بسم الله الرحمن الرحيم

BioChemistry | FINAL 2

Peptide & Proteins pt.2

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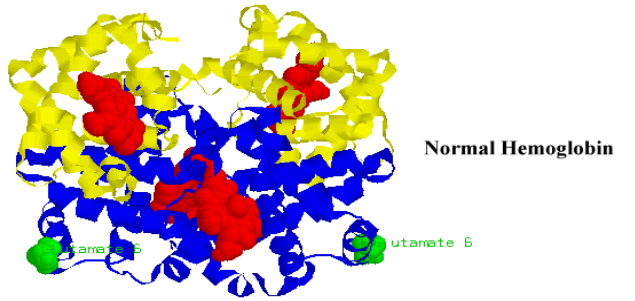
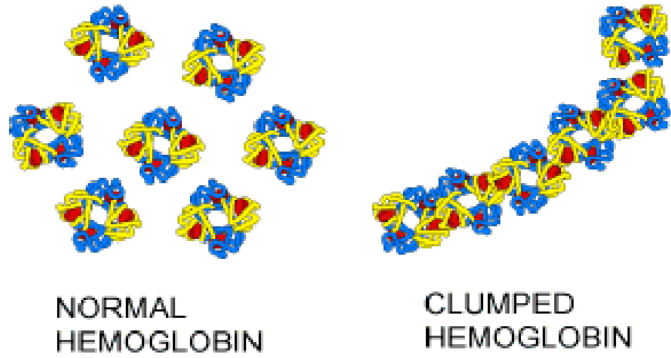
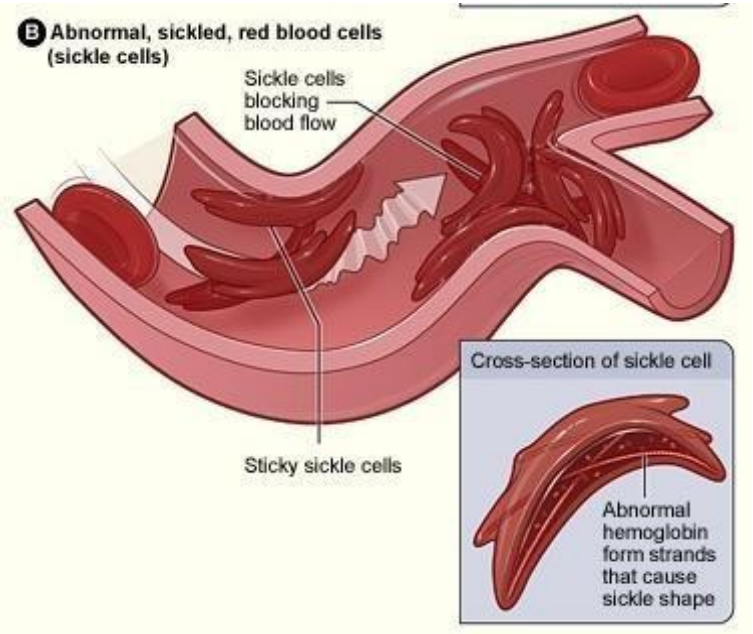
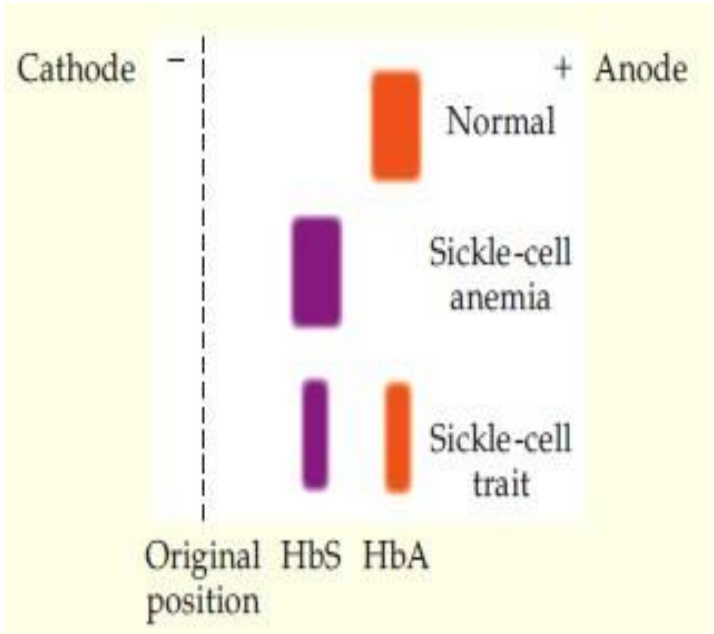
SICKLE CELL HEMOGLOBIN (HBS)

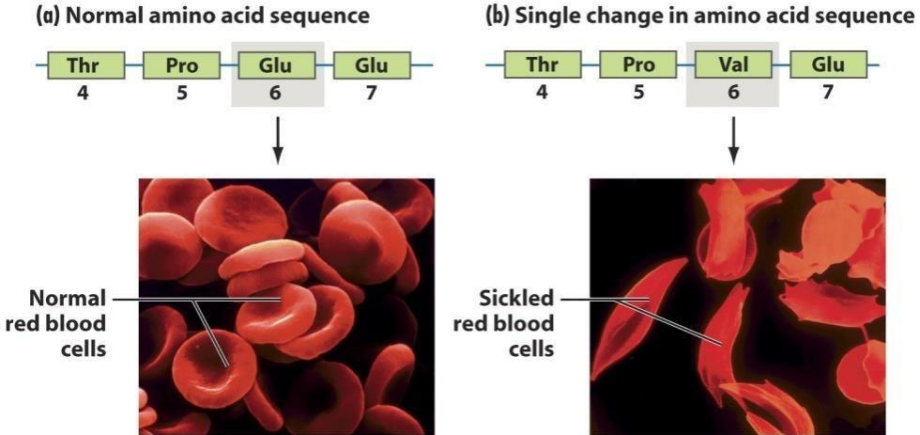
- It is caused by a change of amino acids in the 6th position of β globin (Glu to Val)
- The mutation results in: 1) arrays of aggregates of hemoglobin molecules, 2) deformation of the red blood cell, and 3) clotting in blood vessels and tissues







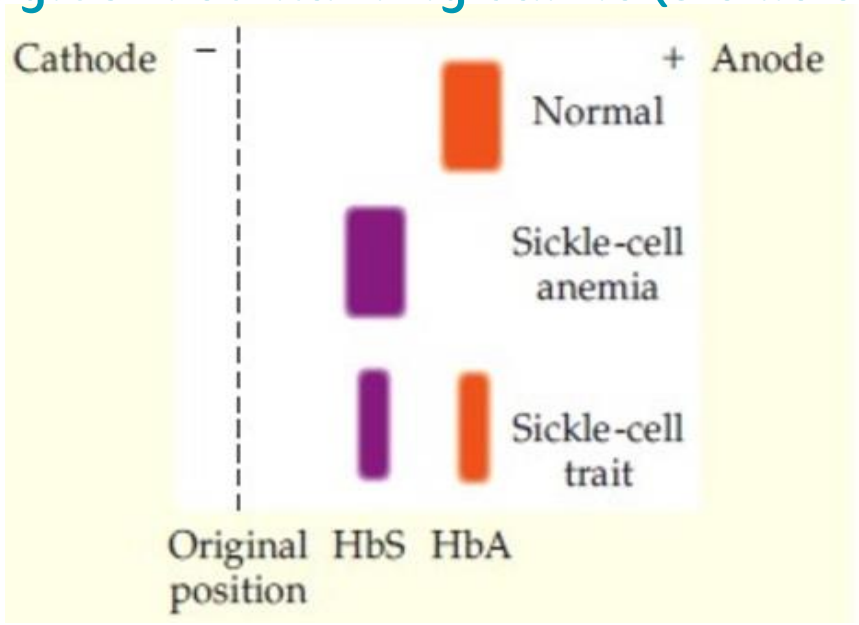




-Gel electrophoresis is a method by which we subject a gel to an electric current. The gel could contain different proteins or DNA molecules. The gel is porous so that molecules can move through the pores. We apply a charge on the protein on top of the charge it carries. We then place the gel into an electric current . Proteins will move from the negative electrode toward the positive electrode.

-As you can see, the normal hemoglobin moves at a faster rate towards the positive electrode than the sickle cell hemoglobin. That's because the normal hemoglobin has negatively charged Glutamic acid whereas the sickle cell hemoglobin has valine. This means that normal hemoglobin is more negative than sickle cell hemoglobin, so it gets attracted to the positive electrode faster.

-The third graph shows a sample from a person carrying a hemoglobin containing Glutamic acid (normal hemoglobin), and a hemoglobin containing valine (sickle cell hemoglobin). We call this type of person a carrier

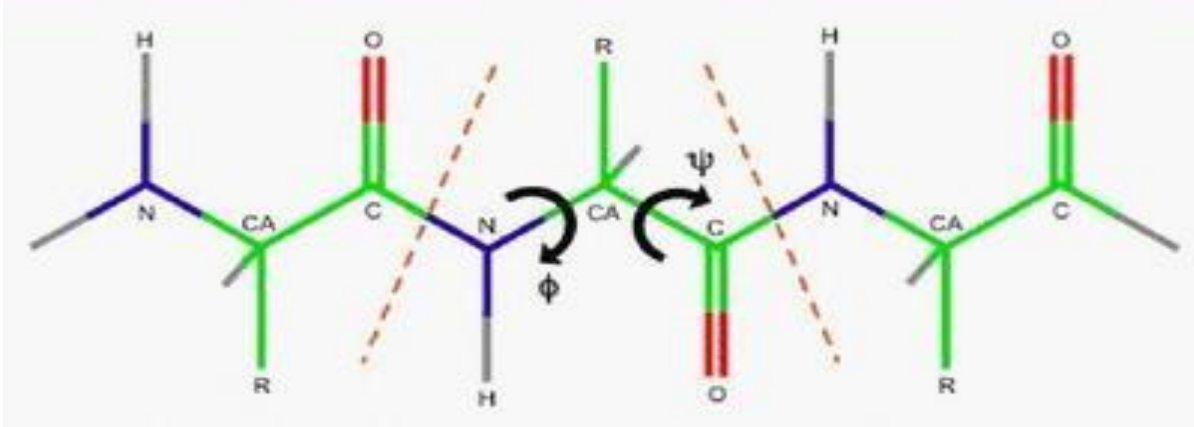


SECONDARY STRUCTURE

WHAT IS IT? HOW IS IT CAUSED?

Rotation around alpha carbon

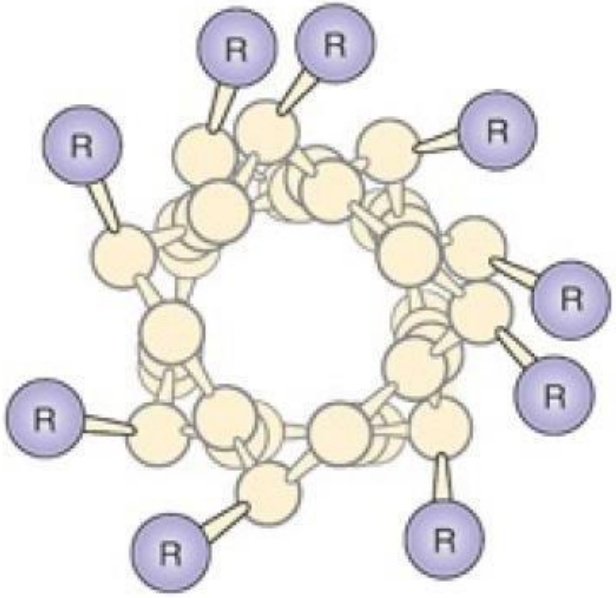
- The two bonds within each amino acid residue freely rotate
  - the bond between the α-carbon and the amino nitrogen (PHI bond)
  - the bond between the α-carbon and the carboxyl carbon (PSI bond)
  - However, no rotation around peptide bond
  - Since backbones are unified among amino acids, there is low variety in the secondary structure.
- A hydrogen-bonded, local arrangement of the backbone of a polypeptide chain
- Polypeptide chains can fold into regular structures such as:
  - Alpha helix
  - Beta-pleated sheet
  - Turns
  - Loops

