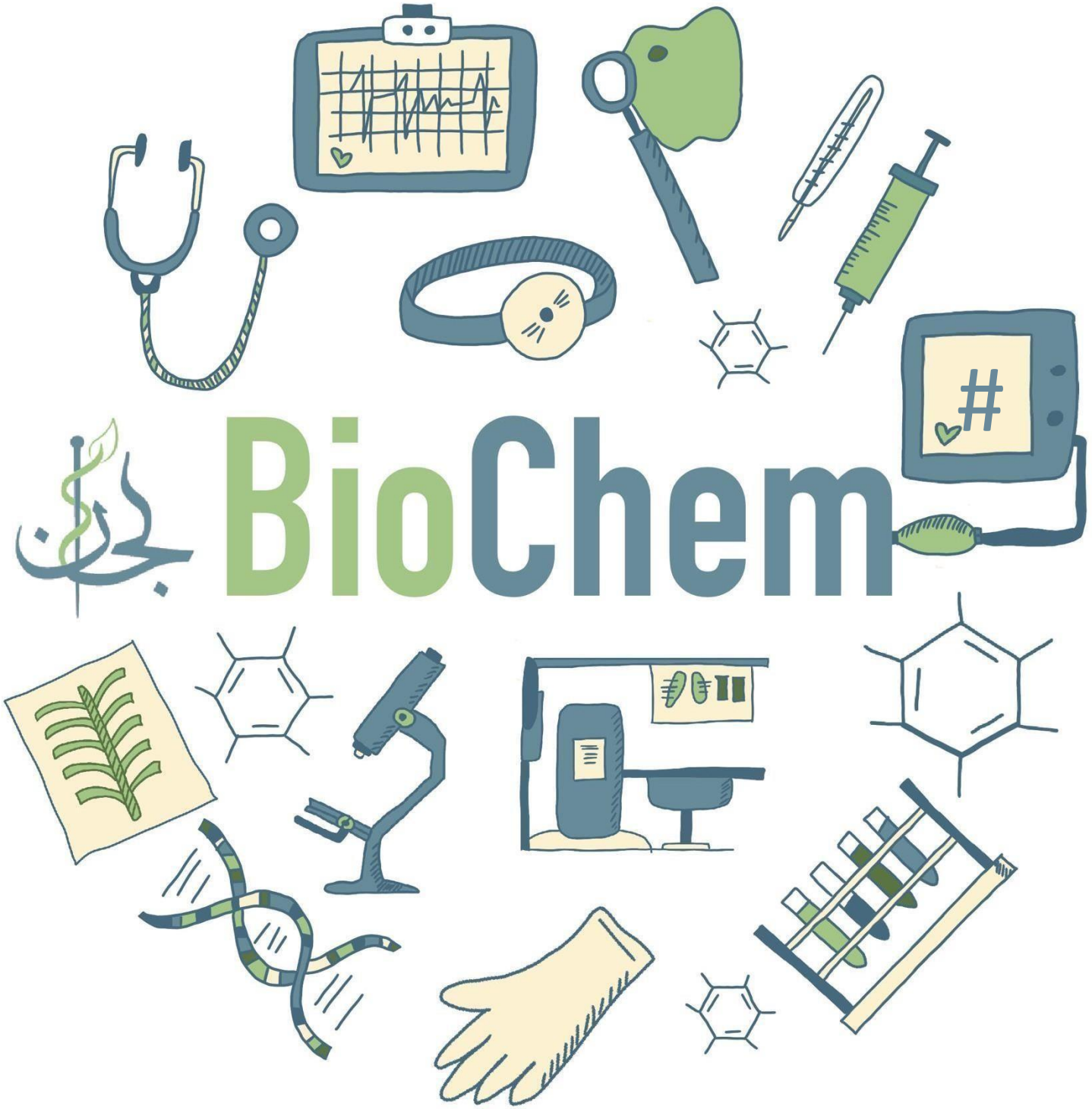


BioChem

Writer: Hanan Alsheikh & Ahmad Qatawneh

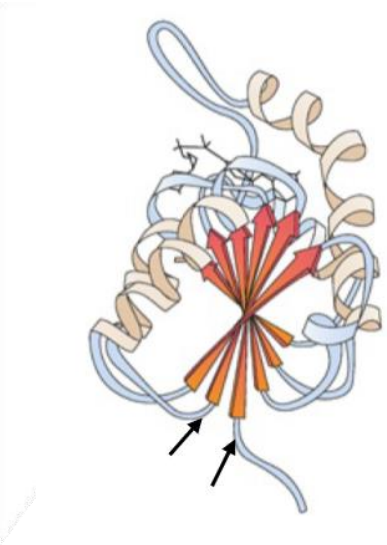
Science: Ahmad Qatawneh

Grammar: Hanan Alsheikh

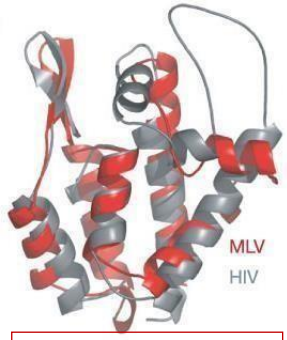
Doctor: Mamoun ahram

Tertiary structure

- The overall conformation of a polypeptide chain.
- The three-dimensional arrangement of all the amino acid residues.
- The spatial arrangement of amino acid residues that are far apart in the sequence (in the primary structure). Look at the picture, the two amino acids pointed out are located far away from each other in the primary structure, but are close to each other in space. So, what matters here is the arrangement of amino acids in space.

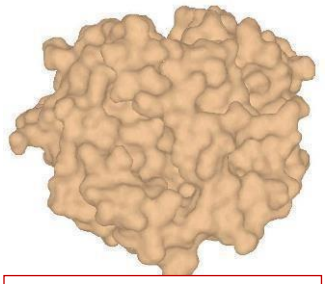


How to look at proteins: different ways to illustrate the tertiary structure of proteins:



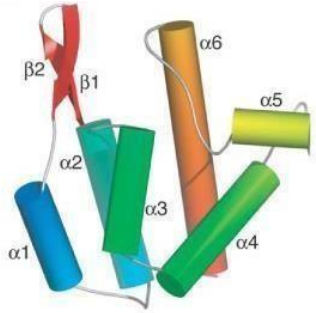
Ribbon structure

Mostly used. α-helices look like ribbons (helical rods) and the strands are illustrated as thick arrows (the end of the arrow indicates the end of the β-strand closer to the C-terminus of the polypeptide).



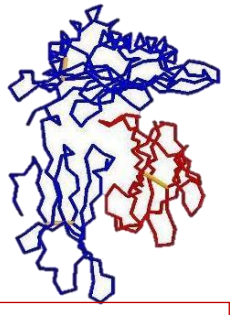
Protein surface map

A modern computer-made model that only shows the protein surface and no interior. It's important in designing drugs because drugs bind to protein surfaces.



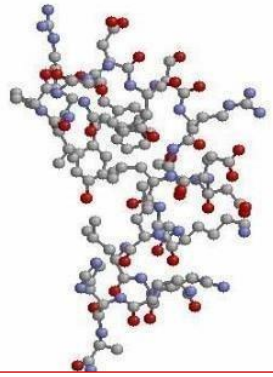
Cylinder structure

There are many ways to illustrate α-helices and they are illustrated as cylinders here. β-strands are represented as arrows similar to the ribbon structure.



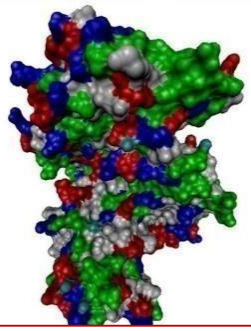
Trace structure

The backbone of the polypeptide can be simply drawn to illustrate the protein structure.



Ball and stick structure

Atoms are small balls represented accurately showing their exact 3D angular orientation. The backbone is also shown.

