Doctor 022

# Biochemistry

Sheet no. 16


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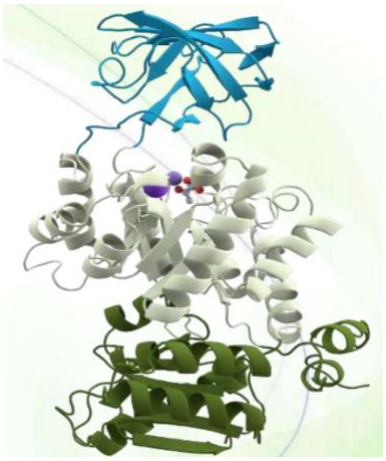
# Domains

**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. They are relatively large structures.**

**An important characteristic is that Domains fold independently of the rest of the protein or of other domains within the same protein.**



**As a result, if I cut the domain from the rest of the protein, it can still fold into its original 3D structure. And still Maintains its function.**

**It is independent in its structure and function (but sometimes, to do its function efficiently it needs other domains).**

**This also explains how Genetic Engineering works. I can genetically engineer proteins, by adding different domains to them.**

**Similar domains can be found in proteins with similar function and/or structure and can be present in different proteins.**

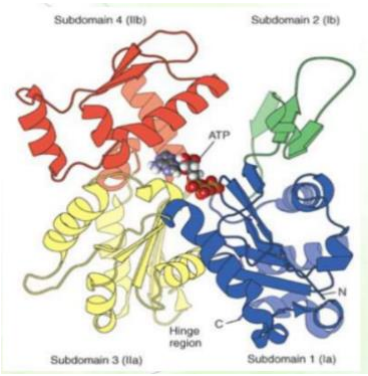
**Domains may also be defined in functional terms:**

- **Enzymatic activity (ex: catalytic domain).**
- **Binding ability (e.g., a DNA-binding domain).**

**In the primary structure of the motifs (the amino acid sequence) the amino acids of the motif must be close to each other in order to be connected in the secondary structure. but in the domain, the amino acids in the primary structure can be far away from each other, then when they form the secondary structure, those secondary structures can be away from each other. But when they fold in the tertiary structure they will come together and form the domain. (This was a question from one of the students, it is okay if you don't understand it).**

# Folds

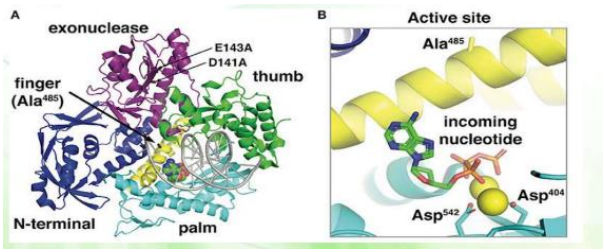
**When large patterns of secondary structures or multiple domains within a protein possess specific functions, they are known as Folds.**




- **The actin fold.**
- **The nucleotide-binding fold.**

**Simply speaking, a fold is a structure that is composed of multiple domains which perform a specific function when merged, they can fold independently. You can think of it as a quaternal structure but in one protein.**

**A fold is one peptide, composed of multiple domains connected to perform a specific function. But quaternary structure is composed of more than one peptide (we will study it later).**



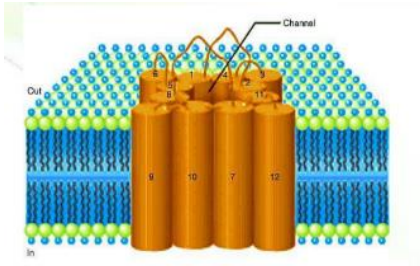

**Sometimes a peptide can be composed of more than one fold.**

# α-helices as transmembrane domains

**Membrane-spanning proteins contain a transmembrane domain that is an α–helix made of hydrophobic amino acids.**

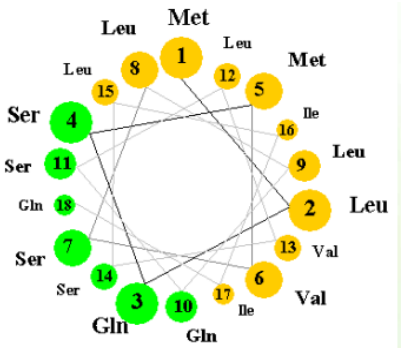
**Some membrane proteins contain several transmembrane domains that are also α-helices.**

**And when multiple a-helices domains gather, they can form a channel. Like in the following picture:**



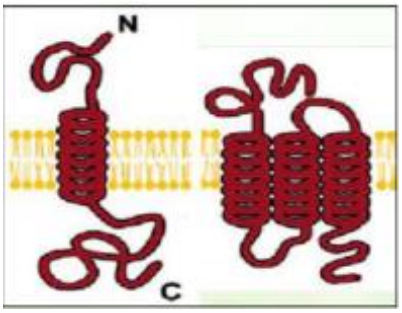

**For receptors, the helices are connected by loops containing hydrophilic amino acid side chains that extend into outside of both sides of the membrane.**

**Membrane ion channels contain amphipathic α–helices.** **They have a hydrophobic domain residing in the membrane and a hydrophilic region exposed to the outside so ions can pass through it without repulsion.**




**If we look at amphipathic a-helix from above, this is what the amino acids composing it looks like:**

**Look at the figure below, one a-helices that spans the membrane will be hydrophobic. But if it is a channel, it will be amphipathic.**





**If you look at the primary structure you will notice that some of them are polar, and some are hydrophobic. When they fold, they will be orientated (hydrophobic outside and polar inside).**

# Denaturation and Renaturation

## 1.Denaturation

**Denaturation is the disruption of the native conformation of a protein via breaking the non-covalent bonds that determine the structure of a protein, so it returns into its original primary structure (peptide).**

**Complete disruption of tertiary structure is achieved by reduction of the disulfide bonds in a protein.**

**The denatured protein loses its properties such as activity and becomes insoluble. There are many.**

### Denaturing agents:

- **Heat: Heat disrupts low energy van der Waals forces, hydrogen bonds, hydrophobic interactions and any non-covalent interaction in proteins by increasing kinetic energy of electrons.**

- **Extreme pH values: change in the charge of the protein's amino acid side chains (electrostatic and hydrogen bonds). Very high or very low pH levels can disrupt charges causing some bonds (salt bridges for example) to break.**

- **Detergents: Triton X-100 (nonionic, uncharged) and sodium dodecyl sulfate (SDS, anionic, charged) disrupt the hydrophobic forces.**

  **The first type is (nonionic, uncharged) detergents, that make a hydrophobic environment around the protein causing internal hydrophobic residues in proteins to become external, changing the shape of the protein, therefore denaturing it. An example of nonionic detergent is Triton X-100.**

  **The second type is (anionic, charged), which acts the same as nonionic detergents but also gives the protein a charge, which might disrupt electrostatic interactions. An example of anionic detergent is Sodium Sodecyl Sulfate (SDS), which can be found in shampoos.**

- **Urea and guanidine hydrochloride: they disrupt hydrogen bonding and hydrophobic interactions.**

- **Reducing agents: β-mercaptoethanol (β-ME) and dithiothreitol (DTT), They reduce disulfide bonds leading to complete denaturation of protein.**

## 2.Renaturation:

**Renaturation is the process in which the native conformation of a protein is reacquired. Renaturation can occur quickly and spontaneously, and 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.

**What determines the structure of the protein is basically The Energy.**

**The polypeptide (the primary structure of protein) folds and changes its shape, angles and orientation, forming non-covalent bonds to get the most stable structure, which is the structure with least energy. These shapes, angles and orientations are determined by the sequence of amino acids in the polypeptide.**

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

- The amino acid sequence (the primary structure), mainly the internal residues.
- The proper angles between the amino acids

the different sets of weak noncovalent bonds that form between the mainly the R groups.

Non-protein molecules. **Non protein groups might also be needed to give the protein its structure and function. Like metals (iron in hemoglobin), or lipids (lipoproteins), or nucleotides (ribosomes).**

## Chaperones:

**As we said before, renaturation is spontaneous, but that's only true for small polypeptides. Some big or highly hydrophobic polypeptides need assistance to refold, which is provided by Chaperones.**

**Chaperones are** proteins that bind to polypeptide chains and help them fold with the most energetically favorable folding pathway**.** 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.

## Other proteins that help in renaturing polypeptides:

Cis–Trans Isomerase: that converts a trans peptide bond preceding a proline into the cis conformation, which is well-suited for making hairpin turns. And it can convert cis amino acids into trans amino acids.

Protein Disulfide Isomerase: after the protein has folded, breaks and reforms disulfide bonds between the –SH groups of two cysteine residues.

## Problems of Misfolding

When proteins do not fold correctly, their internal hydrophobic regions become exposed and interact with other hydrophobic regions on other molecules, and form aggregates.

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 they are called (amyloid). And they can be lethal.

Both soluble and insoluble aggregates can be toxic to cells.

## Diseases caused by misfolding proteins:

### 1.Prion diseases:

Striking examples of protein folding-related diseases are prion diseases, such as Creutzfeldt-Jacob disease (in humans), and mad cow disease (in cows), and scrapie (in sheep).

Pathological conditions can result if a brain protein known as Prion Protein (PrP) is spontaneously misfolded into an incorrect form called PrPsc. This misfolded protein causes other non-diseased prion proteins (PrP)s to be misfolded into (PrPsc), aggregating and making amyloids inside the brain. Then spreading to other tissues, it can be infectious and inherited (mutations).

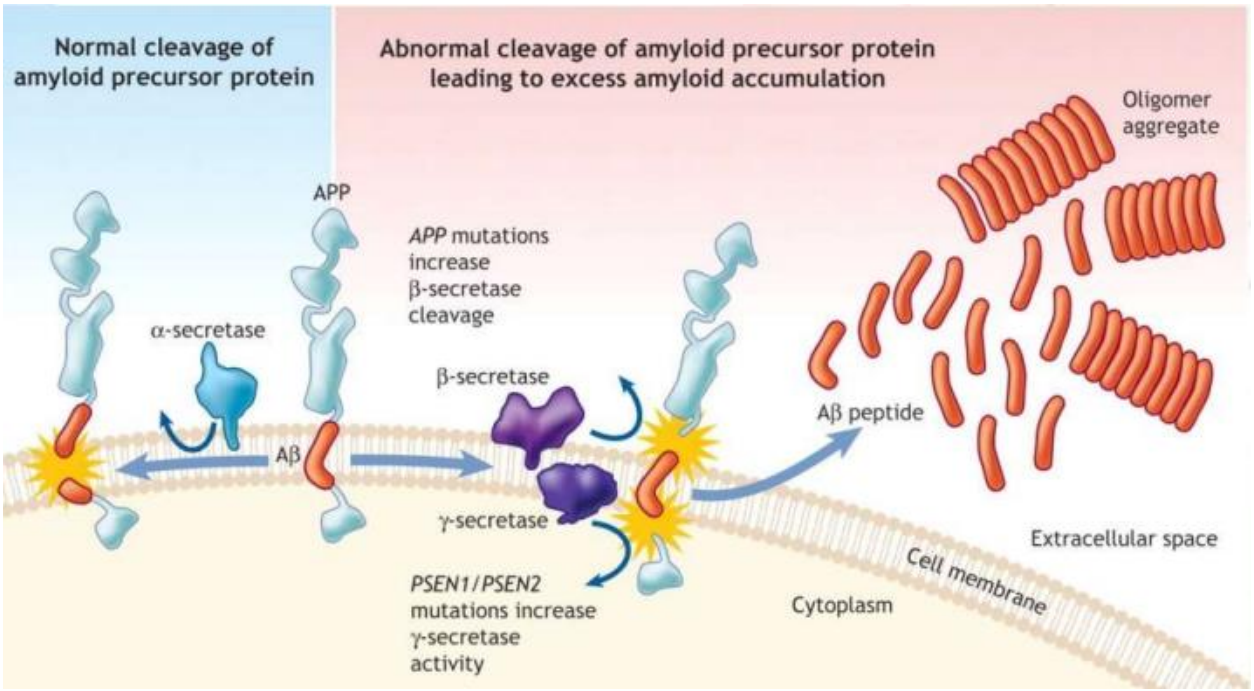
PrPC has a lot of α-helical conformation, but PrPsc has more β strands forming aggregates.

The disease is caused by a transmissible agent.

Abnormal protein can be acquired by Infection, Inheritance or Spontaneous.

## 2.Alzheimer's Disease: Not transmissible between individuals. Extracellular plaques of protein aggregates of a protein called tau and another known as amyloid peptides (Aβ) damage neurons.

**These proteins are hypothesized to follow the same mechanism of PrPsc which is basically protein misfolding, by forming plaques damaging the neurons.**




# Quaternary structure

**Not all proteins have quaternary structure.**

**Quaternary structure exists only in proteins that are made of more than one polypeptide chain, they are mostly transcribed from different genes, some of them are from the same gene.**

**They are oligomeric proteins (oligo (3-10) = a few or small or short; mer = part or unit)**

**The spatial arrangement of subunits and the nature of their interactions.**

Proteins made of

One subunit = monomer

Two subunits: dimer

The simplest: a homodimer

Three subunits: trimer

Four subunit: tetramer ...etc

**The subunits may be counted by alpha, beta, gama.**

**Each polypeptide chain is called a subunit.**

**-Oligomeric proteins are made of multiple polypeptides that are**

**• identical → homooligomers (homo = same).**

**• different → heterooligomers (hetero = different).**

**• Oligomer sometimes refers to a multisubunit protein composed of identical subunits, whereas a multimer (or protomer) describes a protein made of many subunits of more than one type (different subunits)**

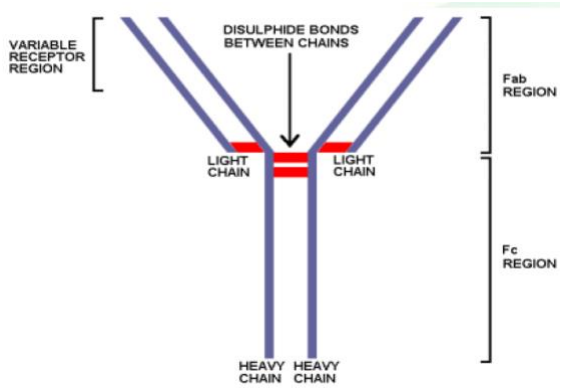
Alpha-Alpha → oligomer

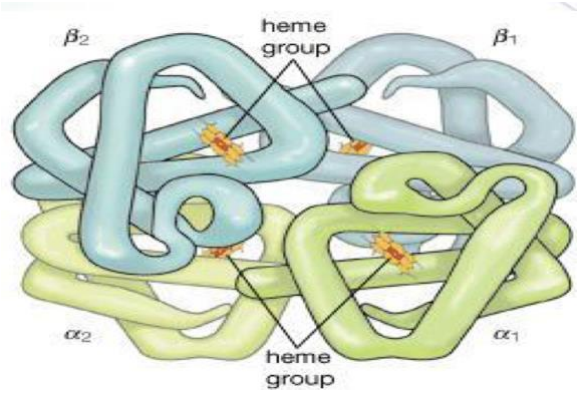
Alpha-Beta → multimer or protomer

- How are the subunits connected?

They are connected covalently (disulfide bonds) or non-covalently(**hydrophobic interactions, van der waals interactions,hydrogen bonding, salt bridges.**)

-non-covalent bonds stabilize interactions between subunits




**-Remember that hemoglobin composed of 4 subunits (2 alpha, 2 beta), they are connected to each other via hydrophobic interactions or disulfide bonds (covalently as immunoglobulin which is composed of four subunits (2 heavy,2 light)), the type of connection depneds on the type of proteins.**

**-The hydrophobic amino acids, which are found on the outer surface, lead to form single protein (As hemoglobin), from its subunits.**

## -Complex protein structures

**-Sometimes, for proteins to be functional, they need non-protein groups.**

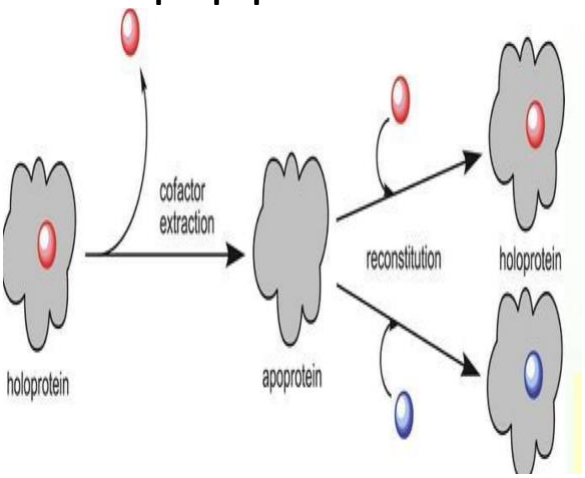
**Ex:Hemoglobin, globin is a protein,heme group (contains iron atom) is a non-protein group.**

**When a protein is conjugated to any associated non-protein components, such as prosthetic groups or metal ions, the protein is known as a holoprotein (AKA a conjugated protein). AKA= also known as.**

**If the non-protein component is removed, the protein is known as an apoprotein, it represents protein part ONLY.**

**In other words, it is the protein portion of a conjugated protein without the attached non-protein group.**

**-In case of lipoprotein, if the lipid (non-protein group) is removed, the structure is called apolipoprotein.**




**Coenzymes: complex organic molecules that assist enzymes in catalyzing biochemical reactions**

**Prosthetic groups: Coenzymes or metals that are tightly (covalently) bound to proteins.**

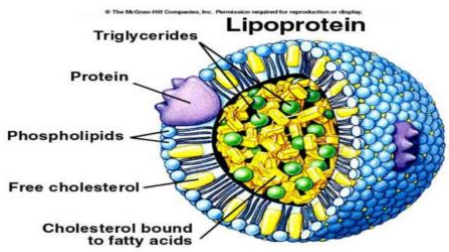
**-Other names of conjugated proteins**

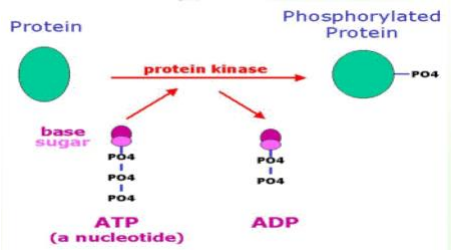
**1)Lipoproteins: Proteins associated with lipids**

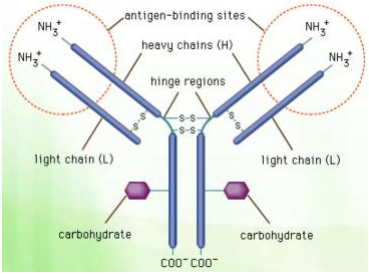
**2)Phosphoproteins: proteins that are phosphorylated**

## 3)Hemoproteins: proteins with heme Nucleoproteins: proteins with a nucleic acid

## 4)Glycoproteins: proteins with carbohydrate groups




**Classes of glycoproteins**

- **N-linked sugars: are carbohydrates (anomeric carbon) that are covalently attached to the nitrogen (N) atom of asparagine (Asn) residues in proteins.**

**The amide nitrogen of the R-group of asparagine(polar amino acid contains nitrogen atom)**

- **O-linked sugars: are carbohydrates (anomeric carbon)that are covalently attached to the oxygen (O) atom of serine (Ser) or threonine (Thr) residues in proteins.**

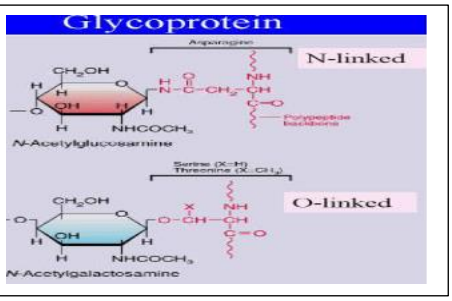
**The hydroxyl groups of either serine or threonine(polar amino acids contain hydroxyl group)**

**Occasionally to hydroxylysine such as in collagen.**

وليس الغنى إلا غنى العلم إنه ... لنور الفتى يجلو ظلام افتقاره

ولا تحسبنَّ العلم في الناسِ منجيا ... إذا نكبت أخلافُهم عن منارهِ

وما العلم إلا النورُ يجلو وجى العمى ... لكن تزيغُ العينُ عند انكساره




إذا رُمتَ أن تحيا سليماً من الأذى ... ودينُكَ موفورٌ وعرضُكَ صَيِّنُ

لسانَكَ لا تذكُرْ بِهِ عَورَةَ امرئٍ ... فَكُلُّكَ عوراتٌ وللناسِ ألسُنُ

وعَينَاكَ إن أبدَت إليكَ مَعايِباً ... فَدَعها وقُل يا عَينُ للناسِ أعيُنُ

***<u>The End of Mid term material, Good Luck.</u>***