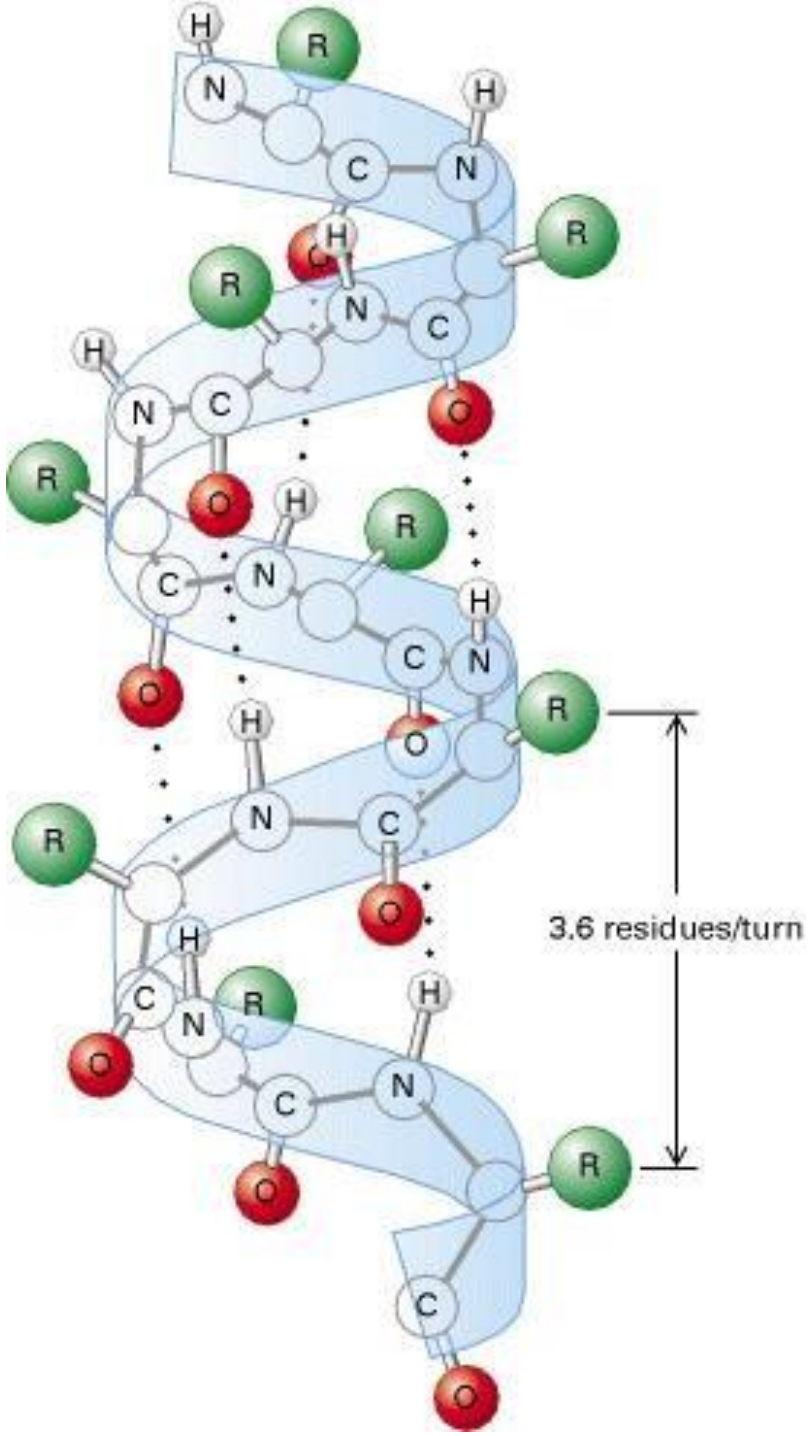


THE α-HELIX

Not any helical structure is called an alpha helix. Alpha helix is a specific type of helical structures.

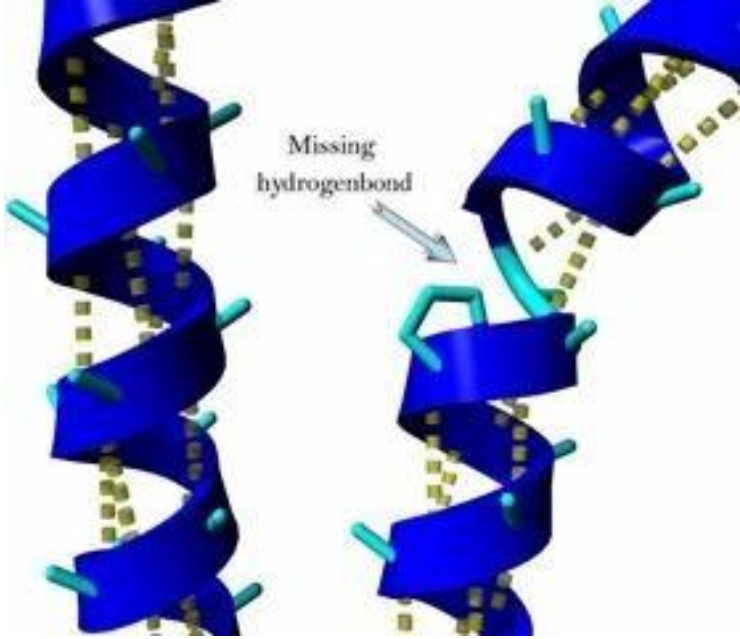
- 3.6 amino acids per turn (amino acid no.1 attaches to no. 4 and it's attached to no.7)
- The pitch of the helix (the linear distance between corresponding points on successive turns) is 5.4 Å
- It is very stable! Because of the huge number of hydrogen bonding.
- Avoiding steric hindrance
- Side chains are projecting outwards as to give the properties of alpha helix.



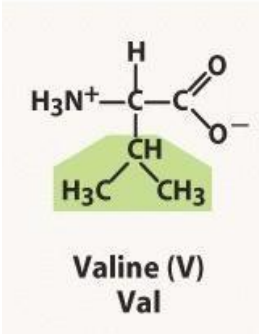


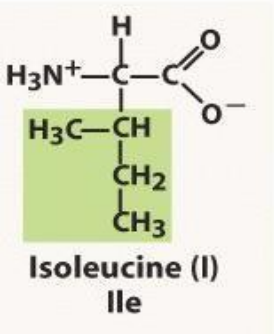
AMINO ACIDS NOT FOUND IN α-HELIX

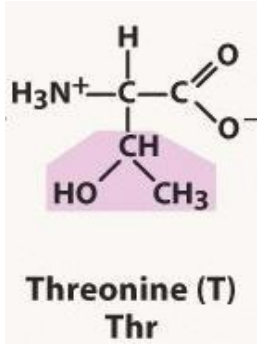
Because they are the breakers of the alpha helix



- Glycine: too small
- Proline
  - No rotation around N-Cα bond
  - No hydrogen bonding of α-amino group
  - It produces kinks
- Close proximity of a pair of charged amino acids with similar charges (or opposite charges)
- Amino acids with branches at the β-carbon atom (valine, threonine, and isoleucine)

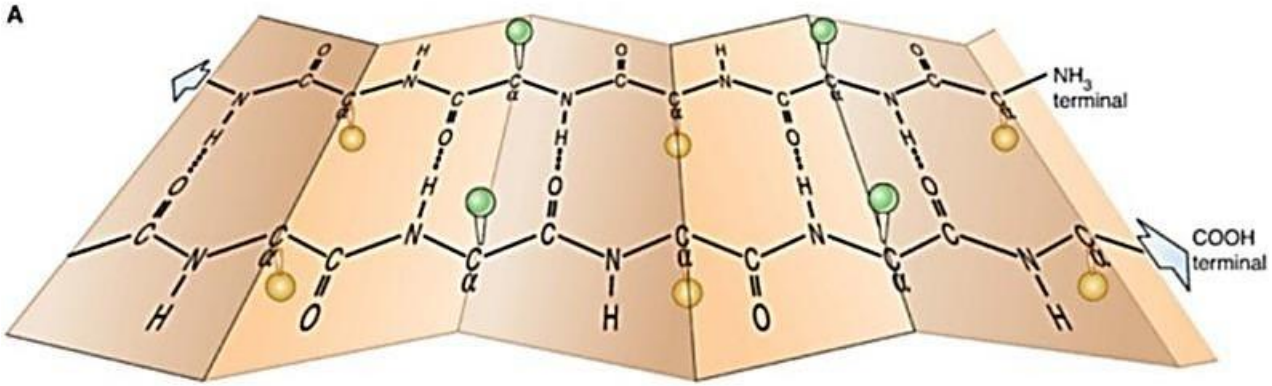


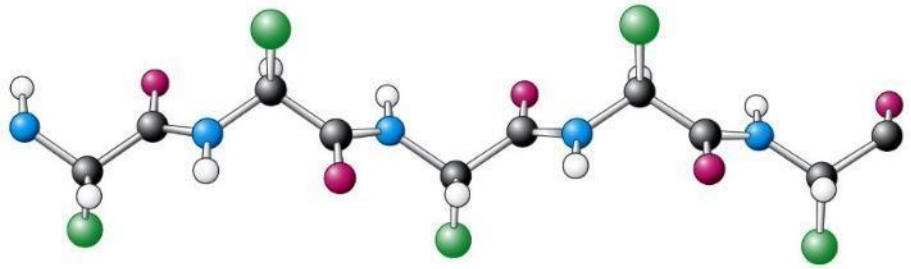




B-PLEATED SHEET (β-SHEET)

- Steric hindrance is not found in beta pleated sheets since side chains are distributed above and below (this means that beta pleated sheets are not affected by close proximity of charges or branching amino acids). (Proline and Glycine are still considered breakers of the beta pleated sheets).
- They are composed of two or more straight chains (β strands) that are hydrogen bonded side by side (typically 4 or 5)
- Optimal hydrogen bonding occurs when the sheet is bent (pleated) To form β-pleated sheets





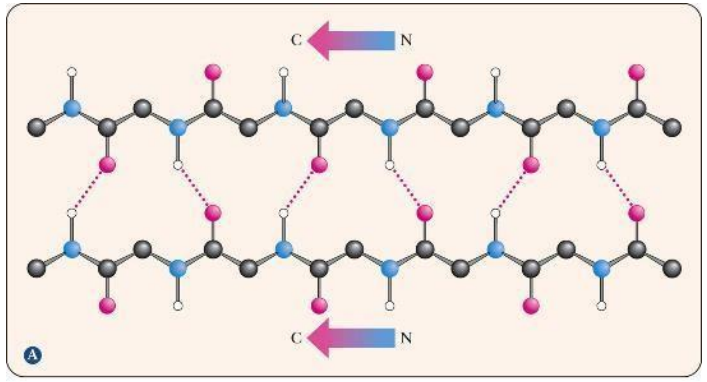
β strand

MORE ON β-SHEETS

Note: In alpha helices, the hydrogen bonds formed were parallel to the long axis of the helix. While hydrogen bonds formed in beta pleated sheets are perpendicular to the long axis of the sheet

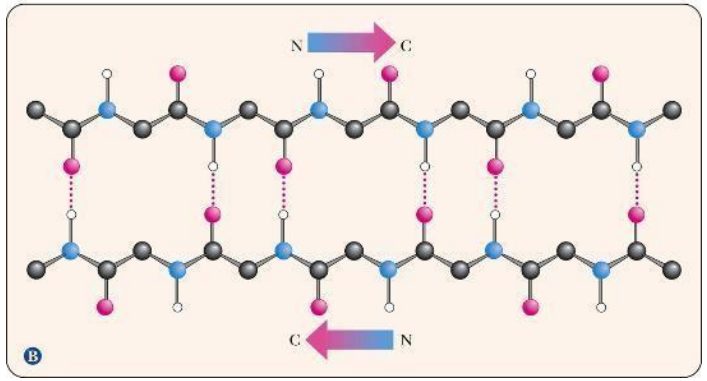
- β sheets can form between many strands, typically 4 or 5 but as many as 10 or more
- Such β sheets can be purely antiparallel, purely parallel, or mixed