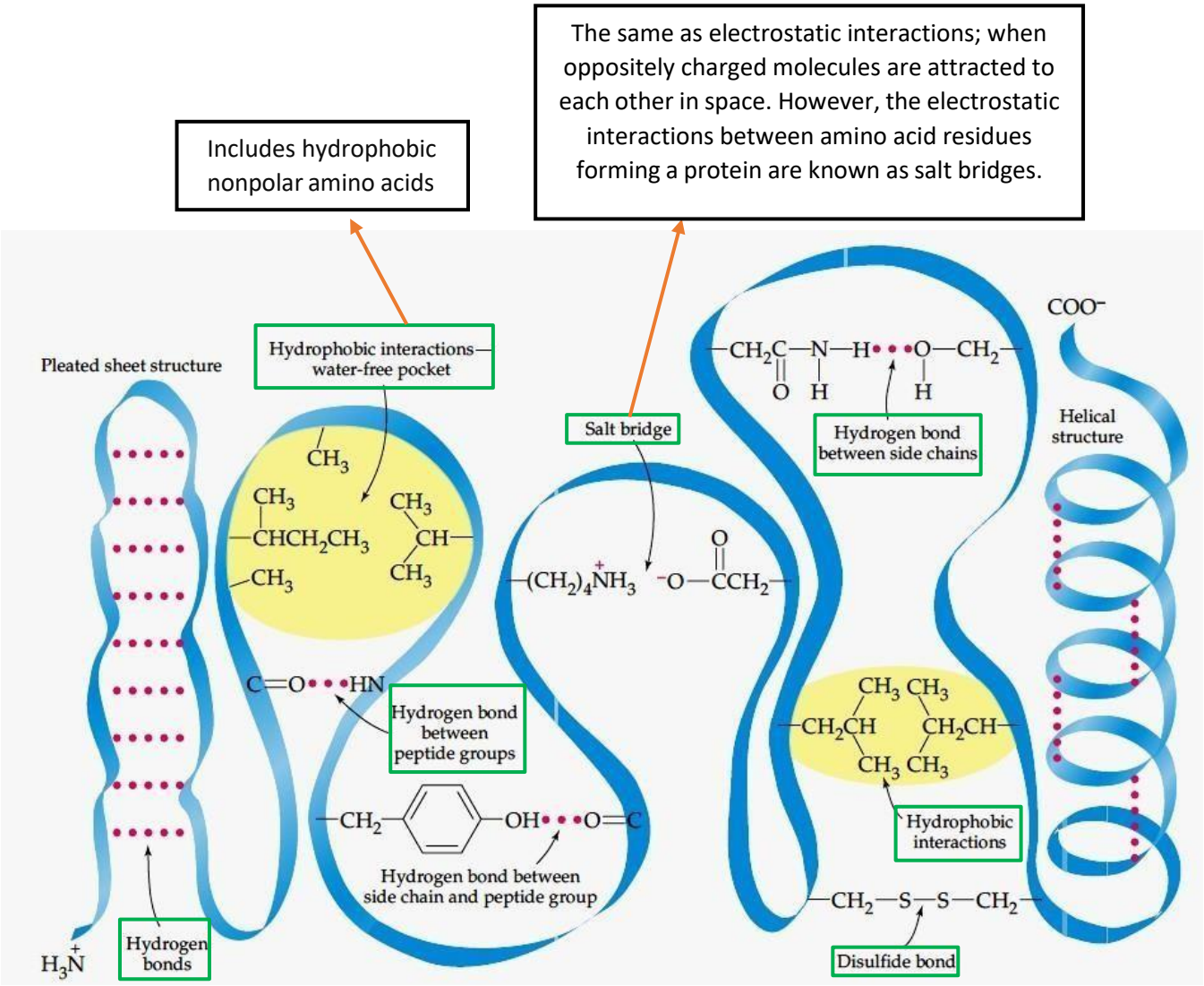


Space filling structure

More complex than the ball and stick structure with larger balls and an unshown backbone.

Shape-determining forces

The tertiary structure of proteins is determined by <u>non-covalent interactions and not peptide bonds</u>. There are 4 types of non-covalent interactions: hydrogen bonds, Van der Waals interactions, electrostatic interactions and hydrophobic interactions. **These are the forces that determine how proteins look like**.

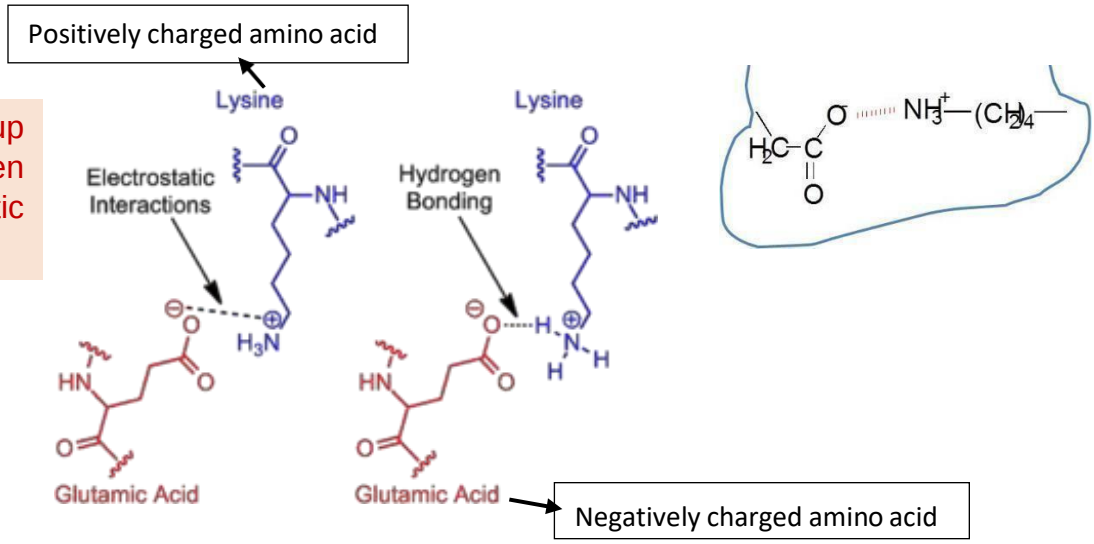


Notice how hydrogen bonds can form between different groups throughout the protein.

Non-covalent interactions

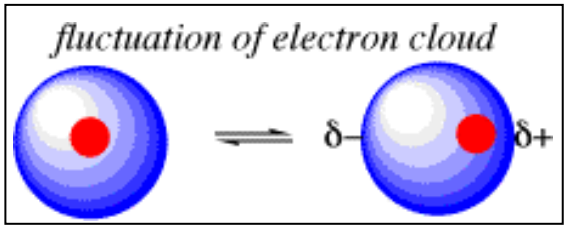
- Hydrogen bonds occur not only within and between polypeptide chains but with the surrounding aqueous medium (the solvent where the protein is dissolved; water and salt solution, a buffer solution, etc).
- Charge-charge interactions (**salt bridges**) occur between oppositely charged R-groups of amino acids.
- Charge-dipole interactions form between charged R groups with the partial charges of water.

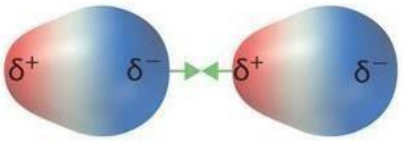
The same charged group can form either hydrogen bonding or electrostatic interactions



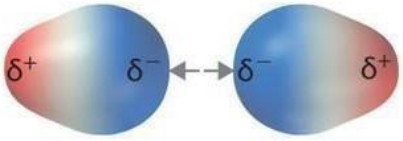
Van der Waals attractions

- Despite them being **the weakest noncovalent forces**, they are extremely important. They are the most transient as they keep changing depending on the distribution of electrons within the atom and since electrons keep moving, they can be unevenly distributed in a fraction of a second generating partial charges which causes different attractions to occur between partially charged molecules (attraction or repulsion) as the pictures bellow illustrates.
- There are both attractive and repulsive van der Waals forces that control protein folding.
- Although van der Waals forces are extremely weak, they are significant because there are so many of them in large protein molecules unlike if you had one or very few Van der Waals interactions their effect would be negligible.





