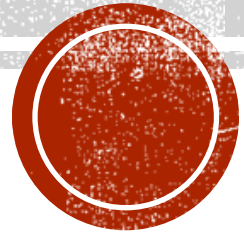
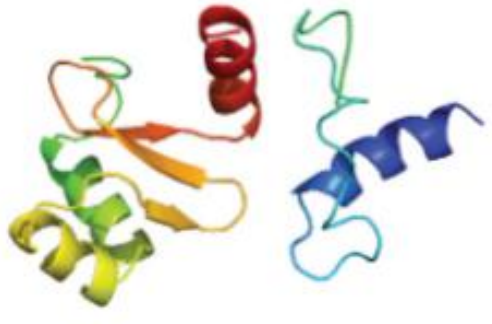
POLYPEPTIDES AND PROTEINS

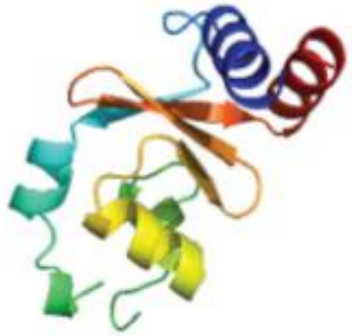
Prof. Nafez Abu Tarboush



PROTEINS

- Native conformation





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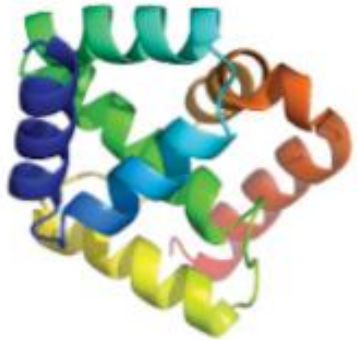

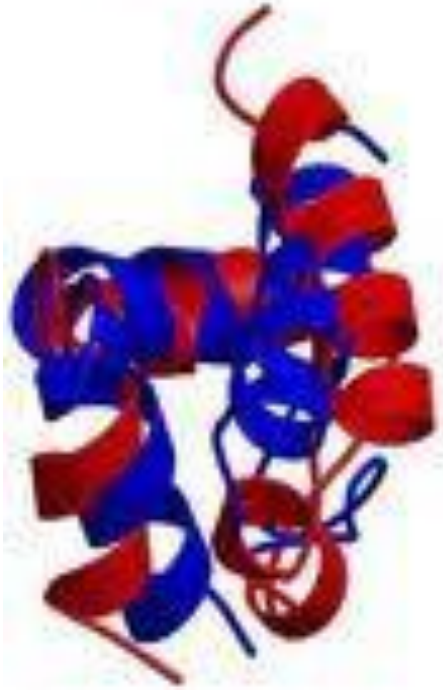

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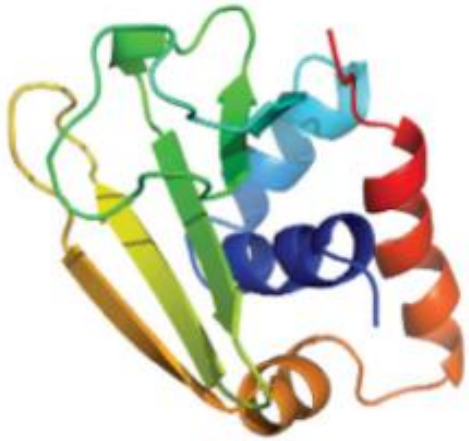
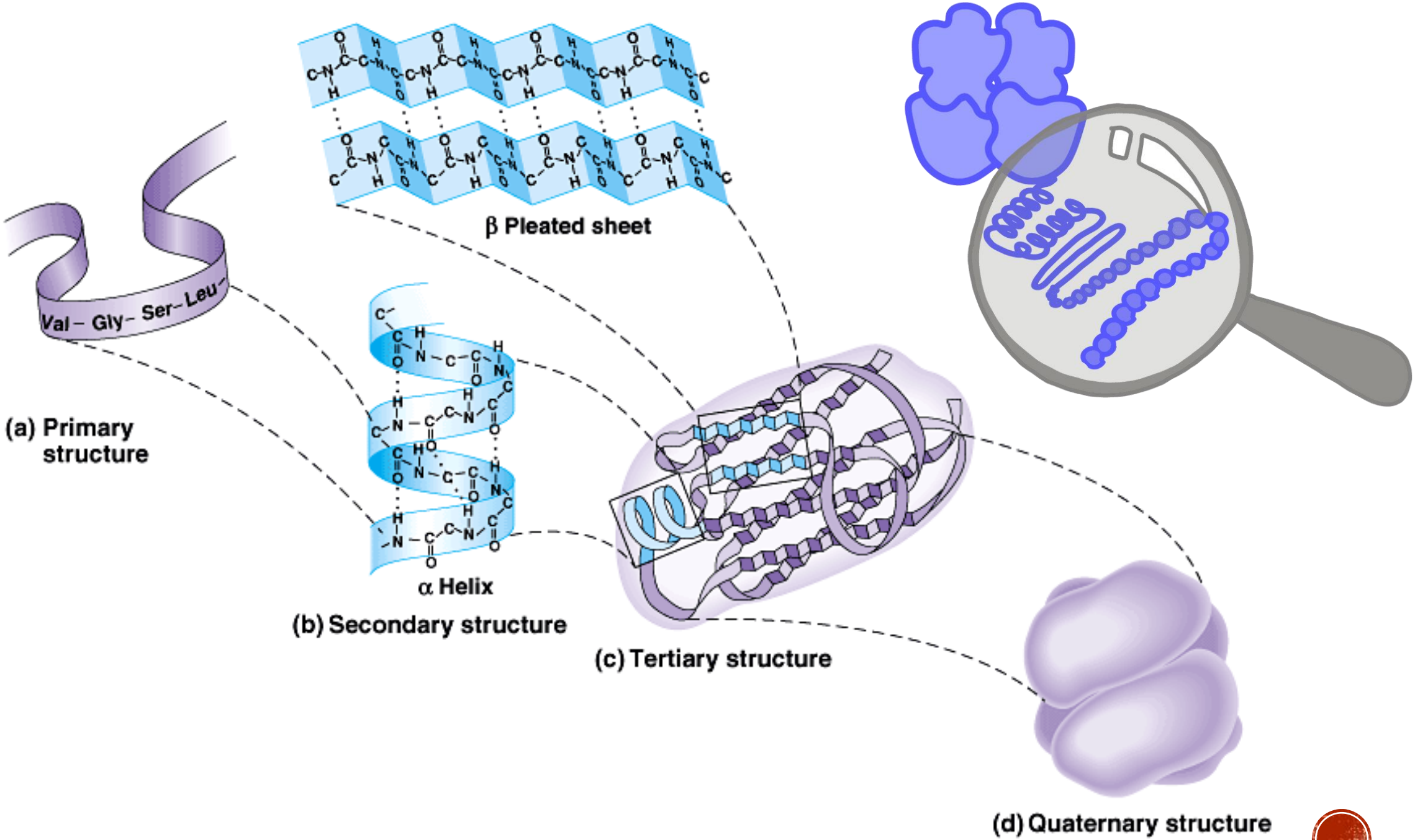


LEVELS OF PROTEIN STRUCTURE

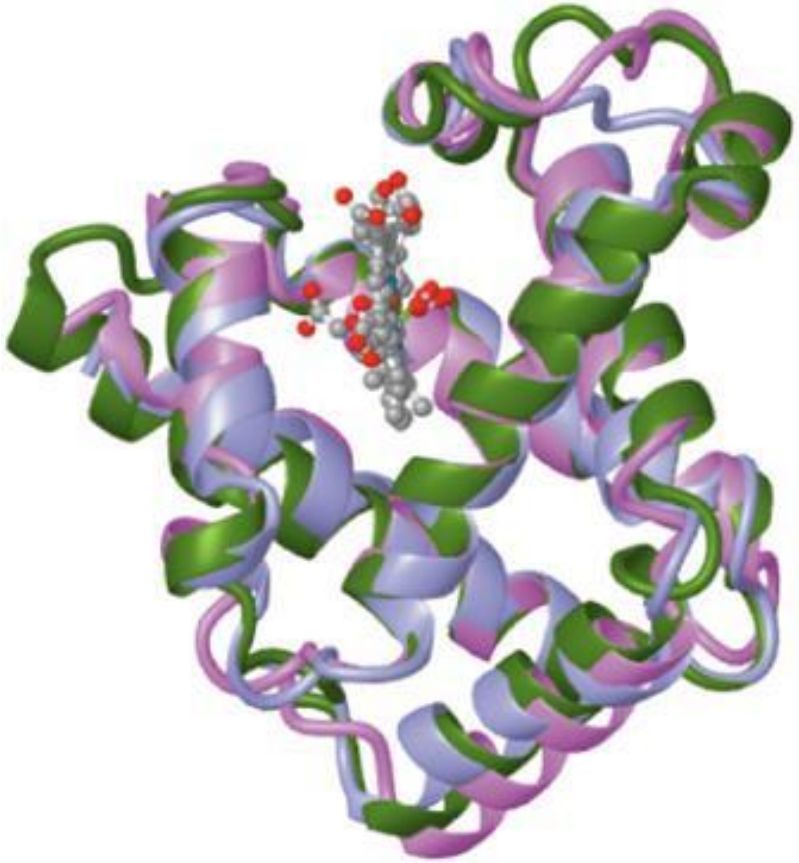
- **Primary structure**: the number, type, and sequence of amino acid residues
- **Secondary structure**: the localized organization of parts of a polypeptide chain
- **Tertiary structure**: the three-dimensional structure and/or arrangement of all the amino acids residues of a polypeptide chain
- Some 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

WHAT IS PRIMARY STRUCTURE

- The primary structure of a protein determines the other levels of structure

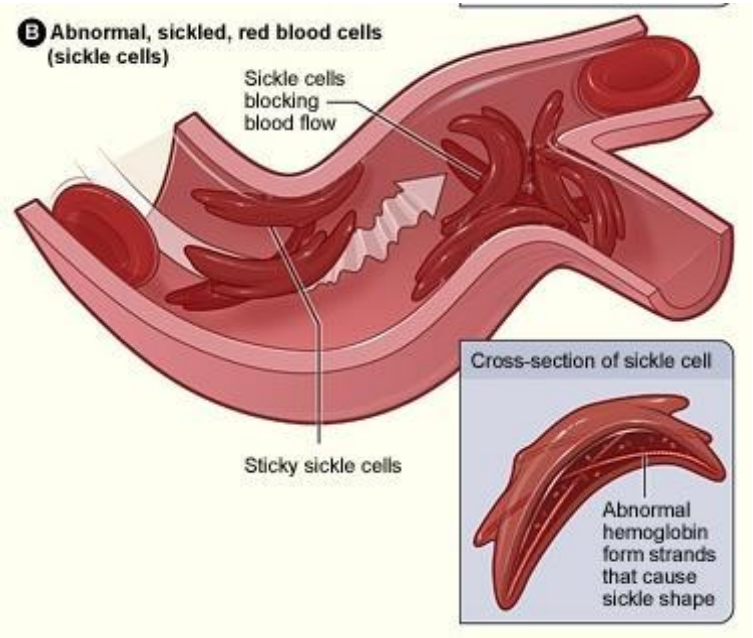


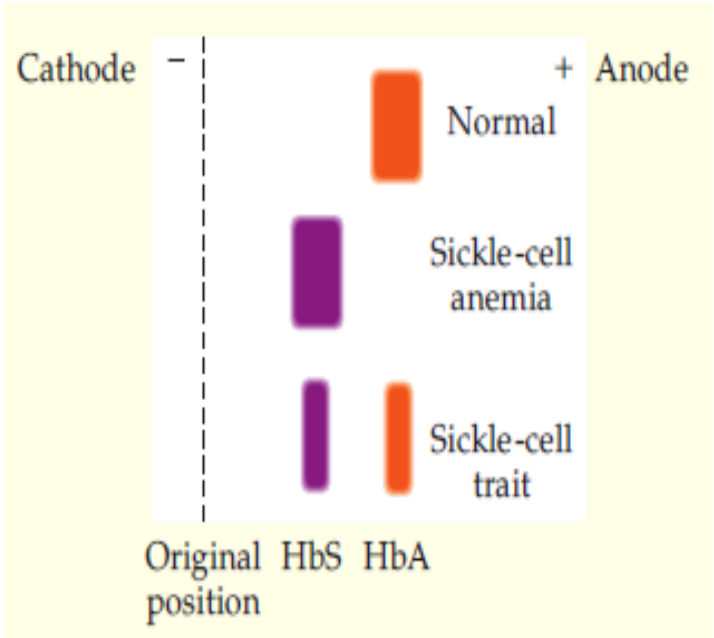
	1	5	10	15
Myoglobin	gly-----	leu-ser-asp-gly-glu-trp-gln-	leu-val-leu-asn-	val-trp-gly-lys-val-
β-chain hemoglobin	val-his-	leu-thr-pro-glu-glu-lys-ser-	ala-val-thr-ala-	leu-trp-gly-lys-val-
α-chain hemoglobin	val-----	leu-ser-pro-ala-asp-lys-thr-	asn-val-lys-ala-	ala-trp-gly-lys-val-
ζ-chain hemoglobin	met-ser-	leu-thr-lys-thr-glu-arg-thr-	ile-ile-val-ser-	met-trp-ala-lys-ile-
γ-chain hemoglobin	met-gly-	his-phe-thr-glu-glu-asp-lys-	ala-thr-ile-thr-	ser-leu-trp-gly-lys-val-

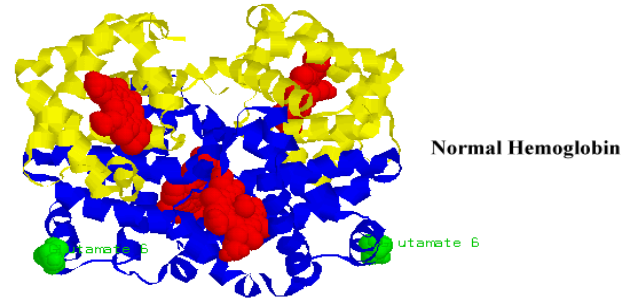
- **α-Globin (blue)**
- **β-Globin (violet)**
- **Myoglobin (green)**

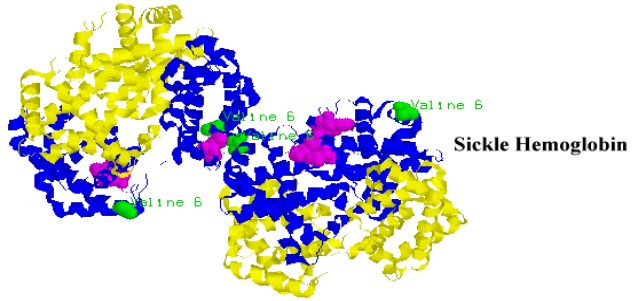
SICKLE CELL HEMOGLOBIN (HBS)