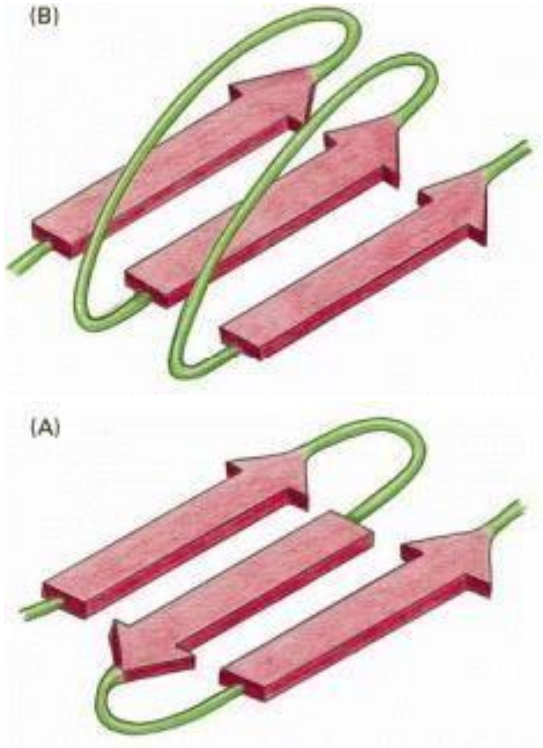
Parallel

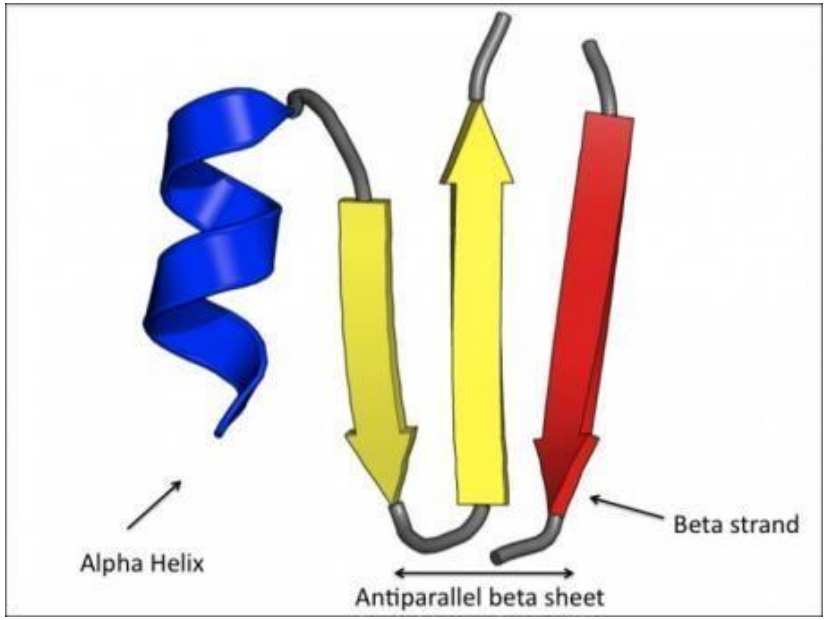


Antiparallel

Here they are connected by short segments





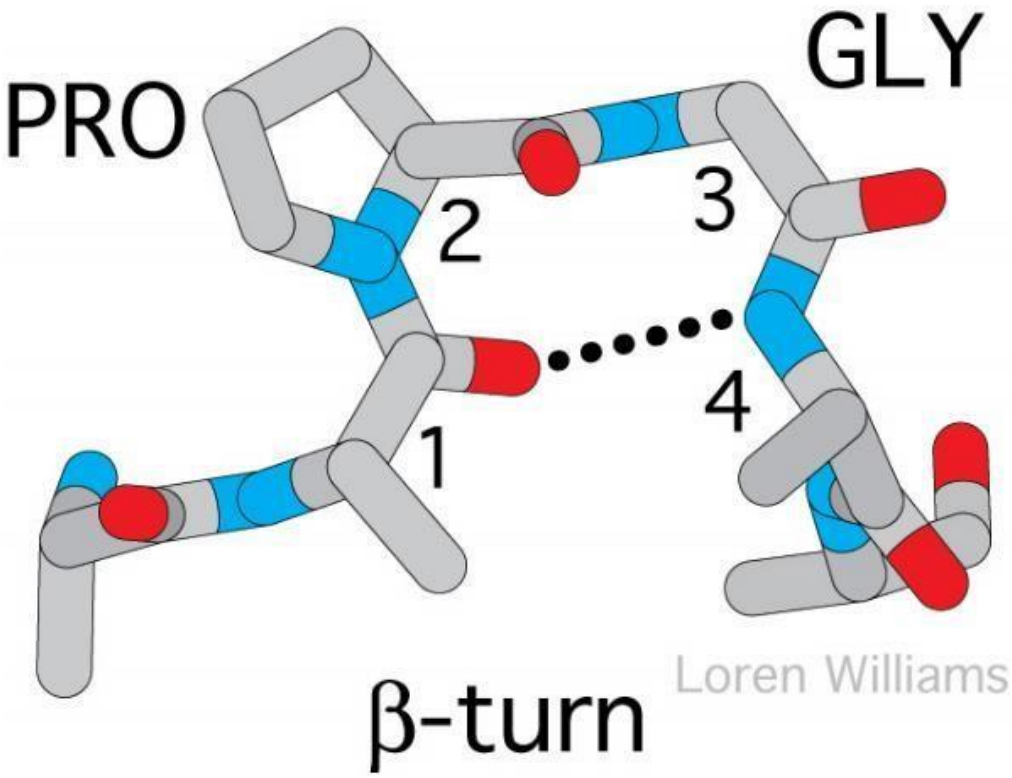


EFFECT OF AMINO ACIDS

- Valine, threonine and Isoleucine with branched R groups at β-carbon and the large aromatic amino acids (phenylalanine, tryptophan, and tyrosine) tend to be present in β-sheets.
- Close proximity of charges doesn't disrupt beta pleated sheet. This is because are found above and below.
- No steric hinderance in beta pleated sheets
- Proline and glycine tend to disrupt β strands

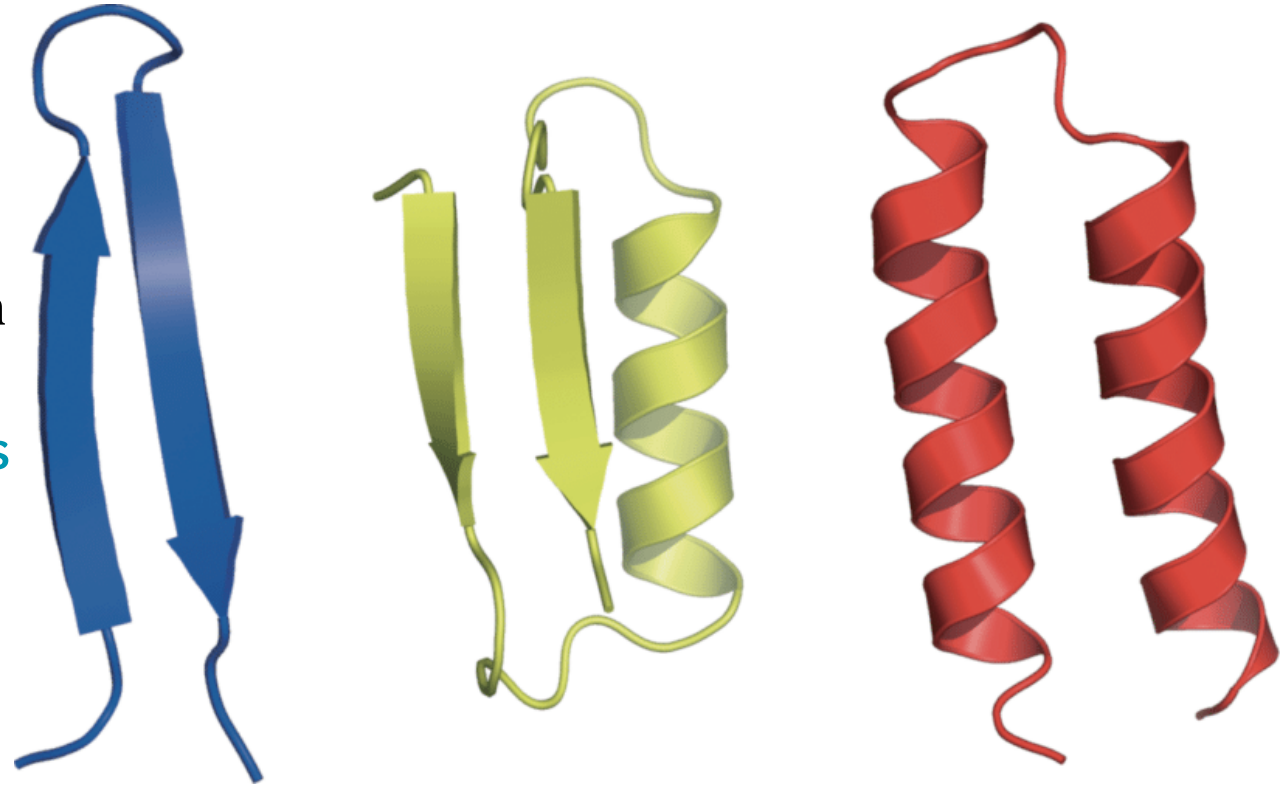
β-TURNS

- Short segments connecting secondary structures together.
- They consist of 4 amino acids where the first and fourth amino acids are connected by hydrogen bonds.
- Turns are compact, U-shaped secondary structures
- They are also known as β turn or hairpin bend
- What are they used for? How are they stabilized?
- Glycine and proline are commonly present in turns
- Why? Proline is found to produce kinking and glycine is found due to its small structure, these 2 amino acids are found in the position of amino acids 2 and 3 of the turn.



LOOPS AND COILS

- They don't have a special regular shape but they are considered longer than beta turns (longer than 4 amino acids)
- Loops are a diverse class of secondary structures in proteins with irregular geometry
- They connect the main secondary structures.
- They are found on surface of molecule (and contain polar residues) and provide flexibility to proteins.
- These loops and coils are also present at the sites of interactions in between protein structures.(antigen with antibody or hormone with receptor). This is because they are easily deflected since they have loose irregular structures
- Amino acids in loops are often not conserved.

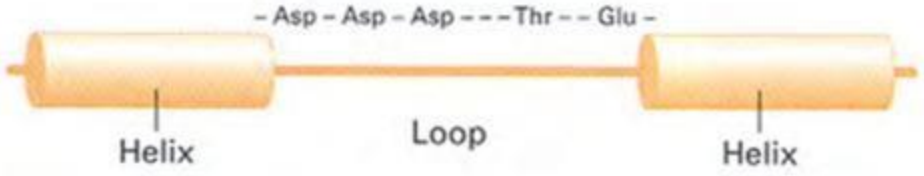


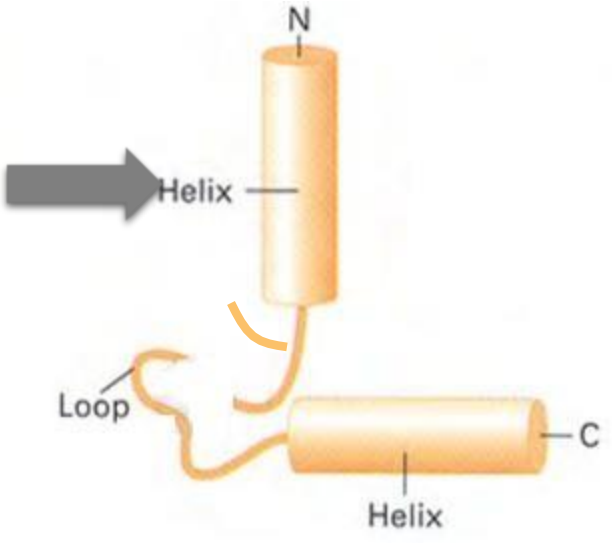
SUPER-SECONDARY STRUCTURES

- They are regions in proteins that contain an ordered organization of secondary structures.
- They are building up of secondary structures together.
- They are repeated in certain proteins.
- Examples:
  - Motifs (if it was remained in its structure)
  - Domains (if it has a specific function)

A MOTIF (A MODULE)

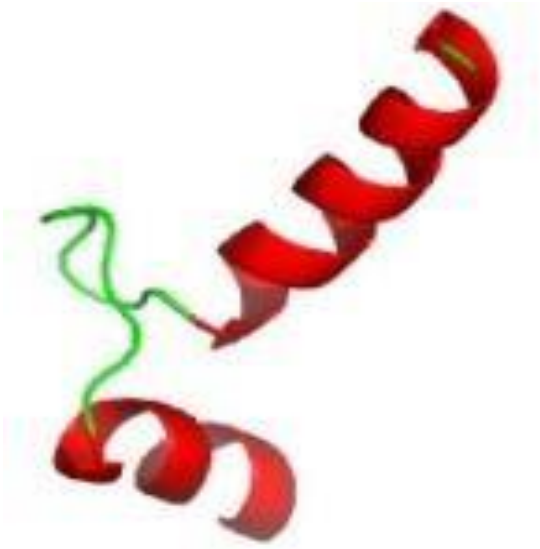
- A motif is made of multiple, repetitive or consecutive (connected) secondary structures, that can be small or large.
- They usually constitute a small portion of a protein (typically less than 20 amino acids).
- In general, motifs may provide us with information about the folding of proteins, but not the biological function of the protein.



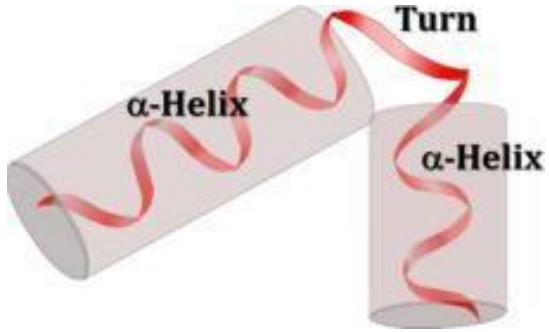


EXAMPLES OF MOTIFS

Helix-loop-helix: Two α-helices connected by a loop. It is found in Ca-binding proteins. (calcium will bind to the loop) Because Calcium is big and bulky, it can cause disruption to the structure .

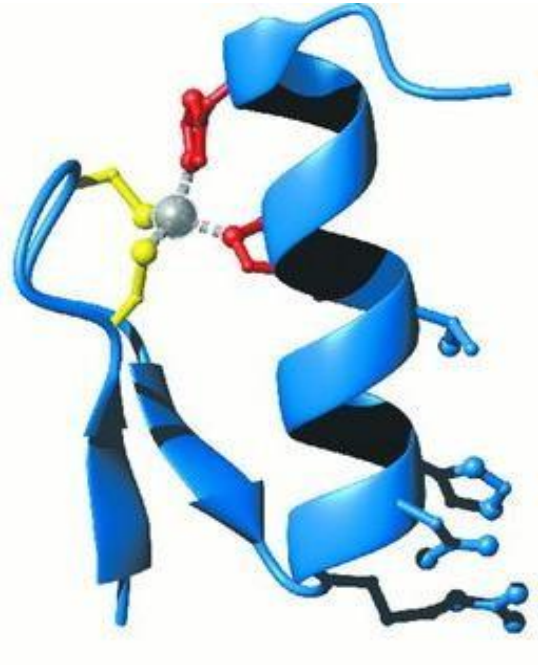


Helix-turn-helix: Two α helices joined by a short strand of amino acids. It is found in DNA-binding proteins.



Zinc finger: Two beta strands with an alpha helix end folded over to bind a zinc ion. Important in DNA binding proteins.

