15

◉ Sheet ○ Slides

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Let's remember these things :

1- **Primary structure:** it's the sequence of amino acids starting from N-terminus toward C-terminus. These amino acids are linked together by the peptide bond.

It determines the secondary and tertiary structures. A change in the primary structure leads to change in secondary and tertiary structures, that results in a nonfunctional protein "like in sickle cell anemia".

2- **Secondary structure:** It's the localized organized structure, results from the hydrogen-bonding interactions of backbone atoms, between the carbonyl group and the amine group (CO ••• HN).

The most common secondary structures are α-helix and β-pleated sheet.

3- **Tertiary structure:** it's the 3-dimentional functional structure, results from all none covalent interactions between R groups.

- it's the final shape of the peptide chain (not always for the whole protein), with the interactions between R groups and with all modifications.
- The tertiary structures of some proteins have covalent bonds such as : disulfide bridges, and covalent interactions with metal ions.

➔ **The native conformation:** is the functional 3-D structure of the polypeptides, it describes the arrangement and organization of all polypeptide components, so it's the tertiary structure.

Properties of Proteins

a. Denaturation :

- It's the disruption of the native conformation of a protein, the characteristic three-dimensional structure that it attains after synthesis.
- It's like stretching the protein from its two terminals, to destruct its folds.
- By denaturation, the protein becomes a polypeptide chain only without a 3-D functional structure, because Denaturation involves the breaking of the noncovalent bonds which determines the structure of a protein.
- It does NOT mean breaking the peptide bonds or other covalent bonds such as disulfide bridges and covalent interactions with metal ions.
- Generally, the denatured protein will lose its properties such as activity and become insoluble, why?

⇨ Many unfolded polypeptides will accumulate together due to the hydrophobic interactions with each other, that causes the Aggregation of polypeptides, then precipitation of them.

- when you boil or fry "heat" an egg, the egg will solidify, and a whitish color appears, this is due to the denaturation of the proteins in the egg.

- Factors of denaturation:

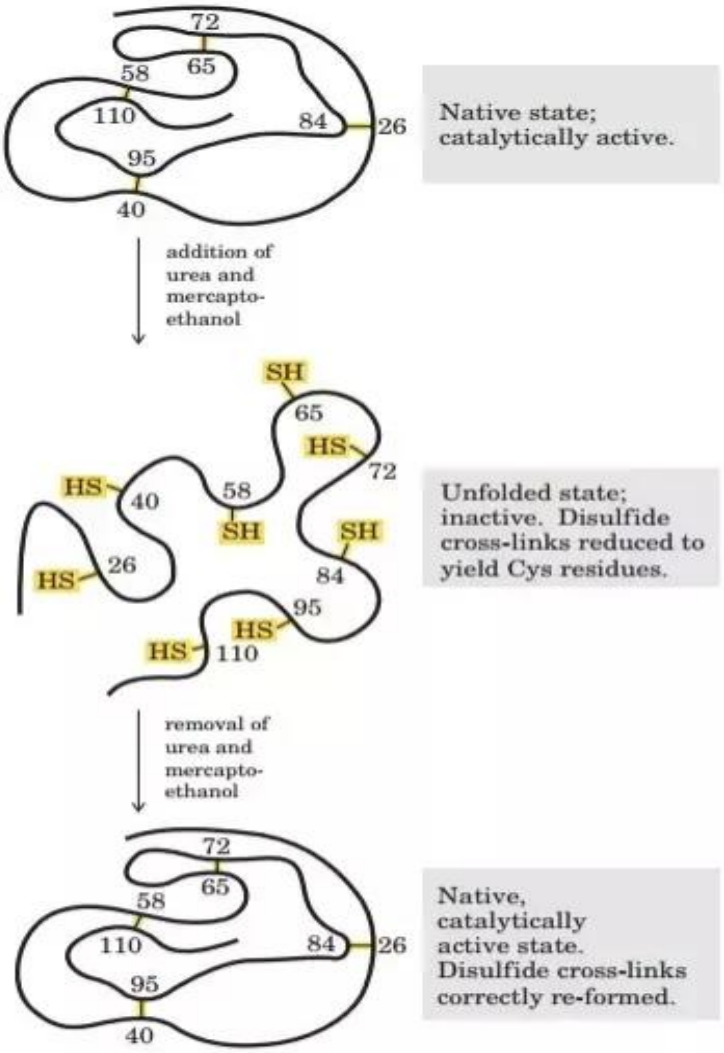
1. **Heat:** It increases the kinetic energy of electrons, so Van der Waals interactions will be disrupted.
   - Although Van der Waals interactions are very weak, you know that a protein have numerous Van der Waals interactions and they are very important to determine the 3-D structure of the protein.
   - Remember that hydrophobic interactions are type of Van der Waals interactions.
2. **Extremes of pH:** Due to a large change in the concentration of hydrogen ions that causes a change in the charge of the protein's amino acids side chain, so the electrostatic and hydrogen bonds are disrupted.
   ----> Electrostatic interactions in the proteins are called salt bridges.
3. **Detergents:** Substances that disturb the hydrophobic interactions within the protein, because they make the environment nonpolar, there are two types:
   a) Non-ionic – Uncharged, such as Triton X-100 which disrupts hydrophobic and electrostatic interactions.
   b) Anionic – Charged, such as:
      - Sodium Dodecyl Sulfate (SDS) which disrupts hydrophobic and electrostatic interactions too.
      - Urea and guanidine hydrochloride which disrupt hydrogen bonding and hydrophobic interactions.
4. **Reducing agents :** reduce disulfide bonds , such as :
   ■ β-mercaptoethanol (βME) ■ Dithiothreitol (DTT).