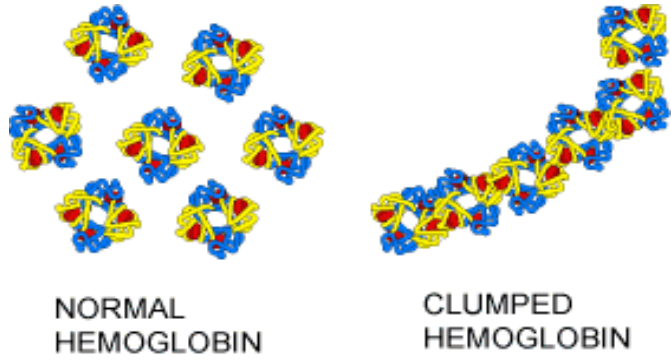
- It is caused by a change of amino acids in the 6th position of β globin (Glu to Val)
- The mutation results in: 1) arrays of aggregates of hemoglobin molecules, 2) deformation of the red blood cell, and 3) clotting in blood vessels and tissues

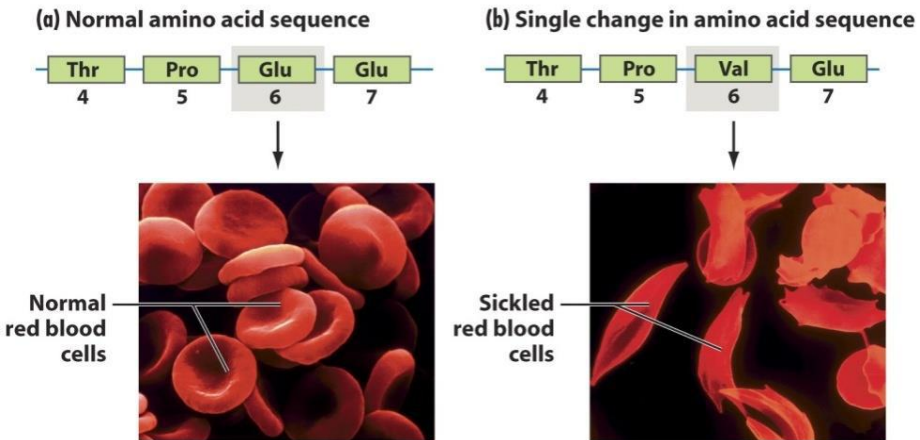


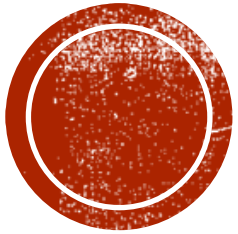








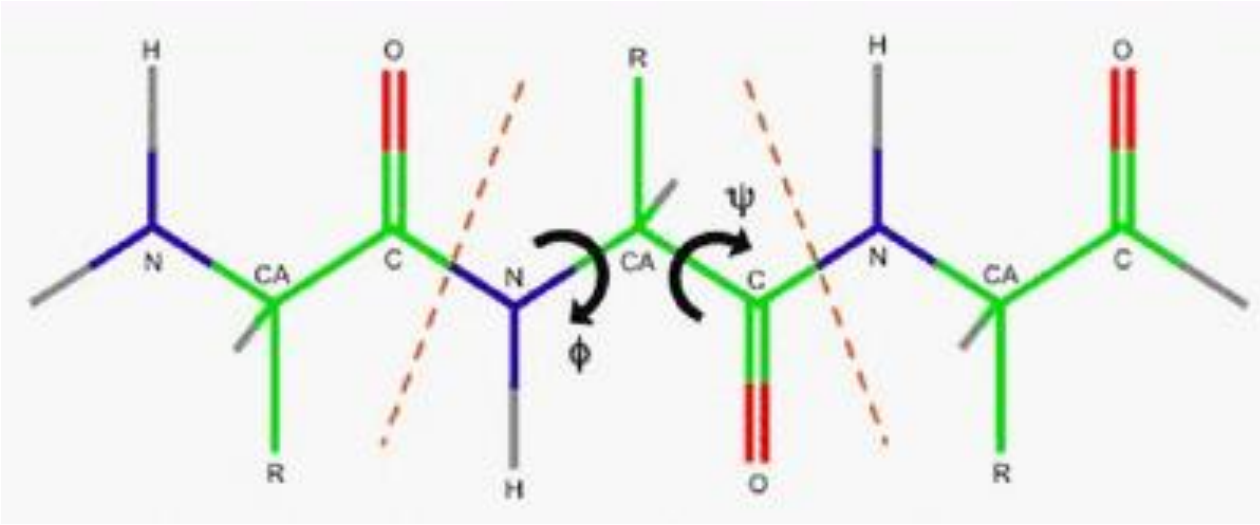




SECONDARY STRUCTURE

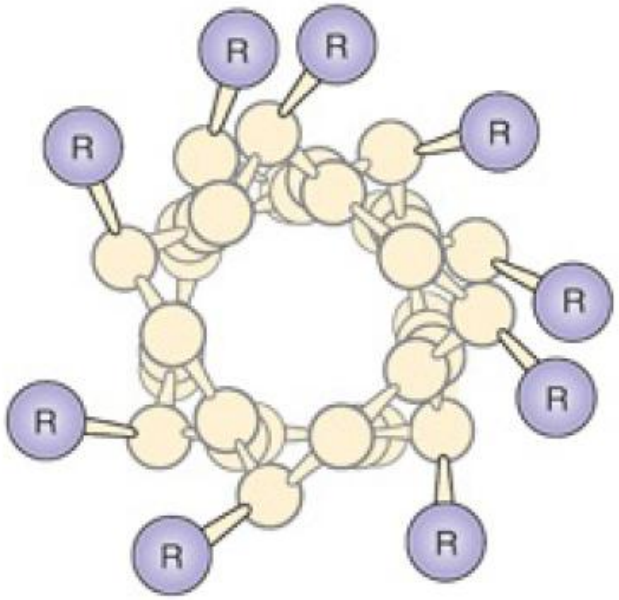
WHAT IS IT? HOW IS IT CAUSED?

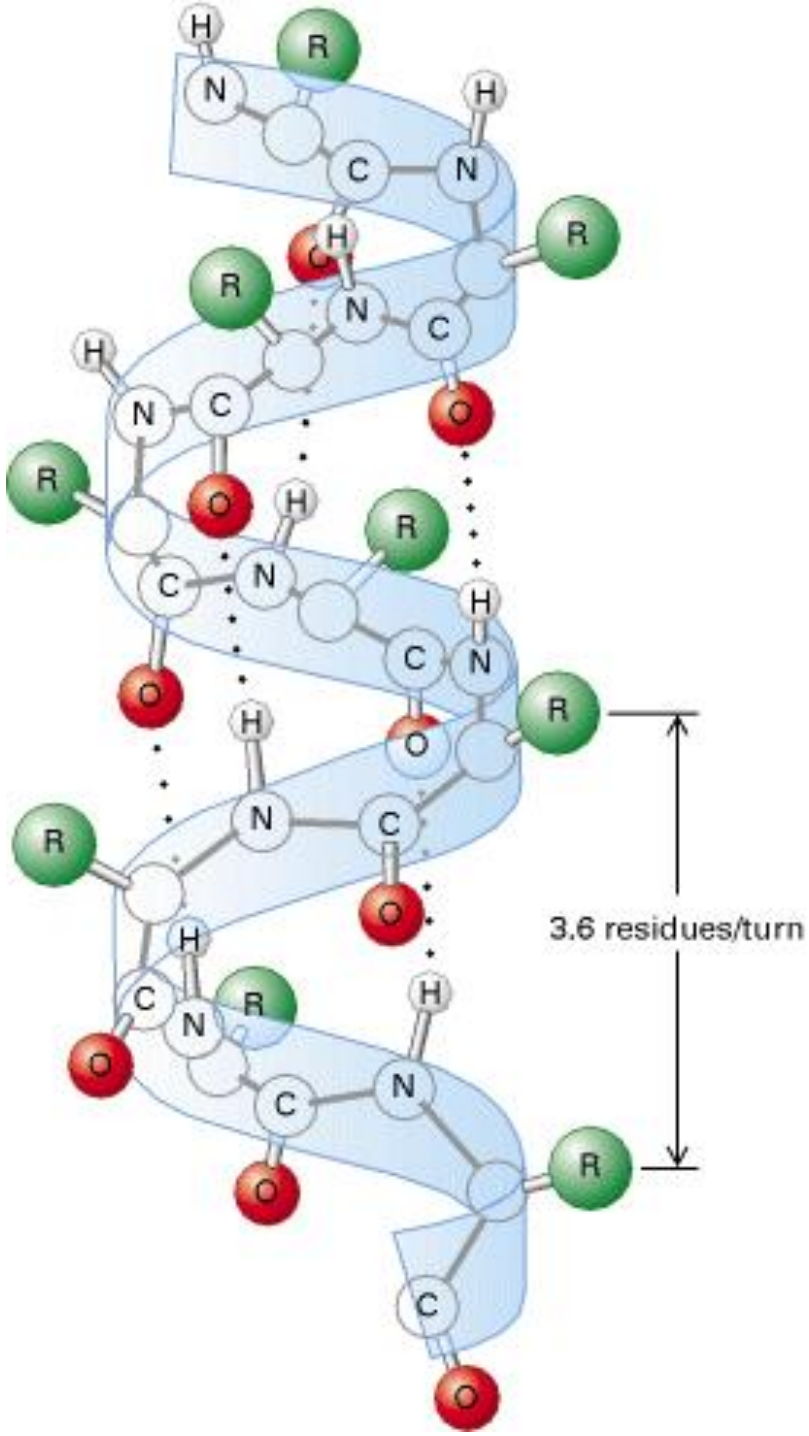
- The two bonds within each amino acid residue freely rotate
  - the bond between the α-carbon and the amino nitrogen
  - the bond between the α-carbon and the carboxyl carbon
- A hydrogen-bonded, local arrangement of the backbone of a polypeptide chain
- Polypeptide chains can fold into regular structures such as:
  - Alpha helix
  - Beta-pleated sheet
  - Turns
  - Loops



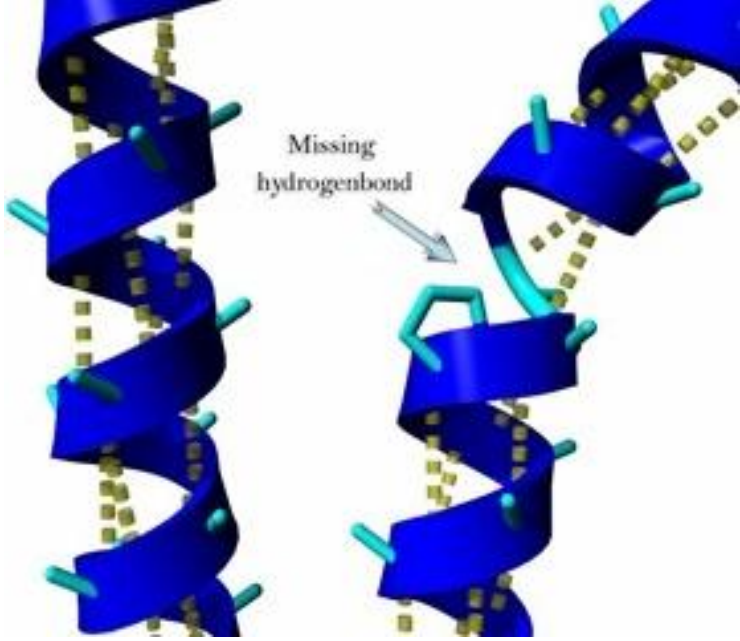
THE α-HELIX

- 3.6 amino acids per turn
- The pitch of the helix (the linear distance between corresponding points on successive turns) is 5.4 Å
- It is very stable!
- Avoiding steric hindrance

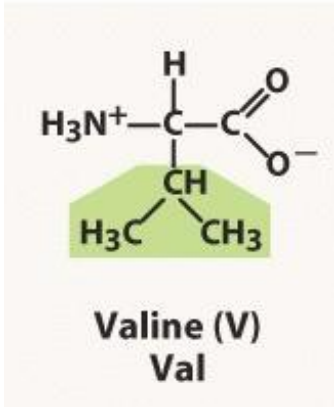


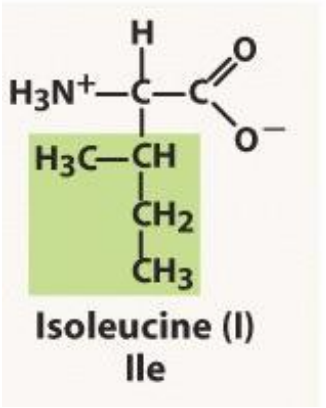


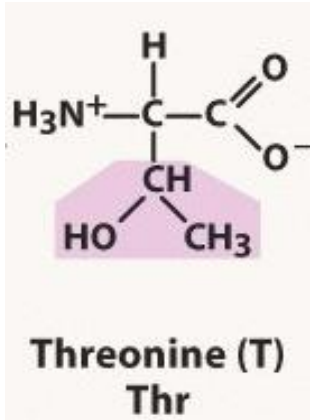
AMINO ACIDS NOT FOUND IN α-HELIX



- 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)

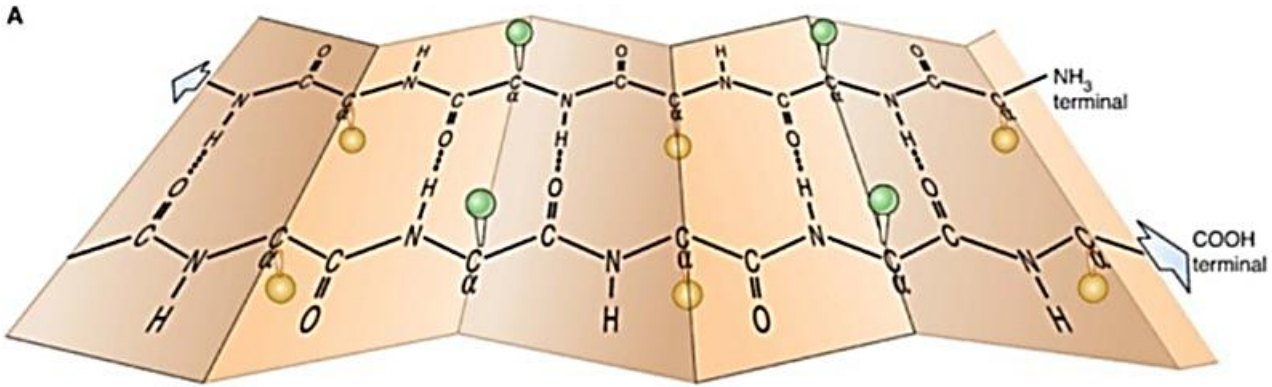


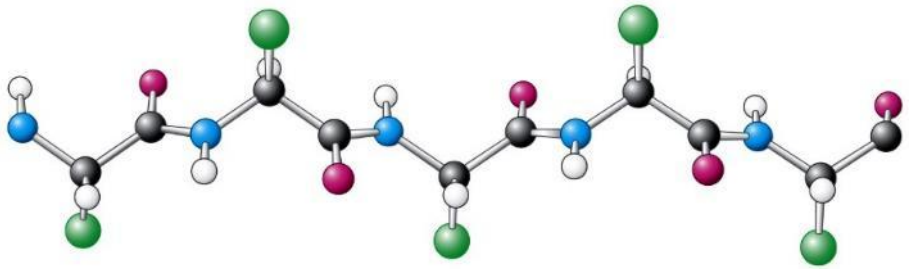




B-PLEATED SHEET (β-SHEET)

- 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



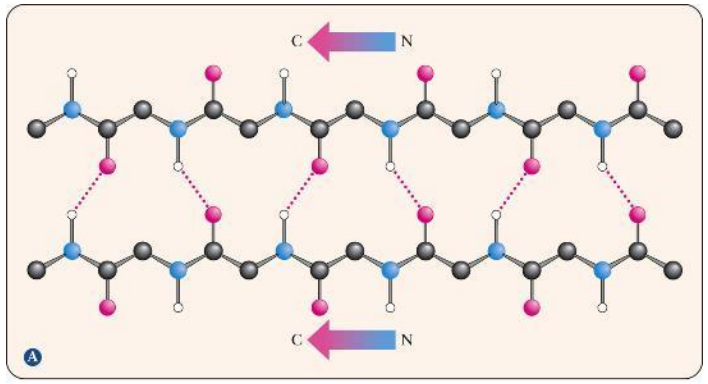


β strand

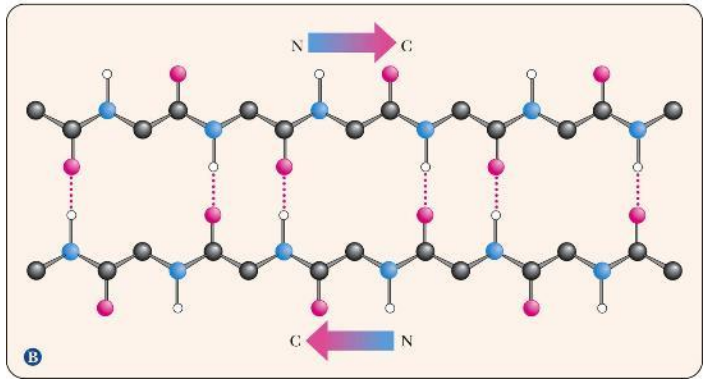
MORE ON β-SHEETS

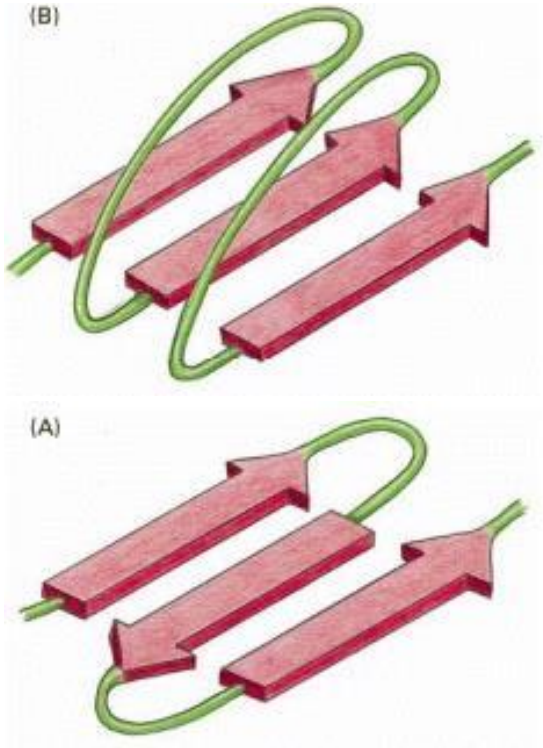
- β sheets can form between many strands, typically 4 or 5 but as many as 10 or more
- Such β sheets can be purely antiparallel, purely parallel, or mixed

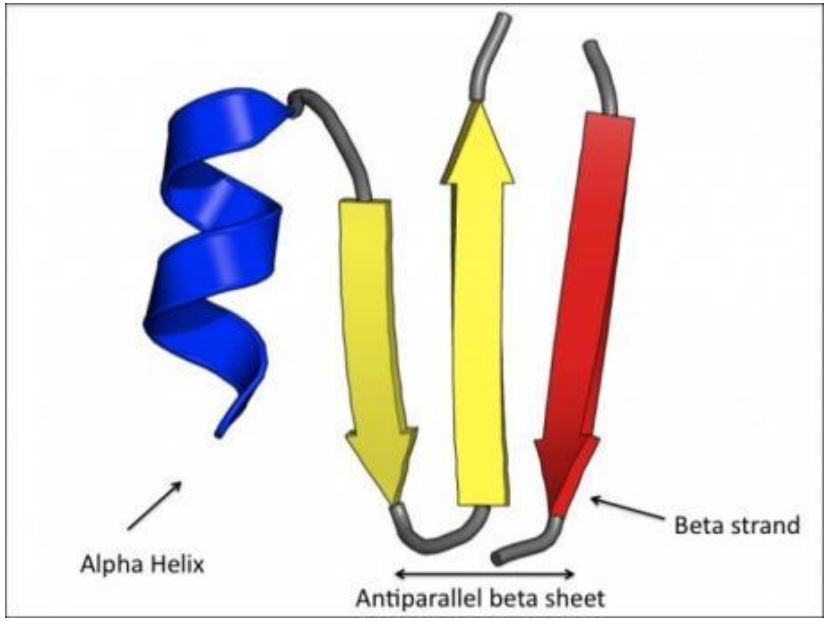
Parallel



Antiparallel





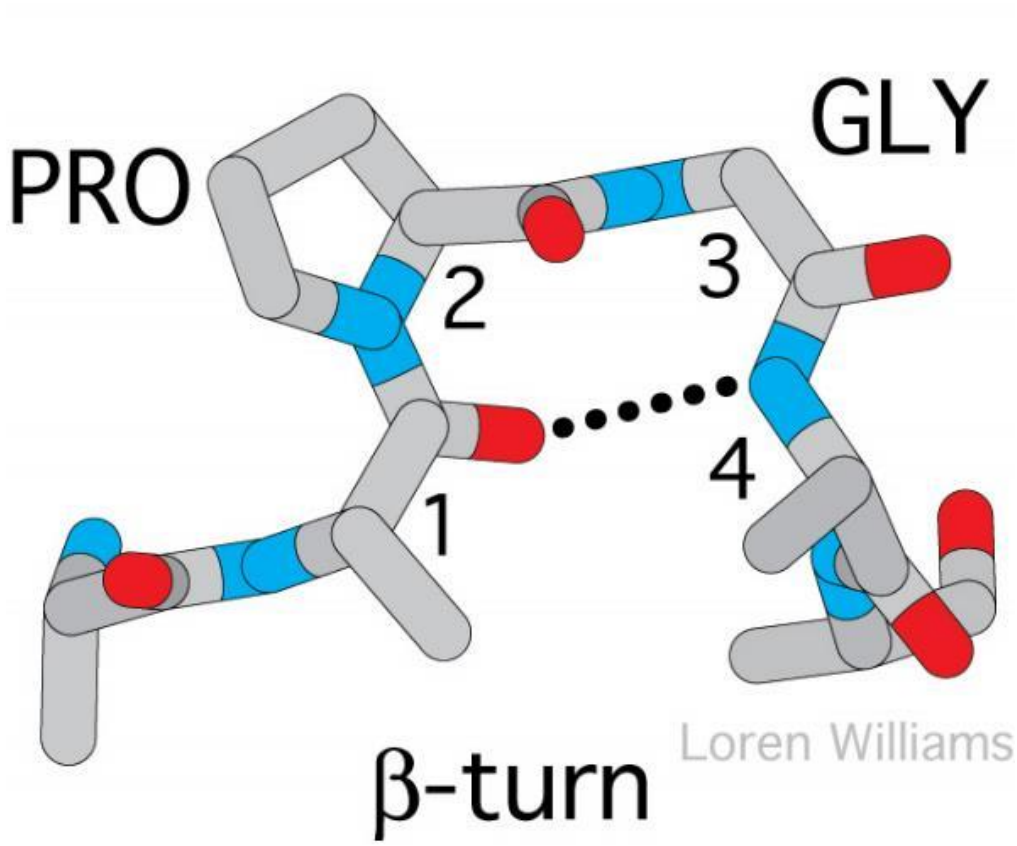


EFFECT OF AMINO ACIDS

- 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 and glycine tend to disrupt β strands

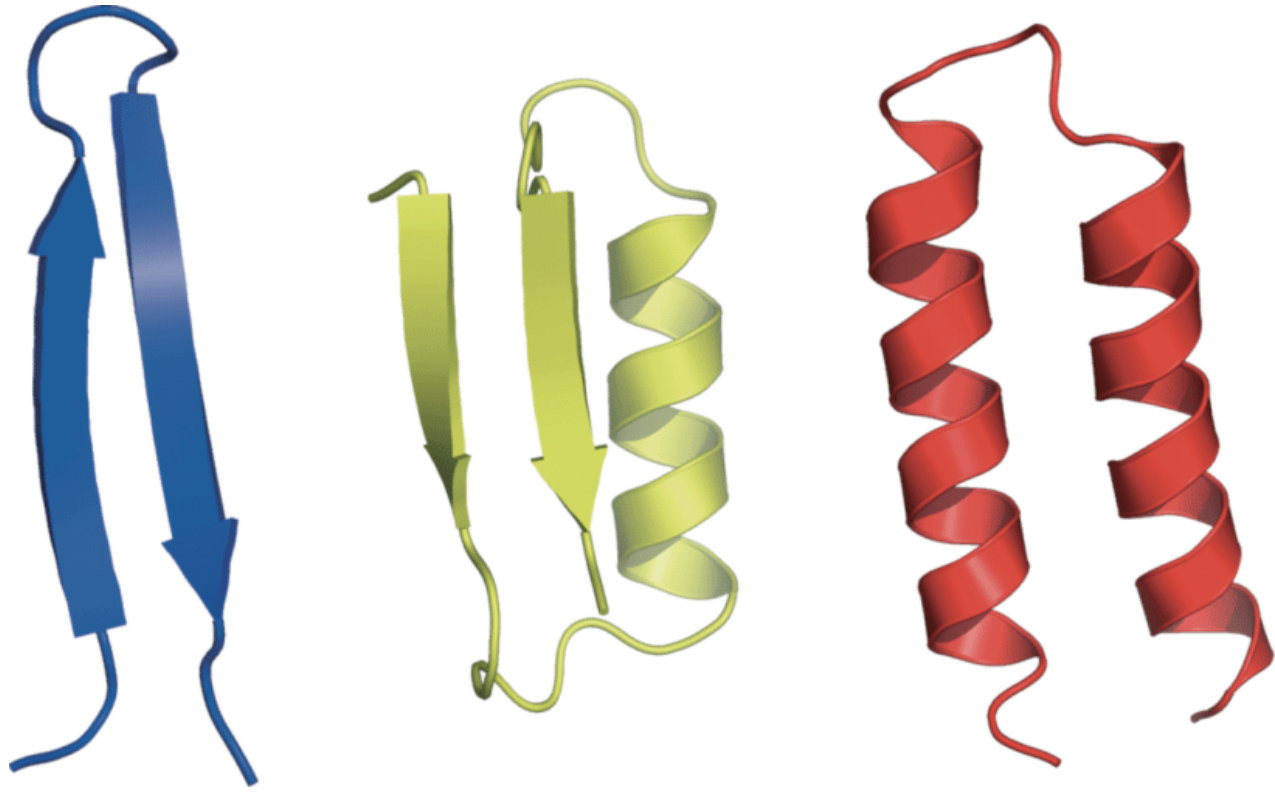
β-TURNS

- 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
- They 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.

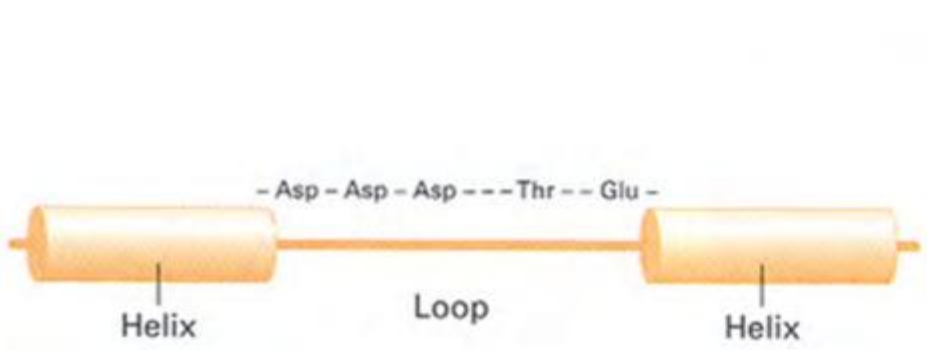


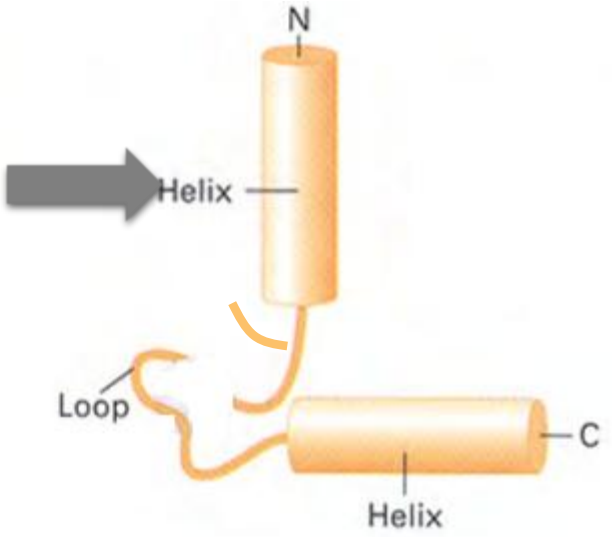
SUPER-SECONDARY STRUCTURES

- They are regions in proteins that contain an ordered organization of secondary structures.
- Examples:
  - Motifs
  - Domains

A MOTIF (A MODULE)

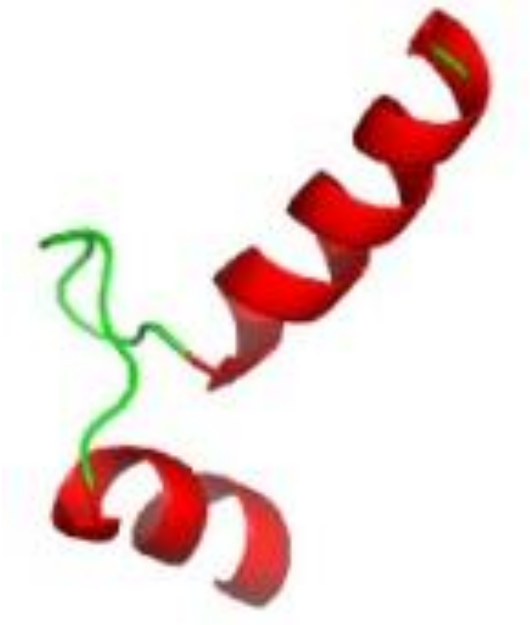
- A motif is made of multiple, repetitive or consecutive (connected) secondary structures, that can be small or large.
- They usually constitute a small portion of a protein (typically less than 20 amino acids).
- In general, motifs may provide us with information about the folding of proteins, but not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



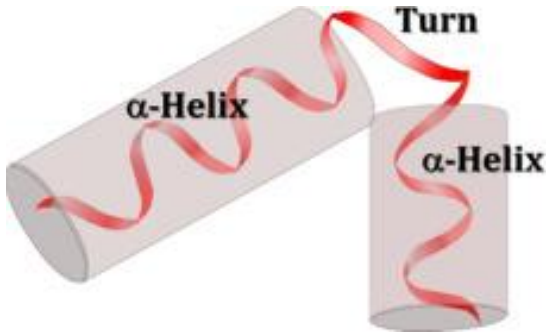


EXAMPLES OF MOTIFS

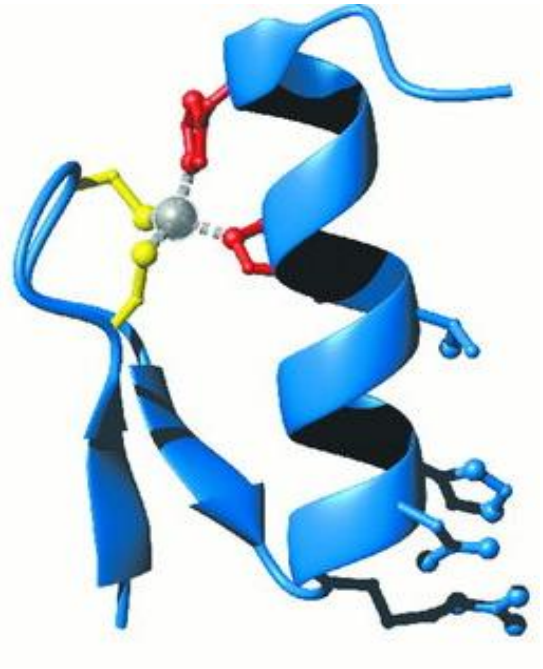
Helix-loop-helix: Two α-helices connected by a loop. It is found in Ca-binding proteins.



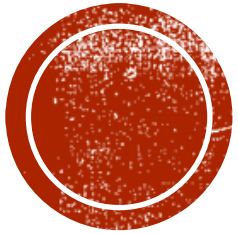
Helix-turn-helix: Two α helices joined by a short strand of amino acids. It is found in DNA-binding proteins.



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



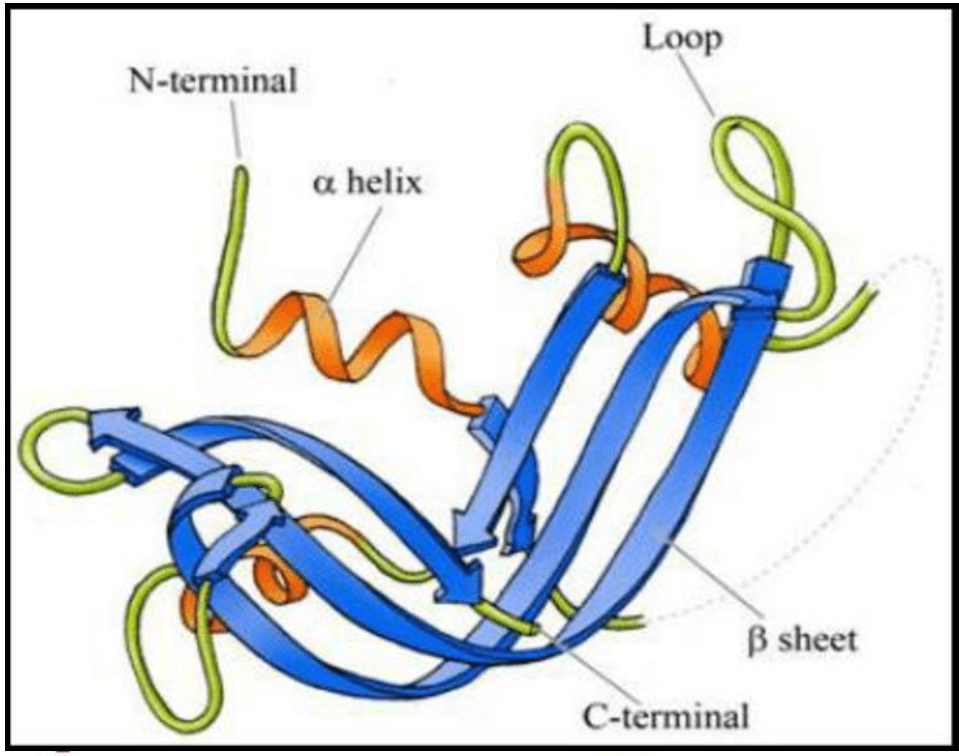
Beta hairpin: Two antiparallel beta strands connected by a turn.

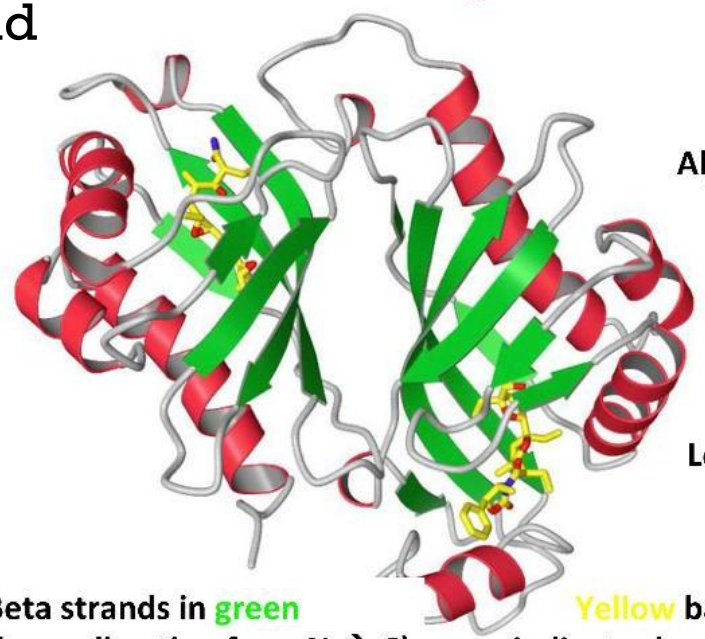


TERTIARY STRUCTURE

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



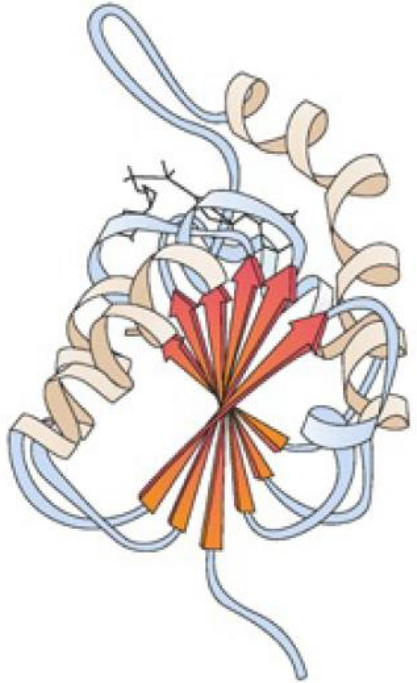


Alpha Helices in red

Loops & turns in grey

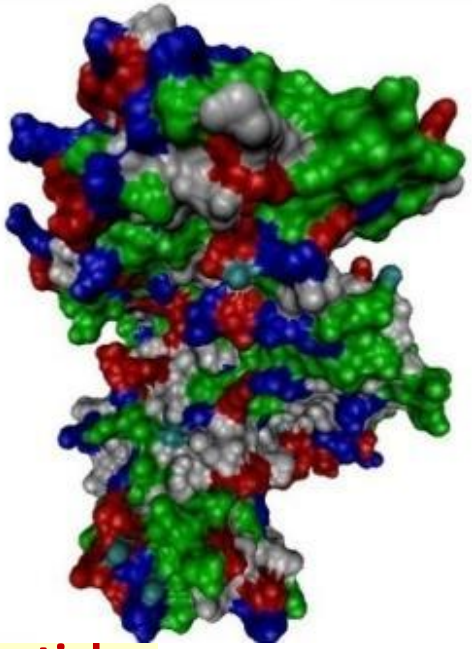
Beta strands in green (arrow shows direction from N → C)

Yellow ball & stick indicates bound molecules

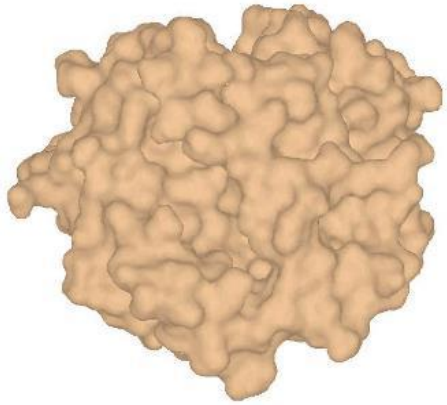


HOW TO LOOK AT PROTEINS...

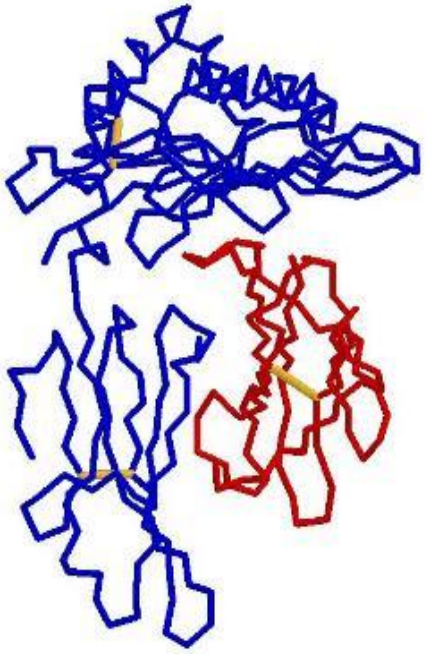
Space filling structure



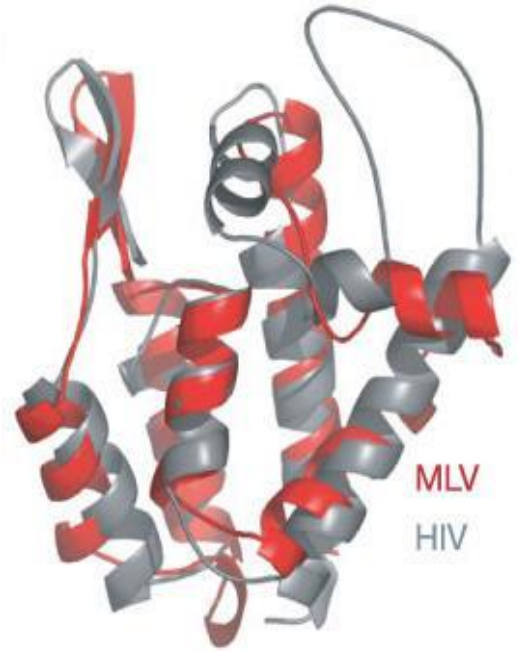
Protein surface map



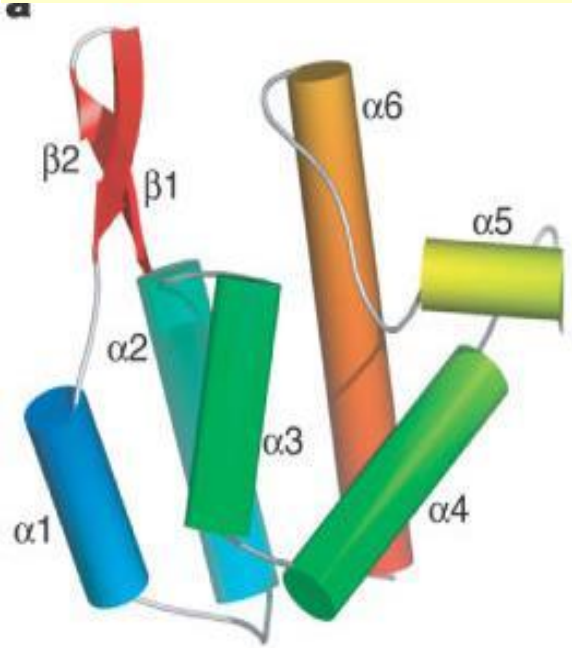
Trace structure



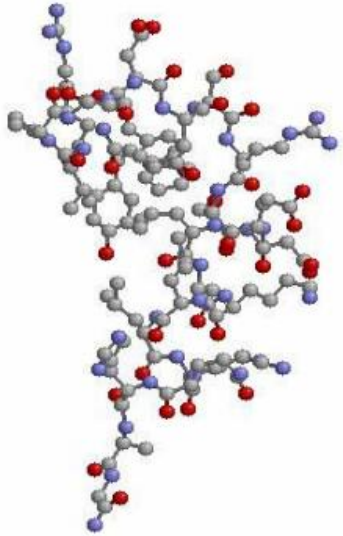
Ribbon structure



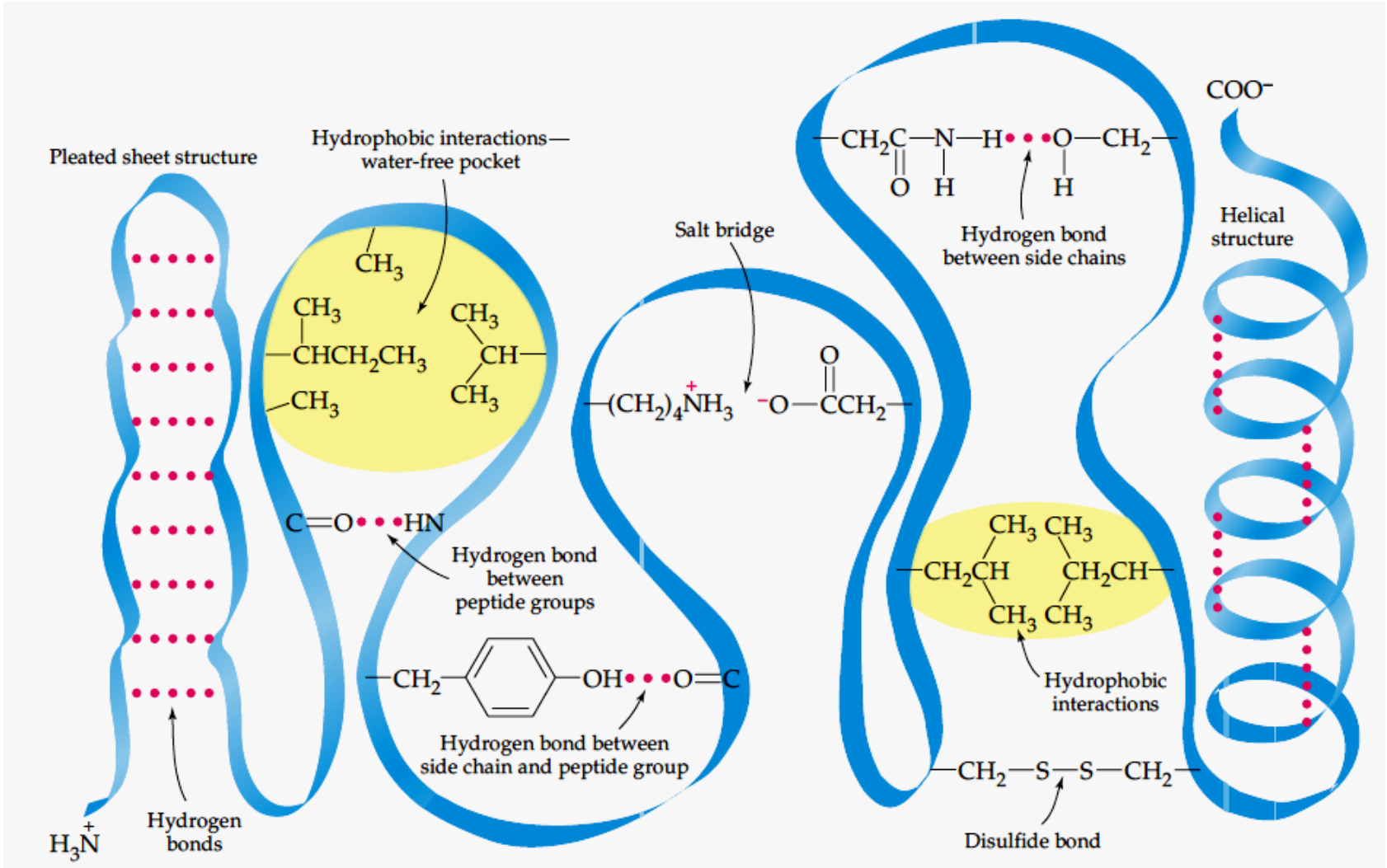
Cylinder structure



Ball and stick structure



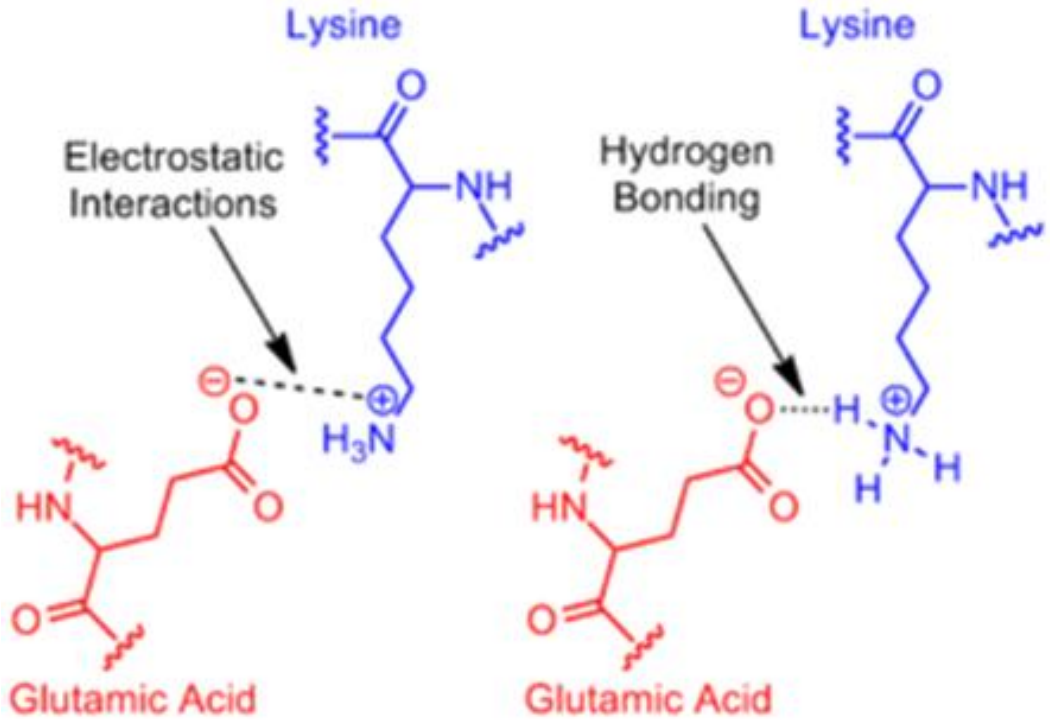
SHAPE-DETERMINING FORCES



NON-COVALENT INTERACTIONS

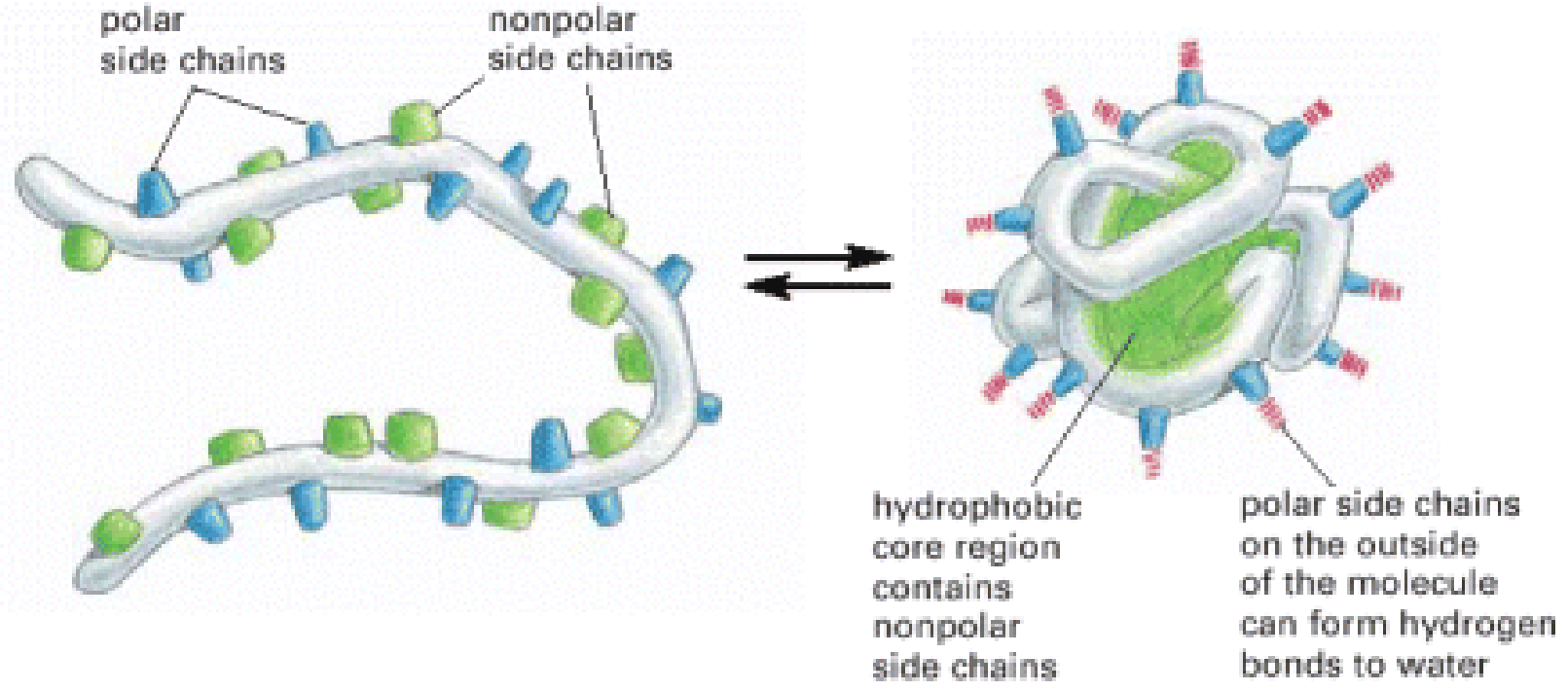
- Hydrogen bonds: 1. within and between polypeptide chains; 2. with the aqueous medium
- Charge-charge interactions (salt bridges, ionic): oppositely charged R-groups
- Charge-dipole interactions: charged R groups with the partial charges of water

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



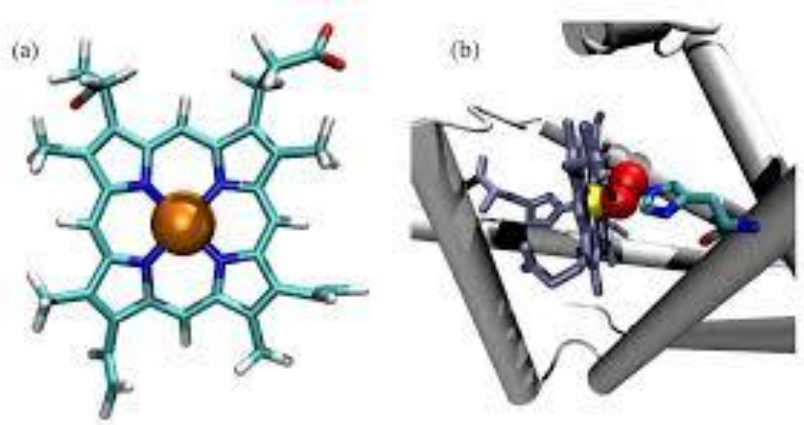
HYDROPHOBIC INTERACTIONS

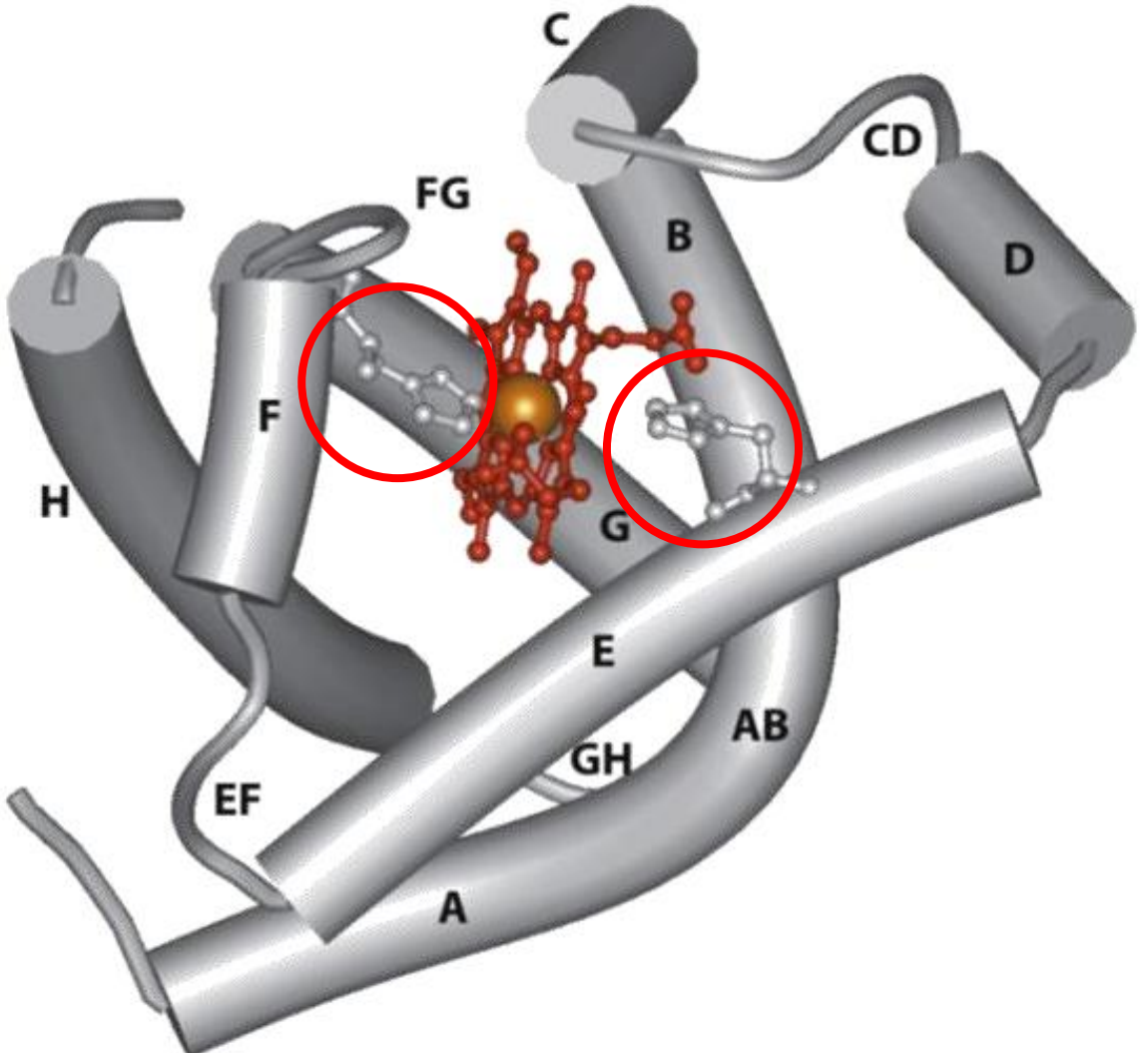
- A system is more thermodynamically (energetically) stable when hydrophobic groups are clustered together rather than extended into the aqueous surroundings.



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

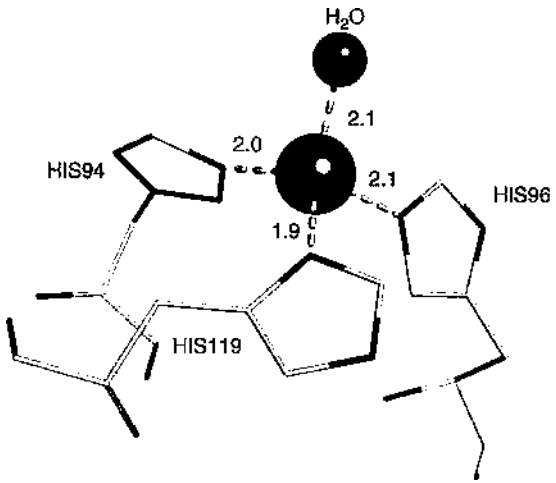
- Polar amino acids can be found in the interior of proteins
- In this case, they form hydrogen bonds to other amino acids or to the polypeptide backbone
- They play important roles in the function of the protein

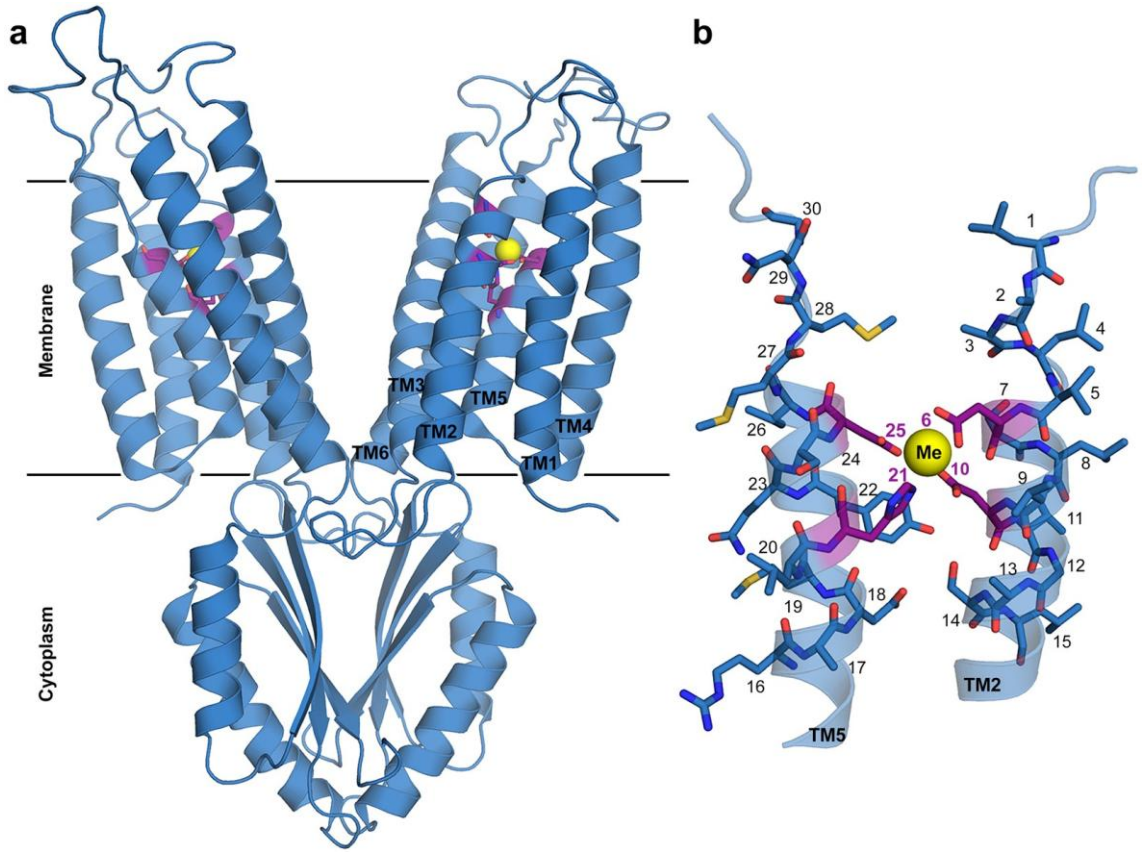


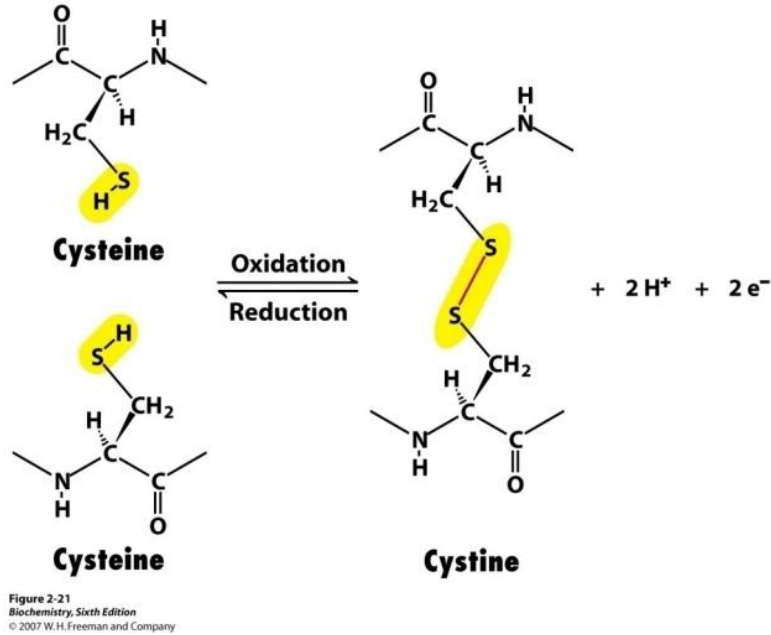


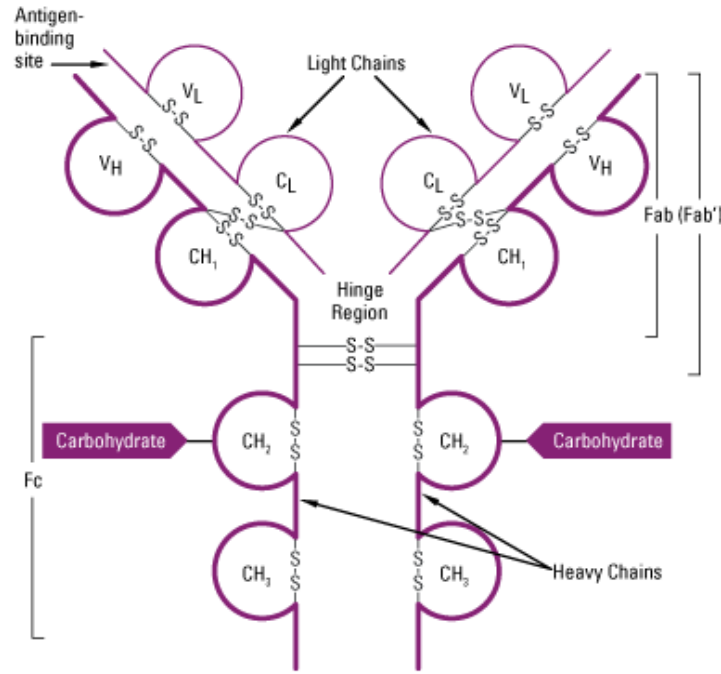
STABILIZING FACTORS

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

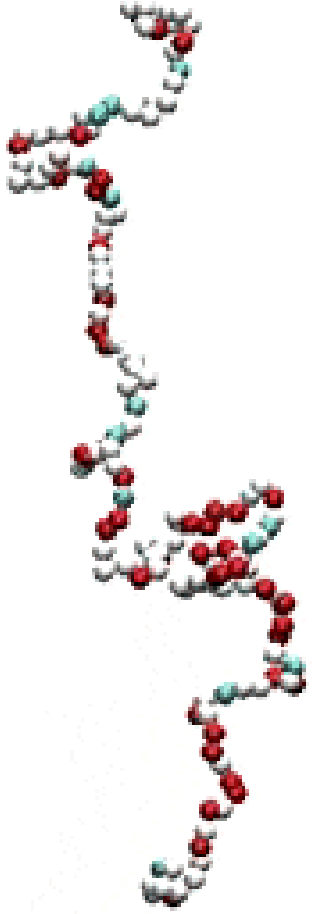


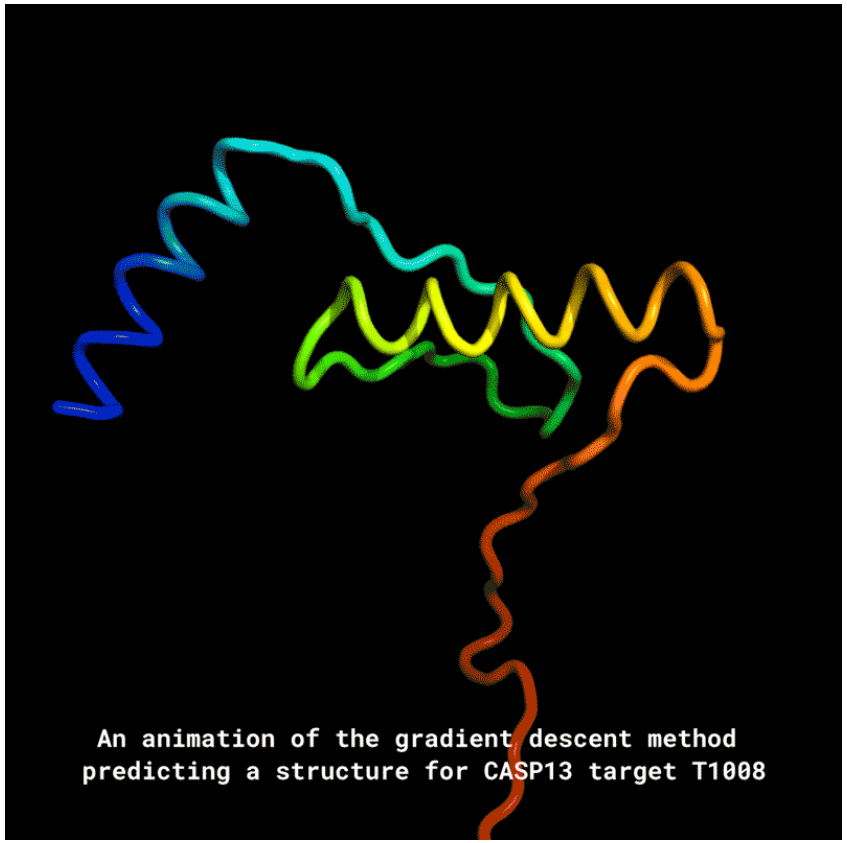




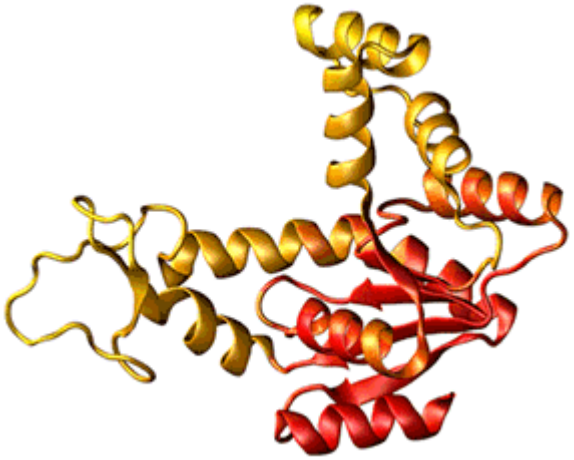


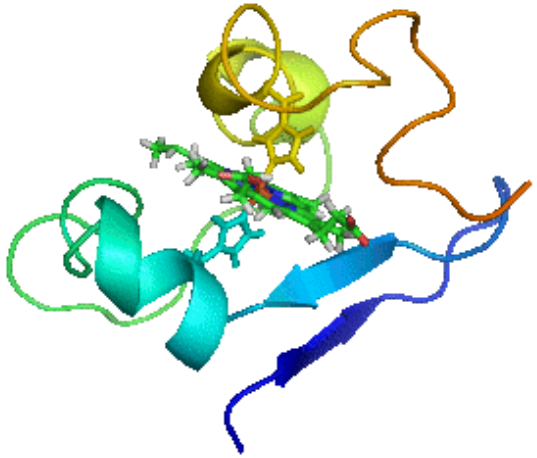
A HYPOTHETICAL LOOK AT PROTEIN FOLDING

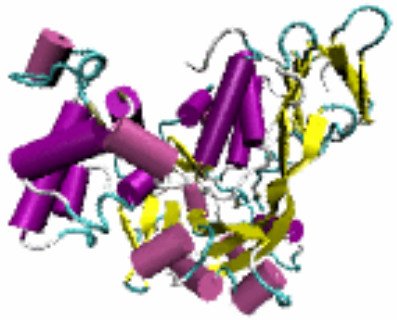


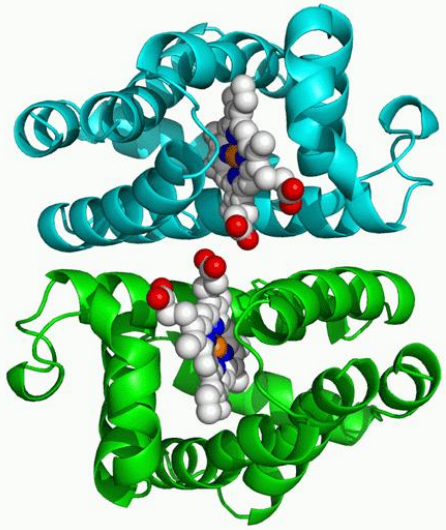


PROTEIN ARE NOT STATIC









QUATERNARY STRUCTURE

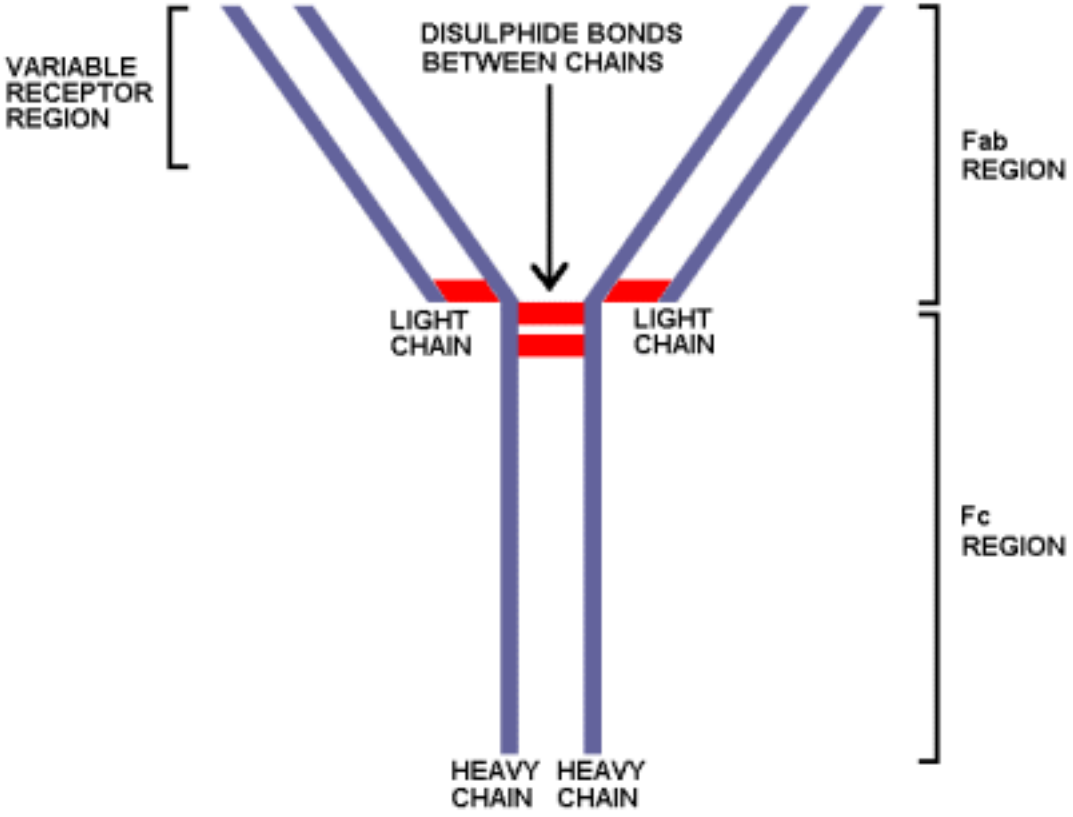
WHAT IS IT?

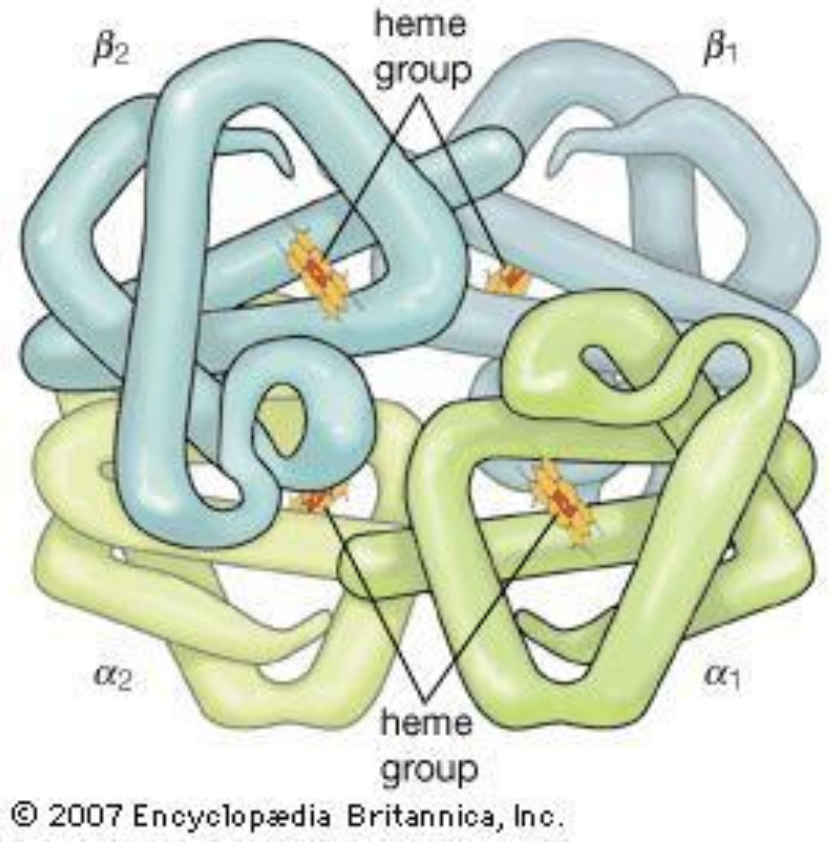
- Proteins are composed of more than one polypeptide chain.
  - They are oligomeric proteins (oligo = a few or small or short; mer = part or unit)
- The spatial arrangement of subunits and the nature of their interactions.
- Proteins made of
  - One subunit = monomer
  - Two subunits: dimer
    - The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

HOW ARE THE SUBUNITS CONNECTED?

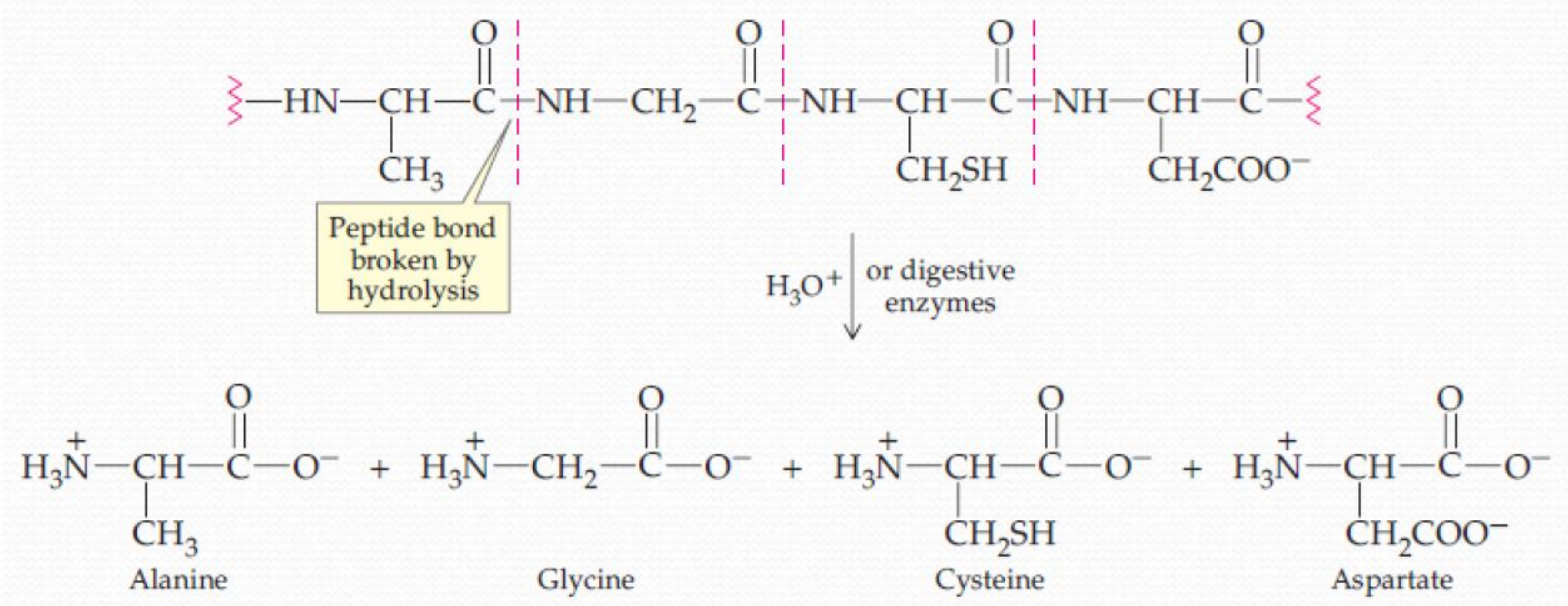
- Sometimes subunits are disulfide-bonded together, other times, noncovalent bonds stabilize interactions between subunits





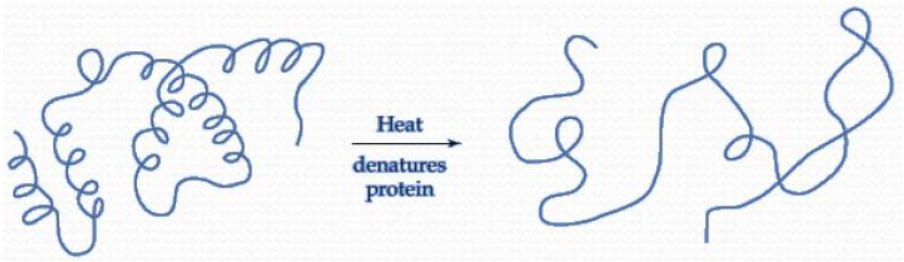
PROPERTIES OF PROTEINS:
PROTEIN HYDROLYSIS

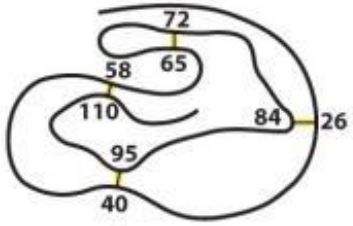
PROTEIN HYDROLYSIS



PROPERTIES OF PROTEINS:
DENATURATION AND RENATURATION

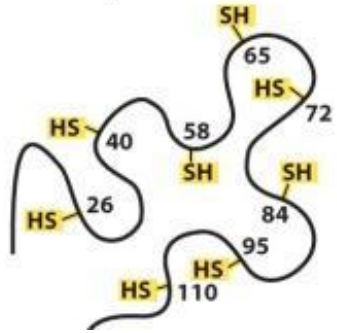
DENATURATION





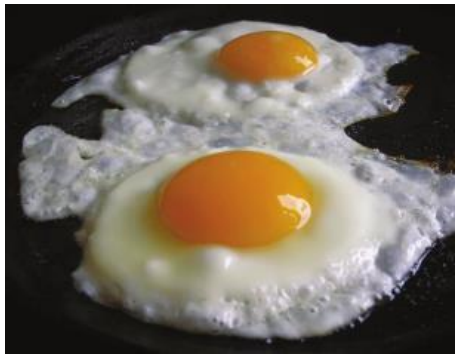
Native state; catalytically active.

addition of urea and mercapto-ethanol



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

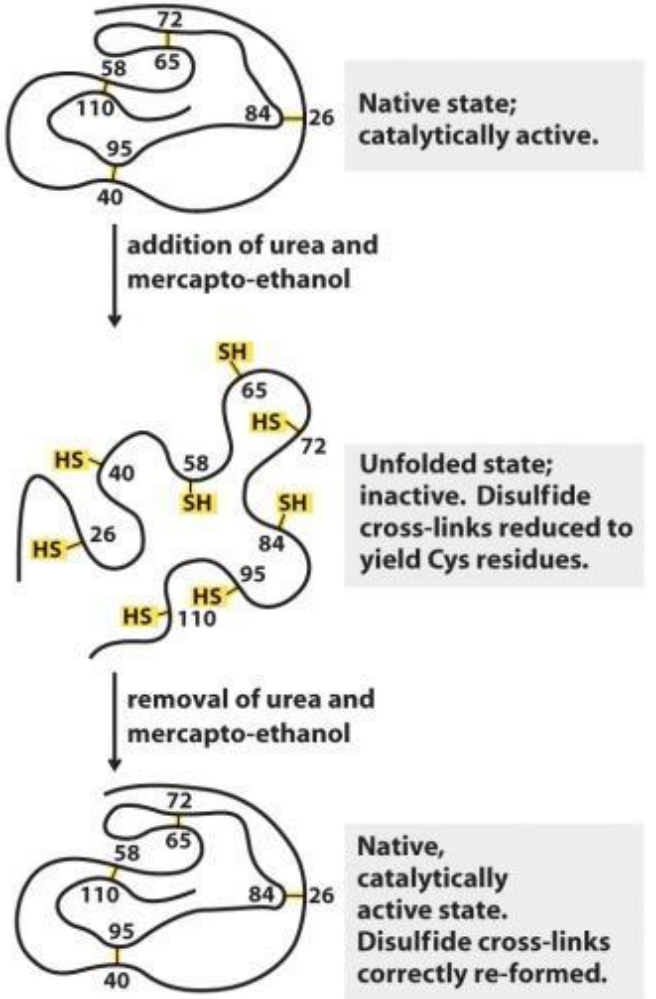
- Solubility
- Heat
- Mechanical
- Extremes of pH



- Organic compounds: acetone, ethanol, bacterial proteins
- 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 such as β-mercaptoethanol (βME) and dithiothreitol (DTT). Both reduce disulfide bonds

RENATURATION

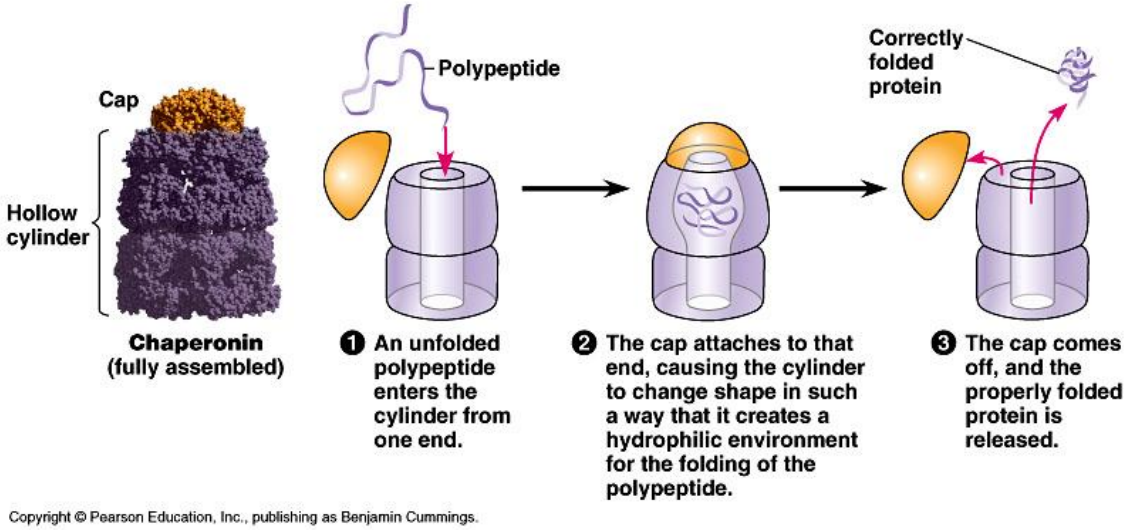
- Renaturation is the process in which the native conformation of a protein is re-acquired
- Most denaturation is irreversible
- Renaturation can occur quickly and spontaneously and disulfide bonds are formed correctly
- If a protein is unfolded, it can refold to its correct structure placing the S-S 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



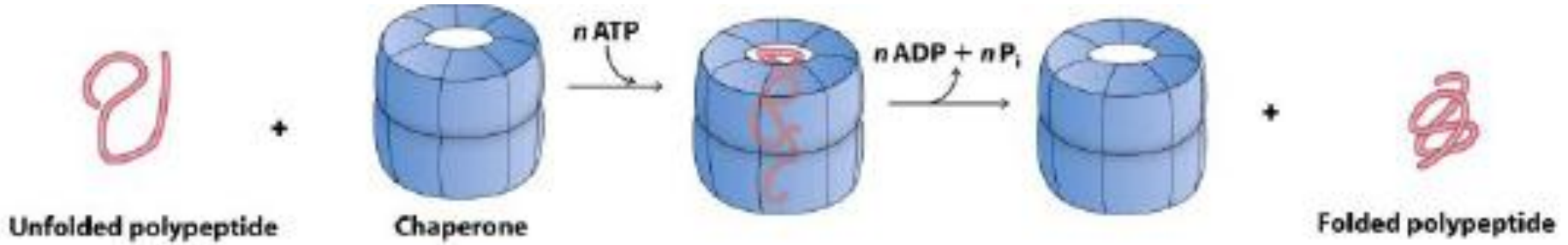
FACTORS THAT DETERMINE PROTEIN STRUCTURE

- 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**

PROBLEM SOLVERS: CHAPERONES



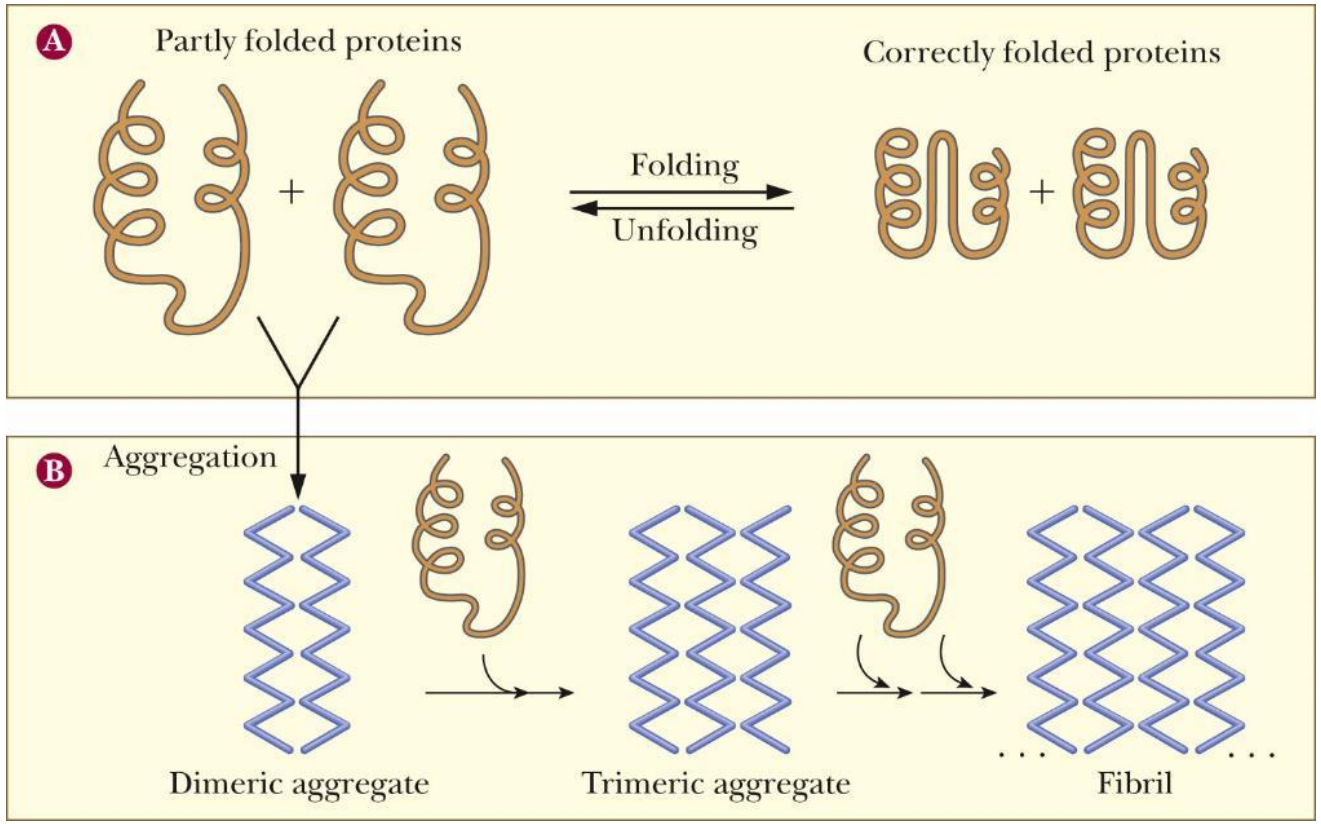
- These proteins bind to polypeptide chains and help them fold with the most energetically favorable folding pathway.
- Chaperones also prevent the hydrophobic regions in newly synthesized protein chains from associating with each other to form protein aggregates .



Many diseases are the result of defects in protein folding

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.

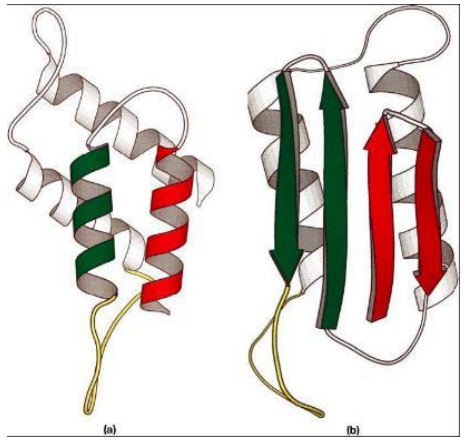


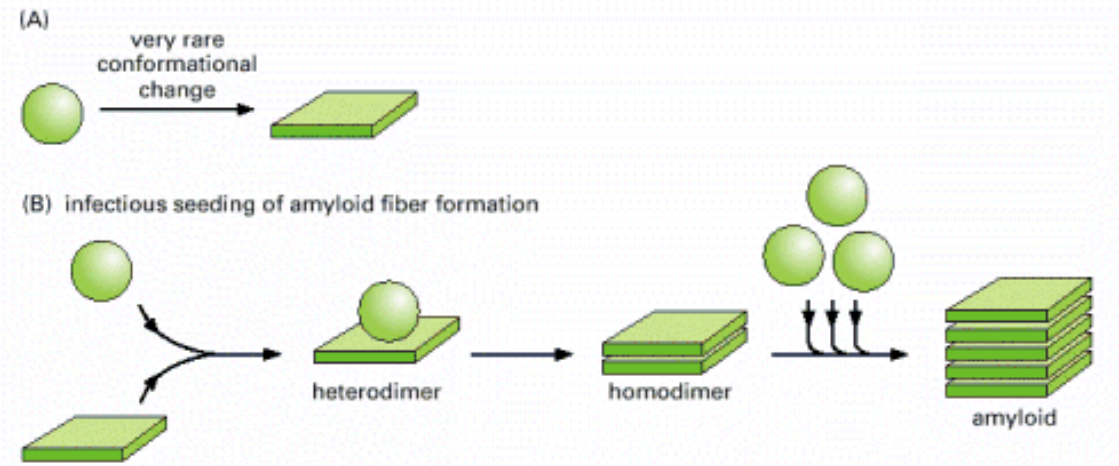
OUTCOME OF PROTEIN MISFOLDING

- 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

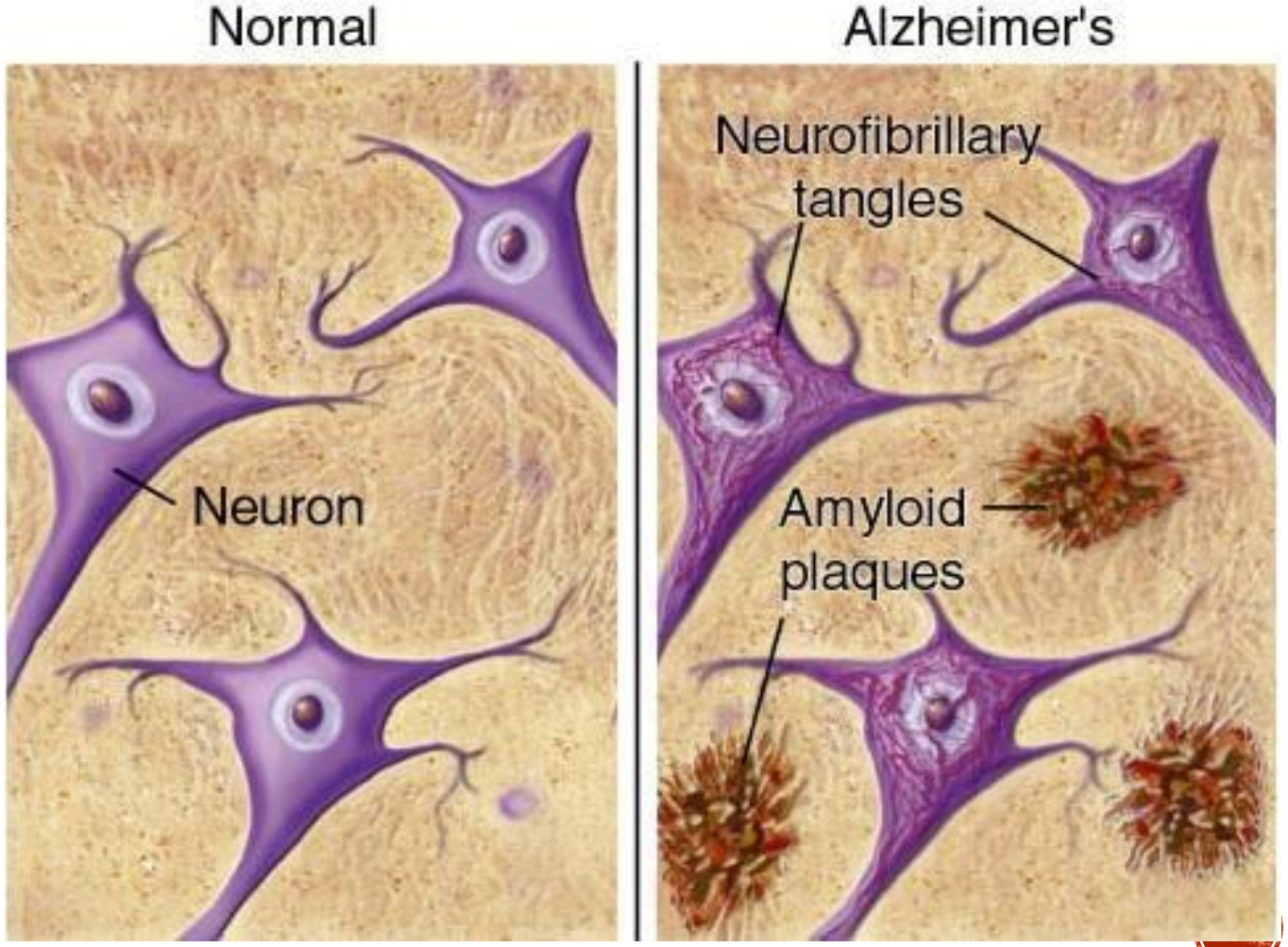
- Striking examples of protein folding-related diseases are prion diseases, such as Creutzfeldt-Jacob disease (in humans), and mad cow disease (in cows), and scrapie (in sheep)
- Pathological conditions can result if a brain protein known to as prion protein (PrP) is misfolded into an incorrect form called PrPsc (Met^{129})
- PrP_C has a lot of α-helical conformation, but PrPsc has more β strands forming aggregates



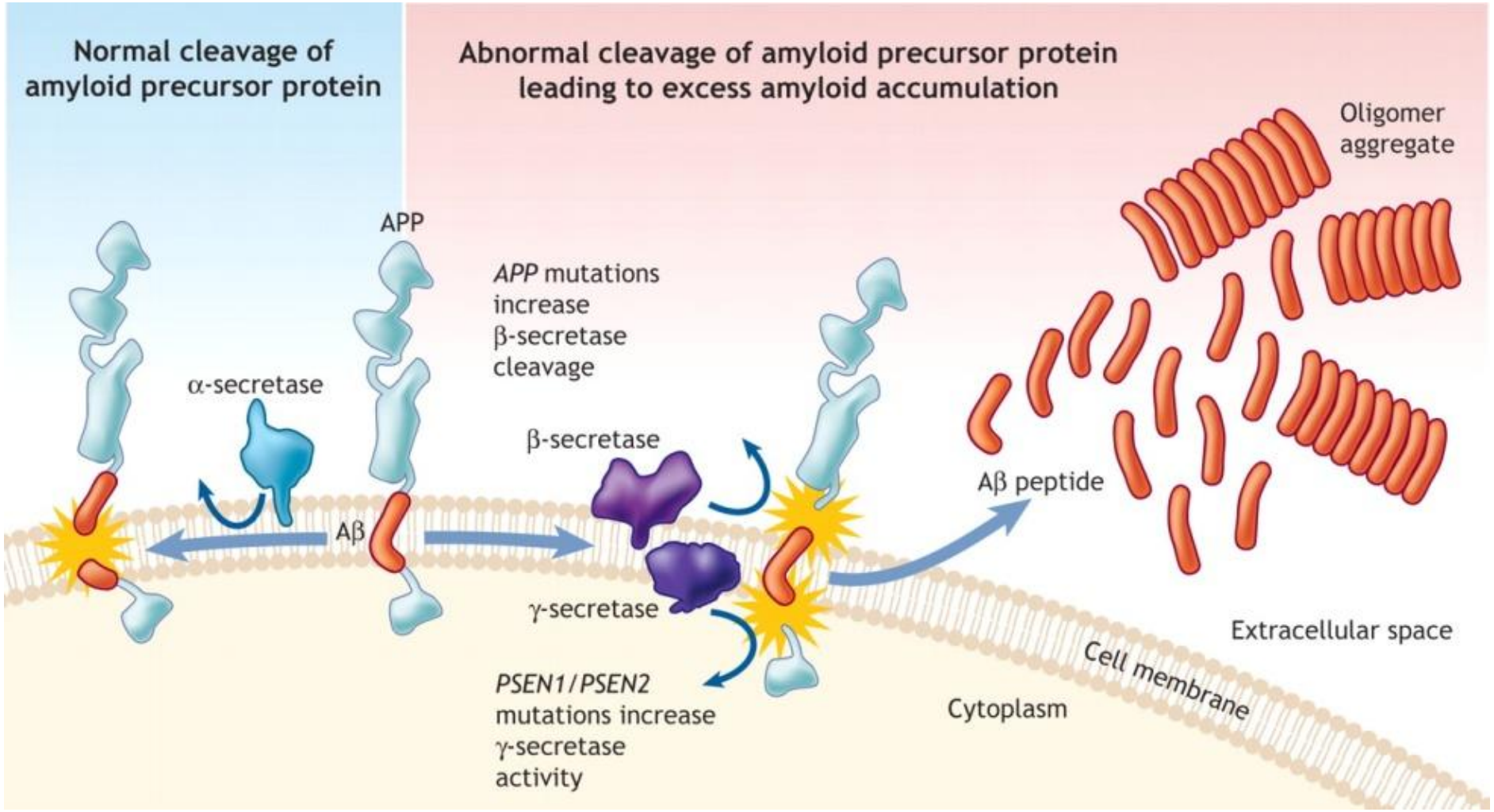


ALZHEIMER'S DISEASE

- 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



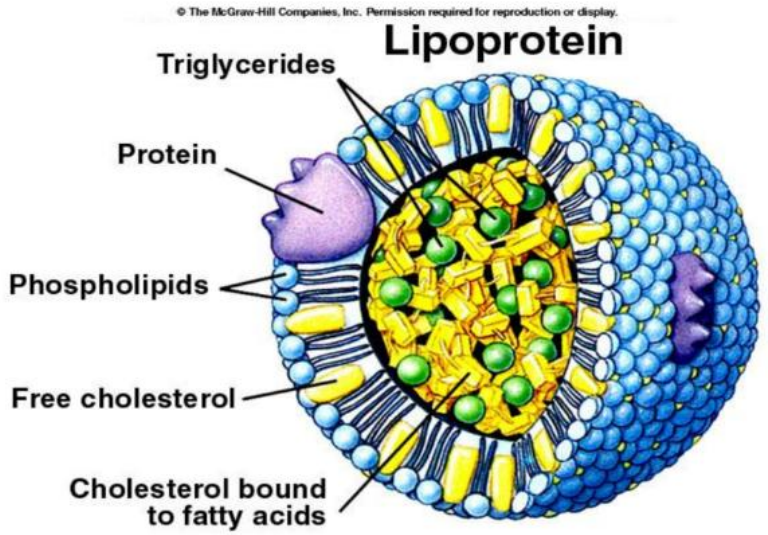
COMPLEX PROTEIN STRUCTURES

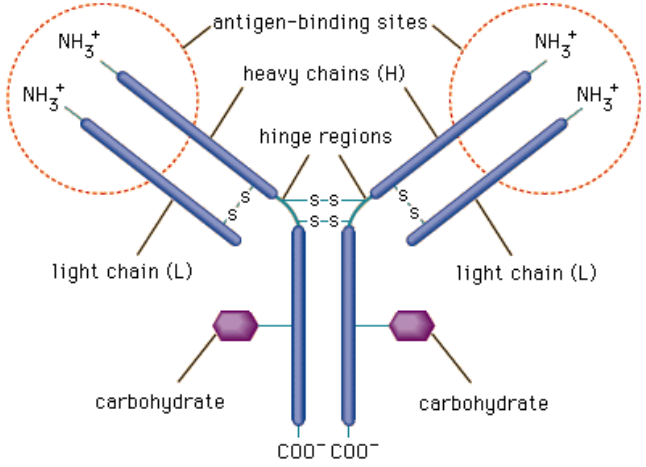
HOLO- AND APO-PROTEINS

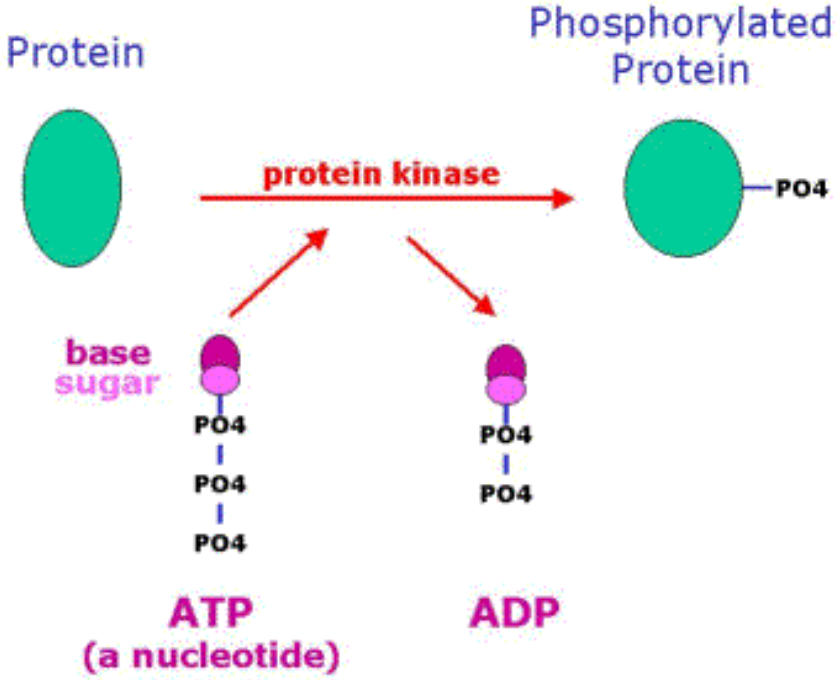
- Proteins can be linked to non-protein groups and are known as **conjugated proteins**
- When a protein is conjugated to a non-protein group covalently, the non-protein group is known as a **prosthetic group** and the protein known as a **holoprotein**
- If the non-protein component is removed, the protein is known as an **apoprotein**

CONJUGATED…

- Lipoproeins: 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