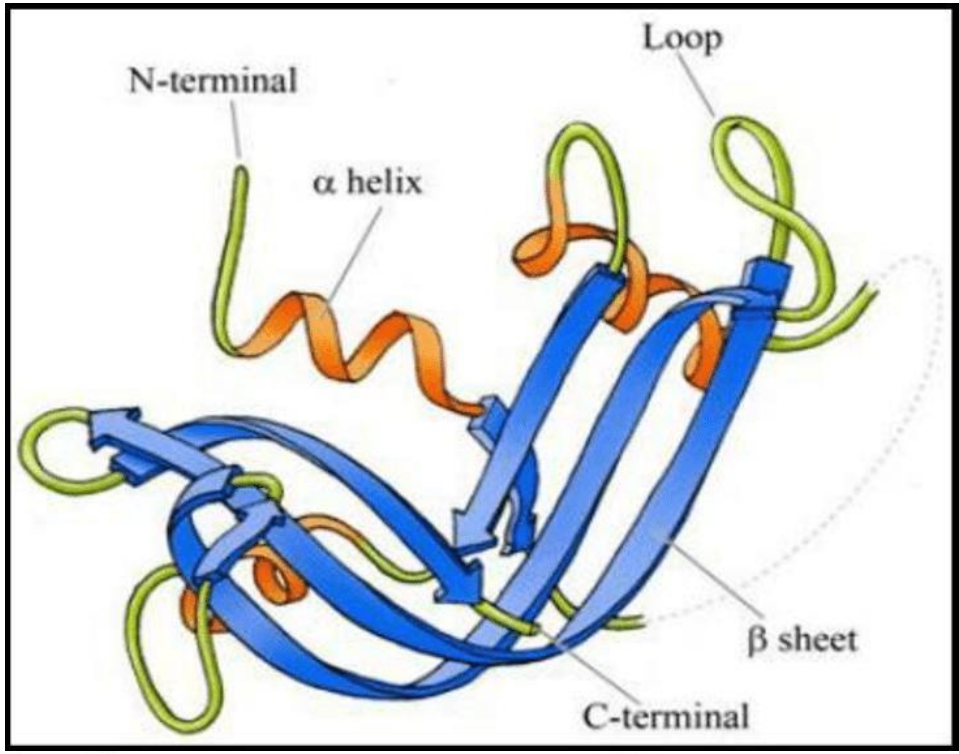
Beta hairpin: (same as beta turn)Two antiparallel beta strands connected by a turn.

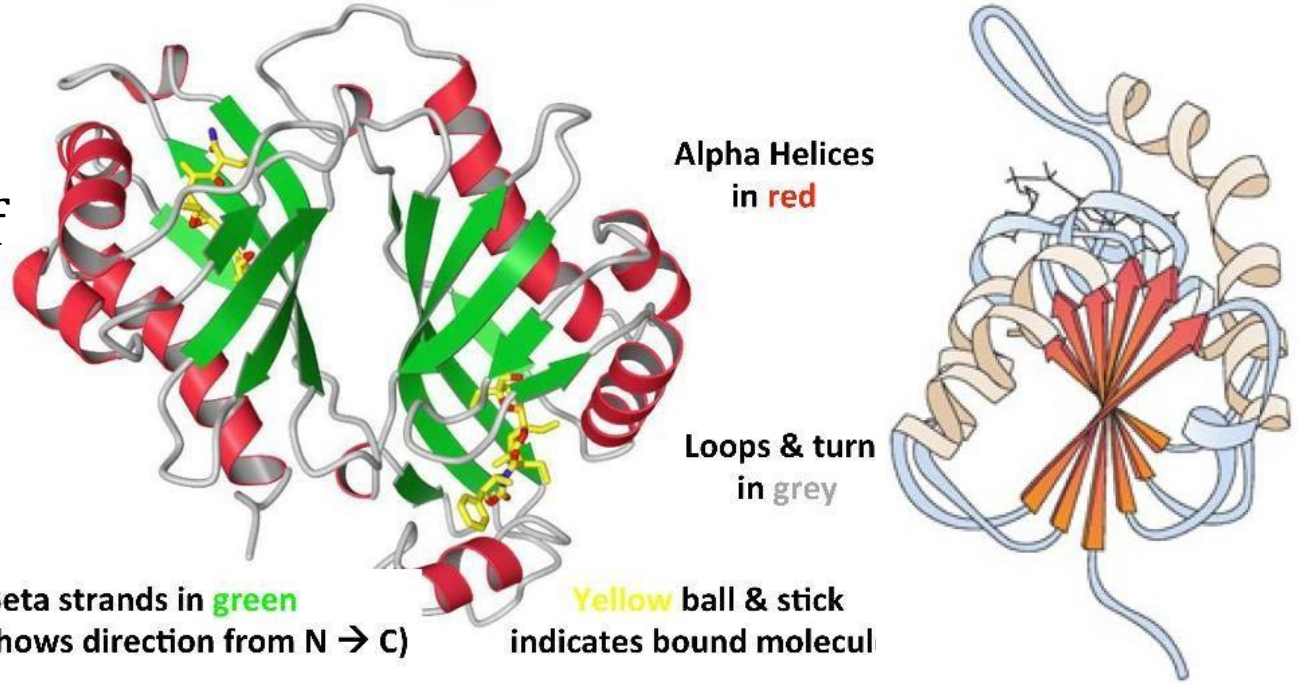


TERTIARY STRUCTURE

WHAT IS TERTIARY STRUCTURE?

- It represents the final form of the protein if it consisted of only 1 polypeptide chain
- It is produced by bonds between the side chains **and** the backbones. (hydrogen bonds, ionic bonds, disulfide bridges as covalent bonds, van der waals, metal interactions, hydrophobic interactions)
- The overall conformation of a polypeptide chain
- The three-dimensional arrangement of all the amino acids residues
- The spatial arrangement of amino acid residues that are far apart in the sequence





What are the types of proteins:

-Fibrous
-Globular

Fibrous proteins

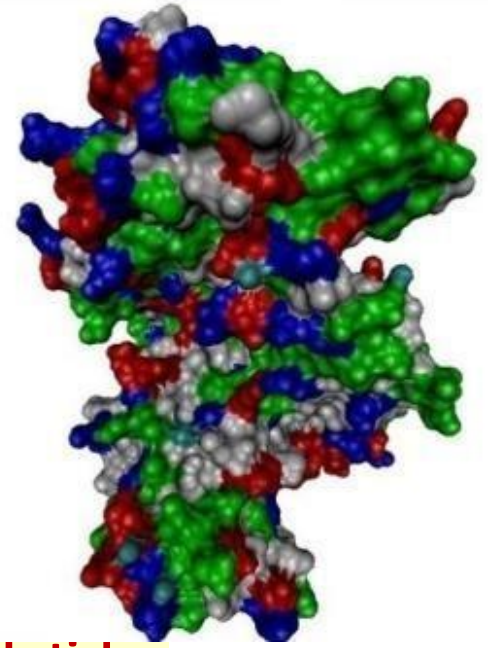
-Their structure is fiber like
-They are insoluble and function in support
-Their variety is limited although they constitute most of the proteins

Globular proteins

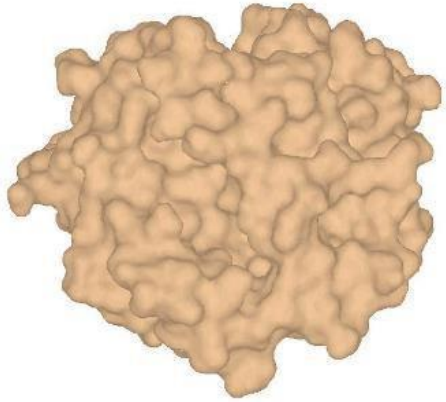
-They are spherical in shape and has more variety than fibrous proteins
-They are soluble, meaning they are found in aqueous media.
-In order for this structure to be produced, hydrophobic amino acids embed themselves inside the protein in order to get away from the surrounding water (hydrophobic interactions), leaving the polar amino acids projecting towards the exterior of the protein, towards water, thus making them soluble in water.
-Primary structure is the foundation for how the protein folding is done, which gives the protein it's final shape.

HOW TO LOOK AT PROTEINS...

