بسم الله الرحمن الرحيم

BIOCHEMISTRY

Lecture 16

protein structure (pt. 3)

"هذا النهجُ ولا تبديل "

31/7

رحم الله الإسماعيلان

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remember from the previous lecture :

4 levels for protein structures :

1)Primary structure : order/sequence of amino acids .
2)Secondary structure : local patterns in proteins (α helix, β sheets ,Turns ,loop).
3)Tertiary structure : how proteins look like in 3D (fibrous ,globular).
4)Quaternary structure.

Multiple **β strands**

DOMAINS :

It is a combination of secondary structures that are far apart from each other, but when the protein folds, these structures come together and form a specific pattern.

Part of tertiary structure , may consist of 100–200 residues.

- Definition : **super secondary structure**, made of combination of multiple secondary structure(The units are slightly separated from each other) (α helices and/or β-sheets) connected to each other by (turns, loops, coils) organized in a specific 3 dimensional structure .
- 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
- Enzymatic activity .
- Binding ability (e.g., a DNA-binding domain).

Domains VS Motif

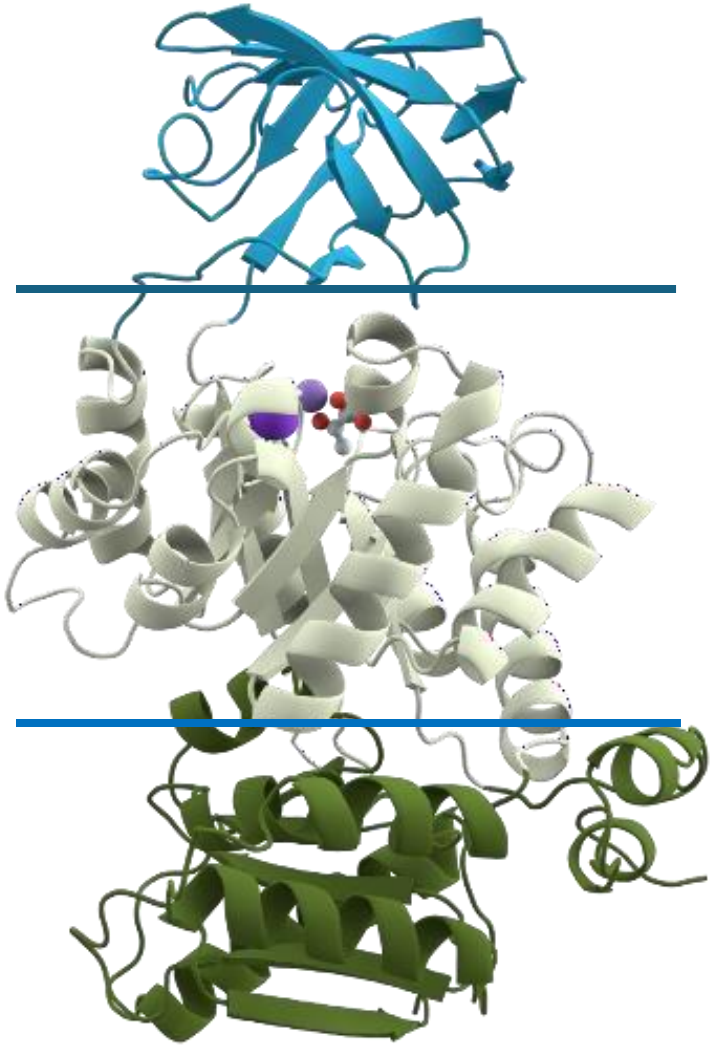
• It is a combination of secondary structures that are far apart from each other, but when the protein folds, these structures come together and form a specific pattern. • 100-200 Larger in size . • Associated with a certain function. • Found in protein that have similar function and purpose ,like Actin-binding proteins (function : to allow the protein to bind to actin and do what ever). • We will see the same domain in several proteins (all of them bind to actin by Actin-binding domain).	• secondary structures are closer to each other .(before folding) • 20-30 amino acid / smaller. • Not necessary to have a function . • May be found in DNA binding protein ,membrane receptor, enzymes . Protein with different function unrelated to each other

Folds independently of the rest of the protein and maintains its function.

- This is a single protein composed of one polypeptide chain, which contains multiple secondary structures, forming a supersecondary structure.
- If I cut part of it and put in an aq solution, the resulting fragment may retain its structure and function.

- In genetic engineering, if a segment of the polypeptide is isolated and placed in solution, it may maintain the same structure and function as it did within the full protein.

مع جين ثاني جزء من جين وبحطه التلاعب بالجينات باخد

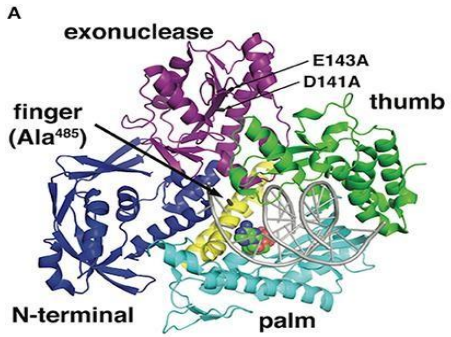


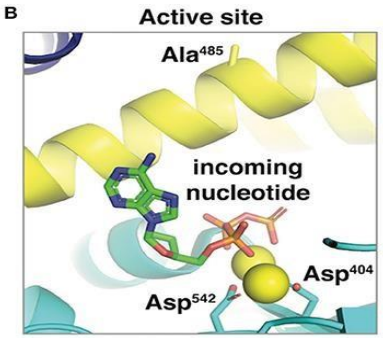
صلّ على الحبيب~

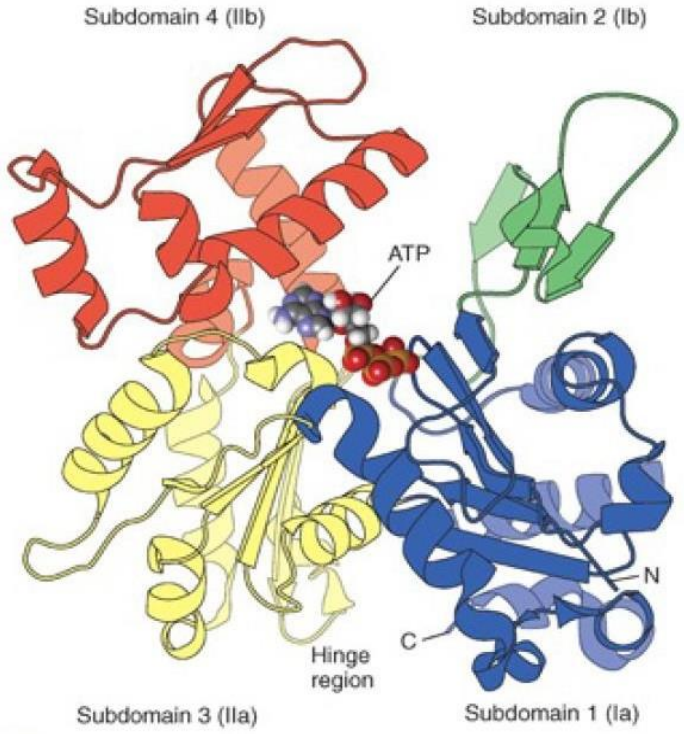
Folds : combination of domains that have a certain function/purpose/structure .

- When large patterns of secondary structures or multiple domains (in 3D) within a protein
  possess specific functions, they are known as **Folds**.
  - The actin fold
  - The nucleotide-binding fold

Tertiary structure: 3D shape of protein , (domains and folds are specific arrangements within this structure).
Super secondary structures : simpler structural motifs formed by the combination of a few adjacent secondary structures (like motifs)
Domains and folds consider as super secondary structures, but domains can be part of tertiary or quaternary protein.





