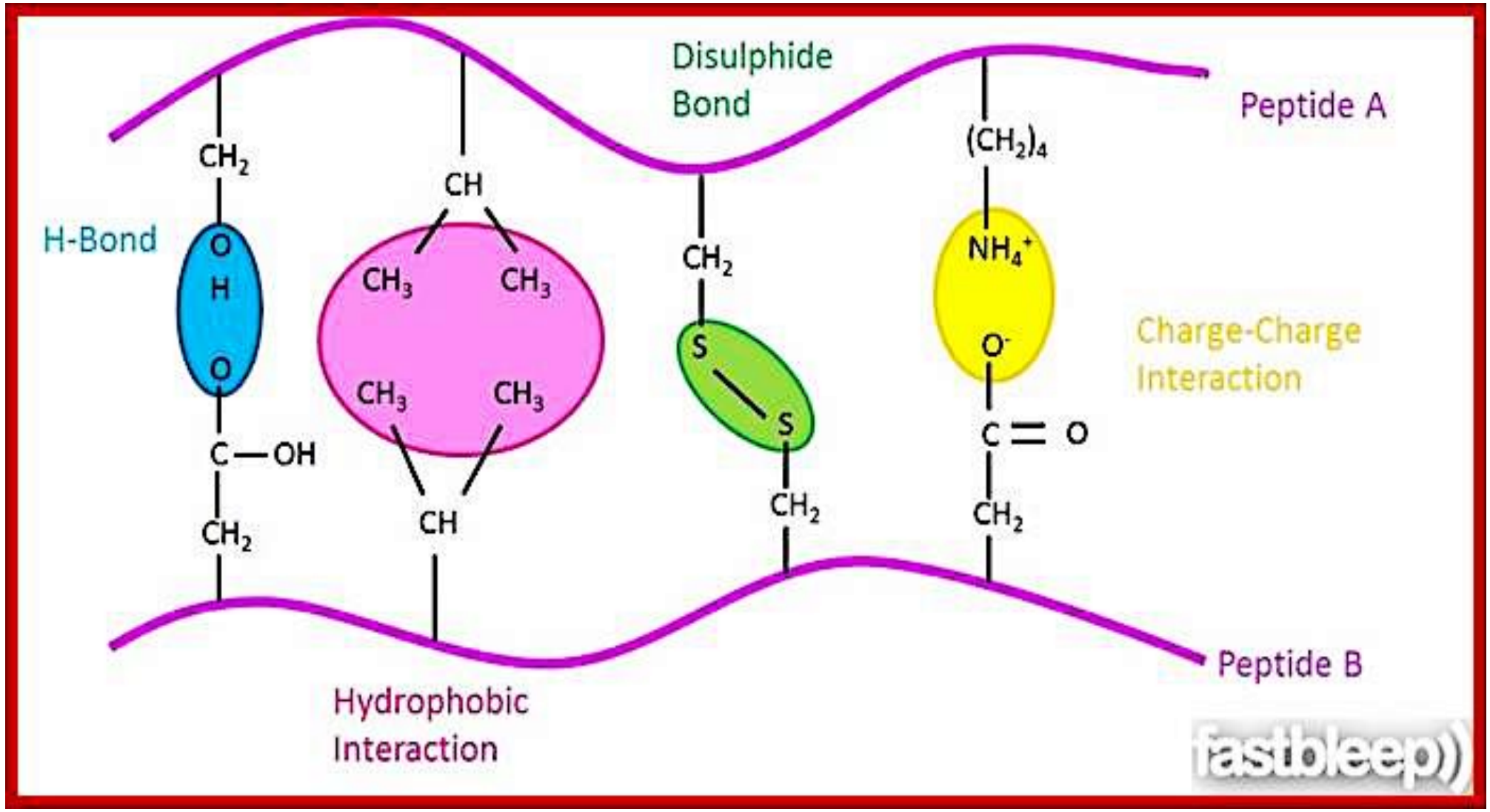
5. **Hydrophobic interactions:** The hydrophobic interactions stabilizee the structures of proteins.
The non-polar groups: Hydrocarbon alkyl groups on Ala, Val, Leu, and Ile interact in this way.