(b) Attraction

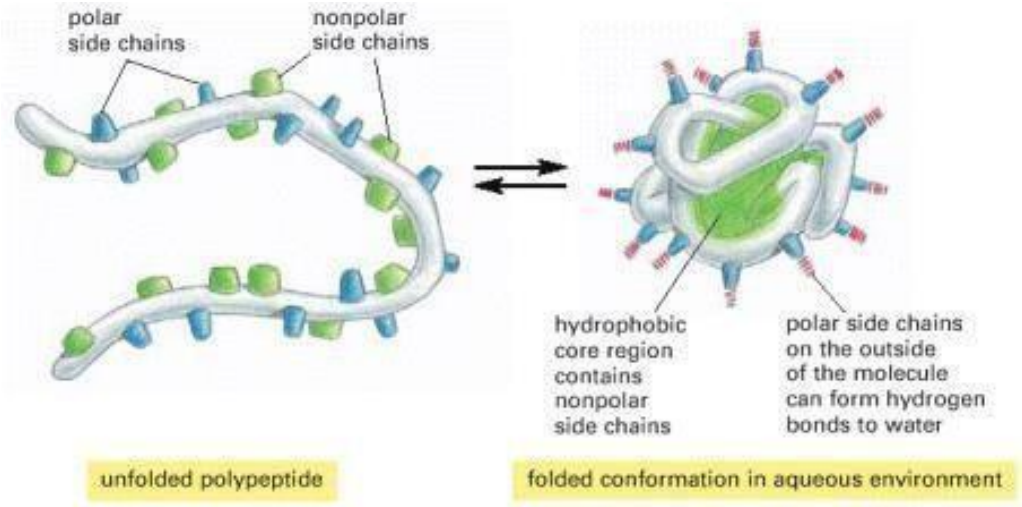


(d) Repulsion

Hydrophobic interactions

The most important forces in determining protein structure. As soon as proteins containing nonpolar hydrophobic amino acids are formed their nonpolar amino acids cluster in the core of the protein away from water while surfaces of globular proteins contain the polar charged amino acids.

- A system is more thermodynamically (energetically) stable when hydrophobic groups are clustered together rather than extended into the aqueous surroundings.



Can polar amino acids be found in the interior? YES

- Polar amino acids can be found in the interior of proteins.
- In this case, they form hydrogen bonds to other amino acids or to the polypeptide backbone.
- They play important roles in the function of the protein. Usually charged polar amino acids that reside in the interior of proteins have certain functions (like histidine residues that are part of the important function of hemoglobin and myoglobin).

Stabilizing factors

There are two forces that do not determine the three-dimensional structure of proteins, but stabilize these structures:

1) Disulfide bonds
2) Metal ions

Holoproteins contain a nonprotein molecule as a part of the protein like (metal ions) and without these groups proteins are considered apoproteins.

Disulfide bonds

Stabilizing forces that do not determine the structure.

- Formed between **two cysteine residues**.
- The side chain of cysteine contains a reactive sulfhydryl group (—SH), which can oxidize to form a disulfide bond (—S—S—) to a second cysteine.
- The crosslinking of two cysteines to form a new amino acid, called **cystine**.
- Disulfide bonds link different polypeptides together forming large proteins and they also form within the protein itself stabilizing its structure.
- Reduction of cystine breaks down disulfide bonds.

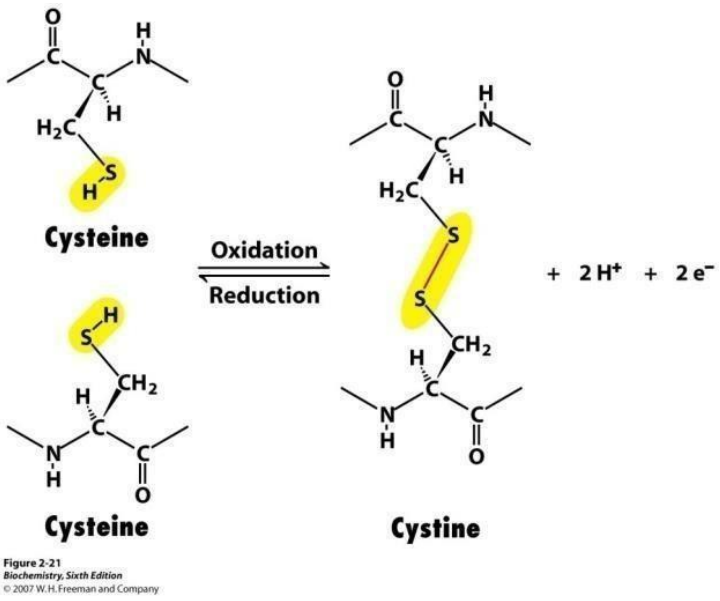


Figure 2-21
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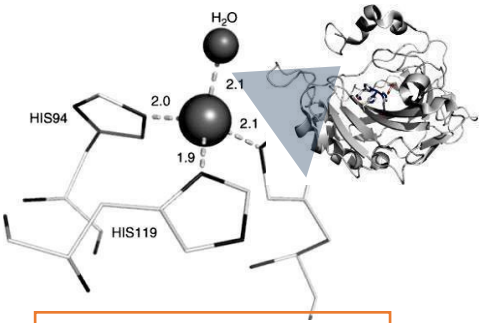
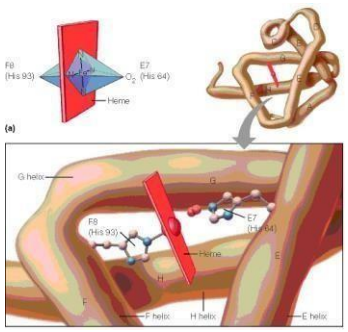
metal ions

- They play an important role in stabilizing protein structures and they play a role in their function as well.
- Several proteins can be complexed to a single metal ion that can stabilize protein structure by forming:
    1) **Covalent** interaction (myoglobin)
    2) Salt bridges (**noncovalent**) (carbonic anhydrase)



Carbonic anhydrase

Zinc is noncovalently bonded to 3 histidine residues stabilizing the enzyme's structure and affecting its function.



Myoglobin

Myoglobin contains one heme group that is covalently linked to a histidine residue.

Domains and folds

Domains are an example of super-secondary structures.

- A domain is a combination of α helices and/or β sheets that are connected to each other via turns, loops, and coils and are organized in a specific three-dimensional structure.

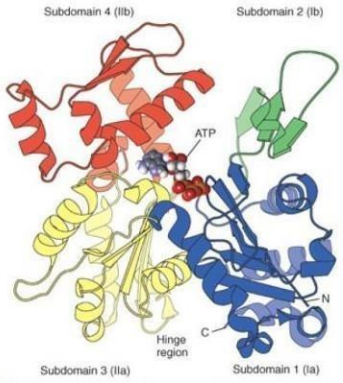
  A domain may consist of **100–200** residues.

- **Domains fold independently** of the rest of the protein or of other domains within the same protein.
- **Similar domains can be found in proteins with similar function and/or structure and can be present in different proteins.**
- Domains may also be defined in functional terms

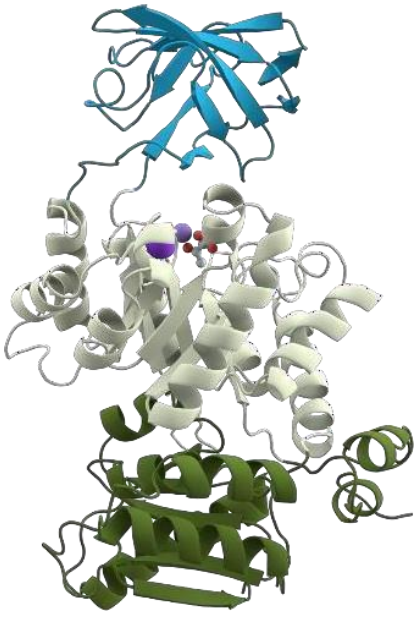
  1) enzymatic activity
  2) binding ability (e.g., a DNA-binding domain)

- **When a domain or multiple domains within a protein possess specific functions, they are known as Folds.**

  - The actin fold.
  - The nucleotide binding fold.