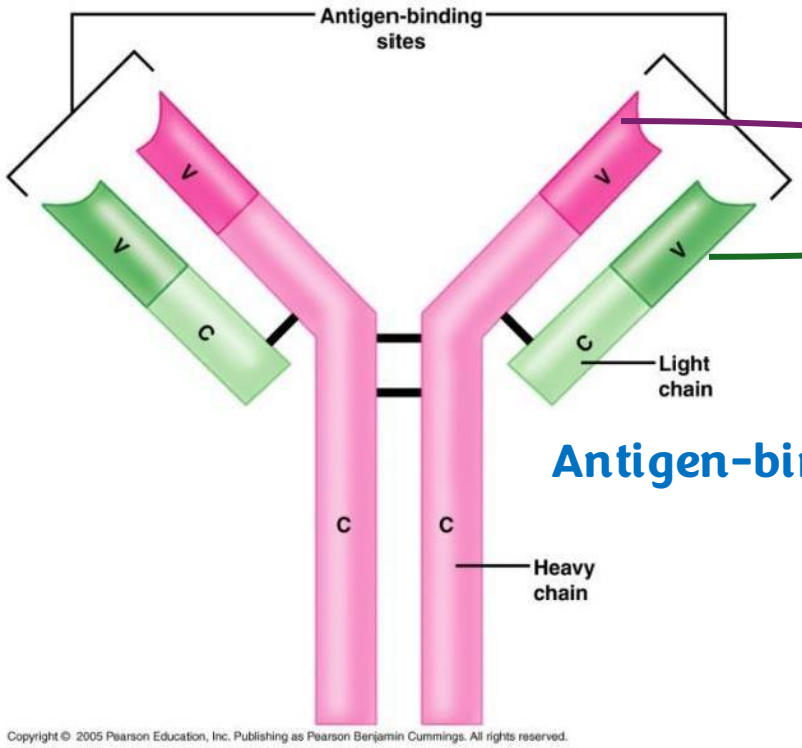


4 Domain
If I took out the red one ~>it would adopt its structure.
If I took out the yellow domain~> it would fold into the same structure as well.
For the protein to perform its function, all four domains must be together.
This is why we refer to them as a fold.
It is **not necessary** for each domain to have an individual function, but they must all be together to perform a collective function.

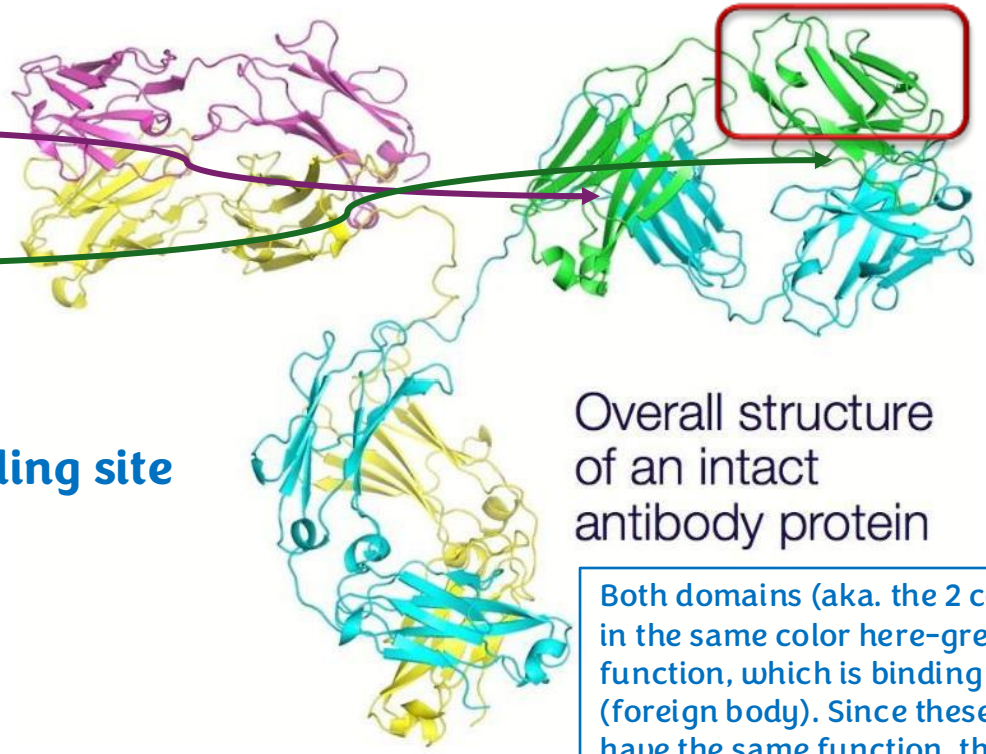
Levels of complexity:
Primary – secondary – super secondary (like motifs , domains, and folds)-tertiary – qurtirnary .

A more complex motif/domain/fold is…

- The immunoglobulin (Antibody) fold or module that enables interaction with molecules of various structures and sizes.
- **immune cells specially B cells secrete it to fight antigens (foreign body)**



Antigen-binding site



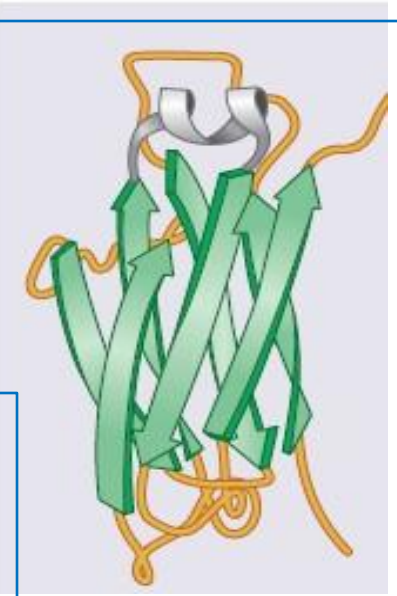
Overall structure of an intact antibody protein

- consecutive multiple secondary structures.

B strand +loop ورا بعض

- By definition it is a motif but it is more complex (so it's called a complex motif), some textbooks call it Domain as well.

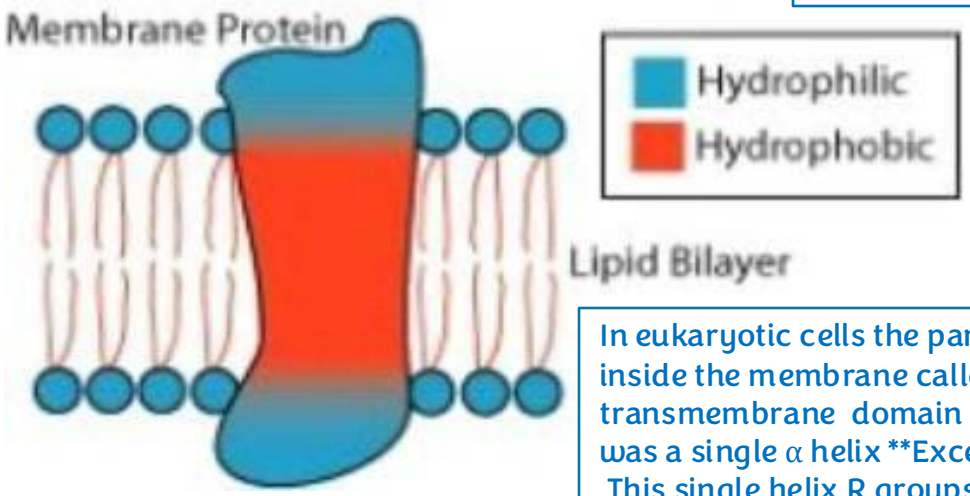
Both domains (aka. the 2 complex motifs in the same color here-green) have a function, which is binding to an antigen (foreign body). Since these two domains have the same function, they are referred to as a fold.