----> Note that complete denaturation needs reducing agents.

b. Renaturation:

- It's the process in which the native conformation of a protein is re-acquired when the circumstances are back to normal.
- Can an unfolded protein re-fold?

- If a protein is unfolded, it can refold to its correct structure by reforming the non covalent bonds, placing the S-S bonds in the right orientation (adjacent to each other prior to formation), then reforming covalent bonds (sulfide bridges and salt bridges). This is particularly true for small proteins, not for large proteins.
- If the unfolded protein is large, it can't be refolded by itself, it needs help from other proteins called chaperones, or protein degradation can occur in the cell.



Factors that determine protein structure

The whole process of formation the protein **depends on Energy.**

Each process in the life depends on Energy !

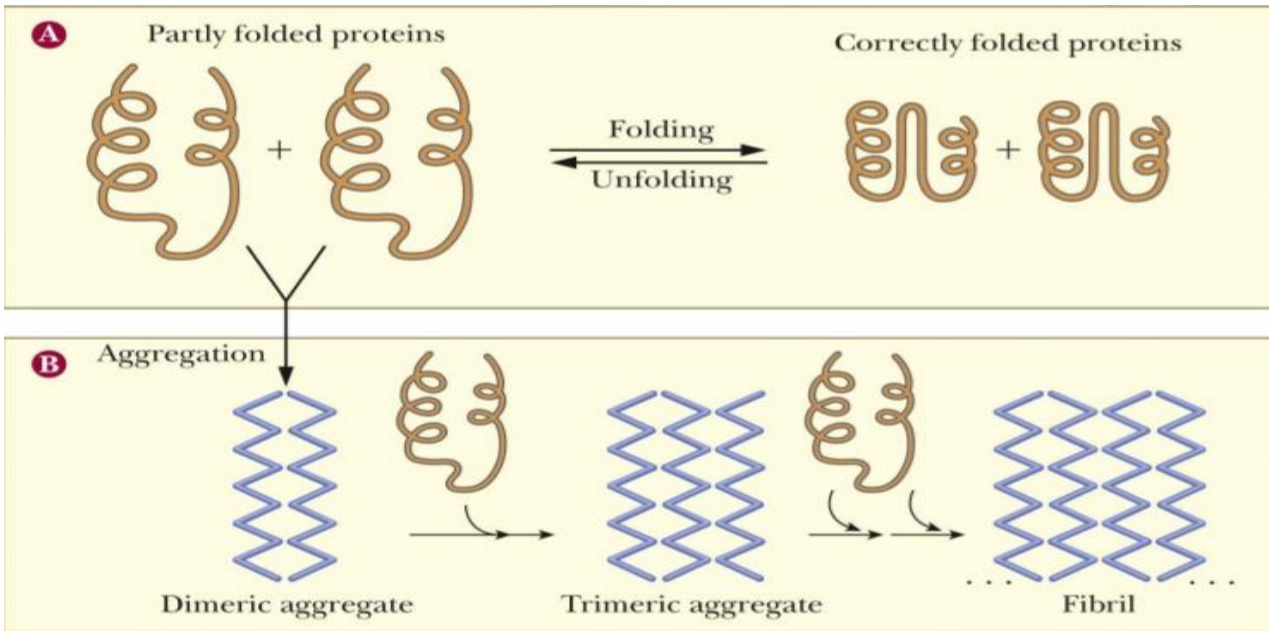
Theoretically millions of structures are possible, but energetically there is one structure that is the most stable and preferable.

