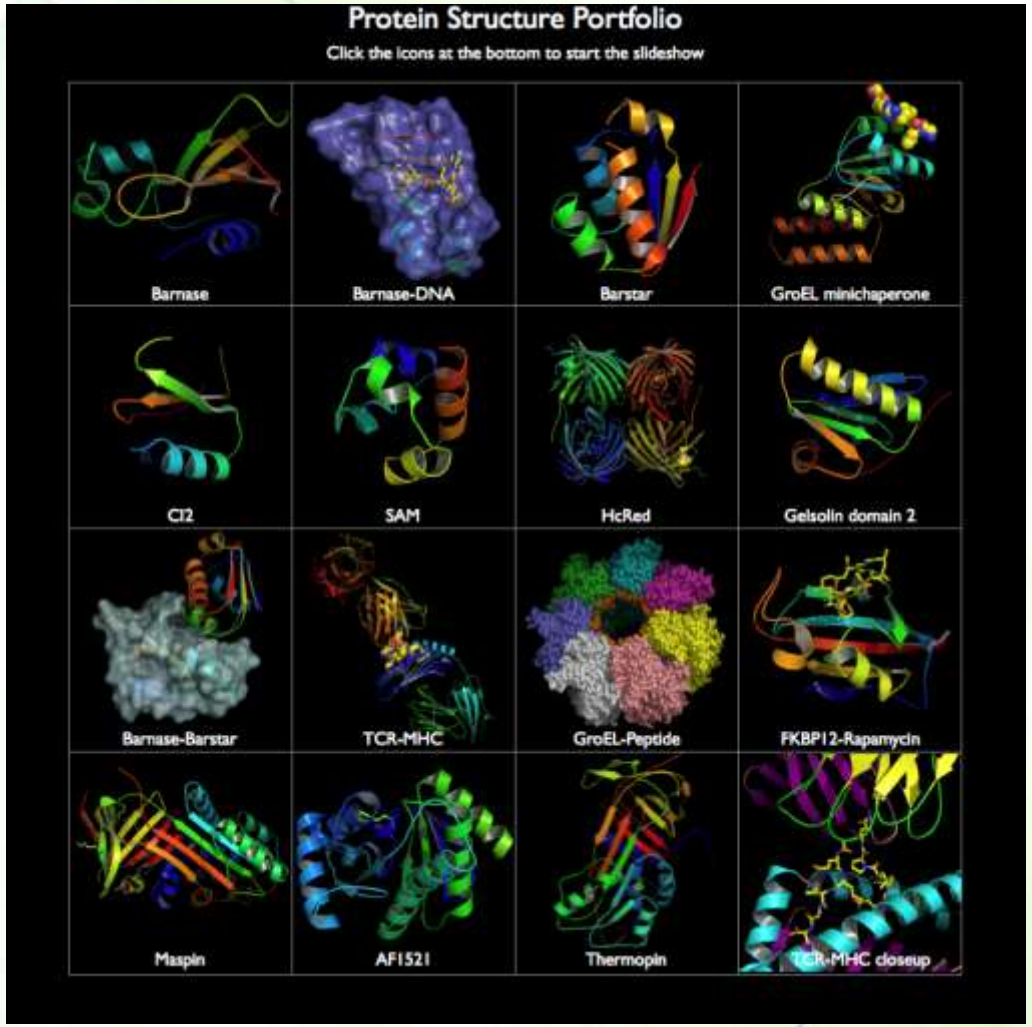
Protein structure

Summer 2023

Overview of proteins

- Proteins have different structures, and some have repeating inner structures, other do not.
- A protein may have *gazillion* possibilities of structures, but a few would be active.
- These active structures are known as native conformations (the 3-dimensioanl structure of a properly folded and functional protein).



Tunyasuvunakool, K., Adler, J., Wu, Z. et al. Highly accurate protein structure prediction for the human proteome. Nature (2021). https://doi.org/10.1038/s41586-021-

Highly accurate protein structure prediction for the human proteome

Kathryn Tunyasuvunakool, Jonas Adler, [...]Demis Hassabis

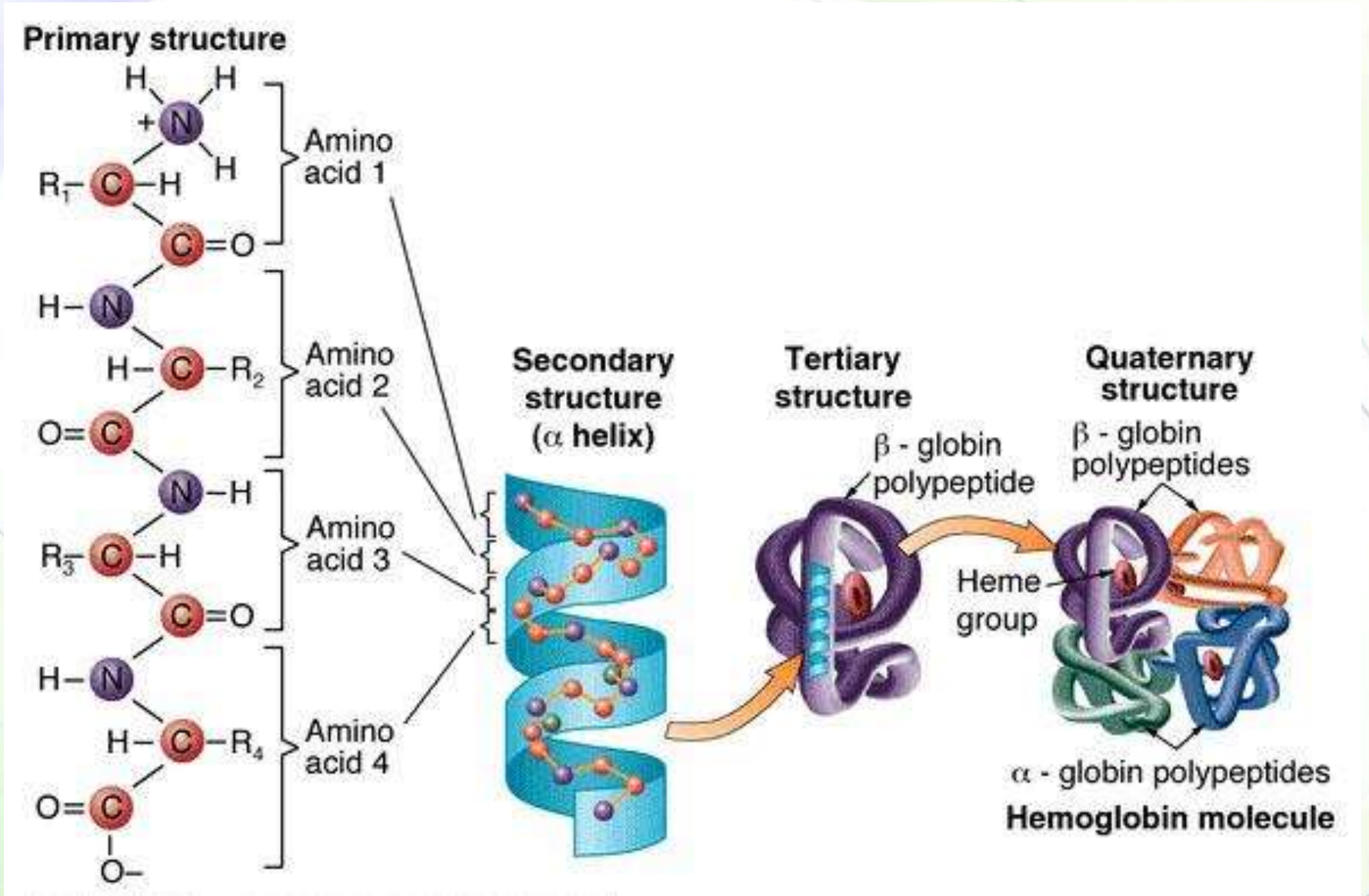
Nature (2021) | Cite this article

New public database of AI-predicted protein structures could transform biology

By Robert F. Service | Jul. 22, 2021 , 11:00 AM

Levels of protein structure

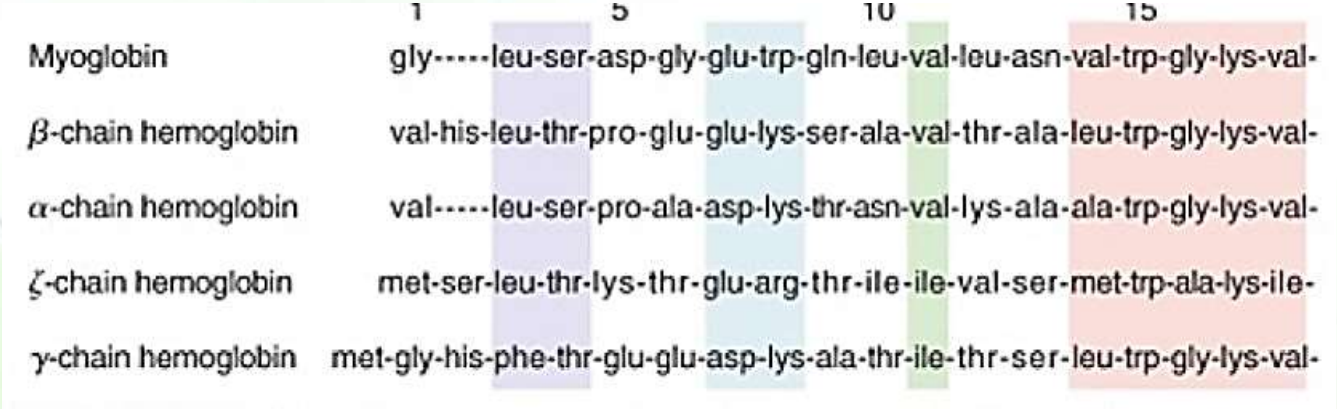
- Primary structure: the sequence of amino acid residues
- Secondary structure: the localized organization of parts of a polypeptide chain
- Tertiary structure: the three-dimensional structure and/or arrangement of all the amino acids residues of a polypeptide chain
- Some proteins are made of multiple polypeptides crosslinked (connected) with each other. These are known as multimeric proteins. Quaternary structure describes the number and relative positions of the subunits in a multimeric protein


Primary structure
Amino acid 1
Amino acid 2
Amino acid 3
Amino acid 4
Secondary structure (α helix)
Tertiary structure
β - globin polypeptide
Quaternary structure
β - globin polypeptides
Heme group
α - globin polypeptides
Hemoglobin molecule

Primary structure

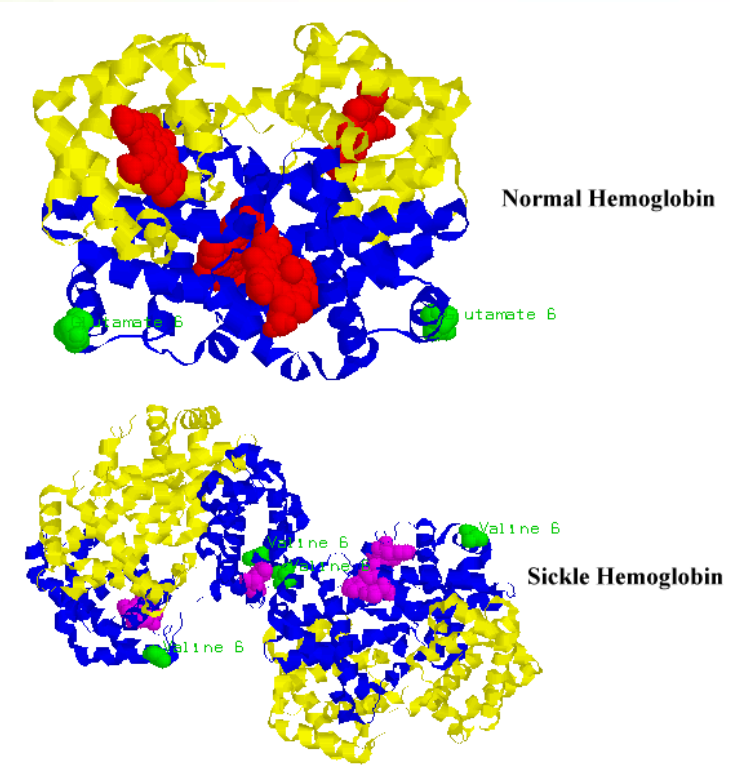
What is primary structure?

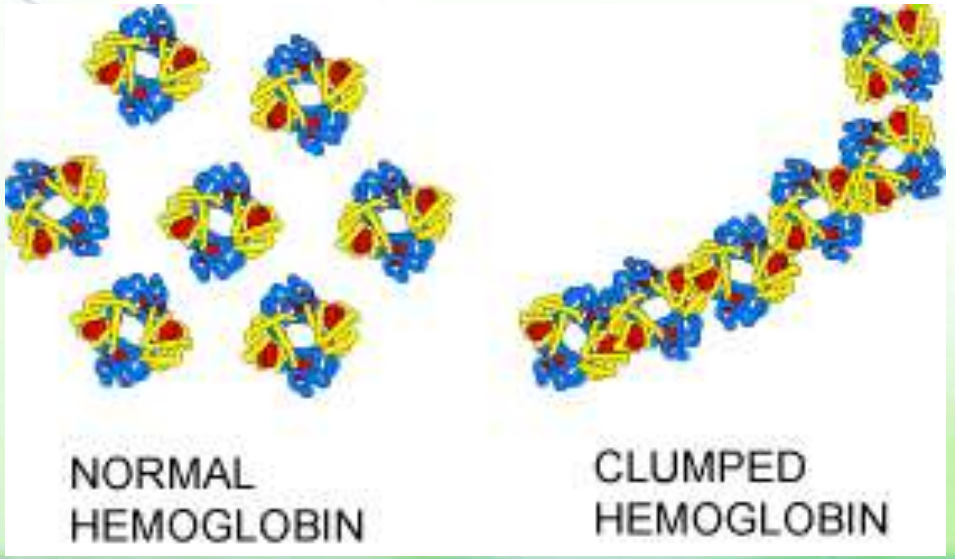
- The order in which the amino acids are covalently linked together.
  - Example: Leu—Gly—Thr—Val—Arg—Asp—His
- The primary structure of a protein determines the other levels of structure.
- Proteins that differ somewhat in primary structure and properties from tissue to tissue, but that retain essentially the same function, are called tissue-specific isoforms or isozymes.

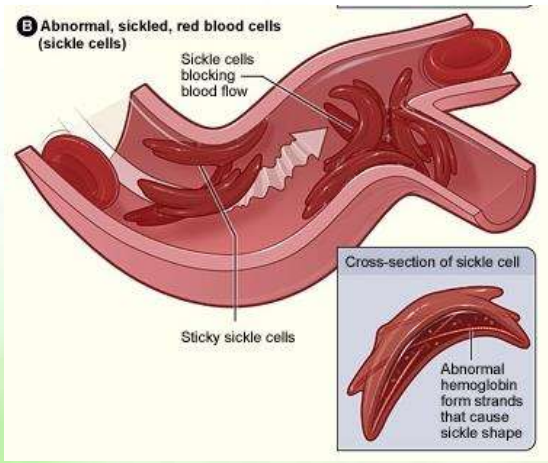


Sickle cell hemoglobin (HbS)

- A single amino acid substitution can give rise to a malfunctioning protein, as is the case with sickle-cell anemia.
- 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.







Secondary Structure

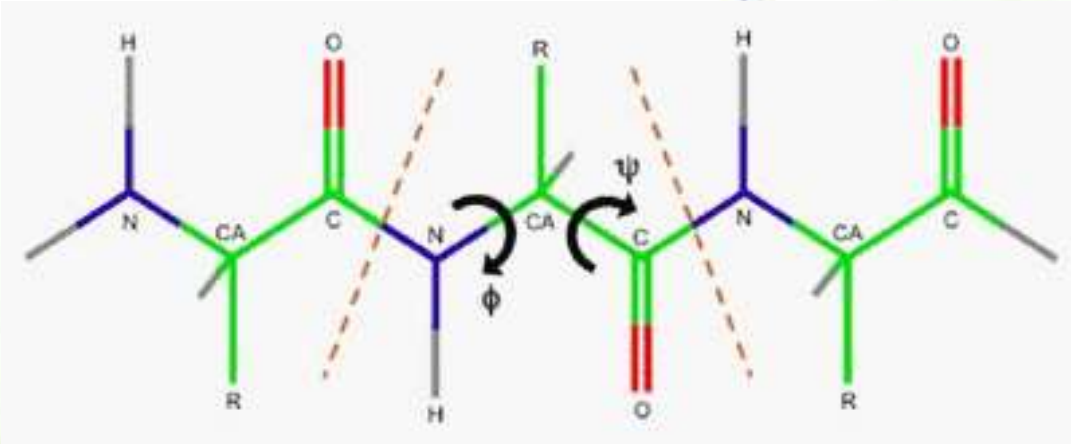
What is it? How is it caused?