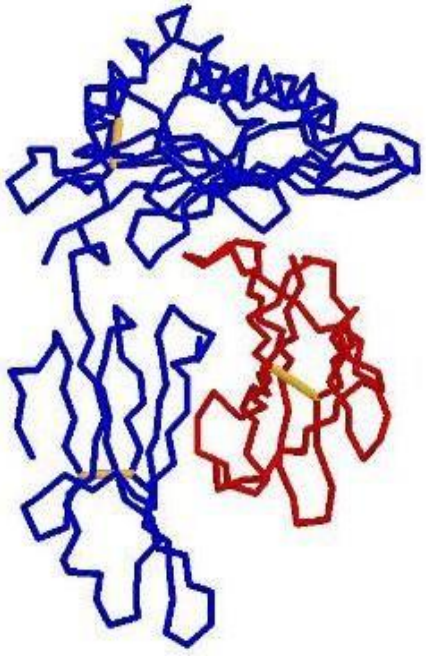
Space filling structure



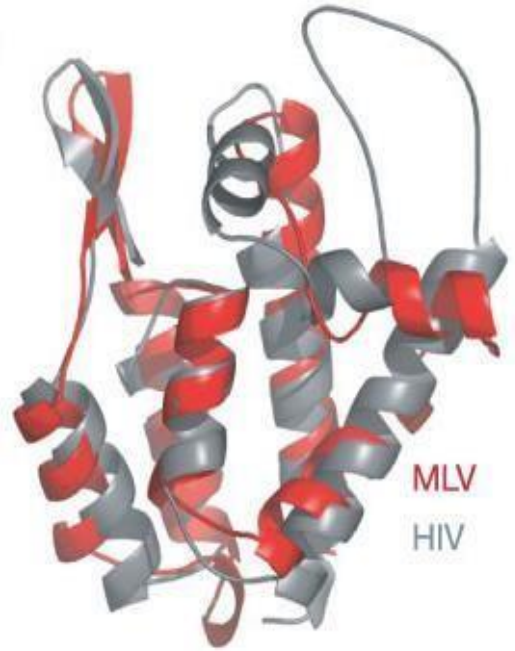
Protein surface map



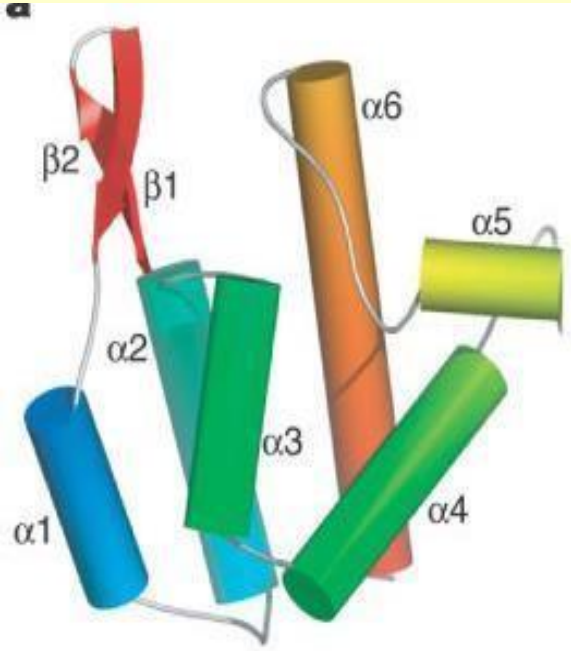
Trace structure



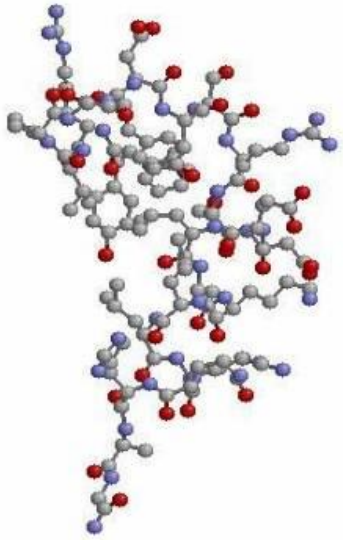
Ribbon structure



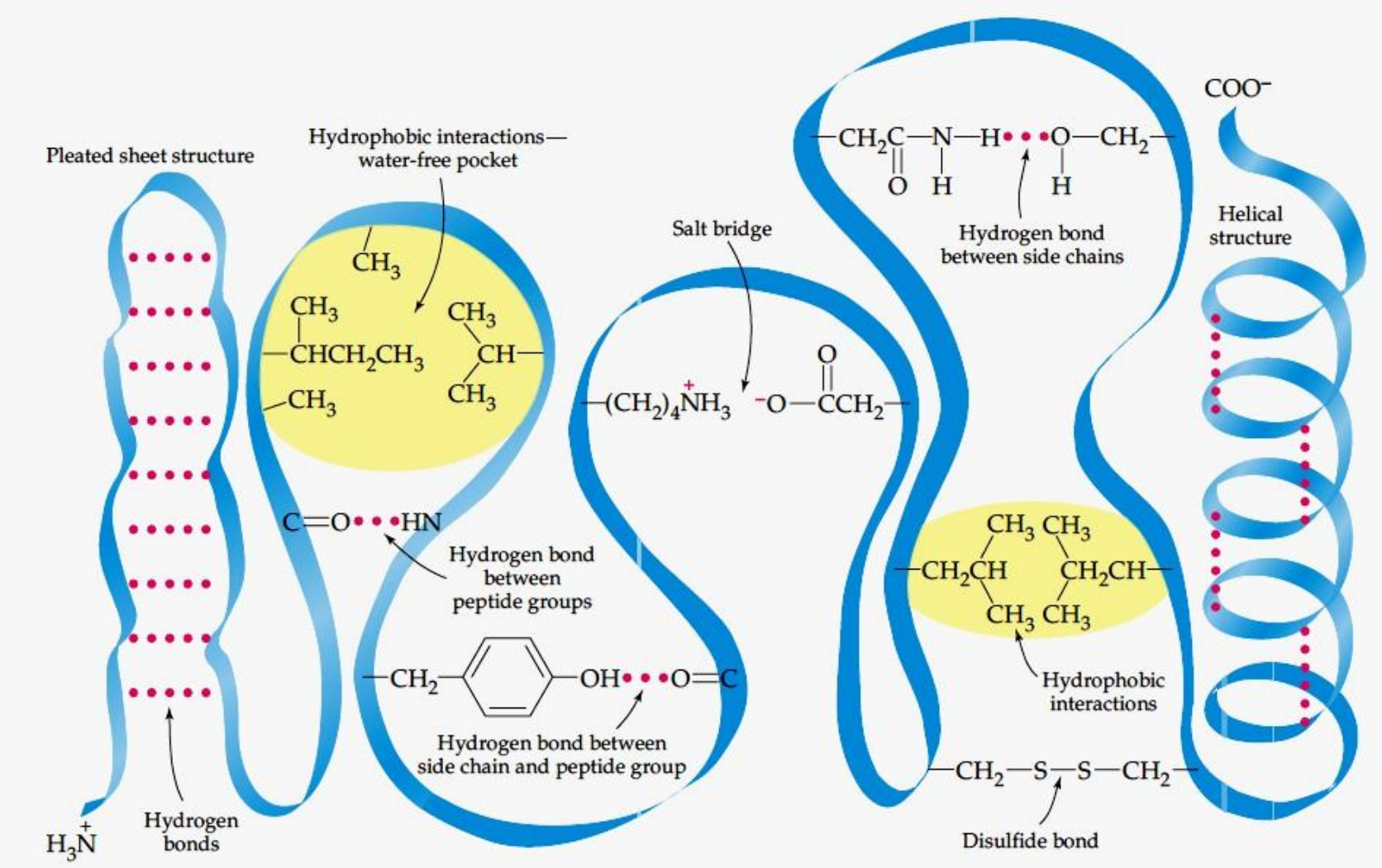
Cylinder structure



Ball and stick structure



SHAPE-DETERMINING FORCES

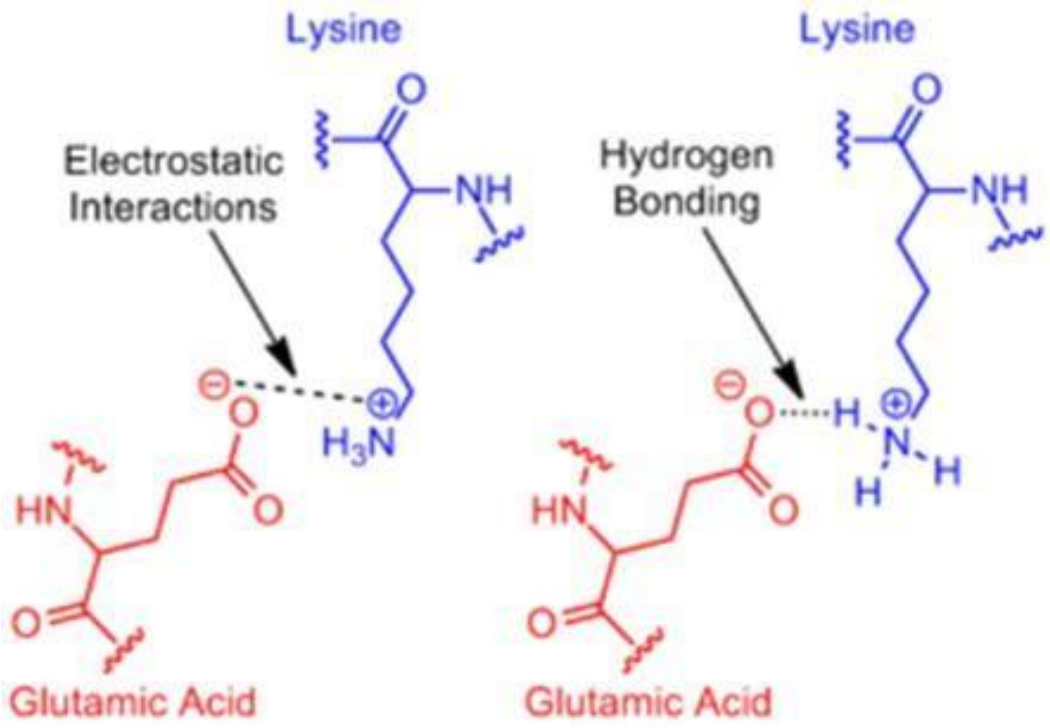


-Metal interactions and disulfide bridges are the strongest interactions among all others, that's why their function is stabilizing the structure (shape stabilizing forces)
-Other interactions (such as hydrophobic interactions, hydrogen bonds, ionic interactions, electrostatic interactions, van der waals) function in determining the final shape (shape determining forces)

NON-COVALENT INTERACTIONS

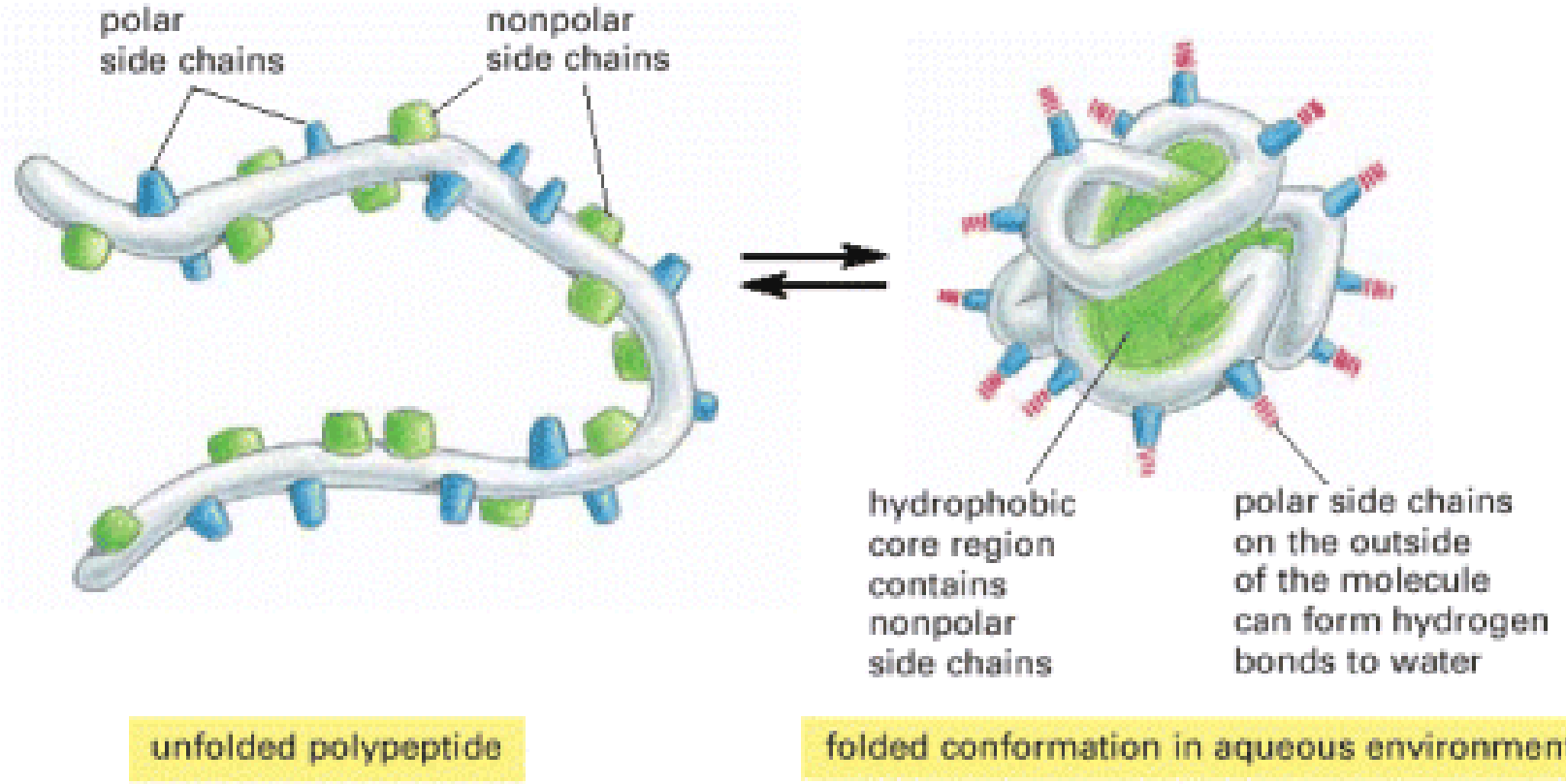
- Hydrogen bonds: 1. within and between polypeptide chains; 2. with the aqueous medium
- Charge-charge interactions (salt bridges, ionic): oppositely charged R-groups
- Charge-dipole interactions: charged R groups with the partial charges of water

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



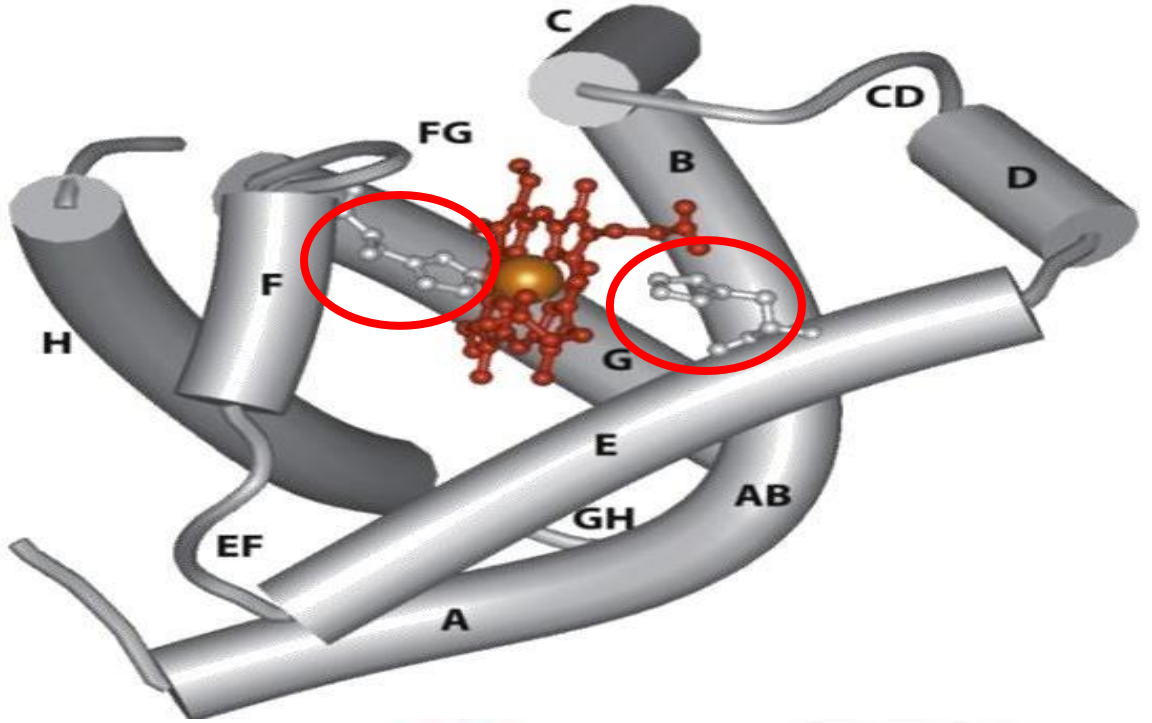
HYDROPHOBIC INTERACTIONS

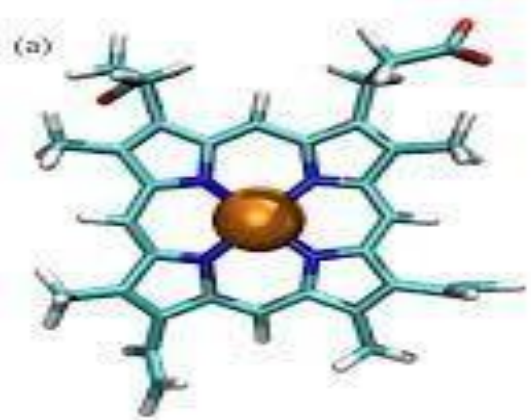
- A system is more thermodynamically (energetically) stable when hydrophobic groups are clustered together rather than extended into the aqueous surroundings.