– <u>The least amount of energy</u> needed to stabilize the protein. This is determined by:

- **The Primary Structure:** The amino acids sequence (mainly the internal residues) with their specific orientations
- **The proper angles between the amino acids.**
- **The different sets of weak non-covalent bonds that form between the mainly the R groups.**
- **Non-protein molecules** such as sugars, metals and lipids (they stabilize the protein structure).

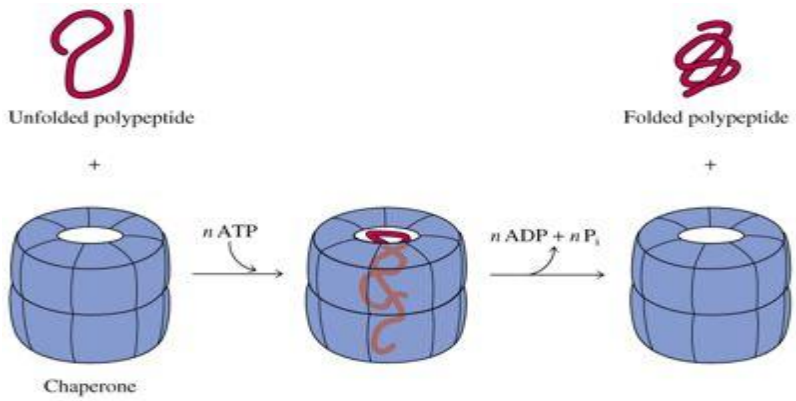
The problem of misfolding

a. When proteins do not fold correctly, their internal hydrophobic regions become exposed and interact with other hydrophobic regions on other molecules, and form aggregates.
b. Partly folded or misfolded polypeptides or fragments may sometimes associate with similar chains to form aggregates.
c. Aggregates vary in size from soluble dimers and trimers up to insoluble fibrillar structures (amyloid).
d. Both soluble and insoluble aggregates can be toxic to Cells.



Problem solvers: chaperones

a. large proteins bind to polypeptide chains and help them fold with the most energetically favorable folding pathway.
b. Chaperones also prevent the hydrophobic regions in newly synthesized protein chains from associating with each other to form protein aggregates.



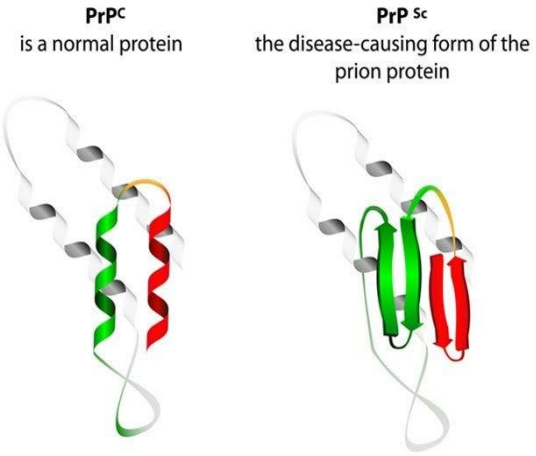
Many diseases are the result of defects in protein folding:

After Pasteur experiments on bacteria, scientists used to think that all diseases are caused by pathogens (bacteria) . But Morgan experiments show that some diseases are inherited (are caused by genetic factors).