In addition, aromatic ring on Phe can "stack" together. In many cases this results in the non-polar side chains of amino acids being on the inside of a globular protein, while the outside of the proteins contains mainly polar groups.



What are the possible interactions between A.As?

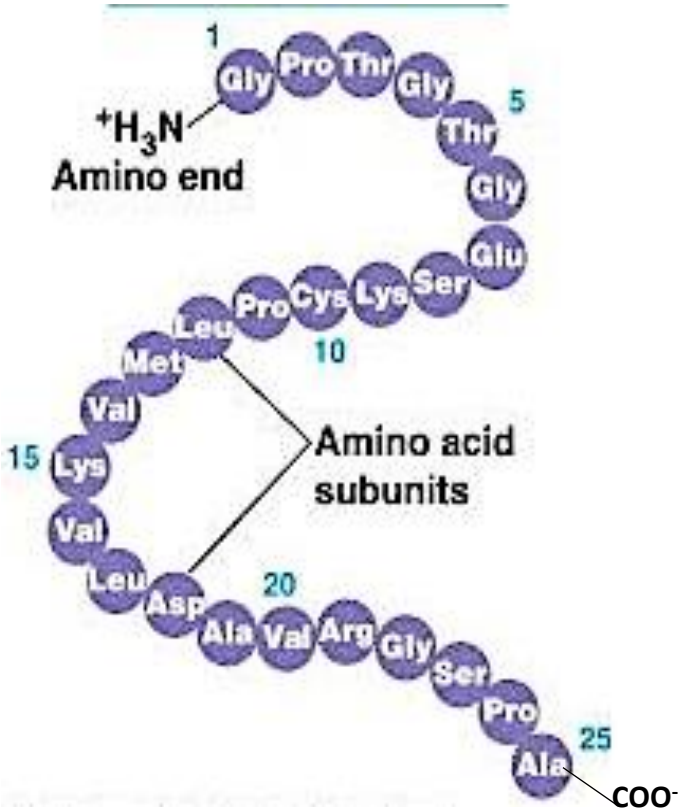


The structures of the protein

1. **The primary structure:**

The sequence of amino acids when linked by peptide bonds.

Simply, the primary structure of a protein is what is encoded in the DNA. Thus, all the properties of the protein are determined by the primary structure.



Oligo peptide (primary structure)