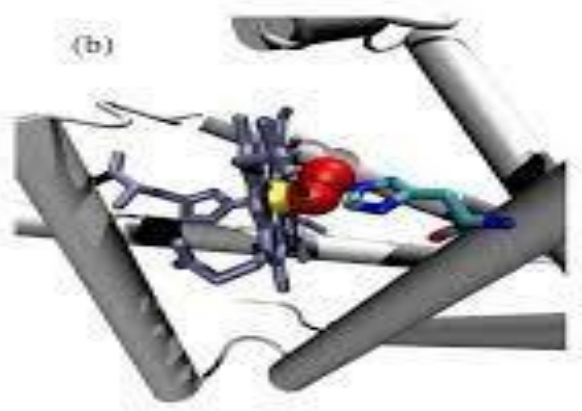


CAN POLAR AMINO ACIDS BE FOUND IN THE INTERIOR?...YES

- Even though they cause instability inside the proteins, but they are found there in order to perform specific functions
- 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
- Nonpolar amino acids can also be found in the exterior of the proteins even though they cause instability, they are found there in order to perform specific functions

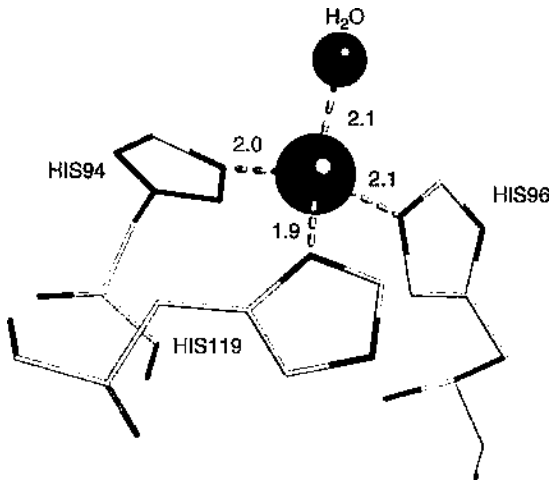


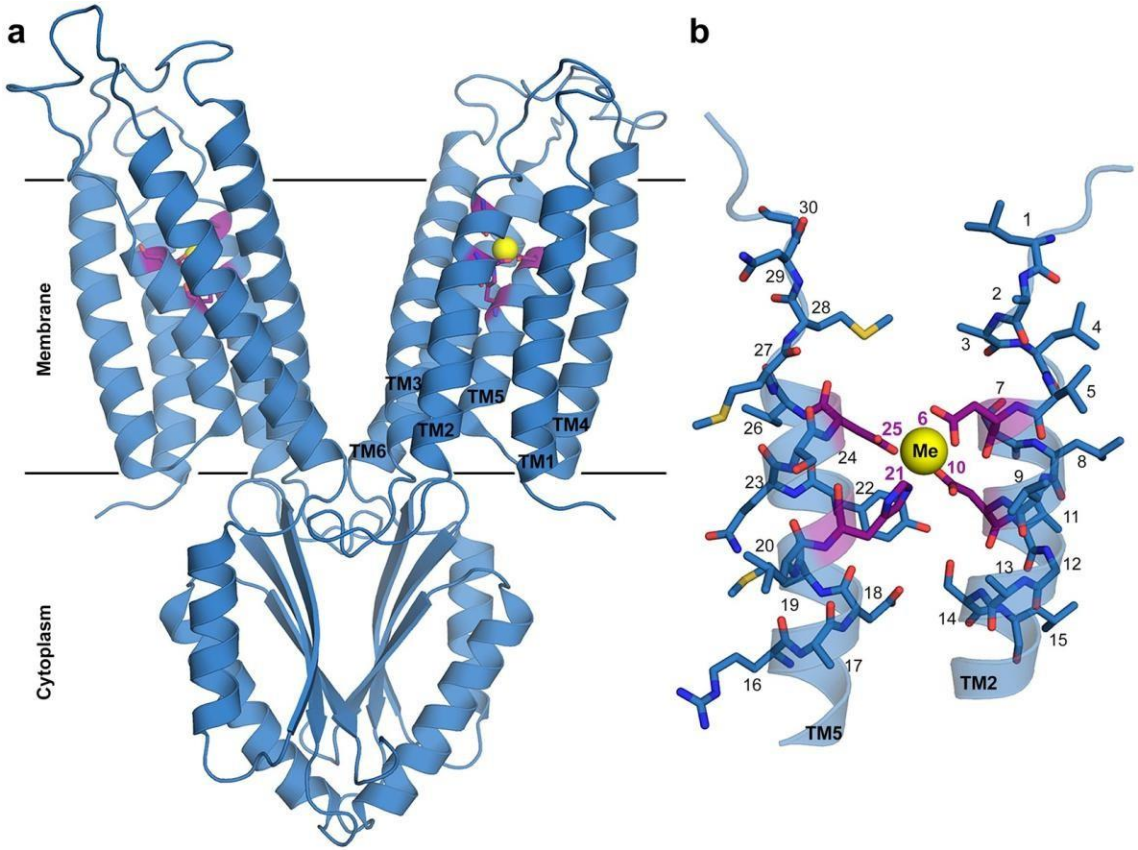


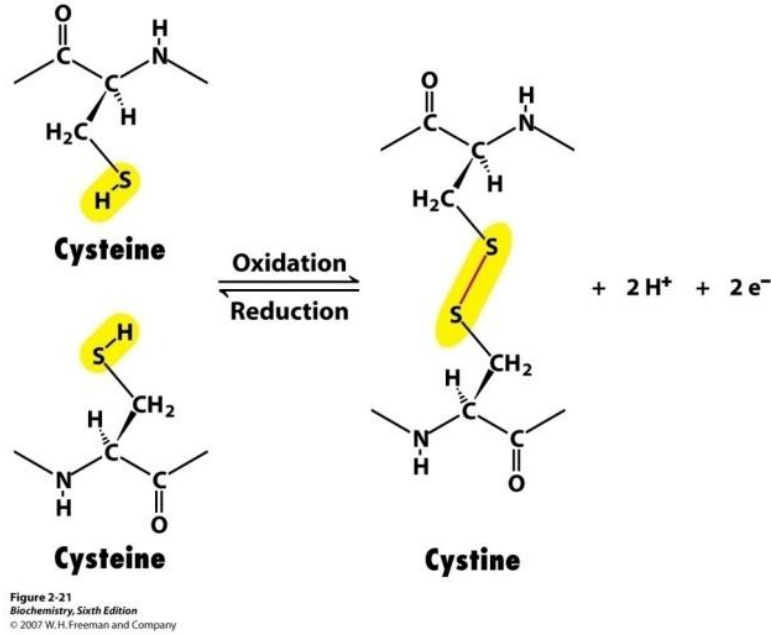


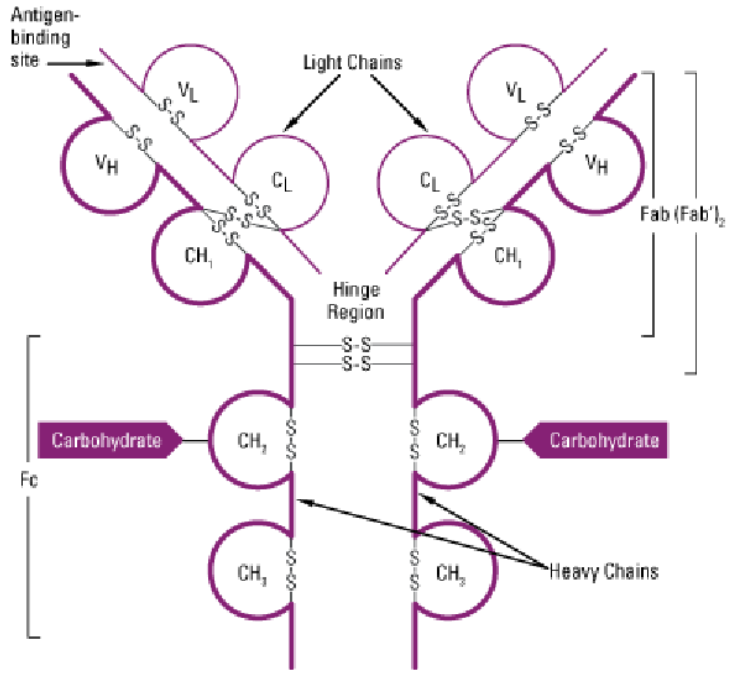
STABILIZING FACTORS

- There are two forces that do not determine the 3D-structure of proteins, but stabilize these structures:
  - Disulfide bonds
  - Metal ions
    - Covalent
    - Salt bridges



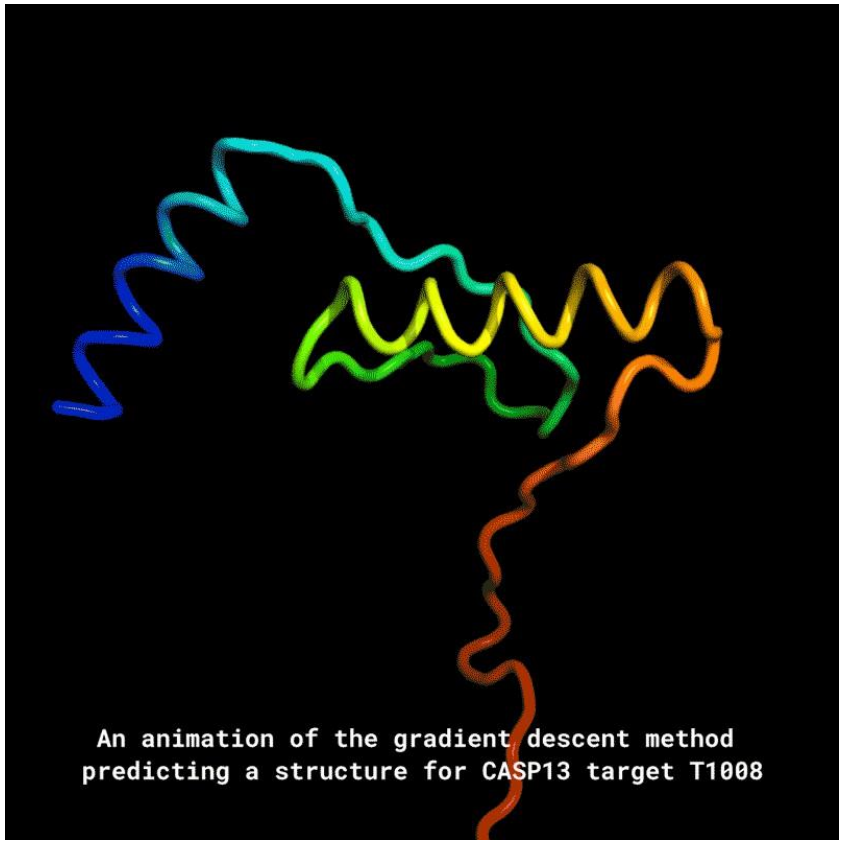




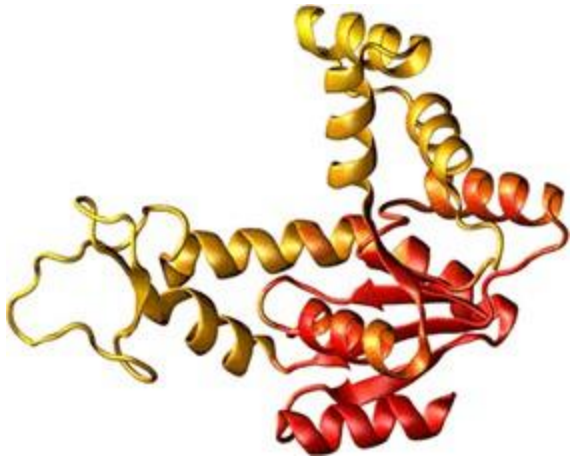


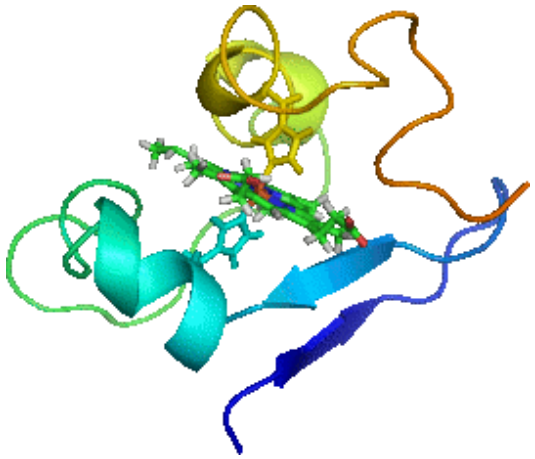
A HYPOTHETICAL LOOK AT PROTEIN FOLDING

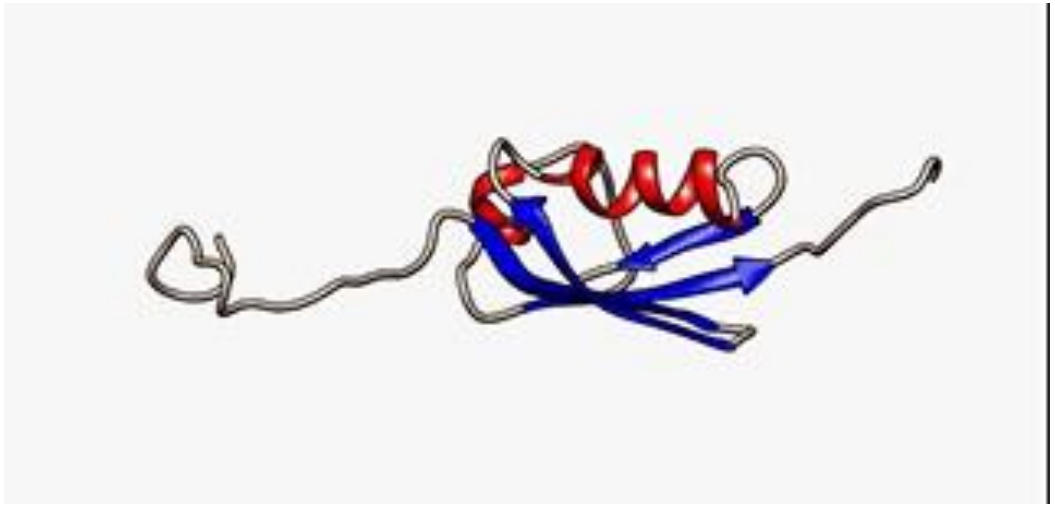
Proteins are dynamic not static (they keep changing their shape, while in aqueous media).
They can be found in different conformations

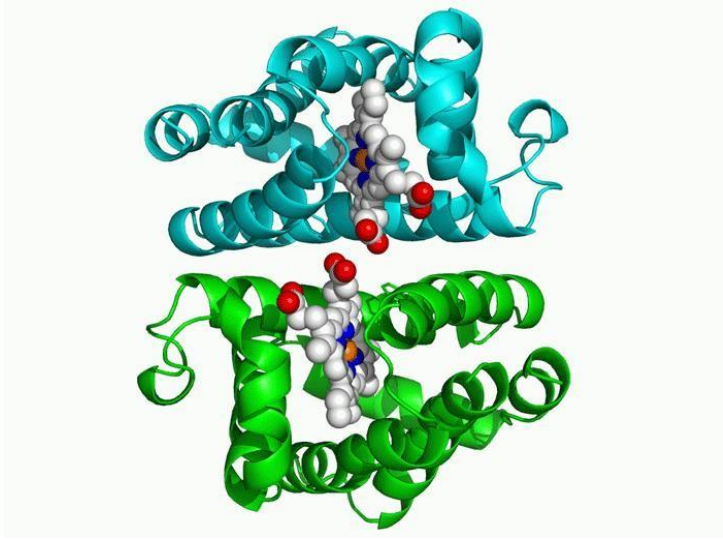


PROTEIN ARE NOT STATIC









Why do we need hemoglobin?