Prion diseases

1. Striking examples of protein folding-related diseases are prion diseases, such as : ■ Creutzfeldt-Jakob disease (in humans), and ■ Mad Cow disease (in cows), ■ Scrapie (in sheep).

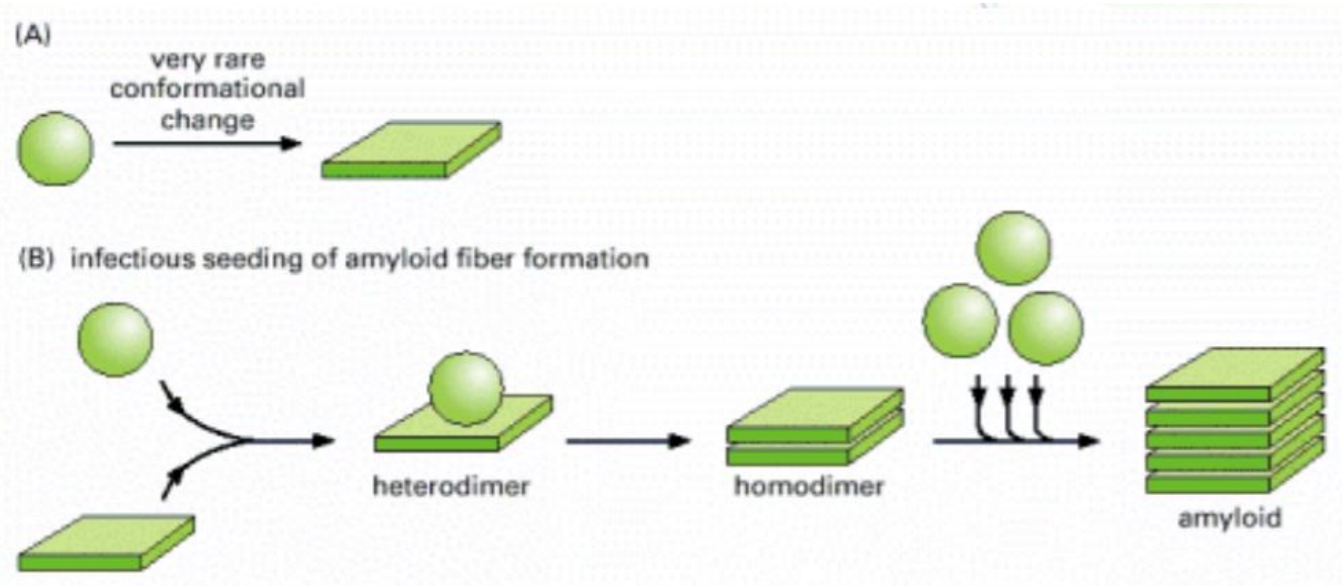
2. Pathological conditions can result if a brain protein known to as prion protein (PrPC) is misfolded into an incorrect form called PrPsc. PrPC has a lot of α-helical conformation, but PrPsc has more β strands forming dimer aggregates which bind to other proteins forming aggregates called amyloid fibers which damage the brain tissue.



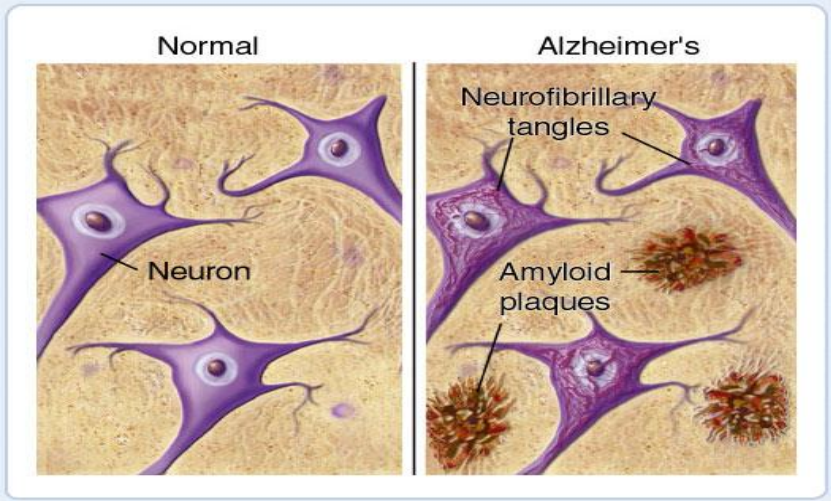
3. The disease is caused by a transmissible agent
Abnormal protein can be acquired by: ● Infection ● Inheritance ● Spontaneously: "Due to a change in the dynamics of the protein's structure formation."