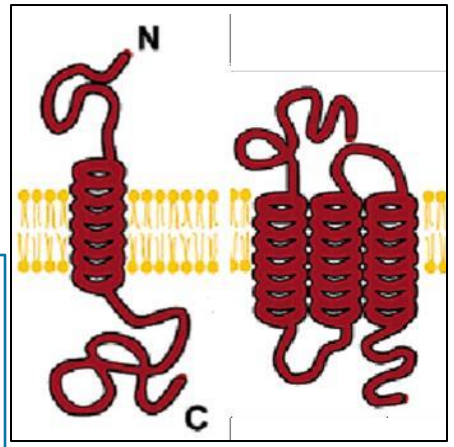


α-helices as transmembrane domains

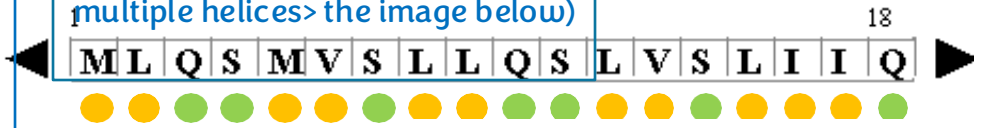
Integral membrane proteins
Or membrane-spanning proteins

- Membrane-spanning proteins contain a transmembrane domain that is an α–helix made of hydrophobic amino acids.
- The α–helices of proteins with multiple transmembrane domains are connected by loops containing hydrophilic amino acids located outside of the membrane.
- Membrane ion channel proteins contain amphipathic α–helices.

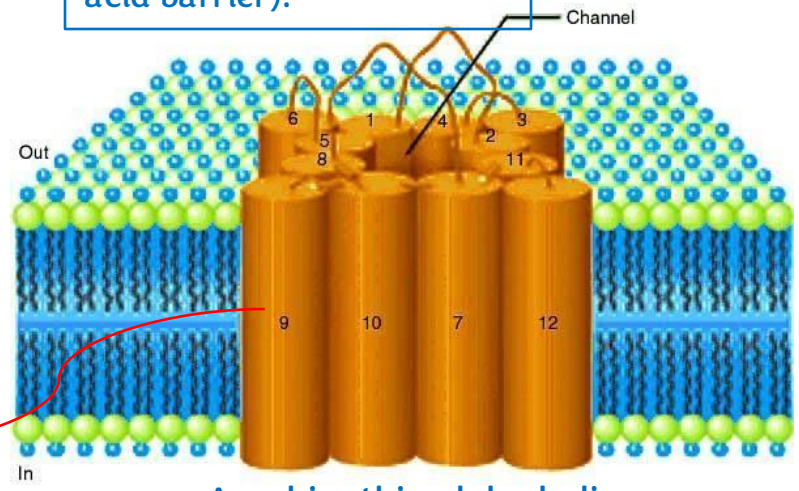




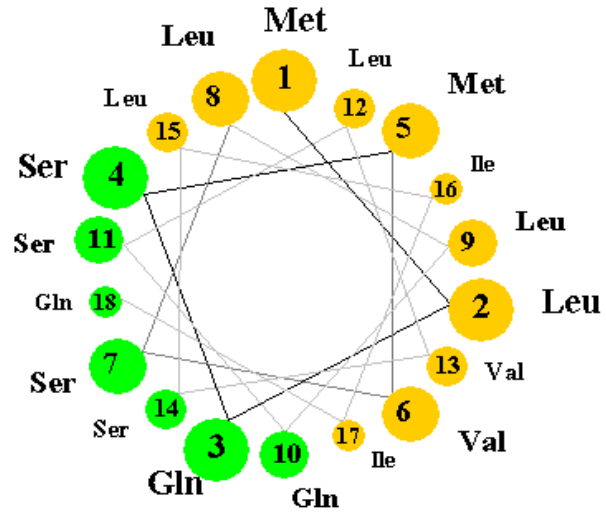
In eukaryotic cells the part inside the membrane called: transmembrane domain even if it was a single α helix **Exception** This single helix R groups will be ONLY hydrophobic (when there's multiple helices> the image below)



Hydrophilic R-groups inside .
Hydrophobic R-groups outside (close to the fatty acid barrier).



Amphipathic alpha helices

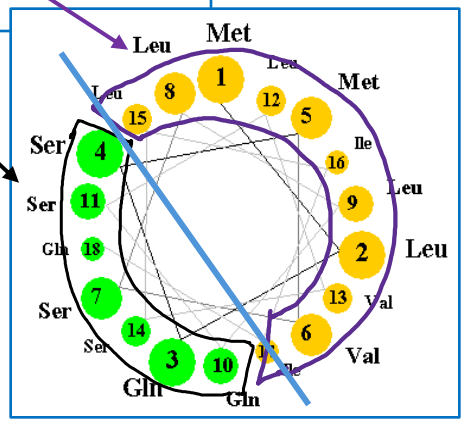


The main function is transporting ions.

- In α-helix, the R groups extend outward and are typically hydrophobic/nonpolar, interacting with fatty acids of the membrane.
- Some proteins have multiple transmembrane domains that span the membrane and extend into the cytosol and outside the cell. Each of these domains is an α-helix, so the R groups in these domains(that span the membrane) are hydrophobic/nonpolar.
- However, the amino acids on the exterior of the cell membrane and within the cytosol are hydrophilic/polar.
- Channels function by allowing ions to pass through, they contain multiple α-helices that make an opening, to help them span the membrane and form openings for ions to enter and exit.

- Alpha helices have a specific orientation of amino acids and R groups.
- We have special α helix look at primary structure it's composed of hydro (philic/ phobic) amino acid .
- When an α-helix rotates, one side has polar amino acids,

while the other side has nonpolar amino acids.



Quaternary structure

All protein have :

- Primary structure .
- secondary structure .
- tertiary structure.

Not all of protein have quaternary structure .

- 3D organization of a proteins that is made by multiple subunits (multiple polypeptides).
- Shows how the polypeptides bind (how they're organized) to each other--> Head to head, tail to tail, head to tail, one on top and the other on bottom.

What is it?

- Proteins have a quaternary structure if they are composed of more than one polypeptide chain.
  - They are oligomeric proteins (oligo = a few or small or short; mer = part or unit) or multimeric proteins.
- *A quaternary structure Is the spatial arrangement of subunits and the nature of their interactions.*

One single polypeptide chain (have primary+ secondary+tertiary)

- For dimers, if the two subunits are :
- identical =homodimer .
- Different = hetrodimer .

- A protein can be a:
  - Monomer (monomeric)

    One subunit
  - Dimer: Two subunits
    - The simplest: a homodimer
  - A trimer: Three subunits A
  - tetramer: Four subunits

    ...etc

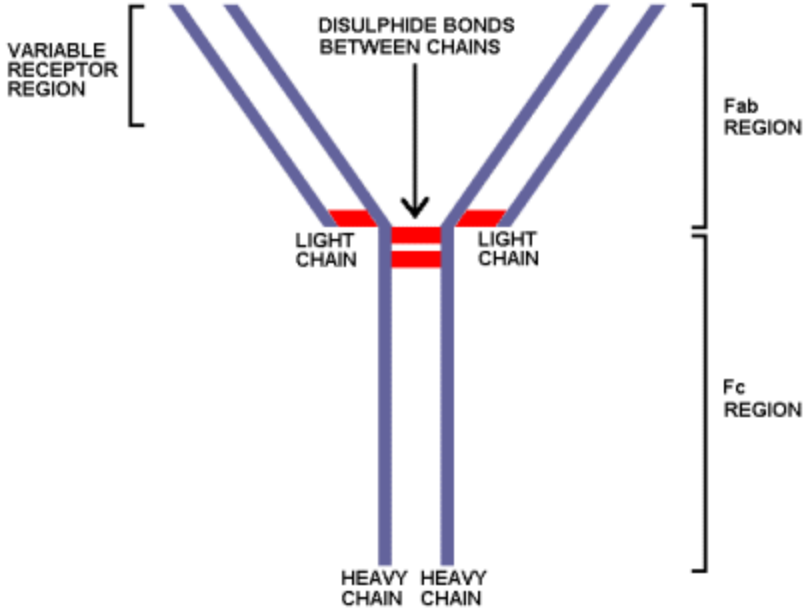
- Each polypeptide chain is called a subunit.
- Oligomeric or multimeric proteins are made of multiple polypeptides that are
  - identical → homooligomers (homo = same)
  - different → heterooligomers (hetero = different)

Each subunit has a name, such as α, β, or γ. If one polypeptide has an α subunit and another one also has an α subunit, they are different and not the same.