-Surface area is very low which means we can't make enough oxygenation in the aqueous media of the blood, which is why we need a protein (hemoglobin) to pick up oxygen with high affinity.

-hemoglobin contains a structure called heme (perforin ring containing iron which binds to oxygen, oxygen binds specifically to iron).

If we add magnesium to this perforin ring, we will get chlorophyll.

If we add cobalt to this perforin ring, we will get cobalamin (vitamin B-12).

The specific color (red in heme, green in chlorophyll) comes from the metal-bound ring reflecting light, not from the protein itself, as proteins are naturally colorless.

-Myoglobin has a higher affinity for oxygen than hemoglobin, so why is hemoglobin found in the blood rather than myoglobin?

Because hemoglobin is a quaternary structure and myoglobin is not, hemoglobin has 4 subunits (4 polypeptide chains).

-so what is unique about quaternary structure? (next slide)

QUATERNARY STRUCTURE

-Why do we need **quaternary structures** (made of more than one polypeptide chain) in proteins, even though tertiary-structured proteins can perform similar functions?

Quaternary structure is important because the interaction between multiple polypeptide chains can affect the protein's function. These interactions form new, **weak non-covalent** bonds between subunits, allowing the subunits to move **freely** and **change** shape. This flexibility enables the protein to perform different functions using the same structure. In contrast, a tertiary structure (with only one polypeptide chain) can perform only one function, that's why hemoglobin (which has a quaternary structure) is used in our blood to transport oxygen, instead of myoglobin (a tertiary structure), even though myoglobin has a higher affinity for oxygen.

-Oxygen binds to proteins containing heme, which contains **iron**, such as hemoglobin and myoglobin.

WHAT IS IT?

Hemoglobin is considered a **hetero-tetramer**;
hetero because it contains different types of subunits: alpha and beta, and tetramer because the total number of subunits is 4 (2 alpha+ 2 beta).
If subunits are the same it's called homo.

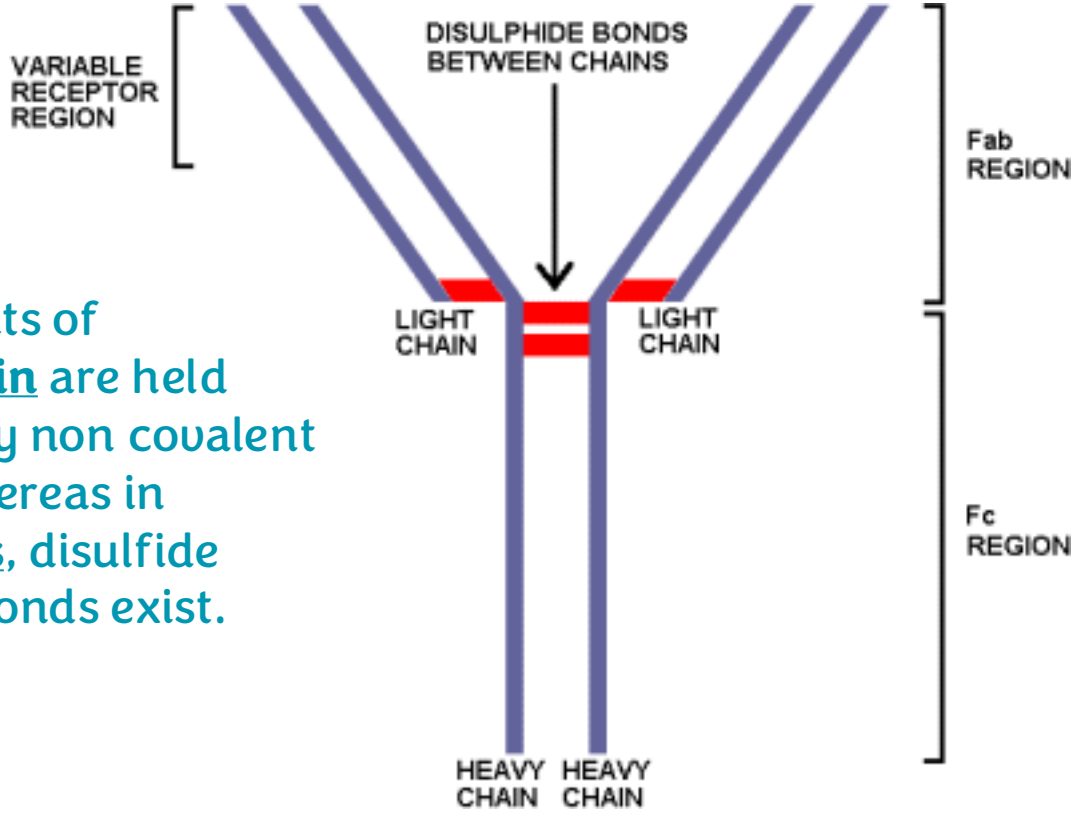
- Proteins are composed of more than one polypeptide chain.
  - They are oligomeric proteins (oligo = a few or small or short; mer = part or unit)
- The spatial arrangement of subunits and the nature of their interactions.
- Proteins made of
  - One subunit = monomer
  - Two subunits: dimer
    - The simplest: a homodimer
  - Three subunits: trimer
  - Four subunit: tetramer
  - Etc..

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

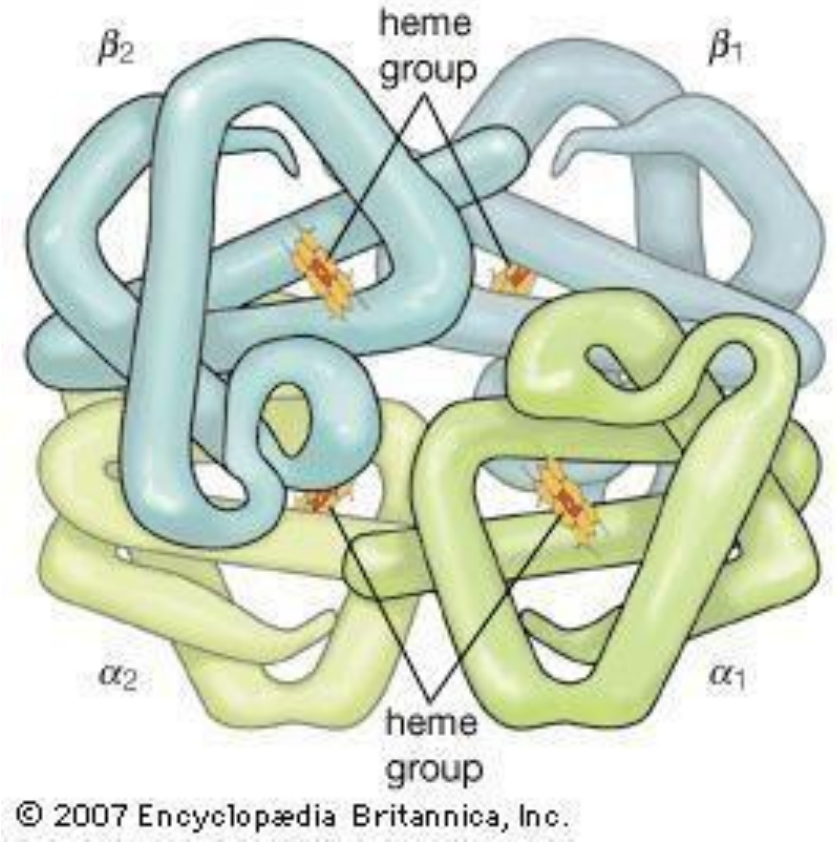
HOW ARE THE SUBUNITS CONNECTED?

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

covalent



The subunits of **hemoglobin** are held together by non covalent bonds, whereas in **antibodies**, disulfide covalent bonds exist.



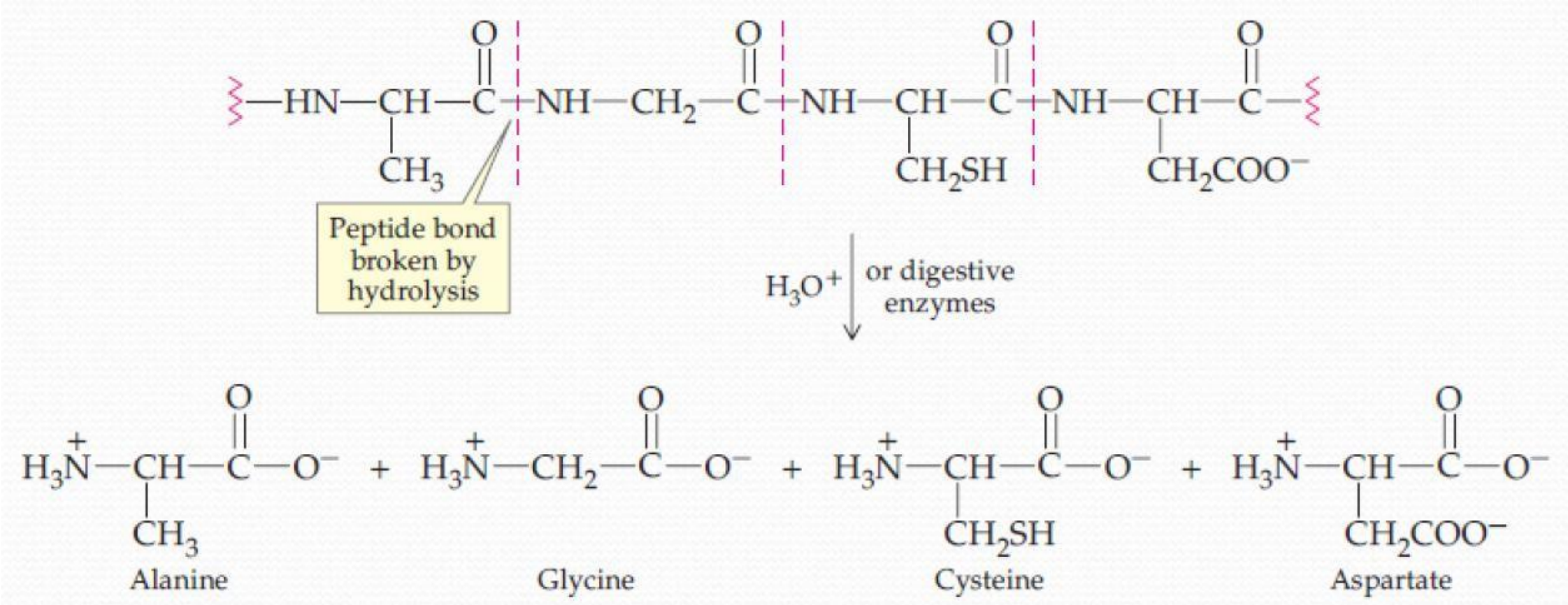
PROPERTIES OF PROTEINS:
PROTEIN HYDROLYSIS

Protein Hydrolysis:

·The backbone of all proteins is made up of amino acids connected by peptide bonds.

·These peptide bonds can be broken through hydrolysis, a process that uses enzymes and water (H_2O).