4. Many cows with this mad cow disease are killed because prions are infectious.

5. Creutzfeldt-Jakob disease (in humans) is found usually in Africans because they eat crude meat (without cooking).



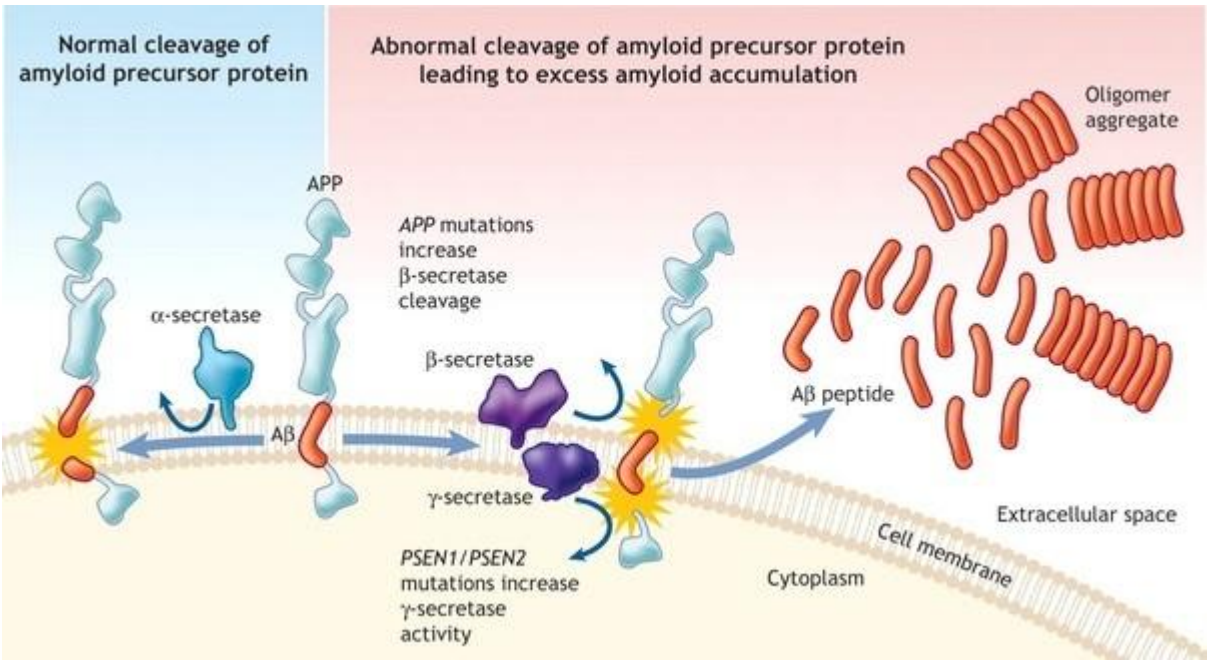
Alzheimer's Disease



1. Scientists found that people with Alzheimer disease have spot-like structures in their brain, these spots are known now as amyloid plaques.

2. It occurs due to aggregates of a protein called tau and another known as amyloid peptides (Aβ) which have hydrophobic domains.

3. How these proteins unfold?
   - ⇨ Surface Proteins in each cell are renewed continuously by turning over the proteins and shedding them after forming new surface proteins.
   - ⇨ During shedding abnormal cleavage can occur in the presence of certain enzymes (shown in the picture), the abnormal cleavage leads to releasing peptides which accumulate in the brain tissue.