How are the subunits connected?

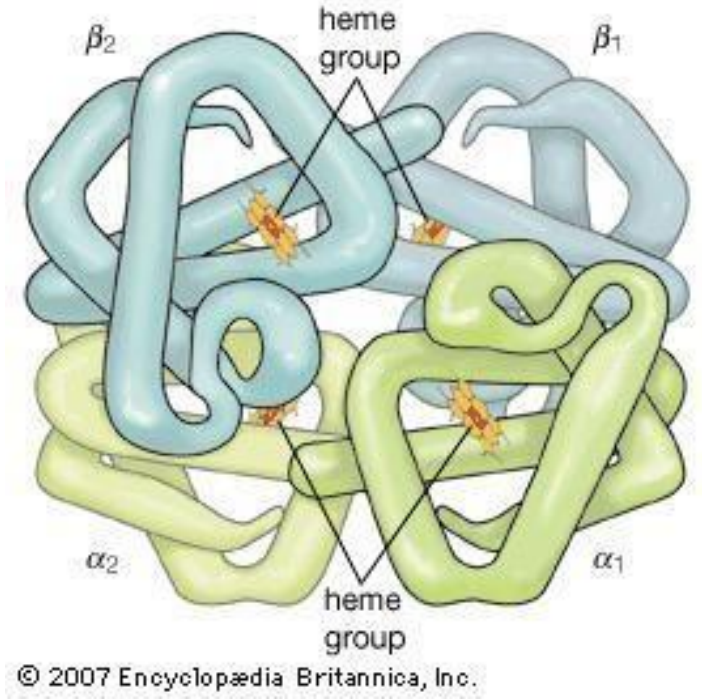
Covalently / noncovalently

- Sometimes subunits are disulfide-bonded together, other times, noncovalent bonds stabilize interactions between subunits



An immunoglobulin consists of four subunits (2 light chains and 2 heavy chains), making it a **heterotetramer**. These subunits are connected **covalently** by **disulfide bonds.**

- The hemoglobin molecule is composed of4 subunits (2 α subunits that are identical to each other and 2 β subunits that are identical to each other). This arrangement is called a heterotetramer.
- The α and β subunits are connected via non-covalent interactions, **(hydrophobic interaction).**
- Normally, hydrophobic amino acids are found inside the protein, while hydrophilic amino acids are on the surface . However, in hemoglobin, hydrophobic amino acids are present on the surface to facilitate these specific interactions.



Properties of Proteins:

Denaturation and Renaturation

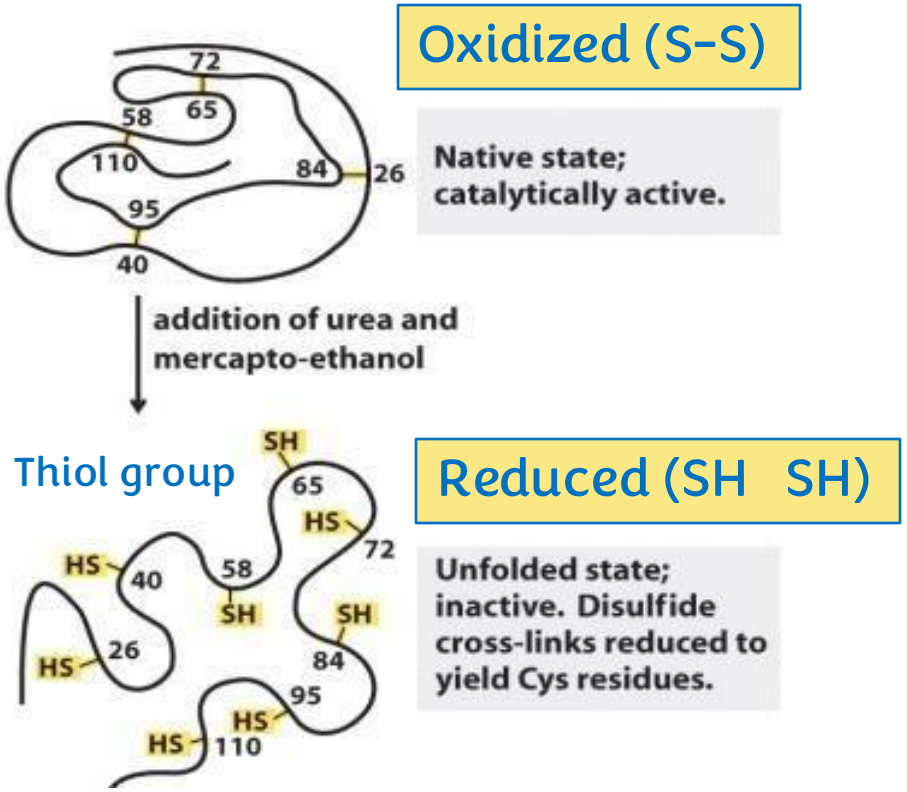
Denaturation

The breakage of **non-covalent** interactions (of all types), which are important for determining secondary, tertiary, and quaternary structures, can lead to the loss of these structures and result in loss of function as well.

- **Denaturation** is the disruption of the native conformation of a protein via breaking the noncovalent bonds that determine the structure of a protein

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

The denatured protein loses its properties such as activity and become insoluble.



- Non-covalent interactions, including hydrogen bonds, are essential for maintaining a protein's secondary and tertiary structures. If these interactions are lost, the protein will lose its 2° and 3° structures. This disruption can also affect the quaternary structure by causing the separation of polypeptide subunits.

Denaturing agents

Hydrophobic interactions are the most important interactions in determining the tertiary structure of a protein.

- **Heat** disrupts low-energy van der Waals forces in proteins
  Increasing the kinetic energy of electrons will disrupt van der Waals interactions.
- **Extremes of pH** (very high & very low PH): change in the charge of the protein's amino acid side chains (electrostatic and hydrogen bonds).

The protonation state of R-groups will change.

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

Fatty acids will move outward because of the new environment

  Detergents create a hydrophobic medium, which disturbs protein structure.
  - SDS also disrupt electrostatic interactions.
- **Urea and guanidine hydrochloride** disrupt hydrogen bonding and hydrophobic interactions.
- **Reducing agents**: β-mercaptoethanol (β-ME) and dithiothreitol (DTT)
  - Both reduce disulfide bonds.

Proteins do not lose their structure solely because of the loss of disulfide bonds; these bonds primarily help stabilize the tertiary structure.

- **Mechanical denaturation**: The protein mechanically loses its function and aggregates or clusters.