- The two bonds within each amino acid residue freely rotate.
  - the bond between the α-carbon and the amino nitrogen
  - the bond between the α-carbon and the carboxyl carbon
- 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

  } Regular secondary structures

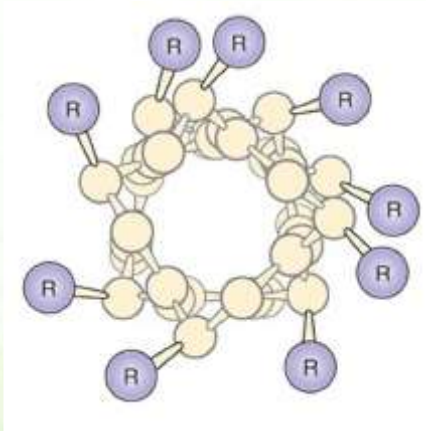
  - Turns
  - Loops
  - Bends
  - Coils

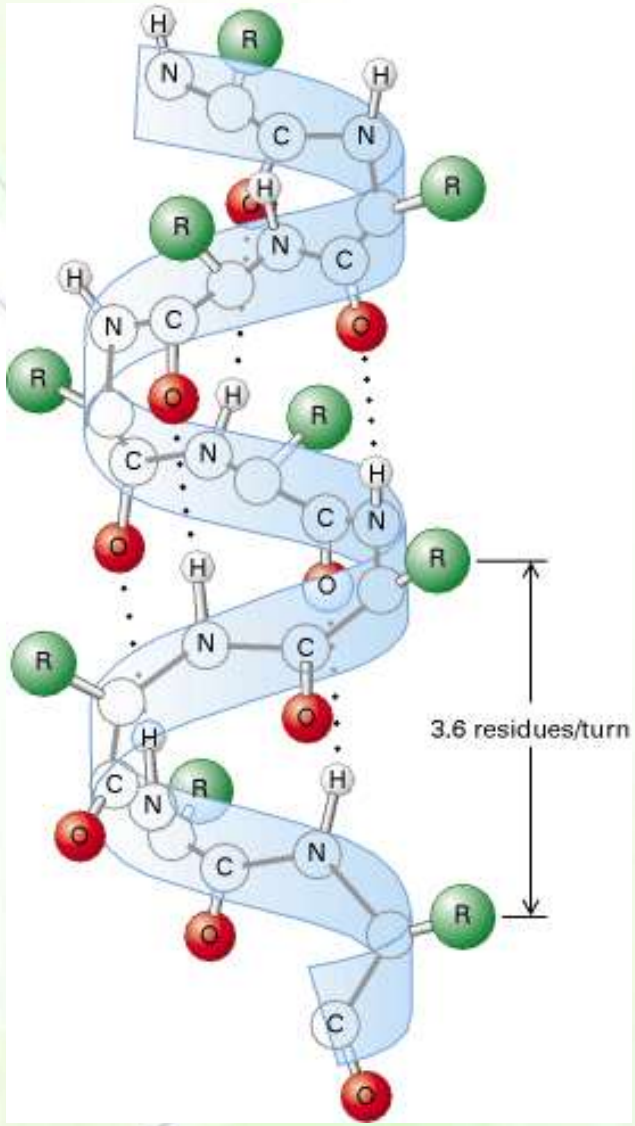
  } Nonregular secondary structures



The α helix

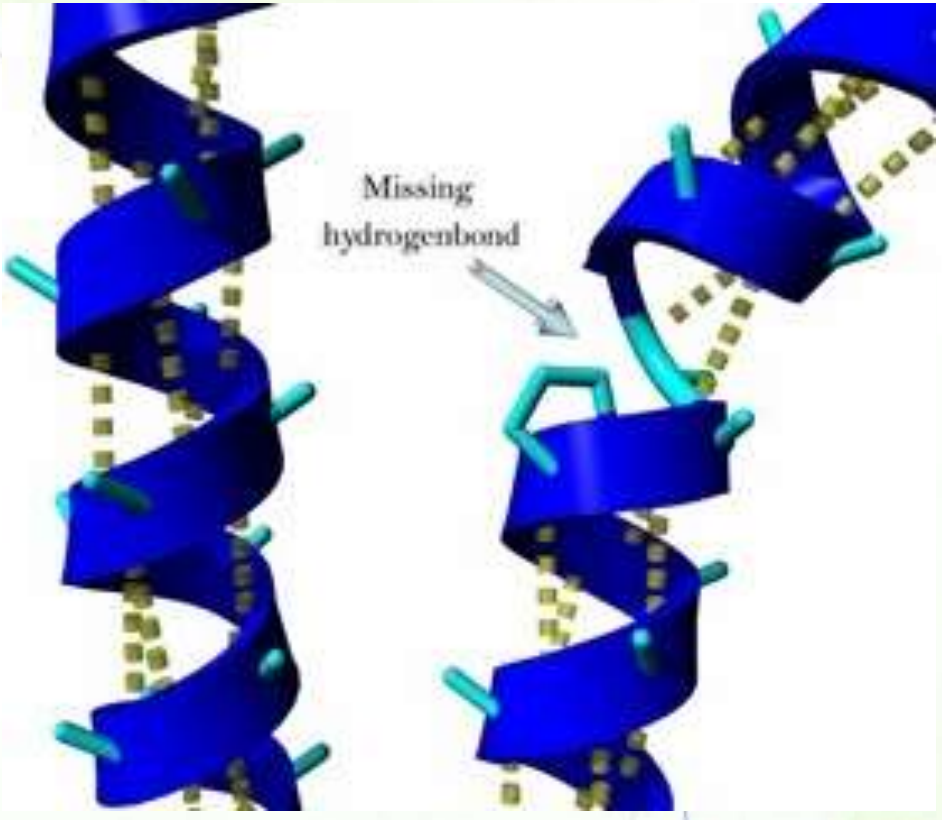
- It looks like a helical rod.
- The helix has an average of 3.6 amino acids per turn.
- The pitch of the helix (the linear distance between corresponding points on successive turns) is 5.4 Å.
  - 1 Å = 10^{-10} m
- It is very stable because of the linear hydrogen bondings.
- The trans side chains of the amino acids project outward from the helix, thereby avoiding steric hindrance with the polypeptide backbone and with each other.

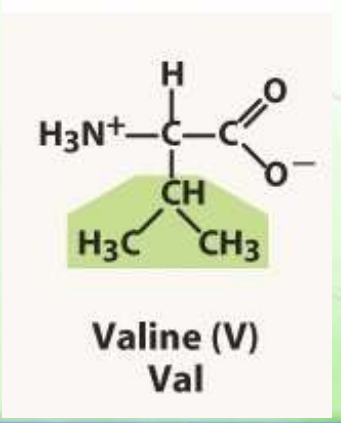


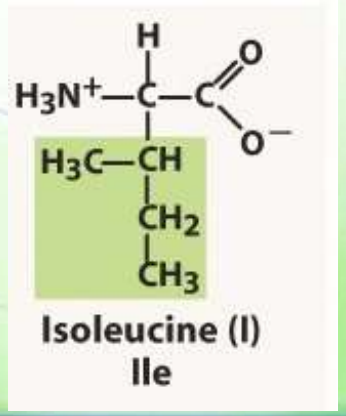


Amino acids NOT found in α-helix

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

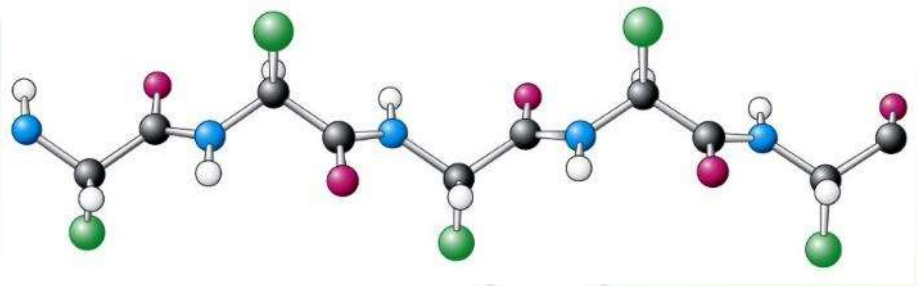






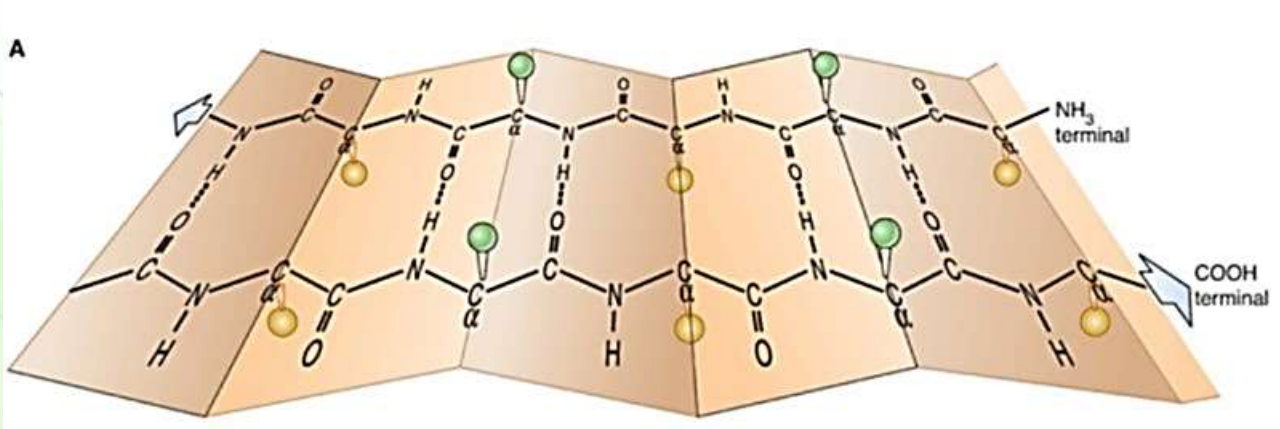
β pleated sheet (β sheet)

- They are composed of two or more straight chains (β strands) that are hydrogen bonded side by side.



β strand

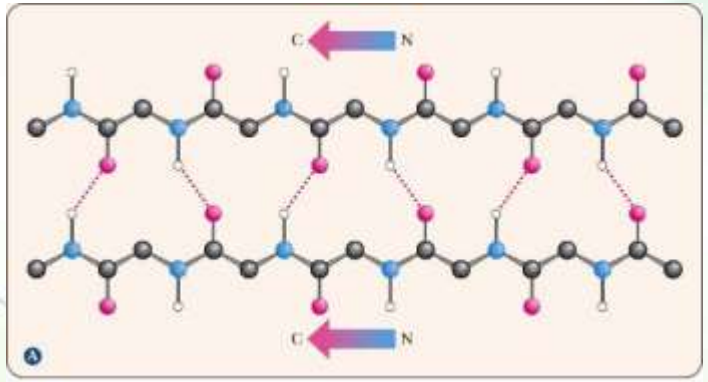
- Optimal hydrogen bonding occurs when the sheet is bent (pleated) to form β-pleated sheets.



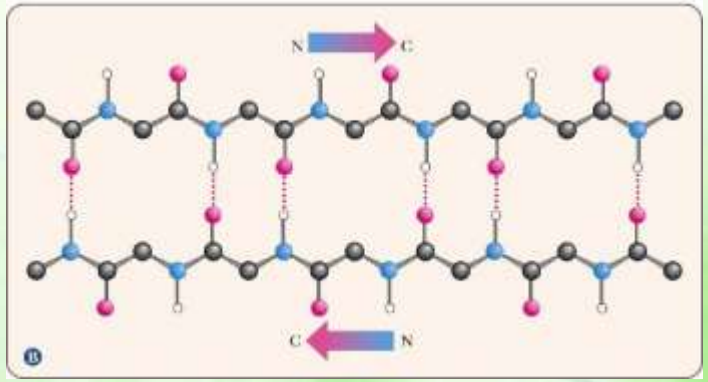
More on β-sheets

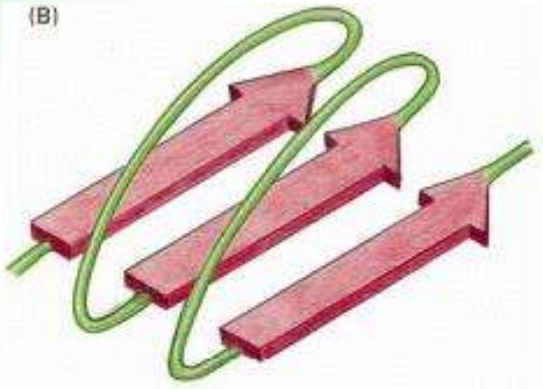
- β sheets can form between many strands, typically 4 or 5 but as many as 10 or more.
- Such β sheets can be purely antiparallel, purely parallel, or mixed.

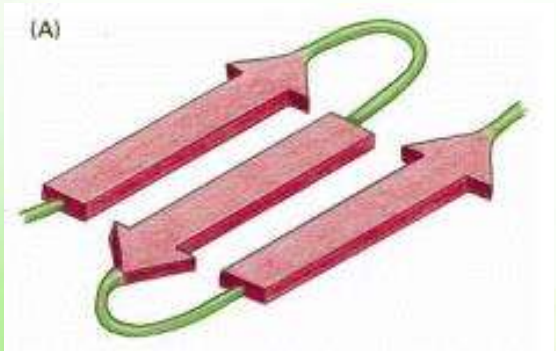
Parallel

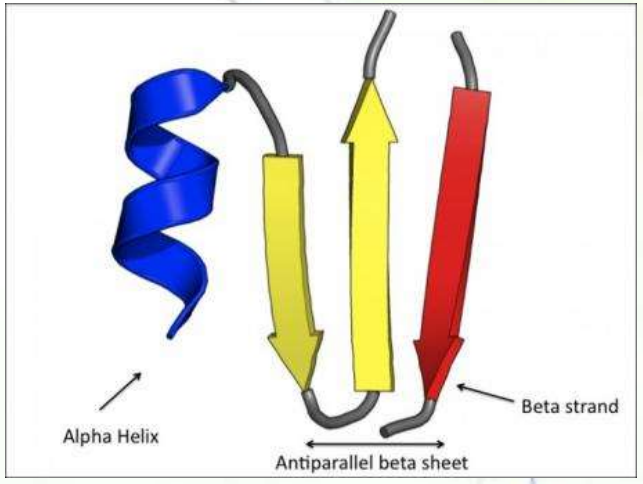


Antiparallel







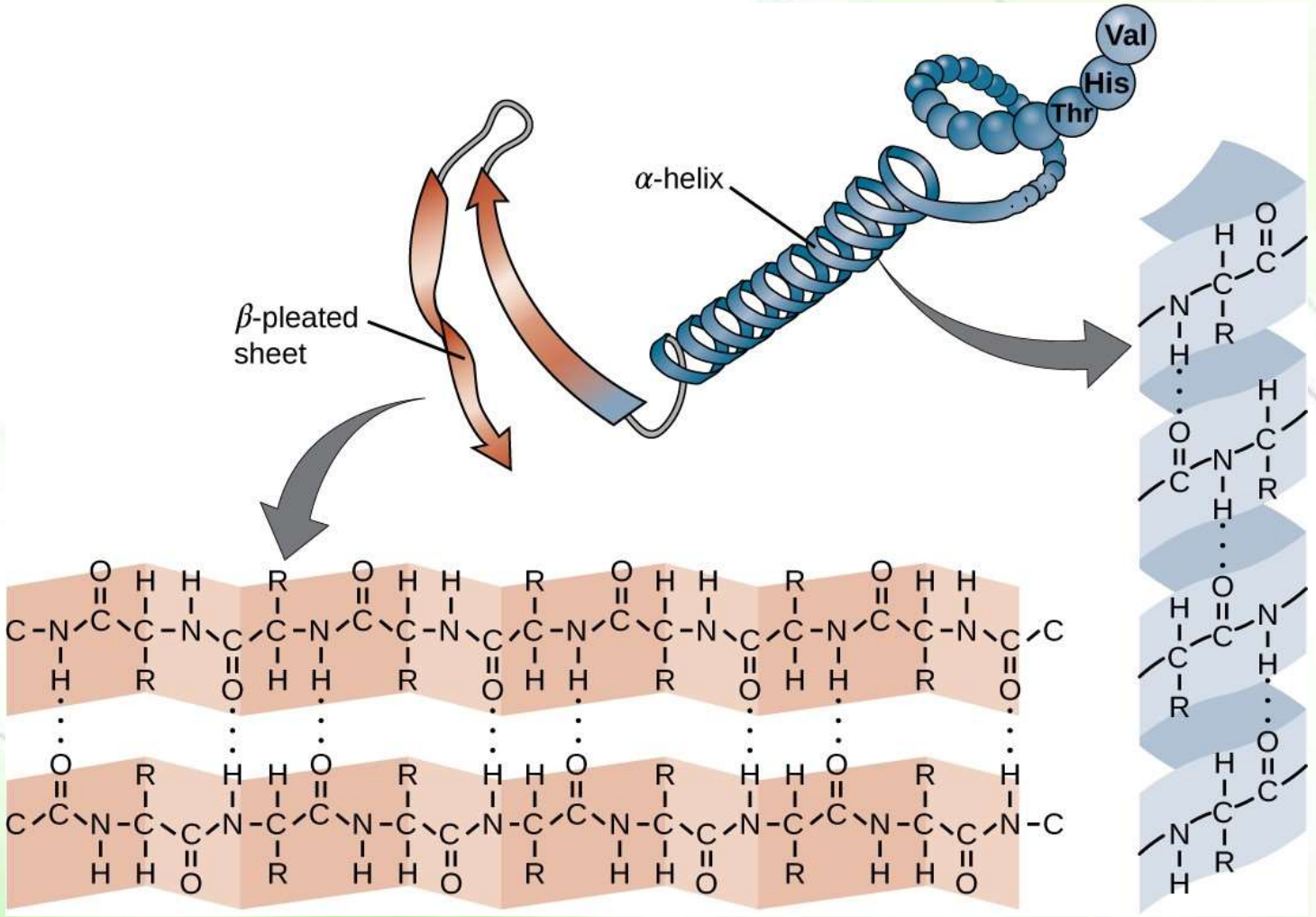


Based on hydrogen bonding pattern, which do you think is more stable: parallel or anti-parallel sheets?

Effect of amino acids

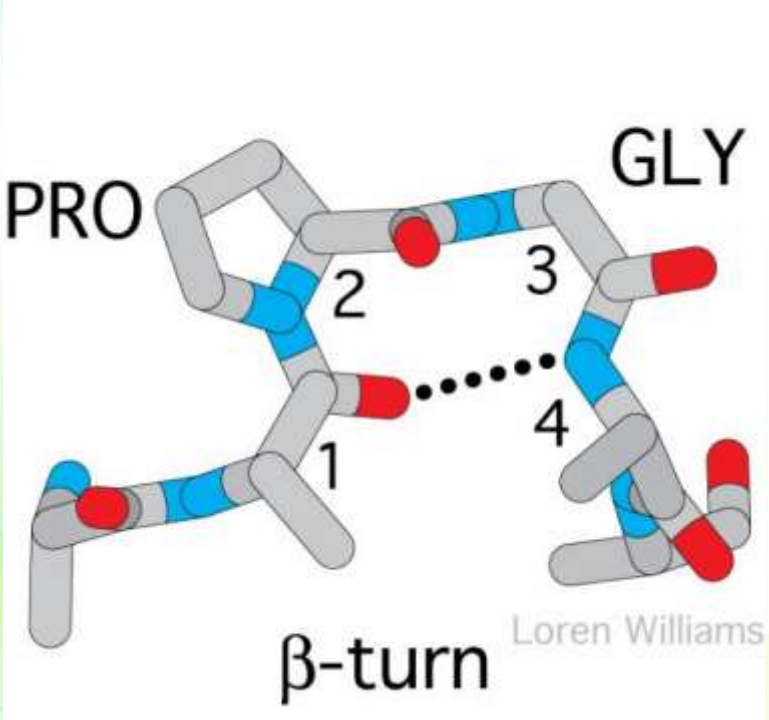
- Valine, threonine and Isoleucine with branched R groups at β-carbon and the large aromatic amino acids (phenylalanine, tryptophan, and tyrosine) tend to be present in β-sheets.
- Proline tends to disrupt β strands

How are they illustrated/drawn?



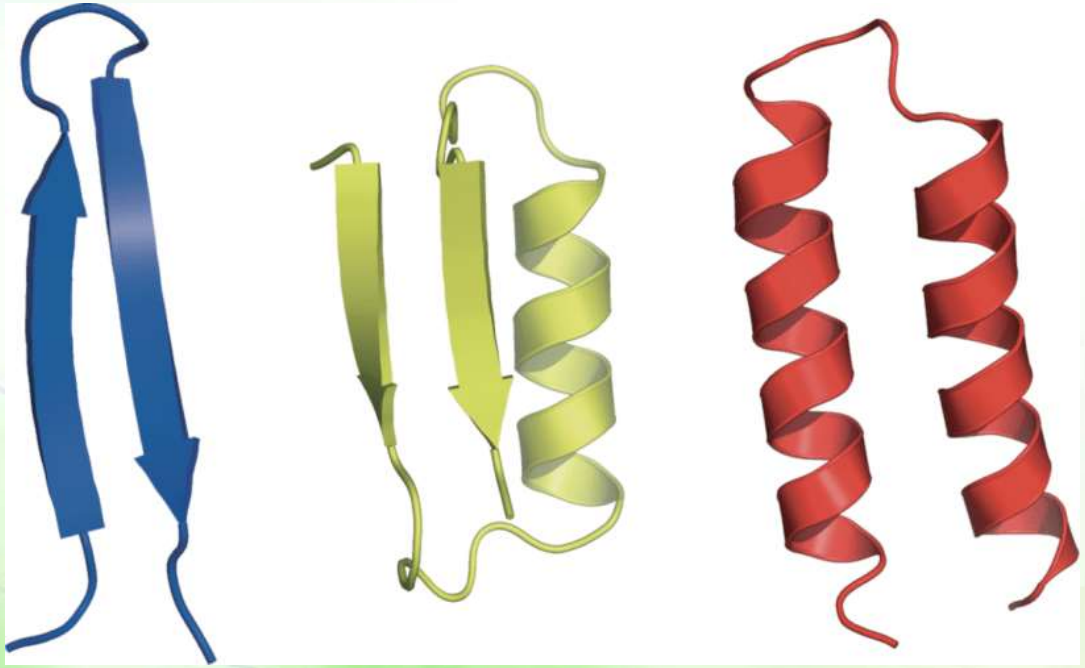
β-turns

- 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?



Loops and coils

- Loops are a diverse class of secondary structures in proteins with irregular geometry and that connect the main secondary structures.
- They are found on surface of molecule (and contain polar residues) and provide flexibility to proteins.
- Amino acids in loops are often not conserved.

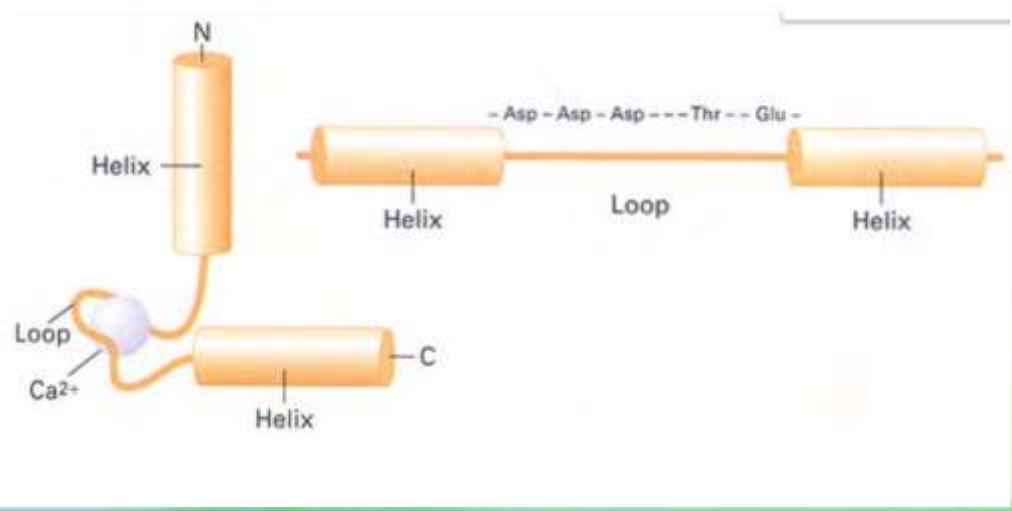


Super-secondary structures

- They are regions in proteins that contain an ordered organization of secondary structures.
- There are at least types:
  - Motifs
  - Domains

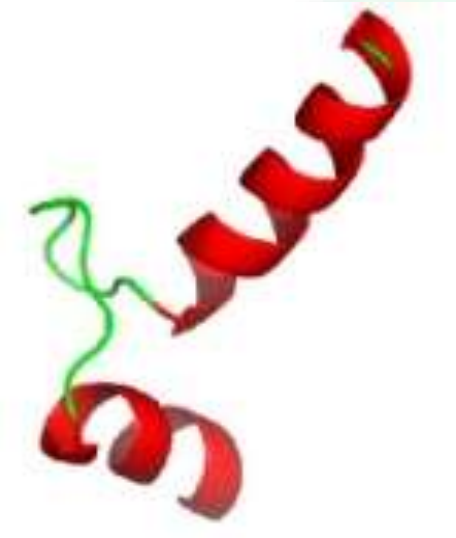
A motif (a module)

- A motif is a repetitive supersecondary structure, which can often be repeated and organized into larger motifs and they can be part of domains.
- It usually constitutes 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.

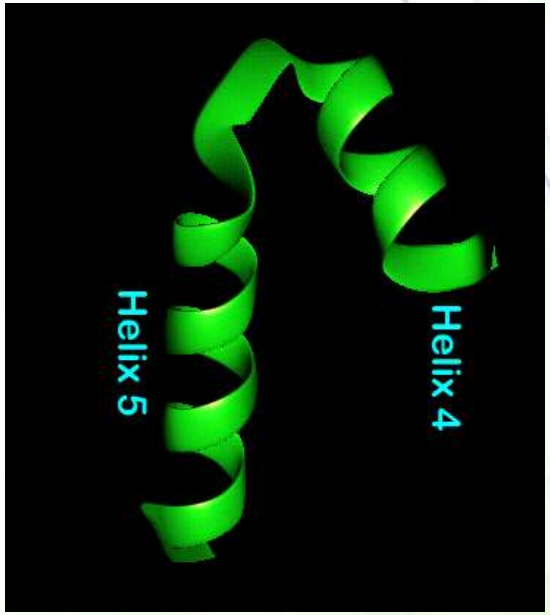


Examples of motifs

Helix-loop-helix is found in many proteins that bind DNA. It is characterized by two α-helices connected by a loop.

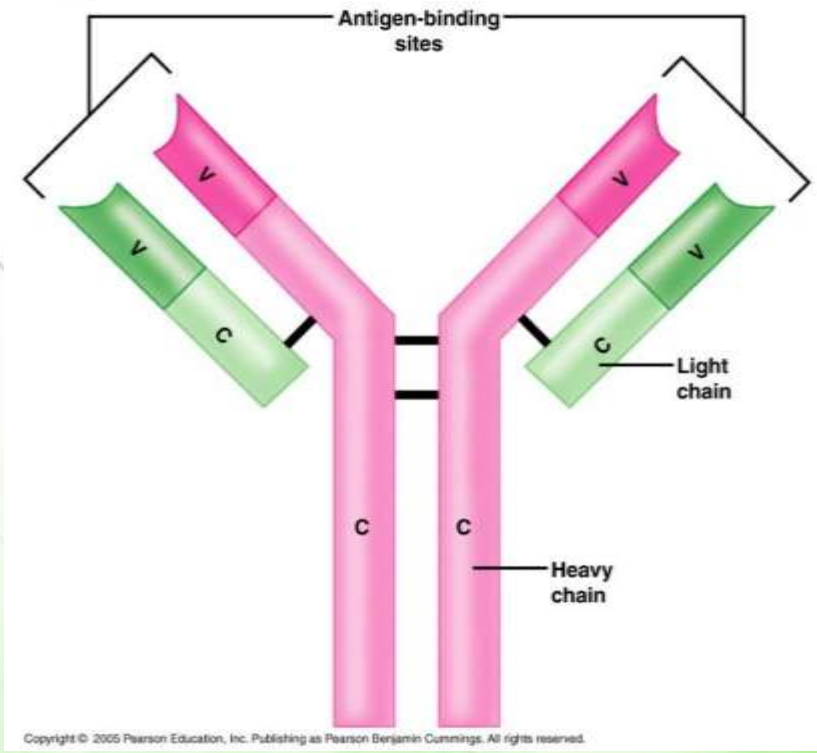


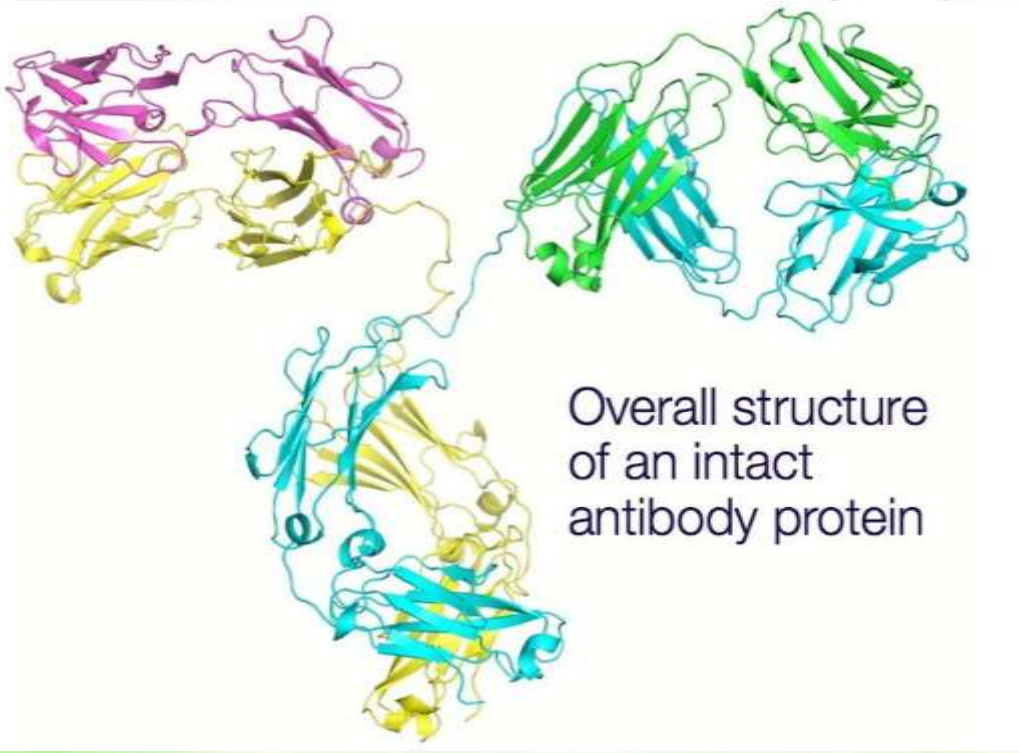
Helix-turn-helix is a structural motif capable of binding DNA. It is composed of two α helices joined by a short strand of amino acids

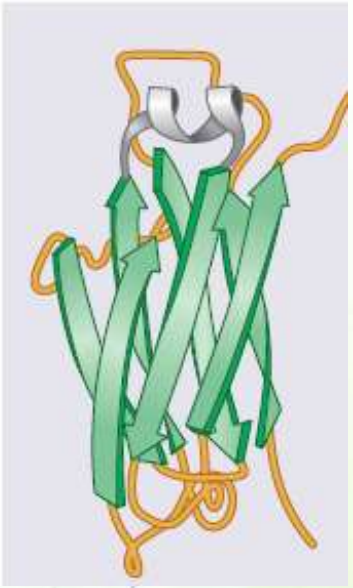


A more complex motif is…

- The immunoglobulin fold or module that enables interaction with molecules of various structures and sizes.



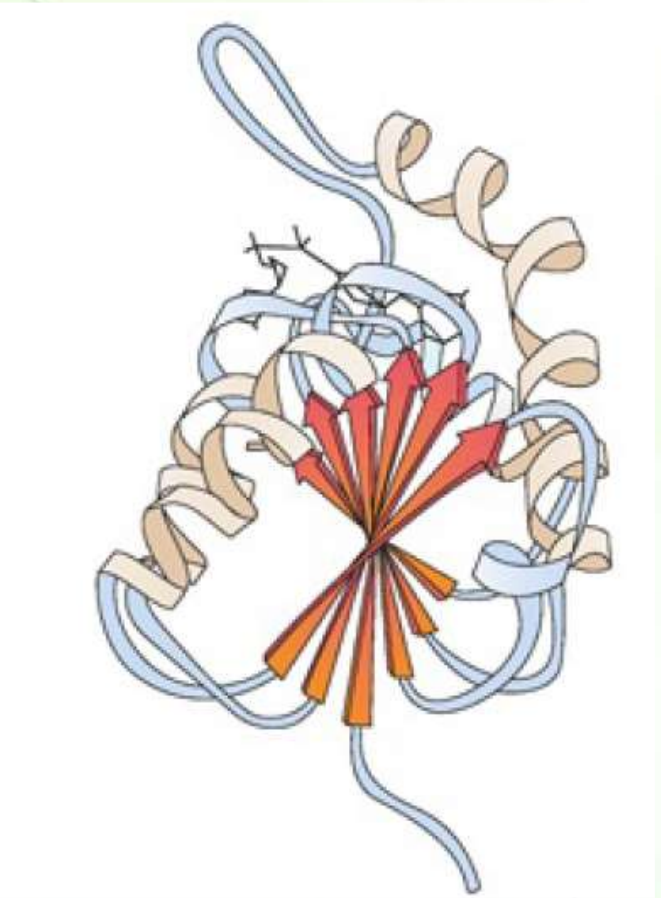




Tertiary structure

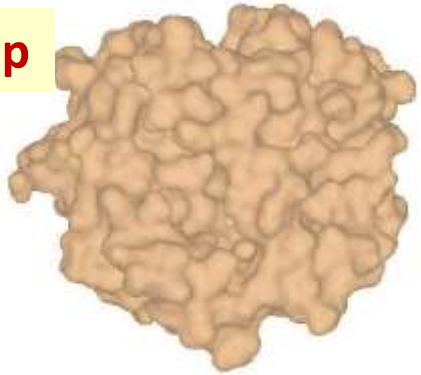
What is tertiary structure?

- 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

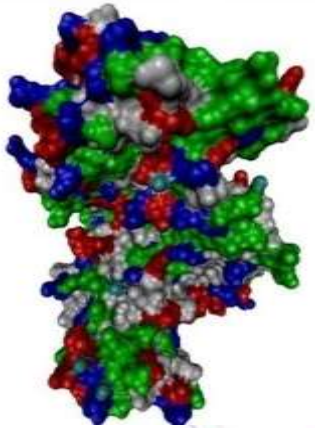


How to look at proteins…

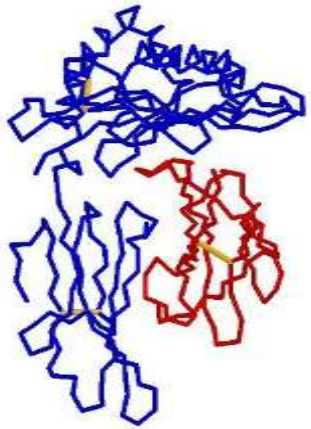
Protein surface map



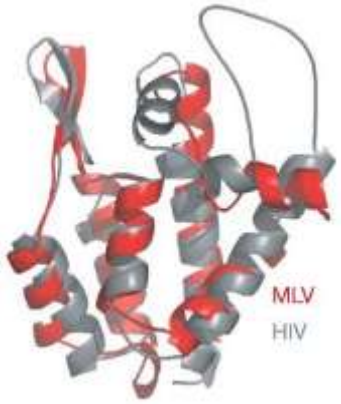
Space-filling structure



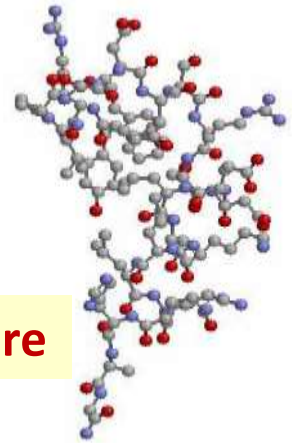
Trace structure



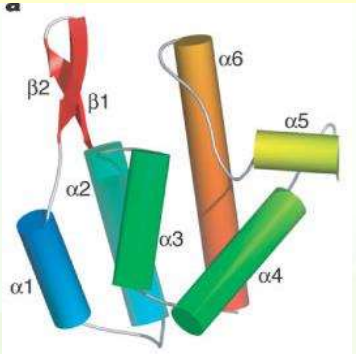
Ribbon structure



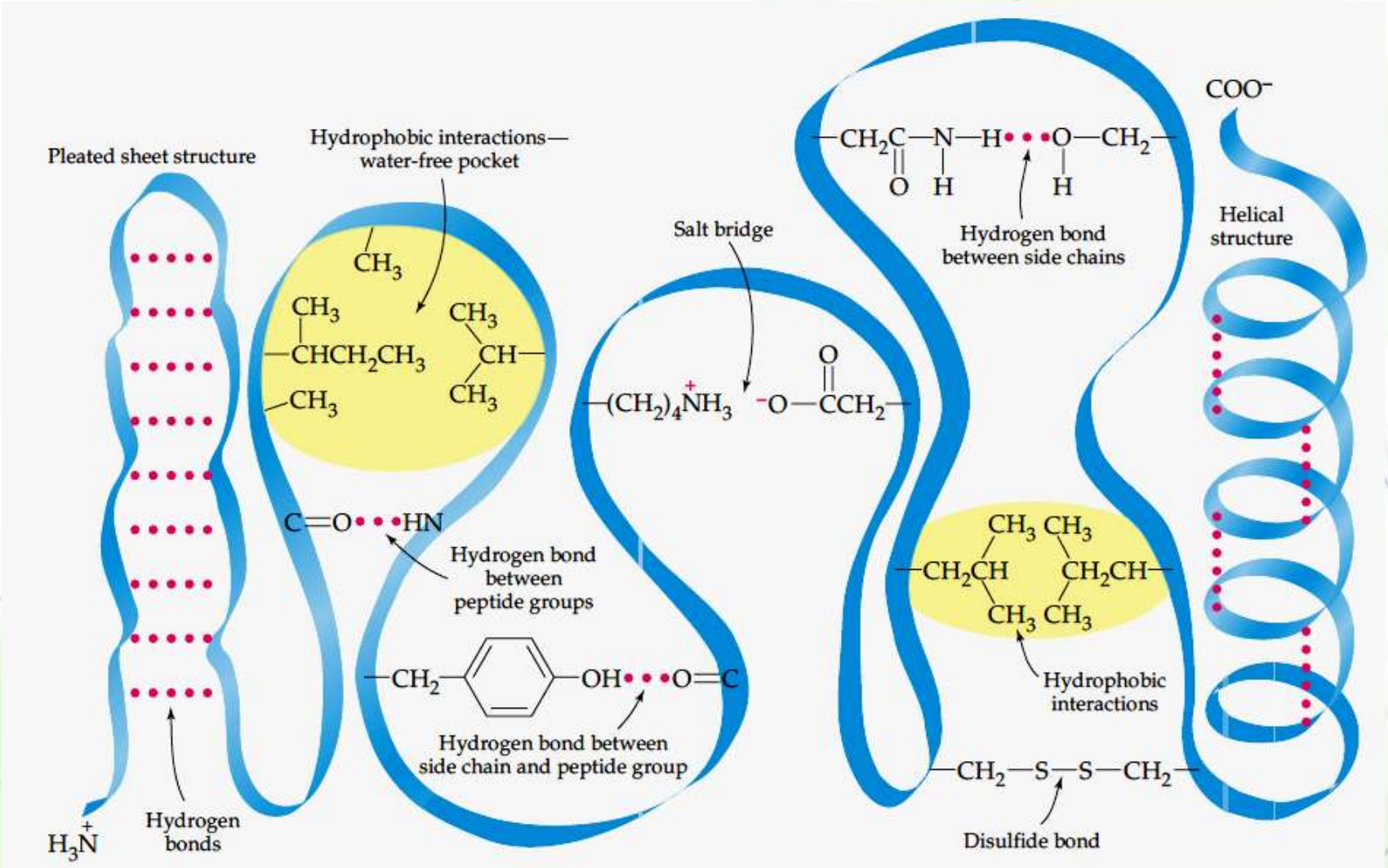
Ball and stick structure



Cylinder structure

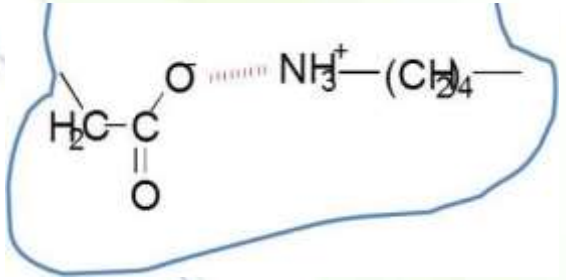


Shape-determining forces

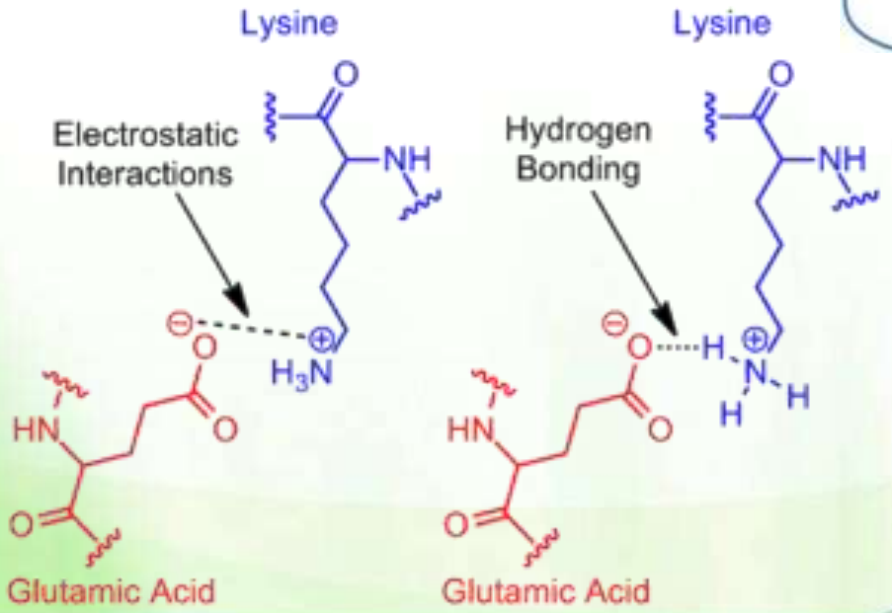


Non-covalent interactions

- Hydrogen bonds occur not only within and between polypeptide chains but with the surrounding aqueous medium.
- 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.

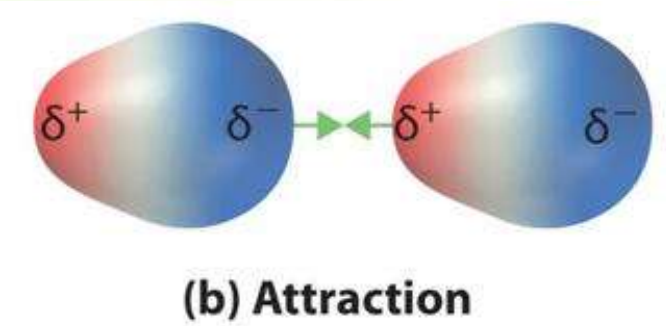


The same charged group can form either hydrogen bonding or electrostatic interactions

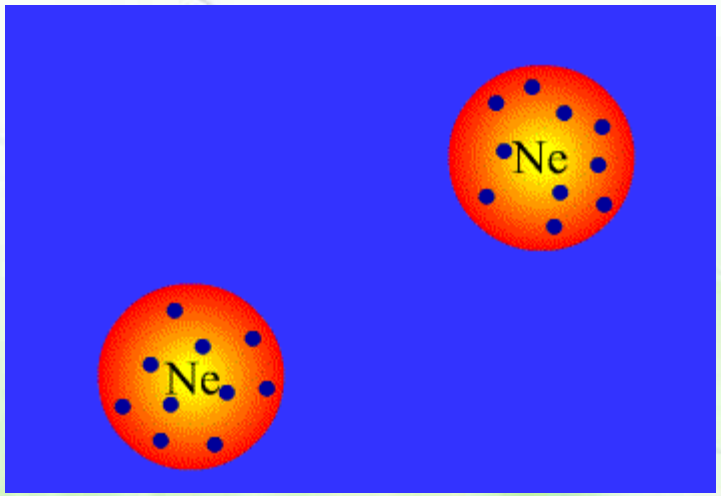


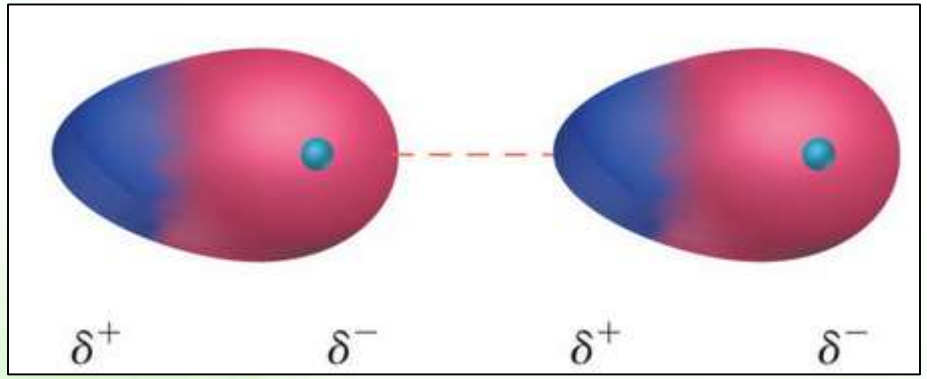
van der Waals attractions

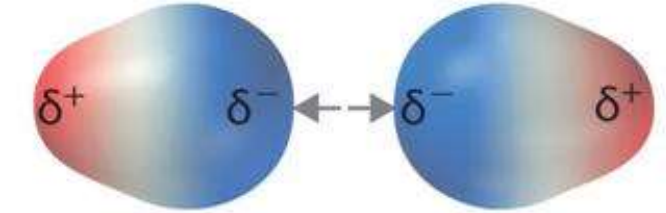
- There are both attractive and repulsive van der Waals forces that control protein folding.
- Although van der Waals forces are extremely weak, they are significant because there are so many of them in large protein molecules.



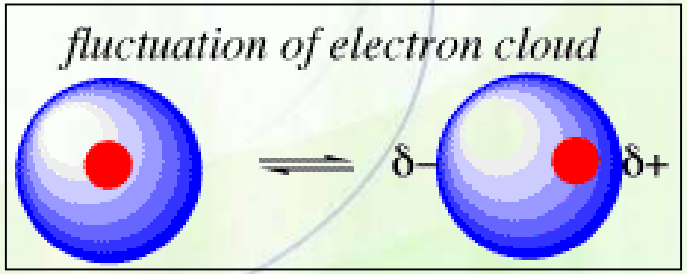
(b) Attraction





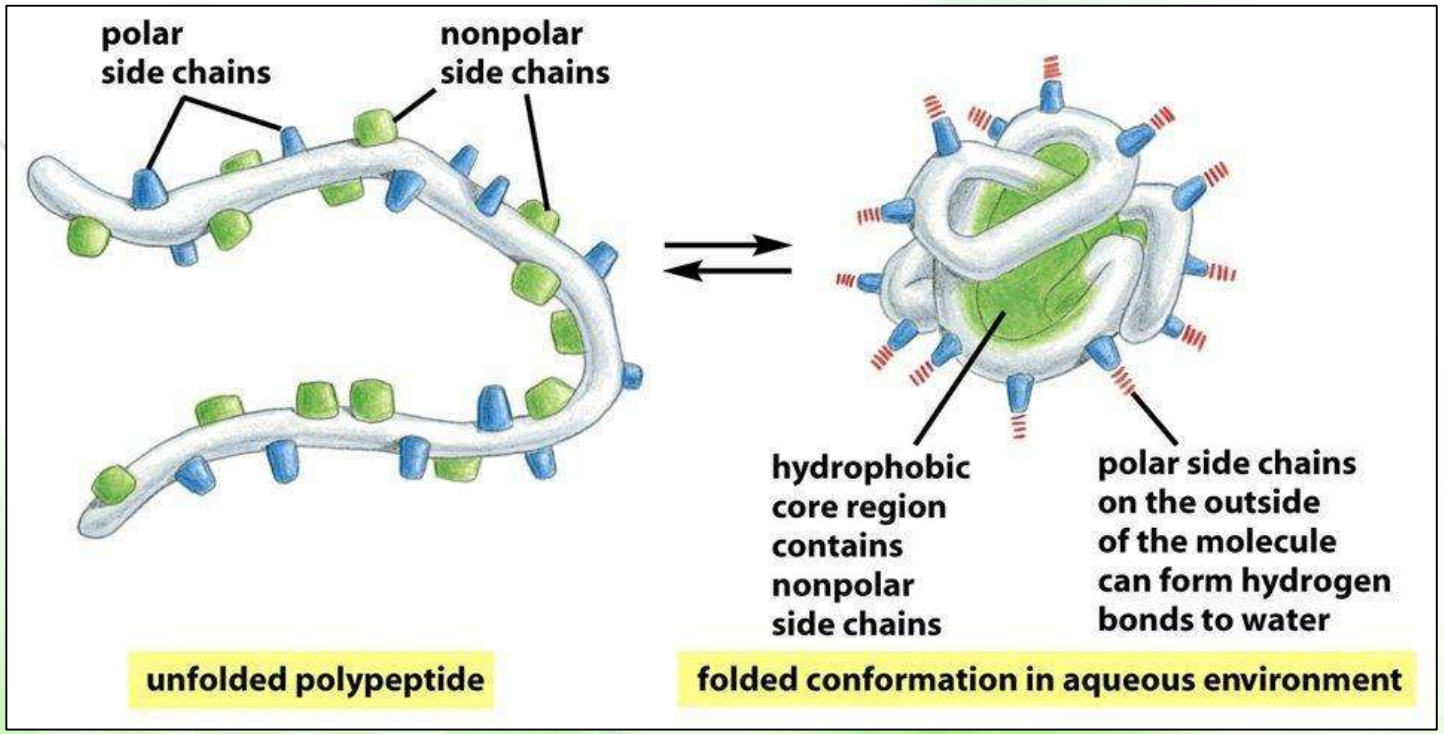


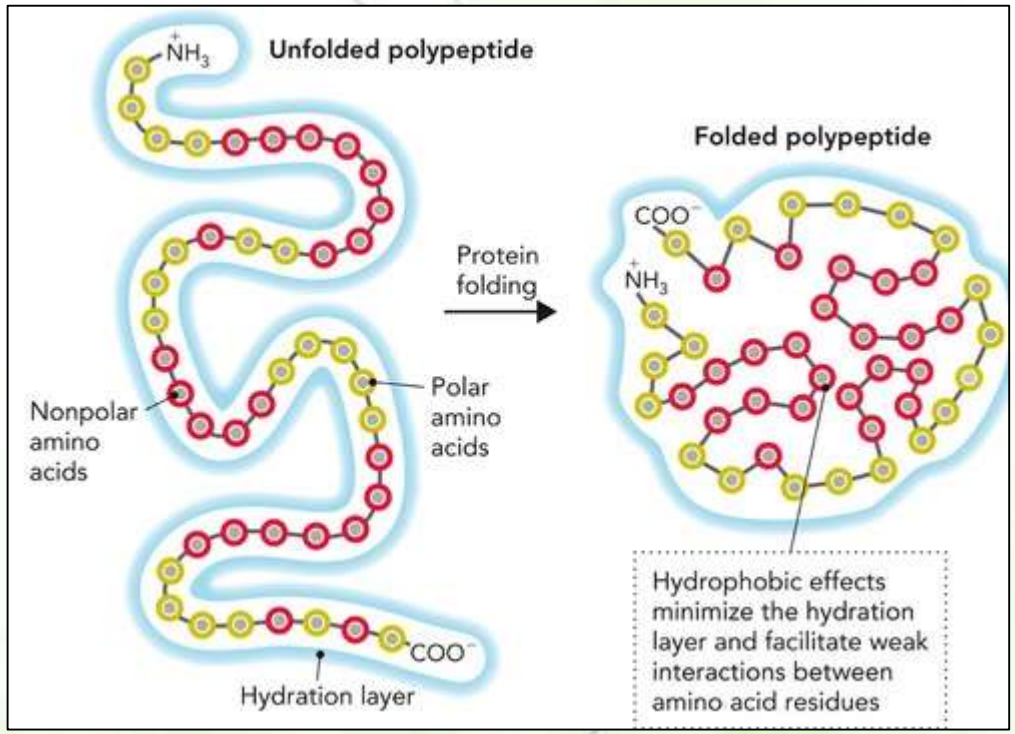
(d) Repulsion



Hydrophobic interactions

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

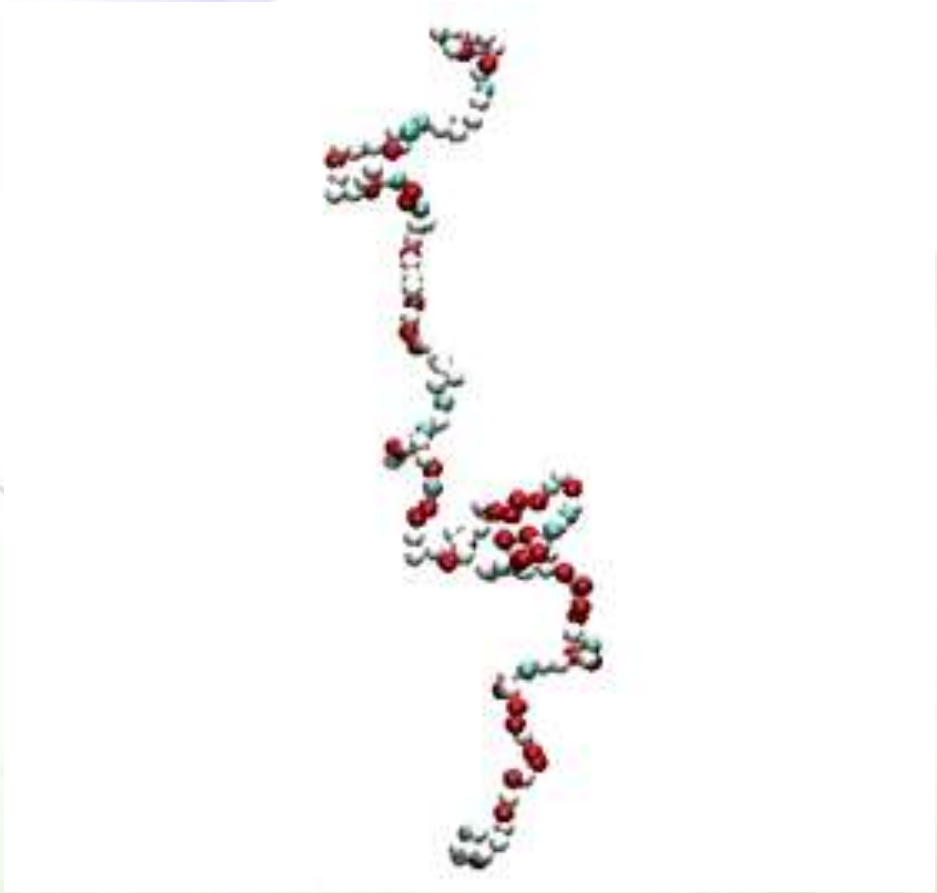


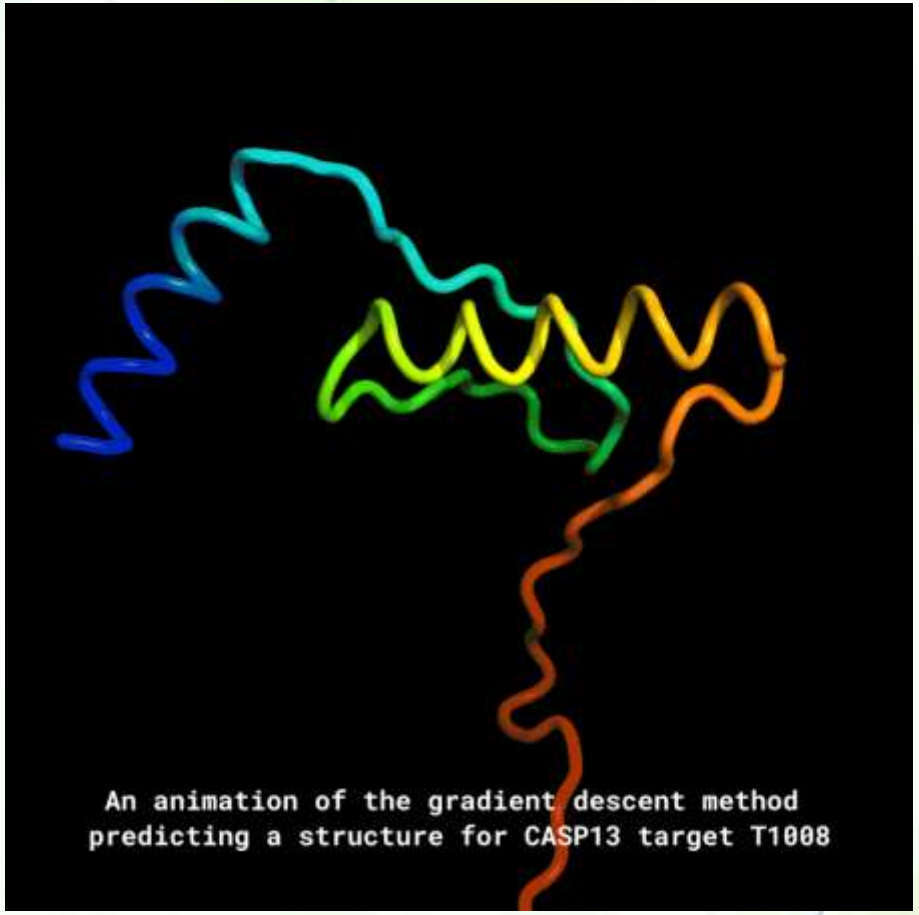


Can polar amino acids be found in the interior?...YES

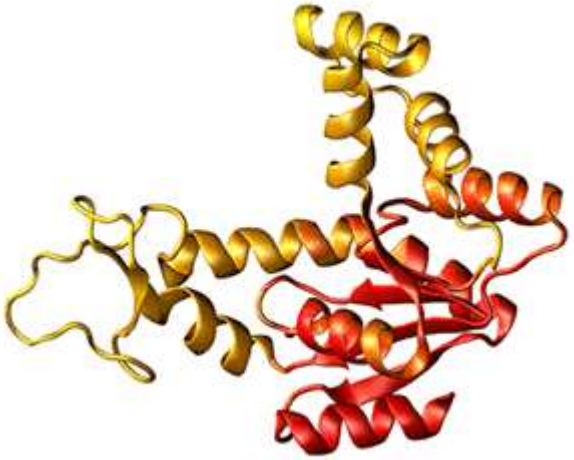
- Polar amino acids can be found in the interior of proteins
- In this case, they form hydrogen bonds to other amino acids or to the polypeptide backbone
- They play important roles in the function of the protein

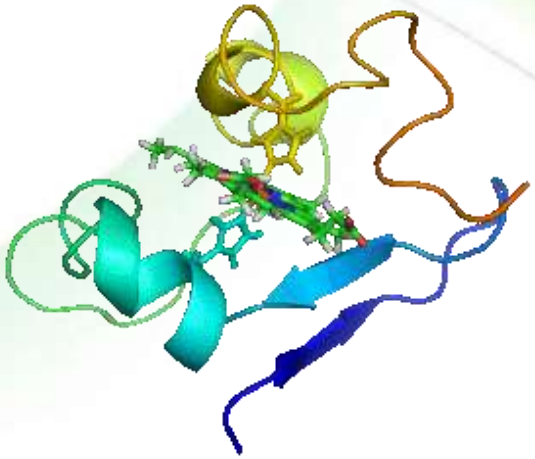
A hypothetical look at protein folding

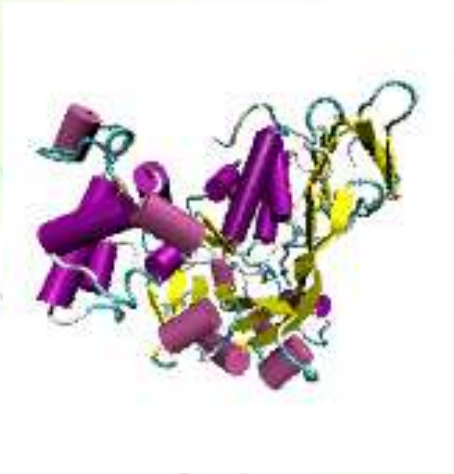


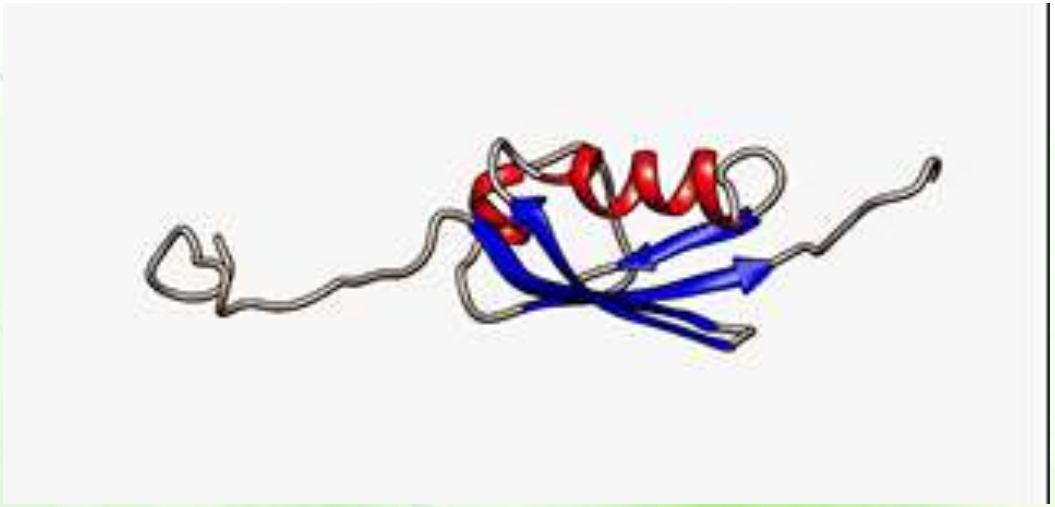


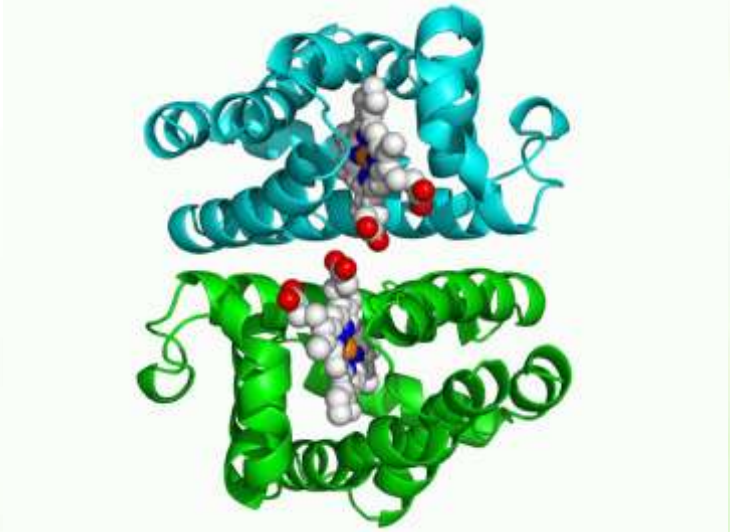
Protein are NOT static











Stabilizing factors

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

Disulfide bonds

- 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.

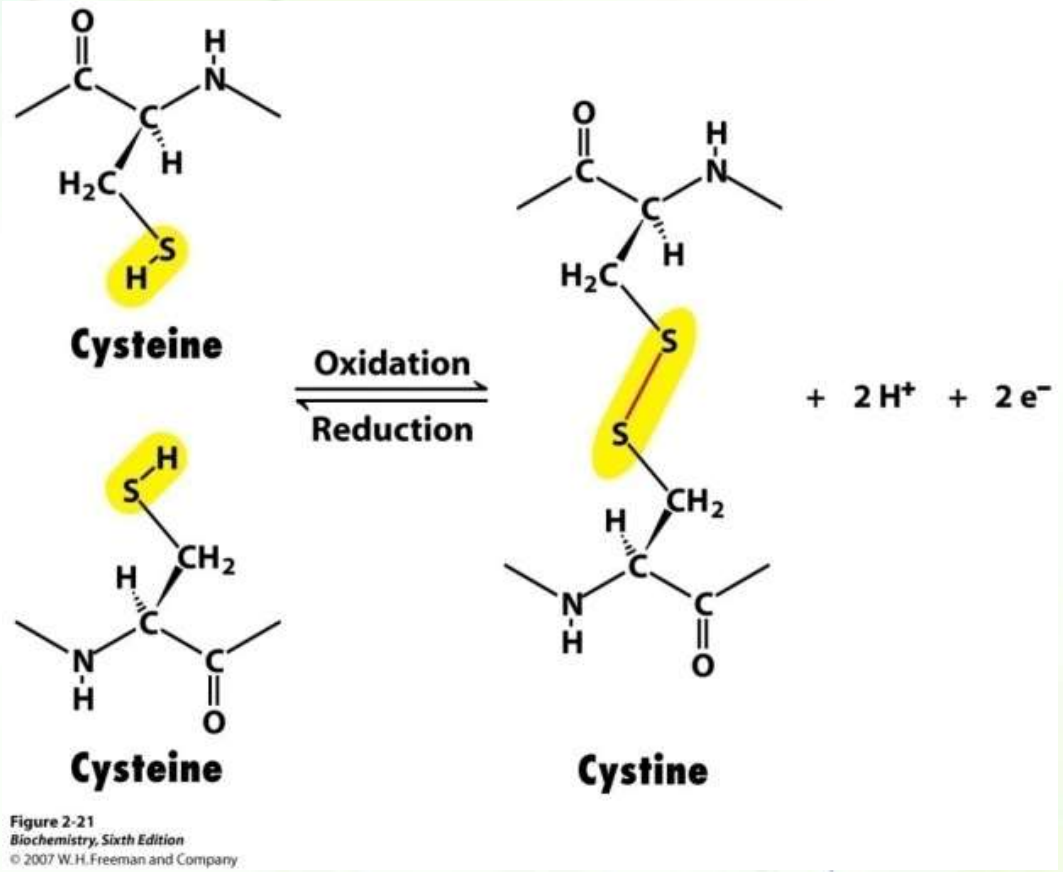
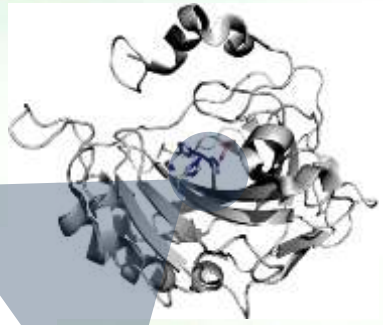


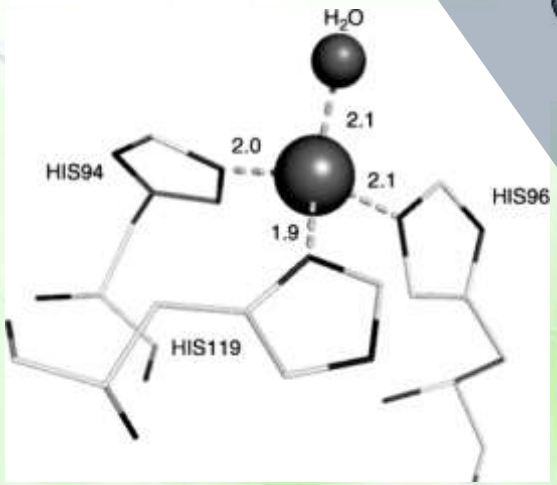
Figure 2-21
Biochemistry, Sixth Edition

metal ions

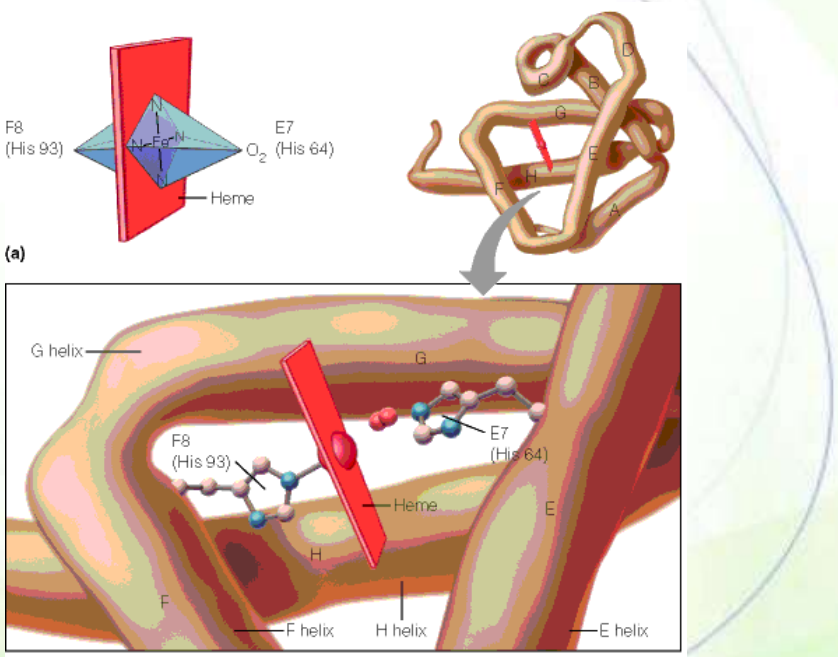
- Several proteins can be complexed to a single metal ion that can stabilize protein structure by forming:
  - Covalent interaction (myoglobin)
  - Salt bridges (carbonic anhydrase)

Carbonic anhydrase



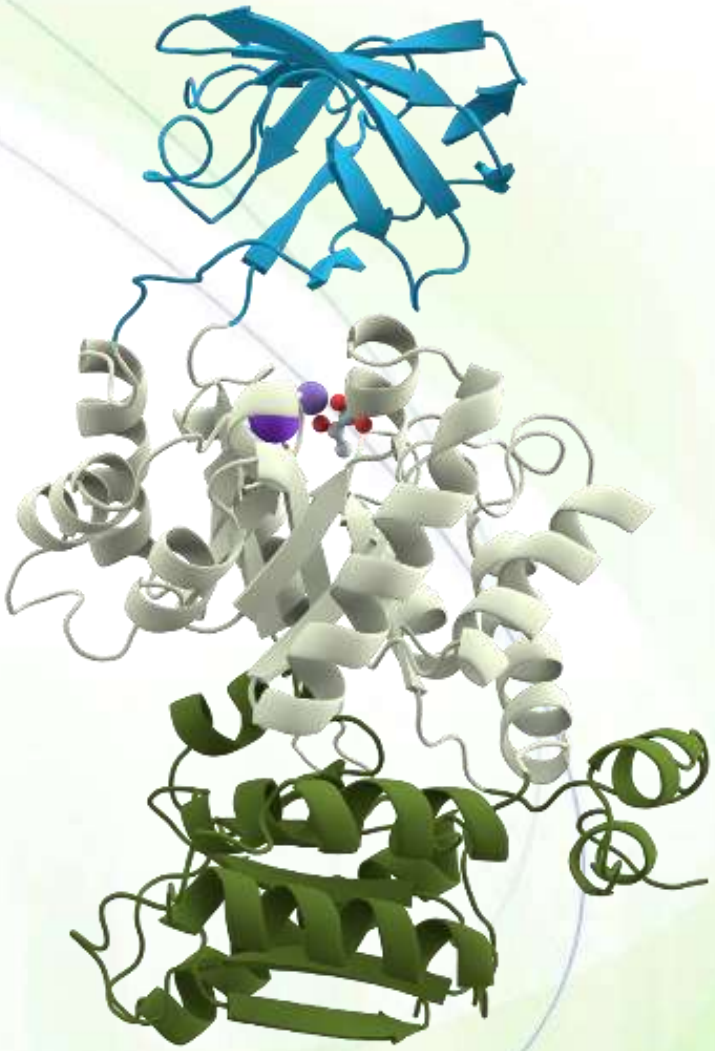


Myoglobin



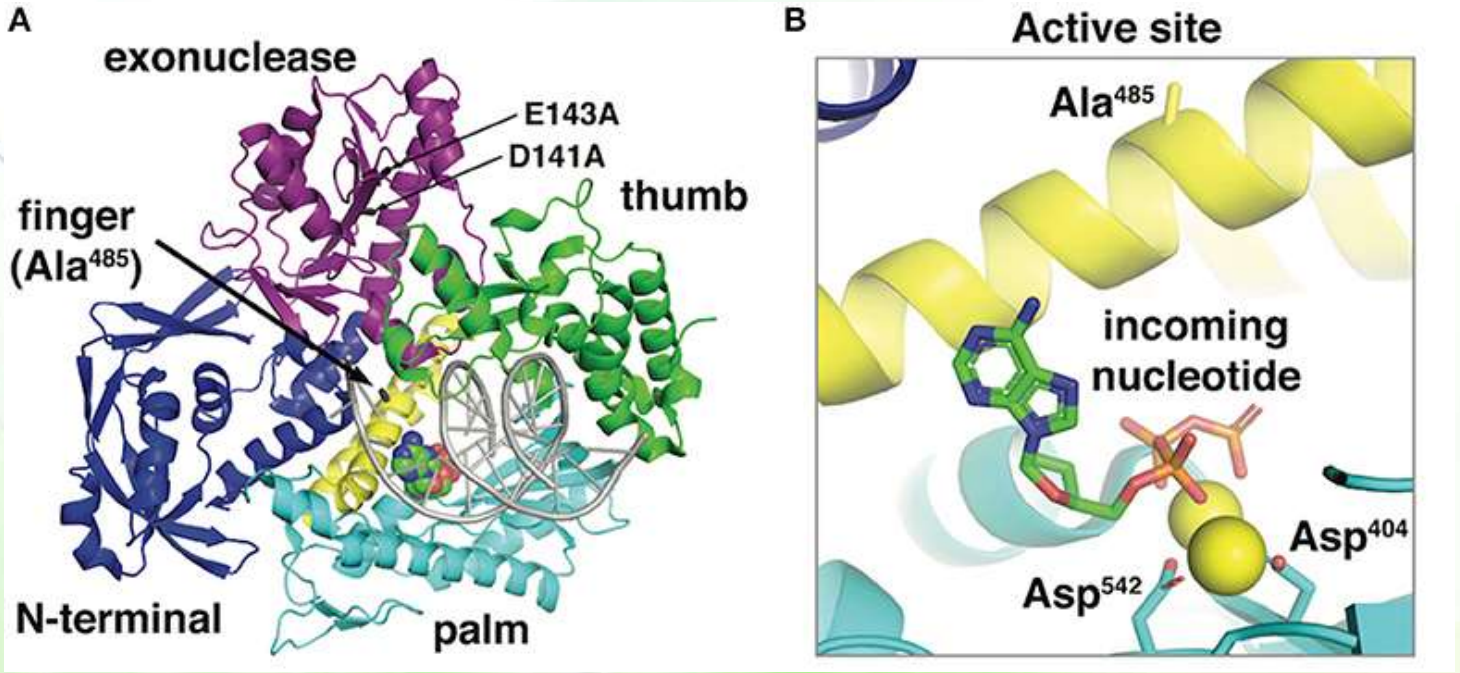
Domains

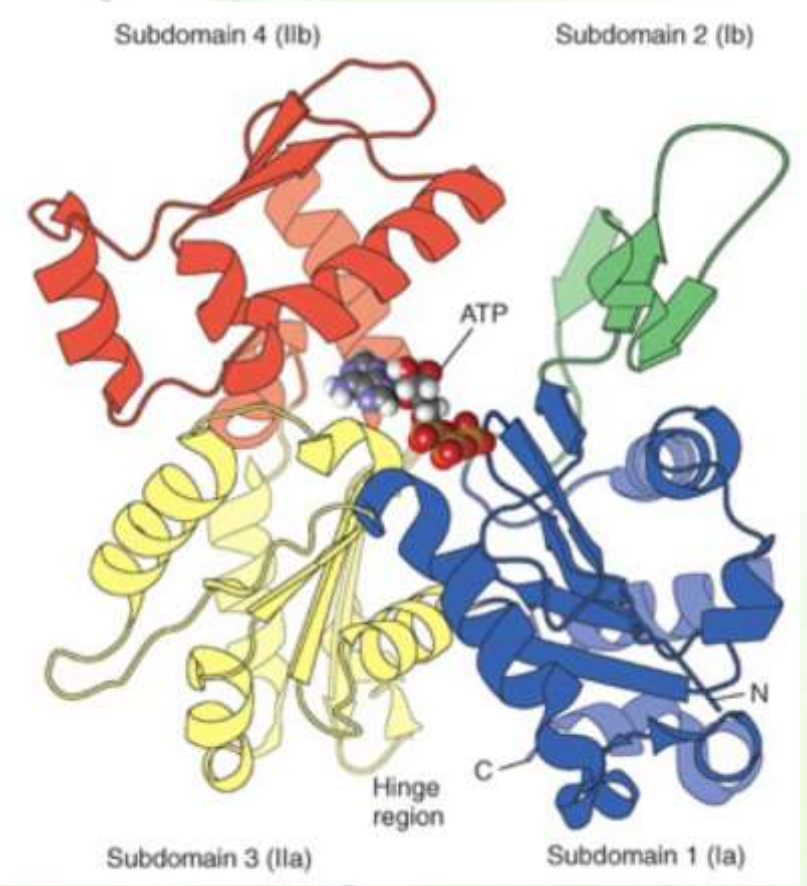
- A domain is a combination of α helices and/or β sheets that are connected to each other via turns, loops, and coils and are organized in a specific three-dimensional structure.
  - A domain may consist of 100–200 residues.
- Domains fold independently of the rest of the protein or of other domains 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)



Folds

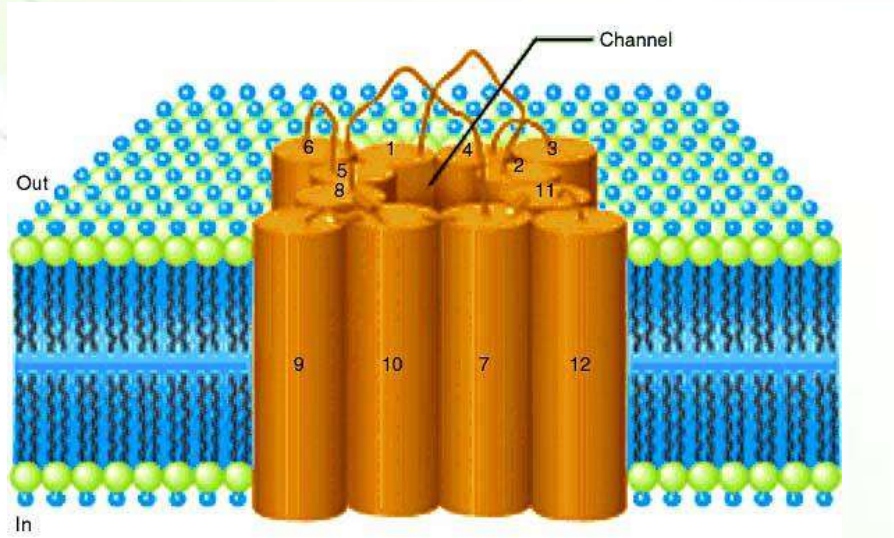
- When large patterns of secondary structures or multiple domains within a protein possess specific functions, they are known as **Folds**.
  - The actin fold
  - The nucleotide-binding fold

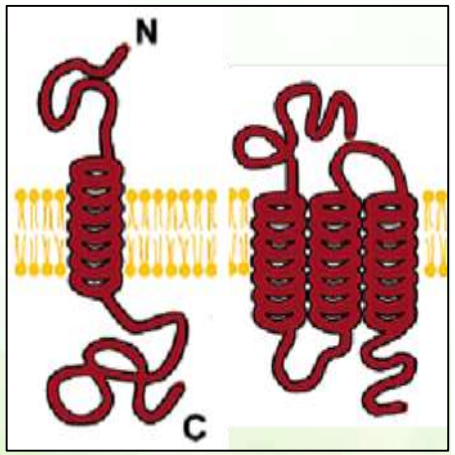


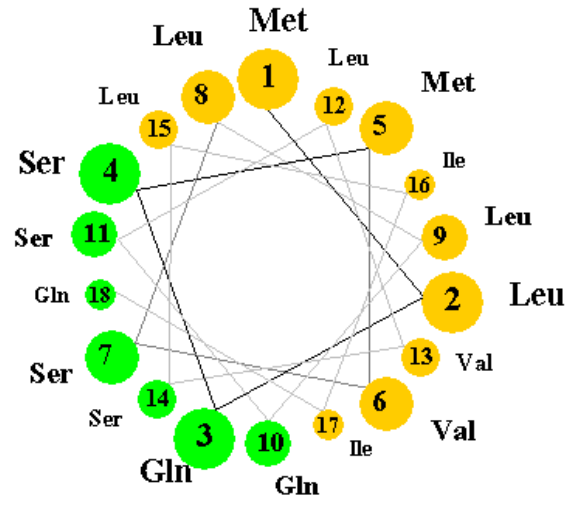


α-helices as transmembrane domains

- 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.
- 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.