4. It's not transmissible between individuals, but it's a progressive disease.

Quaternary Structure

- ✓ Proteins are composed of more than one polypeptide chains. It looks like many buildings in one complex. But One polypeptide isn't functional by itself.
- ✓ They are oligomeric proteins (oligo = a few or small or short; mer = part or unit).
- ✓ The spatial arrangement of subunits relates to the nature of their interactions.
- ✓ Proteins made of: One subunit (monomer), Two subunits (dimer), Three subunits (trimer) or Four subunits (tetramer).
- ✓ Each polypeptide chain in such a protein is called a subunit. In Quaternary proteins the number of subunits may reach sixteen.
- ✓ Oligomeric proteins can be made of multiple polypeptides that are:

• identical: homooligomers (homo = same) like homodimer, homotrimer and so on.

• different: heterooligomers (hetero = different) like heterodimers, heterotrimer and so on.

♧♧ The simplest one is: a homodimer.

- ✓ How are the subunits connected?

Sometimes subunits are disulfide-bonded together, other times, non-covalent bonds stabilize interactions between subunits. For example:

I. *Immunoglobulin* (antibodies) is a Heterotetramer protein with 2 identical subunits and another 2 identical subunits. They are linked covalently by disulfide bonds.

II. *Hemoglobin* is a heterotetramer protein also with 2 alpha subunits and 2 beta subunits. The subunits are linked non covalently by hydrophobic and some electrostatic interactions.

Complex Protein Structures

I. Proteins can be linked (conjugated) to non-protein molecules like sugars, lipids, metals or phosphate group.

II. The non-protein group is important to the protein function.

III. Proteins with their non-protein components are known as **Holoproteins**.

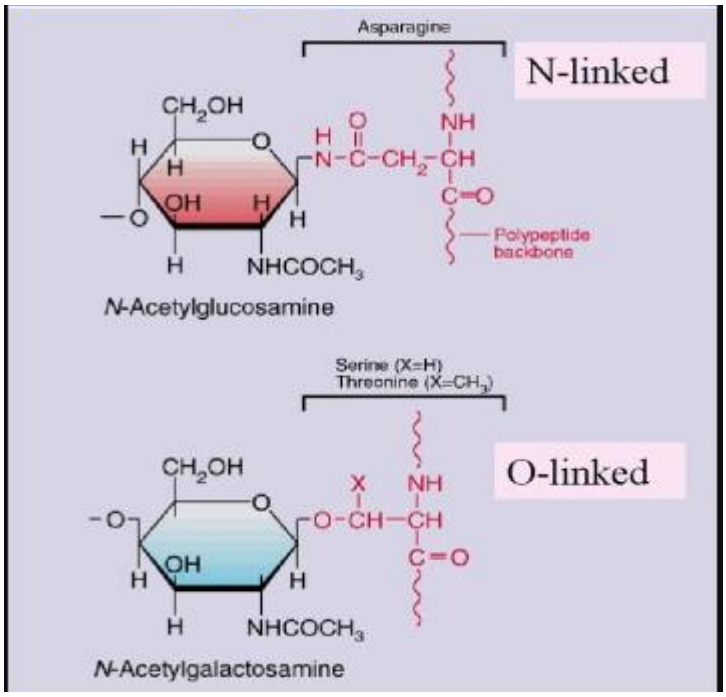
IV. If the non-protein compartment is removed, they are known as an **Apoproteins**.