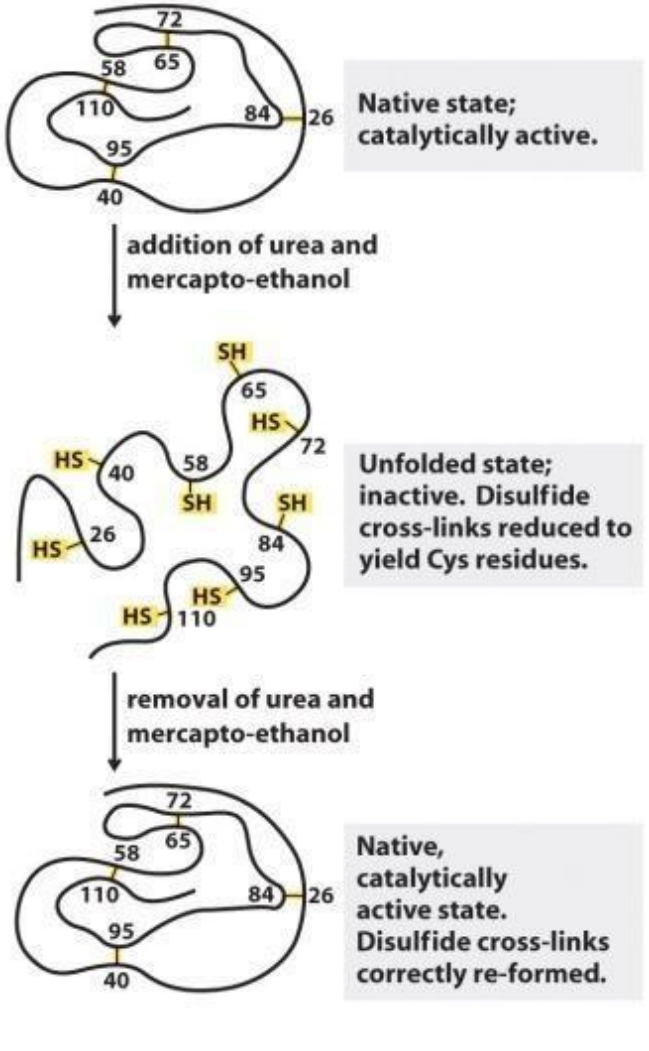
Extra:

- Triton X-100 (nonionic) : Creates a hydrophobic environment, which can lead to changes in the orientation of R-groups.
- SDS (anionic) : Creates a hydrophobic environment and also imparts a negative charge to the protein, affecting electrostatic interactions.

Renaturation

Return to the original stable form and function

- Renaturation is the process in which the native conformation of a protein is re-acquired.
- 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.**



After renaturation, disulfide bonds can reform.

Factors that determine protein structure

The cell aims to synthesize the most energetically stable form of a protein, which depends on the primary structure.

- 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.

Metals for example, they stabilize the structure .

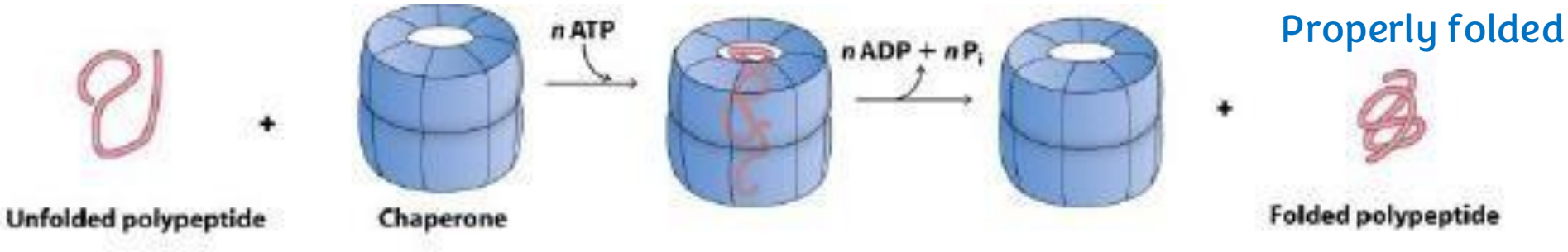
means: companion

Problem solvers: chaperones

Large and hydrophobic proteins do not easily fold independently, **but why ?**

- Large proteins contain a large number of amino acids and secondary structures.
- Hydrophobic regions may avoid the aqueous environment and interact with incorrect amino acids.

- These proteins 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 players

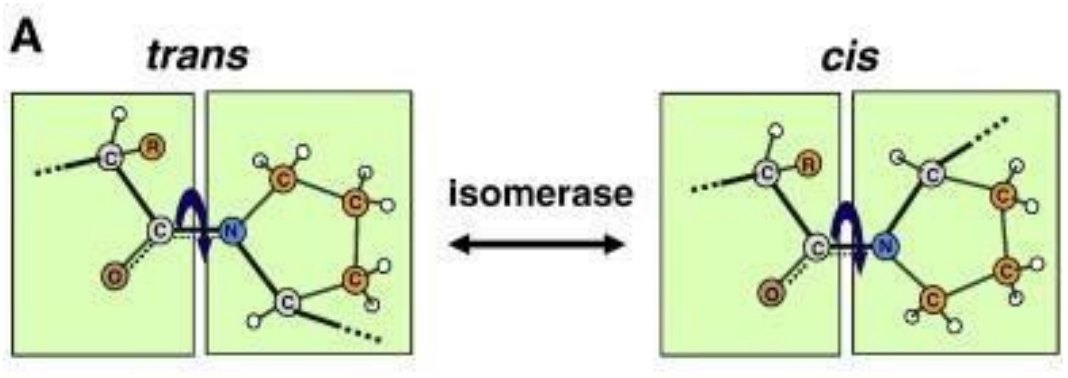
Enzymes that assist in protein folding :

It helps in protein folding.

- A cis–trans isomerase converts a trans peptide bond preceding a proline into the cis conformation, which is well-suited for making hairpin turns.

In both orientations, proline creates steric hindrances.

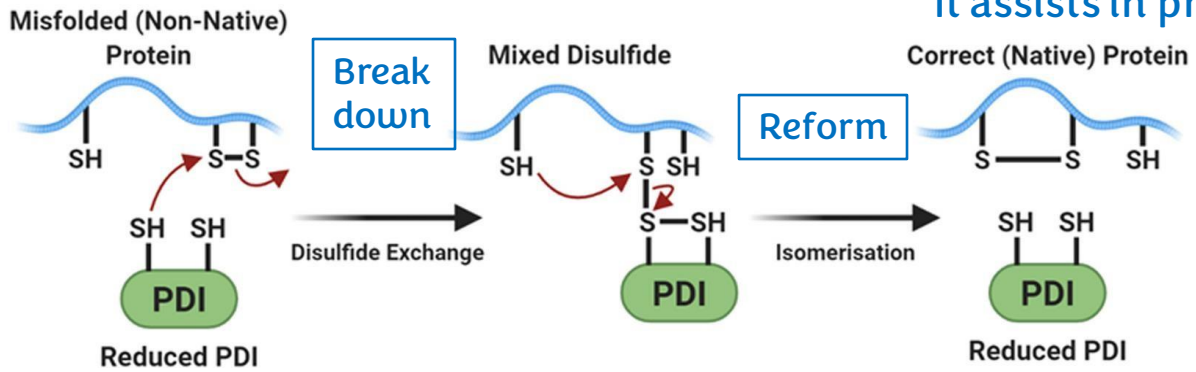
In some proteins, proline must be in the cis configuration, while in others it must be in the trans configuration.



All R groups of amino acids typically follow a rule (they must be in the trans orientation), except for proline, which can be in either the cis or trans orientation.

- A protein disulfide isomerase, after the protein has folded, breaks and reforms disulfide bonds between the –SH groups of two cysteine residues.

It assists in protein stability and folding.



Complex protein structures

Holo- and apo-proteins

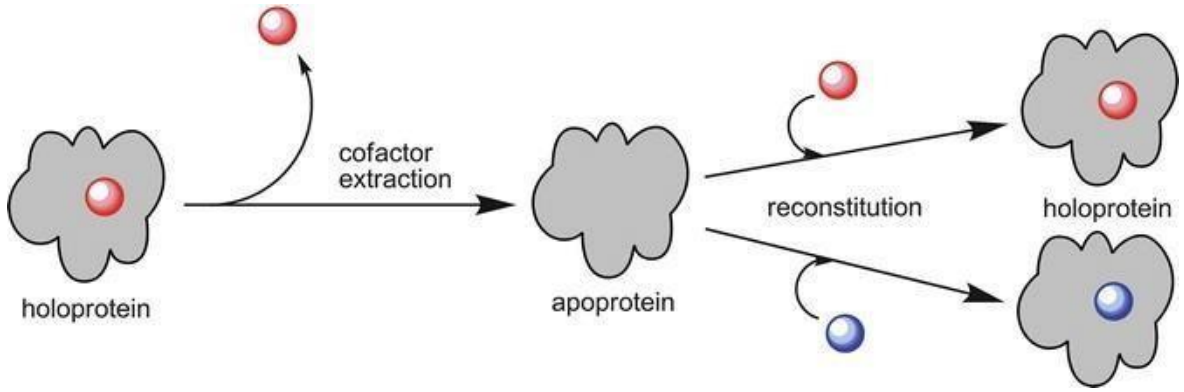
Proteins sometimes need non-protein groups, which help in folding, stabilizing, and maintaining their structure.

Examples of holo proteins are **hemoglobin** and **carbonic anhydrase.**

- 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** (also known as (AKA) a **conjugated protein**).

It is a protein that needs a non-protein group to be associated with it to be functional or to preserve its structure.

- If the non-protein component is removed, the protein is known as an **apoprotein**.
  - In other words, it is the protein portion of a conjugated protein without the attached non-protein group.



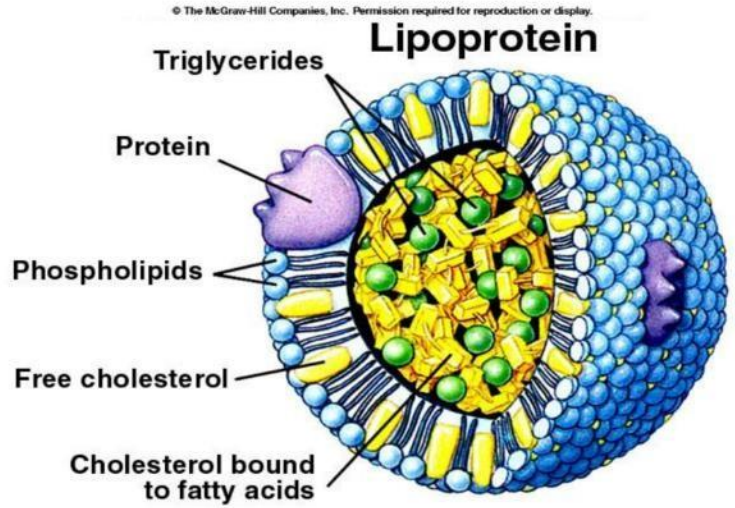
In lipoproteins, the protein portion is called apolipoprotein.

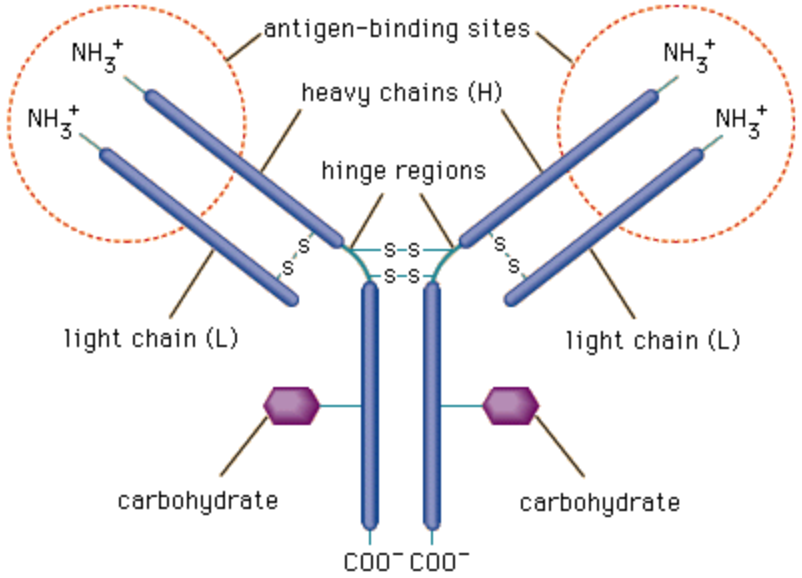
Coenzymes: complex organic molecules that assist enzymes in catalyzing biochemical reactions

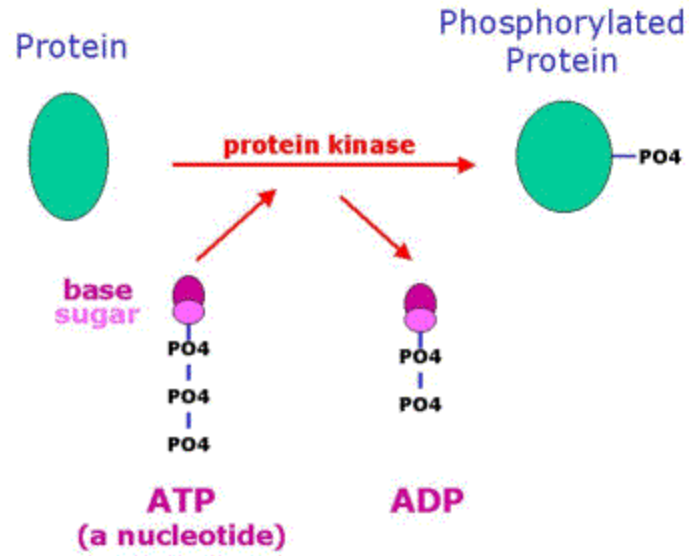
Prosthetic groups: Coenzymes or metals that are tightly (covalently) bound to proteins

Other names of conjugated proteins

- Lipoproteins: Proteins associated with lipids
- Phosphoproteins: proteins that are phosphorylated
- Hemoproteins: proteins with heme
- Nucleoproteins: proteins with a nucleic acid
- Glycoproteins: proteins with carbohydrate groups Like Immunoglobulins.





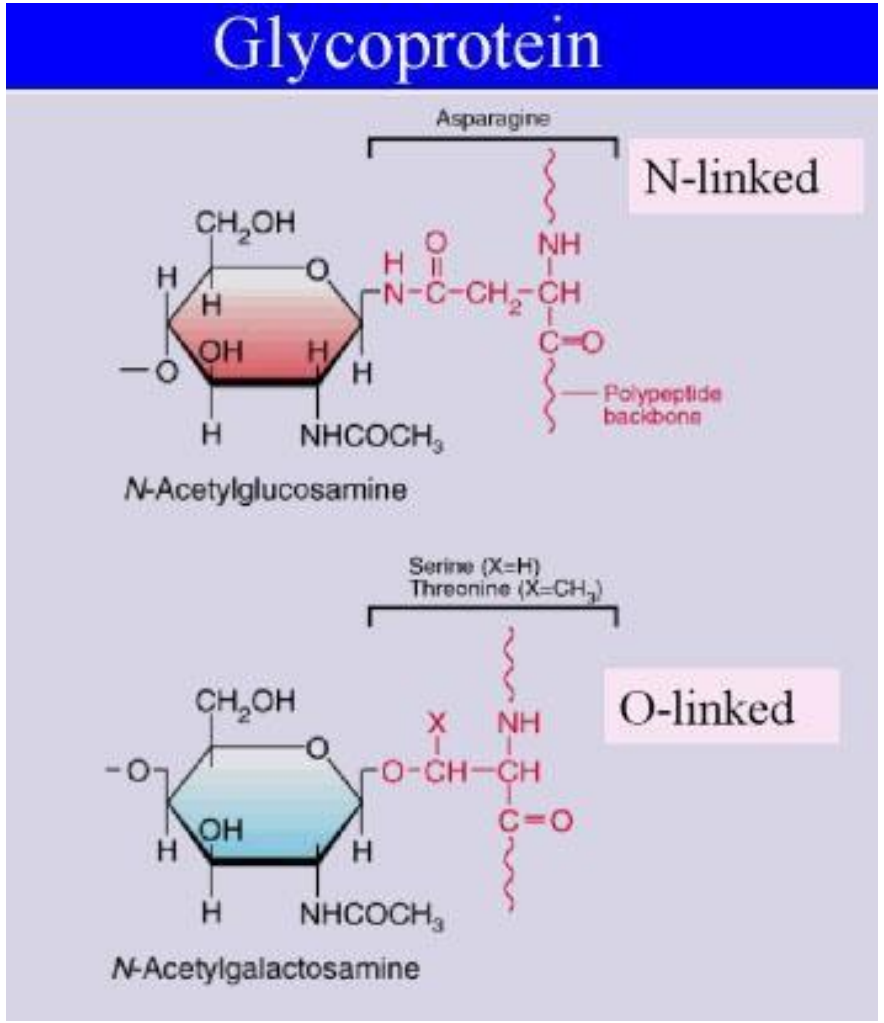


Classes of glycoproteins

Sugars bind to the amino group by a covalent bond, specifically a glycosidic bond.