The structures of the protein

2. Secondary structure:

The local structure of the protein backbone,

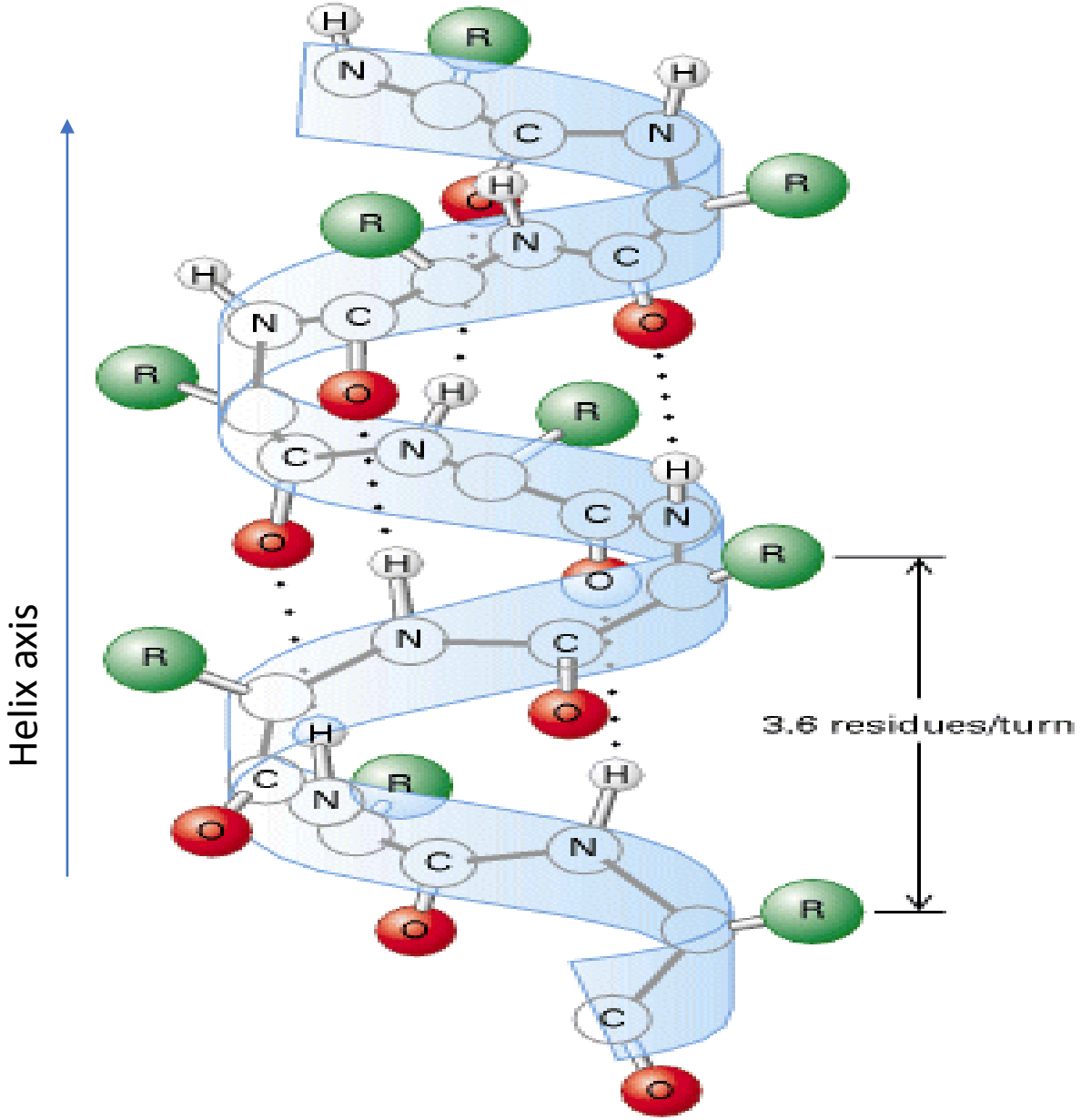
Stabilized by intramolecular and sometimes intermolecular hydrogen bonding of amide groups.

Two types: α-helix and $\mathcal{B}$-sheets.

There are 2 types: α-helix (has a right-handed spiral conformation), in which every backbone N-H group donates a hydrogen bond to the backbone C=O group of the amino acid four residues before it in the sequence.

The other common type of secondary structure is the which is stabilized by hydrogen bonding.

α-helix

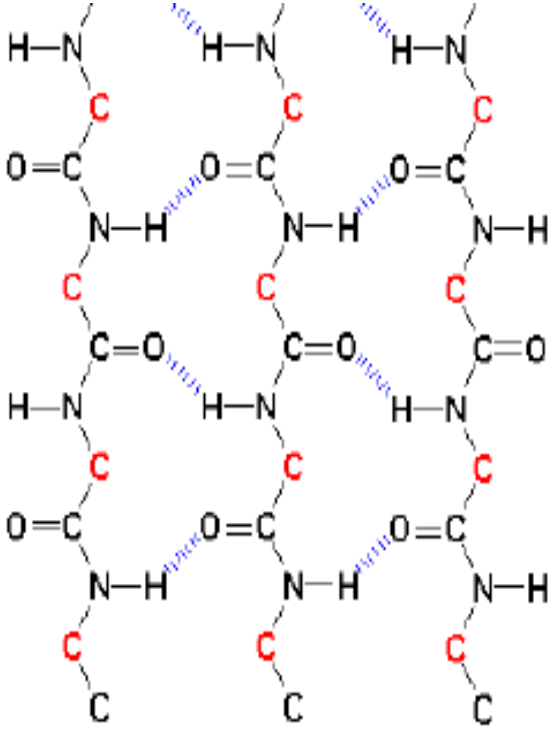


The triple helix of collagen.