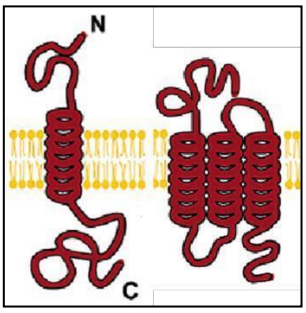
Folds are specified 3D structures that are present in proteins that have different overall functions but share folds and domains. For example, different proteins that contain a nucleotide binding fold can bind to different nucleotides like ATP but they utilize it for different purposes.



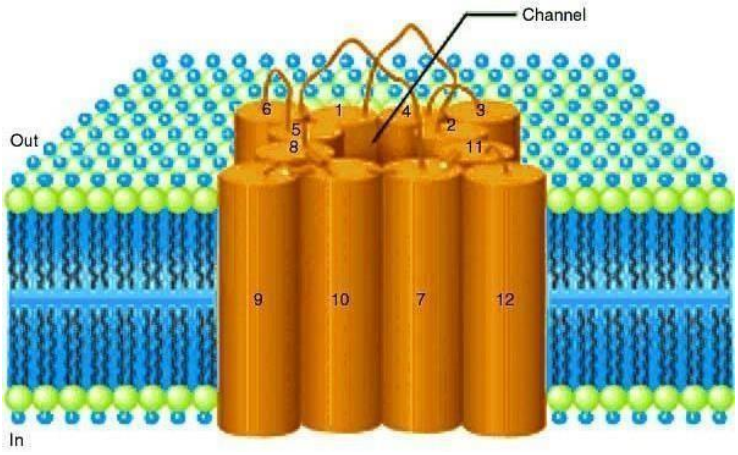
We have 3 different domains in this particular protein and each one is composed of a combination of secondary structures. Domains maintain their structure and function when isolated from their original protein.

α-helices as transmembrane domains

- Membrane-spanning proteins contain a **transmembrane domain that is an α–helix** made of hydrophobic nonpolar amino acids *mainly* so no repulsion occurs between the fatty acids that make up the plasma membrane and the amino acids in the transmembrane domains.



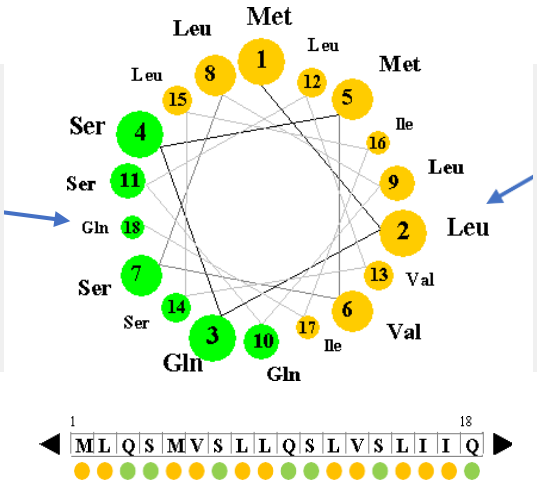
- Some membrane proteins contain several transmembrane domains that are also α-helices that are connected to each other via loops, and turns that usually serve a function like ligand binding.
- For receptors, the helices are connected by loops containing hydrophilic amino acid side chains that extend into outside of both sides of the membrane.
- Membrane ion channels contain **amphipathic** α–helices.



An ion channel with multiple transmembrane domains that are connected to each other via loops. In order for ions to pass through the channel the R groups of amino acids must be polar or charged (with an opposite charge to the ion). However, the R groups that face the fatty acids in the plasma membrane must be hydrophobic.

A top view of the α-helix in this channel

This side of the α-helix is the charged polar side that faces the lumen of the channel.



This side of the α-helix is the hydrophobic nonpolar side that faces the plasma membrane.

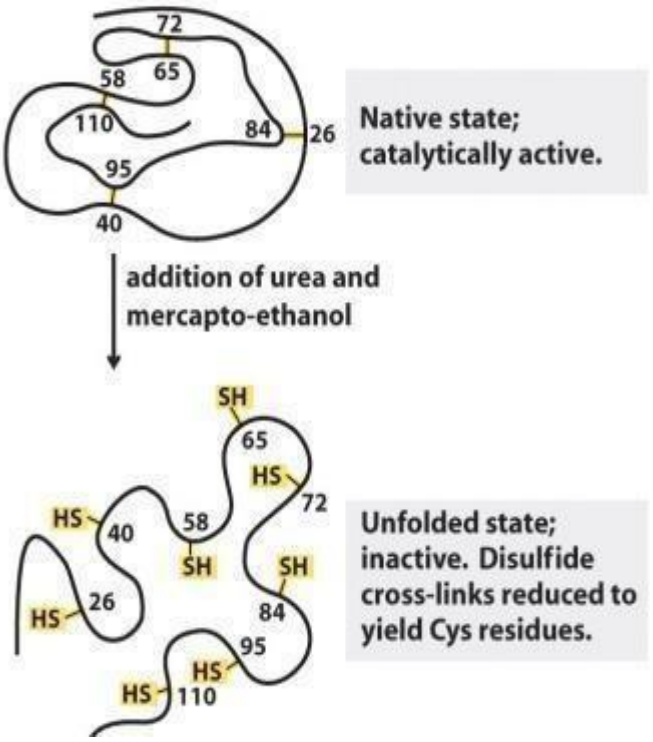
• Denaturation

Just like **DNA denaturation** (The breaking of the non-covalent interactions between the 2 strand of DNA) that we have discussed previously, there is another process known as **Protein Denaturation.**

Protein denaturation is the disruption of the native conformation of a protein via breaking the noncovalent bonds (Hydrogen bonds ,hydrophobic interactions, and electrostatic interactions) that determine the structure of a given protein.

- Complete disruption of the tertiary (3rd) structure can be achieved by reduction of the disulfide bonds via the addition of **Urea Or mercapto-ethanol** in a protein as show in the figure.

- **As a result**, the denatured protein loses its properties such as activity and becomes insoluble.



• Denaturing agents

There are 5 different factors that could lead to denaturation:-

1. **Heat:** Increasing the temperature leads to an increase in the kinetic energy of electrons within proteins thus disrupting low energy van der Waals forces in proteins and due to the large number of these interactions, their disruption could lead to protein denaturation.

2. **Extreme pH**: Extreme values affect the charge of the protein's amino acid side chains and subsequently, disrupting electrostatic interactions (known as salt bridges) as well as hydrogen bonding thus destabilizing (denaturation) the protein.

3. **Detergents:** Introducing hydrophobic substances (a hydrophobic environment) causes the hydrophobic amino acids in the inside (core) of the protein structure to be exposed to the outside leading to disruption in its structure and Hence, Protein denaturation. Two examples on these substances are :-

   - **Triton X-100 (nonionic uncharged detergent):** Due to its hydrophobic nature, it disrupts the hydrophobic interactions leading to protein denaturation.
   - **sodium dodecyl sulfate (SDS, anionic, charged)**:Due to its Amphipathic nature, it disrupts both the hydrogen bonds as well as Hydrophobic forces leading to denaturation.

Side note:- SDS is commonly used Western blotting to plot proteins

4. **Urea and guanidine hydrochloride:** Both disrupt hydrogen bonding and hydrophobic interactions.

