Proteins Structure and Function

University of Anbar/College of Pharmacy
Second semester 2021-2022 / Biochemistry I / 3rd stage

References :

1- Harper’s Illustrated Biochemistry
2- Lehninger Principles of Biochemistry

By
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Peptides and Proteins

20 amino acids are commonly found in protein.

These 20 amino acids are linked together through “peptide bond forming peptides and proteins (what’s the difference?).

- The chains containing less than 90 amino acids are called **“peptides”,** while those containing greater than 90 amino acids are called **“proteins”.**

Peptide bond formation:

α-carboxyl group of one amino acid (with side chain R1) forms a covalent peptide bond with α-amino group of another amino acid (with the side chain R2) by removal of a molecule of water. The result is : **Dipeptide** (i.e. Two amino acids linked by one peptide bond). By the same way, the dipeptide can then forms a second peptide bond with a third amino acid (with side chain R3) to give **Tripeptide.** Repetition of this process generates a polypeptide or protein of specific amino acid sequence.

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Levels of Protein Structure:

- The primary structure of a protein consists of the amino acid sequence along the chain.

• The secondary structure involves α-helices, β-sheets, and other types of folding patterns that occur due to a regular repeating pattern of hydrogen bond formation.

• The tertiary structure (the three-dimensional conformation of a protein) involves electrostatic and hydrophobic interactions, van der Waals interactions, and hydrogen and disulfide bonds.

• Quaternary structure refers to the interaction of one or more subunits to form a functional protein, using the same forces that stabilize the tertiary structure.

• Proteins serve in many roles (e.g., as enzymes, hormones, receptors, antibodies, structural components, transporters of other compounds, and contractile elements in muscle).

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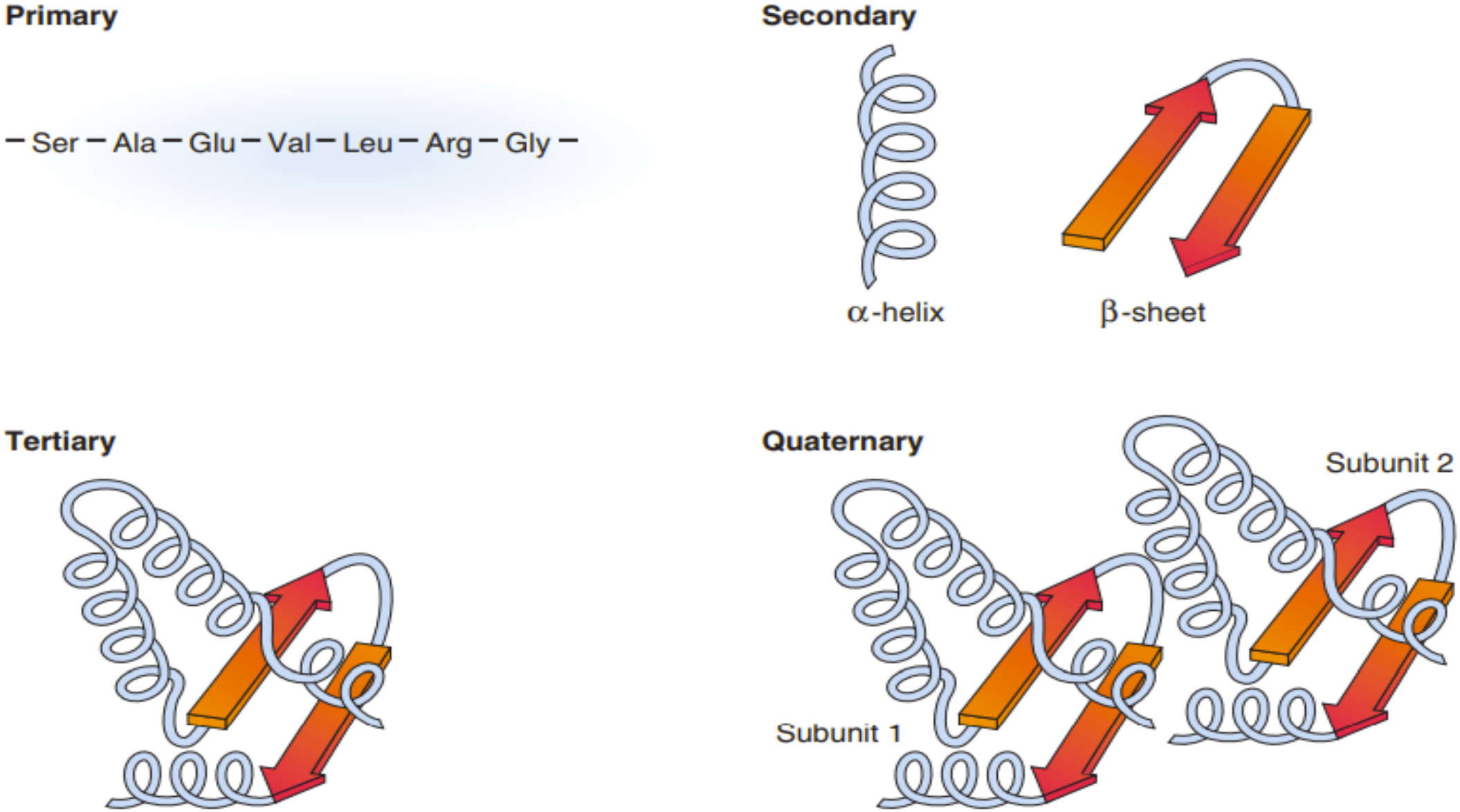

FIGURE 2.7. Schematic diagram of the primary, secondary, tertiary, and quaternary structures of a protein.

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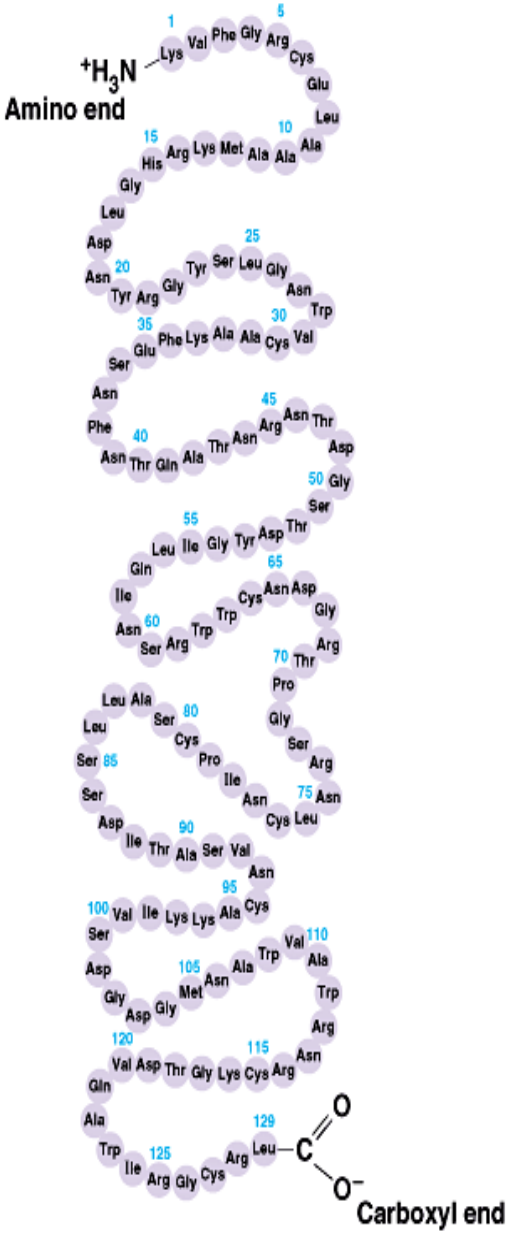
Protein structure:

There are four levels of protein structure (primary, secondary, tertiary and quaternary)

Primary structure:

- The **primary structure** of a protein is its unique sequence of amino acids.
  - Lysozyme, an enzyme that attacks bacteria, consists of a polypeptide chain of 129 amino acids.
  - The precise primary structure of a protein is determined by inherited genetic information.
  - At one end is an amino acid with a free amino group the (the N-terminus) and at the other is an amino acid with a free carboxyl group the (the C-terminus).



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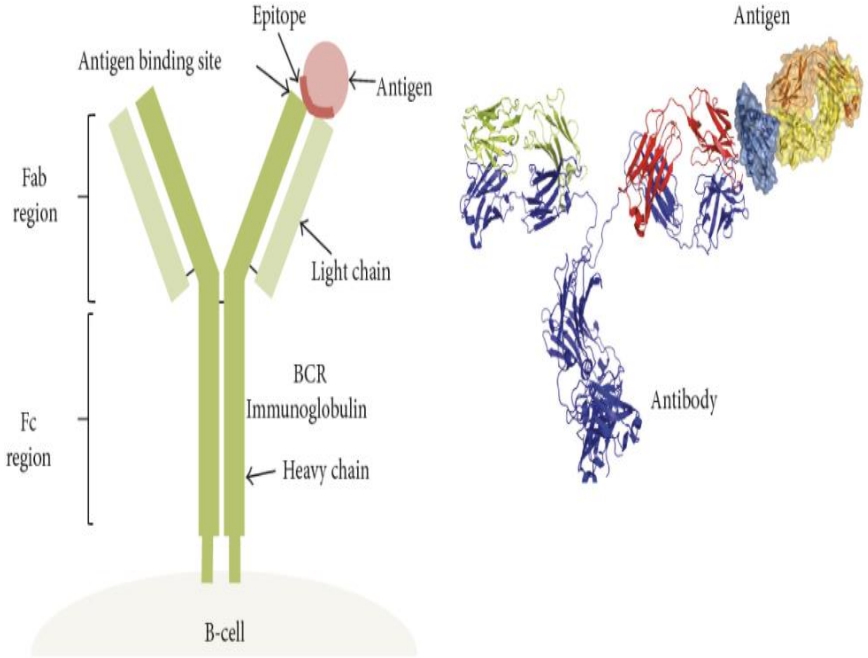
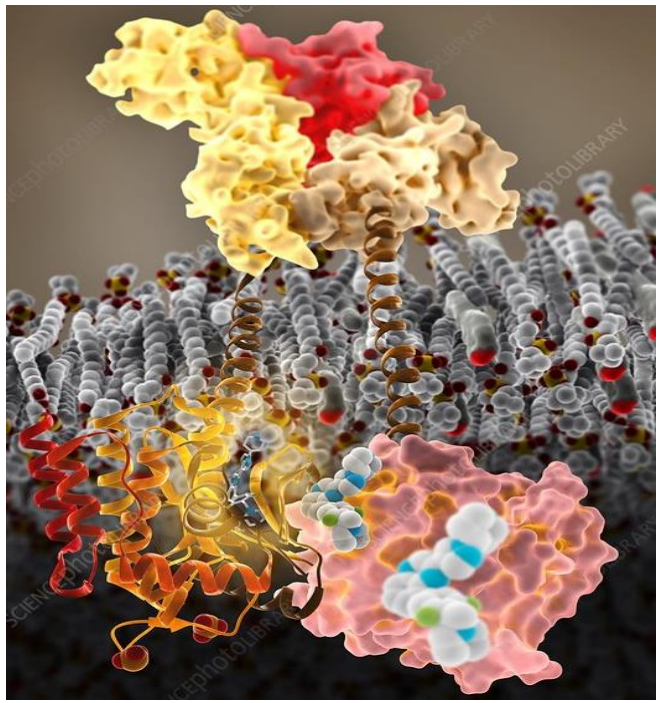
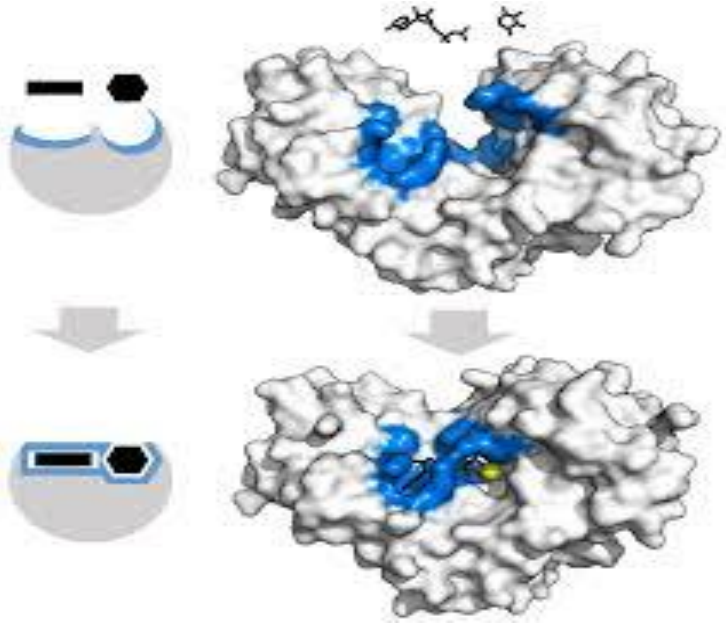
High orders of Protein structure:

A functional protein is **not just** a polypeptide chain, but **one or more** polypeptides precisely **twisted, folded and coiled** into a molecule of unique shape (**conformation**). This conformation is essential for some protein function e.g. Enables a protein to recognize and bind specifically to another molecule e.g. hormone/receptor; enzyme/substrate and antibody/antigen.







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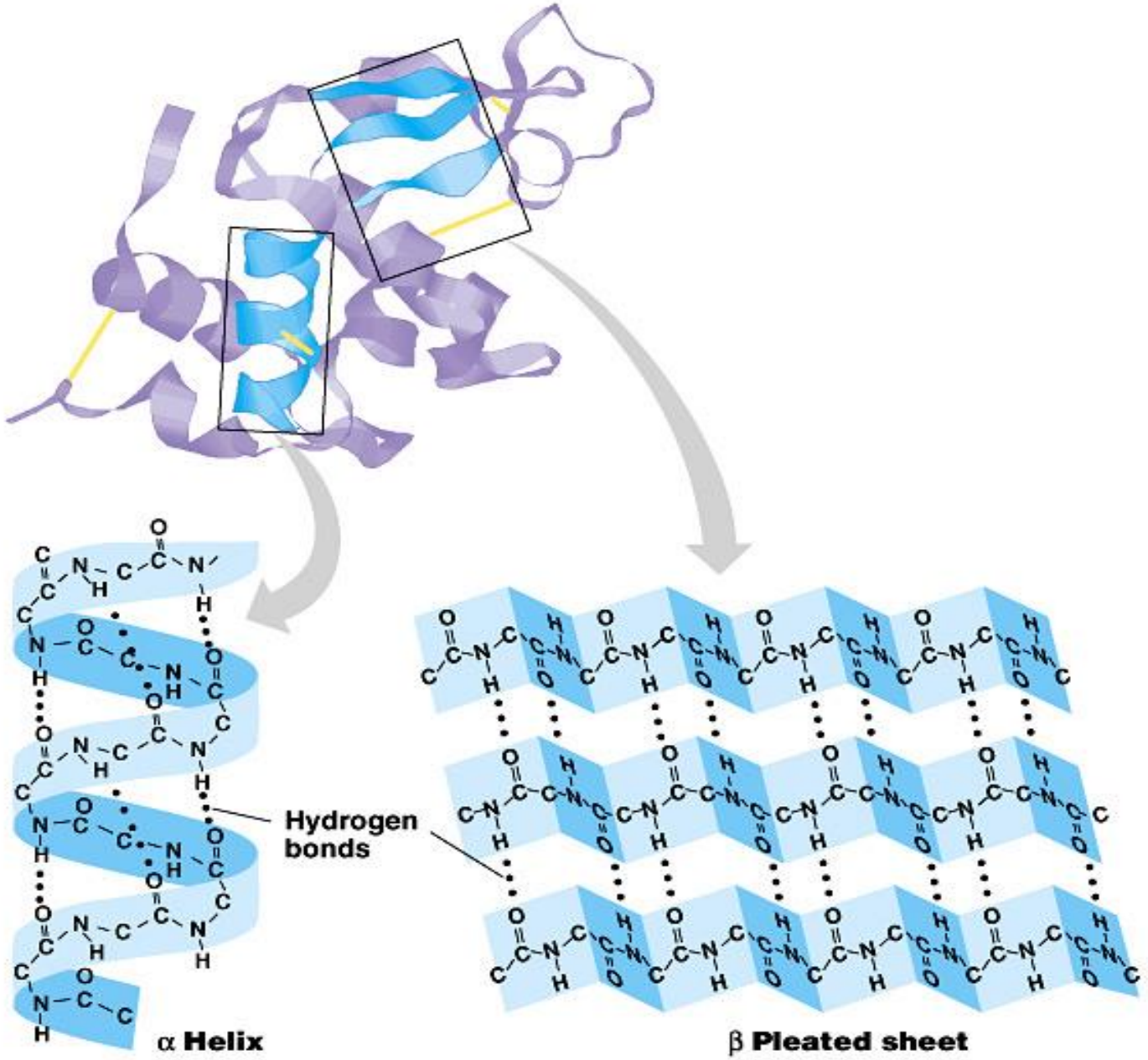
2- Secondary structure:

Results from hydrogen bond formation between hydrogen of –NH group of peptide bond and the carbonyl oxygen of another peptide bond. According to H-bonding there are two main forms of secondary structure:



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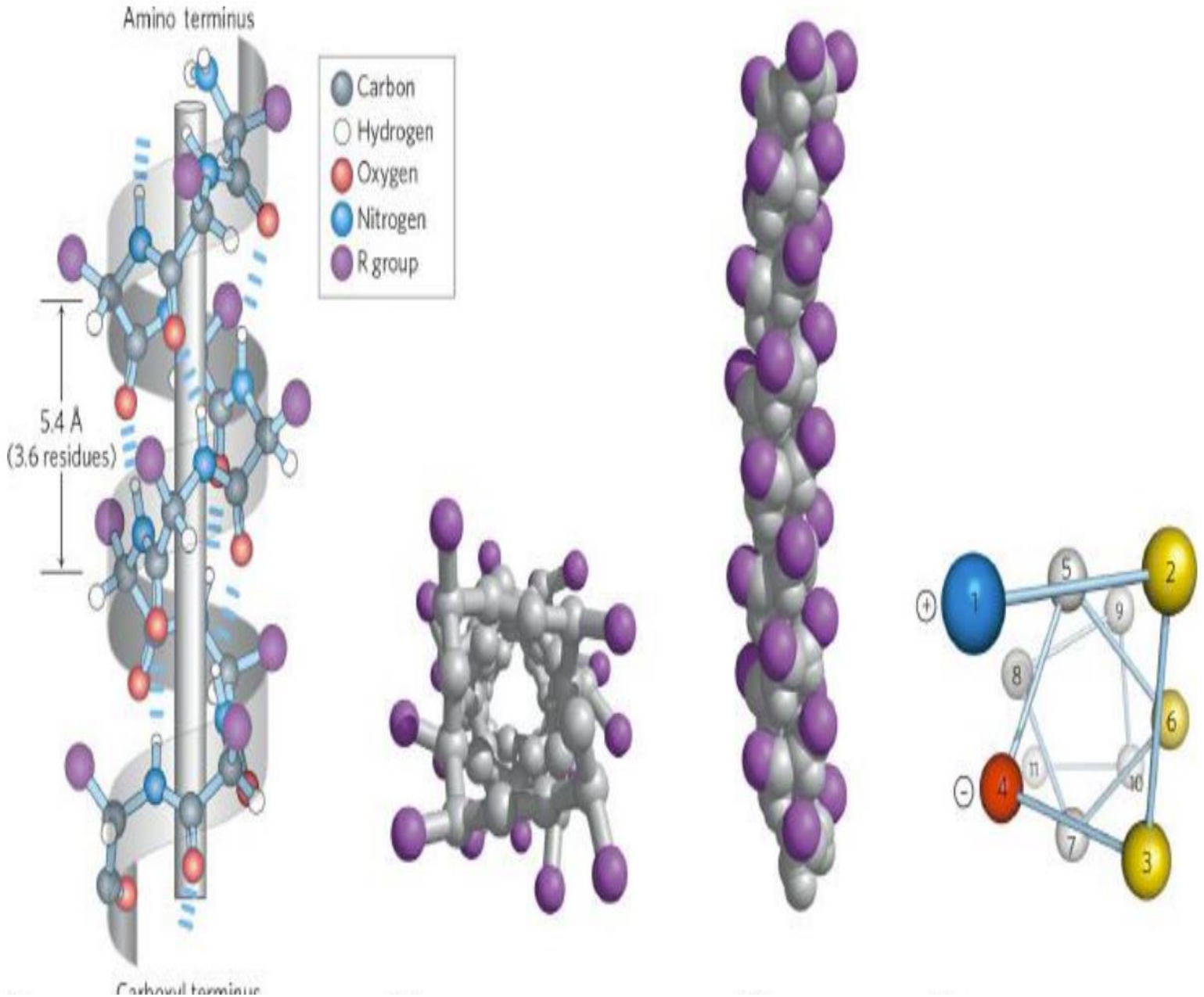
α-helix:

It is a spiral structure resulting from hydrogen bonding between one peptide bond and the fourth one

Q/ What is the length of a polypeptide with 80 amino acid residues in a single, continuous α helix?



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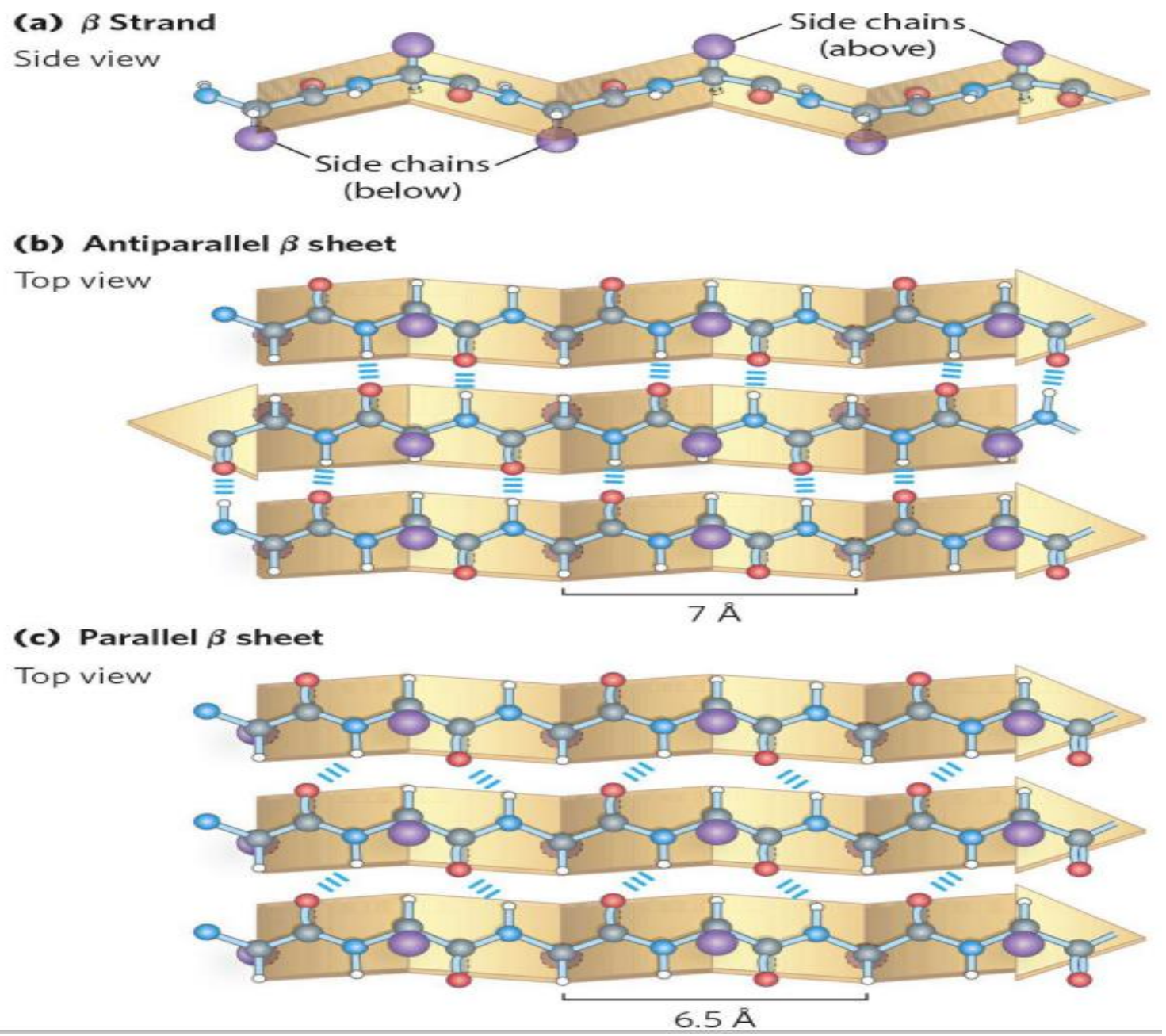
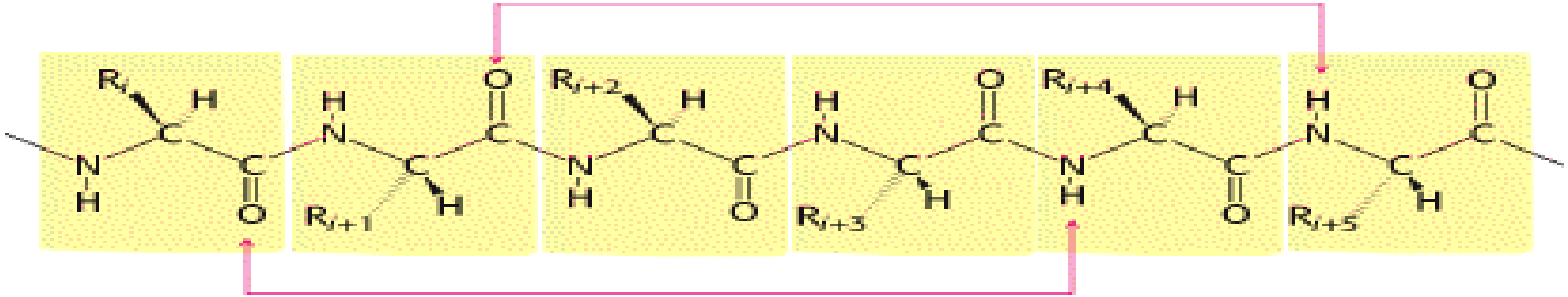
β-sheets:

is another form of secondary structure in which two or more polypeptides (or segments of the same peptide chain) are linked together by hydrogen bond between H- of NH- of one chain and carbonyl oxygen of adjacent chain .





Hydrogen bonding in α-helix: In the α-helix CO of the one amino acid residue forms H-bond with NH of the forth one.

Supersecondary structure or Motifs :

occurs by combining secondary structure.

The combination may be: α-helix- turn- α-helix- turn…..etc

Or: β-sheet -turn- β-sheet-turn……etc

Or: α-helix- turn- β-sheet-turn- α-helix

Turn (or bend): is short segment of polypeptides (3-4 amino acids) that connects successive secondary structures.

e.g. β-turn: is small polypeptide that connects successive strands of β-sheets.

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Ramachandran plots

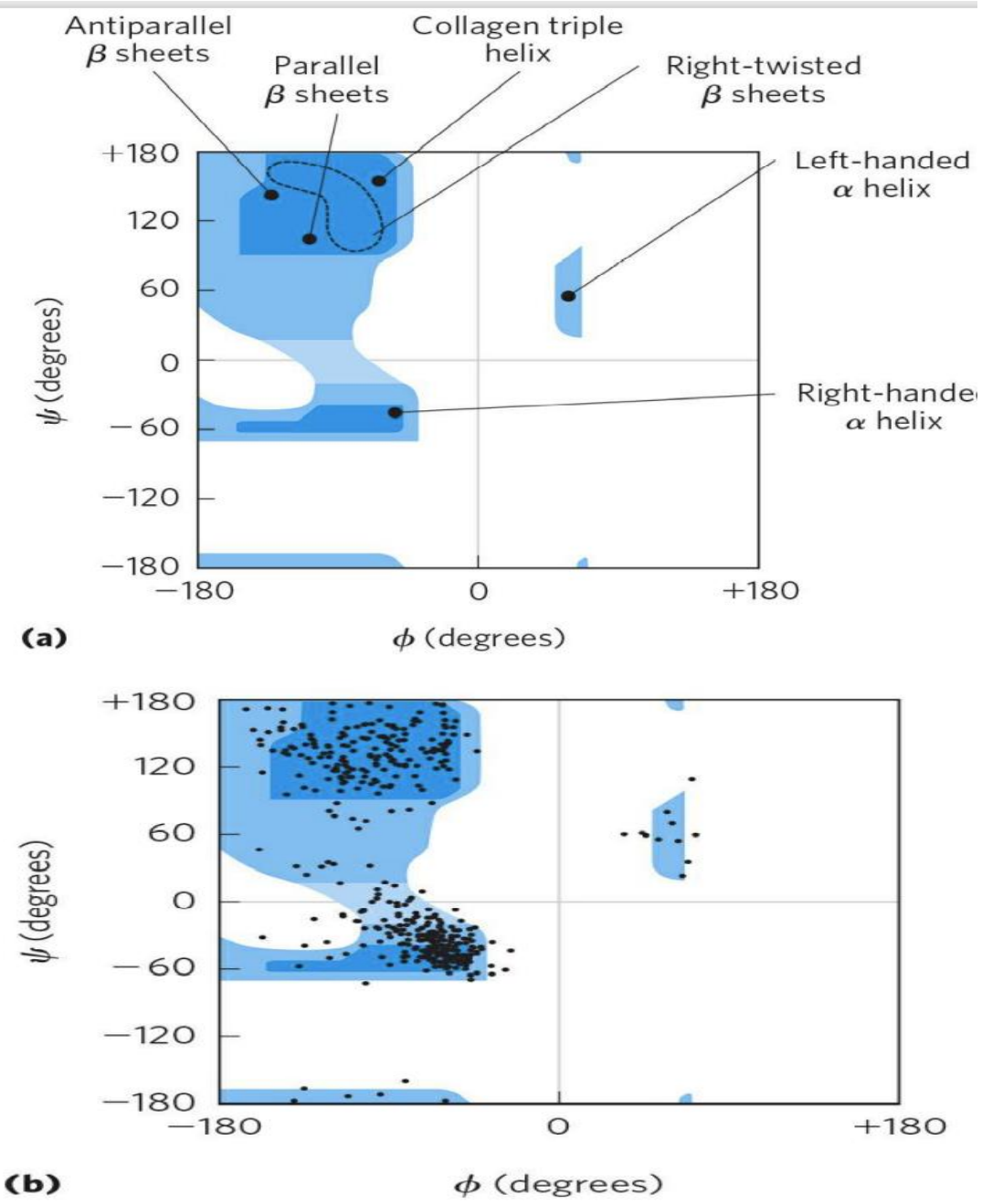
The Ramachandran plot is a plot of the torsional angles - phi (φ)and psi (ψ) - of the residues (amino acids) contained in a peptide.

TABLE 4-1 Idealized ϕ and ψ Angles for Common Secondary Structures in Proteins

Structure	ϕ	ψ
α Helix	−57°	−47°
β Conformation		
Antiparallel	−139°	+135°
Parallel	−119°	+113°
Collagen triple helix	−51°	+153°
β Turn type I		
i + 1[a]	−60°	−30°
i + 2[a]	−90°	0°
β Turn type II		
i + 1	−60°	+120°
i + 2	+80°	0°

Note: In real proteins, dihedral angles often vary somewhat from these idealized values.

[a] The i+1 and i+2 angles are those for the second and third amino acid residues in the β turn, respectively.