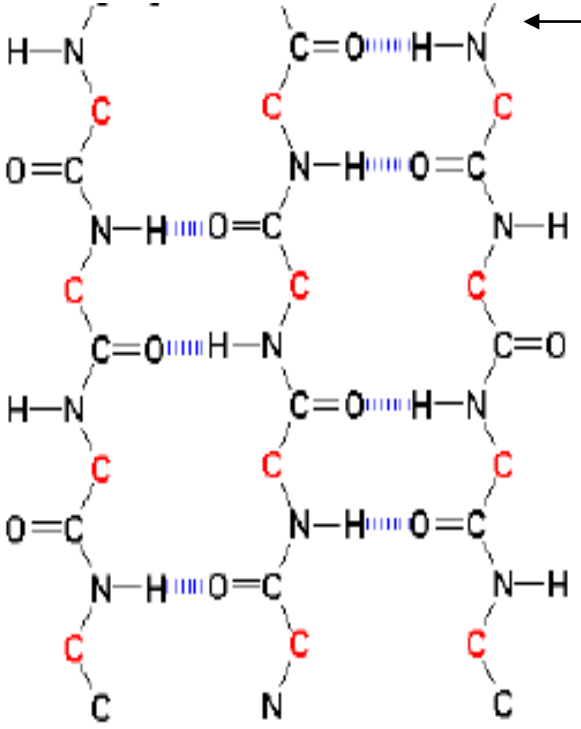
- Shows how left-handed polypeptide helices are twisted together to form a right-handed superhelical structure.
- Individual polypeptide has 3.3 residues per turn and pitch of 10 Å.
- The collagen triple helix has 10 Gly-X-Y units per turn and a pitch of 86.1 Å.

2. Secondary structure:

$\mathcal{B}$-sheets

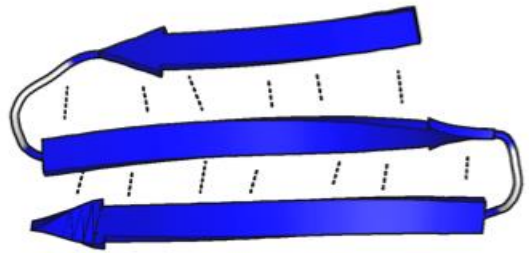


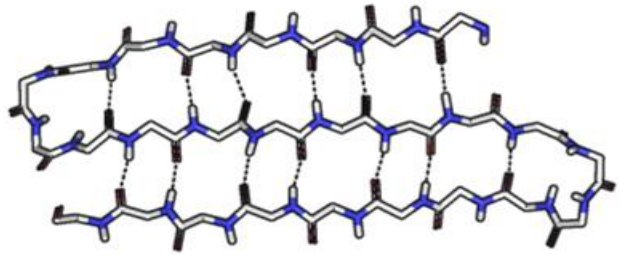
Parellel form

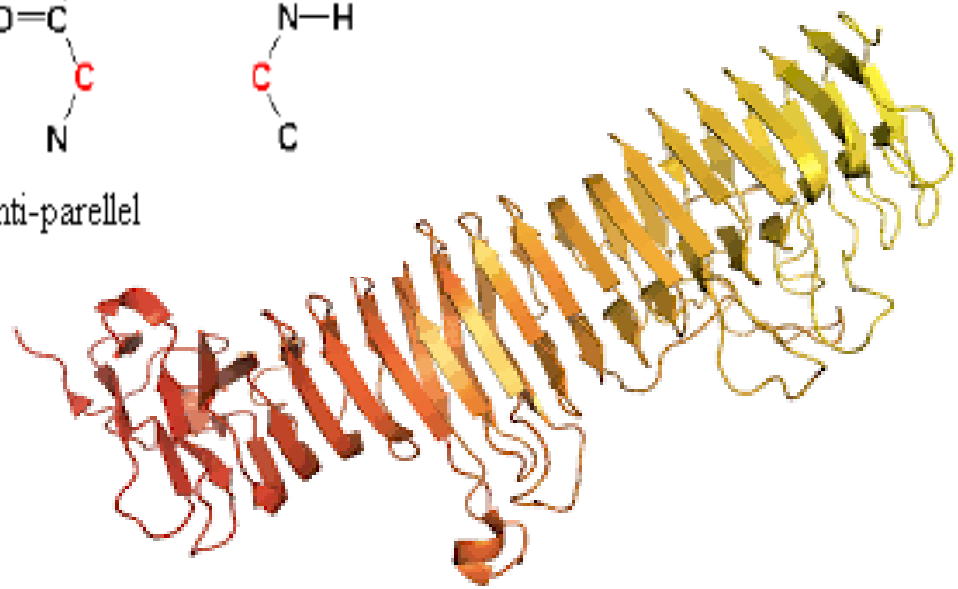


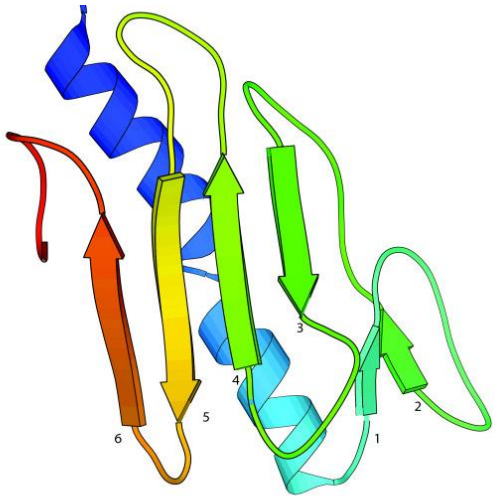
$\mathcal{B}$-strand

Anti-parellel





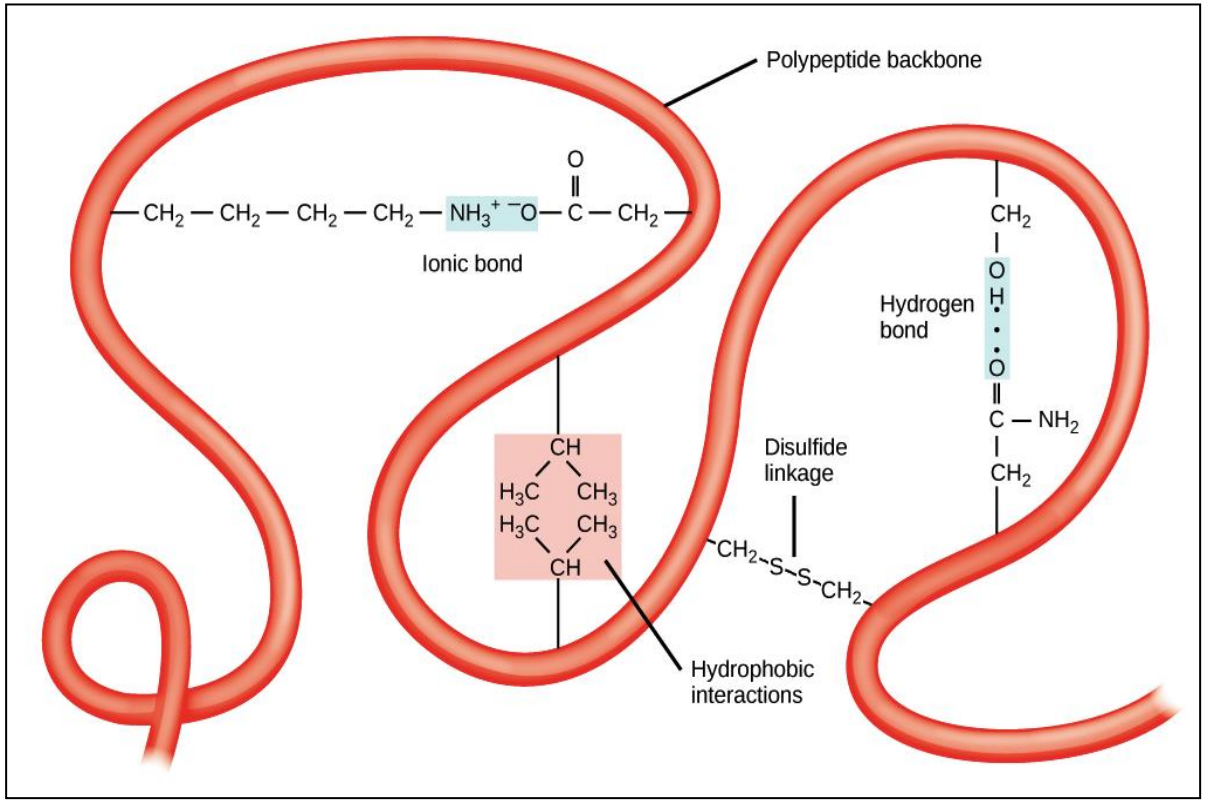




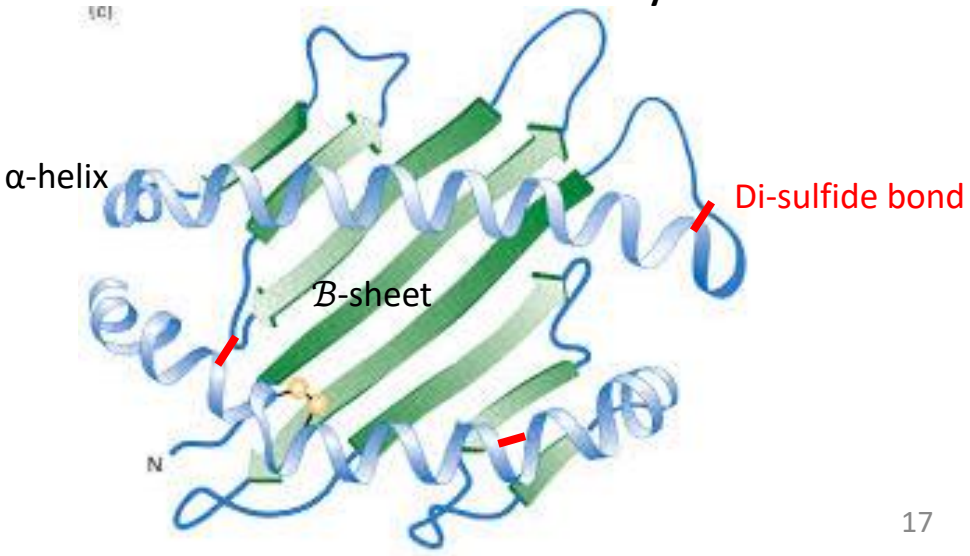
Tertiary structure:

The arrangement of secondary structure elements results in the formation of the tertiary structure.

An example of the tertiary structure is Globular proteins