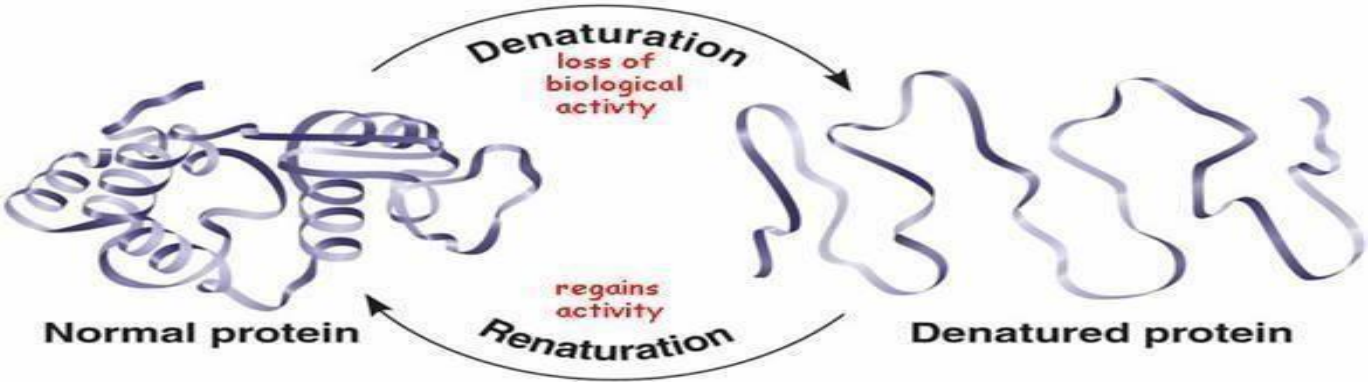
5. **Reducing agents** such as **β-mercaptoethanol (βME) and dithiothreitol (DTT).**Both of which reduce disulfide bonds (bridges) thus destabilizing protein structure.

(**Recall** that even though disulfide bonds aren't necessary for the formation of) the 3D-shape,however their presence further stabilize protein structure.

• Renaturation

The phenomenon of **Renaturation** was discovered when a scientist was experimenting on a **Ribonucleoenzyme** denatured by introducing reducing factors. However, they noticed that after the removal of these factors, the protein re-attained its native 3D-structure by forming non-covalent interactions followed by disulfide bridges

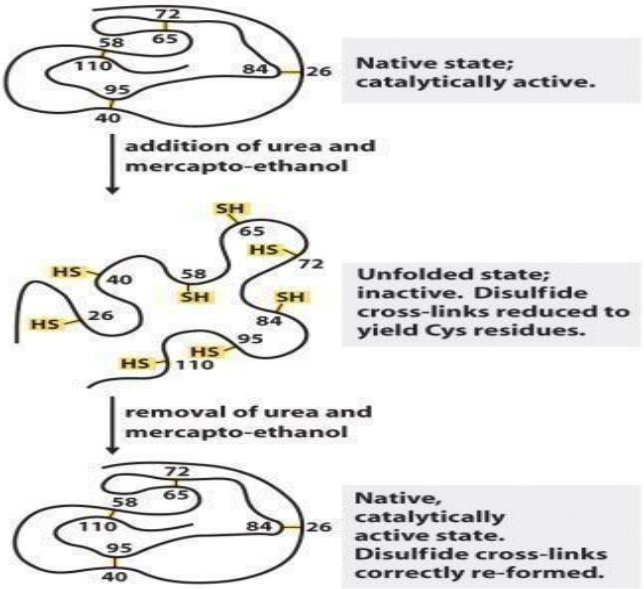
Renaturation is the process in which the native conformation of a protein is re-acquired by removing the denaturation factor and thus regaining the functional activity of this protein



- Renaturation can occur quickly and spontaneously, and after that disulfide bonds are formed correctly.

- If a protein is unfolded, it can refold to its correct structure placing the S-S bonds in the right orientation (adjacent to each other **prior to formation), then the correct S-S bonds are reformed.**

- This is particularly true for small proteins.



Remember: Noncovalent interactions Are the ones responsible for specifying the 3D structure of the protein not disulfide bonds (disulfide-bonds are Involved in stabilization of structure).

• Factors that determine protein structure

The least amount of energy needed (used) to stabilize the protein. This is determined by:

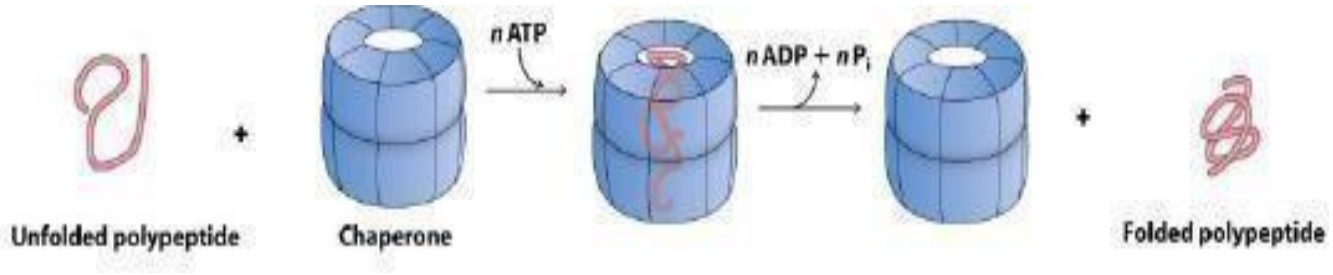
1. **The sequence of Amino acids** (The primary structure) mainly the internal residues. That's why we use informatics (Computer software) to predict the most energetically favorable 3D structure of proteins based on their Primary structure.
2. **The proper angles between the amino acids**, that is where the Amino acids are preferably placed to efficiently form noncovalent interactions starting with hydrophobic interaction.
3. **Non-protein molecules** including the **Heme group, Zinc, and others**. All of which stabilize the 3D structure of protein.

• Problem solvers: chaperones

- Barrel-like proteins having openings within their structure that allow them to bind polypeptide chains effectively helping these chains to fold with the most energetically favorable folding pathway.

- They do so by placing certain regions (**especially hydrophobic regions**) within the same polypeptide chain next to each other thus **preventing the random association of hydrophobic regions of different newly synthesized chains to each other after being** released by the ribosomes.**(i.e., preventing the formation of Protein aggregates)**

- Notice that Chaperones utilize ATP to ensure the correct folding of polypeptides.

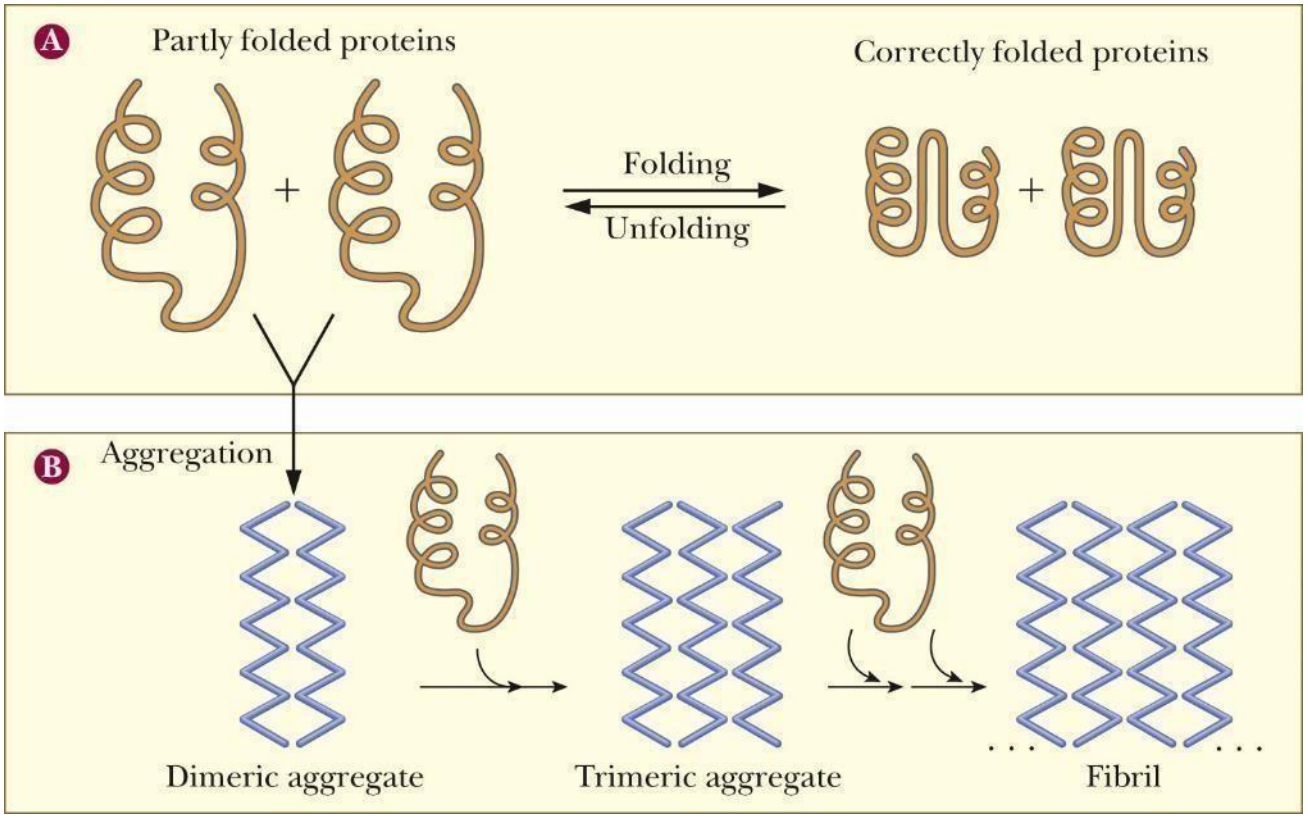


Note: Many diseases are the result of defects in protein folding.