- N-linked sugars
  - The amide nitrogen of the R-group of Asparagine

    Enzymatically, only asparagine can form an N-glycosidic bond with a sugar molecule.
- O-linked sugars
  - The hydroxyl groups of either serine or threonine (No enzyme to link tyr with sugar- chemically it's possible)
  - Occasionally to hydroxylysine such as in collagen Hydroxylation of lysine enables it to bind with sugars.



The problem of misfolding

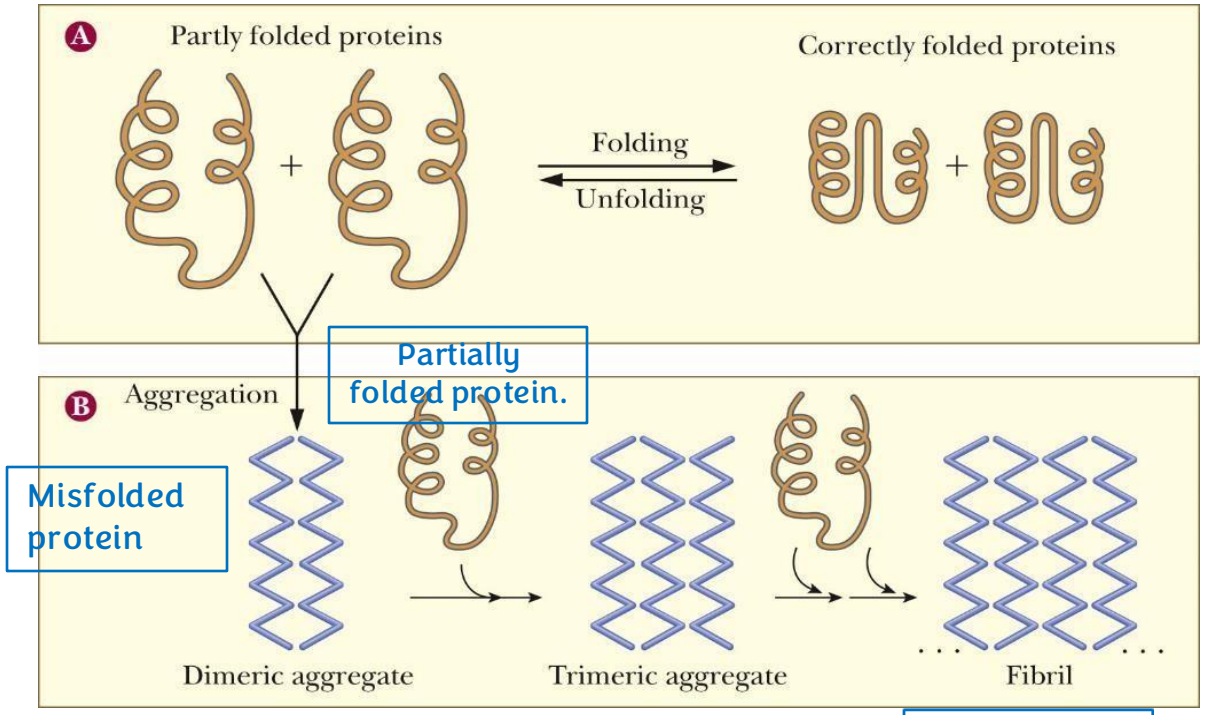
Each protein has the same exact structure . Every time the cell synthesizes the protein, it folds into the same exact structure, which is the most energetically stable form.

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

How does the cell handle protein misfolding?

We have two mechanisms:

- refolding the protein
- degrading it and removing it from the cell.



Sometimes, a misfolded protein can affect a properly folded protein, causing it to become misfolded as well. The reason is not revealed yet .

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 elongated structures (called amyloid).

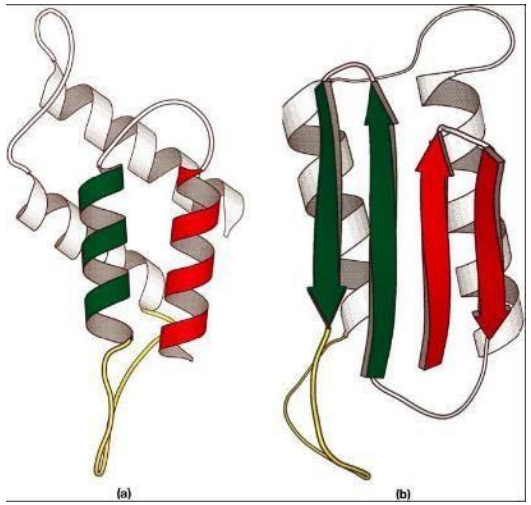
This aggregate is toxic to the cell and can lead to cell death.

- Both soluble and insoluble aggregates can be toxic to cells.

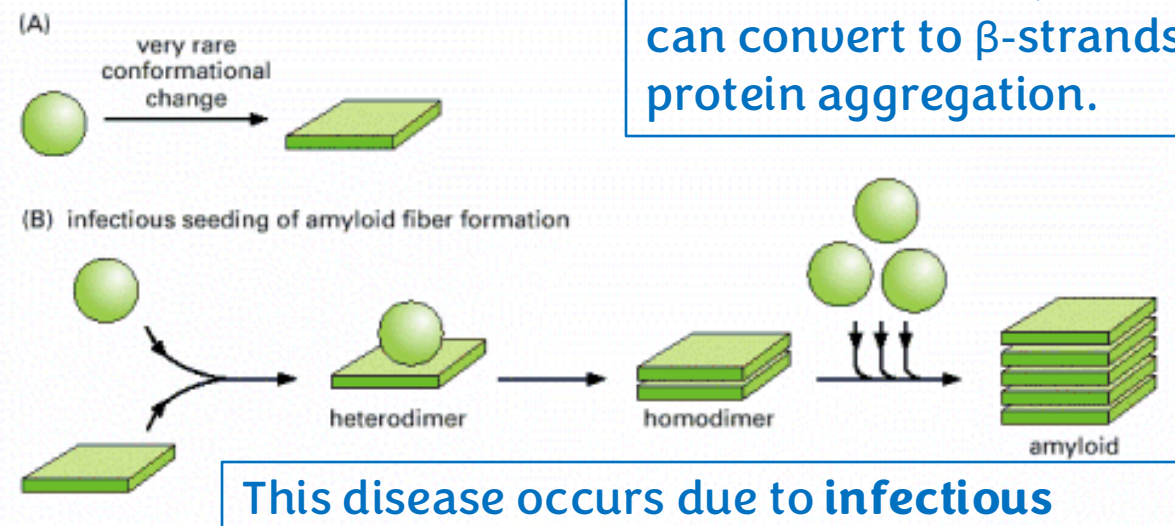
Prion disease

Caused by misfolded protein.

- 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 to as prion protein (PrP) is misfolded into an incorrect form called PrPsc.
- PrPC has a lot of α-helical conformation, but PrPsc has more β strands forming aggregates.



For some reasons, α-helices in prions can convert to β-strands, leading to protein aggregation.



This can occur in brain tissue and neurons.