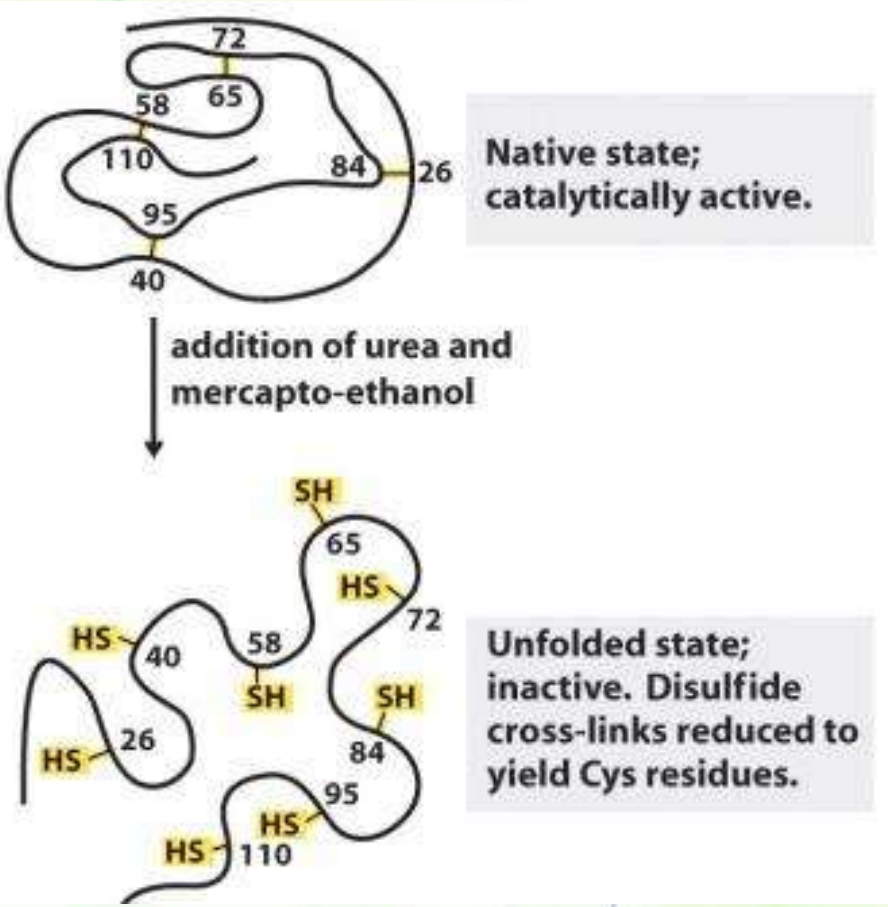


Properties of Proteins:

Denaturation and Renaturation

Denaturation

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



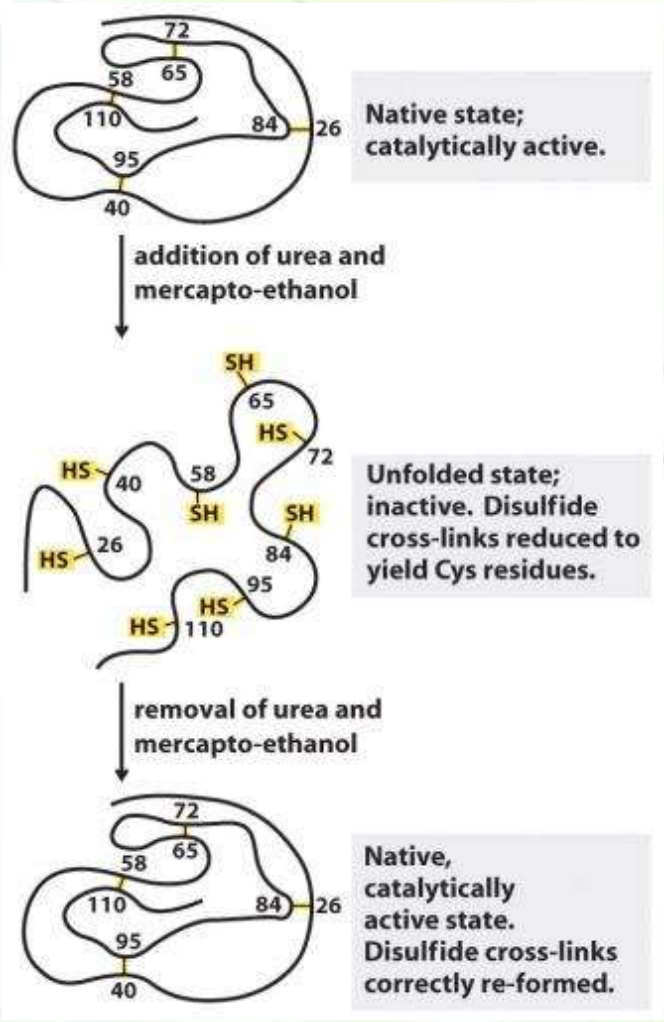
Denaturing agents

- Heat disrupts low-energy van der Waals forces in proteins
- Extremes of pH: change in the charge of the protein's amino acid side chains (electrostatic and hydrogen bonds).
- Detergents: Triton X-100 (nonionic, uncharged) and sodium dodecyl sulfate (SDS, anionic, charged) disrupt the hydrophobic forces.
  - 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.

Renaturation

- 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.

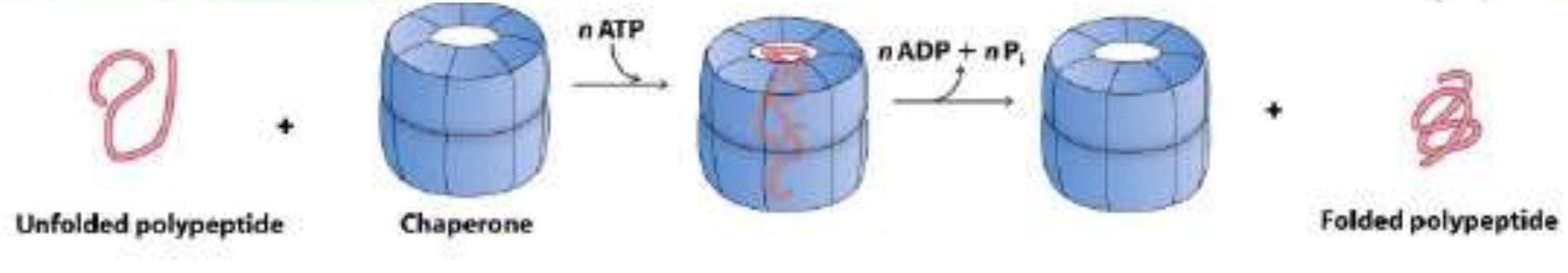


Factors that determine protein structure

- 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 the mainly the R groups.
    - Non-protein molecules.

Problem solvers: chaperones

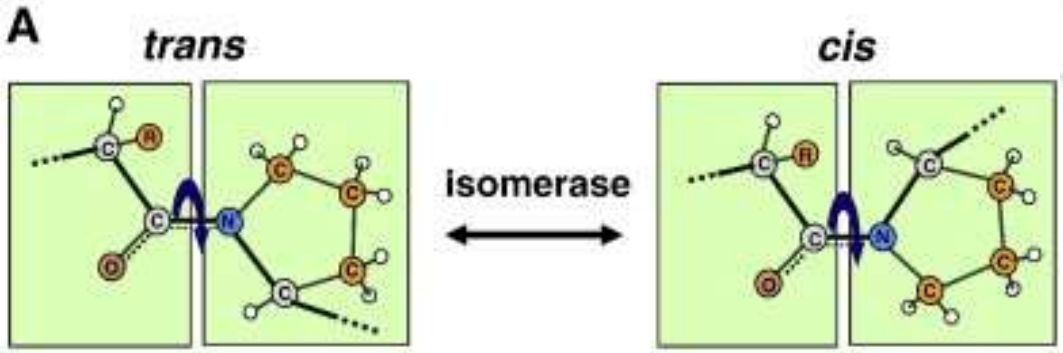
- These proteins bind to polypeptide chains and help them fold with the most energetically favorable folding pathway.
- Chaperones also prevent the hydrophobic regions in newly synthesized protein chains from associating with each other to form protein aggregates .



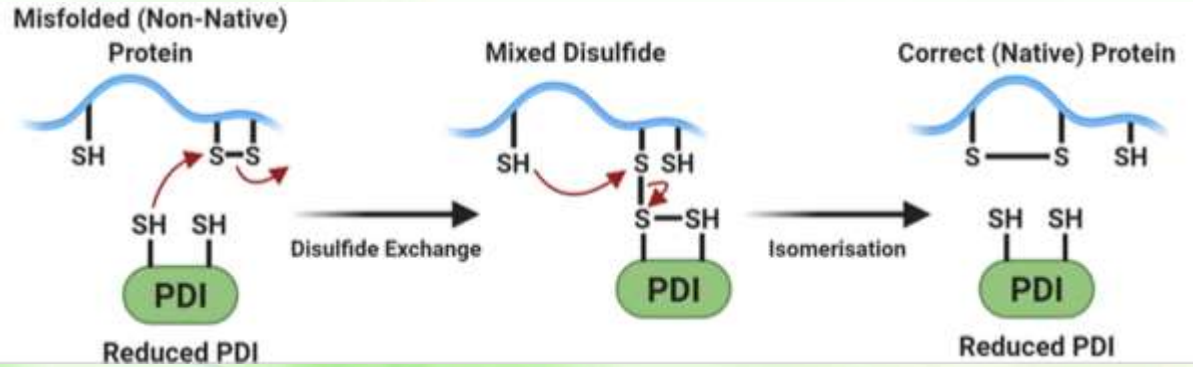
Many diseases are the result of defects in protein folding.

Other players

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

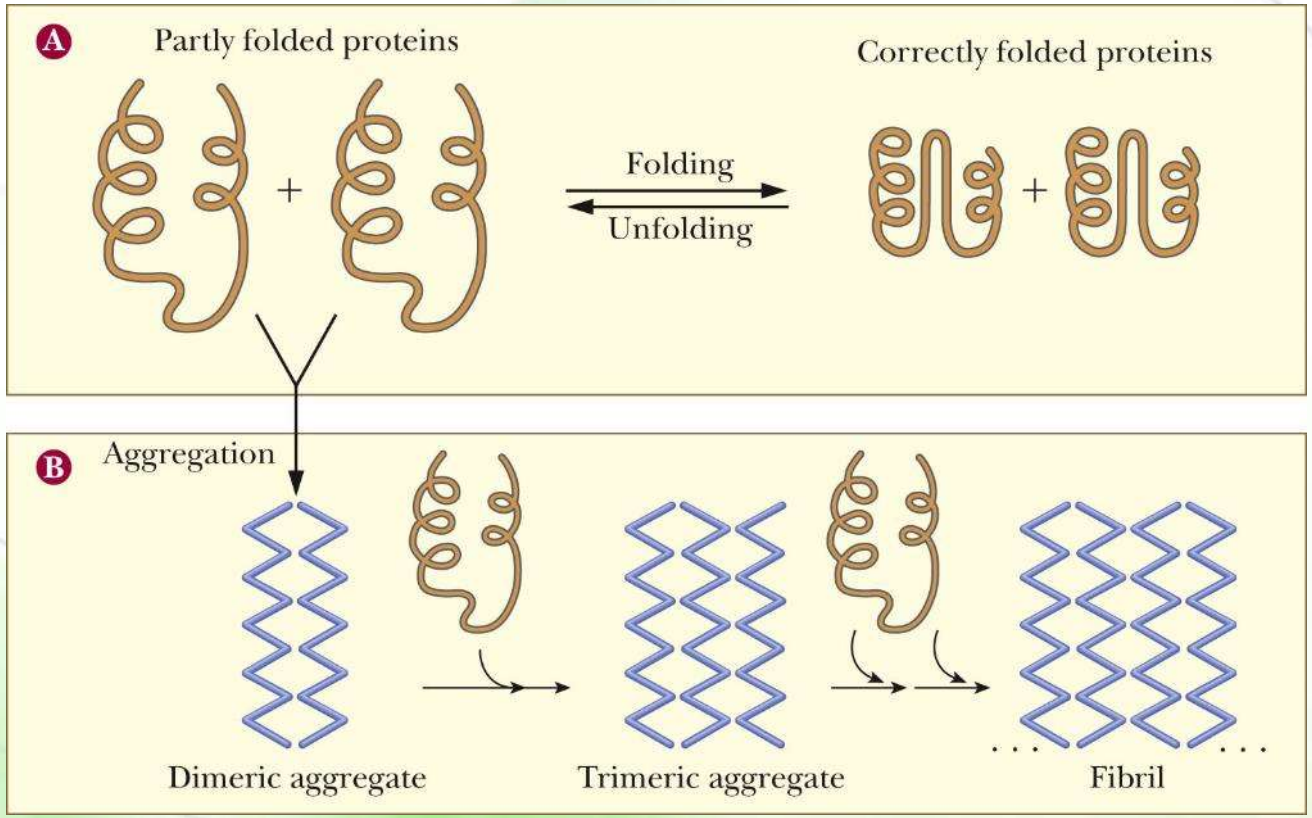


- A protein disulfide isomerase, after the protein has folded, breaks and reforms disulfide bonds between the –SH groups of two cysteine residues.



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.

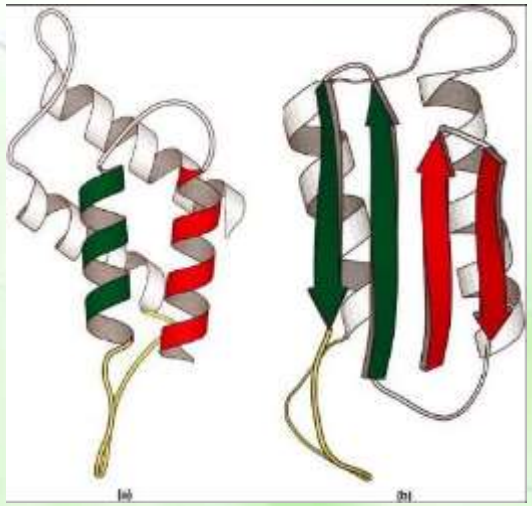


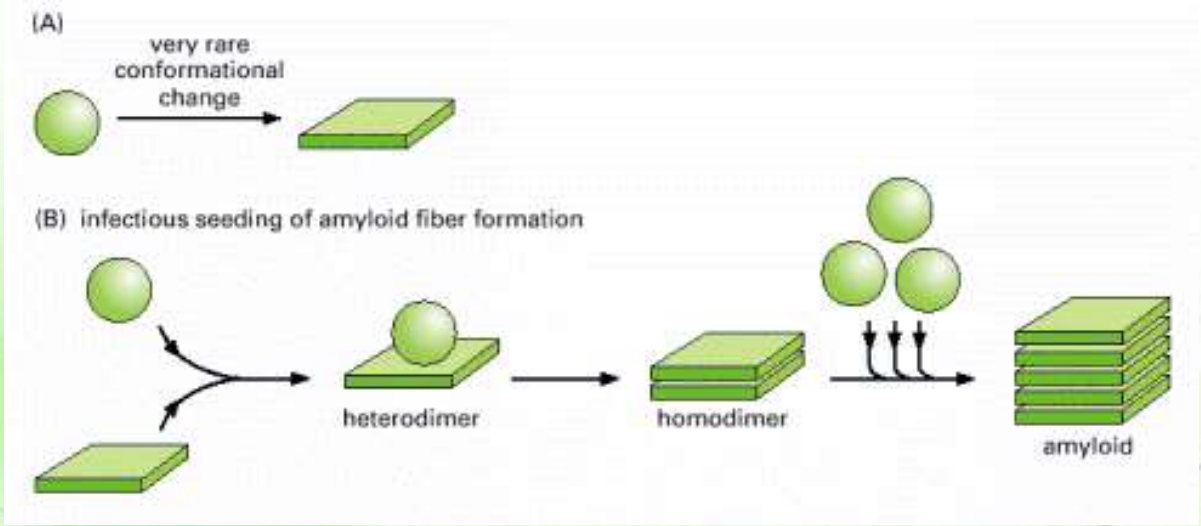
Outcome of protein misfolding

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

Prion disease

- 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.