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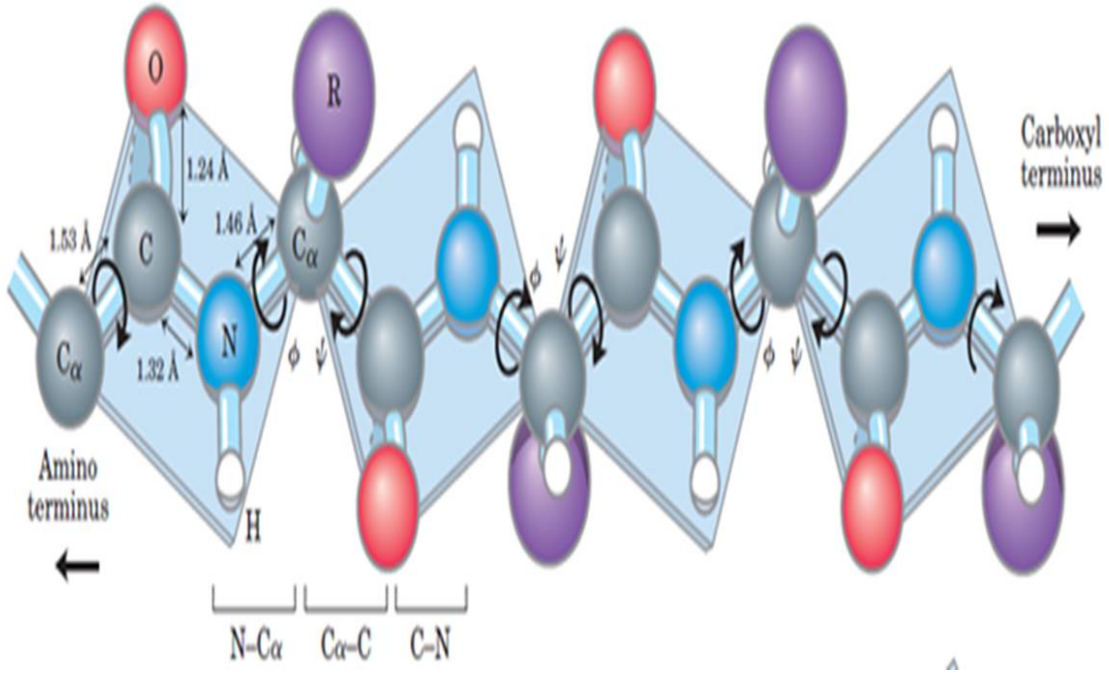
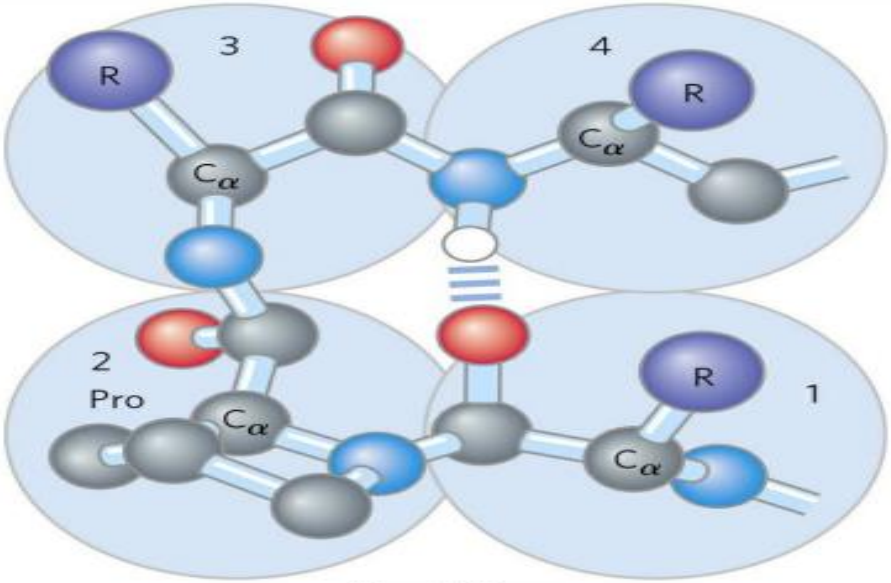
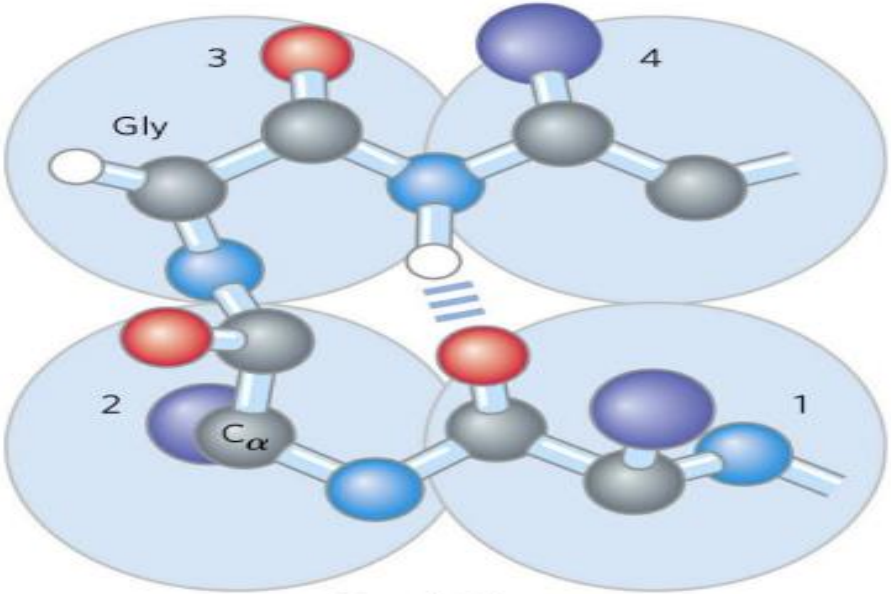
Structures of β turns

The only amino acid residue often found in a conformation outside these regions is glycine. Because its side chain is small, a Gly residue can take part in many conformations that are sterically forbidden for other amino acids.





Type I β turn



Type II β turn

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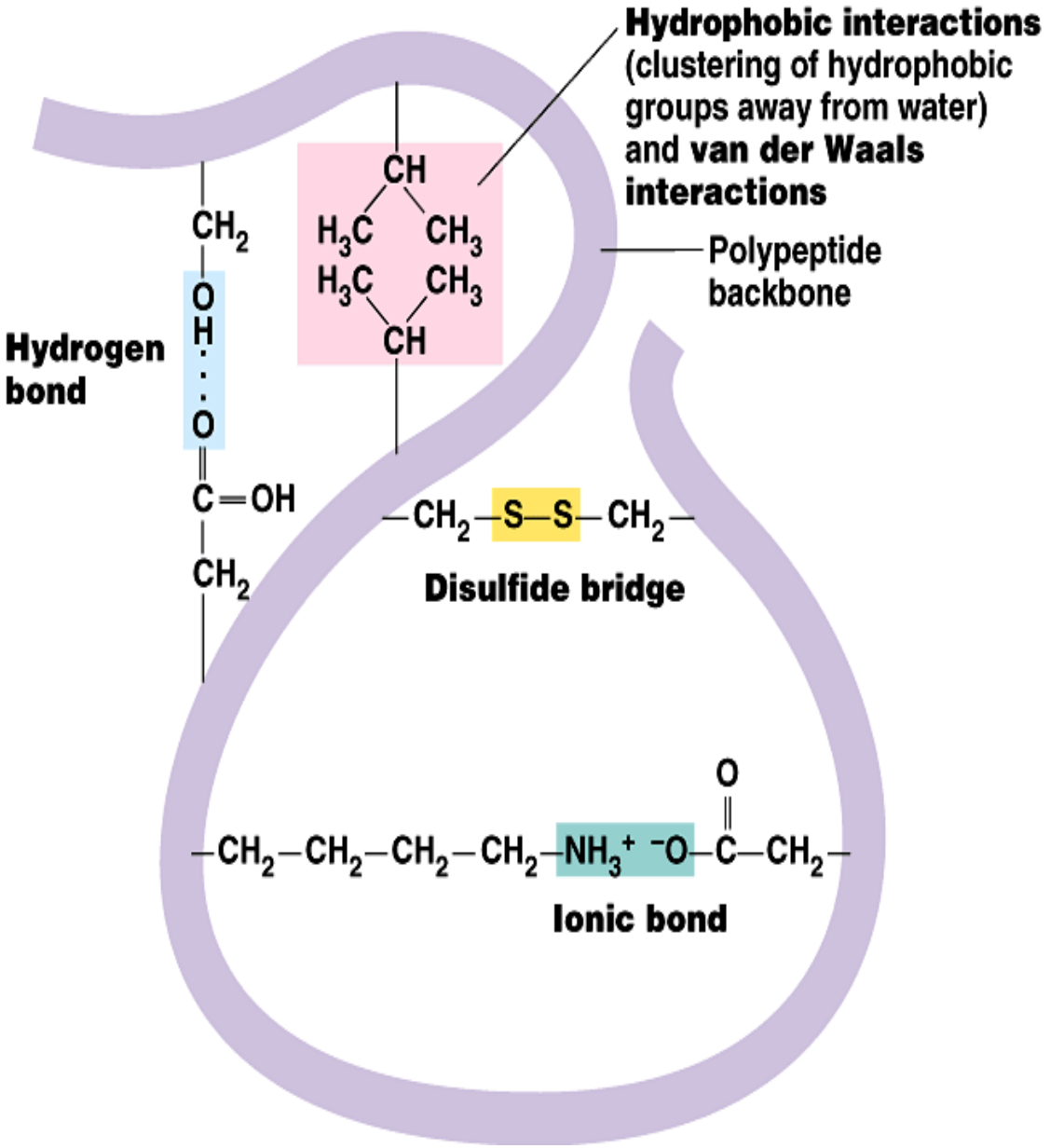
- **Tertiary structure** is determined by a variety of interactions (bond formation) among R groups and between R groups and the polypeptide backbone.
  a. **The weak interactions** include:
  - **Hydrogen bonds** among polar side chains
  - **Ionic bonds** between charged R groups (basic and acidic amino acids)
  - **Hydrophobic interactions** among hydrophobic (non polar) R groups.



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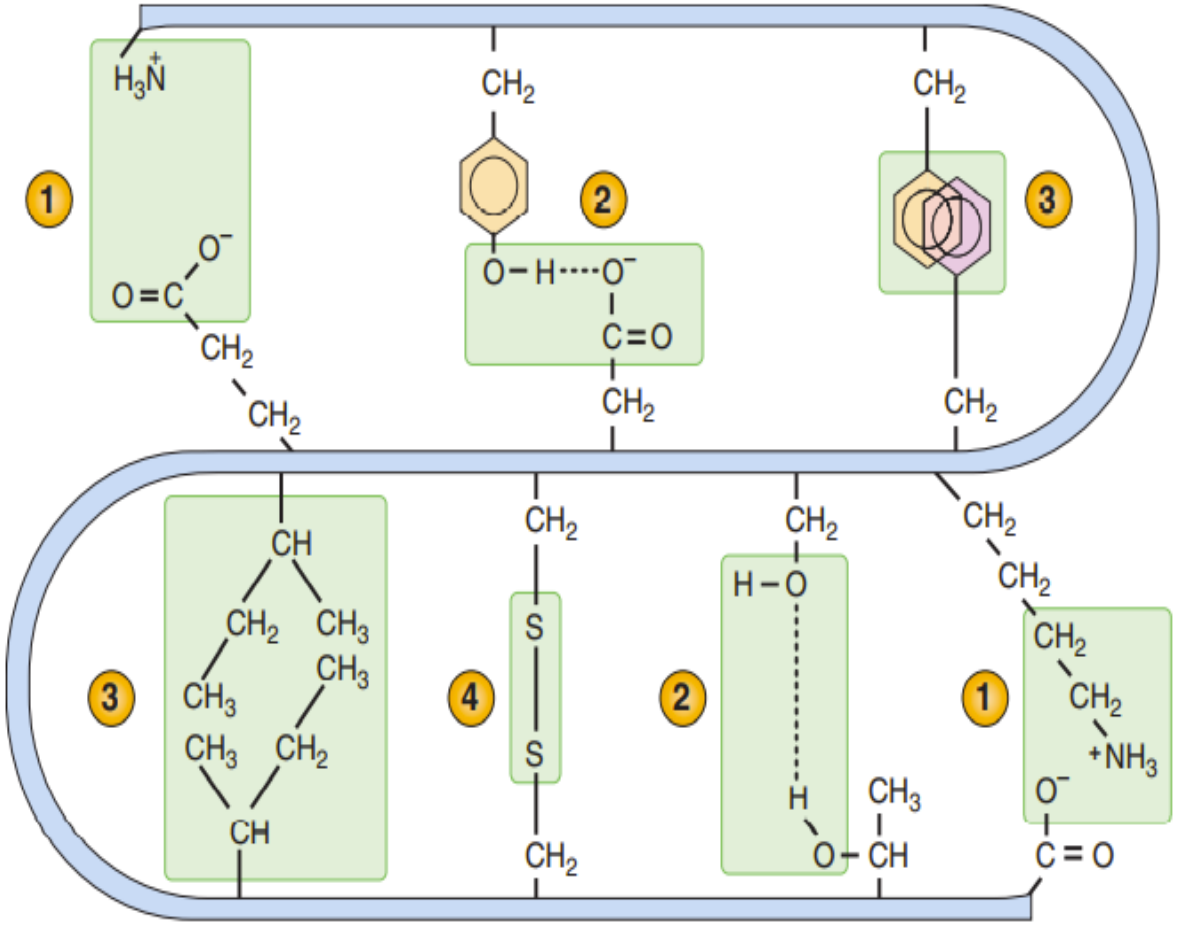
b. Strong covalent bonds include **disulfide bridges**, that form between the sulfhydryl groups (SH) of cysteine monomers, stabilize the structure.



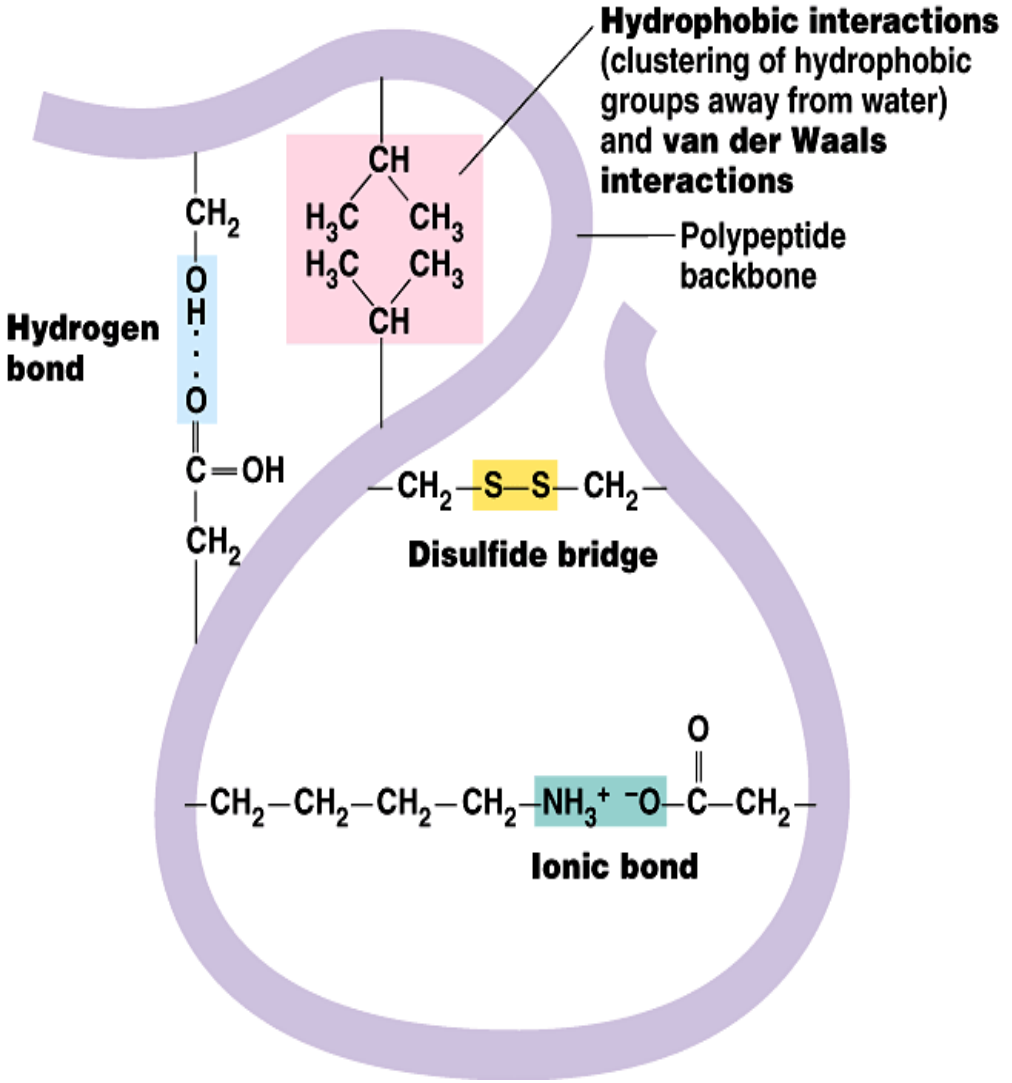


FIGURE 2.10. **Interactions between amino acid residues in a polypeptide chain.** ❶ Electrostatic interactions; ❷ hydrogen bonds; ❸ hydrophobic interactions; ❹ a disulfide bond.

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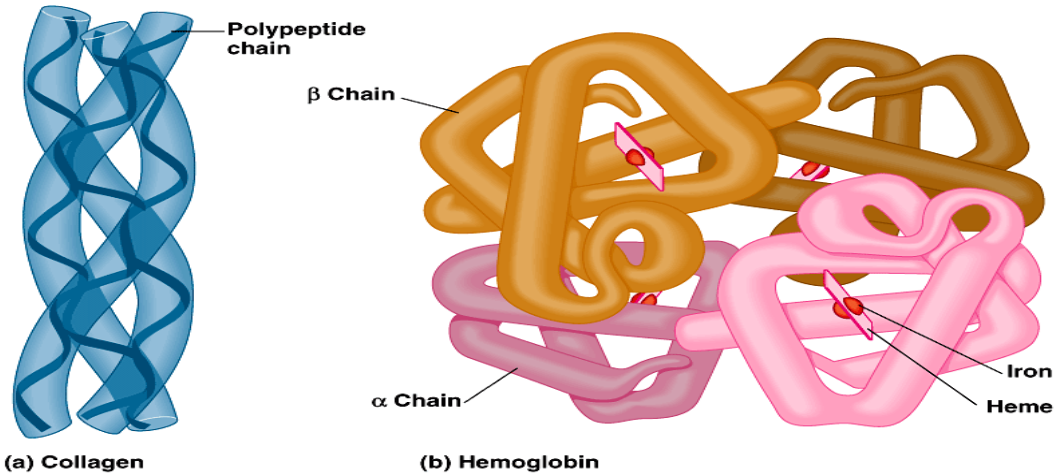
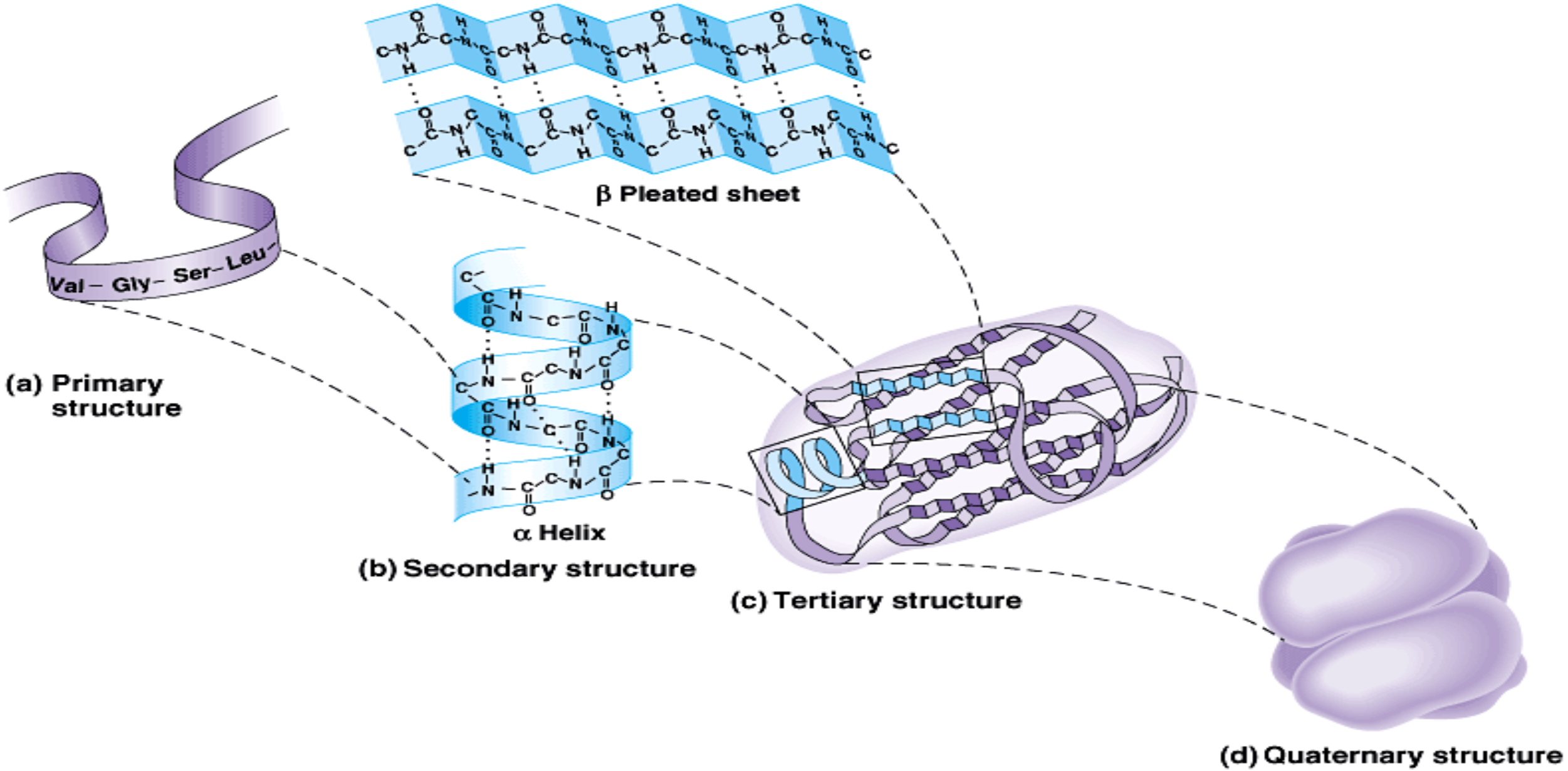
- **Quaternary structure:** results from the aggregation (combination) of two or more polypeptide subunits held together by non-covalent interaction like H-bonds, ionic or hydrophobic interactions.
- Examples on protein having quaternary structure:
  - **Collagen** is a fibrous protein of three polypeptides (trimeric) that are supercoiled like a rope.
  - This provides the structural strength for their role in connective tissue.
  - **Hemoglobin** is a globular protein with four polypeptide chains (tetrameric)
  - **Insulin** : two polypeptide chains (dimeric)




β Pleated sheet
Val – Gly – Ser – Leu –
(a) Primary structure
α Helix
(b) Secondary structure
(c) Tertiary structure
(d) Quaternary structure

Classification of proteins

I- Simple proteins:

i.e. on hydrolysis gives only amino acids

Examples:

1- Albumin and globulins**:** present in egg, milk and blood

They are proteins of high biological value i.e. contain all essential amino acids and easily digested.

2-Types of globulins:

α1 globulin: e.g. antitrypsin

α2 globulin: e.g. hepatoglobin: protein that binds hemoglobin to prevent its excretion by the kidney

β-globulin: e.g. transferrin: protein that transport iron

γ-globulins = **Immunoglobulins** (antibodies) : responsible for immunity.

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2- Globins (Histones): They are basic proteins rich in histidine amino acid.

They are present in : a - combined with DNA

b - combined with heme to form hemoglobin of RBCs.

3- Gliadines are the proteins present in cereals.

4- Scleroproteins: They are structural proteins, not digested.

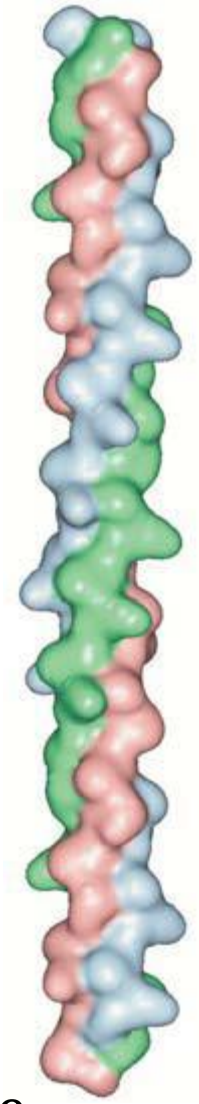
include: keratin, collagen and elastin.

a- α-keratin: protein found in hair, nails, enamel of teeth and outer layer of skin.

- It is α-helical polypeptide chain, rich in cysteine and hydrophobic (non polar) amino acids so it is water insoluble.

b- collagens: protein of connective tissues found in bone, teeth, cartilage, tendons, skin and blood vessels.

- Collagen may be present as gel e.g. in extracellular matrix.
- Collagens are the most important protein in mammals. They form about 30% of total body proteins.
- There are more than 20 types of collagens, the most common type is **collagen** which constitutes about 90% of cell collagens.
- **Structure of collagen:** three helical polypeptide chains (trimeric) twisted around each other forming triplet-helix molecule.
- ⅓ of structure is glycine, 10% proline, 10% hydroxyproline and 1% hydroxylysine. Glycine is found in every third position of the chain. The repeating sequence –Gly-X-Y-, where X is frequently proline and Y is often hydroxyproline and can be hydroxylysine.



Structure of collagen

a. Collagen consists of three chains that wind around each other to form a triple helix.

b. Collagen contains approximately 1,000 amino acids, one-third of which are glycine. The sequence Gly-X-Y frequently occurs, in which X is often proline and Y is hydroxyproline or hydroxylysine.

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Synthesis of collagen:

a. The polypeptide chains of preprocollagen are synthesized on the rough endoplasmic reticulum, and the signal (pre) sequence is cleaved.
b. Proline and lysine residues are hydroxylated by a reaction that requires O2 and vitamin C.
c. Galactose and glucose are added to hydroxylysine residues.
d. The triple helix forms, procollagen is secreted from the cell, and cleaved to form collagen.
e. Cross-links are produced. The side chains of lysine and hydroxylysine residues are oxidized to form aldehydes, which can undergo aldol condensation or form Schiff bases with the amino groups of lysine residues.

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Solubility: collagen is insoluble in all solvents and not digested.
When collagen is heated with water or dil. HCl it will be converted into gelatin which is soluble , digestible and used as diet (as jelly). Gelatin is classified as derived protein.

Some collagen diseases:

1- Scurvy: disease due to deficiency of vitamin C which is important coenzyme for conversion of proline into hydroxyproline and lysine into hydroxylysine. Thus, synthesis of collagen is decreased leading to abnormal bone development, bleeding, loosing of teeth and swollen gum.
2- Osteogenesis Imperfecta (OI): Inherited disease resulting from genetic deficiency or mutation in gene that synthesizes collagen type I leading to abnormal bone formation in babies and frequent bone fracture in children.

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C- Elastin: present in walls of large blood vessels (such as aorta). It is very important in lungs, elastic ligaments, skin, cartilage, ..

It is elastic fiber that can be stretched to several times as its normal length.

Structure: composed of 4 polypeptide chains (tetramer), similar to collagen being having 33% glycine and rich in proline but in that it has low hydroxyproline and absence of hydroxy lysine. is a chronic obstructive lung disease (obstruction of air ways) resulting from deficiency of α1-antitrypsin particularly in cigarette smokers.

Role of α1-antitrypsin: Elastin is a lung protein. Smoke stimulate enzyme called elastase to be secreted form neutrophils (in lung). Elastase cause destruction of elastin of lung.

α1-antitrypsin is an enzyme (secreted from liver) and inhibit elastase and prevent destruction of elastin. So deficiency of α1-antitrypsin especially in smokers leads to degradation of lung and destruction of lung (loss of elasticity of lung, a disease called emphysema.

Conjugated proteins

i.e. On hydrolysis, give protein part and non protein part and subclassified into:

1- Phosphoproteins: These are proteins conjugated with phosphate group. Phosphorus is attached to oh group of serine or threonine.
e.g. Casein of milk.

2- Lipoproteins:

These are proteins conjugated with lipids.

Functions: a- help lipids to transport in blood

b- Enter in cell membrane structure helping lipid soluble substances to pass through cell membranes.

3- Glycoproteins:

proteins conjugated with sugar (carbohydrate)

e.g. – Mucin

- Some hormones such as erythropoeitin
- present in cell membrane structure
- blood groups.

4- Nucleoproteins: These are basic proteins (e.g. histones) conjugated with nucleic acid (DNA or RNA).

e.g. a- chromosomes: are proteins conjugated with DNA

b- Ribosomes: are proteins conjugated with RNA

5- Metalloproteins: These are proteins conjugated with metal like iron, copper, zinc, ……

a- Iron-containing proteins: Iron may present in heme such as in

- hemoglobin (Hb)
- myoglobin (protein of skeletal muscles and cardiacmuscle),
- cytochromes,
- catalase, peroxidases (destroy H2O2)
- tryptophan pyrrolase (desrtroy indole ring of tryptophan).

Iron may be present in free state (not in heme) as in:

- Ferritin: Main store of iron in the body. ferritin is present in liver, spleen and bone marrow.
- Hemosidrin: another iron store.
- Transferrin: is the iron carrier protein in plasma.

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b- Copper containing proteins:

e.g. - Ceruloplasmin which oxidizes ferrous ions into ferric ions.

- Oxidase enzymes such as cytochrome oxidase.

c- Zn containing proteins: e.g. Insulin and carbonic anhydrase

d- Mg containing proteins:e.g. Kinases and phosphatases.

6-Chromoproteins: These are proteins conjugated with pigment. e.g.

- All proteins containing heme (Hb, myoglobin, ……..)
- Melanoprotein:e.g proteins of hair or iris which contain melanin.

Derived proteins

Produced from hydrolysis of simple proteins.

e.g. - Gelatin: from hydrolysis of collagen

- Peptone: from hydrolysis of albumin

Table 3.4
Classes of proteins based on their biological functions

Protein	Biological Function
	Enzymes
Phosphofructokinase	An enzyme in carbohydrate metabolism that catalyzes phosphate group transfer from ATP to fructose-6-phosphate
Trypsin	A digestive enzyme in vertebrates that catalyzes protein hydrolysis
Adenylate cyclase	An enzyme that catalyzes the formation of the second messenger cyclic AMP
RNA polymerase	An enzyme present in all organisms that catalyzes DNA-directed RNA synthesis
Reverse transcriptase	An enzyme found in HIV (the virus that causes AIDS) that catalyzes RNA-directed DNA synthesis
	Structural Proteins
Collagens	Fibrous proteins found in all animals; form cable networks that serve as scaffolding for support of tissues and organs
Elastins	Fibrous proteins found in connective tissue of the lungs and in large blood vessels such as the aorta, which have rubberlike properties that allow them to stretch to several times their normal length
Keratins	Mechanically durable fibrous proteins present in vertebrates as major components of the outer epidermal layer and its appendages such as hair, nails, and feathers
	Defense (Immune) Proteins
Antibodies	Globular proteins produced by the immune system of higher animals that participate in the destruction of biological invaders
Interferons	Proteins, produced by higher animals, that interfere with viral replication
	Transport and Storage Proteins
Hemoglobin	Globular heme-containing protein that carries oxygen from the lungs to other tissues of vertebrates
Apolipoproteins	Components of lipoproteins such as low density lipoprotein (LDL) that participate in triacylglycerol and cholesterol transport
Casein	Protein found in milk that stores amino acids
Ferritin	Widely distributed protein that stores iron
Myoglobin	A heme-containing protein found in vertebrates that binds oxygen
Glucose permease	Carries glucose into erythrocytes
	Regulatory and Receptor Proteins
Lac repressor	Genetic switch that turns off bacterial genes involved in lactose catabolism
Insulin	A protein synthesized in the pancreas that acts as a signal for the fed state in higher animals
Insulin receptor	A membrane protein that binds insulin and sends a message inside the cell to regulate carbohydrate metabolism
	Muscle Contraction and Mobility Proteins
Actin	Component of skeletal muscle
Myosin	Component of skeletal muscle
Dynein	Protein that causes movement of sperm and protozoa by their flagella and cilia

Denaturation and renaturation:

a. Proteins can be denatured by agents such as heat and urea that cause unfolding of polypeptide chains without causing hydrolysis of peptide bonds.

b. The denaturing agents destroy secondary and tertiary structures, without affecting the primary structure.

c. If a denatured protein returns to its native state after the denaturing agent is removed, the process is called renaturation.

Hemoglobin:

1. Structure of hemoglobin

Adult hemoglobin (HbA) consists of four polypeptide chains (two A chains and two B chains), each containing a molecule of heme.

- The ` chains and a chains of HbA are similar in three-dimensional configuration to each other and to the single chain of muscle myoglobin although their amino acid sequences differ.

- Eight regions of `α-helix occur in each chain, labeled A through H.
- Heme fits into a crevice in each globin chain and interacts with two histidine residue

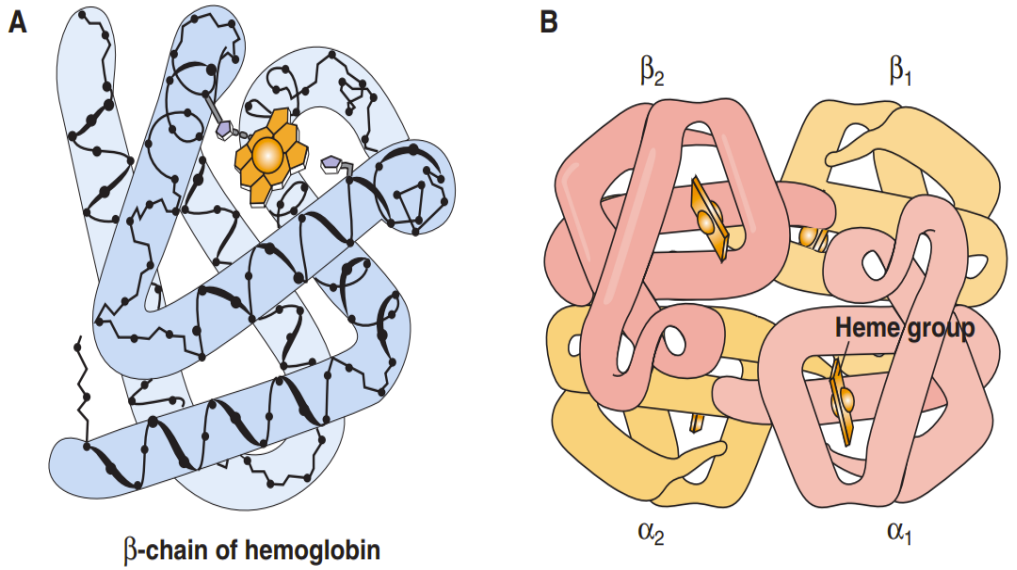
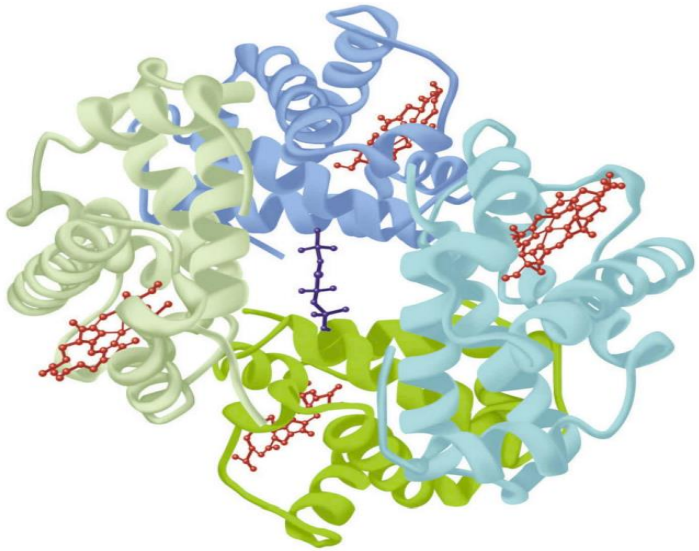


FIGURE 2.12. The structure of the β chain of hemoglobin (panel A) and hemoglobin (panel B). Cylindrical regions contain α-helices. The planar structure near the top center of the polypeptide chain is heme. (From: Ferscht A. *Structure and Mechanism in Protein Science.* New York, NY: W.H. Freeman and Company; 1999, with permission.)



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Function of hemoglobin:

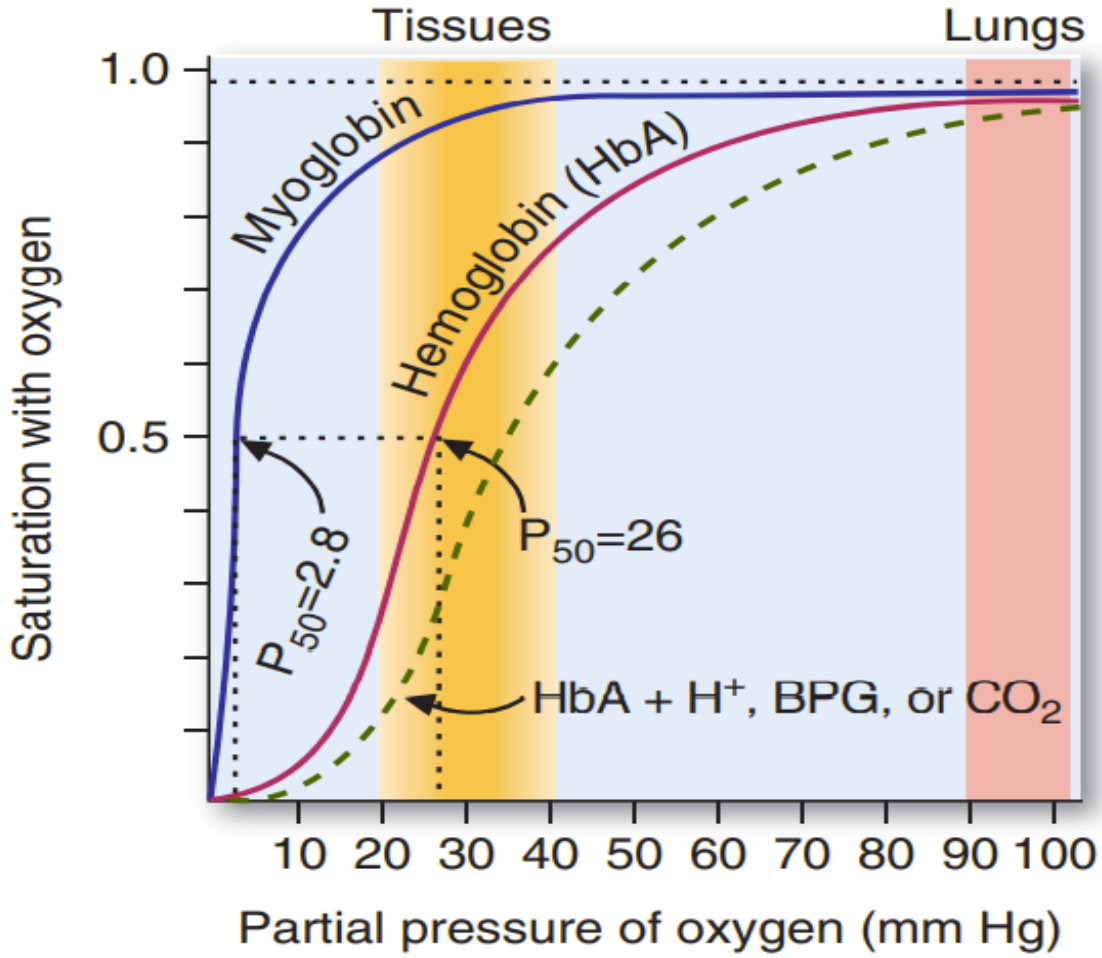
Hemoglobin, found in the red blood cells, carries oxygen from the lungs to the tissues, and returns carbon dioxide and protons from the tissues to the lungs.

A. The oxygen saturation curve for hemoglobin is sigmoidal .

(1) Each heme binds one O2 molecule, for a total of four O2 molecules per HbA molecule.

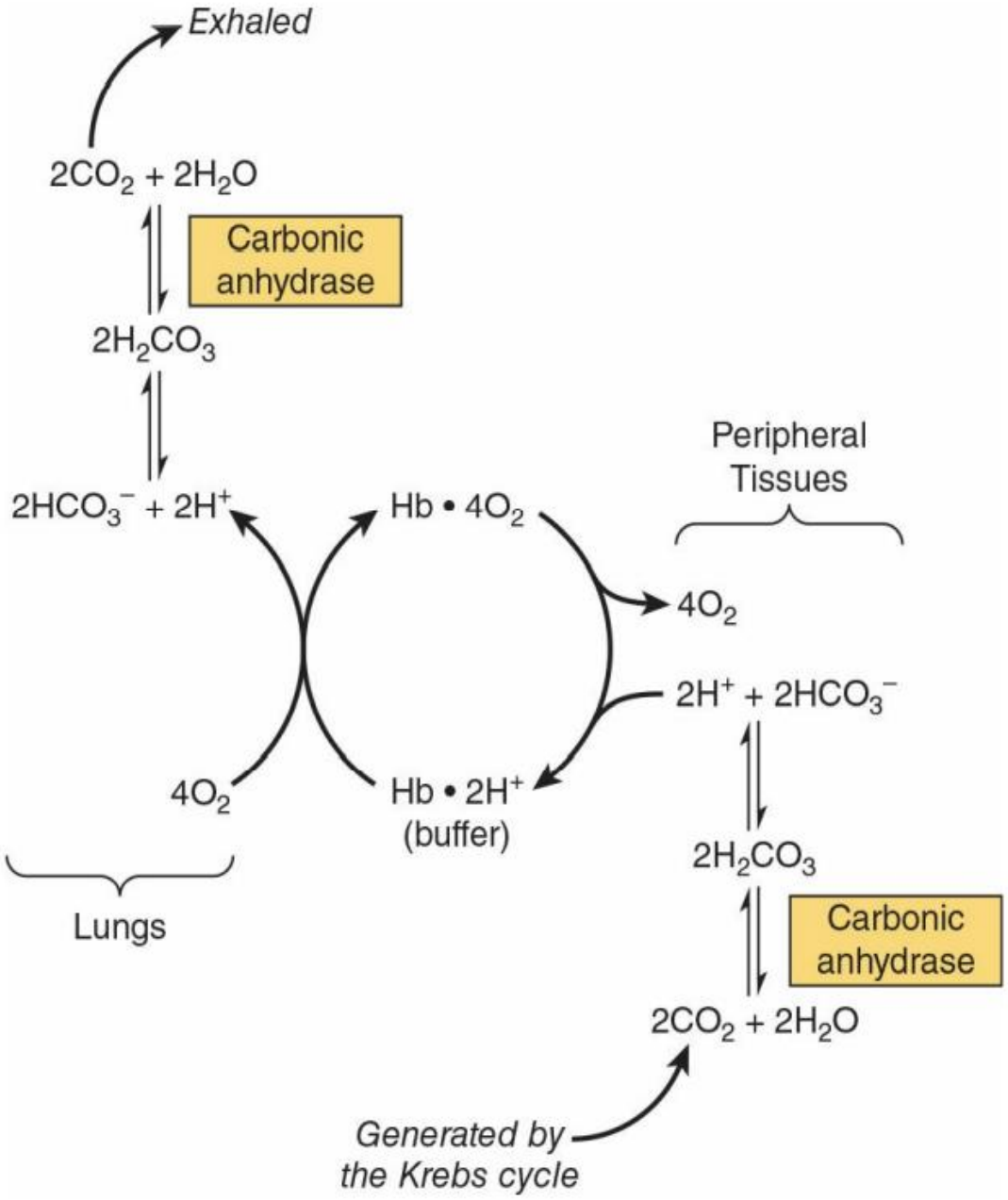
HbA changes from the tense (T) form to the relaxed (R) form when oxygen binds.

(2) Binding of O2 to one heme group in hemoglobin increases the affinity for O2 of its other heme groups.



Oxygen saturation curves for myoglobin and adult hemoglobin (HbA). Myoglobin has a hyperbolic saturation curve. HbA has a sigmoidal curve. The HbA curve shifts to the right at lower pH, with higher concentrations of BPG, or as CO2 binds to HbA in the tissues. Under these conditions, O2 is released more readily. P50 is the partial pressure of O2 at which half-saturation with O2 occurs.

The Bohr effect is a phenomenon first described in 1904 by the Danish physiologist Christian Bohr. Hemoglobin's oxygen binding affinity (see oxygen–haemoglobin dissociation curve) is inversely related both to acidity and to the concentration of carbon dioxide.That is, the Bohr effect refers to the shift in the oxygen dissociation curve caused by changes in the concentration of carbon dioxide or the pH of the environment.

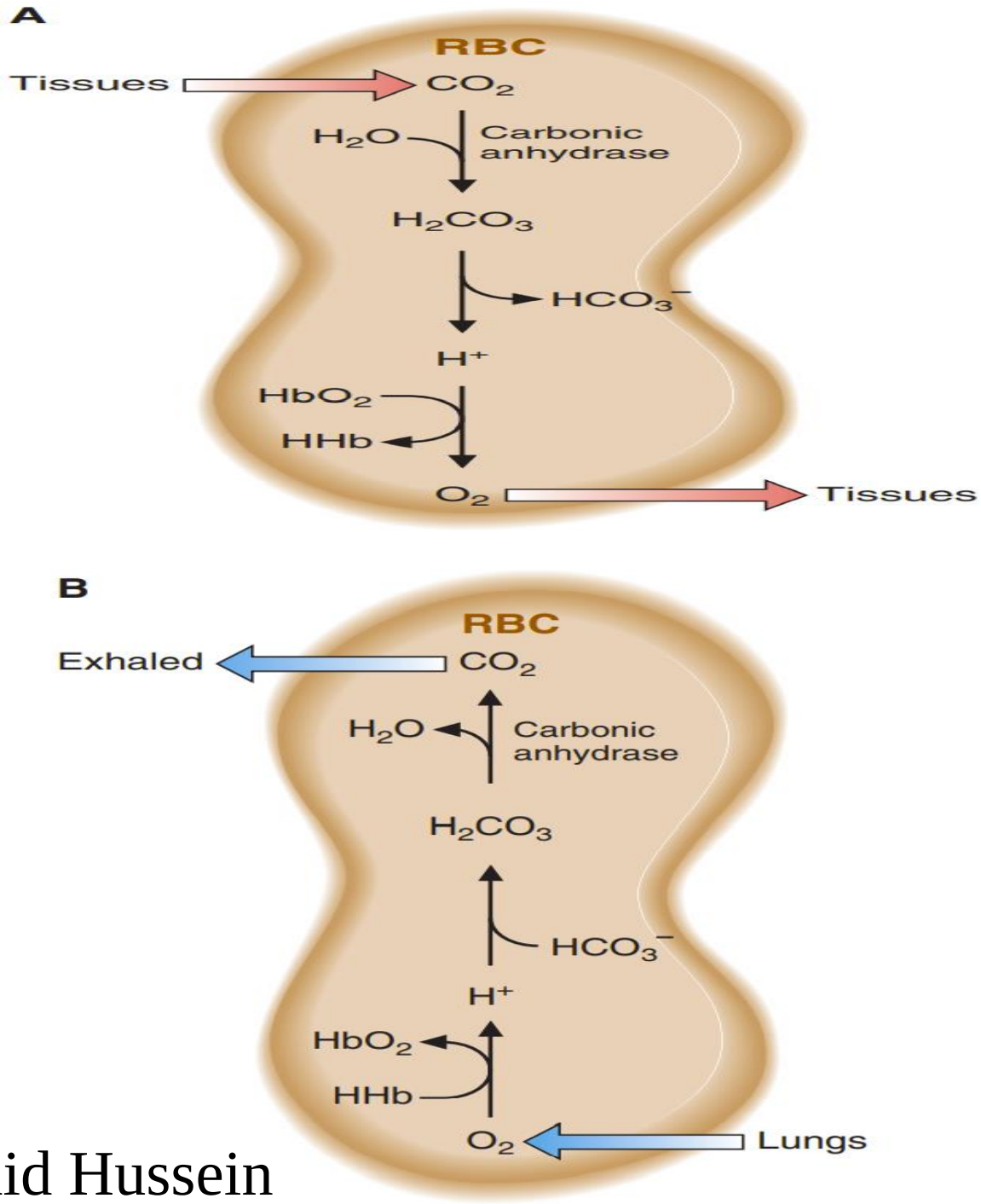


B. The binding of protons to HbA stimulates the release of O2, a manifestation of the Bohr effect . The O2 is readily released in the tissues where [H+] is high due to the production of CO2 by metabolic processes. These reactions are reversed in the lungs. O2 binds to HbA, and CO2 is exhaled.

C. Covalent binding of CO2 to HbA in the tissues also causes the release of O2.

D. Binding of 2,3-bisphosphoglycerate (BPG), a side product of glycolysis in red blood cells, decreases the affinity of HbA for O2. Consequently, O2 is more readily released in tissues when BPG is bound to HbA .

E. Fetal hemoglobin (HbF), composed of two α subunits and and two γ subunits, has a lower affinity for BPG.



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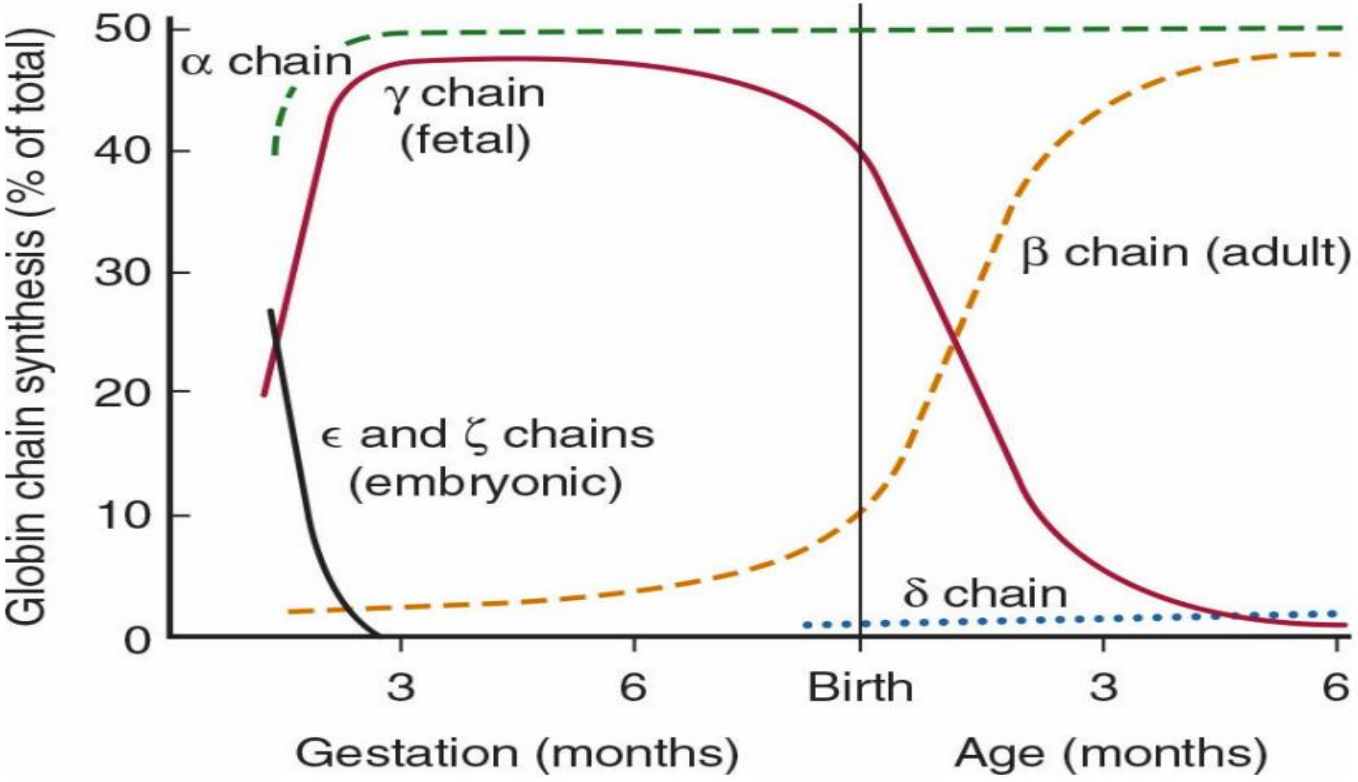
P50 Expresses the Relative Affinities of Different Hemoglobins for Oxygen

- The quantity P50 , a measure of O2 concentration, is the partial pressure of O2 at which a given hemoglobin reaches half-saturation.
- The values of P50 for HbA and HbF are 26 and 20 mm Hg, respectively.
- In the placenta, this difference enables HbF to extract oxygen from the HbA in the mother's blood.



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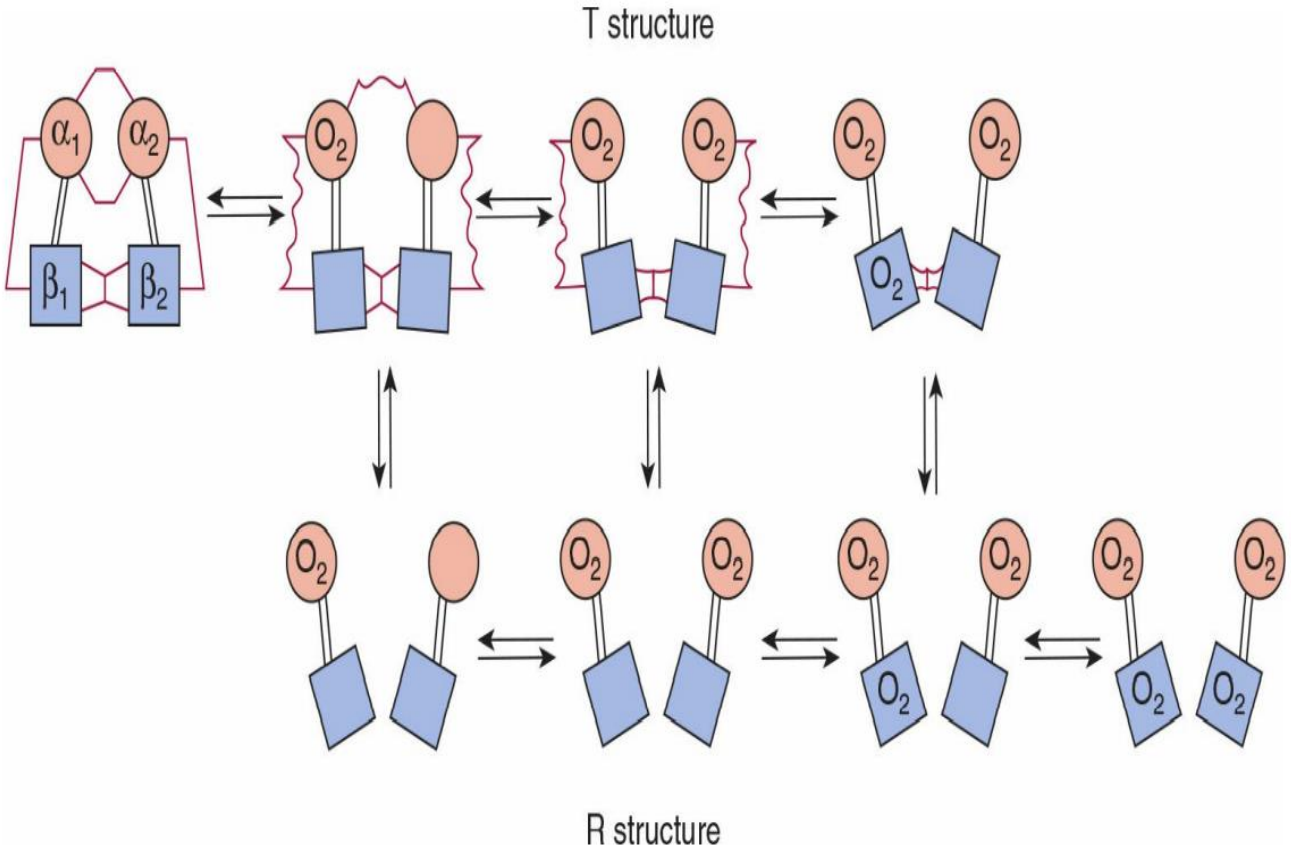
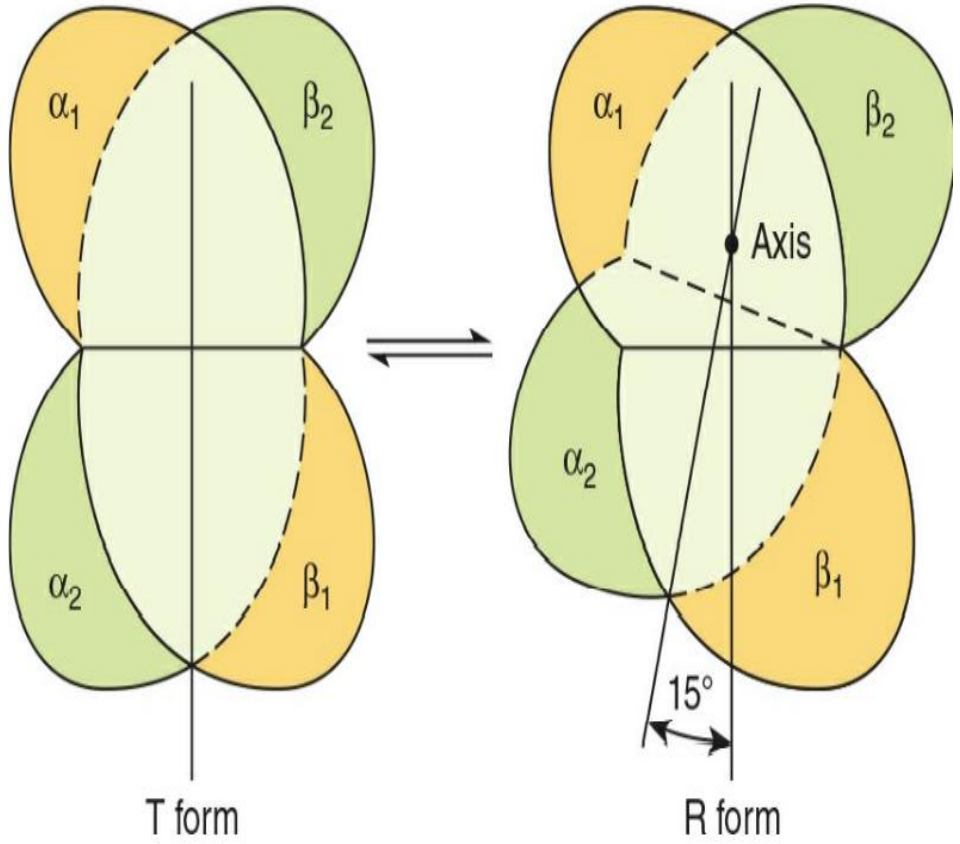
Oxygenation of Hemoglobin Is Accompanied by Large Conformational Changes

- The terms T and R also are used to refer to the low affinity and high-affinity conformations of allosteric enzymes.
- Transition from the T structure to the R structure





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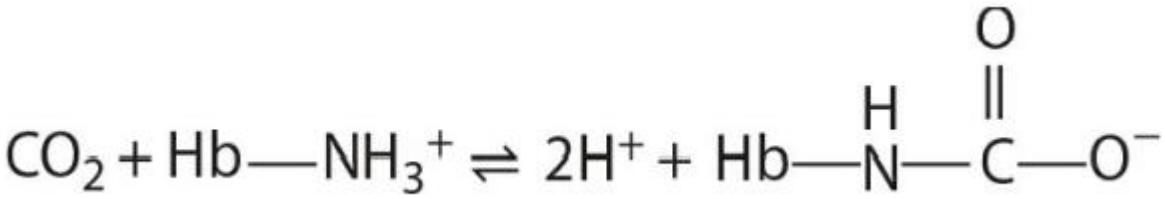
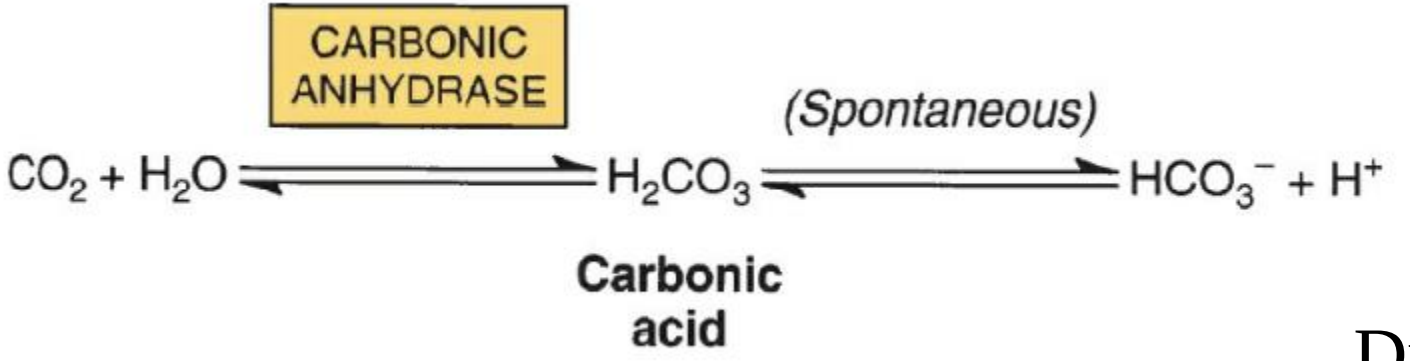
Hemoglobin Assists in the Transport of CO2 to the Lungs

- About 15% of the CO2 in venous blood is carried by hemoglobin as carbamates formed with the amino terminal nitrogens of the polypeptide chains



- The remaining CO2 is carried mostly as bicarbonate, which is formed in erythrocytes by the hydration of CO2 to carbonic acid (H2CO3), a process catalyzed by carbonic anhydrase. At the pH of venous blood, H2CO3 dissociates into bicarbonate and a proton.



BIOMEDICAL IMPLICATIONS

Myoglobinuria:

- Following massive crush injury to skeletal muscle followed by renal damage, released myoglobin may appear in the urine. Myoglobin can be detected in plasma following a myocardial infarction, but assay of serum troponin, lactate dehydrogenase isozymes, or creatine kinase provides a more sensitive index of myocardial injury.

Anemias

- Anemias, reductions in the number of red blood cells or of hemoglobin in the blood, can reflect impaired synthesis of hemoglobin or impaired production of erythrocytes (eg, in folic acid or vitamin B12 deficiency). Diagnosis of anemias begins with spectroscopic measurement of blood hemoglobin levels.

Thalassemias

- The genetic defects known as thalassemias result from the partial or total absence of one or more α or β chains of hemoglobin. Over 750 different mutations have been identified, but only three are common. Either the α chain (α thalassemias) or β chain (β thalassemias) can be affected.

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GLYCATED HEMOGLOBIN (HbA1c)

Blood glucose that enters the erythrocytes can form a covalent adduct with the ε-amino groups of lysyl residues and the N-terminal valines of hemoglobin β chains, a process referred to as glycation.

PROTEINS & PEPTIDES MUST BE PURIFIED PRIOR TO ANALYSIS

Highly purified protein is essential for the detailed examination of its physical and functional properties.
Selective precipitation exploits differences in relative solubility of individual proteins as a function of pH (isoelectric precipitation), polarity (precipitation with ethanol or acetone), or salt concentration (salting out with ammonium sulfate). Chromatographic techniques separate one protein from another based on the difference in their size (size-exclusion chromatography), charge (ion-exchange chromatography), hydrophobicity (hydrophobic interaction chromatography), or ability to bind a specific ligand (affinity chromatography).

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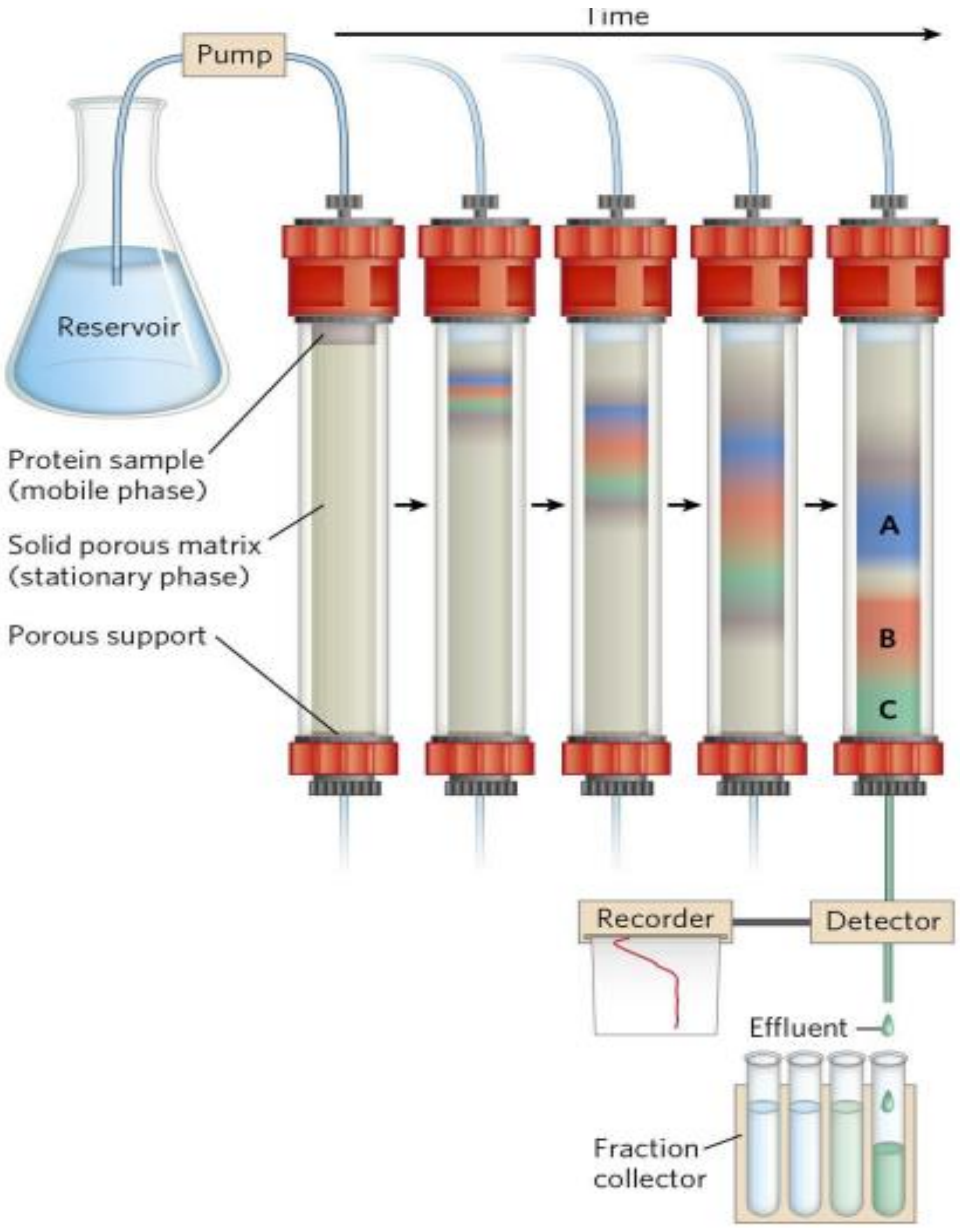
Proteins Can Be Separated and Purified

- HPLC—High-Pressure Liquid Chromatography
- Size-Exclusion Chromatography (gelfiltration)
- Ion-Exchange Chromatography
- Hydrophobic Interaction Chromatography
- Affinity Chromatography
- Protein Purity Is Assessed by Polyacrylamide Gel Electrophoresis (PAGE)
- Isoelectric Focusing (IEF)



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Column Chromatography

Chromatographic methods are typically enhanced by the use of HPLC, or high-performance liquid chromatography. HPLC makes use of high-pressure pumps that speed the movement of the protein molecules down the column, as well as higher-quality chromatographic materials that can withstand the crushing force of the pressurized flow. By reducing the transit time on the column, HPLC can limit diffusional spreading of protein bands and thus greatly improve resolution.

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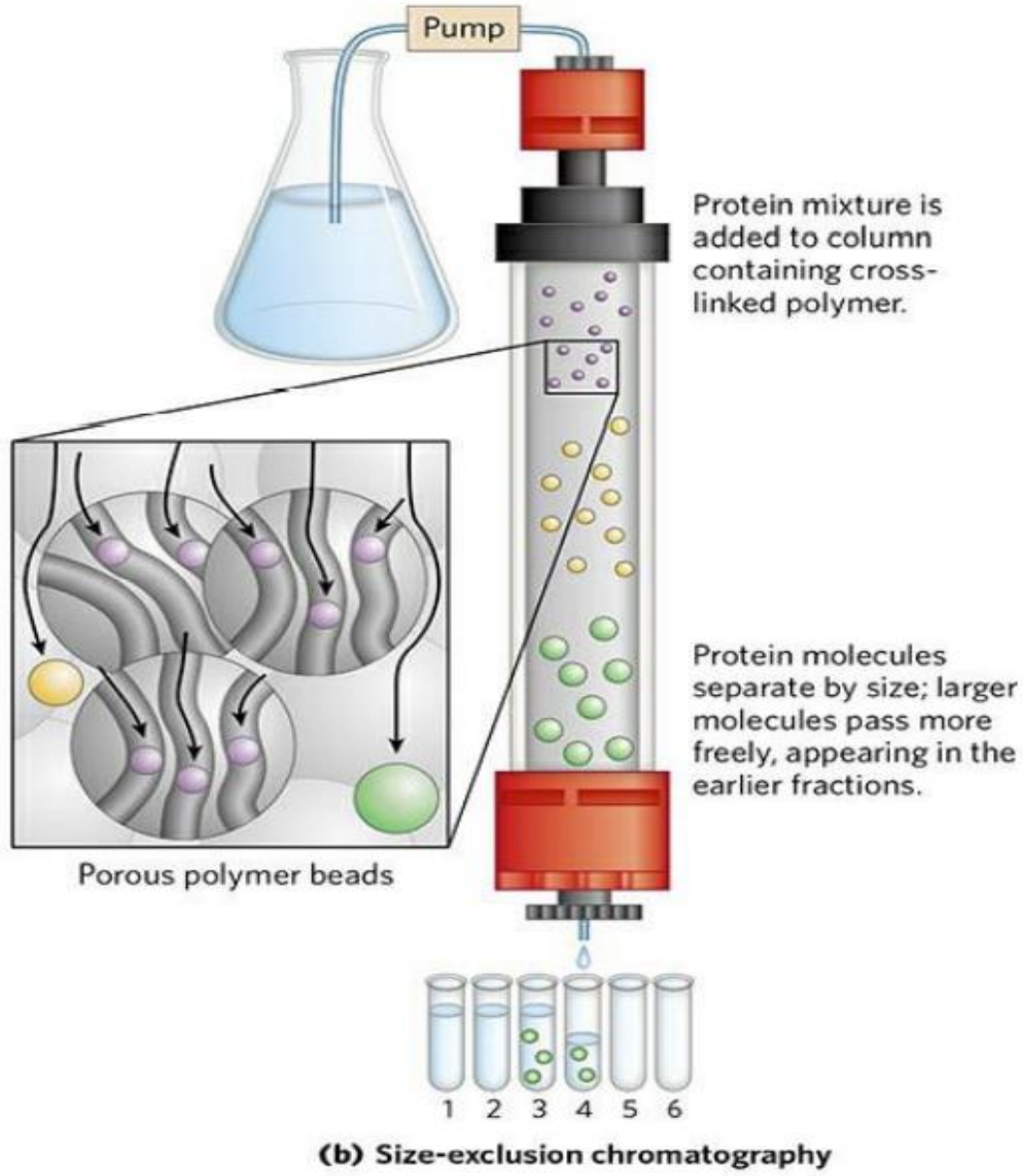
Size-Exclusion Chromatography (gel filtration)

separates proteins according to size. In this method, large proteins emerge from the column sooner than small ones—a somewhat counterintuitive result. The solid phase consists of cross-linked polymer beads with engineered pores or cavities of a particular size. Large proteins cannot enter the cavities and so take a shorter (and more rapid) path through the column, around the beads. Small proteins enter the cavities and are slowed by their more labyrinthine path through the column



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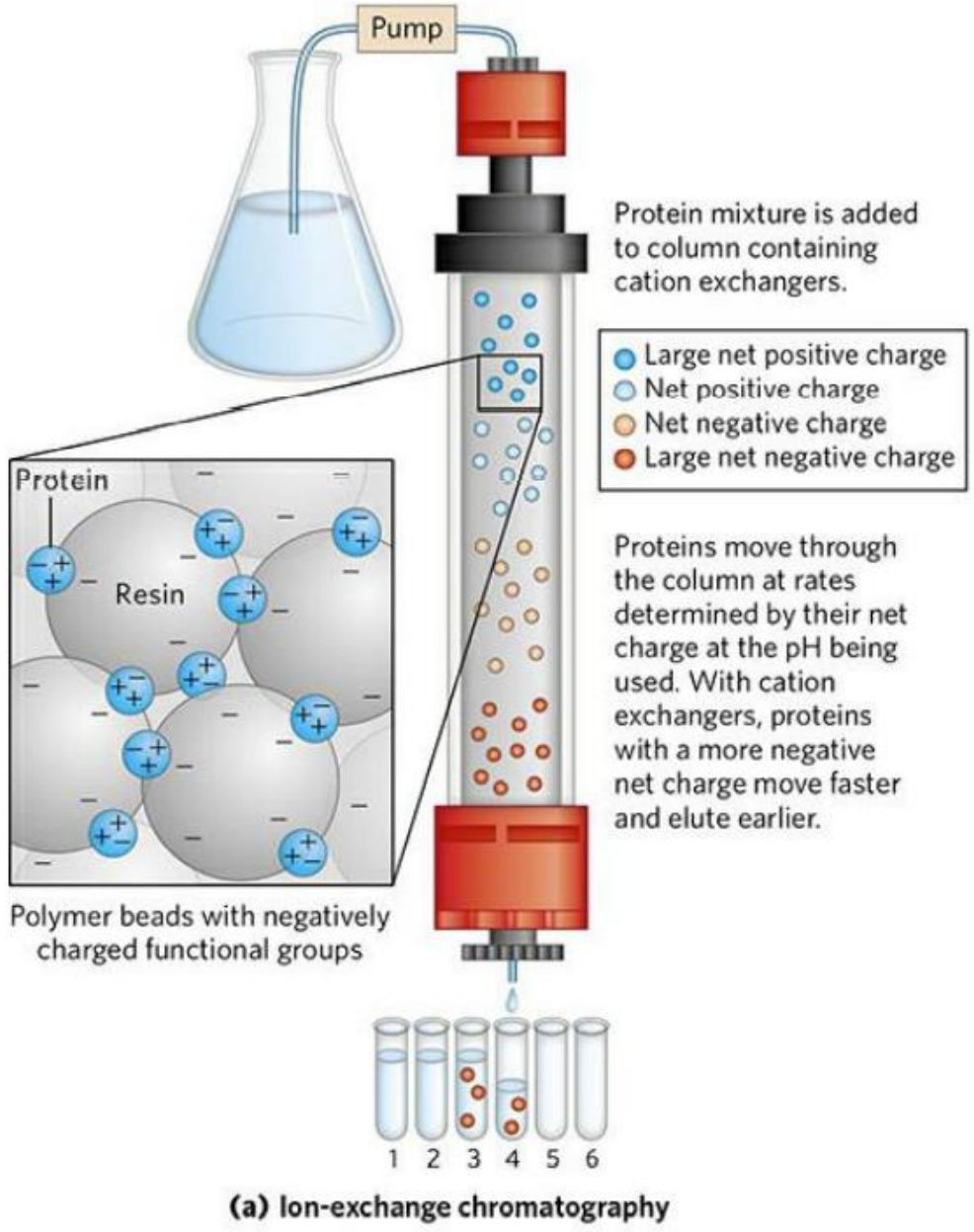
Ion-Exchange Chromatography

- Ion-exchange chromatography exploits differences in the sign and magnitude of the net electric charge of proteins at a given pH.The column matrix is a synthetic polymer (resin) containing bound charged groups; those with bound anionic groups are called cation exchangers, and those with bound cationic groups are called anion exchangers.
- Separation can be optimized by gradually changing the pH and/or salt concentration of the mobile phase so as to create a pH or salt gradient.



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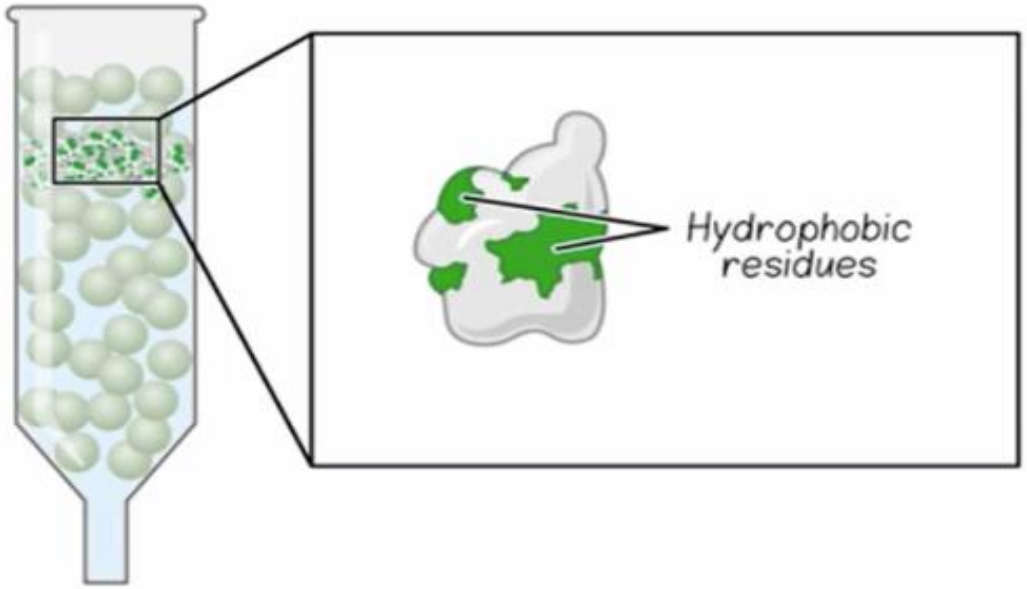
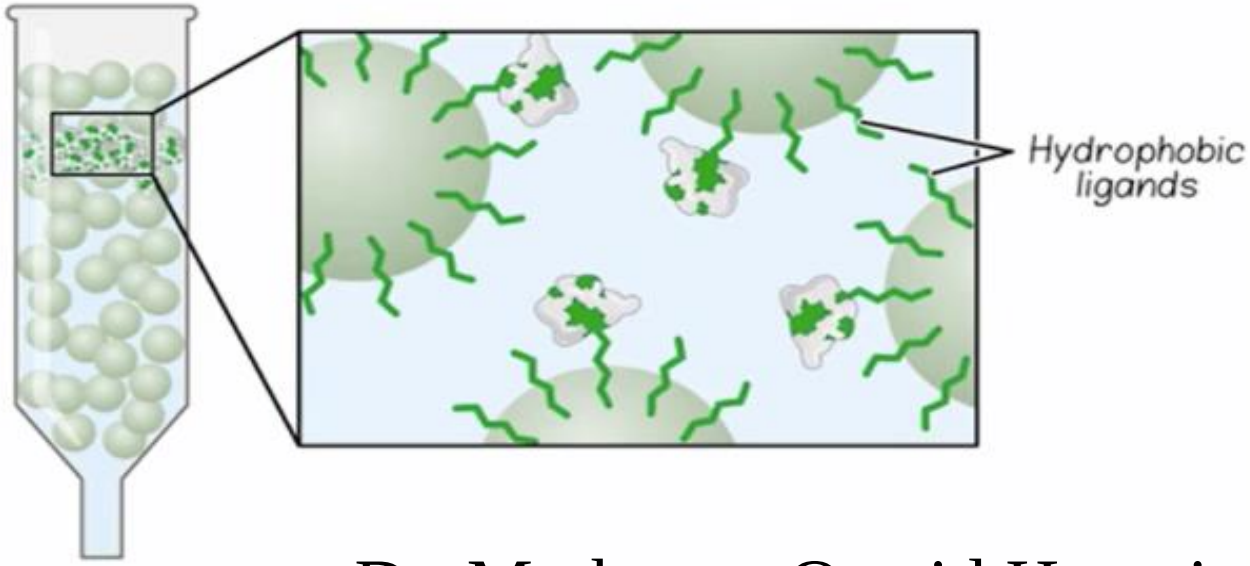
Hydrophobic Interaction Chromatography

Hydrophobic Interaction Chromatography (HIC) is a widely used technique for separation and purification of proteins and peptides. HIC sorts biomolecules by degree of their surface hydrophobicity. Samples are adsorbed to the resin at relatively high salt concentrations and eluted by applying a decreasing salt gradient. The mild conditions used in HIC separation of peptides and proteins typically maintain protein structure and biologic activity. This makes HIC a powerful tool for the process purification of biomolecules





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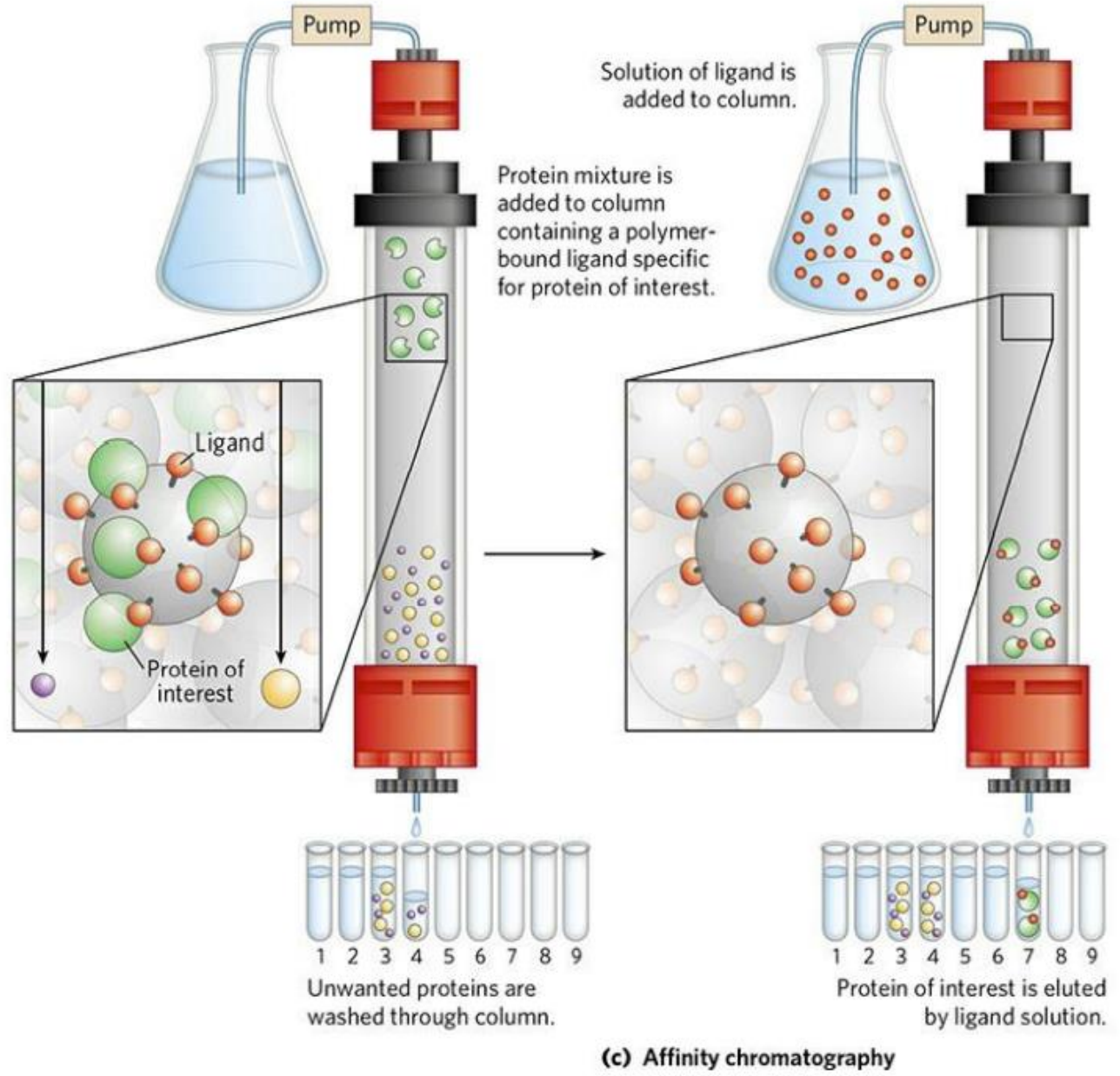
Affinity Chromatography

Affinity chromatography is based on binding affinity. The beads in the column have a covalently attached chemical group called a ligand—a group or molecule that binds to a macromolecule such as a protein. When a protein mixture is added to the column, any protein with affinity for this ligand binds to the beads, and its migration through the matrix is retarded.



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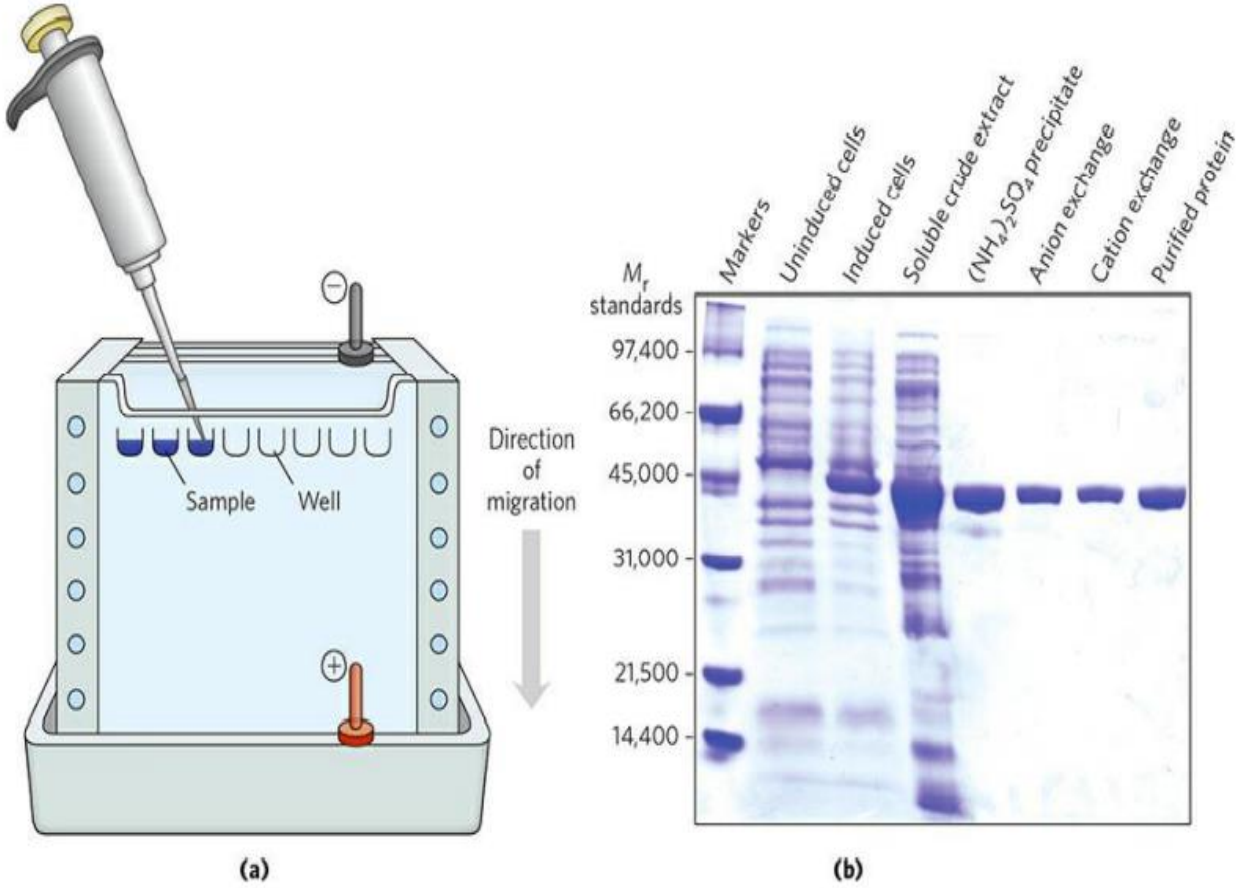
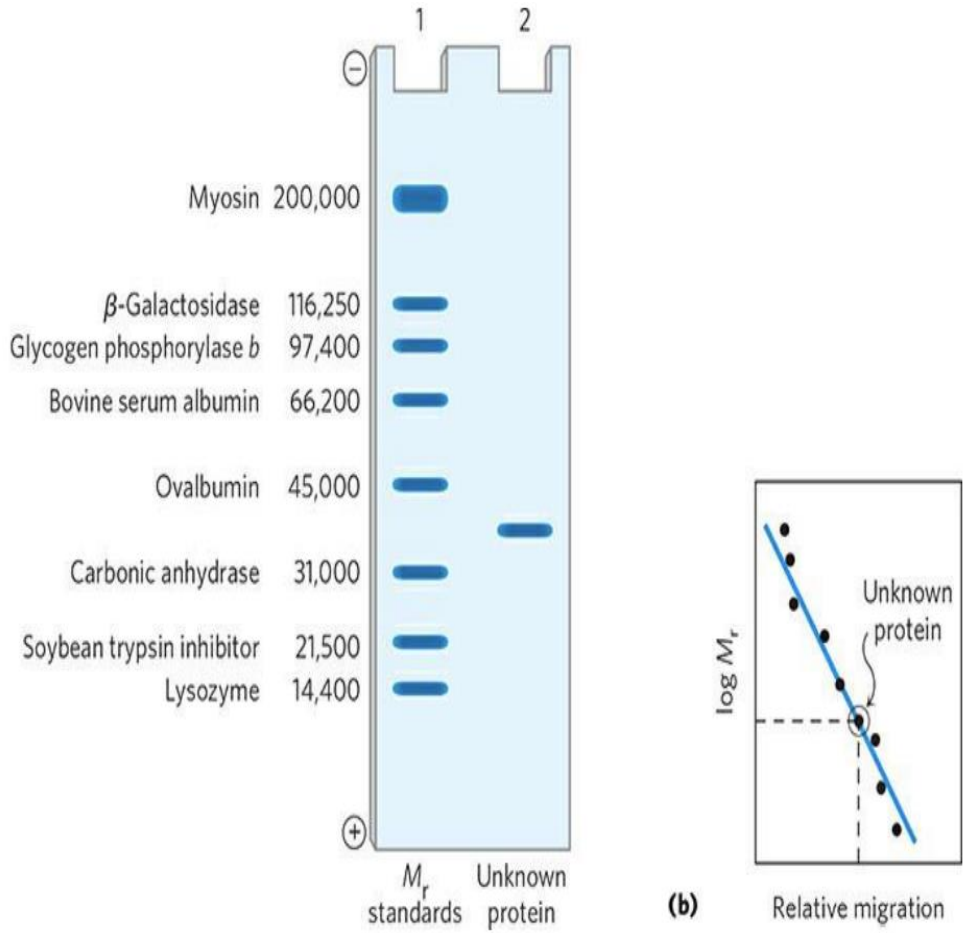
Protein Purity Is Assessed by Polyacrylamide Gel Electrophoresis (PAGE)

Another important technique for the separation of proteins is based on the migration of charged proteins in an electric field, a process called electrophoresis.





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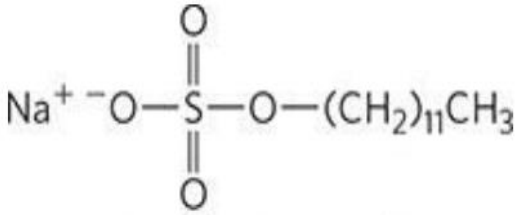
The polyacrylamide gel acts as a molecular sieve, slowing the migration of proteins approximately in proportion to their charge-to-mass ratio. Migration may also be affected by protein shape. In electrophoresis, the force moving the macromolecule is the electrical potential, E. The electrophoretic mobility, μ, of a molecule is the ratio of its velocity, V, to the electrical potential. Electrophoretic mobility is also equal to the net charge, Z, of the molecule divided by the frictional coefficient, f, which reflects in part a protein's shape. Thus:

$$\mu = \frac{V}{E} = \frac{Z}{f}$$

An electrophoretic method commonly employed for estimation of purity and molecular weight makes use of the detergent sodium dodecyl sulfate (SDS)

Electrophoresis in the presence of SDS therefore separates proteins almost exclusively on the basis of mass (molecular weight), with smaller polypeptides migrating more rapidly.

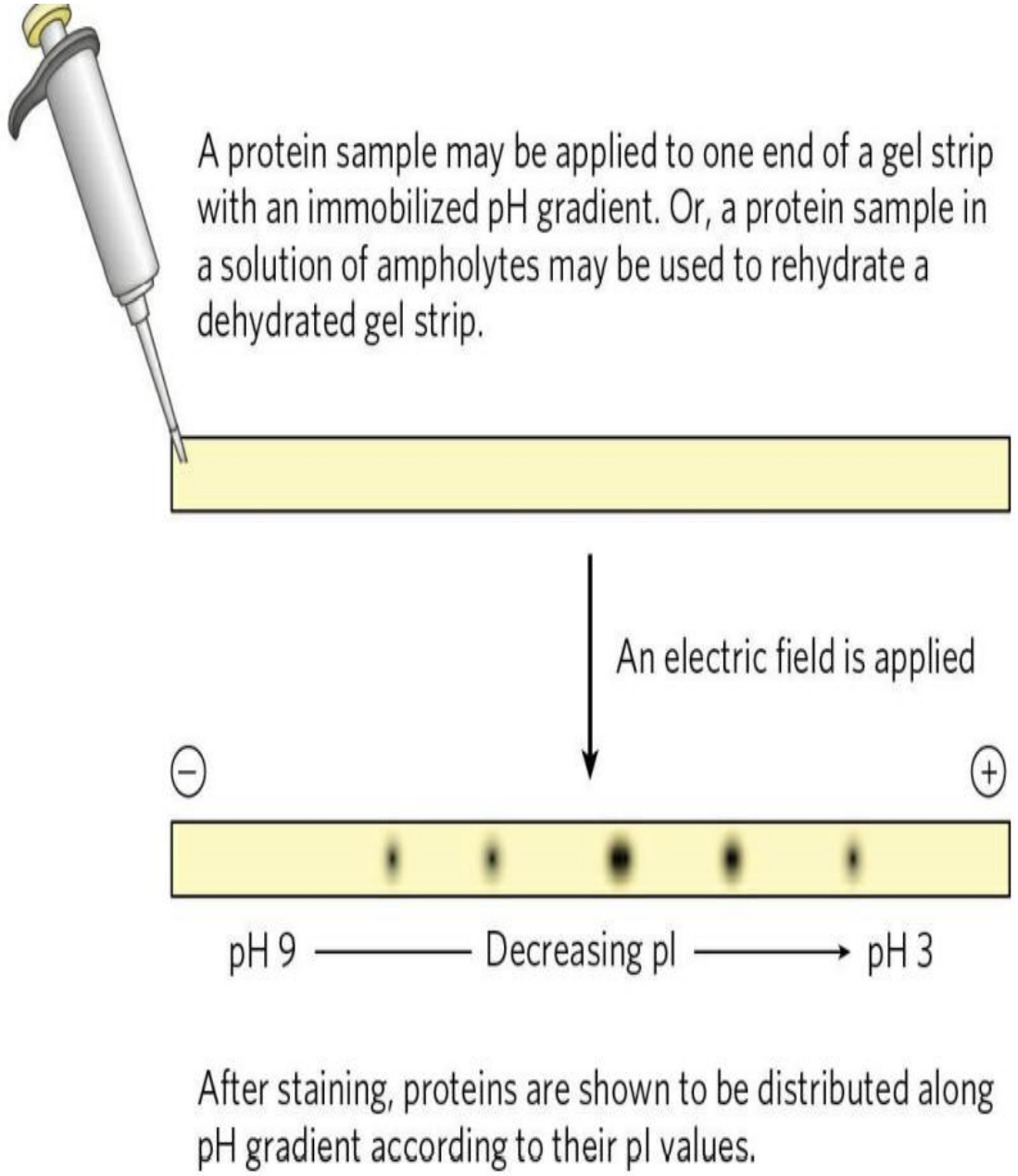


Sodium dodecyl sulfate (SDS)

Isoelectric focusing is a procedure used to determine the isoelectric point (pI) of a protein.

A pH gradient is established by allowing a mixture of low molecular weight organic acids and bases to distribute themselves in an electric field generated across the gel. When a protein mixture is applied, each protein migrates until it reaches the pH that matches its pI. Proteins with different isoelectric points are thus distributed differently throughout the gel.

Combining isoelectric focusing and SDS electrophoresis sequentially in a process called two dimensional electrophoresis permits the resolution of complex mixtures of protein.



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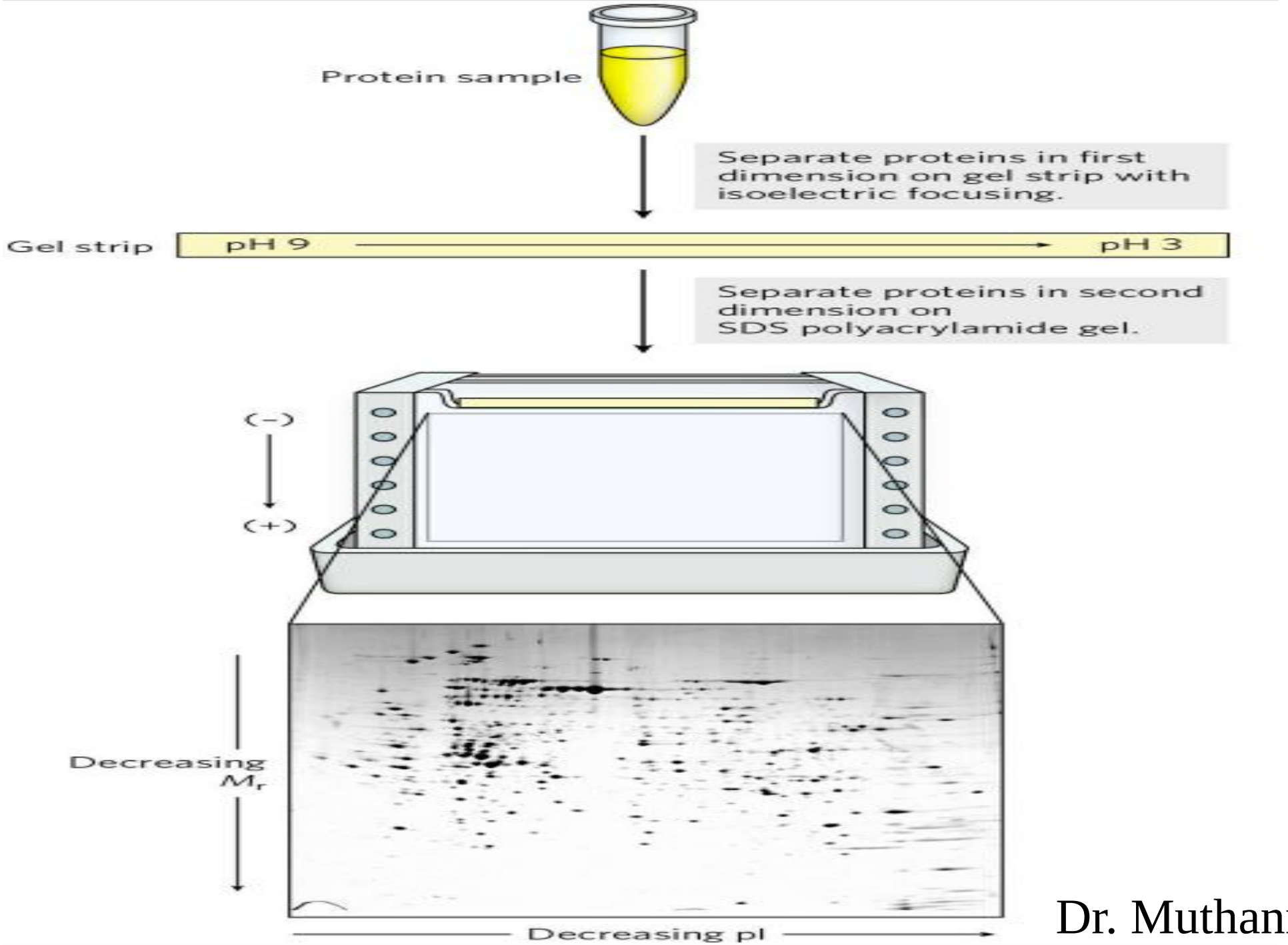

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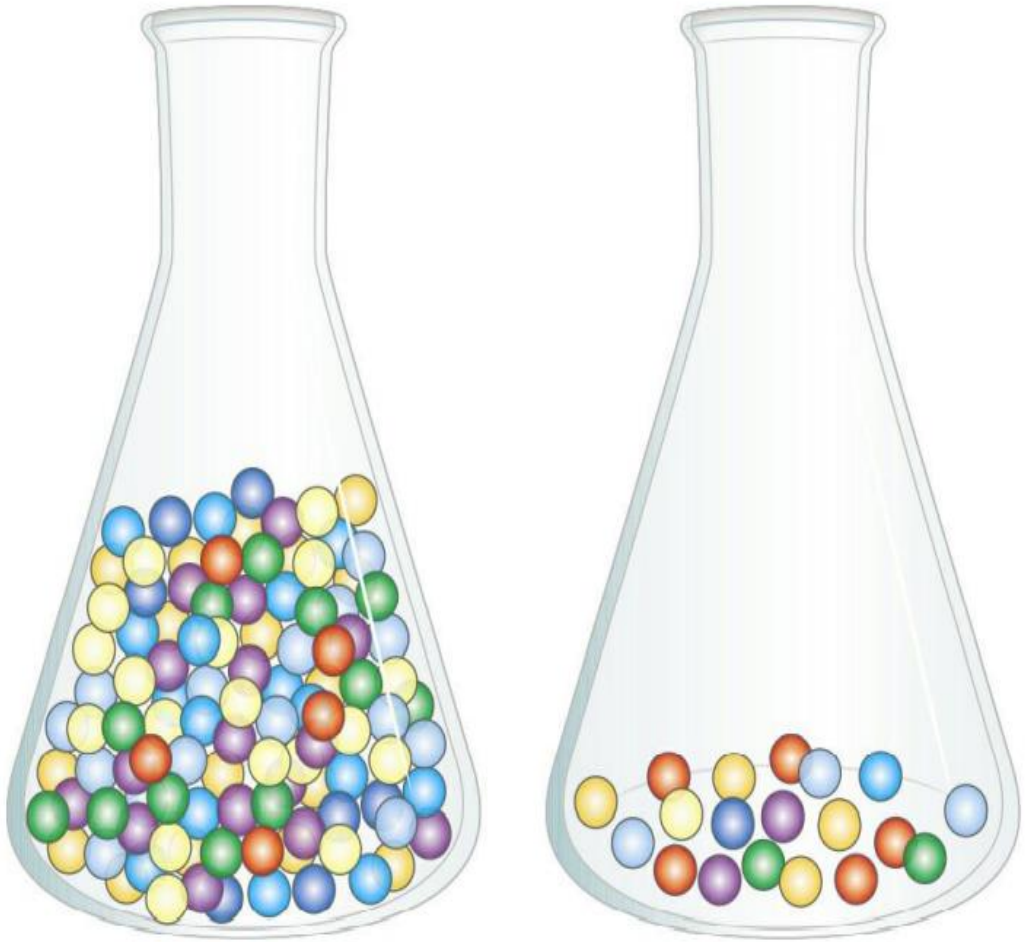
A pure preparation is essential before a protein's properties and activities can be determined. Given that cells contain thousands of different kinds of proteins, how can one protein be purified?

TABLE 3-5 A Purification Table for a Hypothetical Enzyme

Procedure or step	Fraction volume (mL)	Total protein(mg)	Activity (units)	Specific activity(units/mg)
1. Crude cellular extract	1,400	10,000	100,000	10
2. Precipitation with ammonium sulfate	280	3,000	96,000	32
3. Ion-exchange chromatography	90	400	80,000	200
4. Size-exclusion chromatography	80	100	60,000	600
5. Affinity chromatography	6	3	45,000	15,000



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Thanks for listening