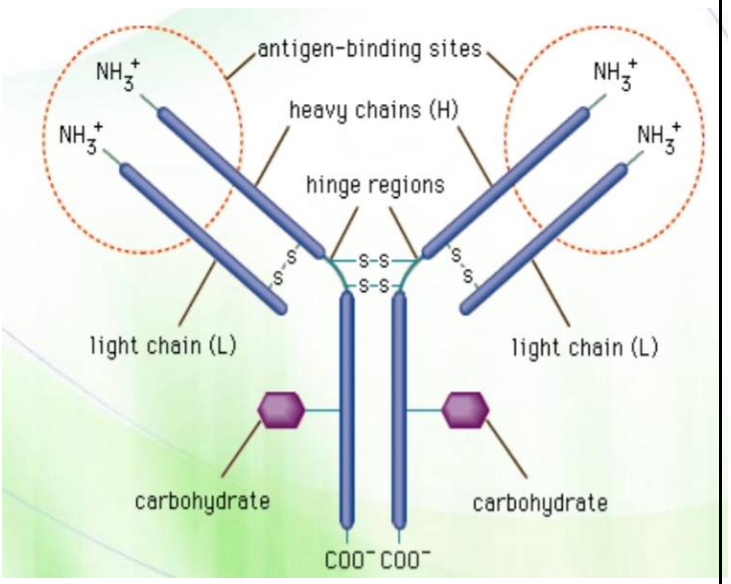
A. Glycoproteins

Proteins covalently conjugated with carbohydrates are termed glycoproteins, which are classified into (depending on where the sugar is associated):

- ➔ N-linked sugars: in which the carbohydrate binds to the *amide nitrogen* of the R-group of asparagine.
- ➔ O-linked sugars: in which the carbohydrate binds to the *hydroxyl group* of either serine or threonine, or occasionally to hydroxylysine.

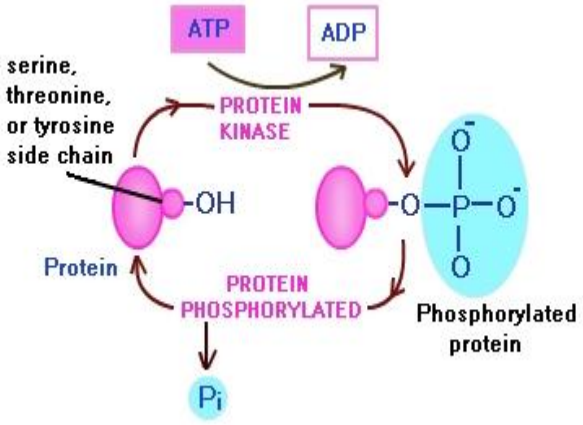
¤ hydroxylysine is a modified amino acid that has OH- group ¤

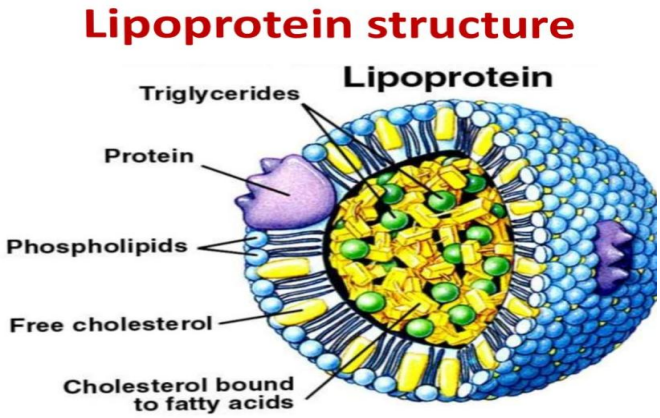




B. Proteins which are associated with lipid are termed *Lipoproteins.*

C. *Phosphorylated Proteins* are called phosphoproteins. Phosphate group can be added to three types of amino acids: *threonine, tyrosine, and serine because they have a hydroxyl group.*





D. Proteins which are associated with metals are called *Metaloproteins.*

Structure-Function Relationship

Biological Functions of Proteins: ** not for memorizing.