PROTEIN HYDROLYSIS



PROPERTIES OF PROTEINS:

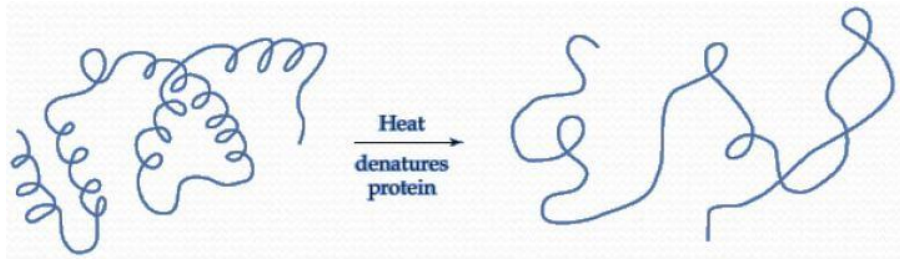
DENATURATION AND RENATURATION

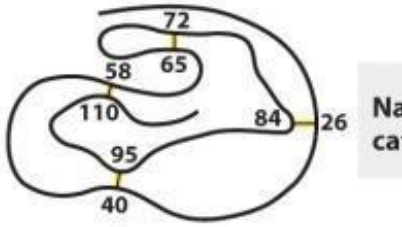
- **Denaturation:** applying a force to disrupt a protein's structure (loss of its final 3d shape). This affects all proteins regardless of their nature. Loss of 3d shape and not degradation, as degradation refers to hydrolysis.
- When a protein is placed **flat**(because of denaturation) in a solution, its **hydrophobic** regions unfold and become exposed to water, which is energetically unfavorable. As a result, these hydrophobic parts stick together, forming **protein aggregates**. In samples with high concentrations of soluble protein, if the proteins became flat this can cause a shift from a liquid to a solid state—such as when heat denatures egg proteins by breaking hydrogen bonds and altering their shape. Mechanical actions, like mixing eggs, can also break bonds and cause the protein to solidify.

- Changing the pH disrupts ionic interactions within the protein.

- In hand sanitizers containing ethanol, rubbing the hands allows ethanol to interact with bacterial proteins, disrupting their normal aggregation. This affects the proteins structure.

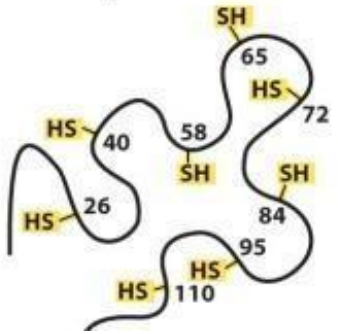
DENATURATION





Native state; catalytically active.

addition of urea and mercapto-ethanol



Unfolded state; inactive. Disulfide cross-links reduced to yield Cys residues.

- Solubility
- Heat
- Mechanical
- Extremes of pH
- Organic compounds: acetone, ethanol, bacterial proteins they bind with proteins from the outside, thus applying forces from the outside of the protein, disrupting the internal forces in the protein accordingly causing denaturation.
- Detergents (Triton X-100 (nonionic, uncharged) and sodium dodecyl sulfate (SDS, anionic, charged)) disrupt the hydrophobic forces.
  - SDS also disrupt electrostatic interactions.

Sodium dodecyl sulfate (SDS) has a hydrophobic tail that inserts into the interior of proteins, while its sulfate group carries a negative charge. This causes each amino acid it binds to gain a negative charge.

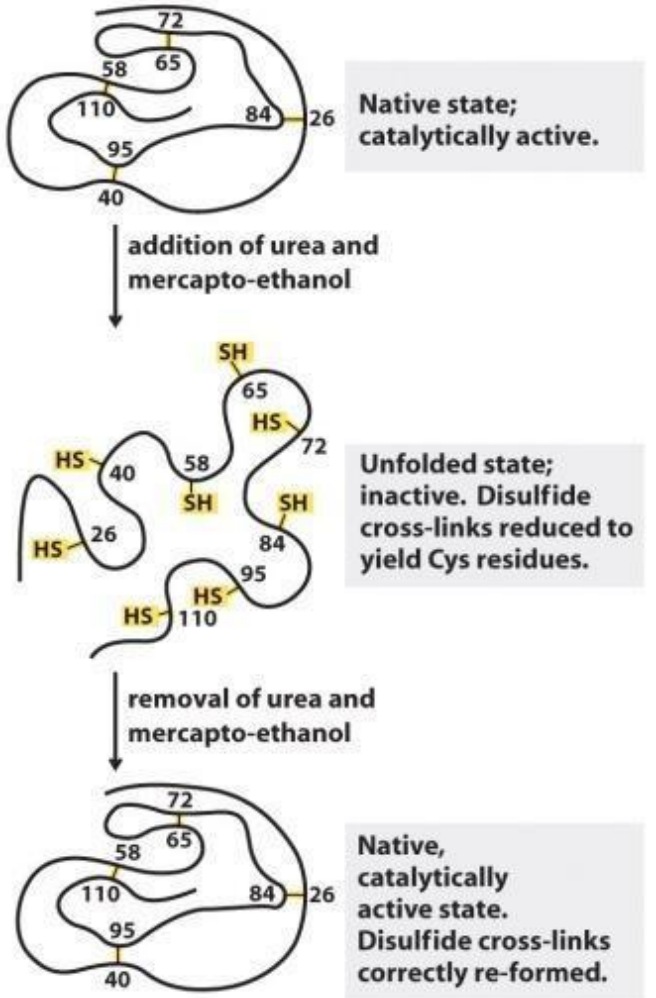
- Urea and guanidine hydrochloride disrupt hydrogen bonding and hydrophobic interactions.
- Reducing agents such as β-mercaptoethanol (βME) and dithiothreitol (DTT). Both reduce disulfide bonds. Thus losing its shape

RENATURATION

- Renaturation is the process in which the native conformation of a protein is re-acquired
- Most denaturation is irreversible

Only few proteins can renature and get back to their original 3d structure.

- 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



FACTORS THAT DETERMINE PROTEIN 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, mainly, the **R groups**
  - **Non-protein molecules**

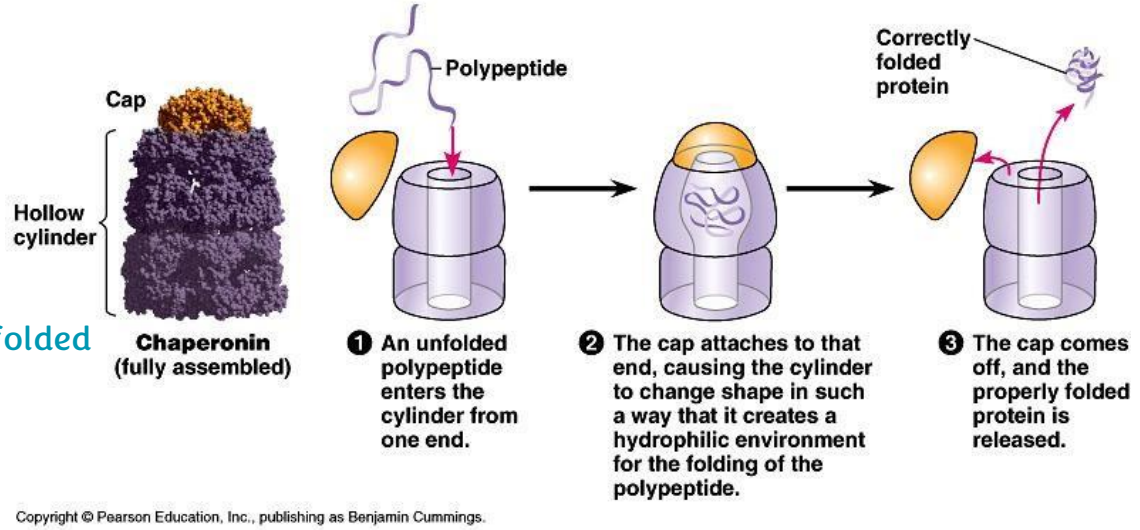
the interactions it causes

And covalent interactions

Such as metals attached to that protein

The **first** chaperon discovered is the **Hsp70** (Heat shock protein 70).

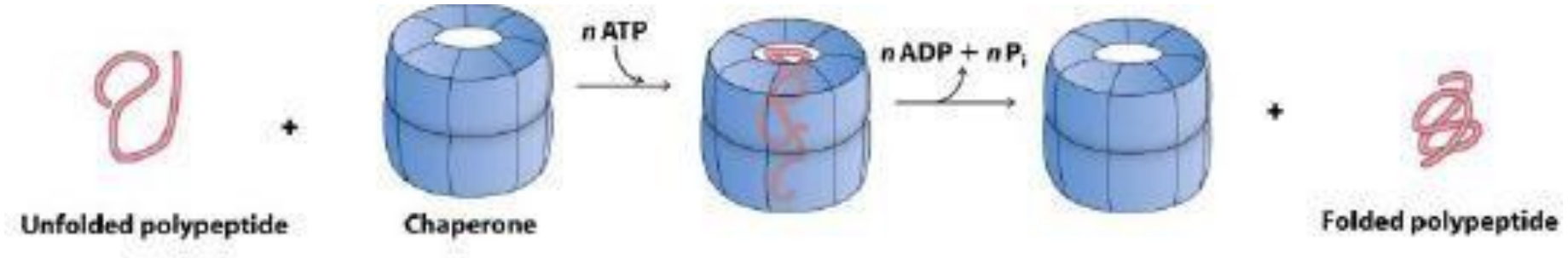
PROBLEM SOLVERS: CHAPERONES



How does **chaperons** work?
Chaperones are barrel-shaped proteins made up of many amino acids. They receive unfolded polypeptide chains from ribosomes and help them fold correctly. The side chains of the chaperone's amino acids interact with the unfolded protein, guiding its proper folding.

Note: Not all proteins require chaperons to fold.

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

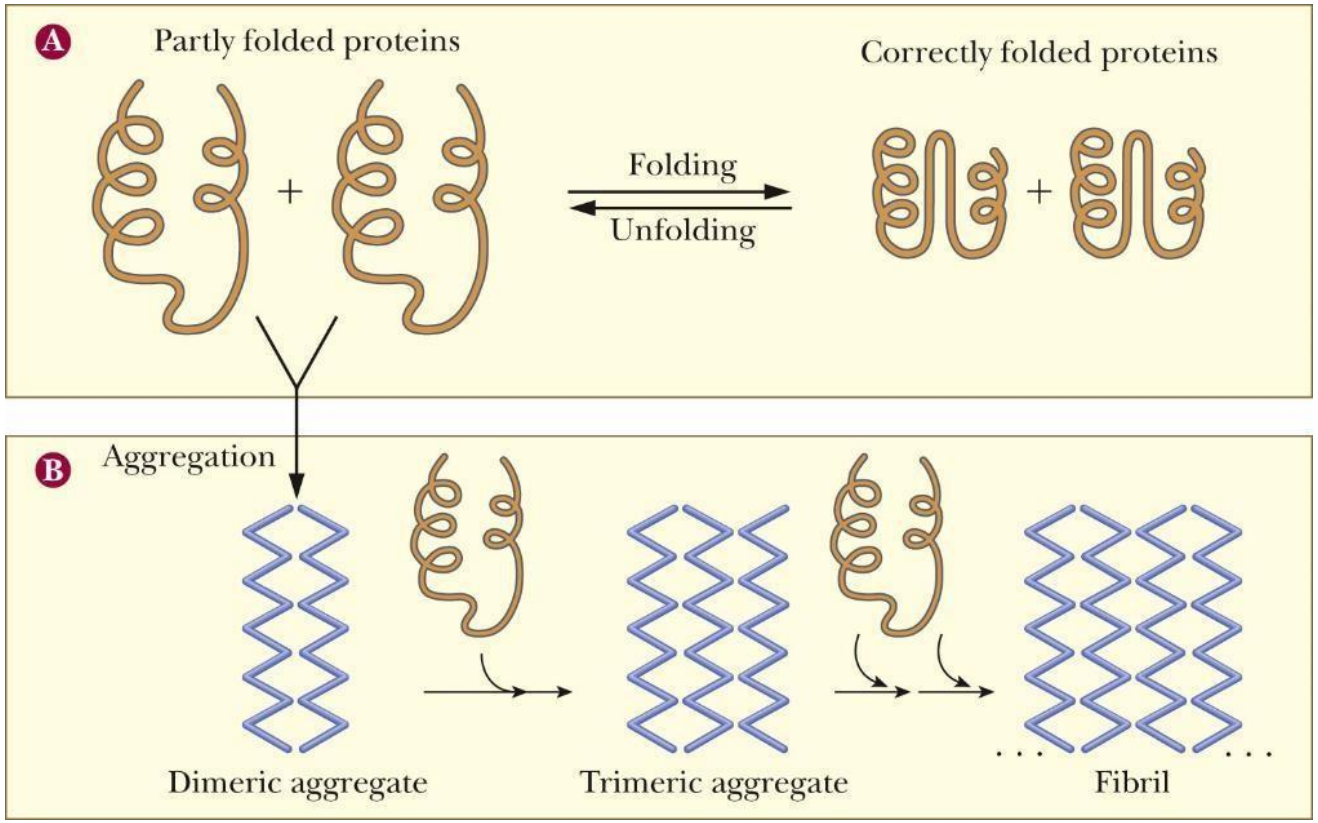


Many diseases are the result of defects in protein folding

In patients with fever, high body temperature can cause denaturation of proteins. To reduce the risk of heat-related damage, especially to the brain, we apply cold compresses(كمادات باردة).This helps lower the temperature and protect brain function, since the brain is highly sensitive to heat.

THE PROBLEM 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.



For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	Slide 33(hand sanitizer)	In hand sanitizers containing ethanol, rubbing the hands allows ethanol to interact with bacterial proteins, disrupting their aggregation. This affects protein function and interfere with cell communication by taking up more space.	In hand sanitizers containing ethanol, rubbing the hands allows ethanol to interact with bacterial proteins, disrupting their normal aggregation. This affects the proteins structure.
V1 → V2			

رسالة من الفريق العلمي: