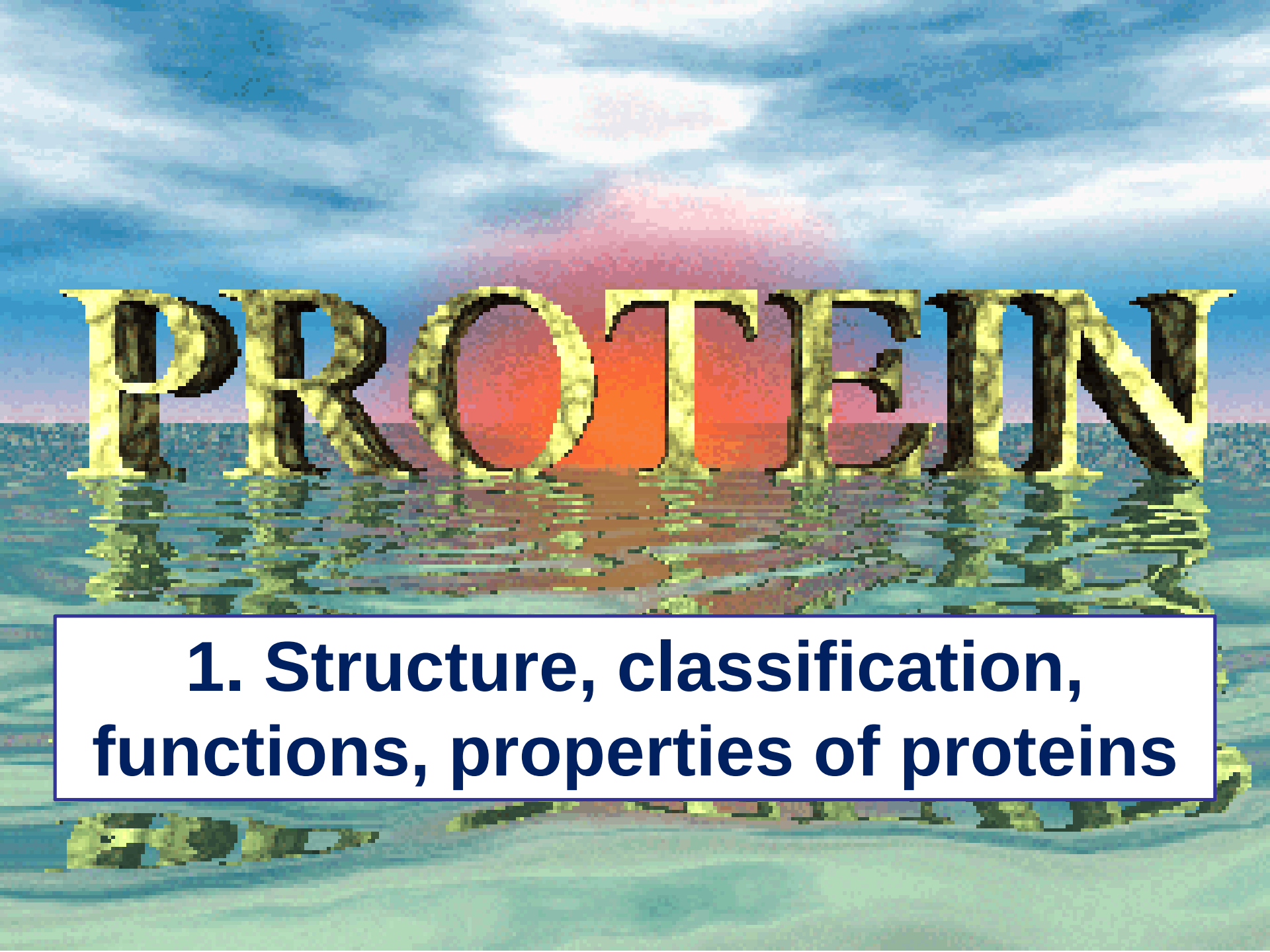


1. Structure, classification, functions, properties of proteins

Proteins are the major components of living organisms and perform a wide range of essential functions in cells.

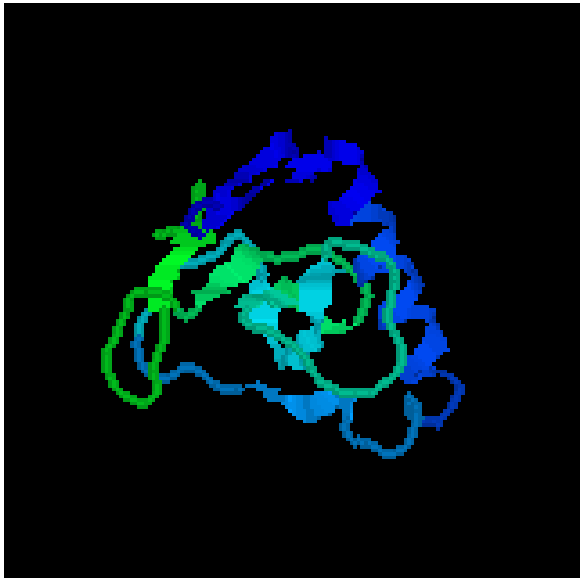
Proteins regulate metabolic activity, catalyze biochemical reactions and maintain structural integrity of cells and organisms.

Classification of Proteins according to the biological function:

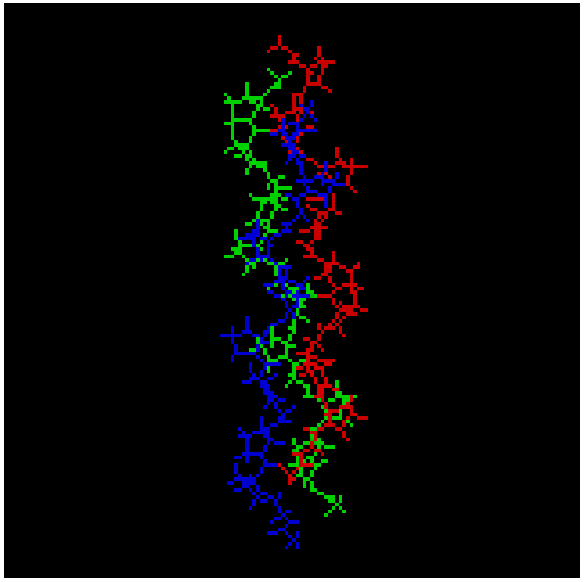
Type:	Example:
1.Enzymes- catalyze biological reactions	DNA-polymerase Dehydrogenases Ribonuclease
2.Hormones	Insulin
3.Transport protein	Hemoglobin
4.Movement proteins	Actin and Myosin in muscles
5.Immune Protection proteins	Immunoglobulins (antibodies)
6.Receptors	Hormone receptors rhodopsin
7.Signalling proteins - regulatory function within cells	Transcription & Translation Factors
8.Structural proteins	Collagen Keratin
9.Storage proteins	Egg ovalbumin milk casein

Proteins are the most abundant and diverse molecules found in living cells.

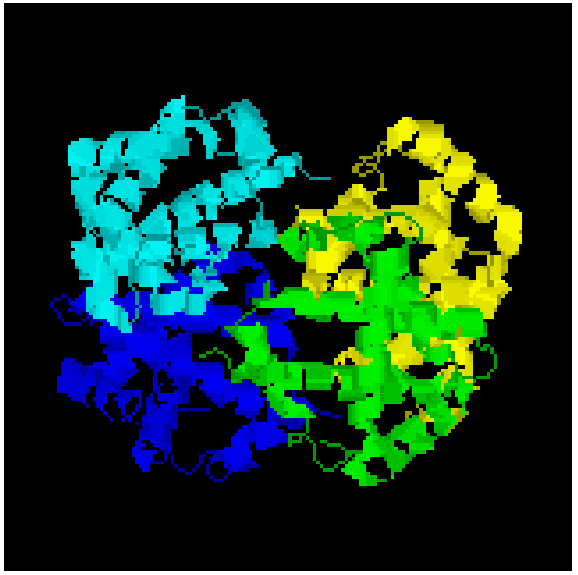
How does one group of molecules perform such a different set of functions? The answer is found in the wide variety of possible structures for proteins.



Ribonuclease



Collagen



Hemoglobin

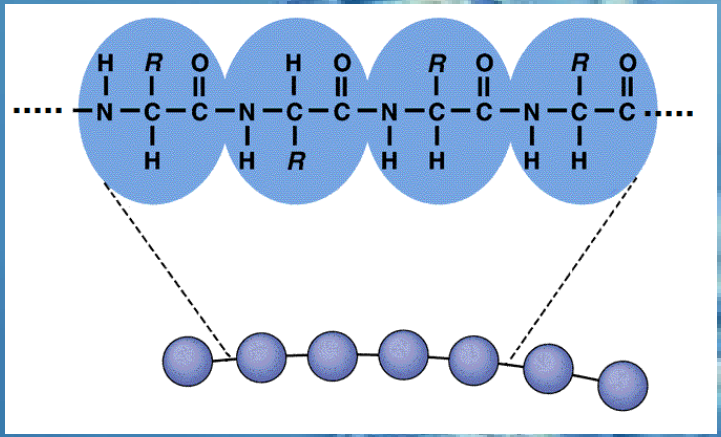
Levels of Protein Structure

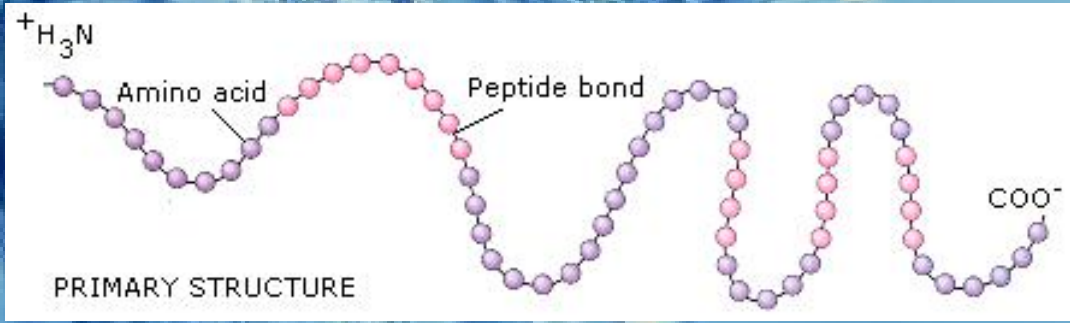
There are 4 levels of the protein structure:

primary
secondary
tertiary
quaternary

The sequence of amino acids in a protein is called:

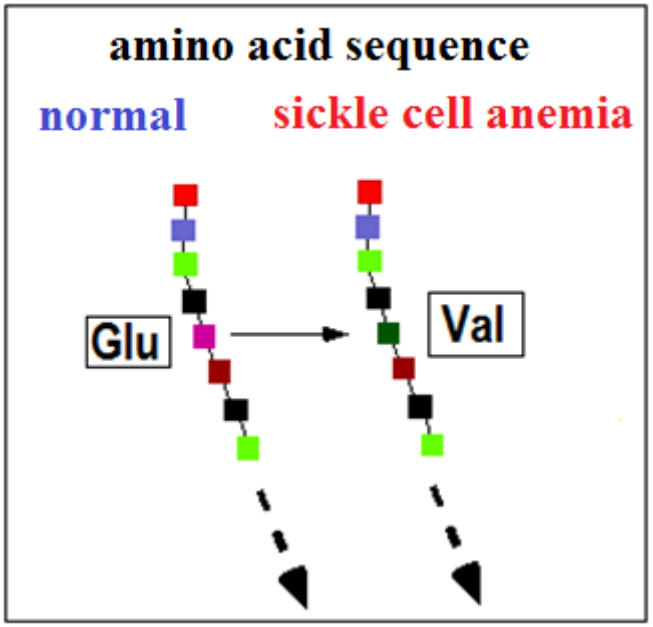
primary structure of protein

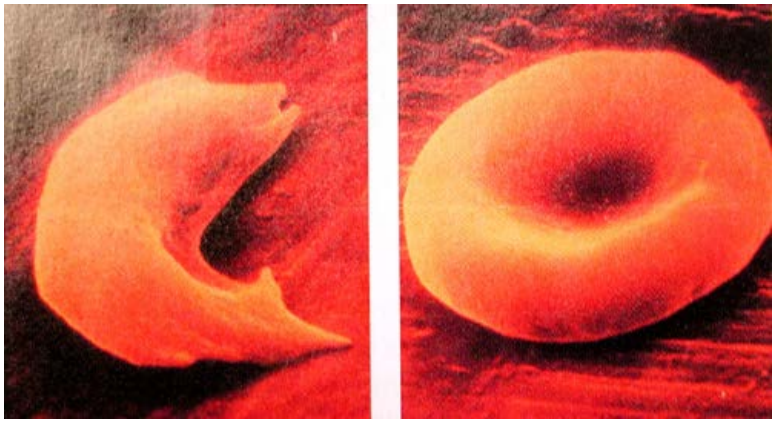


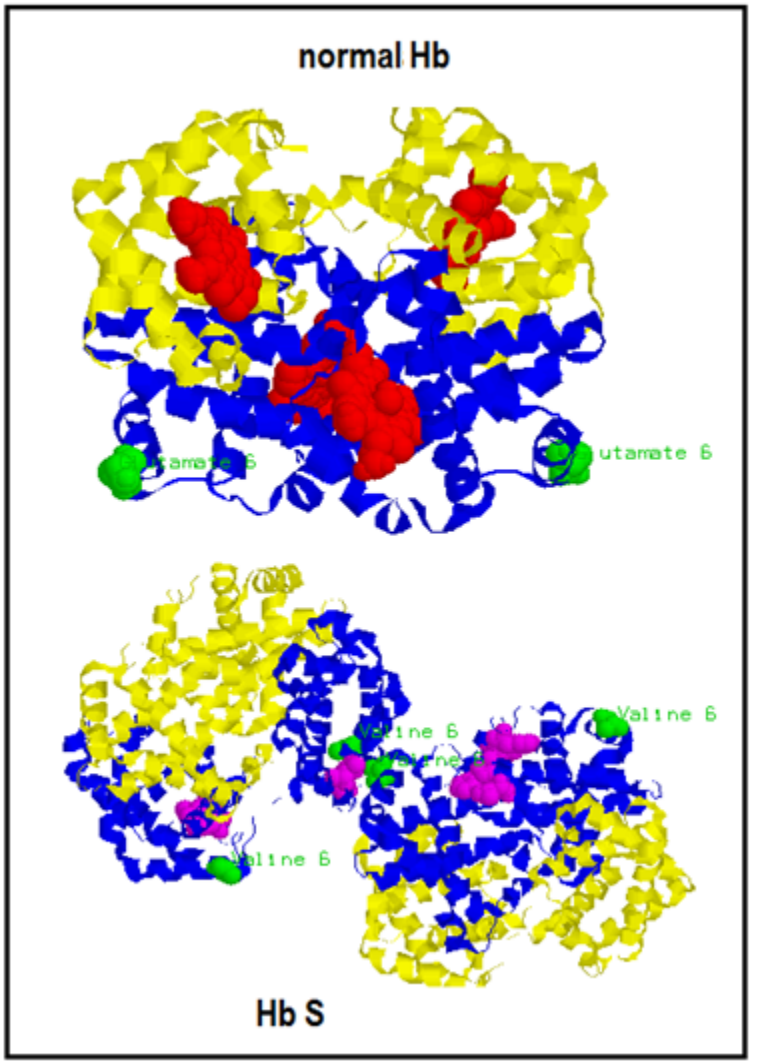


-is genetically determined;
-determines the subsequent protein structures and their properties

The importance of the primary structure







Secondary structure

is a regular arrangement of polypeptides into more compact shapes, stabilized by hydrogen bonds.

The secondary structure describes the relative orientations of amino acids close in sequence. There are three predominant structure

1. alpha-helix
2. collagen-helix
3. beta- sheets.

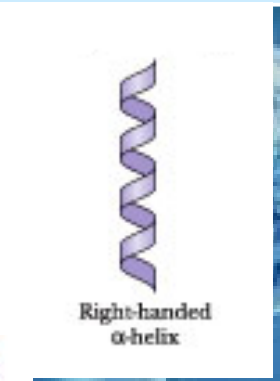
1. The alpha-helix – is a helical structure, a spring-like coil of polypeptide that forms a rigid cylinder of great regularity.

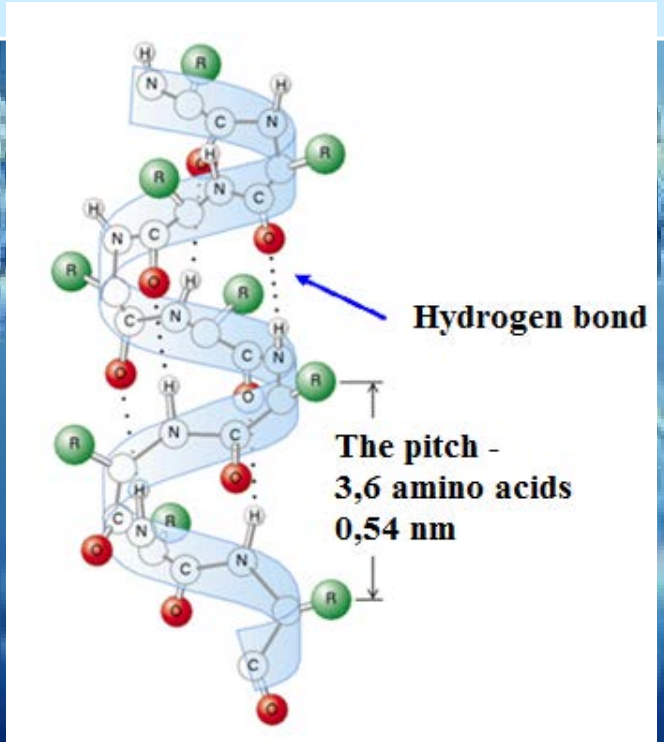
• Alpha helix is a regular structure – it has **3.6 amino acids per turn** of the helix. **The pitch** – the distance separating each turn of the helix is **0,54 nm**.

•The formation of the alpha-helix is spontaneous and is stabilized by **hydrogen bonds**.

• The hydrogen bond forms between **C=O groups** of one amino acid **in the backbone** with **N-H groups** located **four amino acid residues further** along the chain.

• This orientation of H-bonds produces a helical coiling of the peptide backbone such that the **R-groups lie on the exterior of the helix** and perpendicular to its axis.





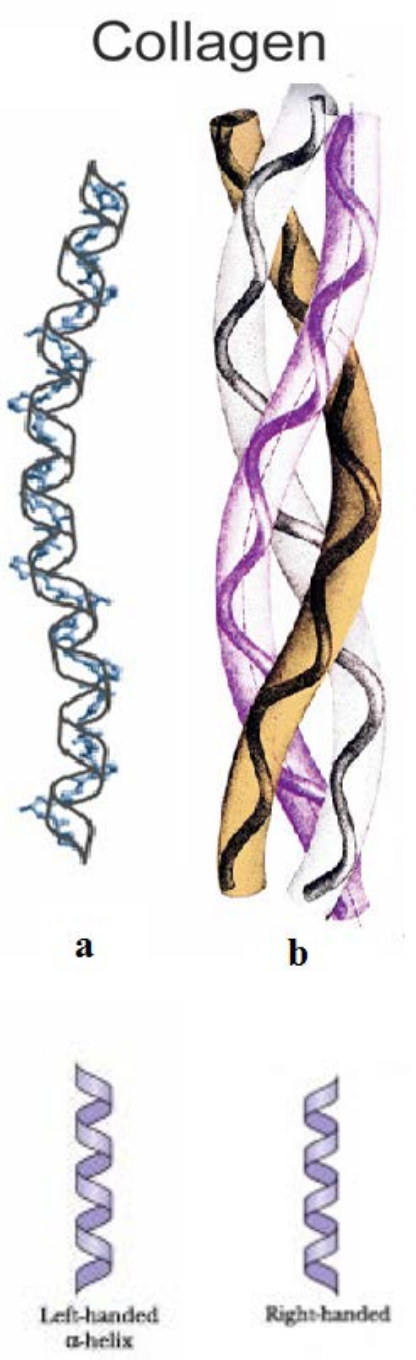
2. Collagen helix.

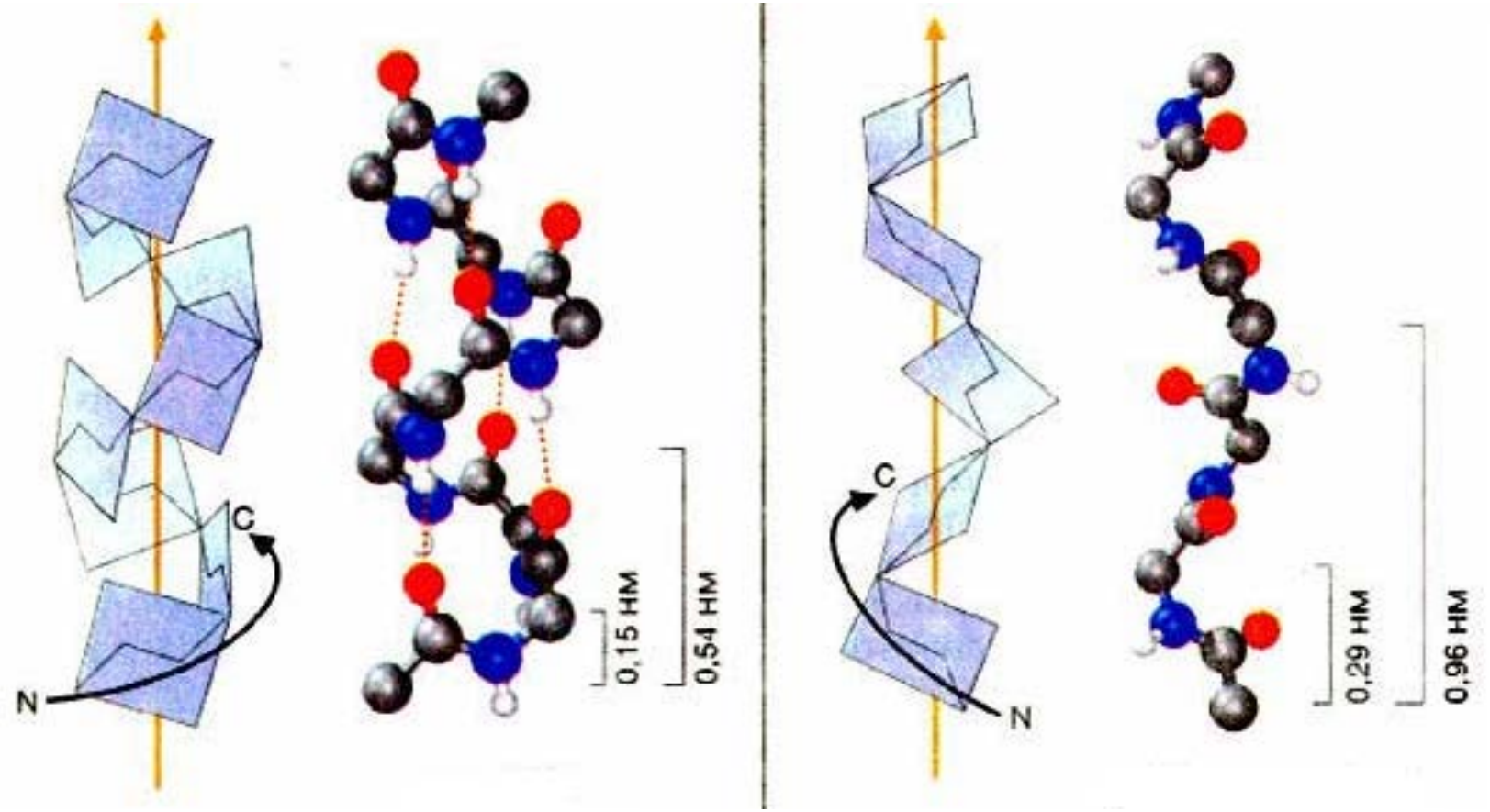
Another type of helix occurs in the collagens, which are important constituents of the connective tissue matrix. The **collagen helix** is **left-handed**, and with a pitch of **0.96 nm and 3.3 residues per turn,** it is steeper than the alpha-helix.

Each collagen polypeptide chains consist of about 1000-1100 amino acids (**a**).

Three linear twisted polypeptide chains (**left-handed helices**) combine together and are further twisted to form a major **right-handed triple helix** - the basic structural unit of collagen - **tropocolagen** (280 nm long, 1.5 nm wide) (**b**).

In contrast to the alpha-helix, hydrogen bonds are not possible within one collagen polypeptide. The triple helix is stabilized by hydrogen bonds formed between peptide groups (-C = O ---- HN =) of different chains.



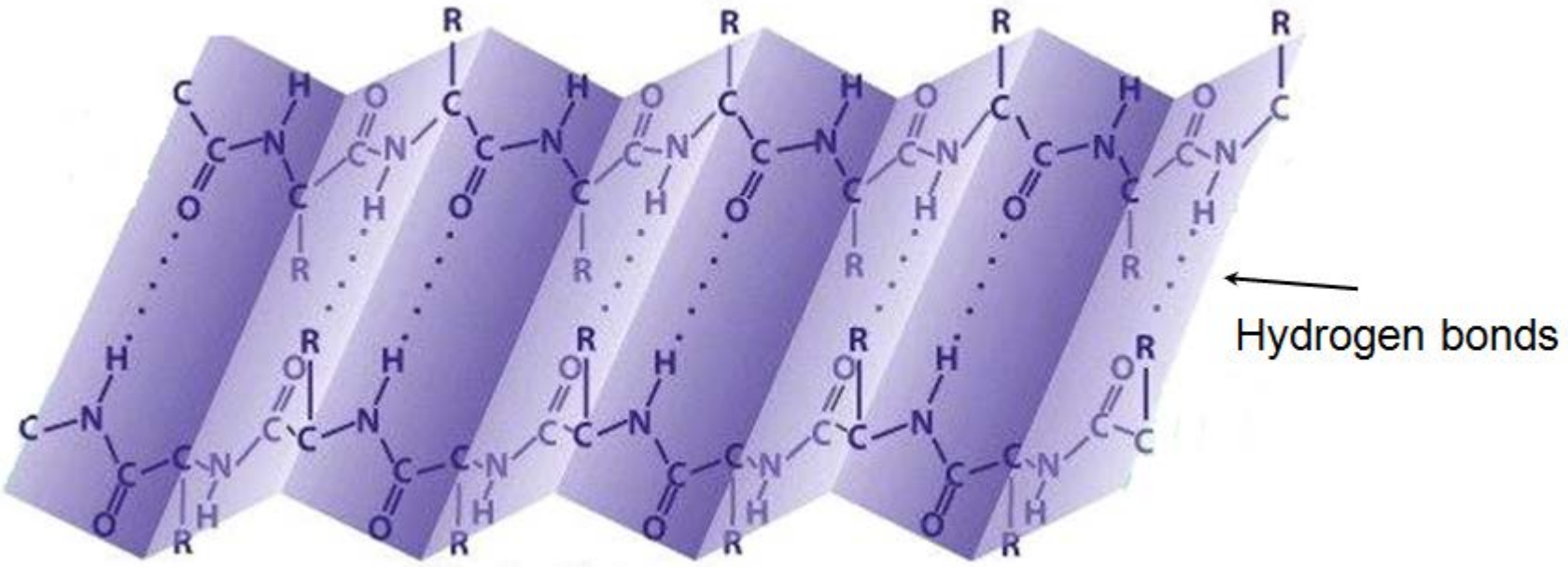


A. Alpha-helix

B.Collagen helix

3. Beta- sheets (pleated-sheet structures)

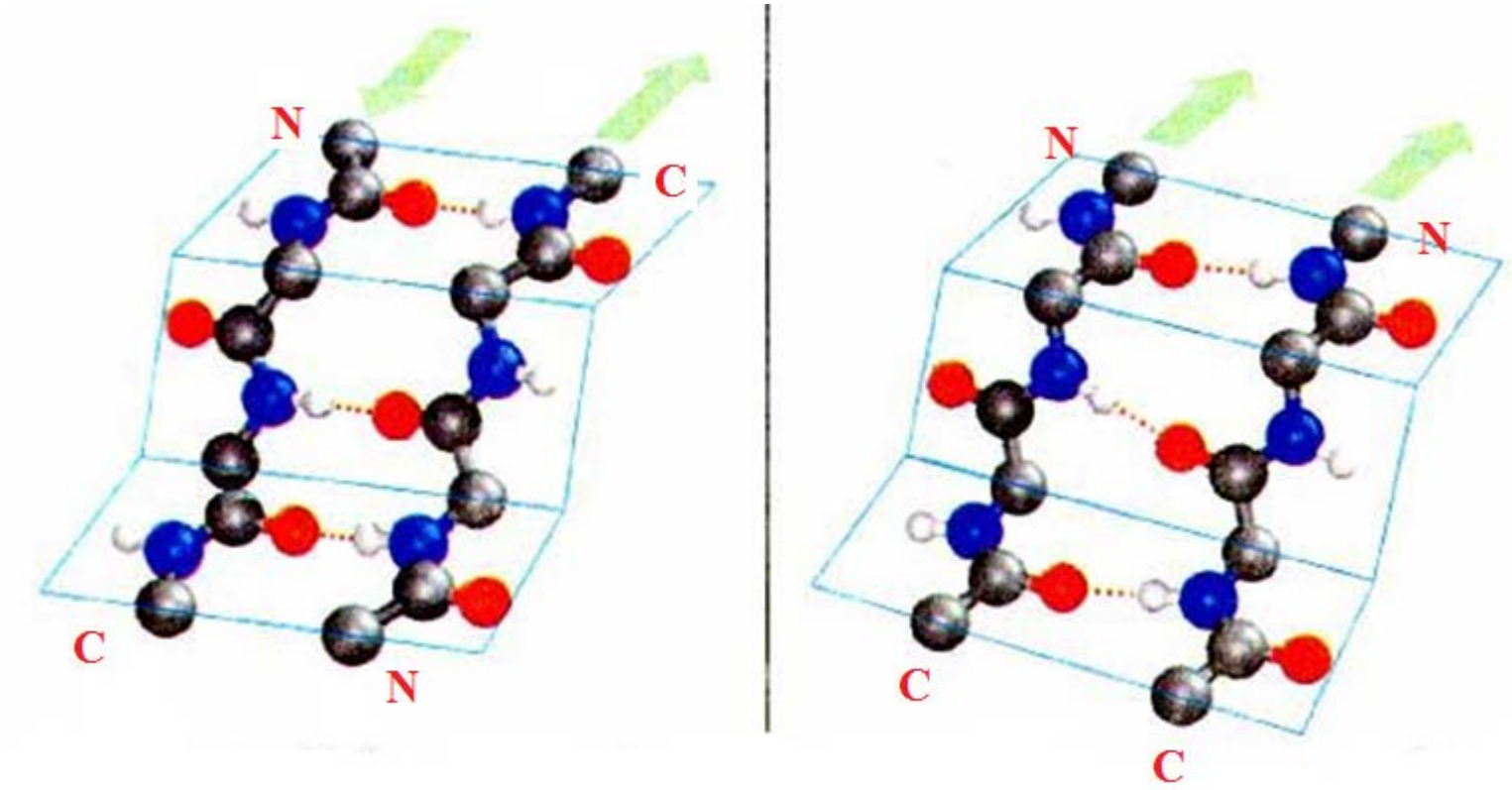
results from the alignment of the polypeptide backbone aside one another. Beta-sheets is stabilized by hydrogen bonds between C=O and N-H groups in different regions of the polypeptide, or even between two different polypeptides.



If extended strands are lined up side by side, H-bonds bridge from strand to strand. Identical or opposed strand alignments make up

parallel or antiparallel beta sheets.

In parallel sheets adjacent peptide chains proceed in the same direction (the direction of N-terminal to C-terminal ends is the same), whereas, in antiparallel sheets adjacent chains are aligned in opposite directions.

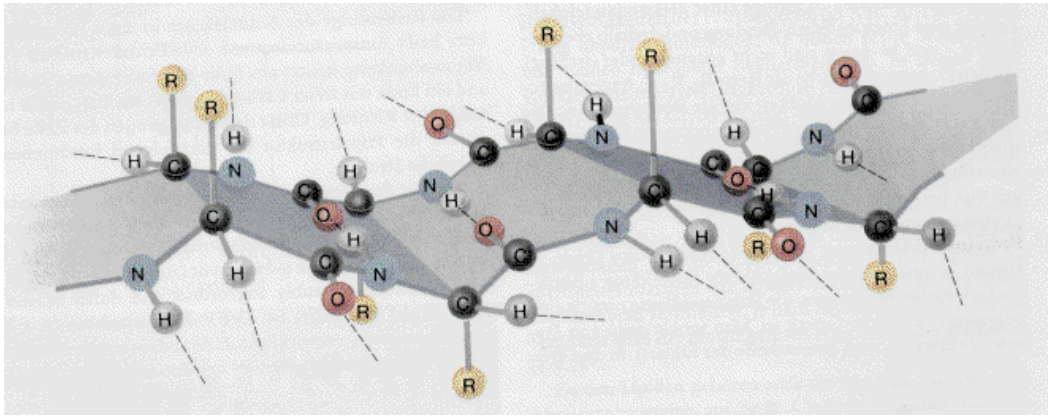


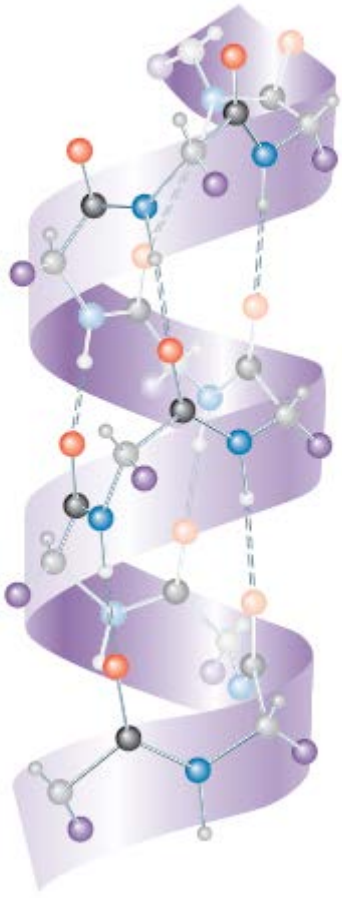
1. Antiparallel beta-sheet

2. Parallel beta-sheet

The side-chain groups (radicals) are not involved in alpha-helix or beta-sheet structure stabilization,

but some radicals can destabilize the regularity of alpha-helix structure, for example – big radicals, loaded with the same electric charge, proline.

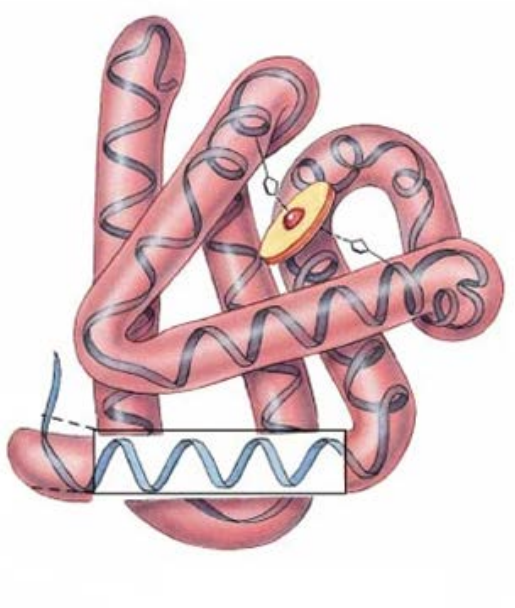




Tertiary protein structure

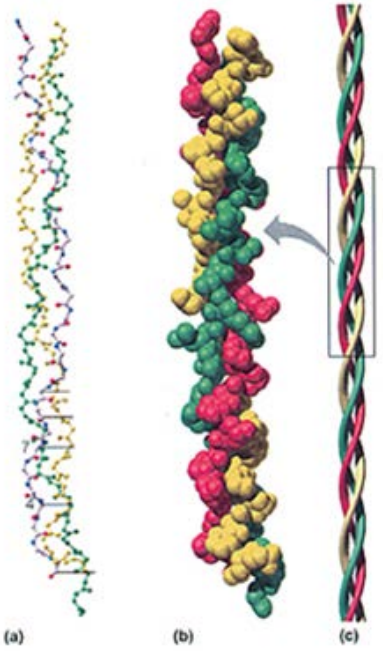
refers to the complete **three-dimensional folding** of the entire polypeptide chain. In general, proteins fold into 2 main types of proteins in dependence of the protein's shape:

Globular proteins are compactly folded and coiled:



Myoglobin

Fibrous proteins are filamentous or elongated:



Collagen fiber

Keratin fiber

Stabilization of the protein's tertiary structure may involve interactions between **the radicals (side chains)** of amino acids located far apart along the primary sequence.
These may include several types of bonds:

Non-covalent
- weak bonds

Covalent
- strong bonds

Non-covalent, weak bonds:

- **Hydrogen bonds** between side-chain groups (if the side groups contain hydroxyl or amino groups).
- **Ionic bonds** between positively and negatively charged amino acid side chains.
- **Hydrophobic interactions (Van der Waals bonds)**, between the nonpolar side groups

Covalent, strong bonds:

- **Disulphide bridges** - covalent bonds between two -SH groups of cystein to form an -S-S linkages:

Cys-SH + HS-Cys ⟶ Cys-S-S-Cys

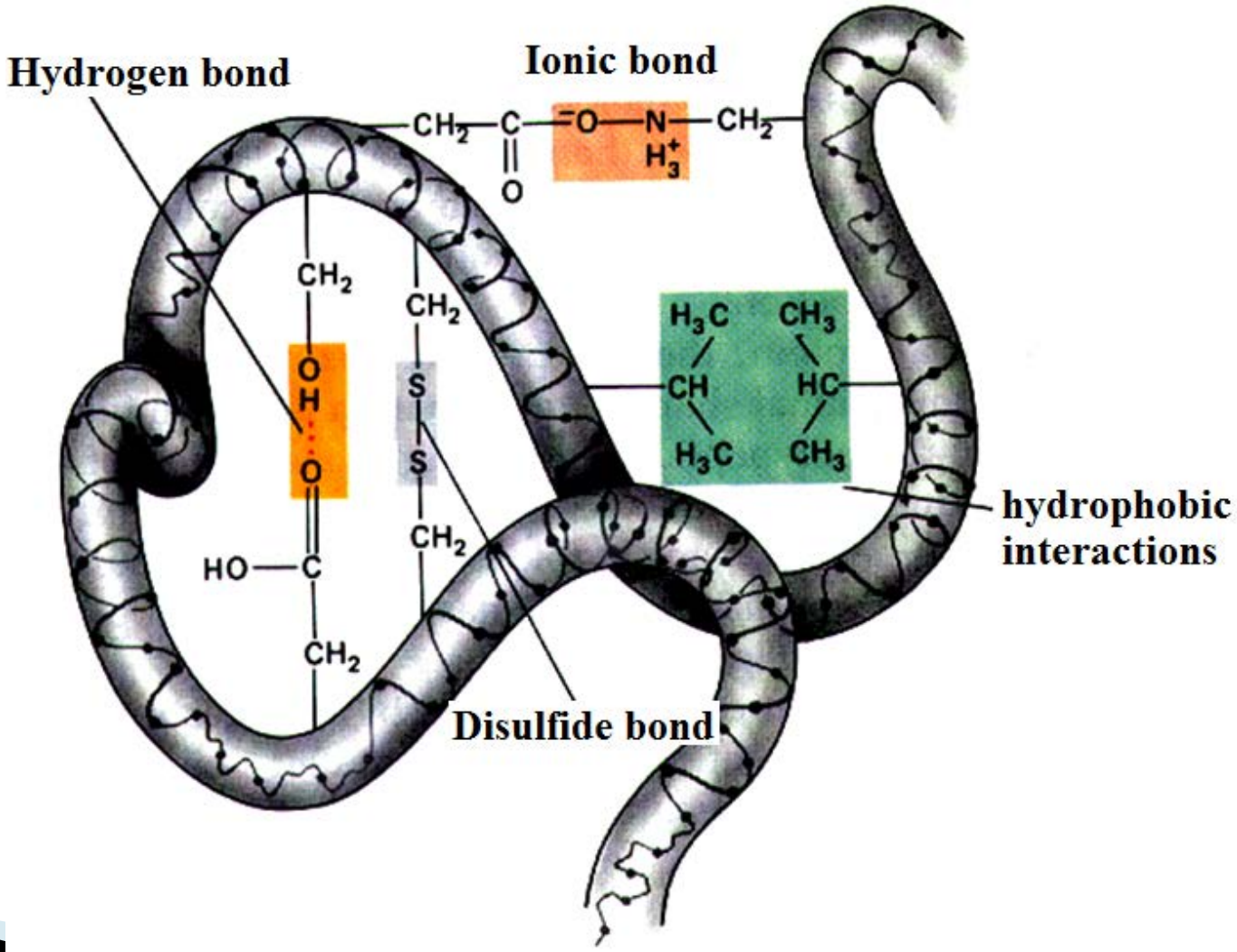
- **Estheric bonds** – between a side-chain carboxylic group (of asp or glu) and a side-chain hydroxyl group (of ser, tre or tir)

Glu-COOH + HO-Ser ⟶ Glu-CO-O-Ser

- **Pseudo peptide bonds** - between a side-chain carboxylic group (of asp or glu) and a side-chain amino group (of lys):

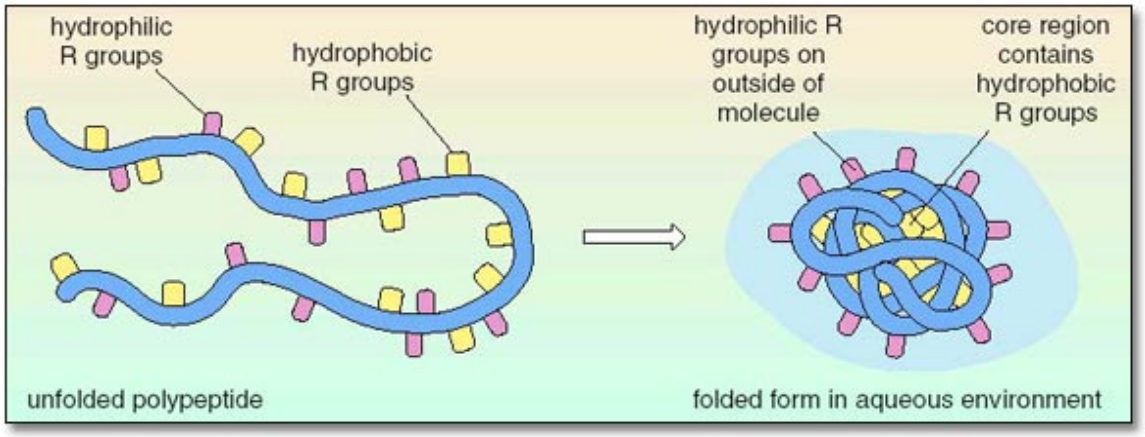
Glu-COOH + H_2N-Lys ⟶ Glu-CO-HN-Lys

Some bonds that stabilized the tertiary structure:

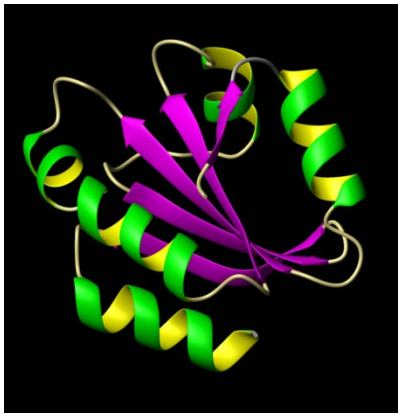


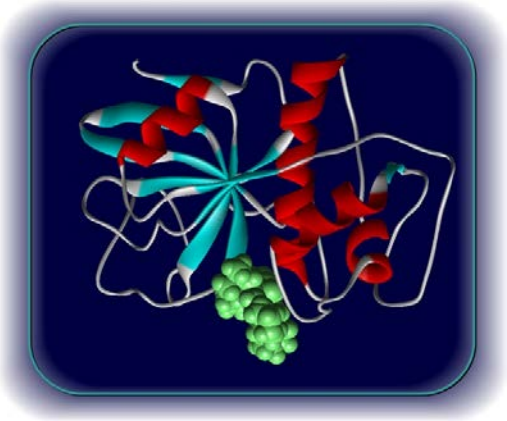
The tertiary structure is determined by the sequence of amino acids in the chain and is the most energetically convenient.

In the tertiary structure of globular proteins in the aqueous medium the **hydrophilic radicals will be oriented to the surface** of the molecule, and **hydrophobic ones will be situated within** the protein molecule.



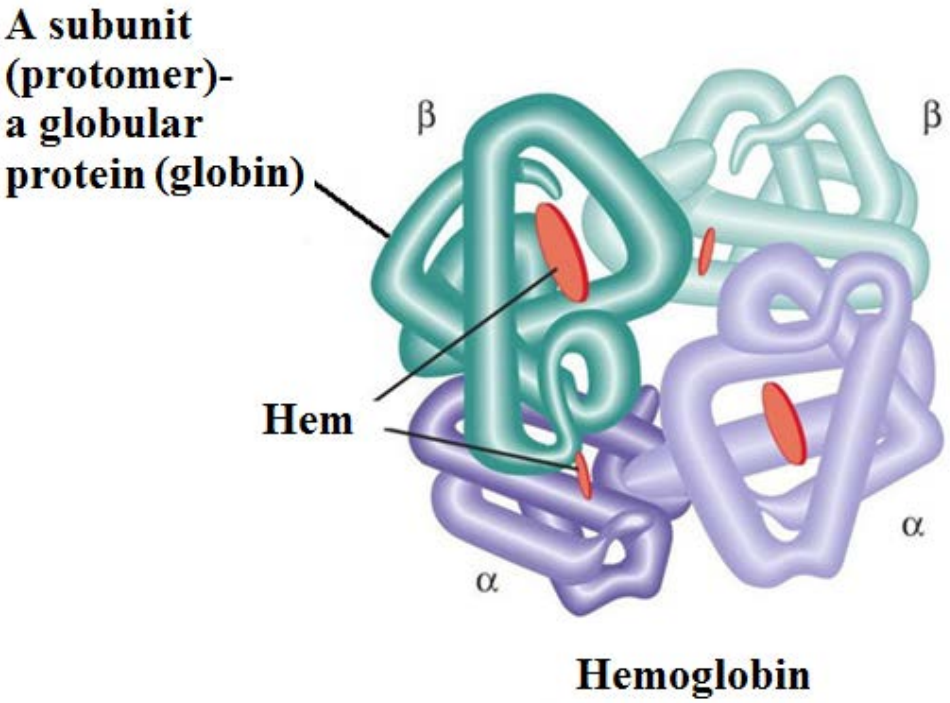
A lot of proteins at this level begin to show their biological properties and are capable of carrying out their designated function.





Quaternary protein structure

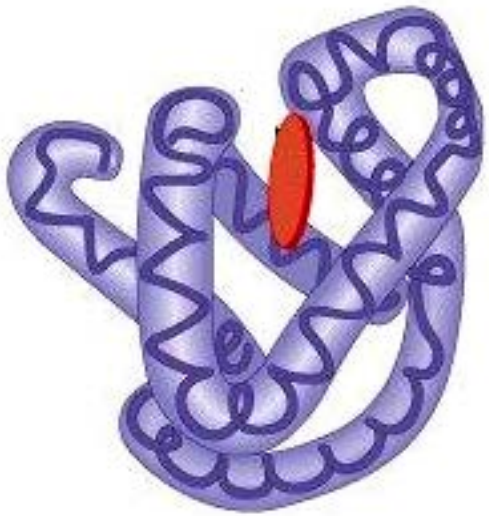
- refers to the regular association of two or more polypeptide chains to form a complex (**olygomer**).
A multi-subunit protein may be composed of two or more identical polypeptides, or it may include different polypeptides (**protomers**).

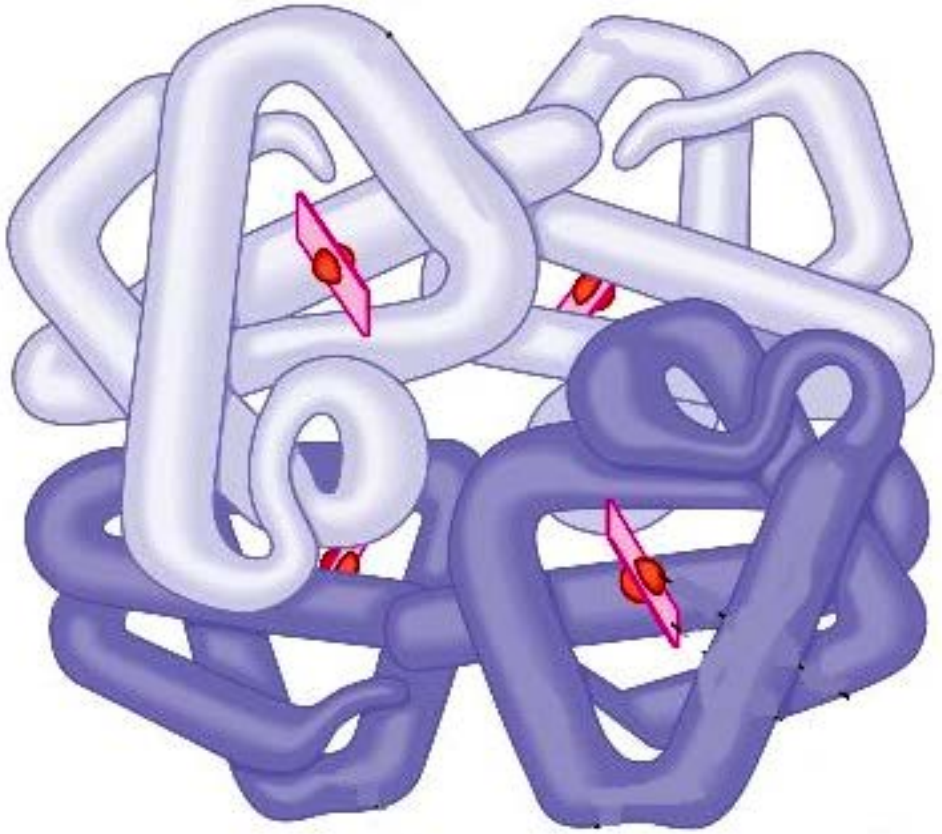


Hemoglobin

Bonds that stabilize the quaternary structure are usually **non-covalent bonds** between contacting surfaces of protomers, less covalent bonds.

Some proteins are already biologically active in tertiary structure, others - only in quaternary structure:

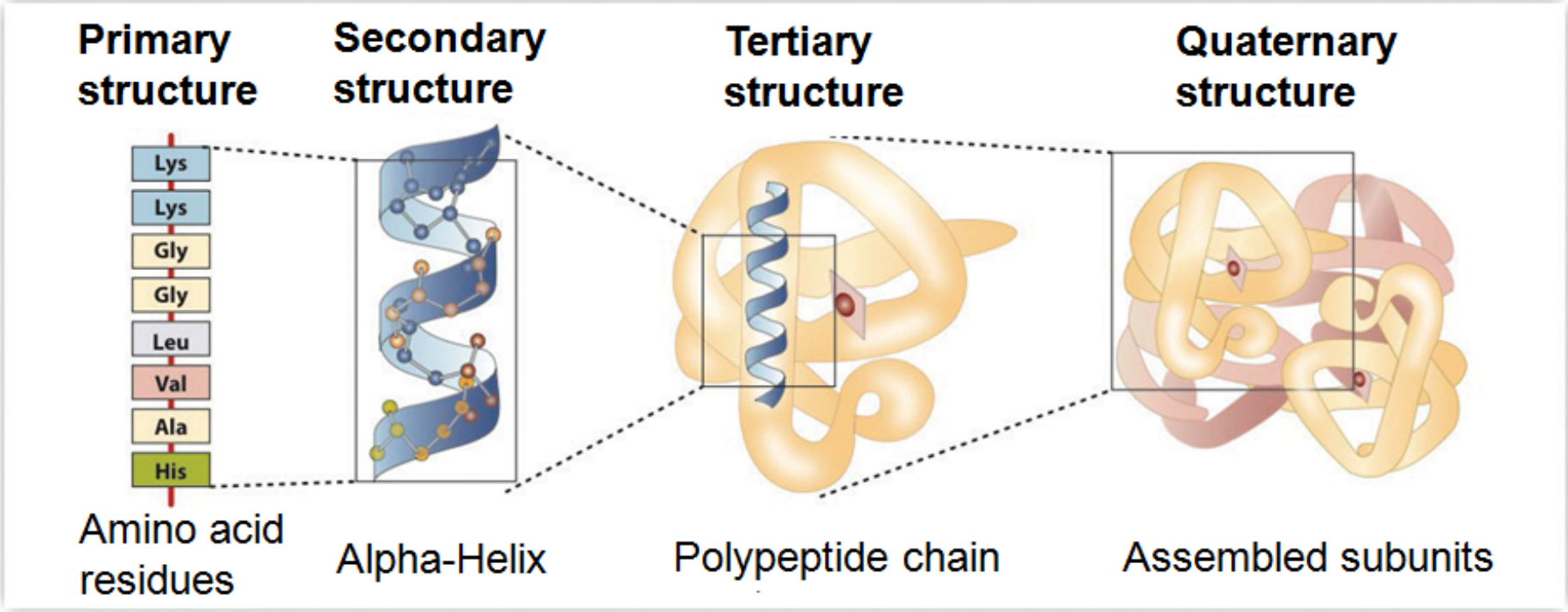




Mioglobin – is biologically activ in tertiary structure

Hemoglobin – is activ in quaternary structure

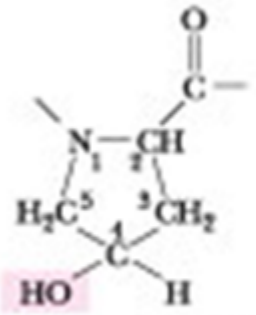
Levels of the protein molecule organization:



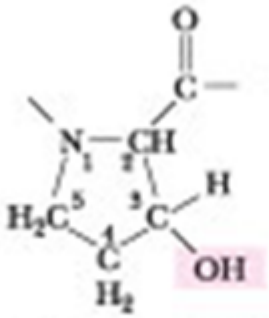
Collagen – peculiarities of structure

- **Collagen** is the most abundant protein in the body (up to 30% dry weight). Collagen fibers are flexible, but very inelastic with extremely **high tensile strength**.
- Collagen has an unique amino acid composition that is crucial to its three-dimensional structure and its characteristic physical properties.
- In the polypeptide chain the sequence a **Gly-X-Y motif** is repeated, where **Gly - glycine (33%), X - proline and hydroxyproline (25%), Y - alanine (11%), lysine, hidroxilysine** or other amino acids. So, nearly one residue of three is a glycine, and the proline content is also unusually high.

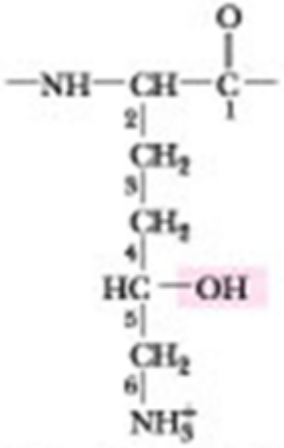
- Unusual modified amino acids are also found in collagen: **hydroxyproline (Hyp) and 5-hydroxylysine (Hyl).**
- Collagen is the only natural protein that contains all of these amino acids.



4-Hydroxyprolyl residue (Hyp)



3-Hydroxyprolyl residue (Hyp)



5-Hydroxylysyl residue (Hyl)

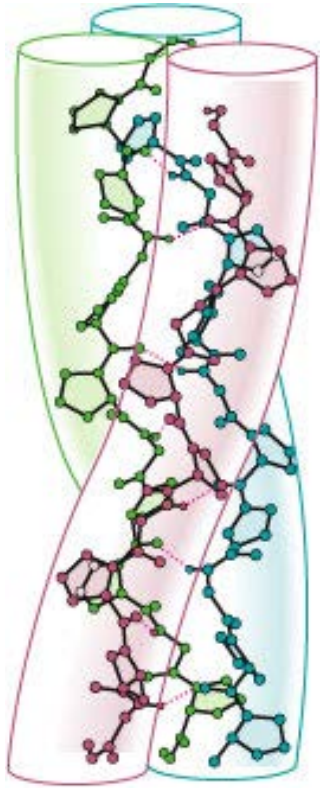
- Collagen does not contain cysteine and tryptophan.

The secondary structure of collagen is the **collagen helix**, which is different from classical α-helix, because collagen helix is much **more extended and is twisted to the left**. These features are determined by the fact that:

- a large number of **proline and hydroxyproline** are present, that decreases the elasticity of polypeptide chain and requires formation of a more extended helix.

- **every 3rd amino acid is glycine –** it is absolutely necessary for the formation of such structures, because none of the radicals of the other amino acids would fit in the space between the three polypeptide chains in triple helix center.

- the triple helix is stabilized by **hydrogen bonds** formed between peptide groups (-C = O ---- HN =) of different chains, as well as hydrogen bonds formed with participation of -OH groups of hydroxyproline. Amino acid radicals in positions X and Y are on the triple helix surface.



- Polypeptide chains are **alpha-chains**, which may be of several types: α_1 (I, II, III, IV), α_2 (up to 30 types), and is distinguished by:
    - hydroxylation at the proline and lysine;
    - amino acid sequence.

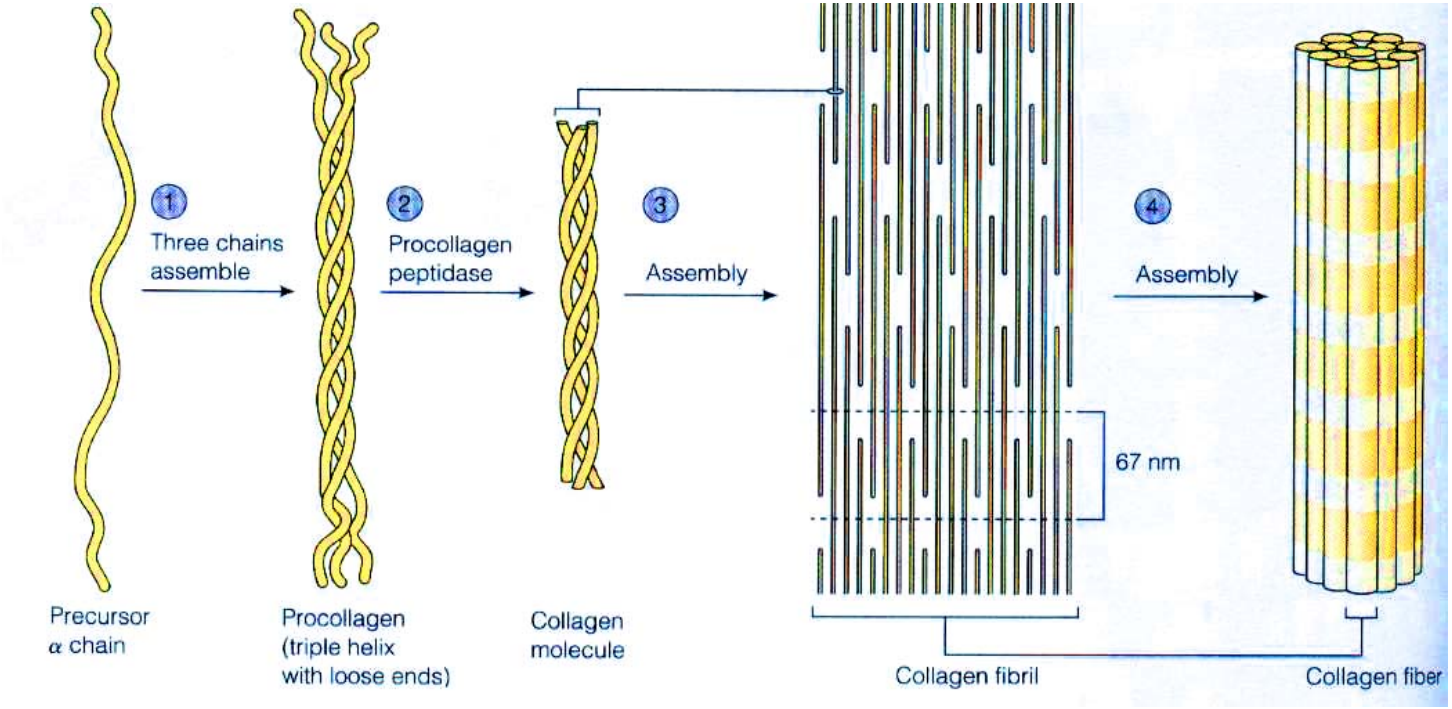
- The distinguished **more than 30 types of collagen**, which differ among themselves by variants of alpha-chains. The most important are the first 5 types of collagen.

- Collagens type **I, II** and **III** are called **fibrillar** because they form fibers, which are used in connective tissues;

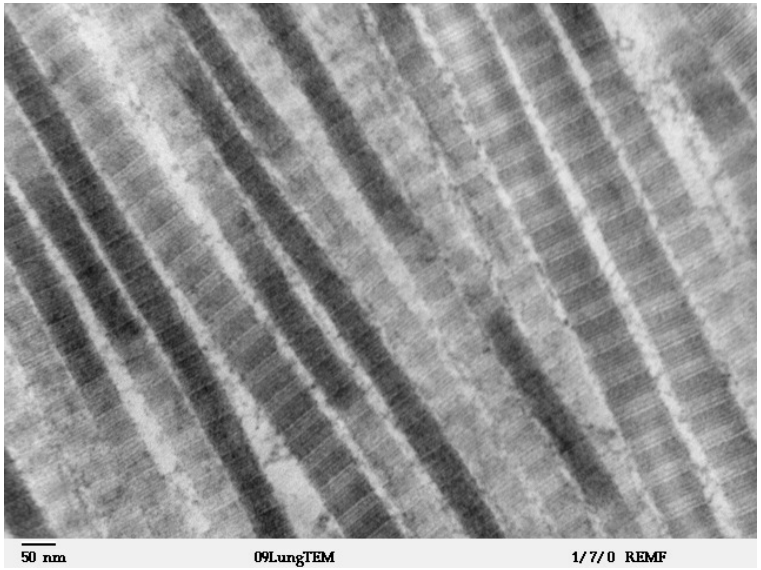
- Collagens type **IV** and **V** are referred to **amorphous** collagens (form the flat networks) – basal lamina.

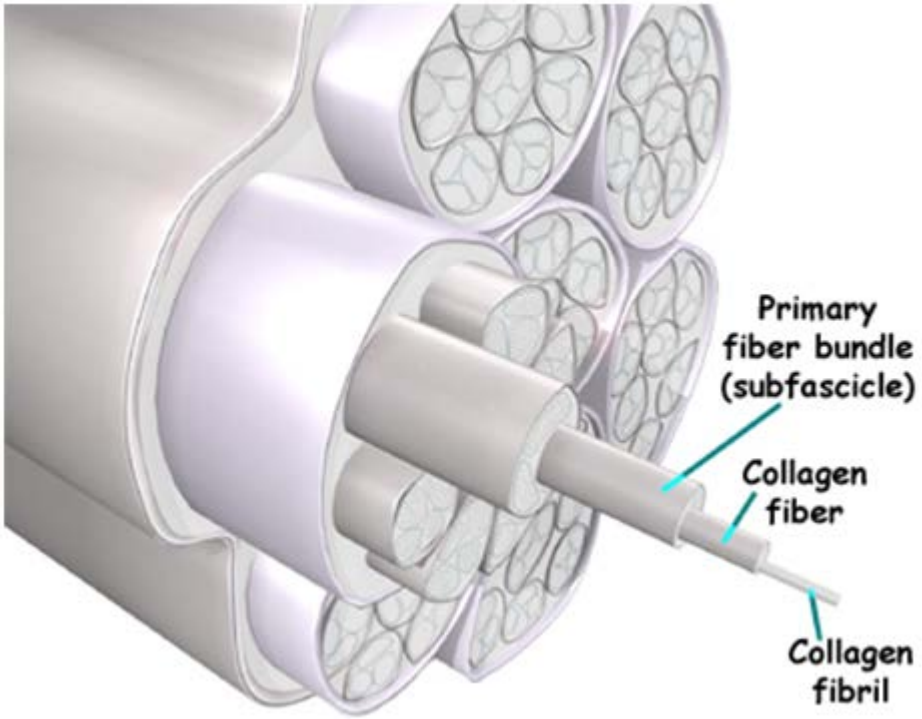
The basic structural unit of collagen is **tropocolagen** (280 nm long, 1.5 nm wide), which polymerize to form collagen fibrils. The tropocollagen molecule consists of **three linear twisted polypeptide chains (left-handed helices)**, which are further twisted to form a major **right-handed helix.**

Each collagen polypeptide chains consist of about 1000-1100 amino acids.



Collagen

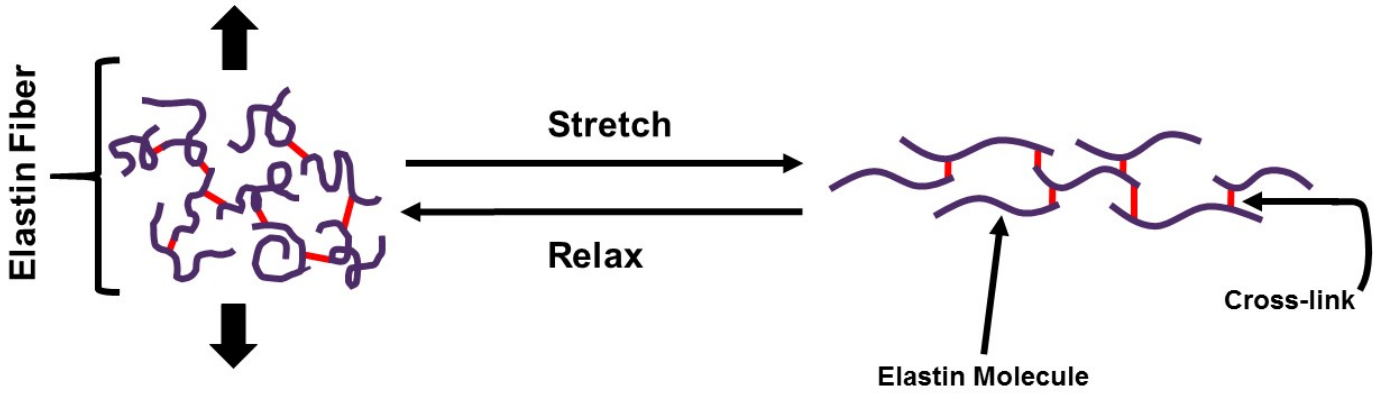




At the ultrastructural level each collagen fibril shows a 64 nm banding (periodicity), which is due to the **stepwise overlapping arrangement** of the rod-like tropocollagen subunits.

Elastin –

- is the main protein component of elastic fibers, making up over 90% of their mass. Unlike collagen, are extensible.
- Elastin contains about 10% proline, 30% glycine, many non-polar amino acids, contains little hydroxyproline and hydroxylysine.
- It is produsedby the fibroblasts, in the extracellular space formed covalently linked aggregates. Such structures have special properties of elasticity (elongation and shortening in different directions).



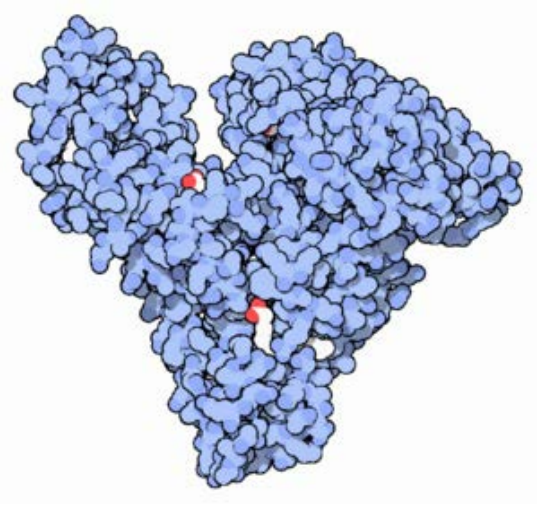
Classification of proteins by chemical composition

- **Simple proteins** - contain only amino acids and no other chemical groups; yield only amino acids upon hydrolysis

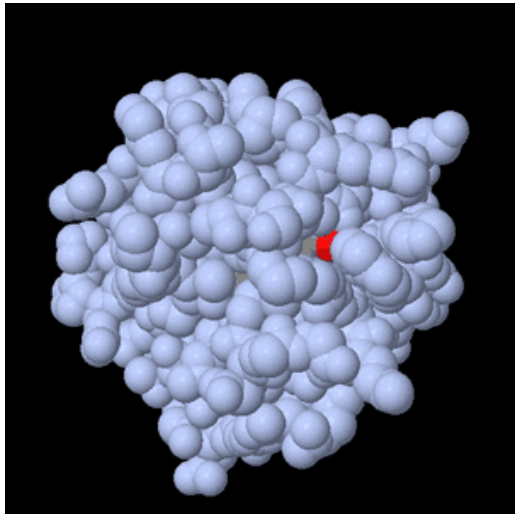
- **Conjugated proteins** - proteins that contain a nonproteic structure called **prosthetic group**. This group is attached by **covalent bonds** or by weak interactions to the proteic part named **apoenzyme**, and is required for the activity of the protein, for example the hem in hemoglobin. Yield, on hydrolysis, some other chemical component in addition to amino acids.

Simple proteins

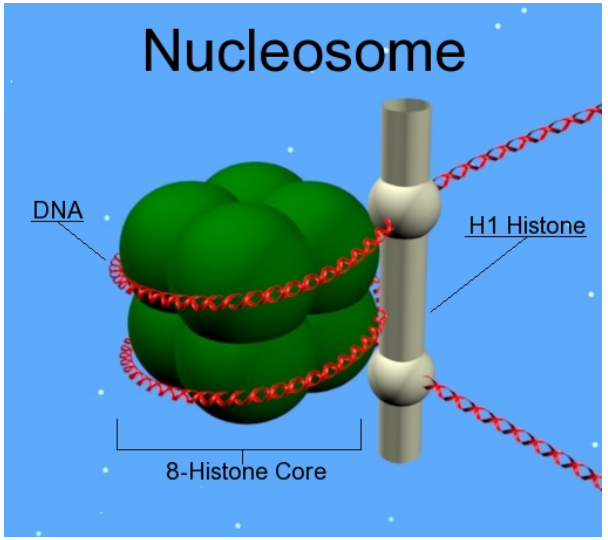
Proteins that yield only alpha-amino acids by hydrolysis: albumins, globulins, histones, glutelins, prolamines, protamines.



Albumin



Globulin



Histones

Conjugated proteins

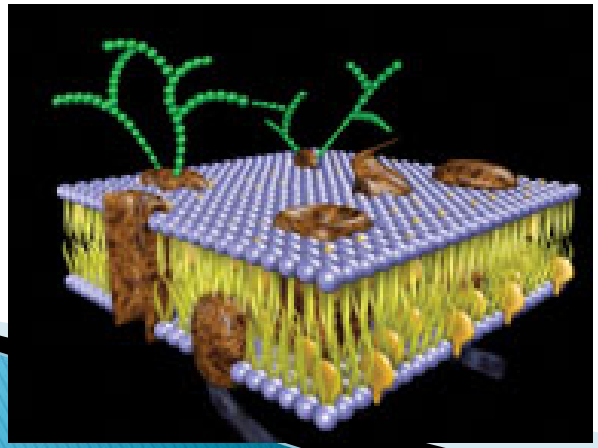
are classified by the chemical nature of their prosthetic groups.

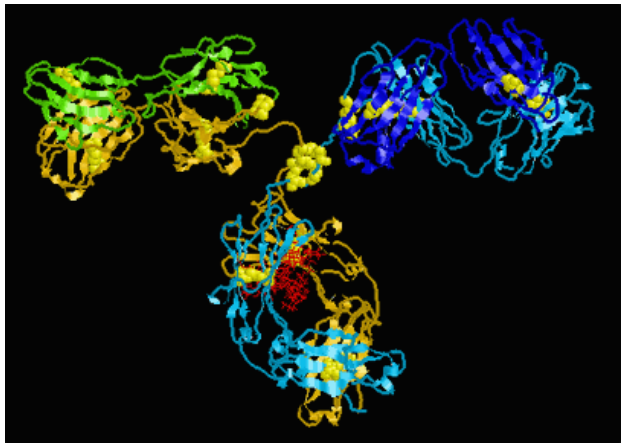
Some examples of conjugated proteins are:

glycoproteins, **lipoproteins**, **phosphoproteins**, **chromoproteins (hemoproteins and flavoproteins)**, **metalloproteins**.

Glycoproteins – the prosthetic group is a carbohydrate (usually some oligosaccharide chains (glycans) covalently attached to their polypeptide side-chains). The protein part predominates (60-80% by weight of the molecule).

Glycoproteins are generally the largest and most abundant group of conjugated proteins. They range from glycoproteins in cell surface membranes that constitute the glycocalyx, to important antibodies produced by leukocytes.





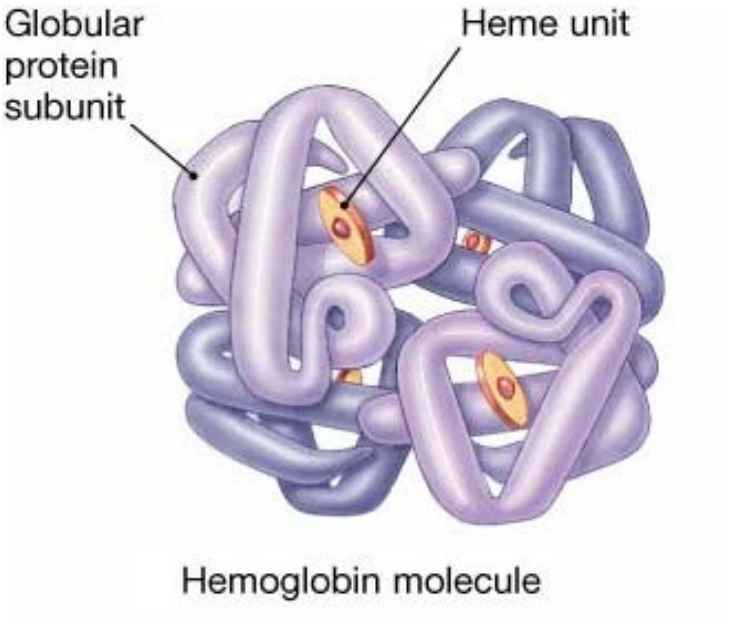
Structural glycoproteins

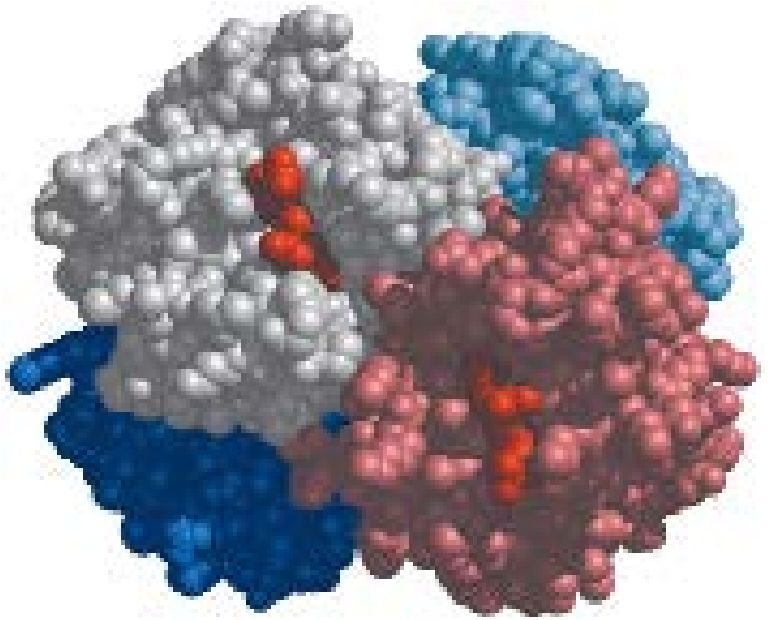
- are non fibrous proteins, which contribute to the basal membranes formation, to the arrangement of fibers in the intercellular substance and intermediate the interactions between cells and extracellular matrix. Regulate tissue morphogenesis, including calcification. Major structural glycoproteins are **fibronectin and laminin**.

Structural glycoproteins

- Carbohydrate component is represented by various hexoses, pentoses (xylose, arabinose, mannose), fucose, sialic acids, N-acetyl-hexozamine.
- Due to carbohydrates glycoproteins are:
  - termostable – have a high and low temperature resistance
  - resistance to proteolytic enzymes
  - well-expressed specificity - ensure the individuality of each glycoprotein.

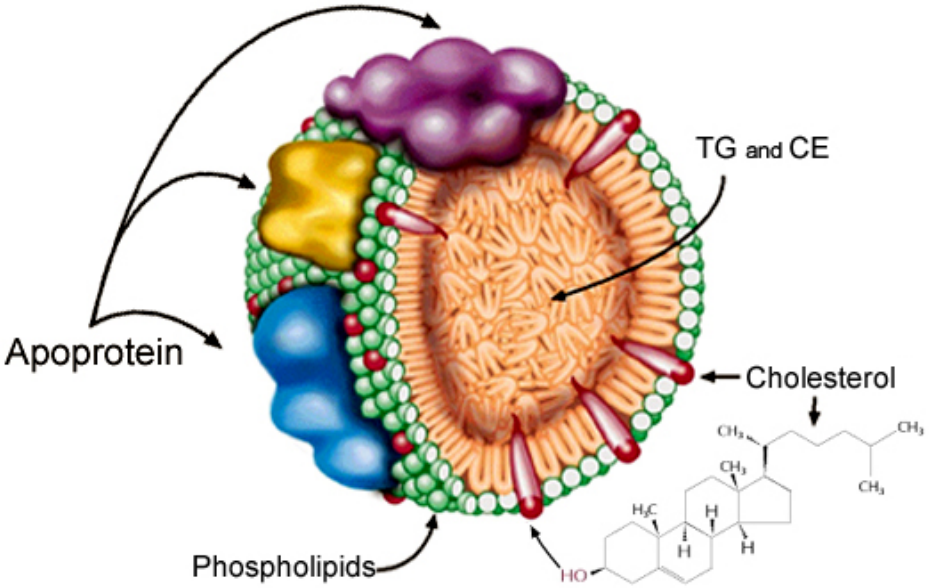
Hemoproteins – the prosthetic group is hem:

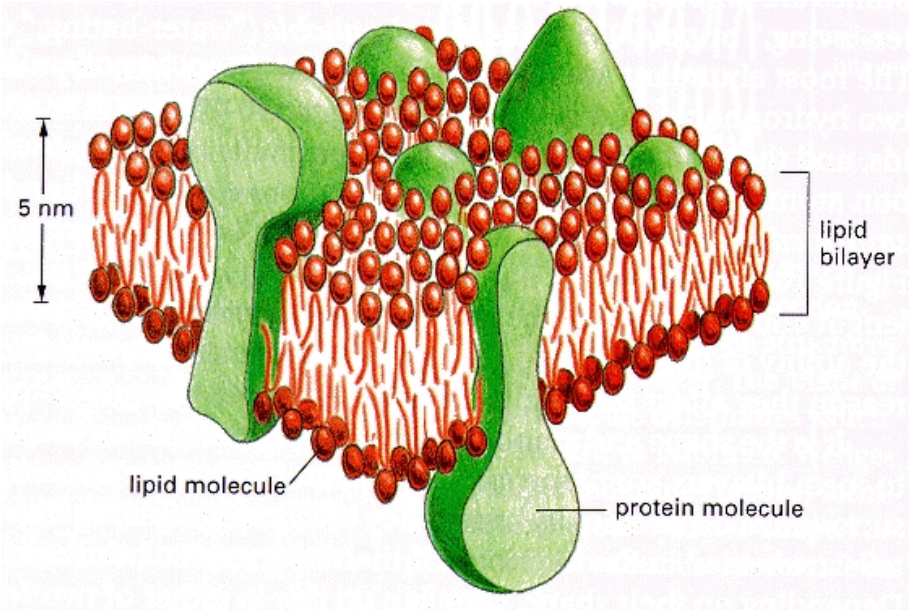




Hemoglobin

Lipoproteins – the prosthetic group is a lipid





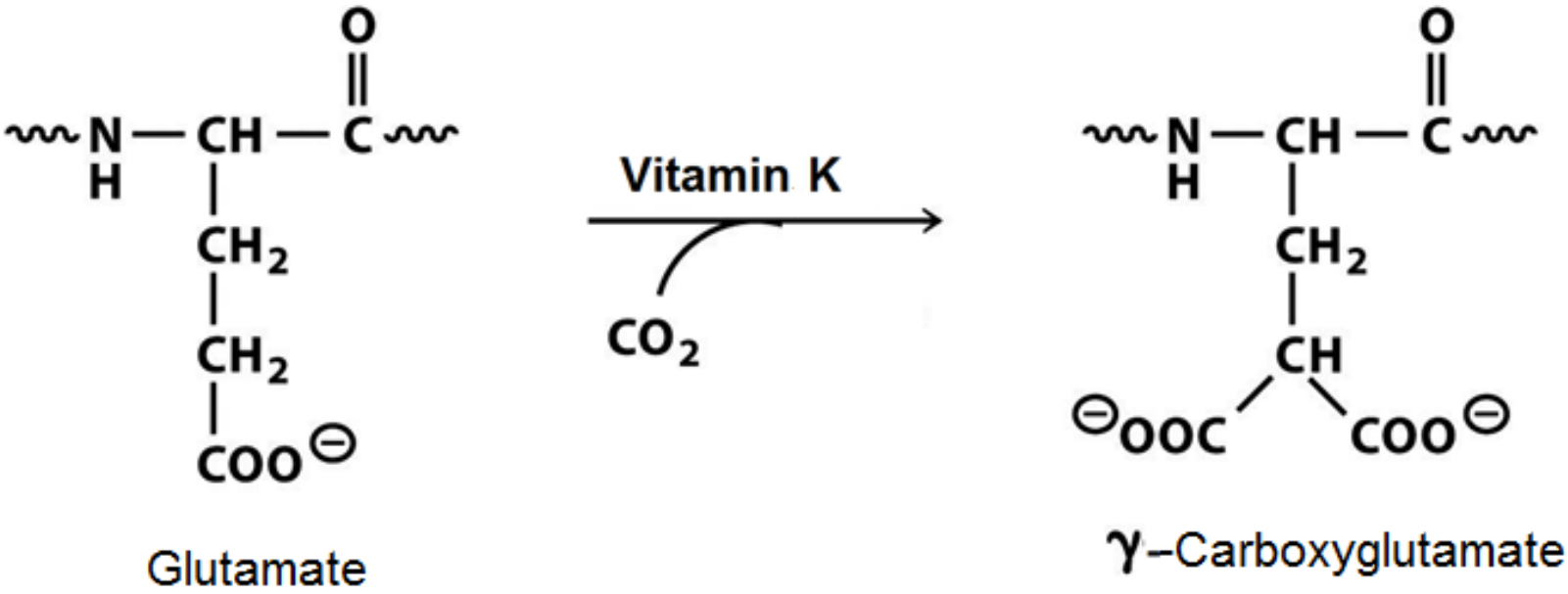
Cell membrane

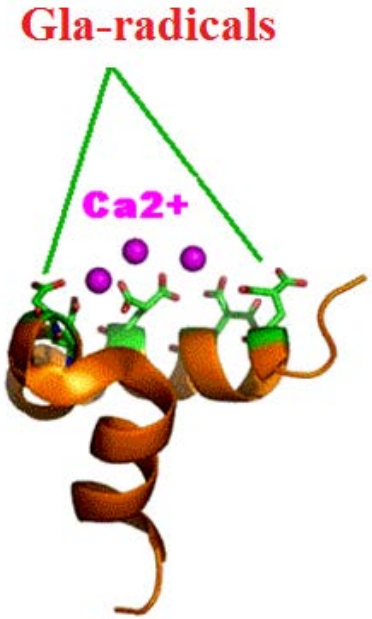
Most lipids are transported in the blood as part of soluble complexes called **lipoproteins**

Calcium-binding proteins

- **Collagen**
- **Gla-proteins (e.g. osteocalcin)**
- **CaBP - intestinal calcium binding protein**
- **Calmodulin**
- **Ca^{2+} -ATP-ase**
- **Some blood clothing factors**

Mechanism of glutamate carboxylation in Ca-binding proteins

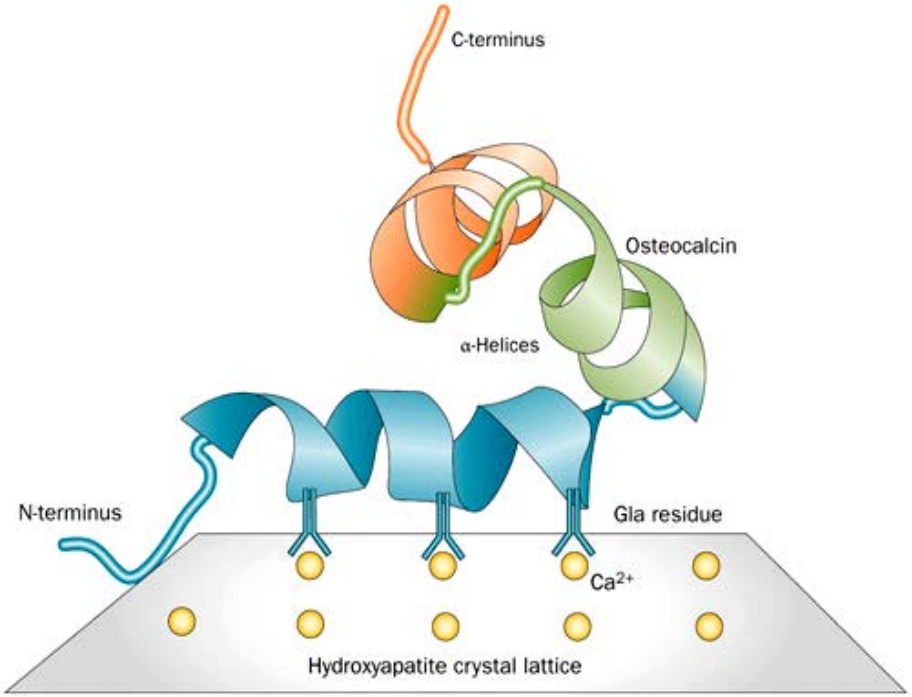




Osteocalcin

- also known as bone gamma-carboxyglutamic acid-containing protein (BGLAP), is a noncollagenous protein found in bone and dentin

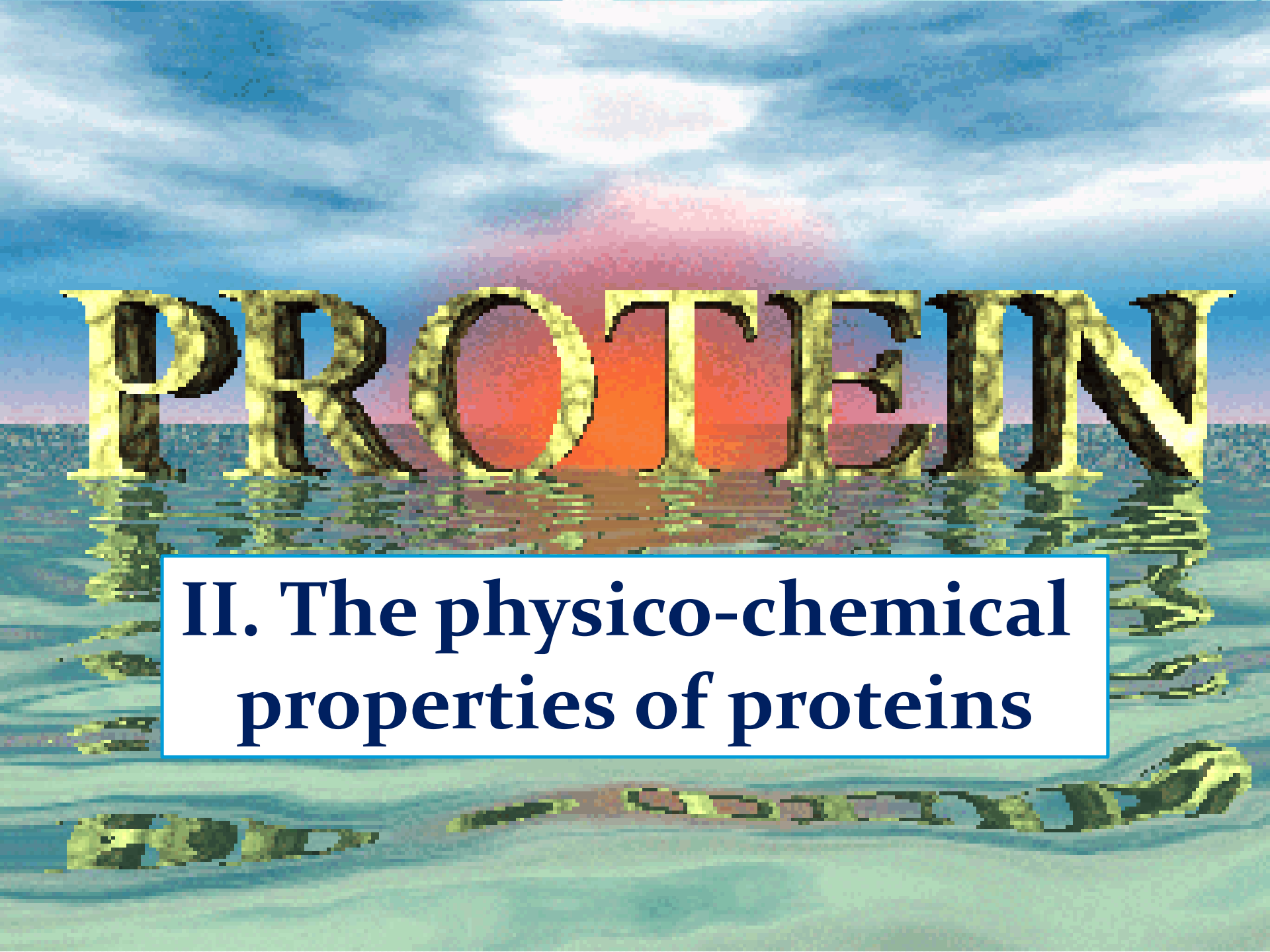
- Osteocalcin is the most abundant non-collagenous protein of the bone tissue, being about 2% of all protein in the human body.



- It has acid radicals of γ - carboxyglutamate (Gla) that are negatively charged and gives the ability to locate calcium ions into hydroxyapatite in positions complementary to those of mineralized tissues.

- Can modulate the shape and growth of hydroxyapatite crystals.

- The C-terminal end promotes adhesion of osteoblasts and osteoclasts.



II. The physico-chemical properties of proteins

Proteins differ by there physical and chemical properties:

- **Molecular mass**
- **Total electrical charge**
- **Termolability**
- **Solubility**

Molecular weight of the proteins

Proteins are **high molecular compounds**

- with molecular weight from **5 000 to 1 000 000 Da** (Daltons)

in dependence of the number of amino acid residues and of the number of protomers.

Molecular weight of some proteins, Da

Protein	Protein source	Molecular weight
Lactalbumin	milk	17.000
Insulin	pancreas	12,000
Hemoglobin	erythrocytes	68.000
Myosin	muscle	850.000
Pepsine	stomach	36.000
Peroxidase	kidney	44.000

Total electrical charge of proteins

- Proteins are amphoteric polyelectrolytes
- The total charge of proteins depends on 2 main factors:

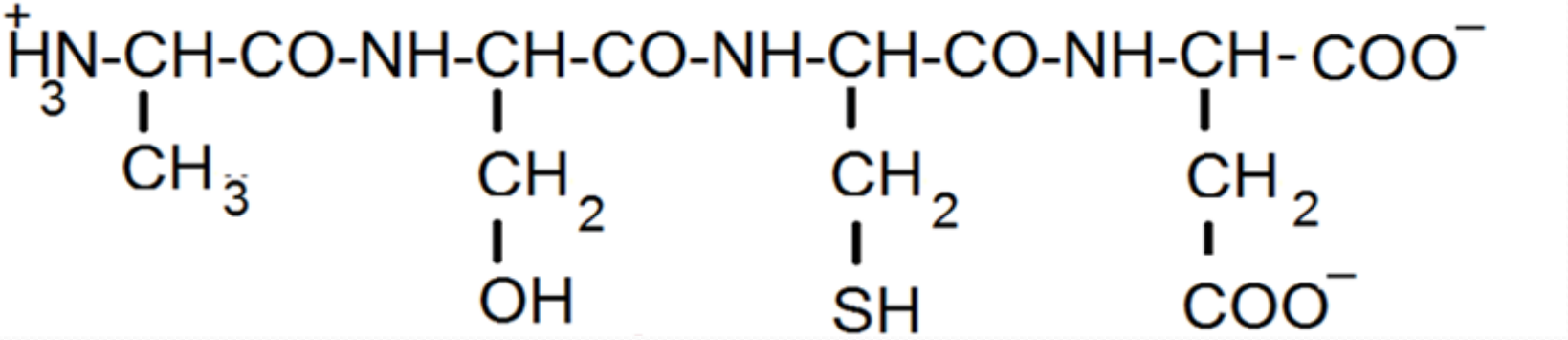
1. amino acid composition

2. pH of the medium

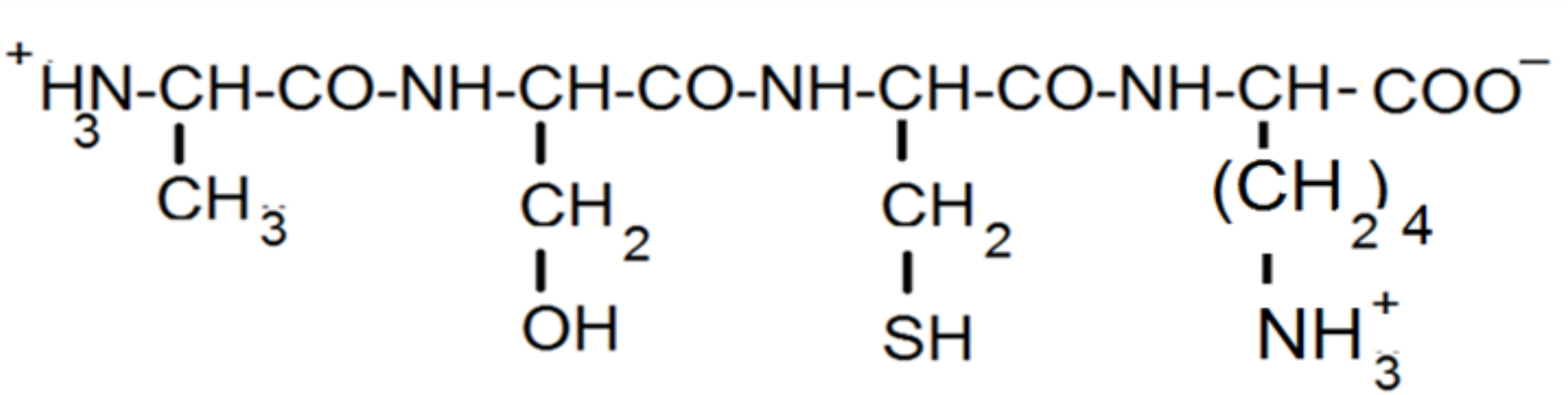
Total electrical charge of proteins:

1. depends on the amino acid composition - on the presence and correlation of charged radicals of amino acids;

- If the protein has more negatively charged amino acids (Glu and Asp)– in aqueous medium its total charge will be negative (for example - albumin).



- If the protein has more positively charged amino acids (Lys, Arg and His)– its charge will be positive (like in histones).



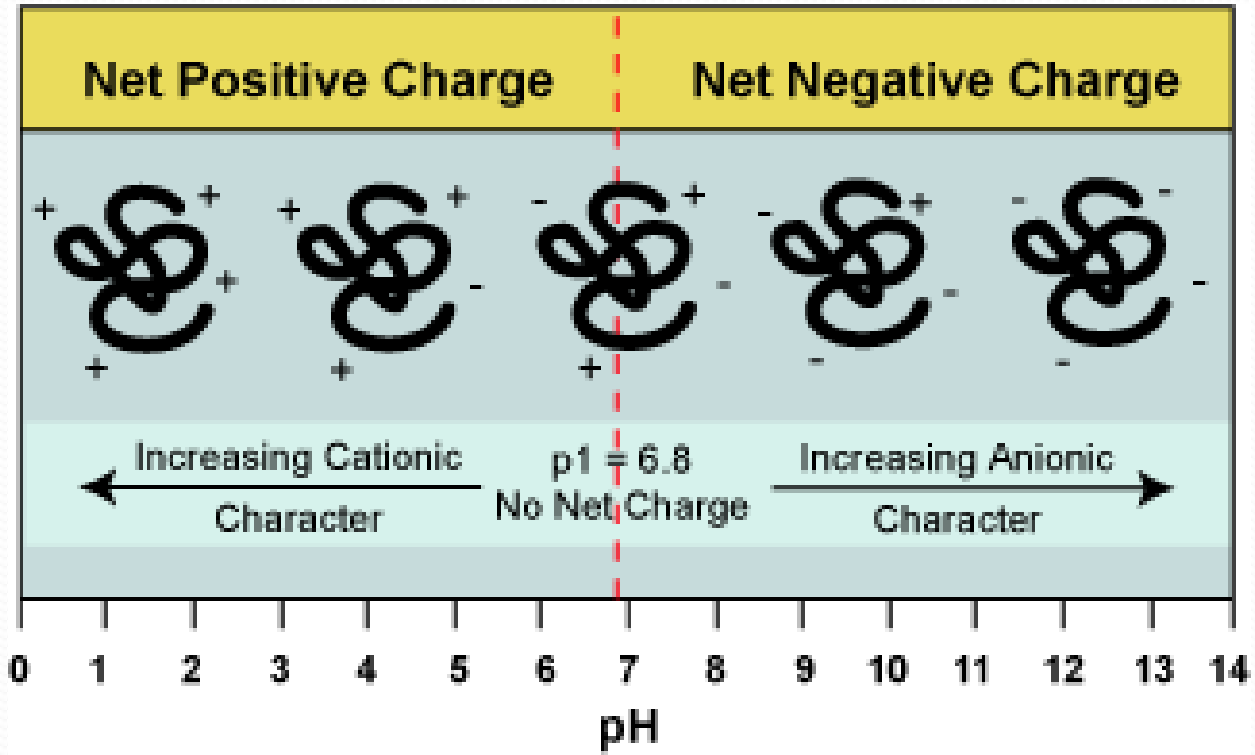
Total electrical charge of proteins:

2. depends on the pH of the medium

In acid medium the concentration of H^+ is high and neutralizes the COO^- - groups of amino acids - the negative charge decreases;

protein becomes positively charged – becomes a cation, and migrates to the cathode in the electric field.

In **basic medium** the concentration of OH^- is high and neutralizes the positive charge of amino groups $-NH_3^+$ - the positive charge decreases, the protein becomes **negatively charged** - becomes **an anion**, and migrates to **the anode** in the electric field.



Isoelectric state and isoelectric point

- The state of the protein when its total electrical charge is equal to zero is called **isoelectrical state**.
- The value of pH when the protein is in the isoelectrical state is called **isoelectrical point.**
- The proteins in the isoelectrical state have a **low solubility** in water medium and can easy precipitate.

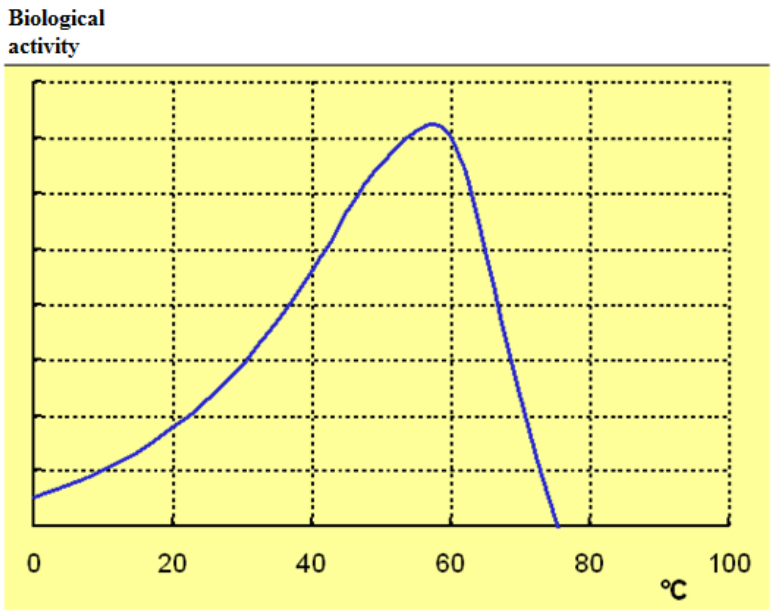
Isoelectric point

- Proteins that contain more acidic amino acids, with negatively charged residues (Glu, Asp) - have the isoelectric point in the acidic medium;

- Proteins that contain more basic amino acids, with positively charged residues (Lys, Arg, His) - have the isoelectric point in the basic medium.

Termolability

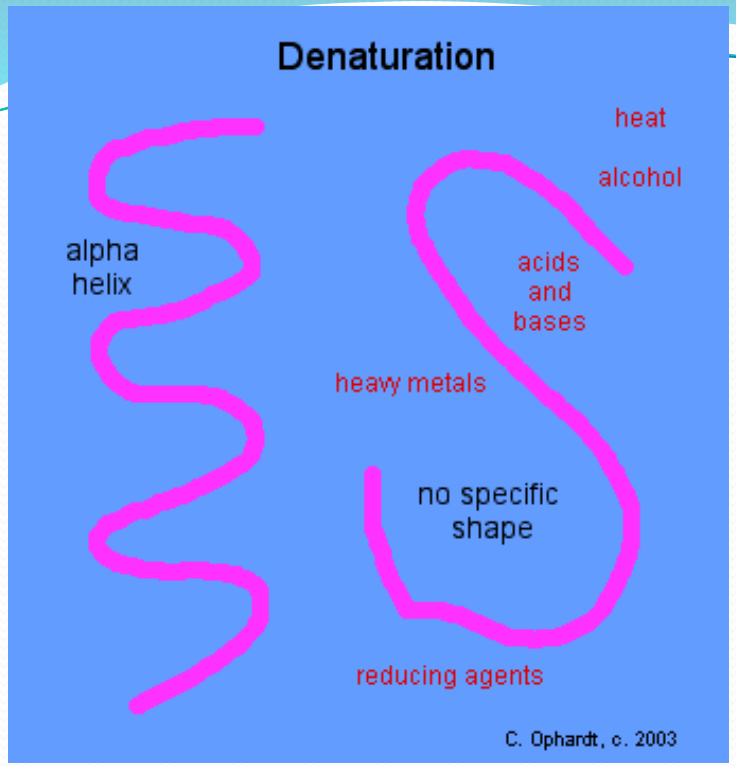
- - is the property of protein to maintain the biological activity in narrow limits of temperature (from 10 to 40°C)
- If the temperature is higher then 50-60°C the protein **denatures** – loses its native conformation. The destruction of all the structural levels of protein (except the primary) takes place.



Termolability

- There are several exceptions – **the termostable proteins** (tripsin, lyzozime, tag-polymerase) – stable at high temperature.

- If the temperature is low - the protein structure doesn't change, but the protein becomes biologically inactive.



Denaturation

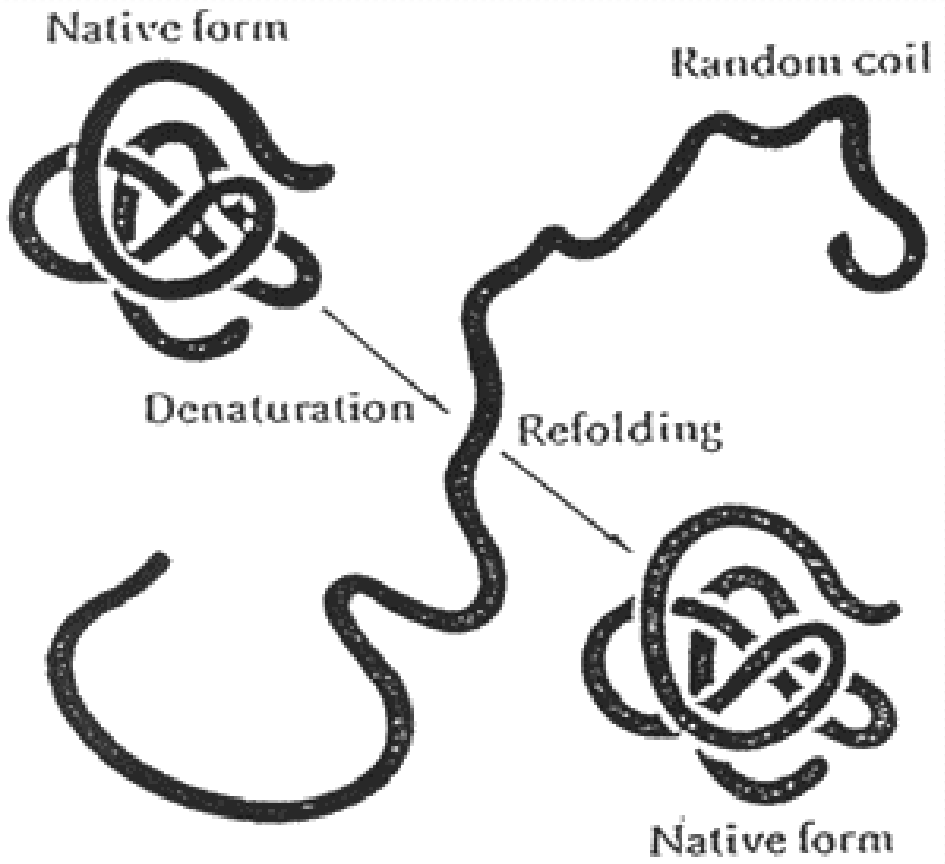
The native conformation of proteins can be lost as the result of **denaturation**: the destruction of the secondary, tertiary and quaternary structures at extreme pH values, at high temperatures, and in the presence of organic solvents, detergents, and other denaturing substances.

Since denaturation reactions are not strong enough to break the peptide bonds, the primary structure (sequence of amino acids) remains the same after a denaturation process.

Effects of Denaturation

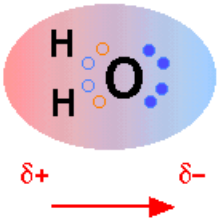
- Loss of biological activity
- Decreased solubility

Refolding or renaturation

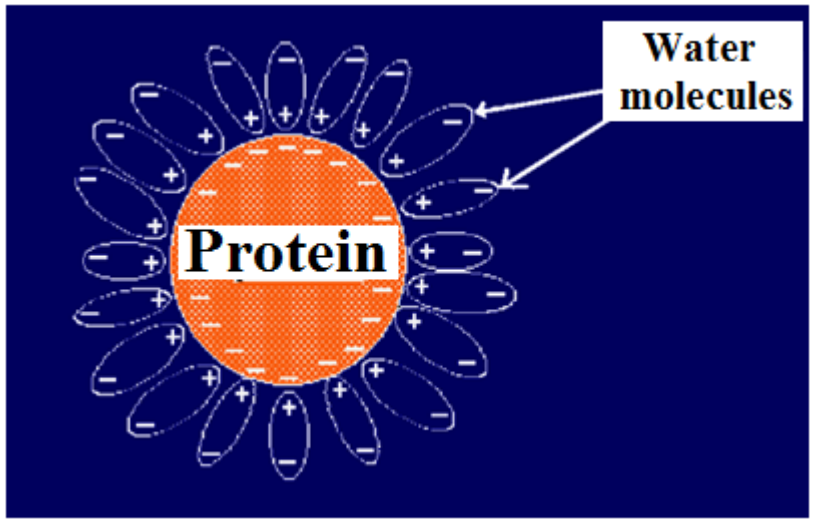


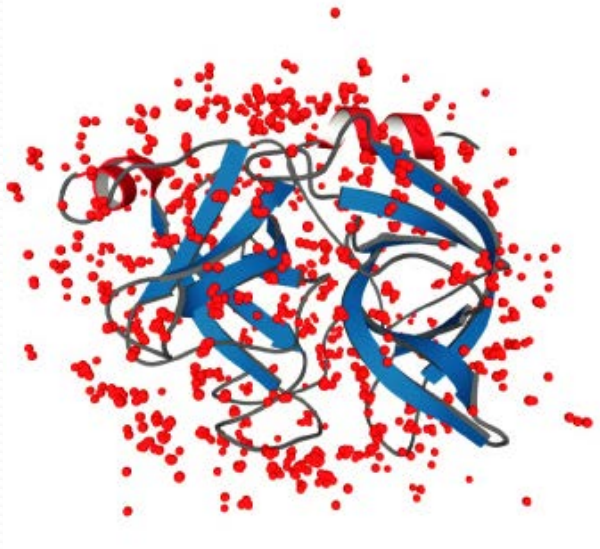
The denatured protein can spontaneously return to its native conformation – **renaturation** can takes place, but only if the denaturating agent **was not strong enough and its action was of short duration**.

Solubility of proteins



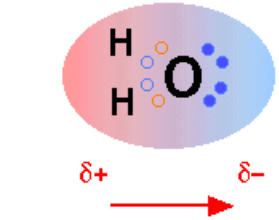
- The most of proteins are hydrophilic compounds and are soluble in water.
- Water interacts with the polar groups of proteins and forms an aqueous membrane - **hydration shell** - at the surface of the protein.





Hydration shell

- - is formed from interaction of polar groups of protein with water dipoles:
- -COO^- group interacts with 4 molecules of H_2O,
- -NH_3^+ group interacts with 3 molecules of H_2O;
- -OH and -NH groups interact with 2 molecules of H_2O.
- Water that enters in the composition of the hydration shell is called "**bound water**"



Solubility of proteins depends on:

1. presence of the **polar groups,** including those with electric charge;
2. presence of the **hydration shell**;
3. **shape of the molecule** – the globular proteins are more soluble then fibrous;

Solubility of proteins depends on:

4. **Solvent** – for example: albumins are soluble both in water and salt solution of different concentration, but globulins are not soluble in water and soluble only in weak salt solution;
5. **pH of the medium** – pH influence on the charge of the protein; in the isoelectrical point the solubility decreases;
6. **Temperature.**

The protein solution are **colloidal solution**

Classification of solutions based on:
- **molecular mass (M);**
- **diameter (Φ) of the dispersed phase particles**

Molecular:	$M < 10^3$ Da, $\Phi < 10$ Å
Colloidal:	10^3 Da $< M < 10^8$ Da, 10 Å $< \Phi < 10^3$ Å
Suspensions:	$M > 10^8$ Da, $\Phi > 10^3$ Å

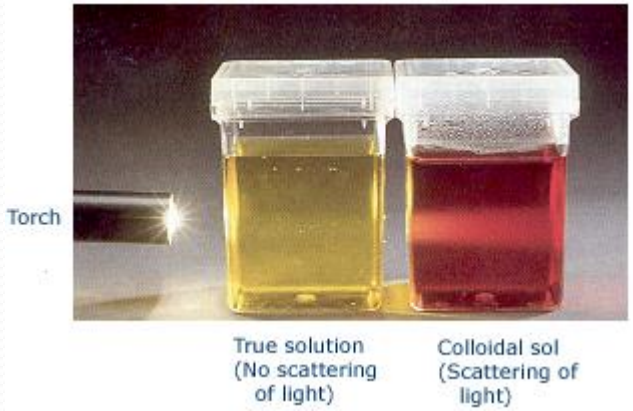
- In contrast to the other colloidal solutions, protein solutions do not require the presence of the stabilizer. Protein solutions are stable and do not precipitate over time.

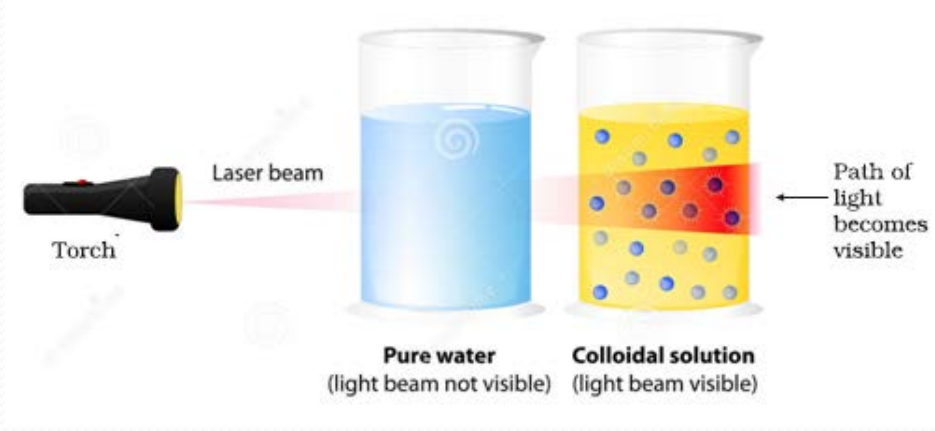
Properties of protein solutions as colloidal solutions:

- Optical properties – Tindal effect
- A low speed of diffusion
- Osmotic (oncotic) properties
- A high viscosity of the solutions
- A capacity to form gels – structural grating with water inside

Optical properties

- When the protein solution is illuminated, the light beam becomes visible, forming a light cone – the **Tindal effect**. This effect is explained by the scattering of light beam by particles in the solution.





The ability of proteins to disperse light is used for:

- the quantitative determination of protein by nephelometric method
- the microscopic study of cellular structures.

Low speed of diffusion

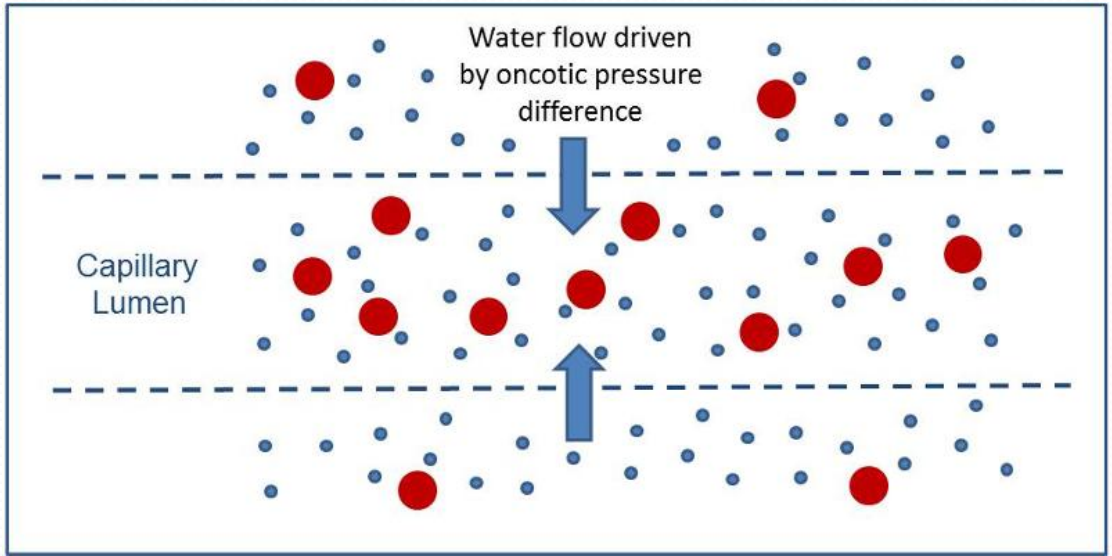
- **Diffusion** – a spontaneous movement of the solute molecules due to the concentration gradient (from regions with higher concentrations toward those with lower concentrations).

- **The speed of proteins diffusion depends more on the shape of the molecule, than on its mass.**

- The intracellular distribution of proteins occurs by diffusion. Since the diffusion rate is low, the speed of the processes is limited by diffusible proteins.

Osmotic (oncotic) properties

- the protein macromolecules is not able to diffuse through the semipermiabile membrane; it results in the phenomenon of **osmosis** (movement of H_2O molecules through the membrane towards the protein in solution). The movement of water is restricted by the hydrostatic pressure called the **osmotic pressure**;
- Osmotic pressure depends on the molar protein concentration and temperature.
- The osmotic pressure caused by proteins is called **oncotic pressure**.

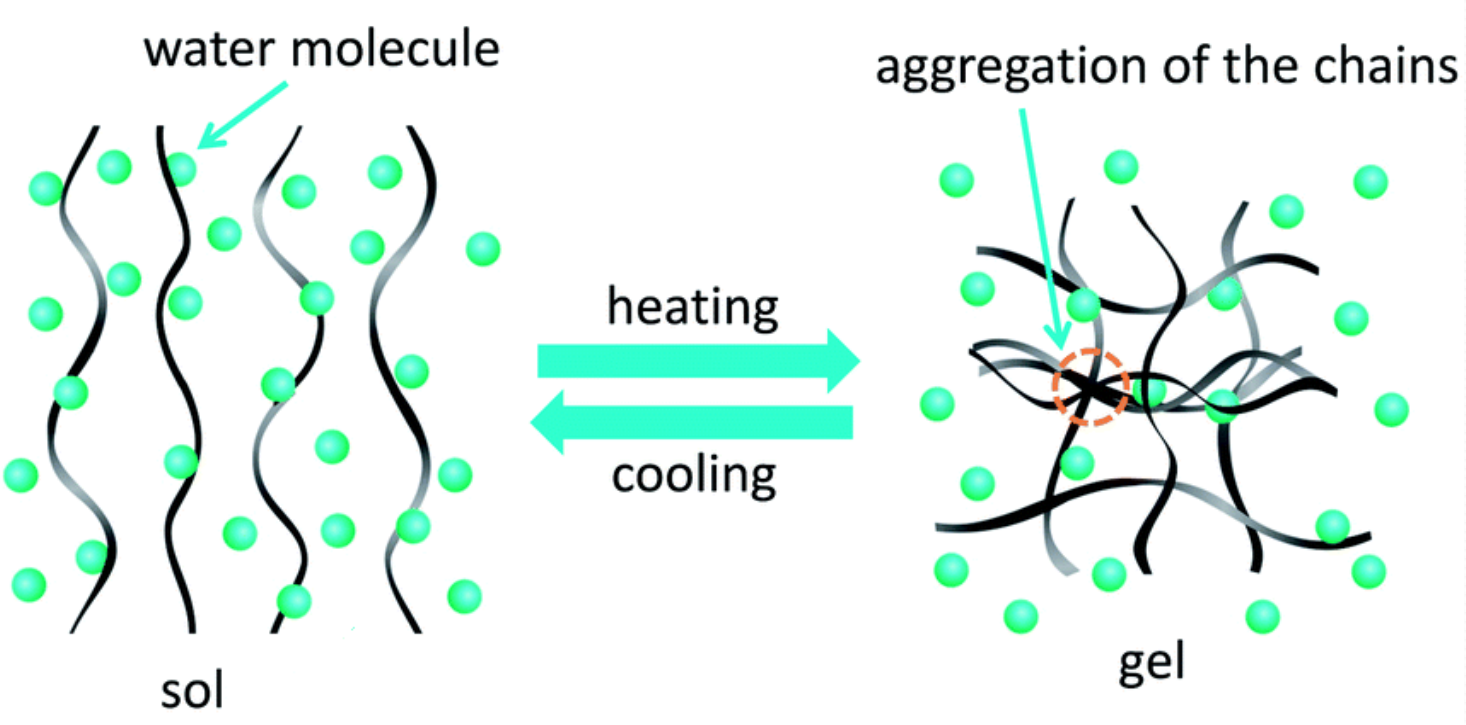


High viscosity of the solutions

- **Viscosity** - the force of cohesion between protein molecules - is dependent on the mass and shape of molecules
- A high protein concentrations lead to a high solution viscosity
- The fibrillar proteins are more viscous than the globular.
- Viscosity is dependent on:
  - **temperature** (at high temperature the viscosity decreases);
  - **presence of electrolytes** (eg. Ca^{2+}salts increase the viscosity by the formation of Ca^{2+}bridges)

Capacity to form gels

- Protein molecules interact one with another to form structural networks with immobilized water inside.
- Gelatinization occurs easier in fibrous protein solutions



- Gel formation is observed in blood coagulation (fibrin network formation).
- In aging gels **syneresis** takes place - the expulsion of water molecules due to contraction of the molecules of the network.
- **Gel formation depends on:**

  - concentration of the solution;
  - temperature (lower temperature favors gel formation);
  - the concentration of hydrogen ions (in the isoelectric point the rate of gel formation is maximal);
  - the presence of electrolytes

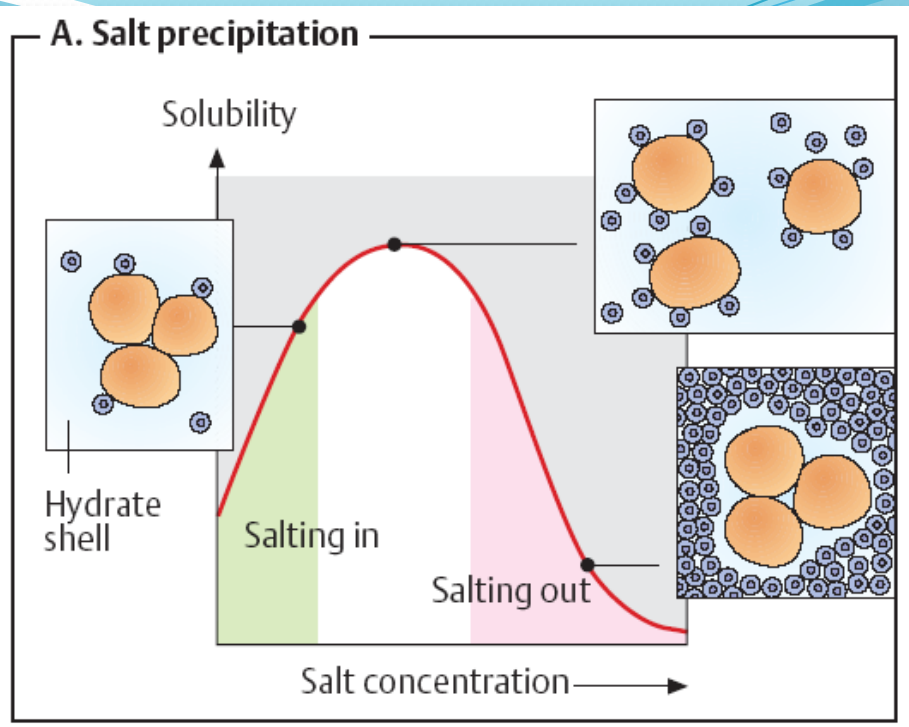
Xerogel-

- it is a dry (waterless) gel.
- is obtined by the **liophilic drying up** of the colloidal solutions
- The **liophilic drying up** is the water removing under vacuum from the frozen colloidal solution.
- Can be kept for a long time – it has a practical importance in the industry of proteic medicines production (e.g.: different proteins - albumin, gamma-globulins and others).

Methods of purification, fractionation and analyzing proteins:

- Salt precipitation
- Dialysis
- Electrophoresis
- Gel-filtration

Salt precipitation



- it is a method of precipitating proteins from the solution under the action of neutral salts in high concentrations (ammonium sulfate, etc.)
- it is a reversible process
- the protein doesn't lose the activity.

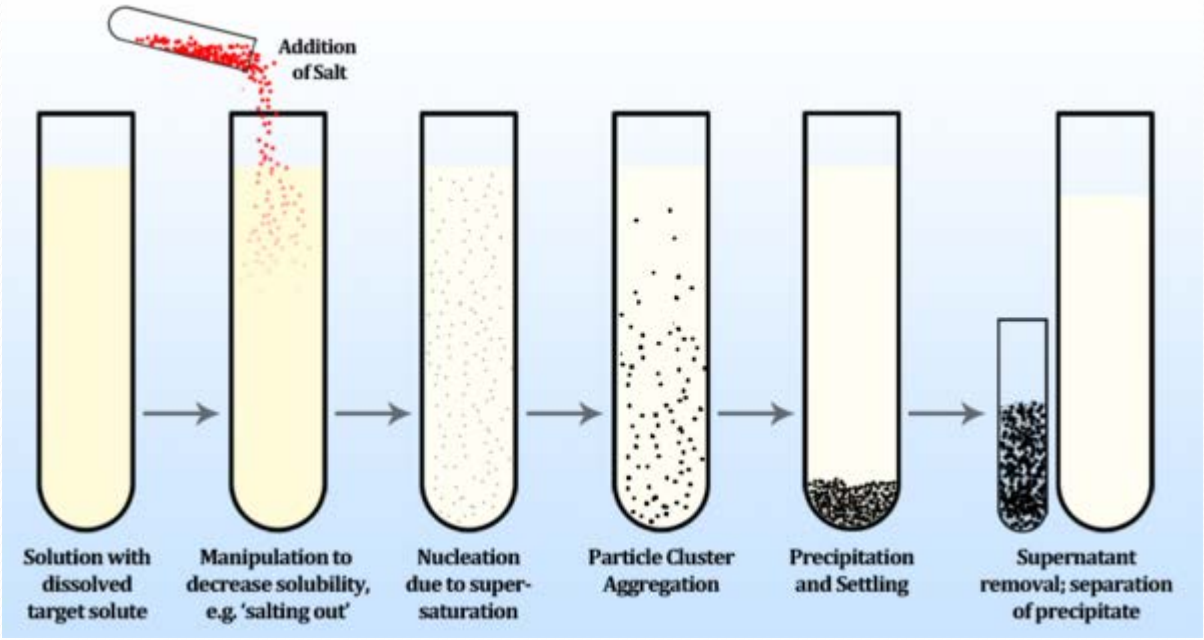
The mechanism of salt precipitation:

- breakdown of the hydration shell
- removing the electric charge.

Salt precipitation

- The solubility of proteins is strongly dependent on the salt concentration (*ionic strength*) of the medium.
- Proteins are usually poorly soluble in pure water. Their solubility increases as the ionic strength increases, because more and more of the well-hydrated anorganic ions are bound to the protein's surface, preventing aggregation of the molecules (**salting in**).

- At very high ionic strengths, the salt withdraws the hydration shall from the proteins and thus leads to aggregation and precipitation of the molecules (**salting out**). For this reason, adding salts such as ammonium sulfate $(NH_4)_2SO_4$ makes it possible to separate proteins from a mixture according to their degree of solubility (**fractionation**).



- The rate of precipitation of proteins by salt precipitation depends on a number of factors such as hydrophilic properties of the protein, its molecular weight, electric charge; thus the salt precipitation of various proteins occurs in various salt concentrations.

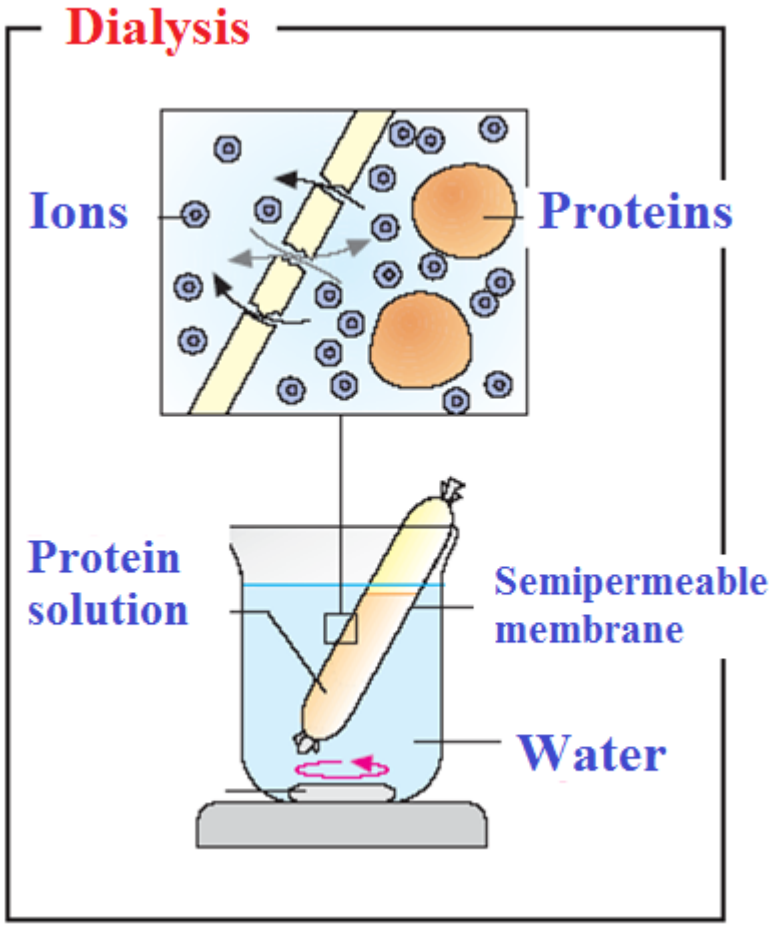
- For example, albumin is precipitated in a saturated solution of ammonium sulphate, whereas globulins - the half-saturated

Dialysis-

- – is the process of separating molecules in solution by the difference in their rates of diffusion through a semipermeable membrane

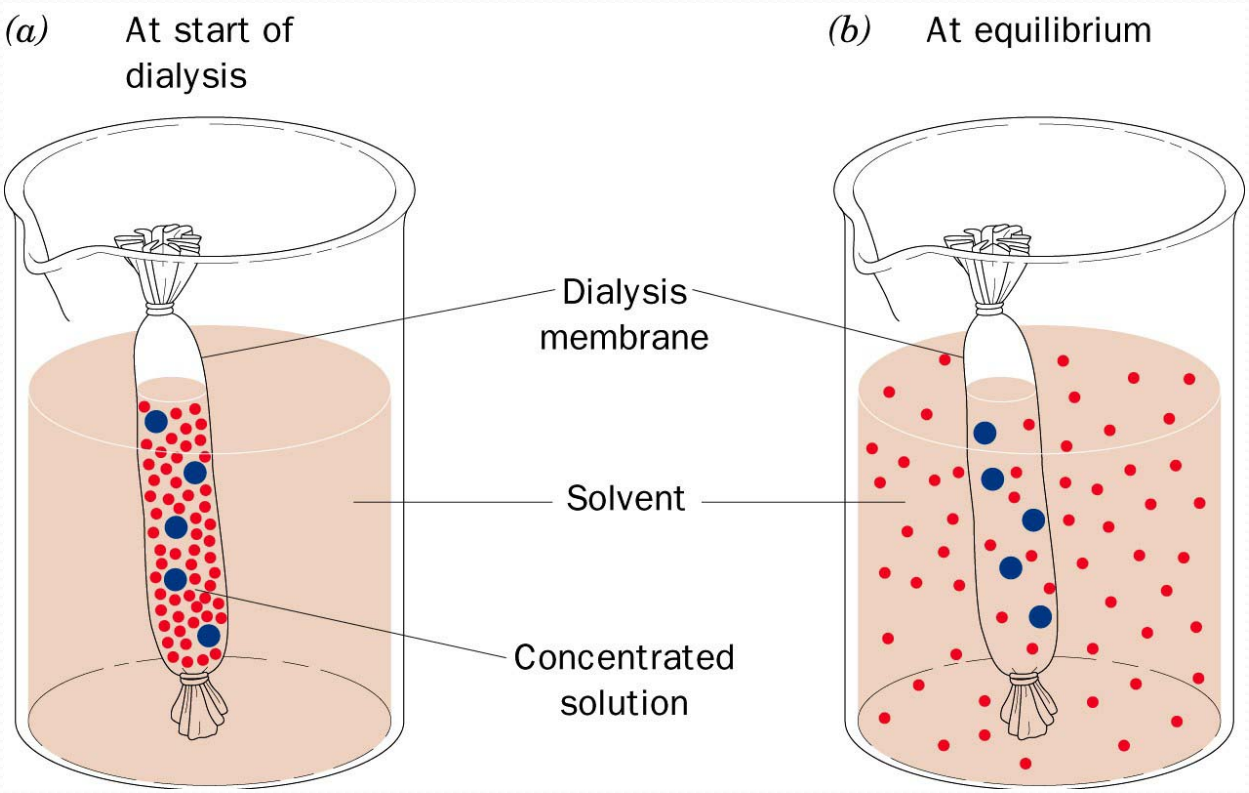
- - method is used for macromolecular compounds (proteins) separation and purification of micromolecular compounds with the help of semipermeable membrane (cellophane, parchment, etc.).

- - through the pores of this membrane can pass only micromolecular compounds that have low molecular weight and small size.

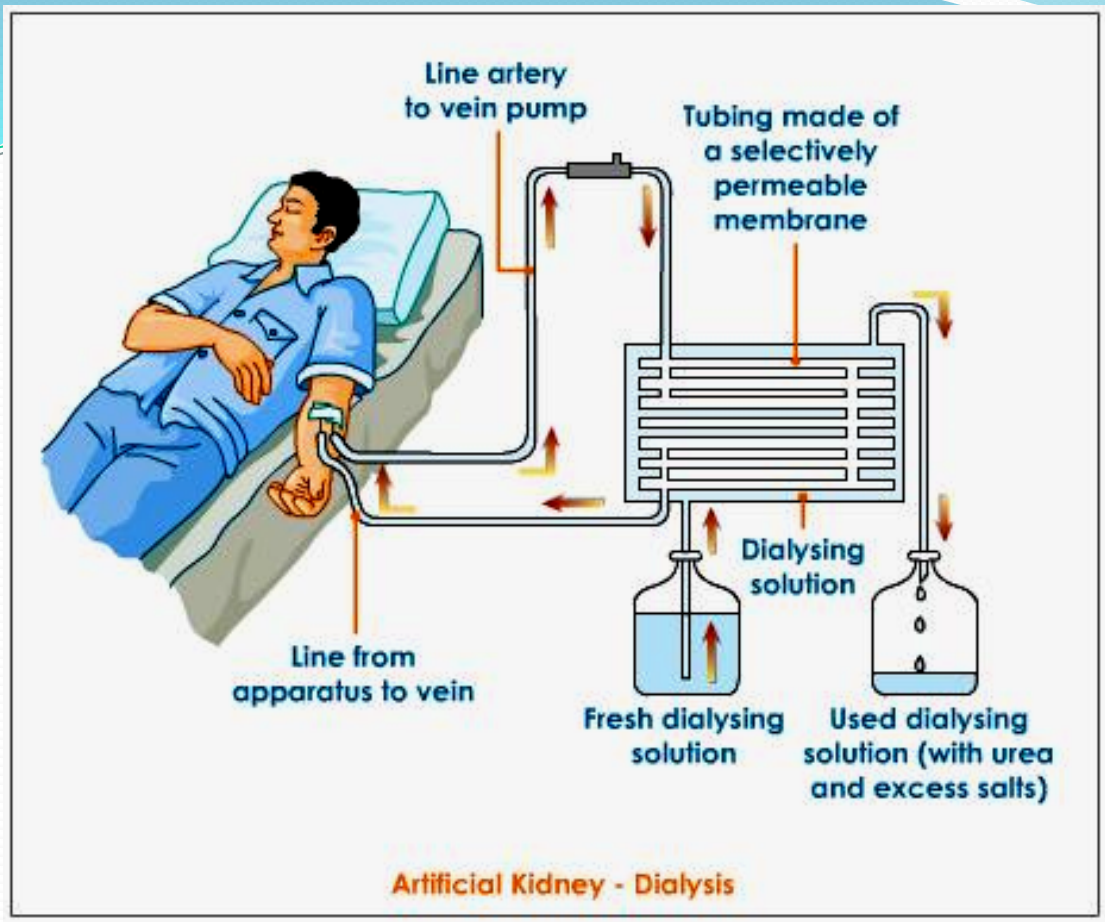


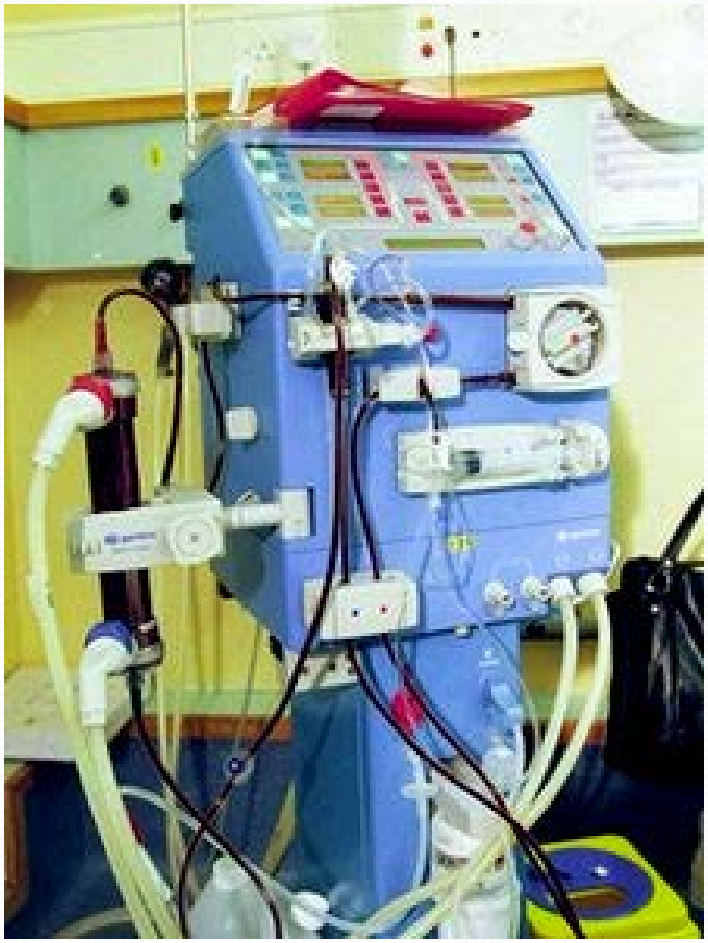
Dialysis

Due to their size, protein molecules are unable to pass through the pores of a **semipermeable membrane**, while lower-molecular substances are able. Thus, dialysis can be used to remove lower-molecular components from protein solutions.



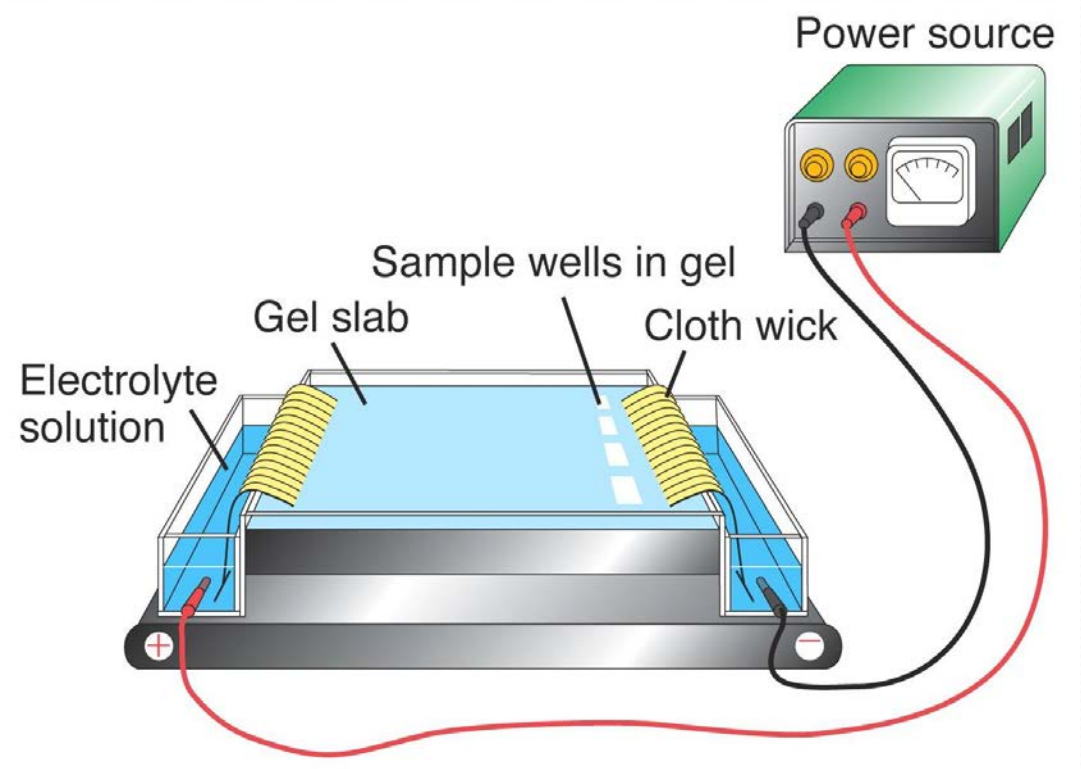
Dialysis in medicine





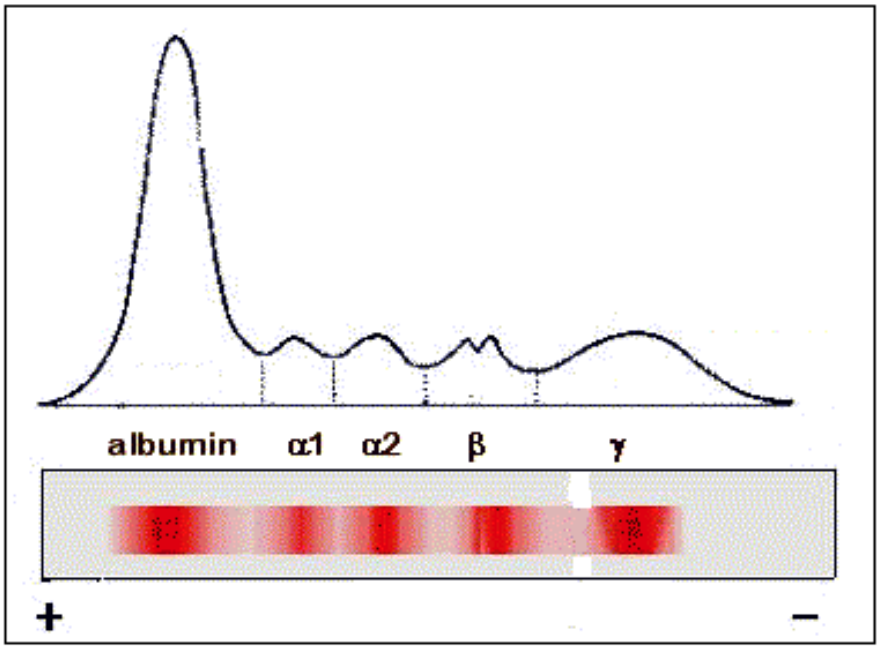
Electrophoresis

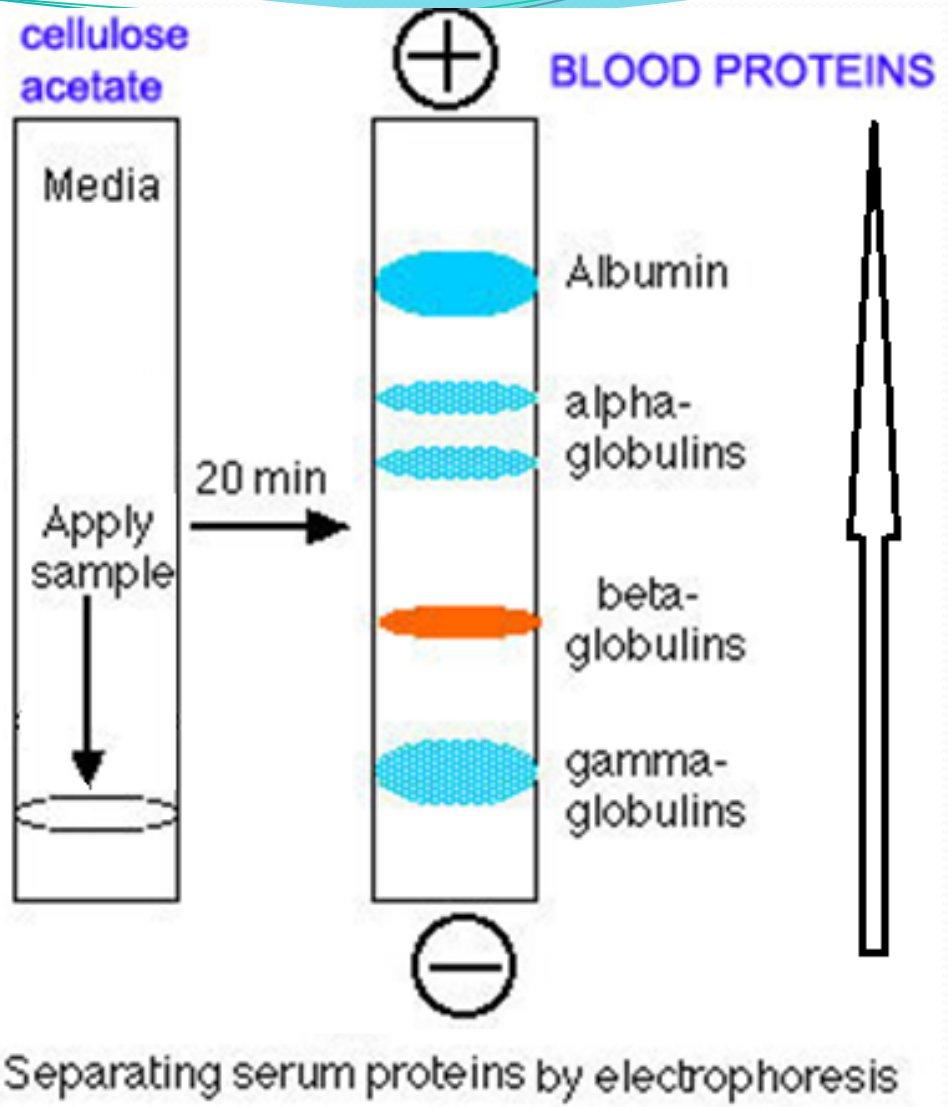
- is based on the ability of particles possessing electric charge, including proteins, to migrate in continuous electric field.



Electrophoresis

- is a technique used to separate different elements (fractions) of a blood sample into individual components. Serum protein electrophoresis is a test that measures the major blood proteins by separating them into five distinct fractions: albumin, alpha1, alpha2, beta, and gamma proteins.





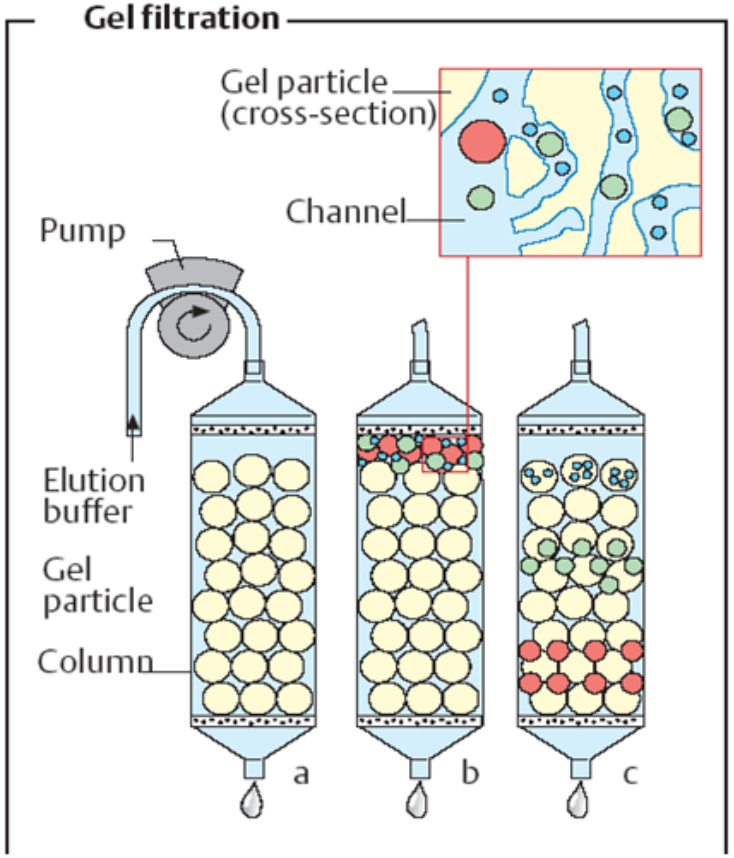
Separating serum proteins by electrophoresis

Gel-filtration (gel-chromatography)

- is one of the main chromatographic methods for the proteins purification.
It requires:
- **Stationary phase: molecular sieve**
- **Mobile phase: buffer**

• The molecular sieves consist of granules of inert hydrated polysaccharide gel. The granules have pore with different diameter.

• Small size micromolecules penetrate these pores, the macromolecules – don't.



• The speed of micromolecules migration through the column is less than of macromolecules – it allows to purify proteins of micromolecular compounds.

• The speed of proteins migration through the column depends on their mass and size - **those which have larger mass and size move faster .**