• The problem of misfolding

When proteins do not fold correctly, their internal hydrophobic regions become exposed and therefore interact with other hydrophobic regions on other molecules, thus forming **Protein aggregates**.

Protein aggregates are large (Can be dimeric, trimeric or Fibril as shown below) leading to their accumulation and precipitation in tissues and therefore, causing tissue damage.



• Outcome of protein misfolding

- Partly folded or misfolded polypeptides or fragments may sometimes associate with similar chains to form aggregates.

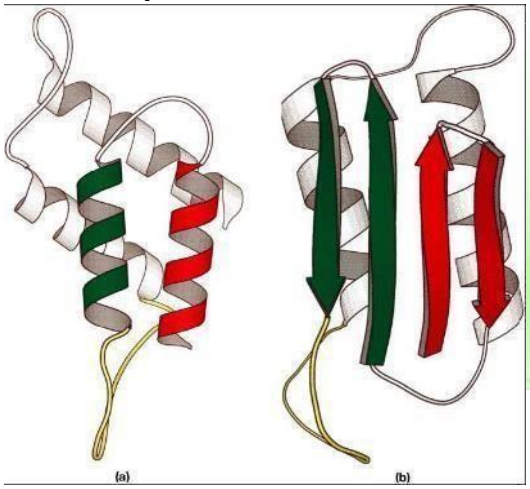
- Aggregates vary in size from **soluble dimers and trimers** up to **insoluble fibrillar structures**. All of which all collectively known as **Amyloids**.

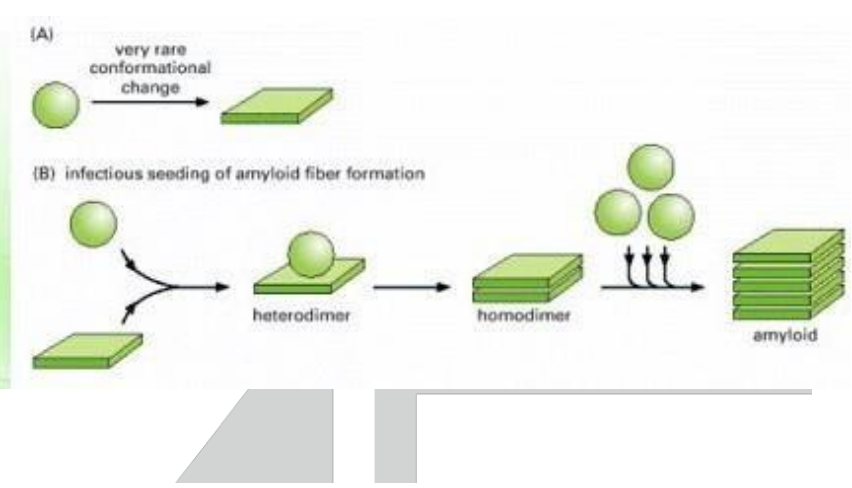
- Both soluble and insoluble aggregates can be toxic to cells leading to different harmful diseases such **Prion diseases** as well as **Alzheimer disease**

• Prion disease

Prion disease, also known as **Creutzfeldt-Jacob disease** (in humans), **and mad cow disease** (in cows), and **scrapie** (in sheep), is a pathological condition that can result if a brain protein known as **cellular prion protein (PrP or PrPc**) is misfolded into an incorrect form called **PrPsc.**

- The difference is that **PrPC has a lot of α-helical conformation**,whereas **PrPsc has more β strands** that lead to the formation of protein aggregates.



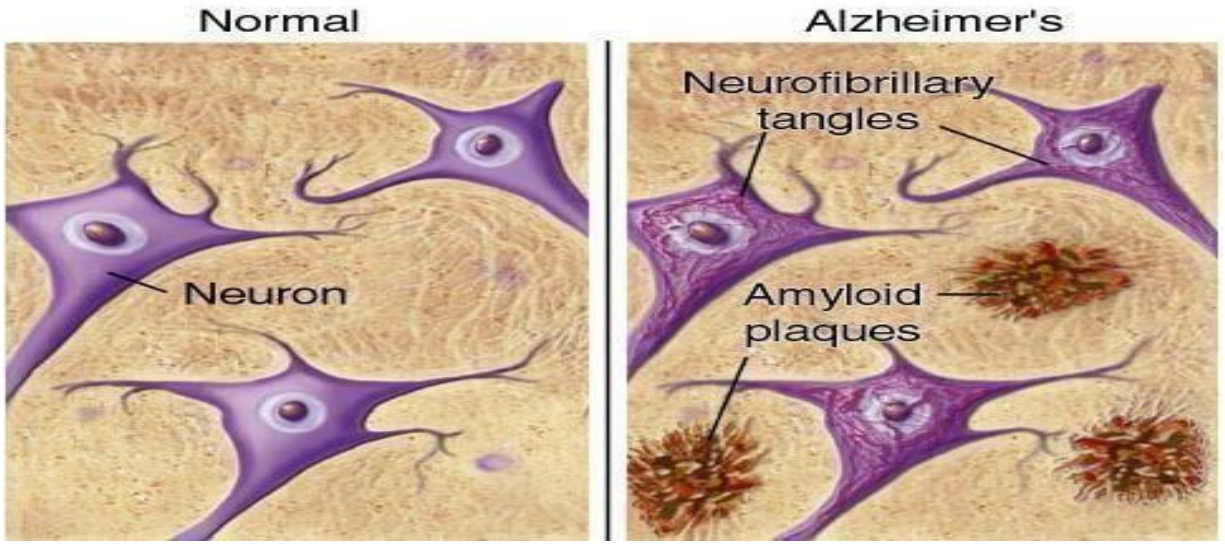


- **In this disease**, a misfolded protein (PrPsc) would bind to a correctly folded protein (PrPc) causing the correct one to misfold leading to protein aggregation and subsequently, the formation of an **Amyloid** in the brain.

- This disease is caused by a **transmissible infectious agent** (A misfolded protein not a pathogen) which can be acquired from:
    1. **Infection** (Receiving these "infectious' proteins from Eating meat infected by mad cow disease)
    2. **Inheritance (Mutations)**
    3. **Spontaneously**

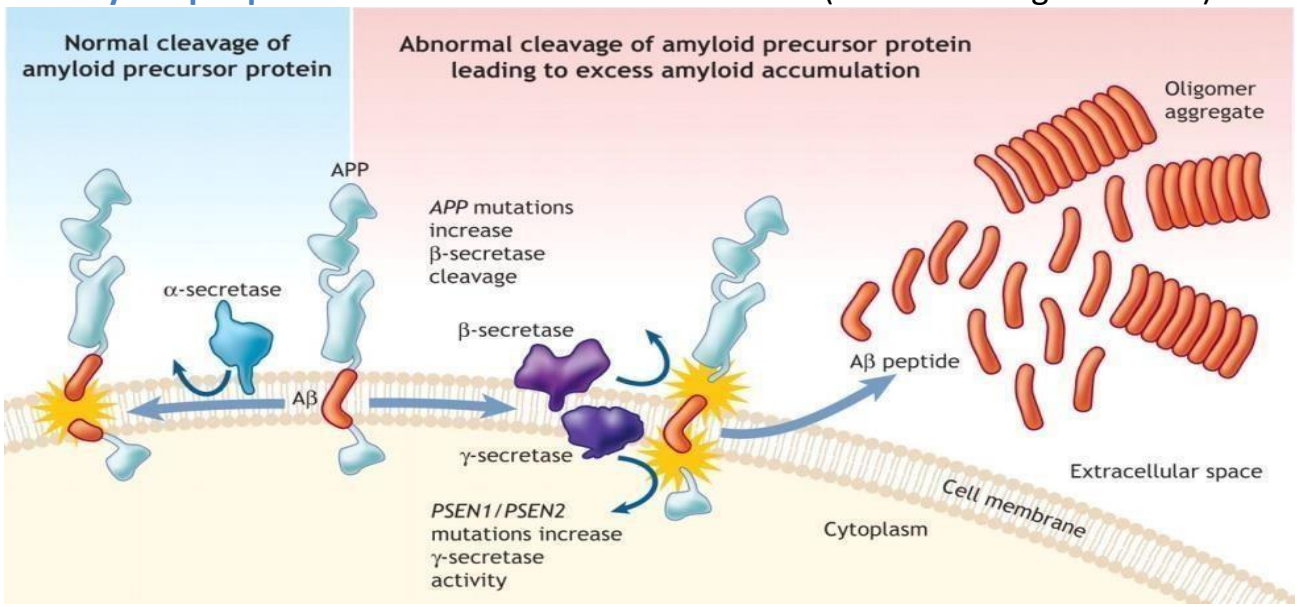
• Alzheimer's disease

A non-transmissible disease that results from the misfolding of certain proteins causing tissue damage in the brain (Memory loss). In which **Extracellular plaques of protein aggregates** of a misfolded protein known as **tau** and another one known as **amyloid peptides (Aβ)** damage neurons.



- The Amyloid peptide (**Aβ)** is present on the cell surface having a transmembrane domain. In normal conditions, cell surface proteins don't stay there for long but instead, they are cleaved (shed) in a process known as **Protein-shedding** via the activity of enzymes known as **Proteases (** such as **α-secretase)** allowing for protein renewal (Replacement).

- **In Alzheimer's disease however**, there is an abnormality in the protein-shedding process resulting in the release of the **Hydrophobic transmembrane regions** of the Amyloid molecules followed by subsequent aggregation of these regions into **Amyloid plaques** in the Extracellular environment. (Look at the figure below)

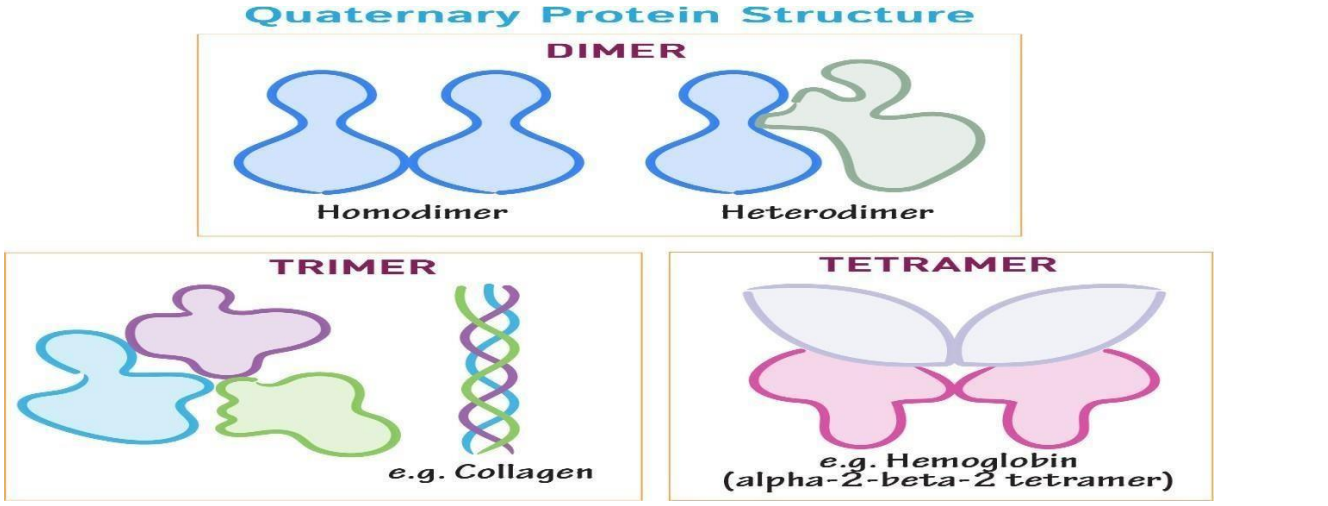


• Quaternary structure

➢ Some Proteins are composed of more than one polypeptide chain thus having a 4th level of organization known as the **Quaternary structure**. They are **oligomeric proteins** (oligo = a few or small or short; Mer = part or unit).

➢ **the Quaternary structure** of a protein is the spatial arrangement of subunits and the nature of their interactions.

Example on the arrangement of subunits: An Oligomeric protein can be composed of 3 polypeptide subunits **(α, β and γ),**In which the alpha subunit is connected to beta and beta is connected to gamma (There is no direct connection of Alpha and gamma).



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➢ **Oligomeric proteins can be composed of** :-

1. **Two polypeptide subunits** : Dimer with the simplest one being a homodimer (2 identical subunits)
2. **Three subunits**: Trimer
3. **3 subunits, 4 subunits …. etc.**

The number of subunits within the structure of an oligomeric protein can reach up to 60 subunits, which is the case for a protein known as **Pyruvate dehydrogenase.**

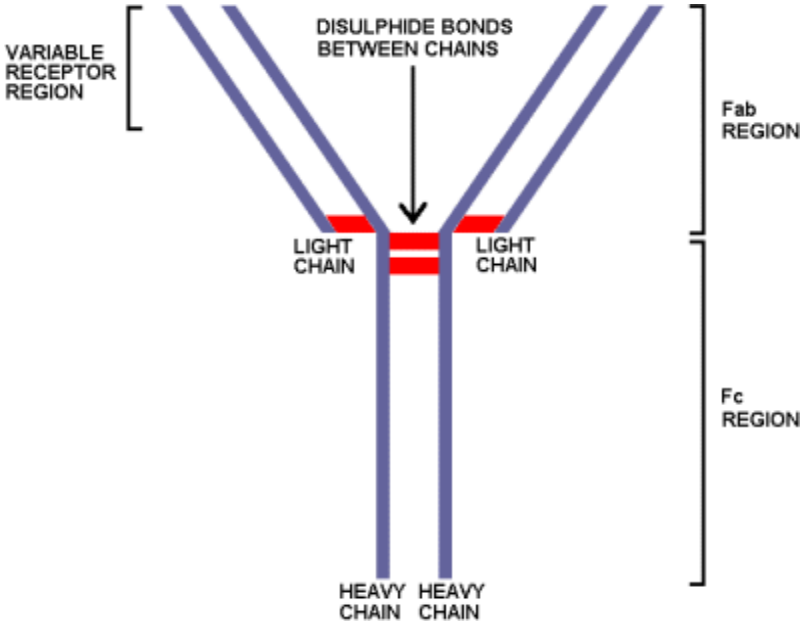
To summarize :-

- Each polypeptide chain is called a **subunit**.
- Oligomeric proteins are made of multiple polypeptides that are
  - identical → homooligomers (homo = same), or
  - different → heterooligomers (hetero = different)
- **Oligomer** sometimes refers to a multisubunit protein composed of identical subunits, whereas a **multimer** describes a protein made of many subunits of more than one type.