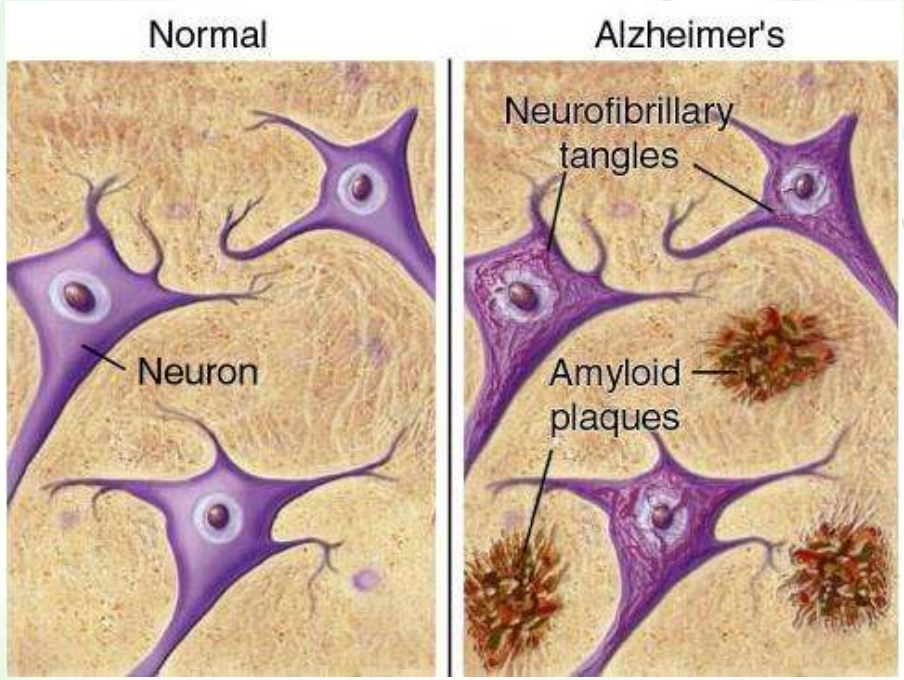
The prion protein

- The disease is caused by a transmissible agent
- Abnormal protein can be acquired by
  - Infection
  - Inheritance
  - Spontaneously

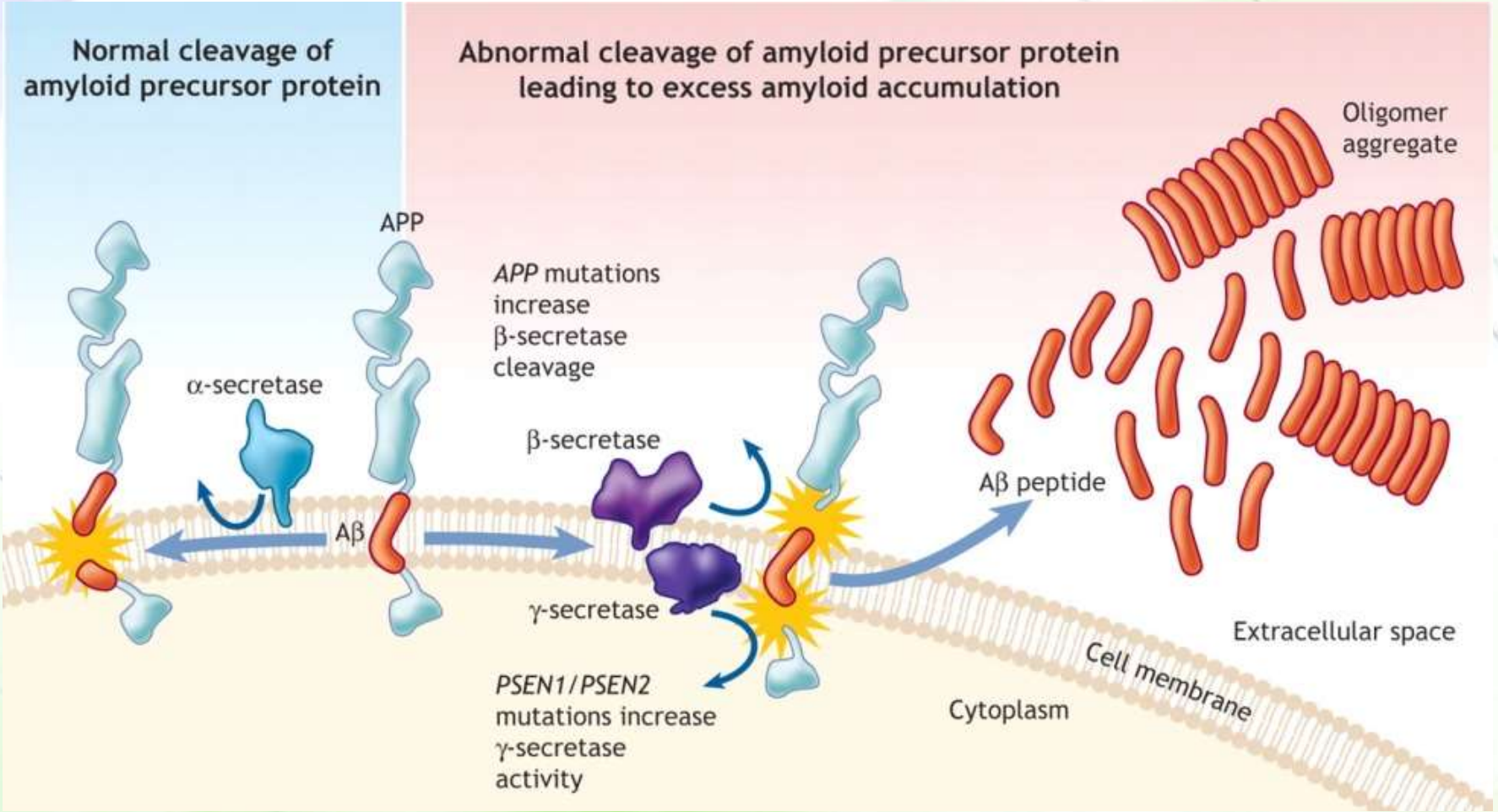
Alzheimer’s Disease

- Not transmissible between individuals

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



Formation of plaques



Quaternary structure

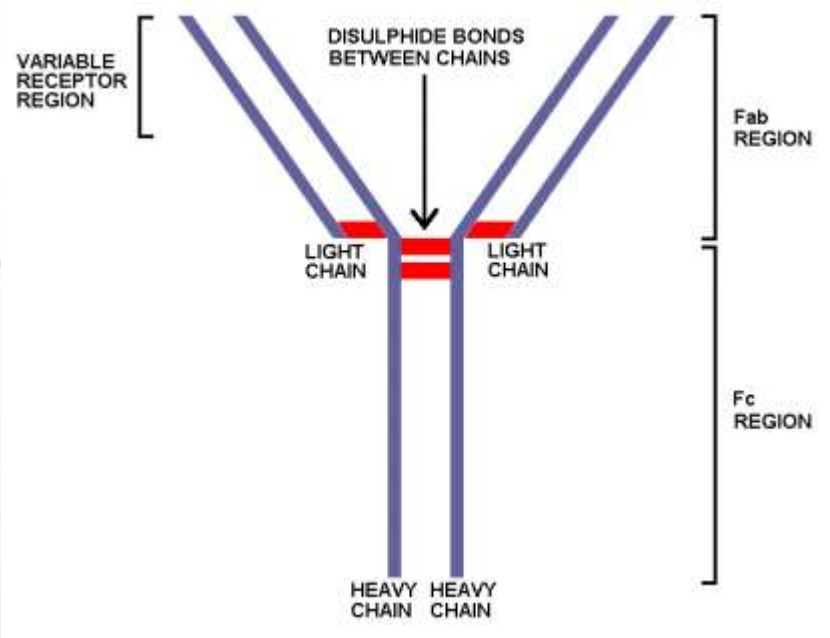
What is it?

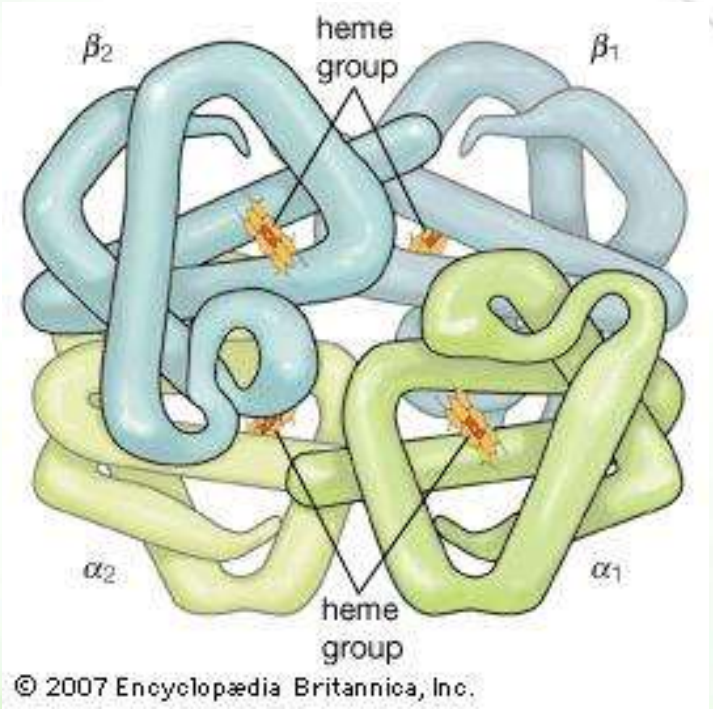
- Proteins are composed of more than one polypeptide chain.
  - They are oligomeric proteins (oligo = a few or small or short; mer = part or unit)
- The spatial arrangement of subunits and the nature of their interactions.
- Proteins made of
  - One subunit = monomer
  - Two subunits: dimer
    - The 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 → homooligomers (homo = same), or
  - 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.

How are the subunits connected?

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

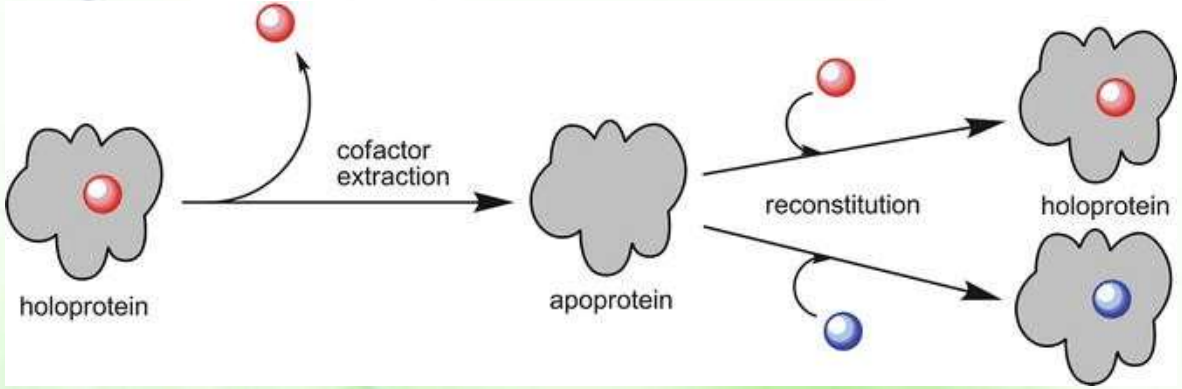




Complex protein structures

Holo- and apo-proteins

- 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).
- 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.

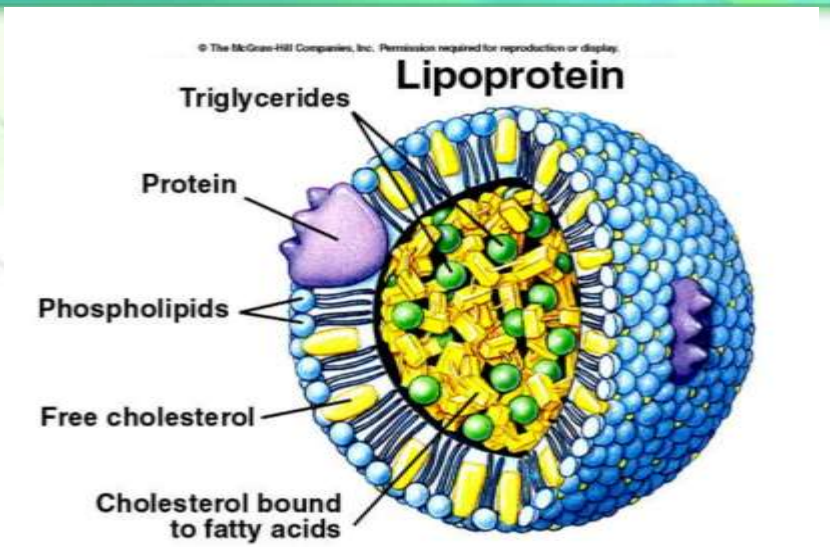


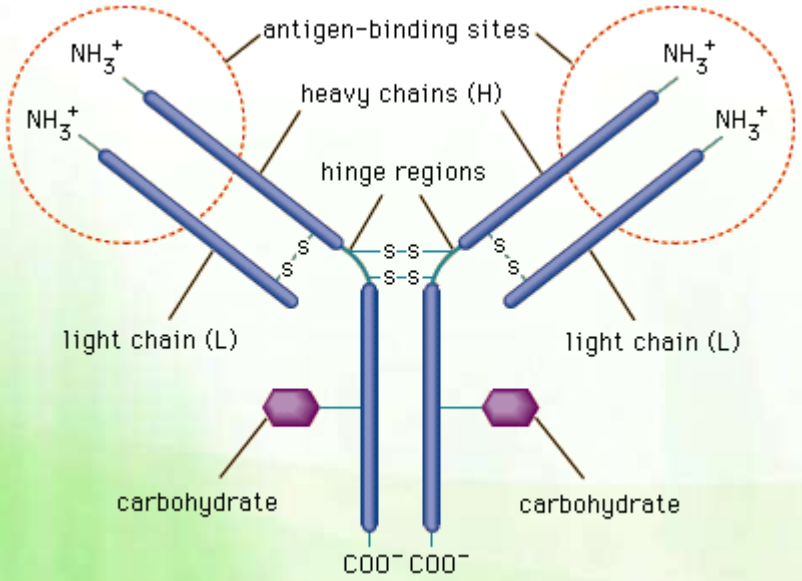
Coenzymes: complex organic molecules that assist enzymes in catalyzing biochemical reactions

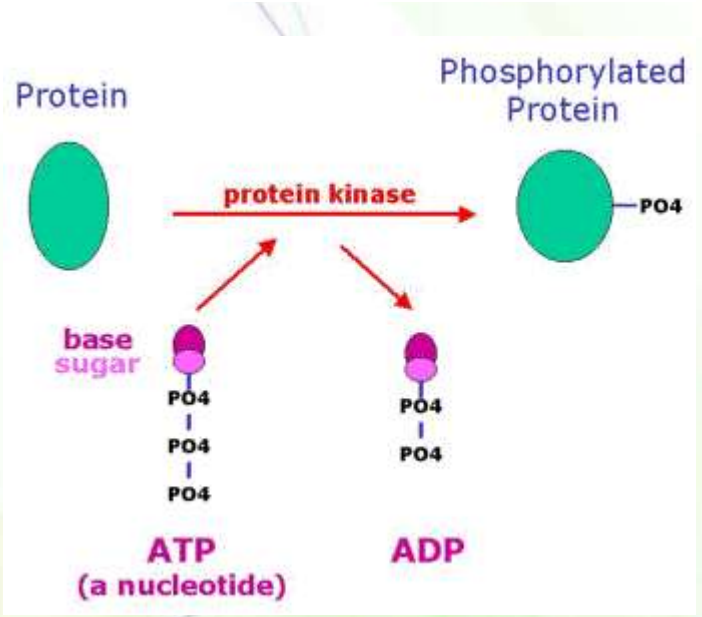
Prosthetic groups: Coenzymes or metals that are tightly (covalently) bound to proteins

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







Classes of glycoproteins

- N-linked sugars
  - The amide nitrogen of the R-group of asparagine
- O-linked sugars
  - The hydroxyl groups of either serine or threonine
  - Occasionally to hydroxylysine such as in collagen