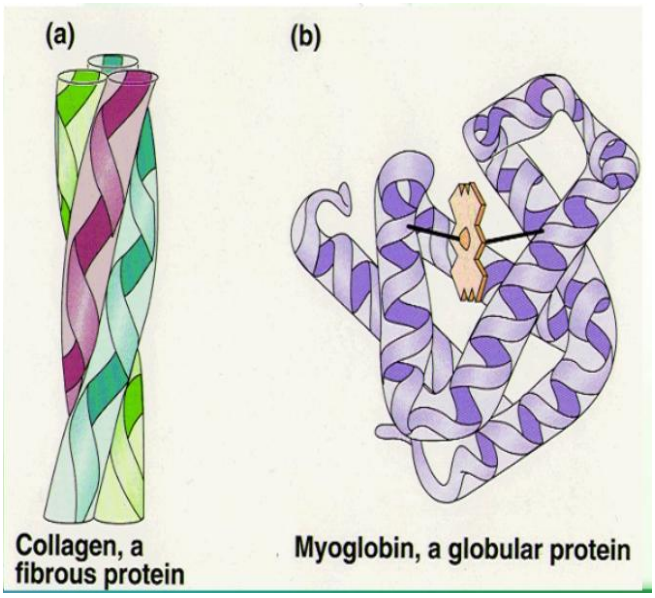
1. Enzymes: like catalysts for reactions.
2. Transport molecules: like hemoglobin; lipoproteins, channel proteins.
3. Contractile/motion: like myosin; actin.
4. Structural: like collagen; keratin, actin.
5. Defense: like antibodies.
6. Signaling: like hormones, receptors.
7. Toxins: like diphtheria; enterotoxins.

Types of proteins

Proteins can be divided into two groups according to structure:

a) FIBROUS (fiber-like with an elongated uniform *secondary-structure only*).
Such as: **collagens, elastins, and keratins.**

b) GLOBULAR (globe-like with rounded *three-dimensional compact structures*).
Such as: **myoglobin, hemoglobin, and immunoglobulin.**



Fibrous proteins

The extracellular matrix

The extracellular space is largely filled by an intricate network of macromolecules including proteins, polysaccharides, and proteoglycans that assemble into an organized meshwork in close association with cell surface. It connects cells together and stabilizes the tissue.

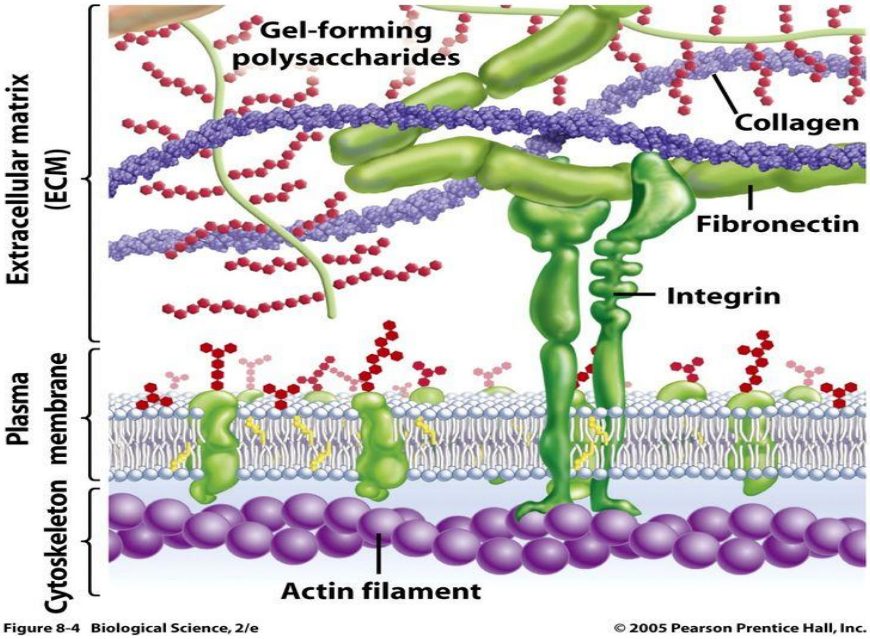


Figure 8-4 Biological Science, 2/e

Collagens and their properties

1) The collagens are a family of fibrous proteins with 25 different types found in all multicellular animals.
2) They are the most abundant proteins in mammals, constituting 25% of the total protein mass in these animals.
3) Collagen molecules are named as type I collagen, type II collagen, type III collagen, and so on.
4) The main function of collagen molecules is to provide structural support to tissues.
5) Hence, the primary feature of a typical collagen molecule is its stiffness to make the tissue solid and rigid.
6) They are found in bone, skin, connective tissue, and cornea in the eye. Notice that you can’t tear the skin easily because it contains collagen fibers.