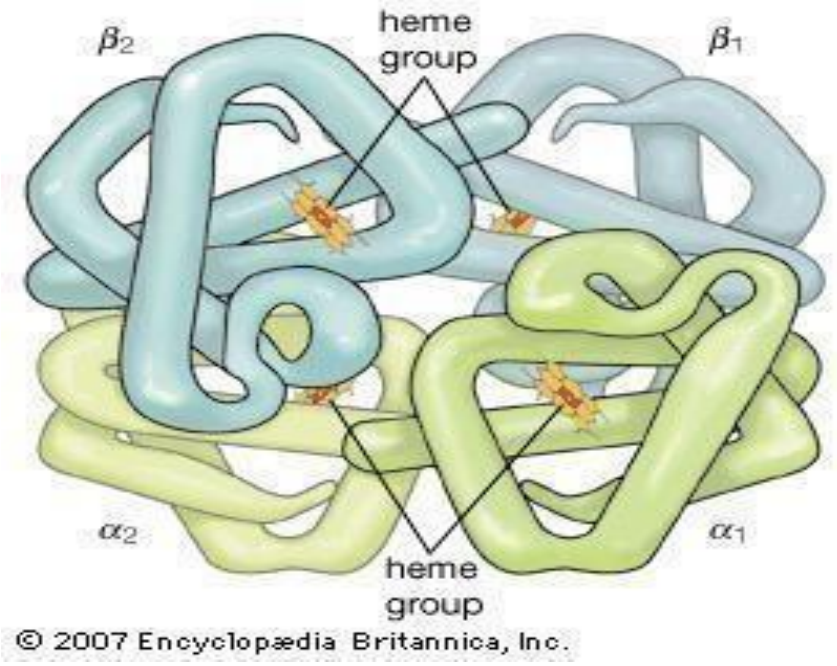
• How are the subunits connected ?

In principle, the type of the linkage holding the subunits together within the quaternary structure depends on the protein itself.

- Sometimes the subunits are linked via **covalent disulfide bonds**. An example is the tetrameric protein known as **Immunoglobulin** (composed of two heavy chains and two light chains).



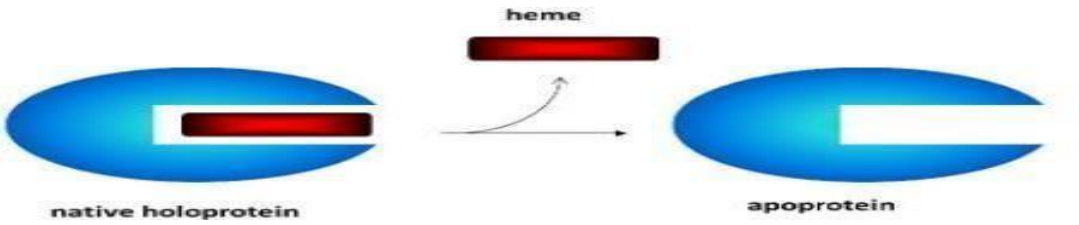
- In other cases, the subunits are connected via **noncovalent interactions**. An example is the tetrameric protein known as **Hemoglobin** responsible for Oxygen transport, which is composed of two alpha subunits, two beta subunits linked together by noncovalent interactions.



• Complex protein structures

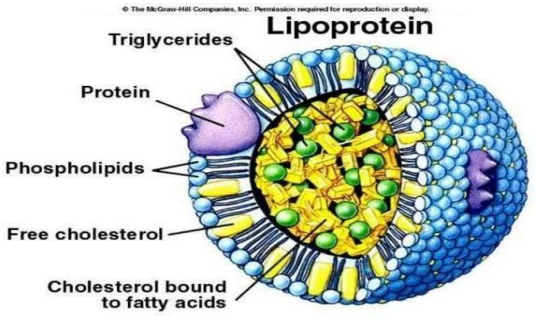
Are Proteins associated (covalently linked) with another non-protein groups, known as **Conjugate proteins.**

- When a protein is conjugated to a non-protein group **covalently**, the non-protein group is known as a **prosthetic group** and the conjugated protein known as a **Holoprotein**.
- If the non-protein component is removed, the protein is now referred to as an **Apoprotein.**

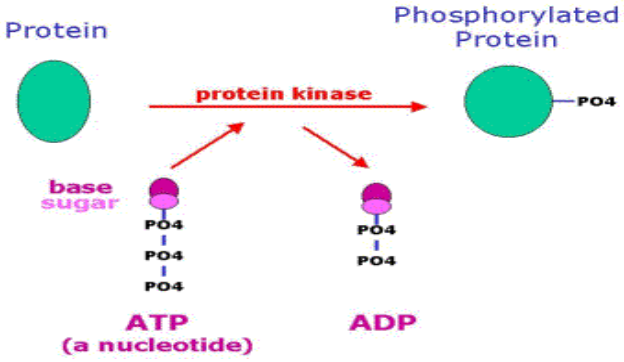


• Examples of complex protein structures

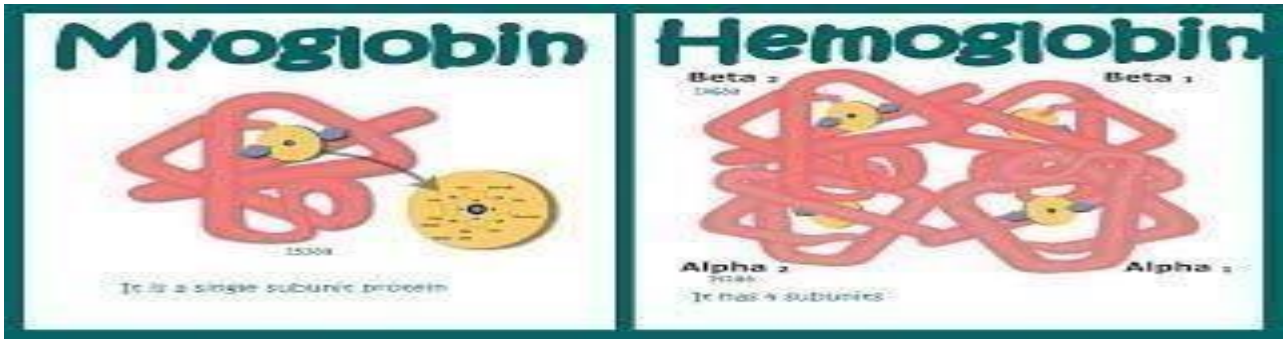
- **Lipoproteins**: Proteins associated with lipids. However, if we remove the lipid component, we refer to them as **Apolipoproteins**.



- **Phosphoproteins**: Proteins that are phosphorylated via enzymes known as **Protein kinases**.

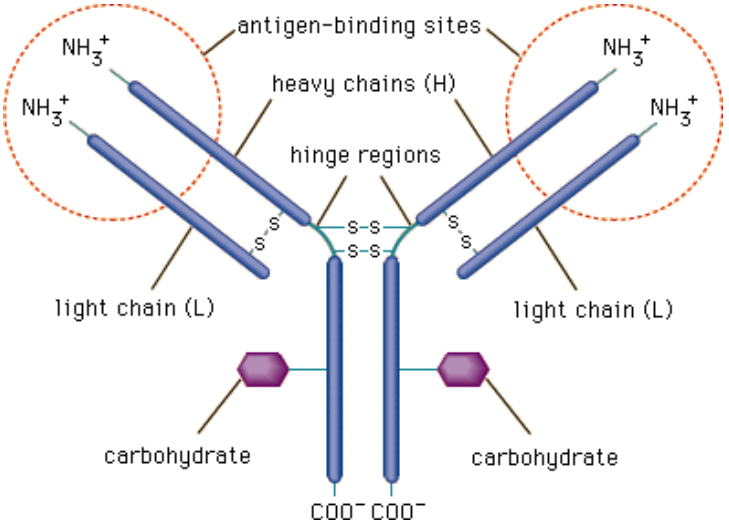


Hemoproteins: Proteins with a heme group such as **Hemoglobin and myoglobin**.



Nucleoproteins: proteins with a nucleic acid.

Glycoproteins: Proteins that are associated with Carbohydrate groups such as **Immunoglobulins**.



- ## Classes of Glycoproteins

- **N-linked sugars:** These are linked via the nitrogen atom of the amide group of **Asparagine** (Asn) residues**.**
- **O-linked sugars**: These are linked via the oxygen atom of the hydroxyl group of both **Serine, Threonine** and occasionally, **modified lysine residues** known as **hydroxylysine**.