Primary str.



Forces hold the tertiary structure

Van der Waals=R groups,

H-bonding= the polar R group, C=O…..H-N

Disulphide bond =S- + S- groups

Ionic bonds= Oppositely charged R groups

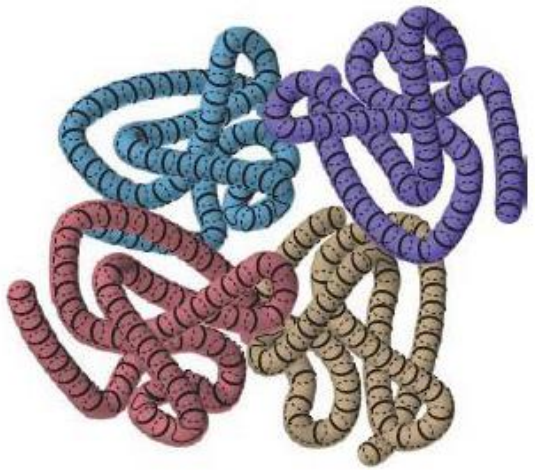
4. The Quaternary structure:

Some proteins form assemblies (units) with other molecules, this is called the quaternary structure,

such as

haemoglobin = four globular proteins

and the actin microfilament, composed of many thousands actin molecules.