This disease occurs due to **infectious proteins.**

The prion protein

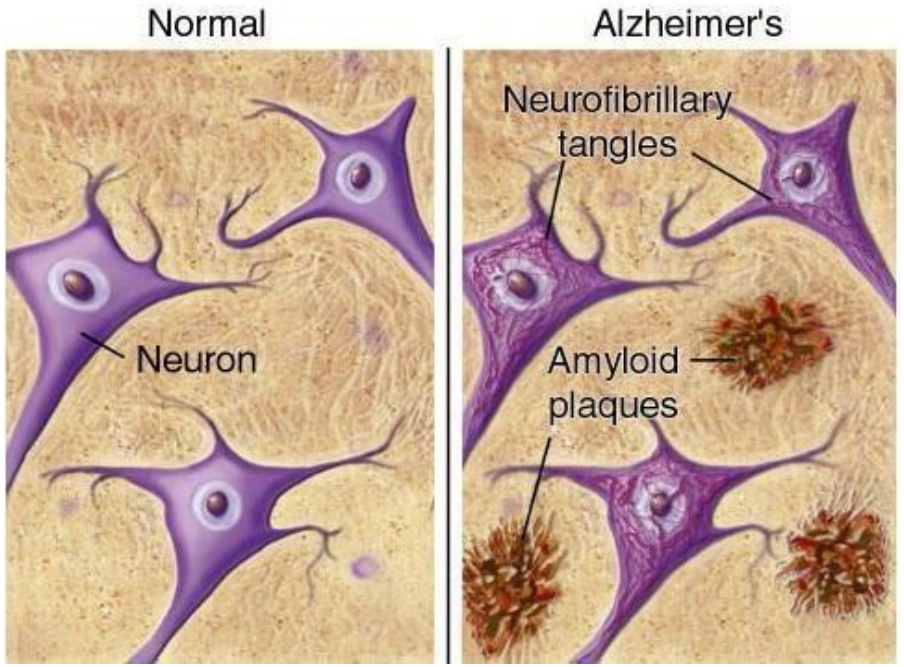
- The disease is caused by a transmissible agent
- Abnormal protein can be acquired by
  - Infection
  - Inheritance (genetic reasons- mutation in the prion gene – different secondary structure (β sheets instead of α helices)
  - Spontaneously

Alzheimer's Disease

It is a form of dementia The risk factor is aging .

- Not transmissible between individuals

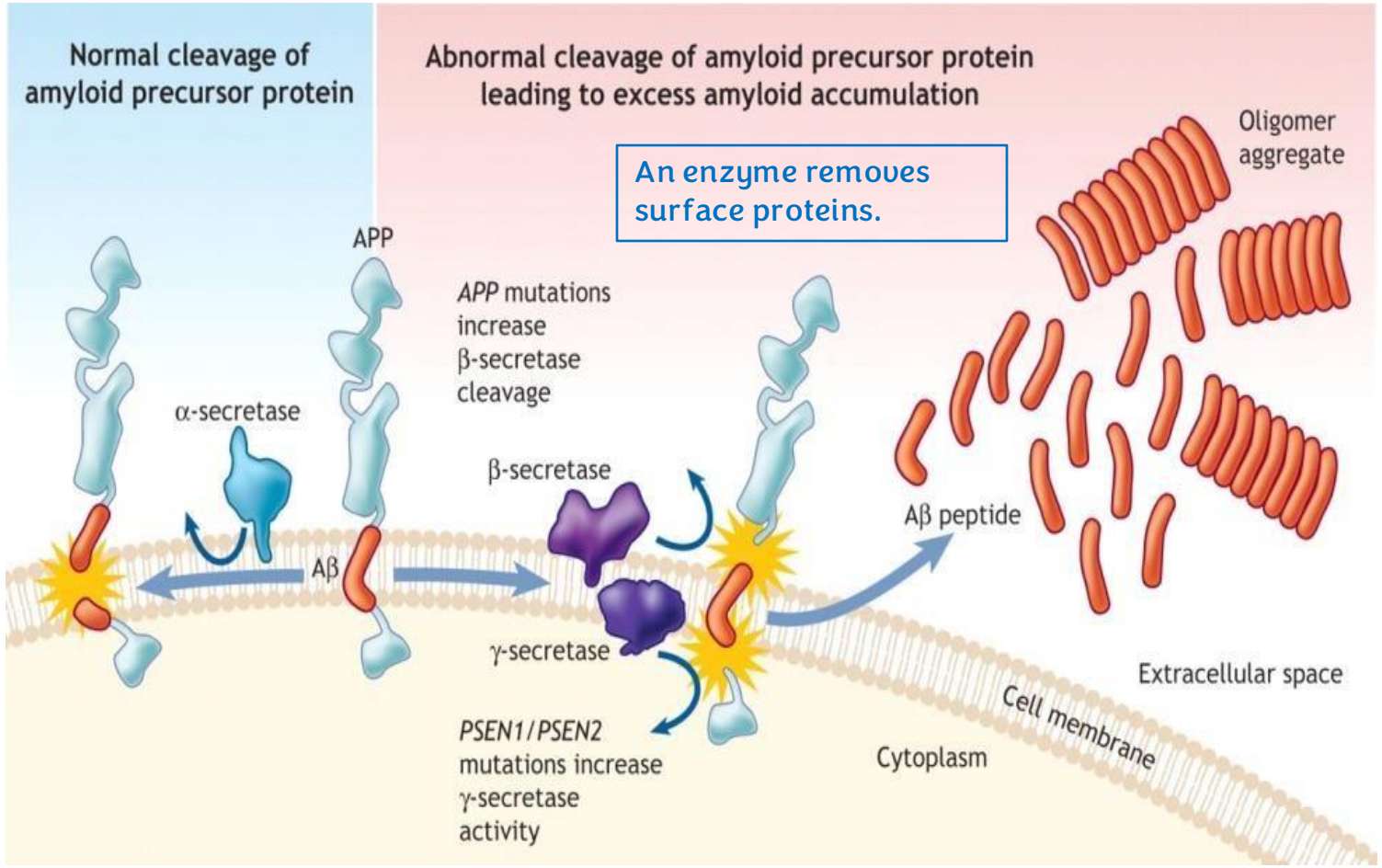
  - Extracellular plaques of protein aggregates of a protein called tau and another known as amyloid peptides (Aβ) damage neurons.



**Two misfolded proteins cause amyloid plaques/ fibrillar aggregates
1. Amyloid
2. Tau

Amyloid plaque: abnormal protein aggregates that accumulate in the brain.

Formation of plaques



The cell renews its surface proteins (amyloid being one of them) through a process called shedding, which involves removing and replacing old or damaged proteins from the surface.

Sometimes, by mistake, the enzyme removes a hydrophobic region . Shedding of hydrophobic proteins from the cell leads to aggregation. This could explain the cause of Alzheimer's disease and amyloid plaques.

سبحانكَ اللهم وبحمدك، أشهدُ أن لا إله إلا أنت، أستغفرك وأتوبُ إليك

For any feedback, scan the code or click on

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V1 → V2	6	Folds and domains are super secondary structures + we add a purple paragraph to explain the idea.	-
	7	Secrete it to find	To fight
	24	Rearrange some information	-
	25	Properly folded	Partially folded
V2 → V3			

Additional Resources Used:

رسالة من الفريق العلمي:

1. https://youtu.be/a7wOprWcYXA?si=I1tCEvyGKtbCG7an

فَمَن يُرِدِ اللَّهُ أَن يَهْدِيَهُ يَشْرَحْ صَدْرَهُ لِلْإِسْلَامِ

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اللهم لك الحمد على نعمة الإسلام، اللهم ثبتنا على دينك الحق حتى نلقاك .

اللهم استعملنا لنصرةِ دينك، ورفعة أمتك، وارحم شهدائنا واغفر لهم وأسكنهم جنانك الطيبة

لا تنسوا أخواننا المسلمين في غزة:

اللهم أنصر أخوانا المستضعفين في غزة ، اللهم إنهم جوعى فأطعمهم وخائفون فآمنهم، اللهم انهم مكسورين فاجبر كسرهم، اللهم ارحم ضعفهم، وآمن روعهم، وأخذل من خذلهم، وانصرهم على عدوك وعدوهم يا قوي يا عزيز .