quaternary structure

Forces hold the quaternary structure

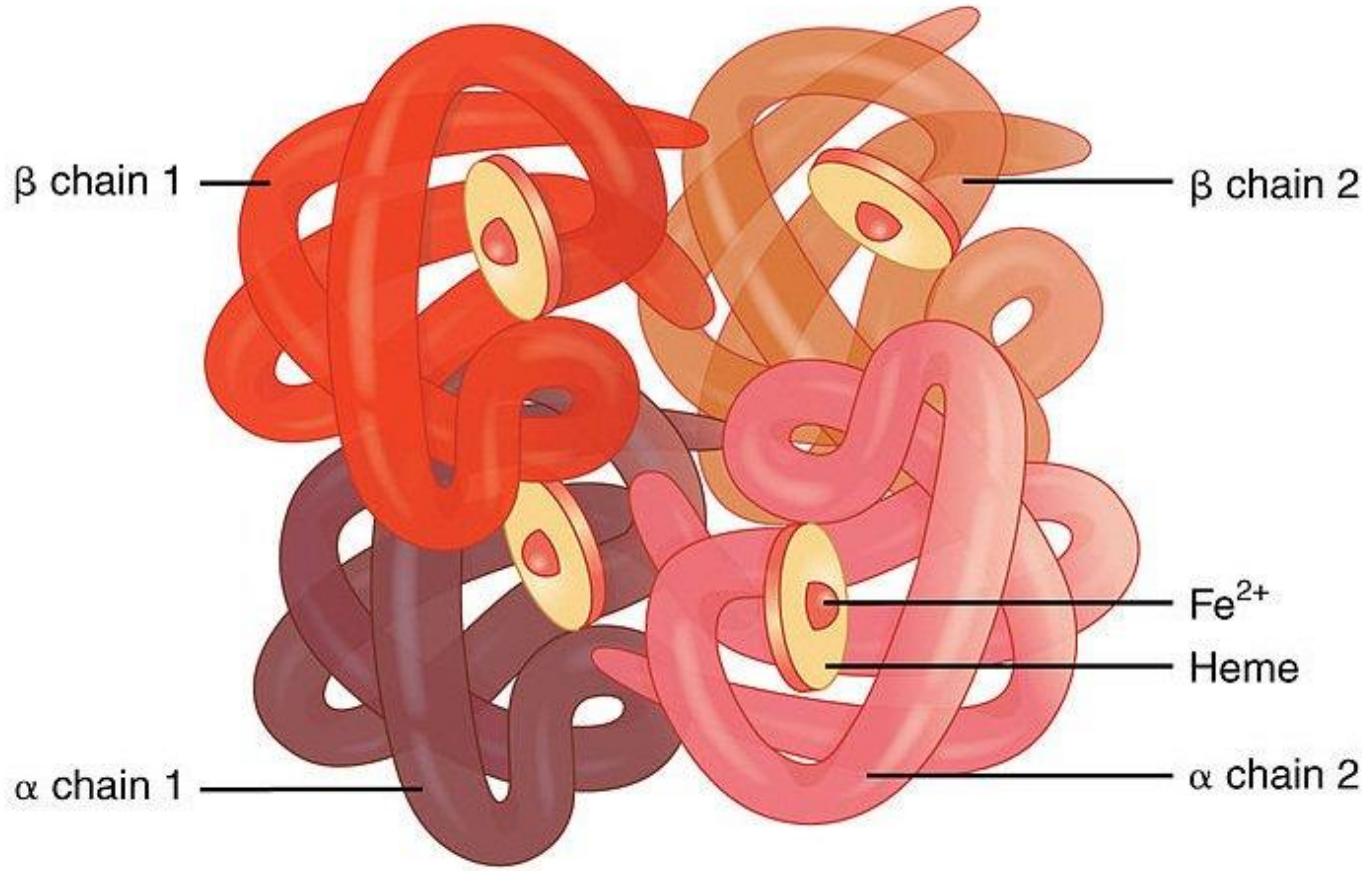
Van der Waals=R groups,

H-bonding= the polar R group, C=O…..H-N

Disulphide bond =S- + S- groups

Ionic bonds= Oppositely charged R groups

Hemoglobin molecule is a functional protein



Forces hold the quaternary structure

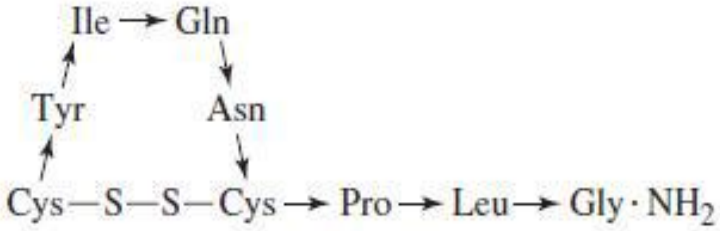
Van der Waals=R groups,

H-bonding= the polar R group, C=O…..H-N

Disulphide bond =S- + S- groups

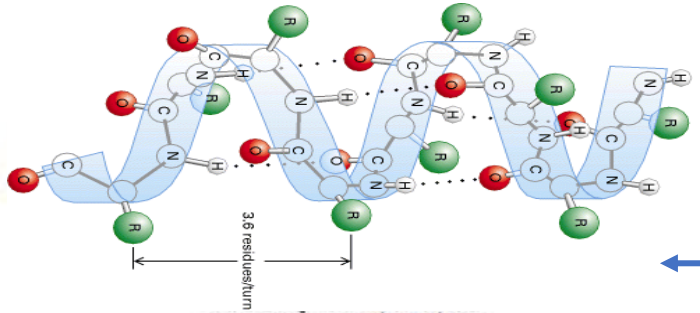
Ionic bonds= Oppositely charged R groups

Why 3 D structure?



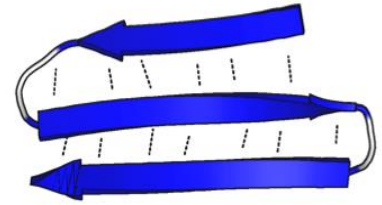
primary structure

α-helix

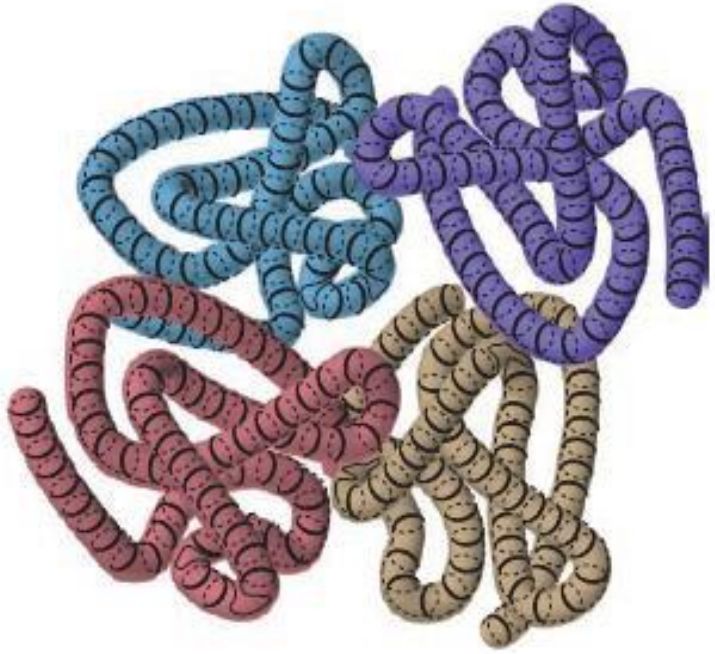


secondary structure

$\mathcal{B}$-sheet



tertiary structure



quaternary structure

Protein Purification

- Protein purification is the separation of a specific protein from contaminants in a manner that produces a useful end product.

- **Why purify proteins?**

➢ **In research,** proteins must be purified in order to determine their structure and study their biochemical properties.

➢ **In industry settings,** proteins are purified on a large scale in order to be sold as products such as drugs, vaccines, diagnostic tools or food additives.

Goals of protein purification

- Obtain a particular protein free of others and other cell components
  - obtain a good yield (absolute amount and proportion of starting amount)
  - maintain the activity of the protein
    - Problems:
      - denaturation
      - proteolysis
      - in vitro mixing
  - a measurement of how well the process worked
  - some characterization of the purified protein

Separation principles

Sizing

- Principle is based on exclusion of larger molecules from pores in resin; smaller molecules require longer times of transit
- sizing resins = "gel filtration"
- different matrices have different size ranges
- examples: Ultrogel, some HPLC resins

Separation principles

Ion exchange

- Separate on the basis of net charge
- wash and elute with higher salt concentrations
- determine highest [salt] that your protein binds to the resin, and use this to load or to pre-wash before eluting your protein
- Example : some HPLC resins

Separation principles

Affinity chromatography

- Separate based on binding to residues specific or semi-specific to the protein you are purifying
- Separation is based on a specific binding interaction between an immobilized ligand and its binding partner.
- Mainly used

END