GENERAL BIOCHEMISTRY MODULE 1

- **Biological macromolecules:**

✓ **Carbohydrates**

✓ **Lipids**

✓ **Amino acids and Peptides**

✓ **Proteins**
  - **myoglobin and hemoglobin**
  - **enzymes and enzymology**

NUCLEIC ACIDS – GENERAL BIOCHEMISTRY
MODULE 2

- **Structure and Function of Nucleic Acids**
- **Genes and Chromosomes (summary)**
- **DNA metabolism (replication)**
- **RNA metabolism (synthesis and transcription)**
- **Protein Synthesis (translation)**

BIOCHEMISTRY LABORATORY

- December 19th 2025, 9-11 AULA MAGNA DERMATOLOGIA -Sant'Orsola
  Prof. Vadim Viviani «Bioluminescence color modulating luciferases for cellular biosensing and environmental analysis»

- January 9th 2025, 11-13 AULA MAGNA DERMATOLOGIA -Sant'Orsola
  Clinical case presentations (4/5 people per group- G1 and G2)

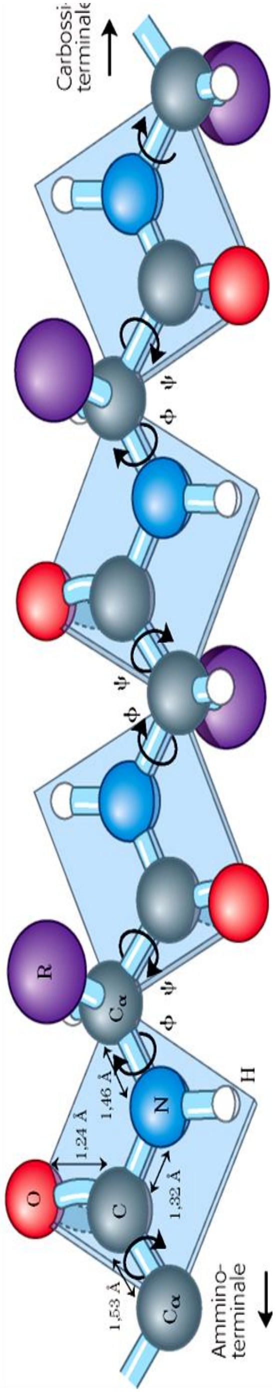
- January 13th 2025, 9-11 AULA MAGNA DERMATOLOGIA - Sant'Orsola
  Clinical case presentations (4/5 people per group- G3 and G4)

- January 15th 2025, 11-13 AULA MAGNA DERMATOLOGIA - Sant'Orsola
  Prof. Antonio Pannuti «RNA in diagnostics and therapy»

- January 17th 2025, 9-11 AULA MAGNA DERMATOLOGIA - Sant'Orsola
  «Mock exam – Chemistry and Biochemistry (with results discussion)»

- January 27th 2025, 9-11 AULA MAGNA DERMATOLOGIA - Sant'Orsola
  Dr. Akram Ghantous «Epigenomics and Big Data: Linking the Environment with Health and Disease”

PROTEINS



- Linear polymers consisting of amino acids linked together by **peptide bonds.**
- Some amino acids are more abundant than others (the most abundant is leucine); **tryptophan, and cysteine are quite rare in proteins.**
- Some proteins contain several polypeptide chains as subunits, called **oligomeric proteins**, which may be identical or different from each other (ex. Hemoglobin).
- **Proteins fold in space, assuming a three-dimensional structure.**

PROTEIN FUNCTIONS

Proteins perform multiple biological functions, closely related to their chemical structure:

Catalysts (enzymes)

Storage

Transporters (albumin)

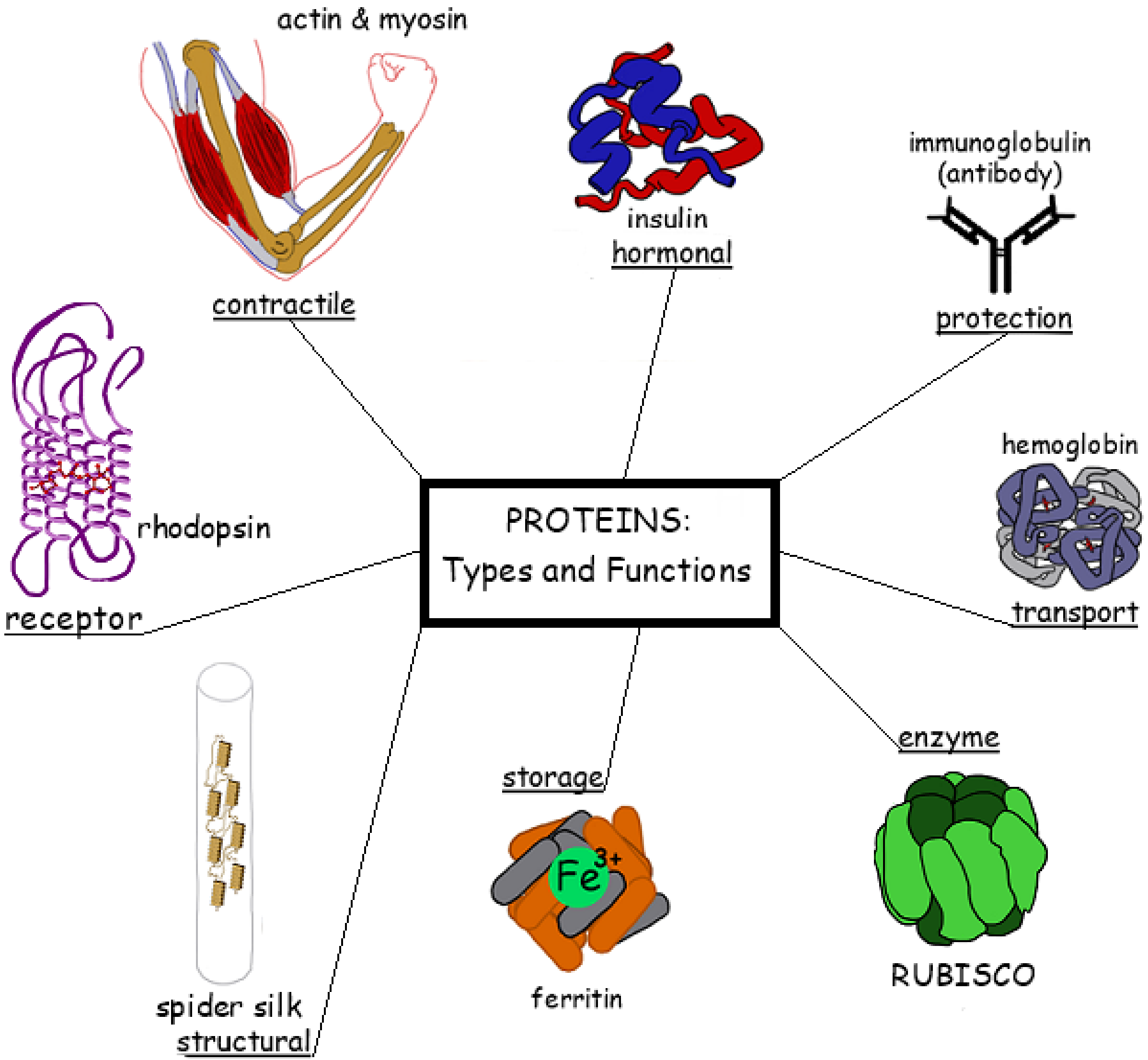
Messangers (hormones)

Antibodies

Regulation (receptors)

Structural (collagen)

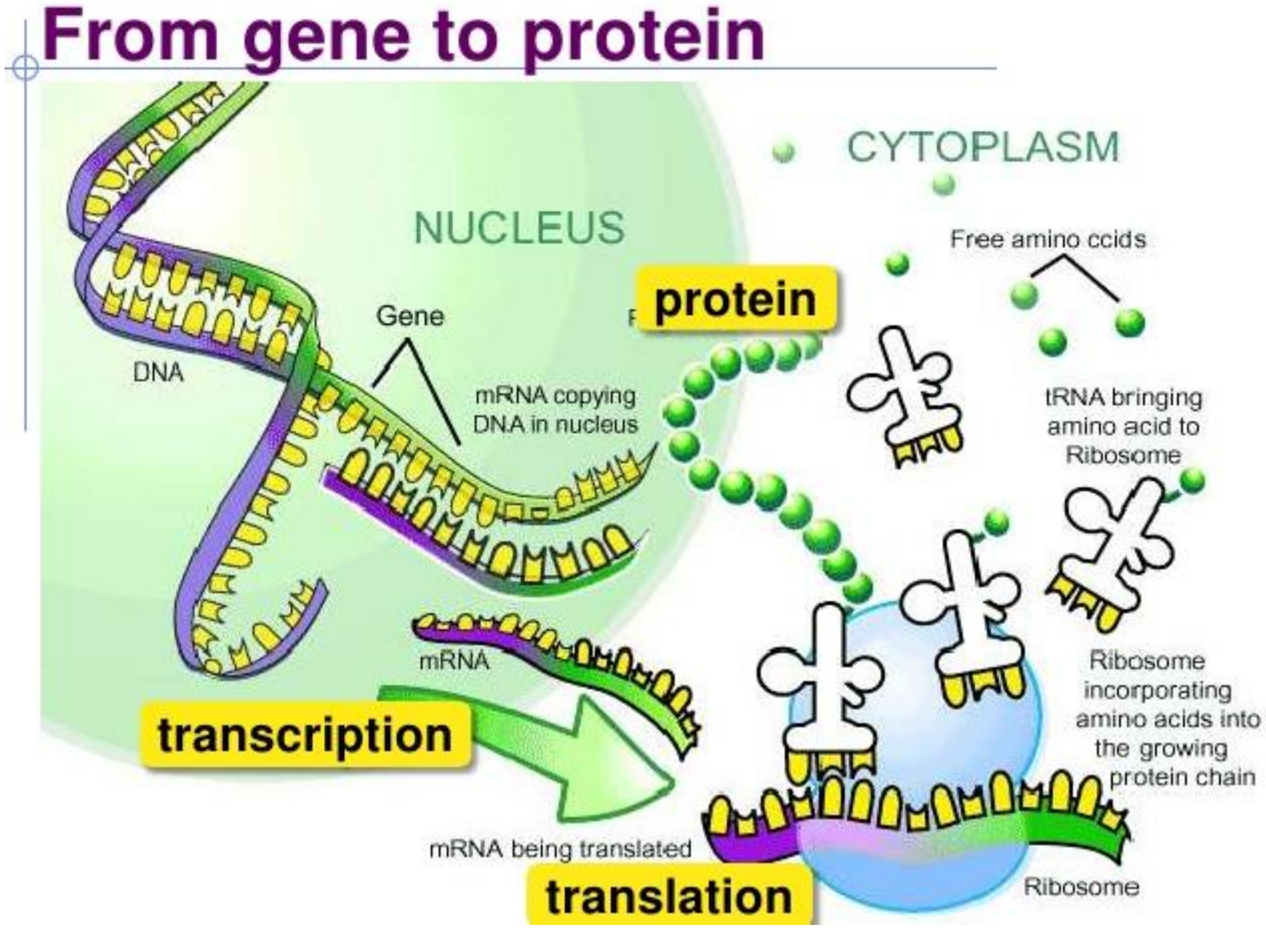
Toxic (toxins)



PROTEIN SYNTHESIS

Proteins are synthesized on ribosomes present in the cytosol, on the basis of the information given by the DNA and translated on mRNA.

The genetic code allows the interpretation of the message contained in the RNA, expressed in nucleotides, in a sequence of amino acids.



THE STRUCTURE OF PROTEINS

- ✓ A variety of intramolecular interactions enables the amino acid chain to fold in a specific way to give the protein a three-dimensional structure and shape, called **NATIVE STRUCTURE**. This structure is critical for its activity and function.

- ✓ Several amino acids can be entangled and connected via *disulfide bridges*.

- ✓ Parts of the amino acid chain can be organized into *α-helices* or *β-sheets*.

- ✓ Globular proteins like enzymes and antibodies are more folded and coiled whereas fibrous proteins (collagen) are more filamentous and elongated.

- ✓ To describe the complex structure of proteins, **four levels of organization** are distinguished: ***primary, secondary, tertiary*** **and *quaternary* structures**.

LEVELS OF PROTEIN STRUCTURE

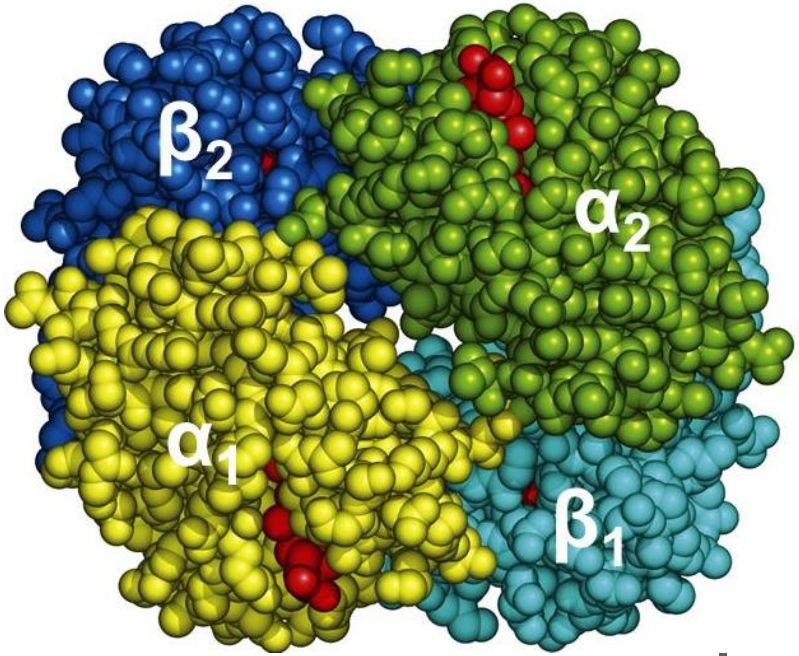
- **Primary:** the sequence of the amino acids in the chain.
- **Secondary:** refers to particularly stable arrangements of amino acid residues giving rise to recurring structural patterns (*i.e.* hydrogen bonding). Examples are α-helix and β-pleated sheet.
- **Tertiary:** three-dimensional folding of a polypeptide.
- **Quaternary:** association of two or more peptide chains to form a protein.

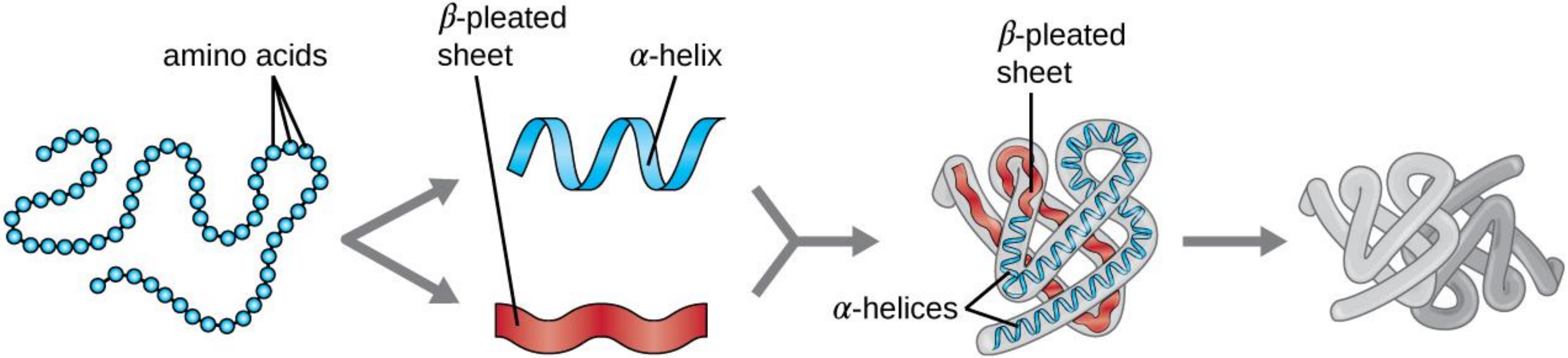
Multisubunit proteins

The individual polypeptide chains in a multisubunits protein may be identical or different.

Multisubunit proteins are called **oligomeric**, if they contain al least two identical units (consisting of one or more polypeptide chains); the identical units are referred as **protomers**.

Hemoglobin has four polypeptide subunits: two identical α chains and two identical β chains, all four held together by noncovalent interactions. Each α subunit is paired in an identical way with a β subunit within the structure of this multi-subunit protein, so that hemoglobin can be considered either a **tetramer of four polypeptide subunits or a dimer (oligomer) of αβ protomers.**





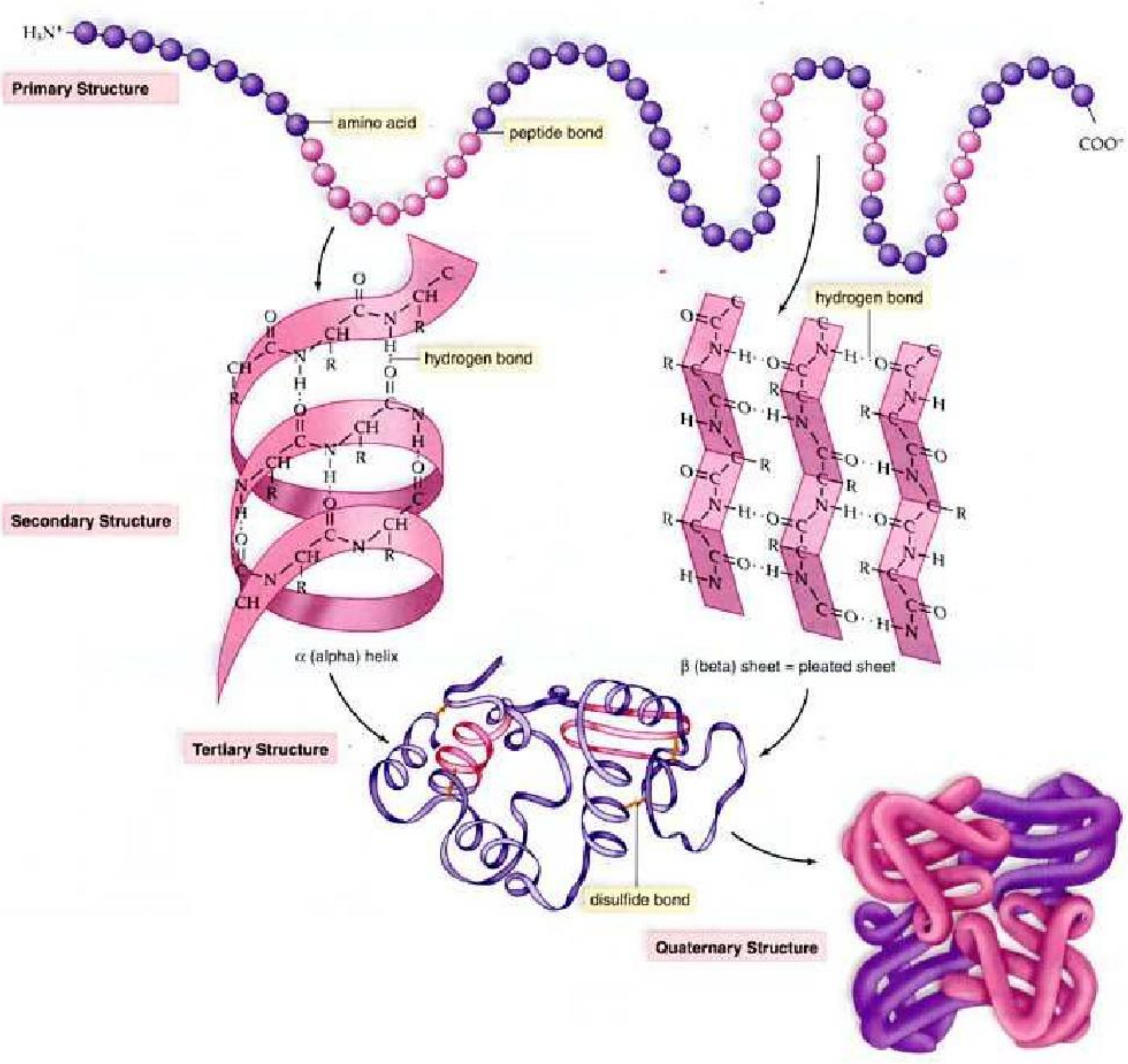
Primary Protein Structure
Sequence of a chain of amino acids

Secondary Protein Structure
Local folding of the polypeptide chain into helices or sheets

Tertiary Protein Structure
three-dimensional folding pattern of a protein due to side chain interactions

Quaternary Protein Structure
protein consisting of more than one amino acid chain

For all proteins of all organisms, weak interactions are very important in the folding of polypeptide chains into their tertiary structures. The association of multiple polypeptides to form quaternary structures also relies on noncovalent interactions.



FACTORS DRIVING PROTEIN FOLDING

What causes a protein folding? To explain this phenomenon, it is necessary to remember the principles of thermodynamics: the native conformations assumed by proteins are those that have the **lowest value of Free Energy (G).**

Δ*H*: favorable (negative) due to non-covalent, intramolecular forces (H-bonds, van der Waals forces)

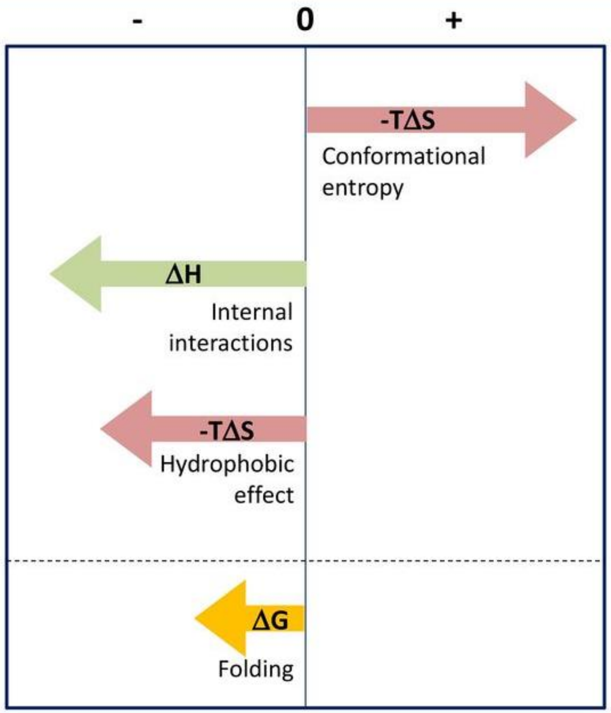
ΔS:

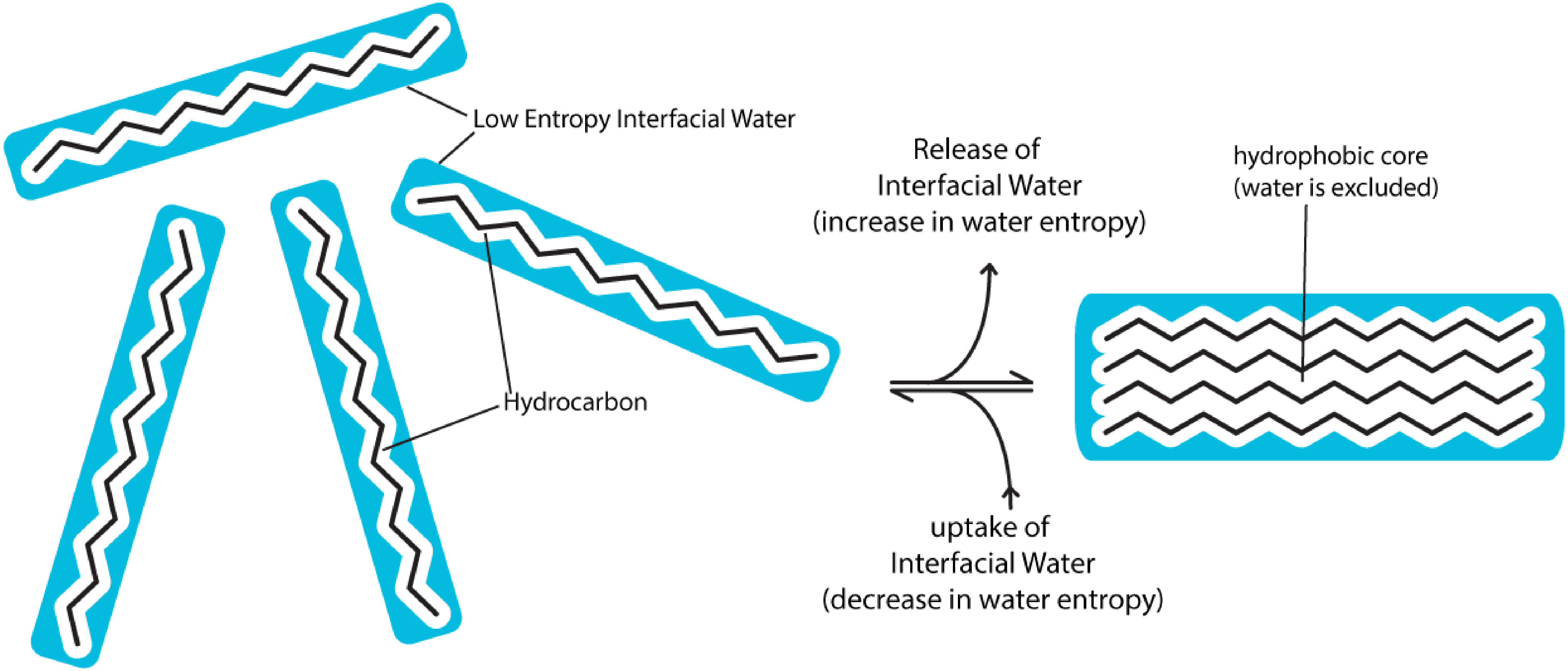
- **unfavorable (negative) because entropy decreases during folding as conformational freedom is restricted.**
- **favorable (positive) contribution of the hydrophobic effect.**

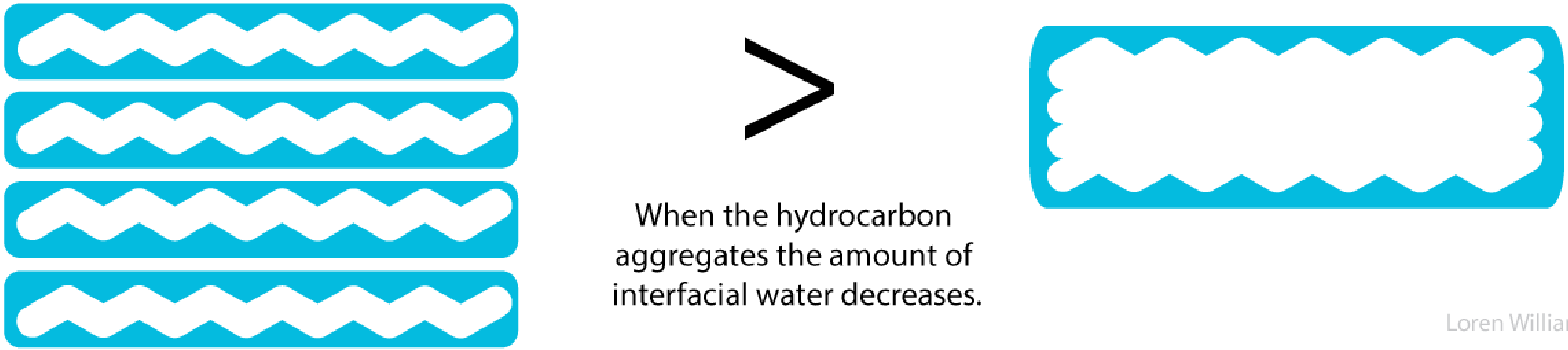
The net free energy of folding will therefore have contributions from two opposing entropy (TΔS) terms, plus an enthalpy (ΔH) term and the net Δ*G is negative.*

$$\Delta G = \Delta H - T\Delta S$$

Favorable free energy of folding is a net result of thermodynamic forces



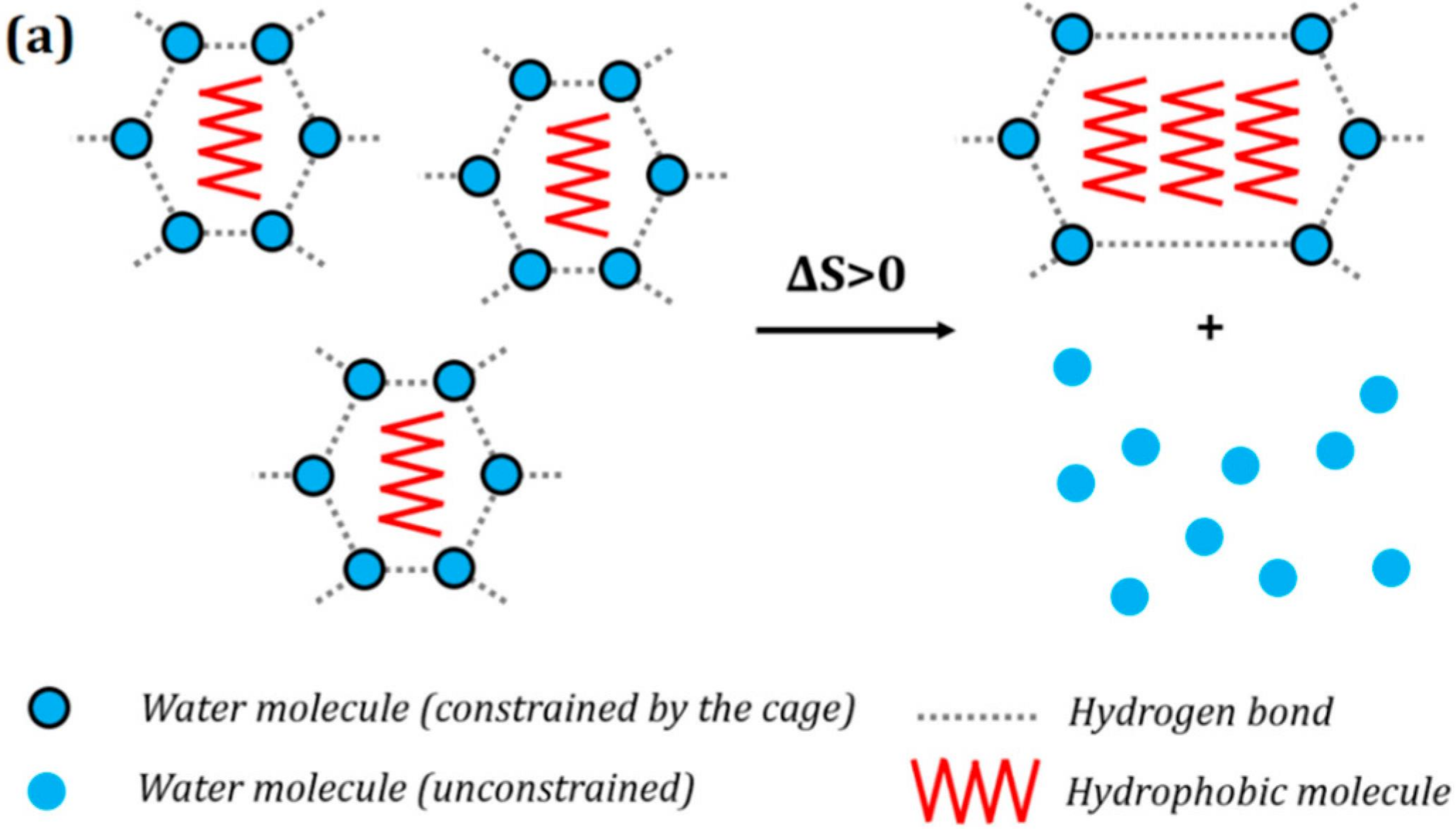




When the hydrocarbon aggregates the amount of interfacial water decreases.

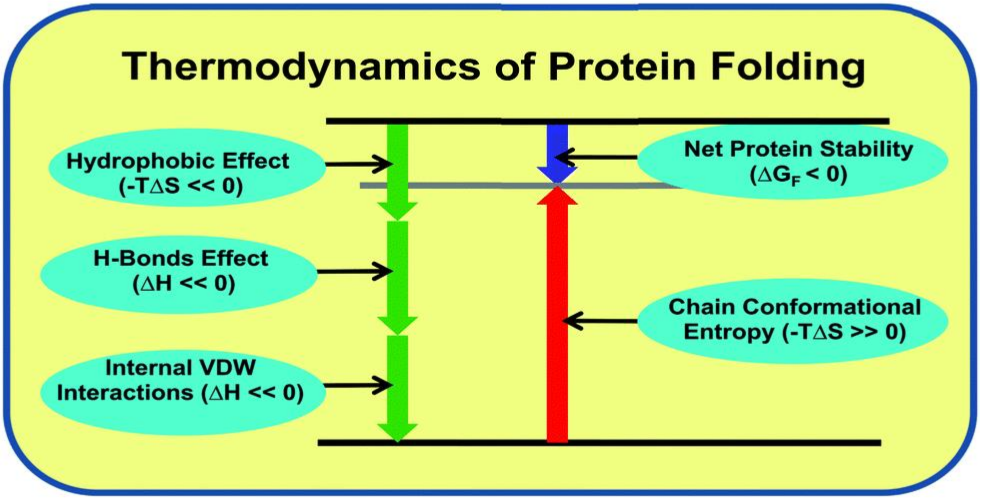
Loren Williams

HYDROPHOBIC EFFECT



There is a favourable entropy change (+ ΔS) from the burial of hydrophobic R-groups within the interior of the folded protein because there is a decrease in the order of water.

PROTEIN FOLDING IS THERMODINAMICALLY FAVOURABLE



Thermodynamics of Protein Folding

Hydrophobic Effect ($-T\Delta S << 0$)

H-Bonds Effect ($\Delta H << 0$)

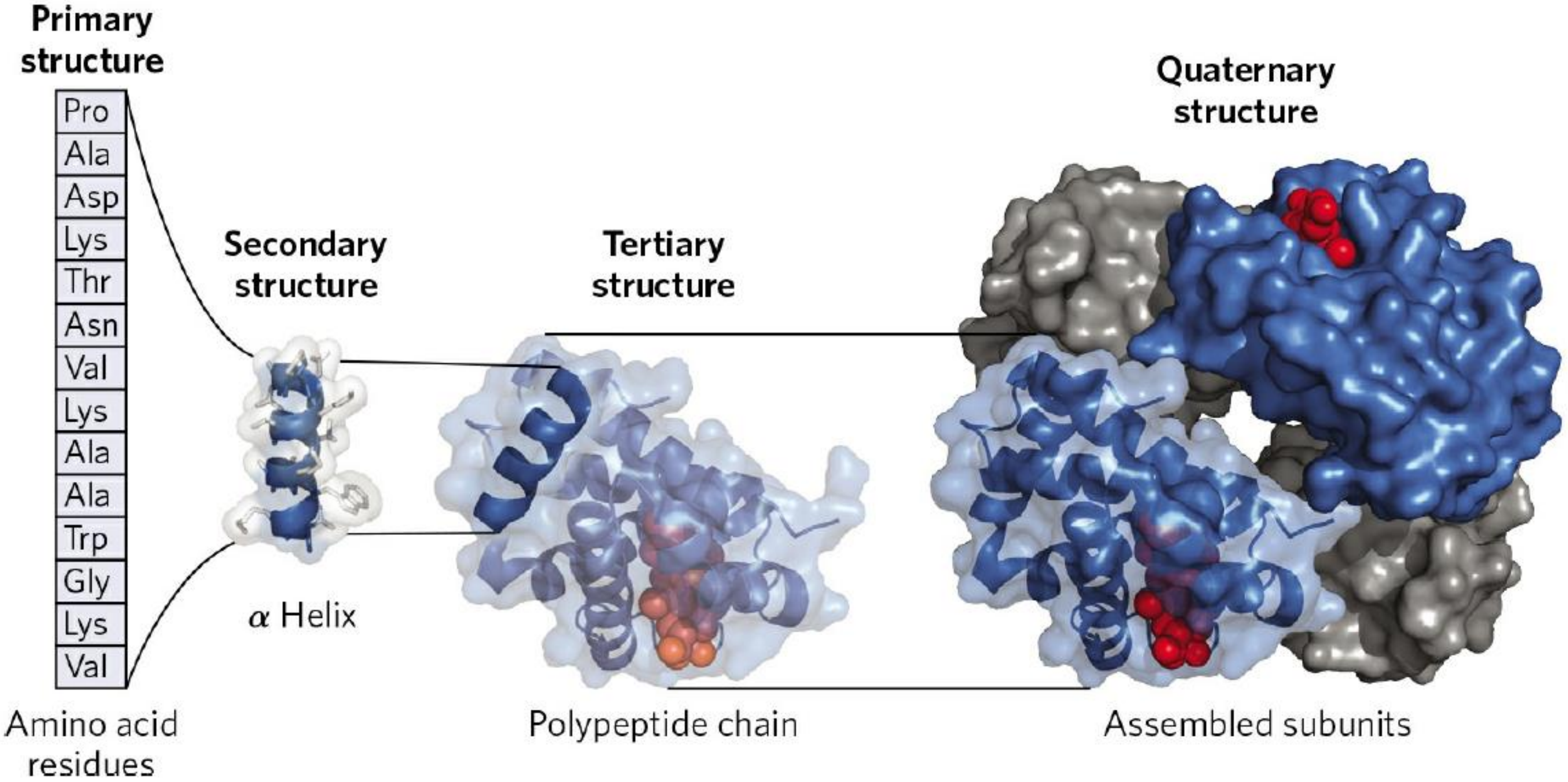
Internal VDW Interactions ($\Delta H << 0$)

Net Protein Stability ($\Delta G_F < 0$)

Chain Conformational Entropy ($-T\Delta S >> 0$)

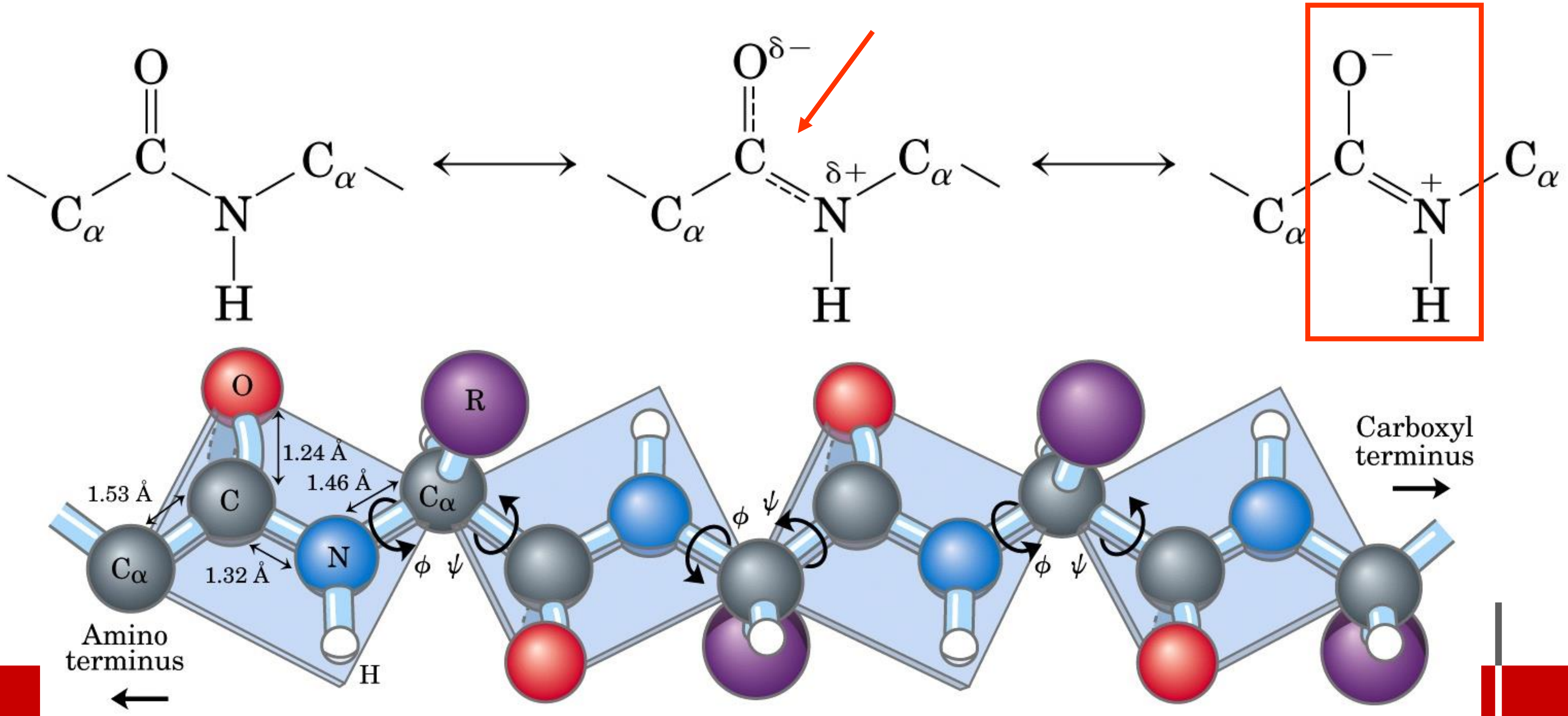
- **Hydrophobic effect**
  - Release of water molecules from the structured solvation layer around the molecule as protein folds increases the net entropy
- **Hydrogen bonds**
  - Interaction of N-H and C=O of the peptide bond leads to local regular structures such as α-helices and β-sheets
- **London dispersion**
  - Medium-range weak attraction between all atoms contributes significantly to the stability in the interior of the protein
- **Electrostatic interactions**
  - Long-range strong interactions between permanently charged groups
  - Salt-bridges, esp. buried in the hydrophobic environment strongly stabilize the protein

Overall, a decrease in entropy due to the hydrophobic effect and enthalpically favourable weak interactions promote protein folding.

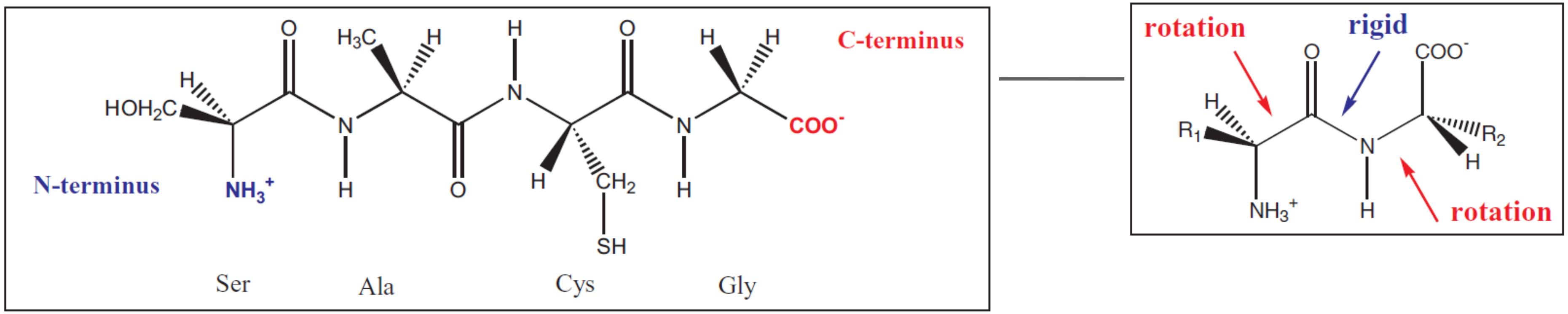

Primary structure
Pro
Ala
Asp
Lys
Thr
Asn
Val
Lys
Ala
Ala
Trp
Gly
Lys
Val
Amino acid residues
Secondary structure
α Helix
Tertiary structure
Polypeptide chain
Quaternary structure
Assembled subunits

PRIMARY STRUCTURE

- Peptide bond does NOT rotate freely, as it has a partial double bond character
- The bond between the carboxylic **Cα and C** (**Ψ psi**) and the one between **N and Cα (Φ fi)** can rotate freely, depending on the steric size of R-residue (R groups)



PRIMARY STRUCTURE



- ✓ The amino acids are connected to each other in a “**head-to-tail” fashion** by formation of a peptide bond, the condensation of a carboxylic and an amino group with the elimination of water.

- ✓ **The C−N bond cannot rotate due to its partial double bond character (the peptide unit NH–CO is rigid). The bonds to the neighbouring Cα-C and Cα-N can rotate within steric constraints and play an important role in folding of the protein.**

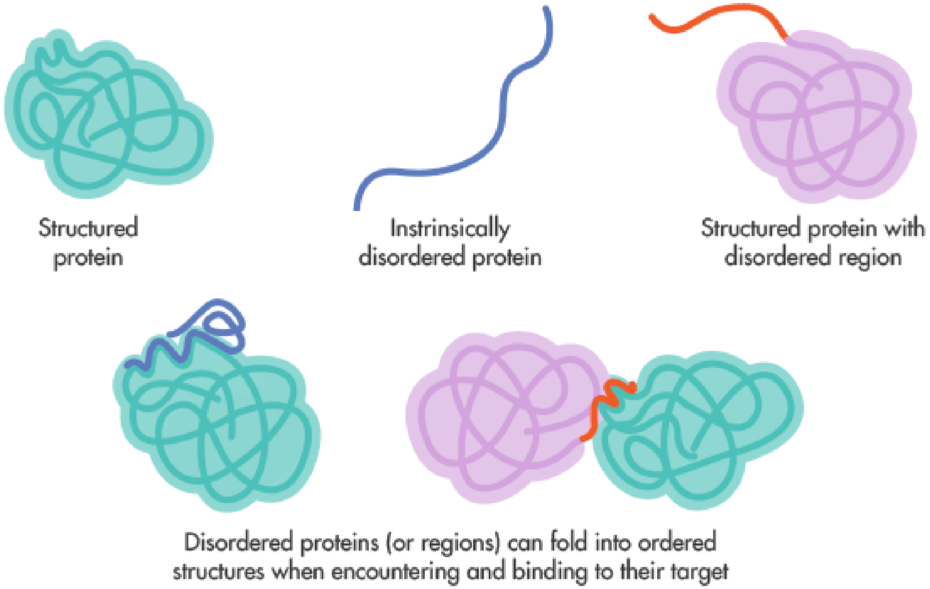
- ✓ The peptide units together with the tetrahedral C-atoms form the backbone of a protein, while the R substituents are referred to as side chains. The first amino acid residue is always the one with the free amino group, the N-terminus, while the last in the chain is the C-terminus with the free carboxyl group.

Few considerations on protein structure

- The three-dimensional structure of a protein is determined by its amino acid sequence (primary sequence)
- The function of a protein depends on its structure
- An isolated protein usually exists in one or a small number of stable structural forms
- The most important forces stabilizing the specific structures maintained by a given protein are noncovalent weak interactions (hydrogen bonds and hydrophobic weak interactions)

Few considerations on protein structure – part II

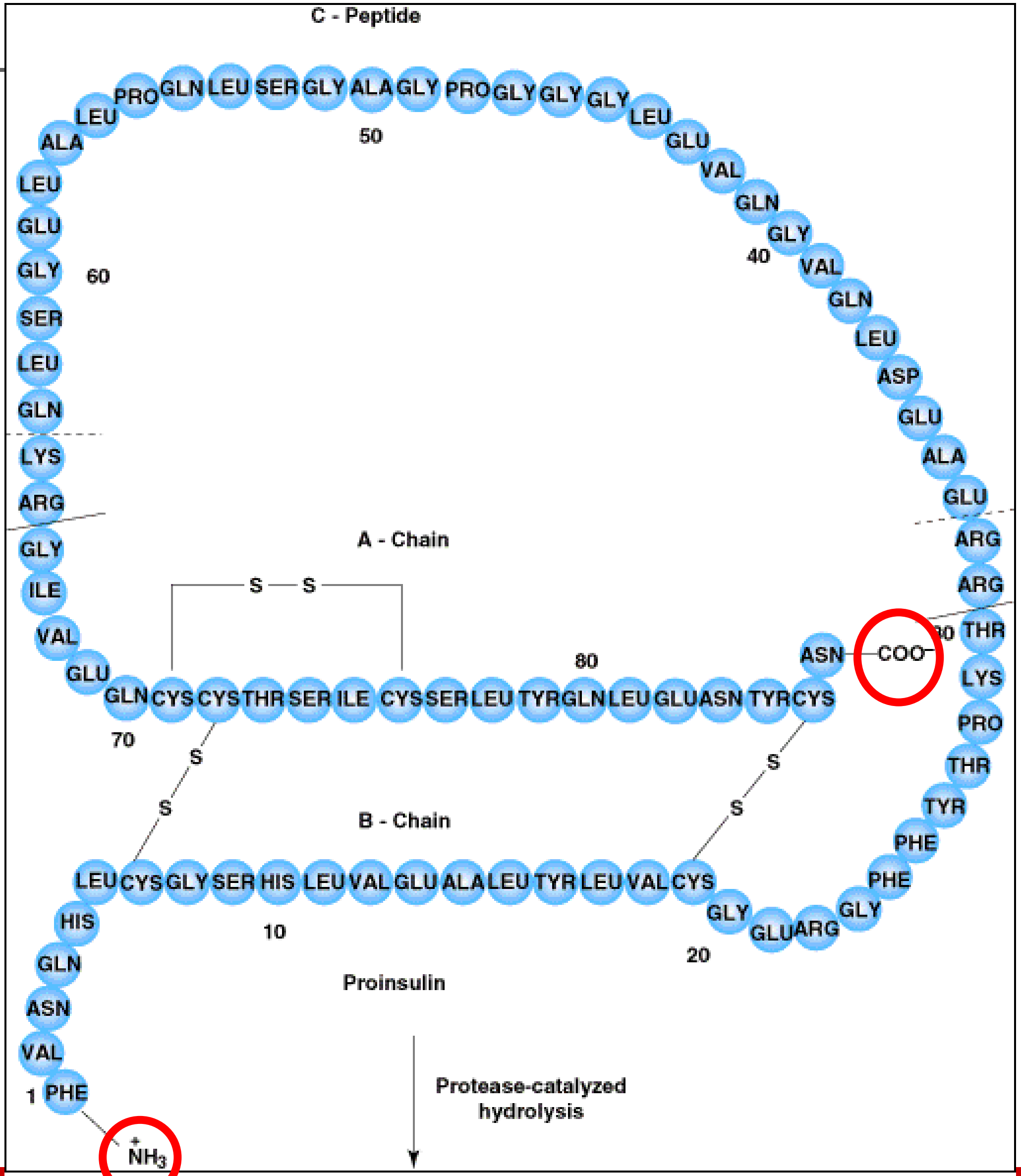
- **Protein structures are not frozen and undergo changes that can be small or very big**
- **A typical protein usually has one or more stable three-dimensional structures, or conformations, that reflect its function.**
- **Some proteins have functional domains that are unstructured, or intrinsically disordered**



Disordered proteins (or regions) can fold into ordered structures when encountering and binding to their target

PRIMARY STRUCTURE

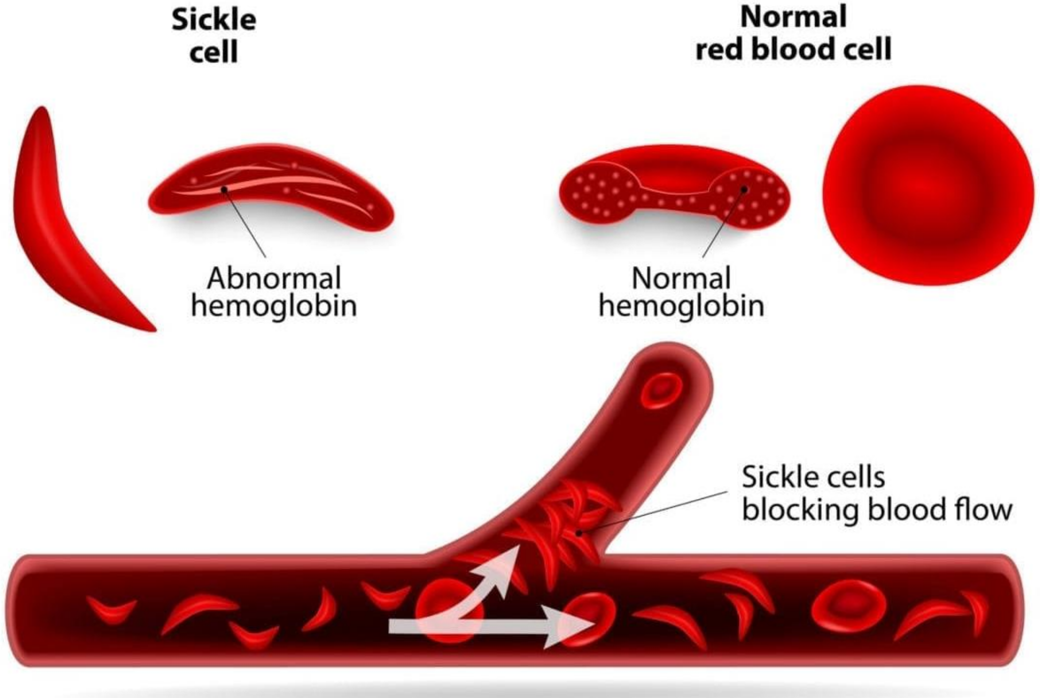
- The amino acid sequence of proteins are determined by DNA.
- Variation of even a single amino acid in the primary structure has consequences that vary between negligible effects or total loss of activity, depending on the protein and the amino acid replaced.
- A mutant form of hemoglobin with only one amino acid mutation causes sickle cell anemia.



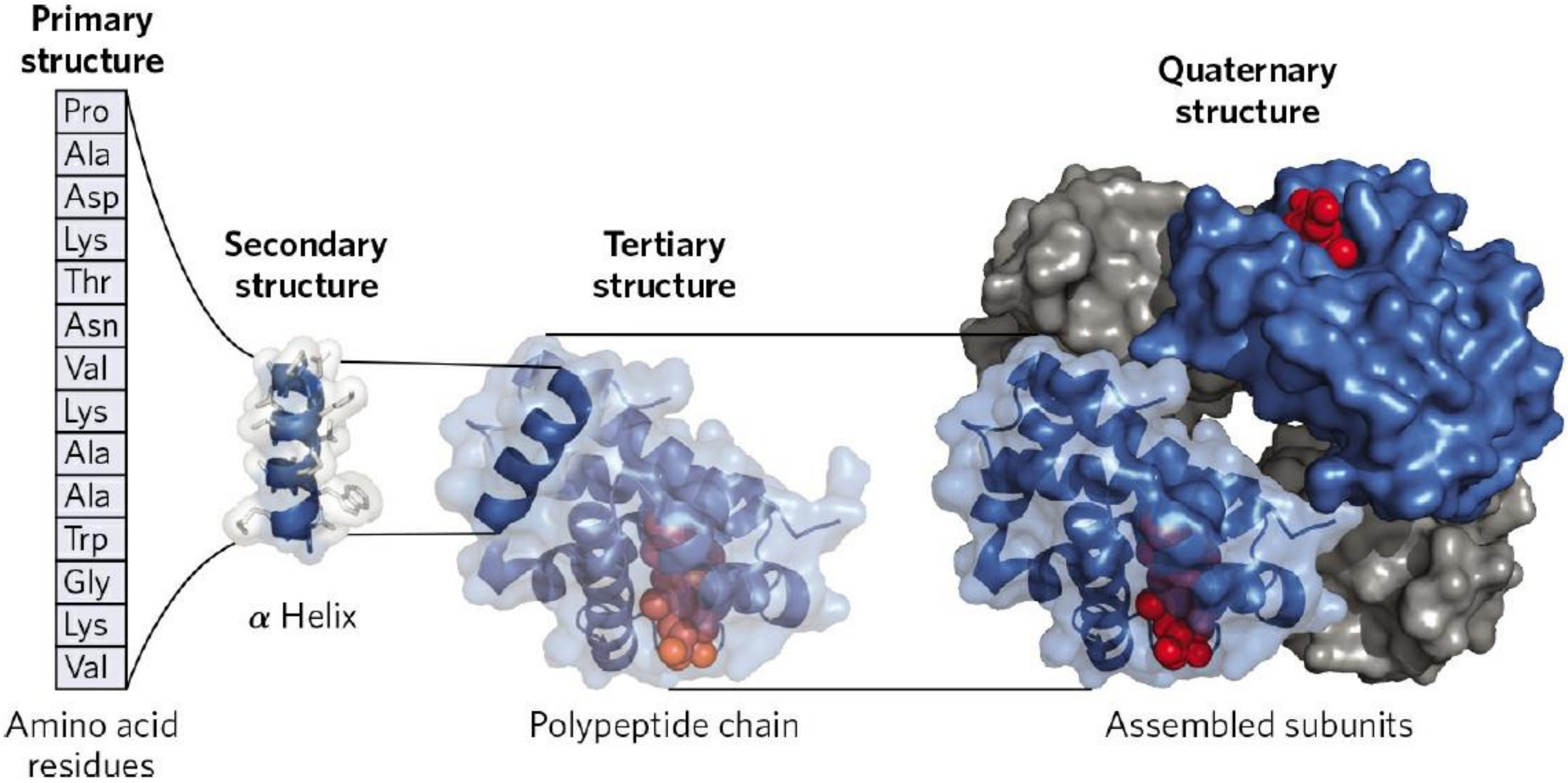
SICKLE CELL ANEMIA

- A mutant form of hemoglobin (called HbS) with only one amino acid mutation causes deoxyHbS molecules to associate abnormally with each other, forming the long, fibrous aggregates characteristic of this disorder.

- The altered properties of HbS result from a single amino acid substitution, a Val (non polar aliphatic aa) instead of a Glu (polar charged aa) residue at position 6 in the two β chains.

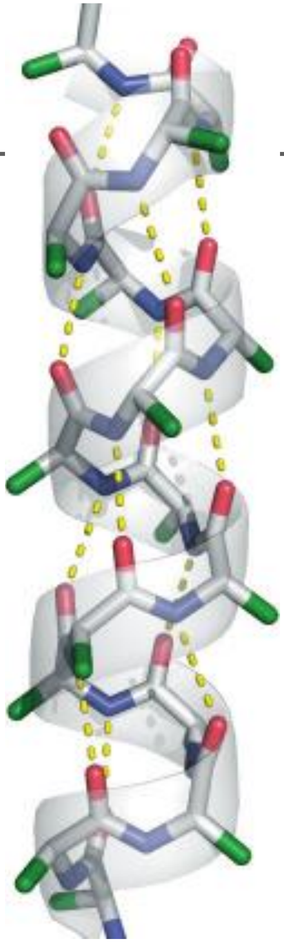


When HbS is deoxygenated, it becomes insoluble and forms polymers that aggregate into tubular fibers. Normal hemoglobin (hemoglobin A, or HbA) remains soluble when deoxygenated. The insoluble fibers of deoxygenated HbS cause the deformed, sickle shape of the erythrocytes; capillaries become blocked by the long, abnormally shaped cells, causing severe pain and interfering with normal organ function—a major factor in the early death of many people with the disease.


Primary structure
Pro
Ala
Asp
Lys
Thr
Asn
Val
Lys
Ala
Ala
Trp
Gly
Lys
Val
Amino acid residues
Secondary structure
α Helix
Tertiary structure
Polypeptide chain
Quaternary structure
Assembled subunits

Secondary structure

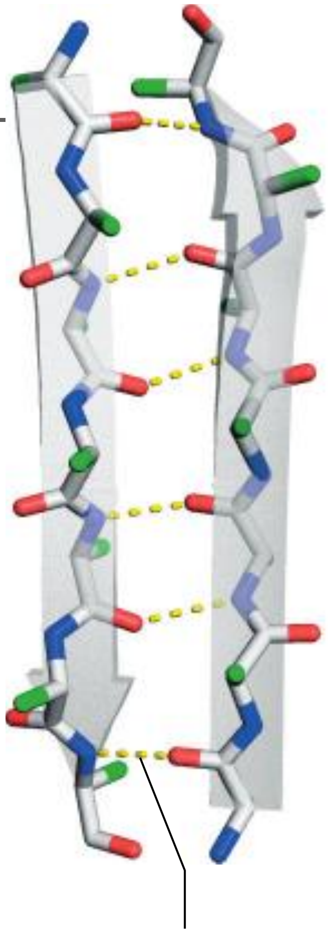
- The term *secondary structure* refers to any chosen segment of a **polypeptide chain** and describes the local spatial arrangement of its main-chain atoms, **without regard to the positioning of its side chains or its relationship to other segments.**
- It depends by **hydrogen bonds formation** between the oxygen of a carboxyl group involved in the peptide bond and the hydrogen of the amine group involved in another peptide bond.
- Secondary structures are regular elements such as ***α-helices*** and ***β-pleated sheets*** (another common type is the ***β turn***), which are formed between relatively small parts of the protein sequence.
- Where a regular pattern is not found, the secondary structure is sometimes referred to as a **random coil**.



N-terminus

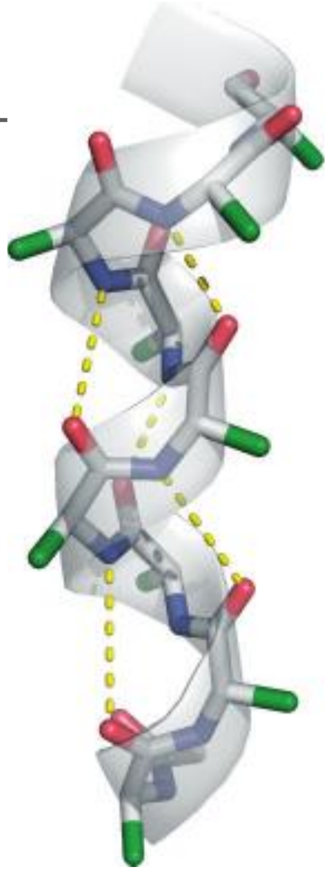
C-terminus

α-HELICES



hydrogen bond

β-PLATED SHEETS



β-TURN

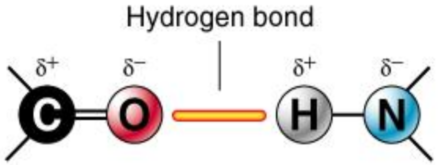
α-HELICES

(L. Pauling e R. Corey, 1951)

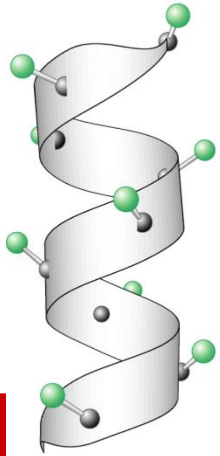
On average, 32-38% of the amino acids in a protein are wrapped in α-helix.

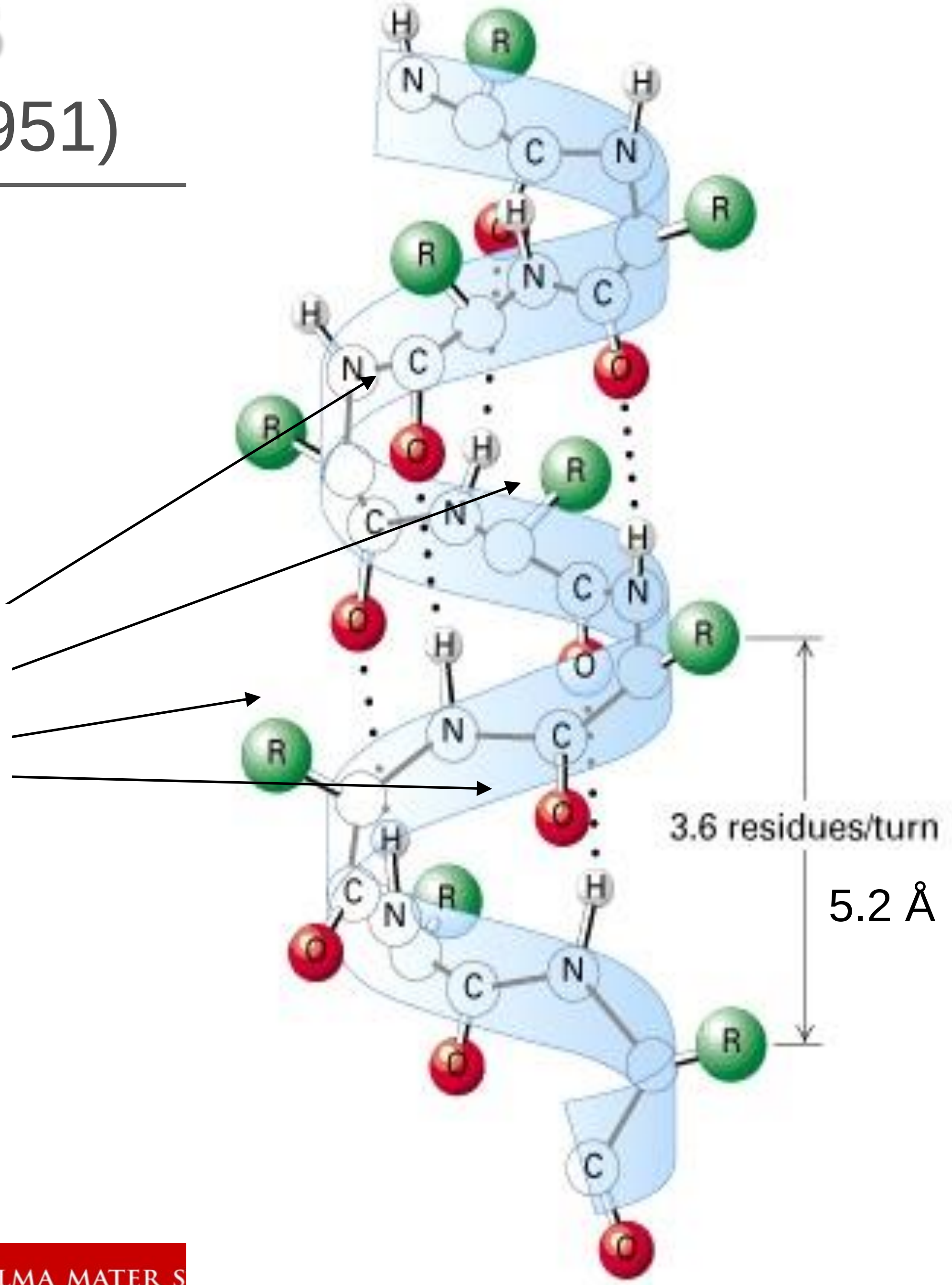
Nobel Award for Chemistry, 1954

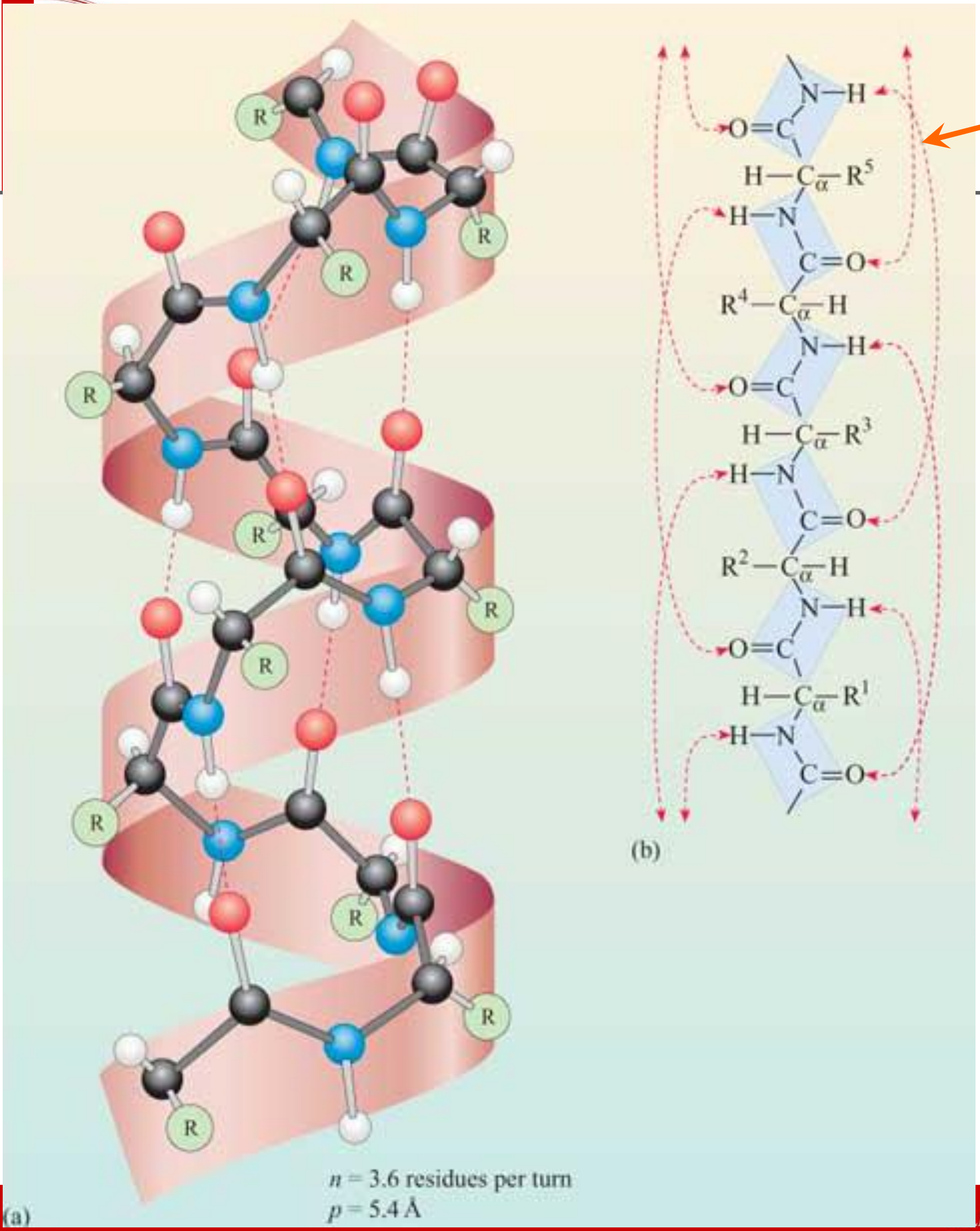
1901-1994



Groups R pointing outwards



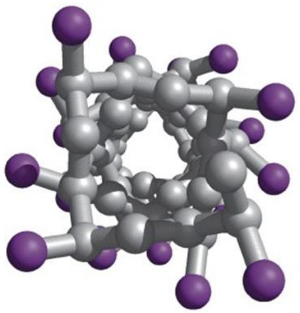




Hydrogen bond

α-helices

Each carbonyl oxygen forms an hydrogen bond with an N-H hydrogen on the next turn of the coil
(every 3.6 aa residues)

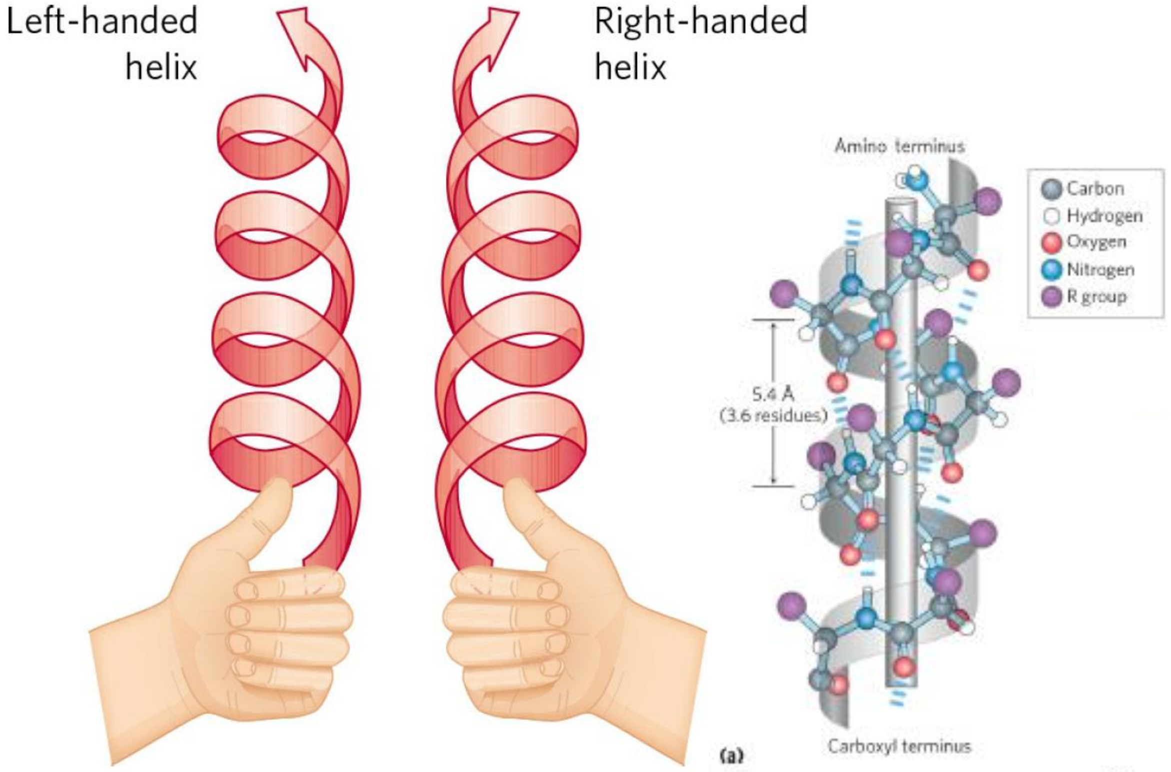


https://www.youtube.com/watch?v=PeFdl6KmxYM&t=9s

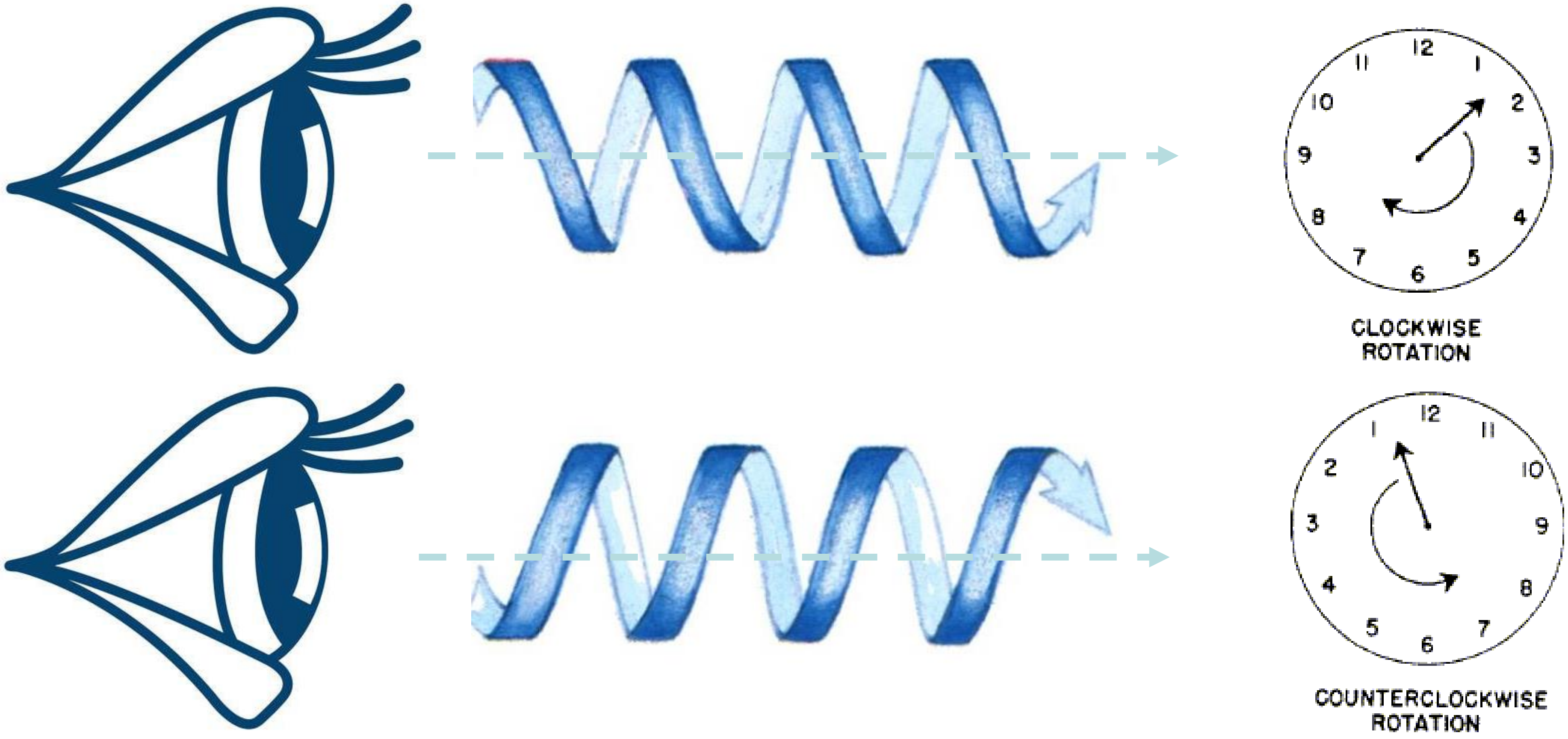
α-helical structure is right-handed or left-handed?

Make fists of your two hands with thumbs outstretched and pointing away from you. Looking at your right hand, think of a helix spiraling up your right thumb in the direction in which the other four fingers are curled as shown (clockwise). The resulting helix is right-handed. Your left hand will demonstrate a left-handed helix, which rotates in the counterclockwise direction as it spirals up your thumb.

- **Only extended right-handed α-helices are found in proteins.**
- **Extended left-handed helices are rare in proteins.**
- **31 verified left-handed helices in a set of 7284 proteins.**



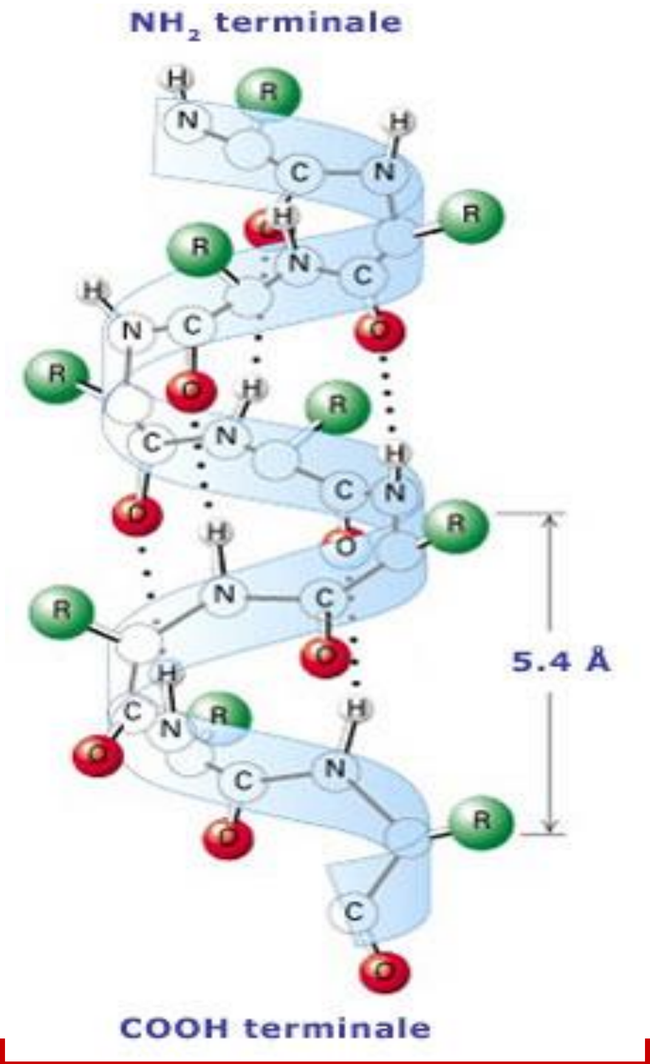
Right-handed



Left-handed

https://www.youtube.com/watch?v=eUS6CEn4GSA

α-Helix



- C=O and N-H functional groups are engaged in hydrogen bonds; C=O from an amino acid binds N-H of an amino acid 4 residues further on.
- Not all polypeptides can form a stable *α* helix. Each amino acid residue in a polypeptide has an intrinsic propensity to form an *α* helix, reflecting the properties of the R group.
- One-fourth of all amino acids in proteins are found in α-helices (the exact fraction varies from one protein to another).

Amides

Structure and Physical Properties

Most amides are solids at room temperature. They have very high boiling points, and the simpler ones are quite soluble in water. Both of these properties are a result of strong intermolecular hydrogen bonding between the N-H bond of one amide and the C=O group of a second amide.

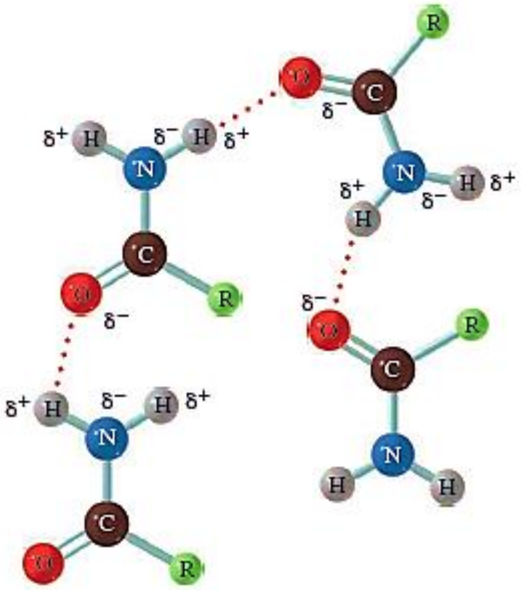
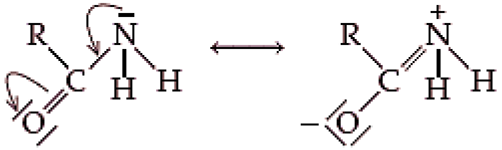


Figure 15.5 Hydrogen bonding in amides.

Unlike amines, amides are not bases (proton acceptors). The reason is that the highly electronegative oxygen atom of the carbonyl group causes a very strong attraction between the lone pair of nitrogen electrons and the carbonyl group. As a result, the unshared pair of electrons cannot “hold” a proton.

Because of the attraction of the carbonyl group for the lone pair of nitrogen electrons, the structure of the C-N bond of an amide is a *resonance hybrid.* In the structures below, lines are used to represent pairs of electrons.



Sequence Affects Helix Stability

- Small hydrophobic residues such as Ala and Leu facilitate α-helix formation
- Alanine shows the greatest tendency to form α helices in most experimental model systems.

- Proline acts as α helix breaker because the rotation around the N-C_{α} bond is impossible.
- Gly acts as a helix breaker because the tiny R-group supports other conformations (Gly better support left-handed α-helices), because is too flexible.
- Stretches of aa with the same charge (e.g. Glu-Glu,.. Or Lys-Lys..) prevent helix formation due to repulsive forces.
- The bulkiness of adjacent R groups.

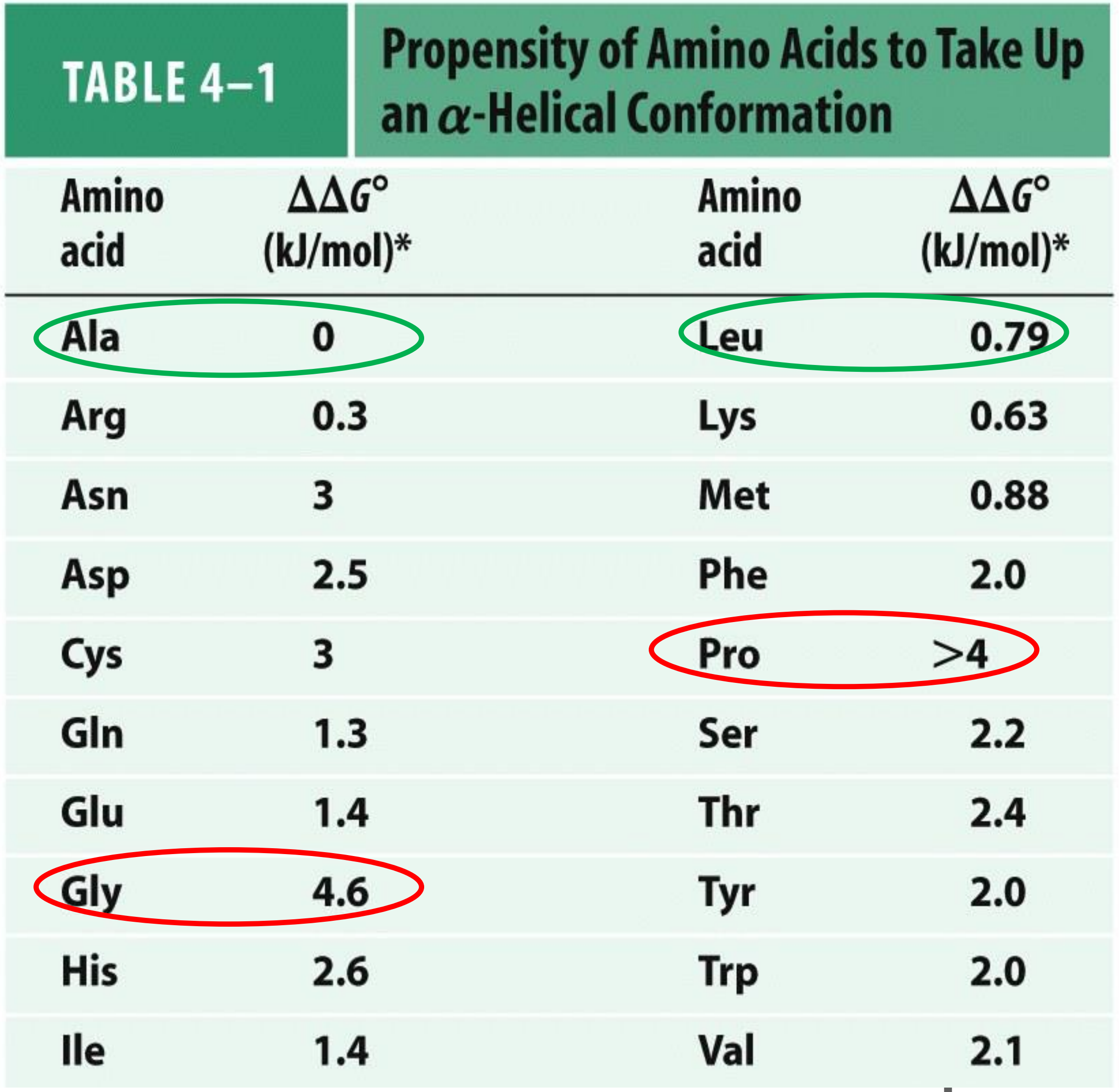
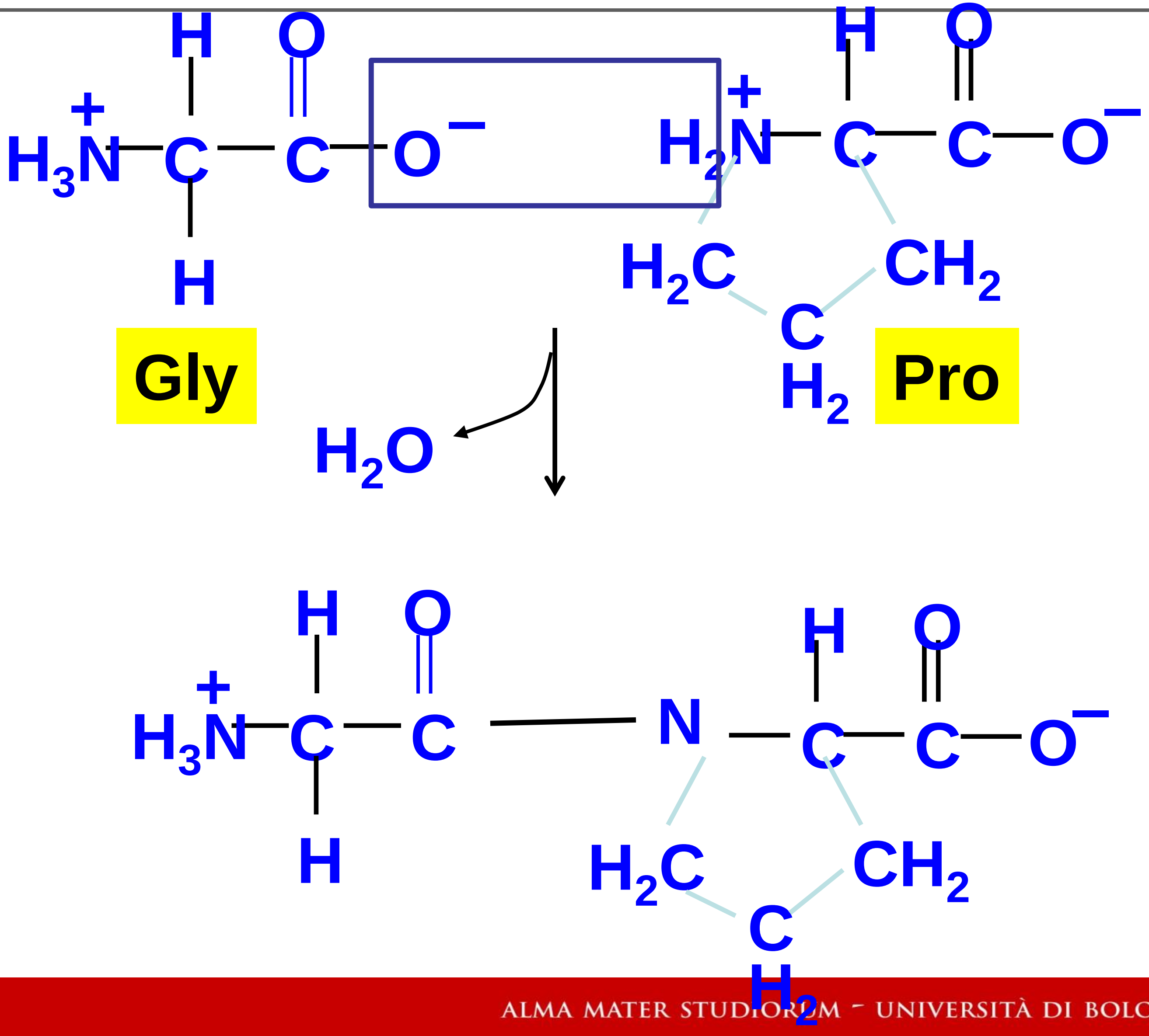
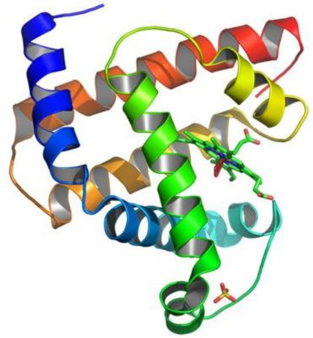


TABLE 4–1 Propensity of Amino Acids to Take Up an α-Helical Conformation

Amino acid	ΔΔG° (kJ/mol)*	Amino acid	ΔΔG° (kJ/mol)*
Ala	0	Leu	0.79
Arg	0.3	Lys	0.63
Asn	3	Met	0.88
Asp	2.5	Phe	2.0
Cys	3	Pro	>4
Gln	1.3	Ser	2.2
Glu	1.4	Thr	2.4
Gly	4.6	Tyr	2.0
His	2.6	Trp	2.0
Ile	1.4	Val	2.1

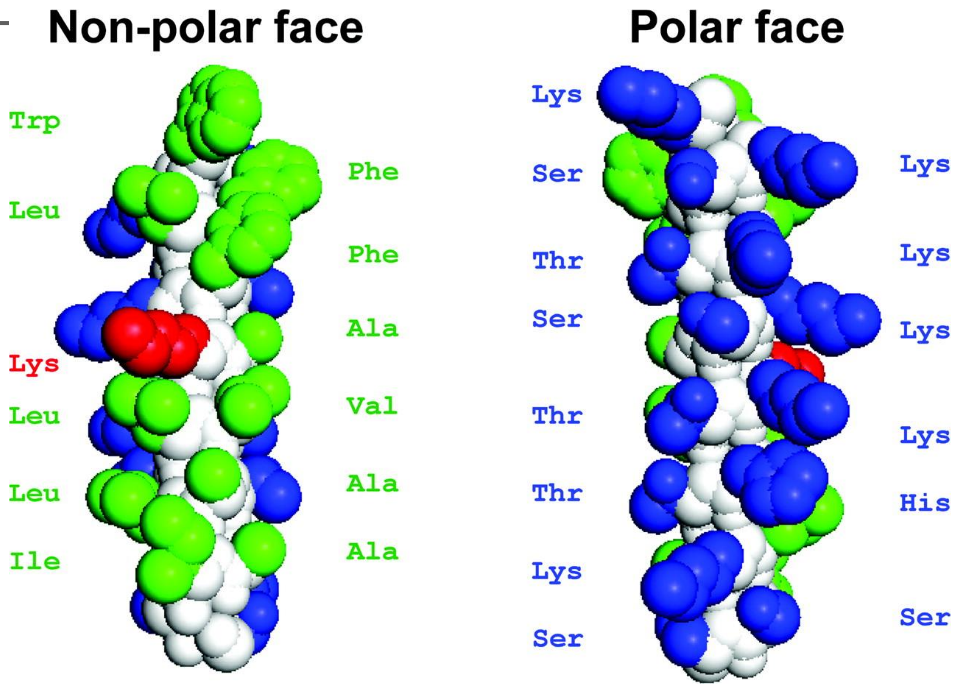
Pro acts as a helix breaker because the peptide group can not form hydrogen bonds

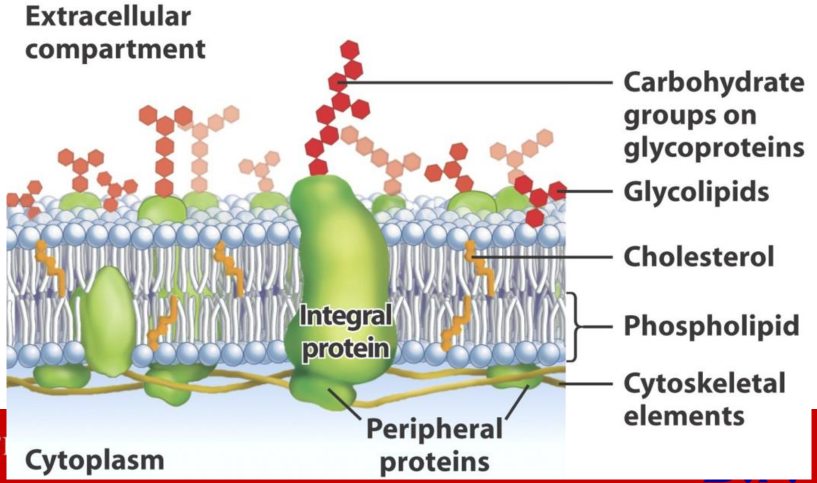




α-HELICES

- The content of α-helices varies greatly from protein to protein, from 0 to 100% (on average 32-38%)
- Generally, 25% of soluble proteins have α-helices (hydrophilic R groups outside, globular proteins)
- Even the proteins completely immersed in the biological membranes have α-helices which cross several times the double phospholipid layer.
- These proteins contain mostly nonpolar amino acids, the R-groups of which point outside the α-helix.
- This structural organization allows the protein to be placed in a totally hydrophobic environment.





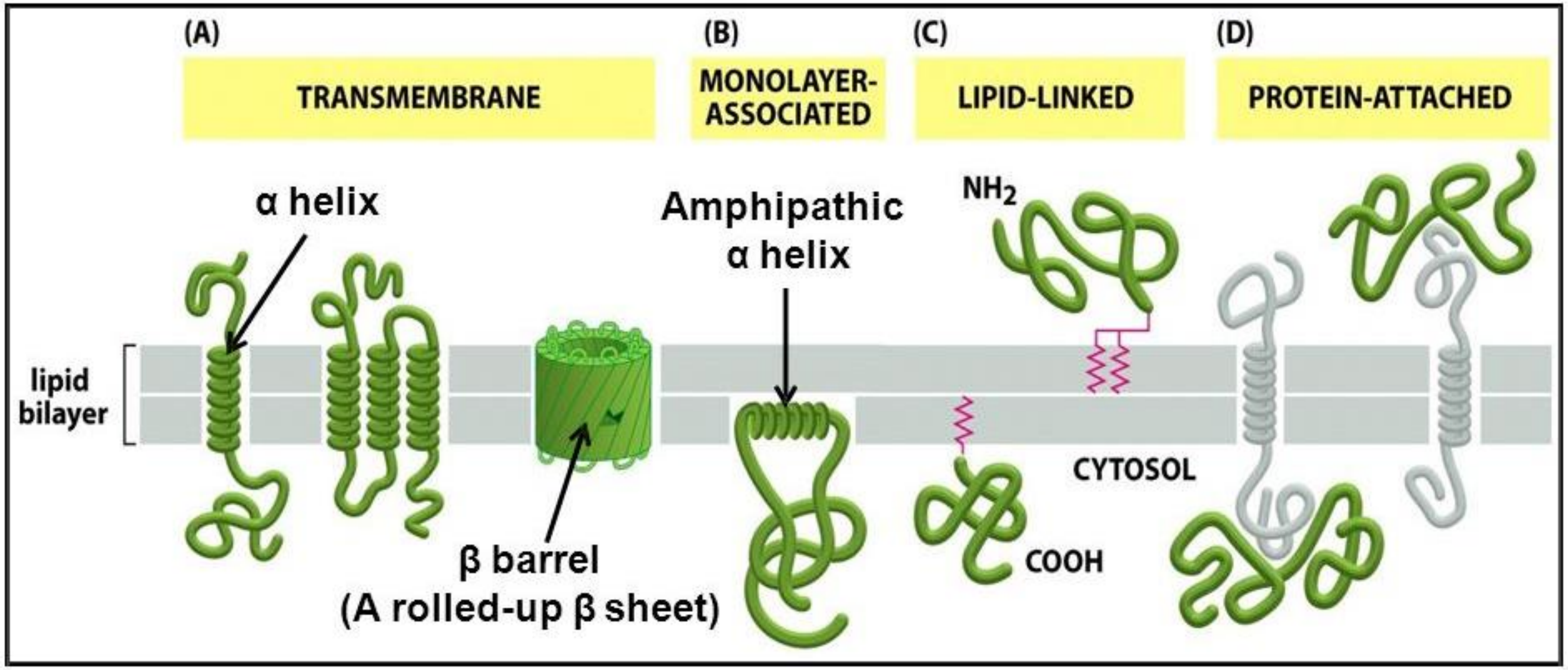
Membrane proteins associate with lipid bilayer in different ways

(A) Transmembrane proteins can extend across the bilayer as a single α helix, as multiple α helices, or as a rolled-up β sheet (called a β barrel).

(B) Membrane proteins anchored to cytosolic surface by an amphipathic α helix.

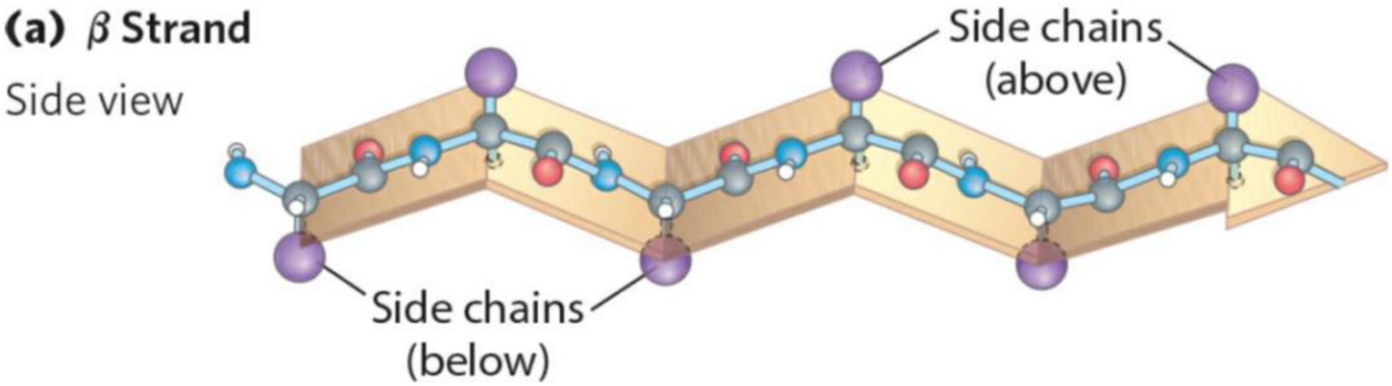
(C) Others are attached to either side of the bilayer solely by **a covalent attachment to a lipid molecule** (red zigzag lines).

(D) Finally, many proteins are attached to the membrane only by relatively weak, noncovalent interactions with other membrane proteins.



β (pleated) Sheets

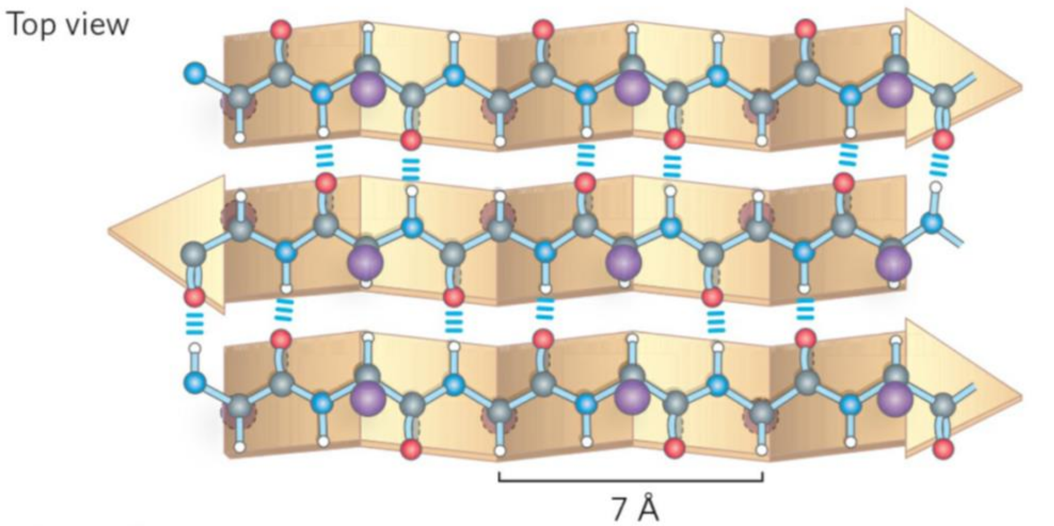
- The β-strand is the structural element of the β-sheet secondary structure.
- For most designed β-strands, the primary structure has an alternating pattern of hydrophobic and polar amino acids. This leads to an extended structure where the amino acid side-chains alternate between the two faces of the strand, forming a zigzag structure.
- The R groups of adjacent amino acids protrude from the zigzag structure in opposite directions, creating the alternating pattern.
- The individual segments (β-strand) that form a β sheet are usually nearby on the polypeptide chain but can also be quite distant from each other; they may even be in different polypeptide chains.



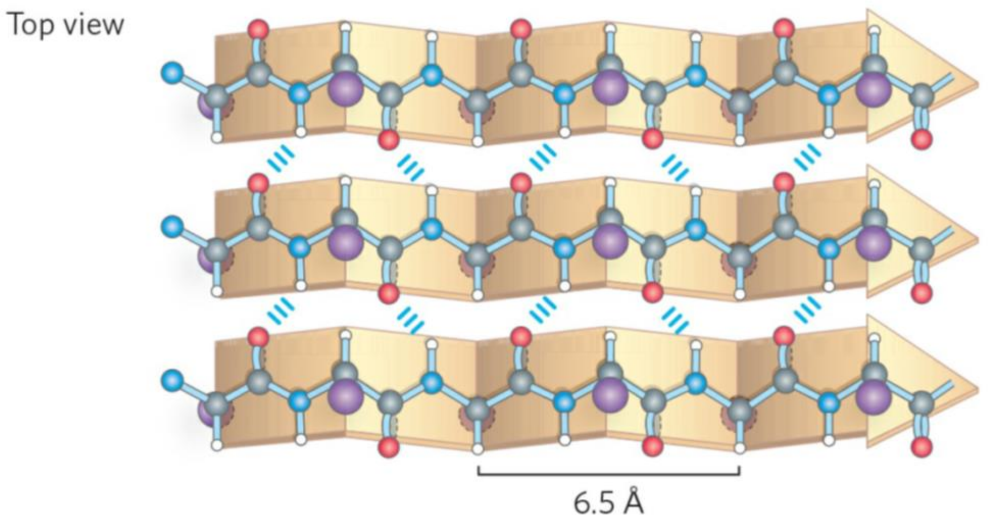
β (pleated) Sheets

- In a β sheet conformation, the polypeptide chain has hydrogen bonds as in α-helices, but between amino acids located quite far along the chain.
- It can be either parallel or antiparallel (having the same or opposite amino-to-carboxyl orientations, respectively).
- The inter-strand hydrogen bonds are essentially in-line in the antiparallel β sheet, whereas they are distorted or not in line for the parallel variant.
- β-sheets come in two varieties: parallel and antiparallel.

(b) Antiparallel β sheet

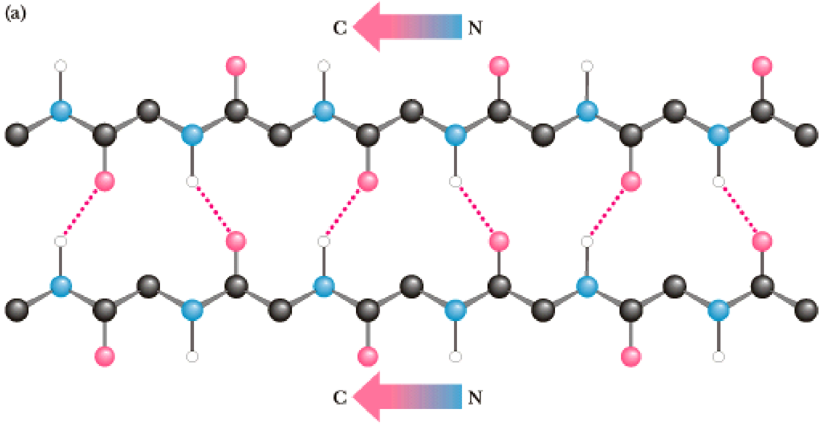


(c) Parallel β sheet

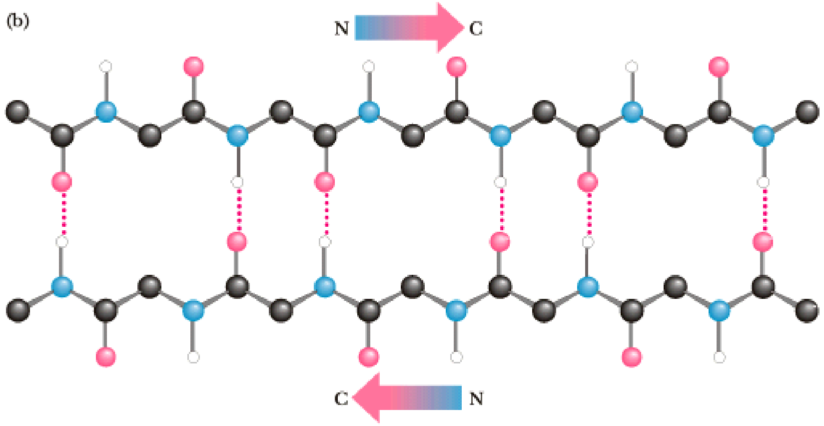


β Sheet: 2 Orientations

Parallel ; Not optimum H-bonds; less stable



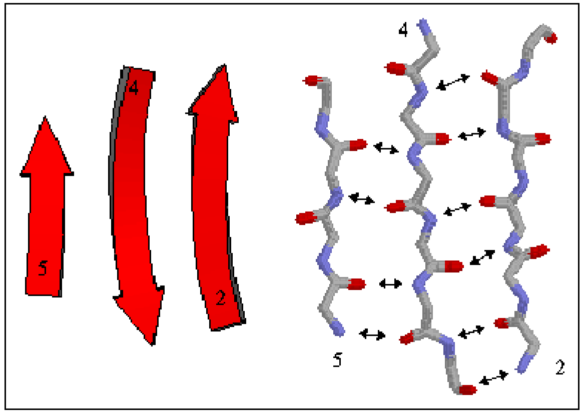
Anti-parallel; Optimum H-bonds; more stable

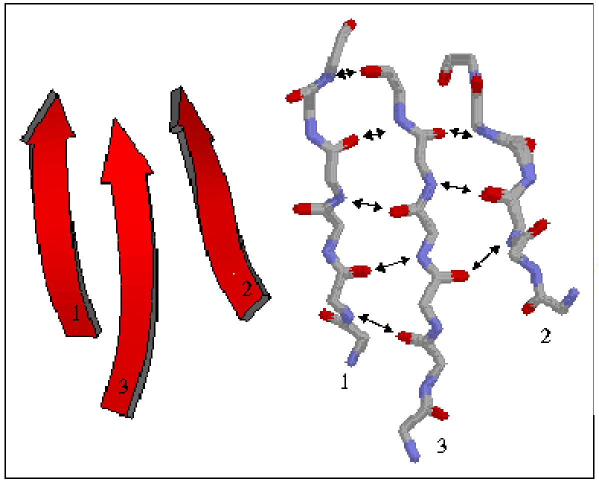


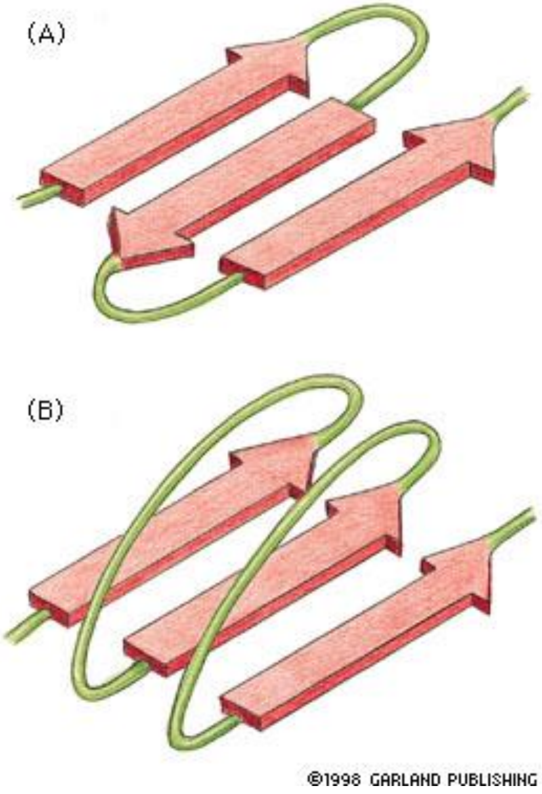
https://www.youtube.com/watch?v=5_MJkj1mG5M

https://www.youtube.com/watch?v=jT1XvChhJ8Y

β sheet-forming sequences can be close or far from one another, or even in different polypeptide chains







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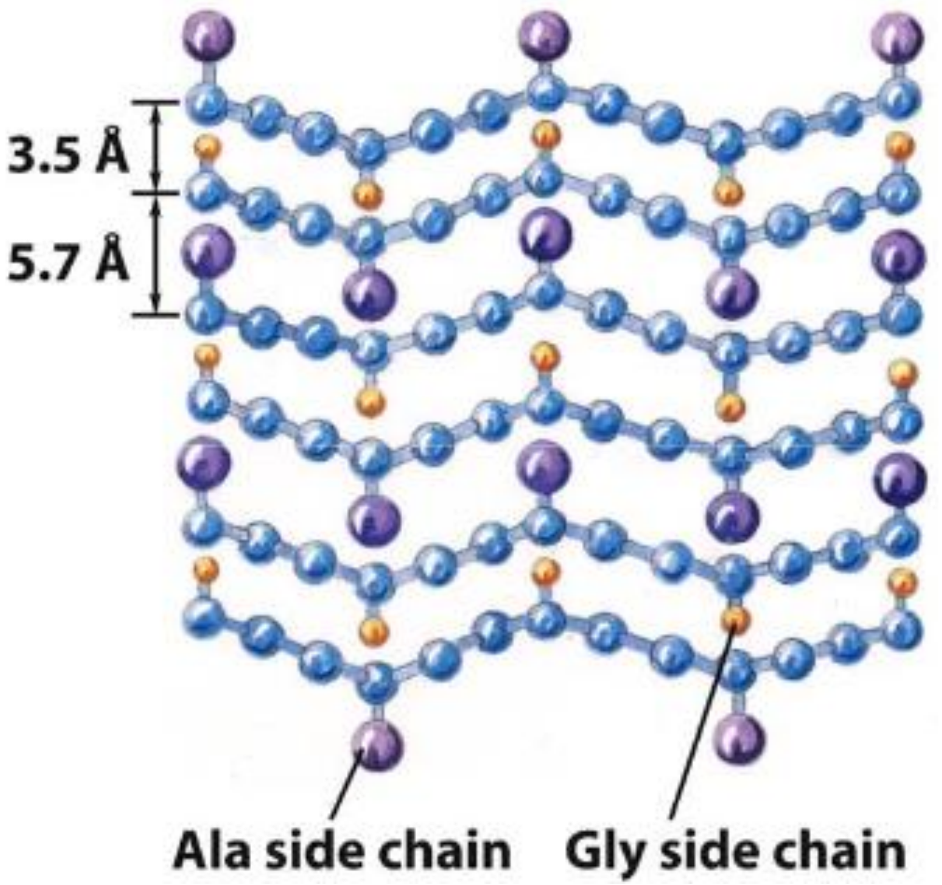
- Small R groups are preferred when β-sheets are layered together within a protein (Gly).

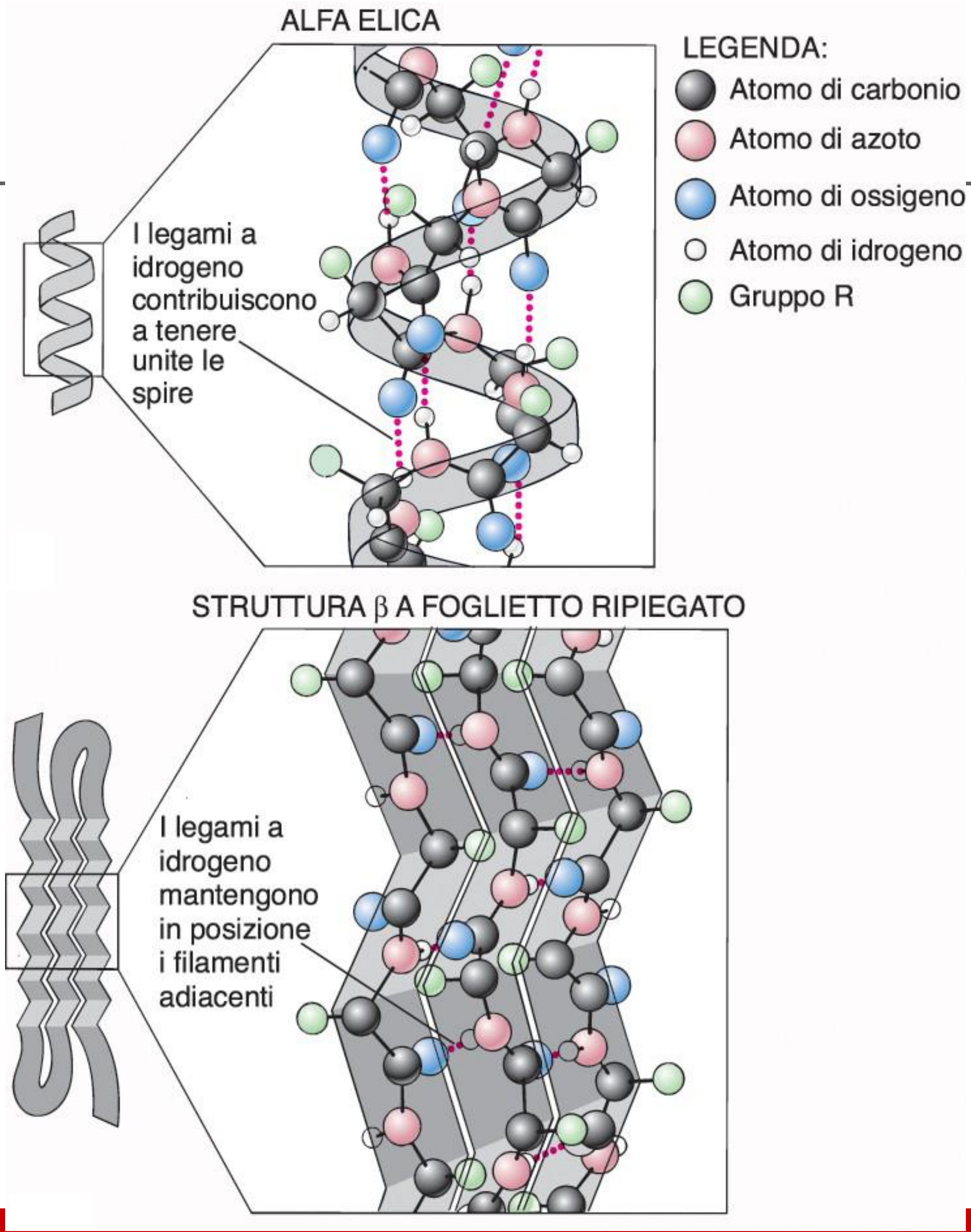
DESTABILIZING FACTORS

- Large dimensions of the R-units
- Repulsion or attraction between charged R-units

Fibroin of silk



- Fibroin has the highest β-sheet content, thanks to the presence of Gly - enriched regions.
- Multiple β-plated sheets provide toughness and rigidity to the silk.
- However, not all of the protein exists in neatly arranged β-sheets.

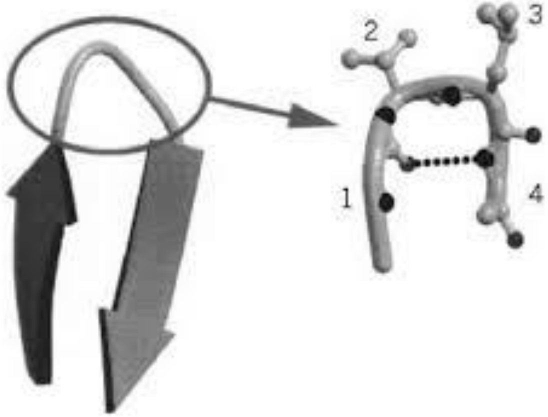

ALFA ELICA
LEGENDA:
● Atomo di carbonio
● Atomo di azoto
● Atomo di ossigeno
○ Atomo di idrogeno
● Gruppo R
I legami a idrogeno contribuiscono a tenere unite le spire
STRUTTURA β A FOGLIETTO RIPIEGATO
I legami a idrogeno mantengono in posizione i filamenti adiacenti

β Turns

Regular secondary structure with repetitive element:

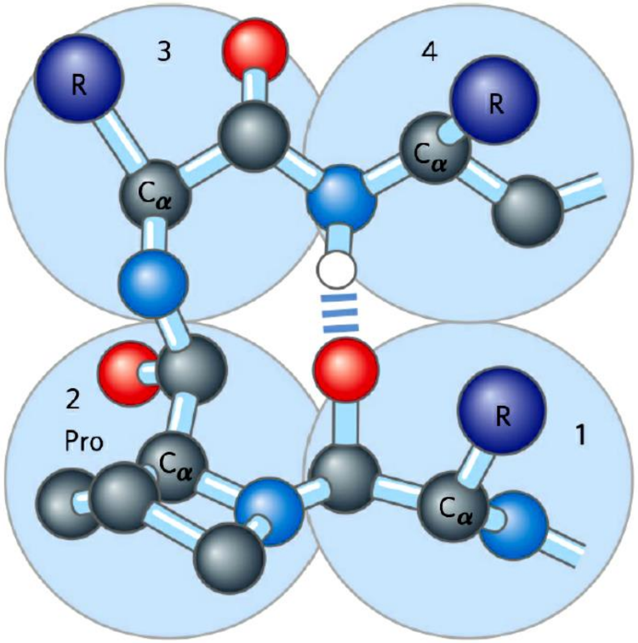
α-helice ; β sheet ; β turn

In globular proteins, which have a compact folded structure, some amino acid residues are in turns or loops where the polypeptide chain reverses direction. These are the connecting elements that link successive runs of α helices or β sheets. Particularly common are β turns that connect the ends of two adjacent strands of an antiparallel β sheet.

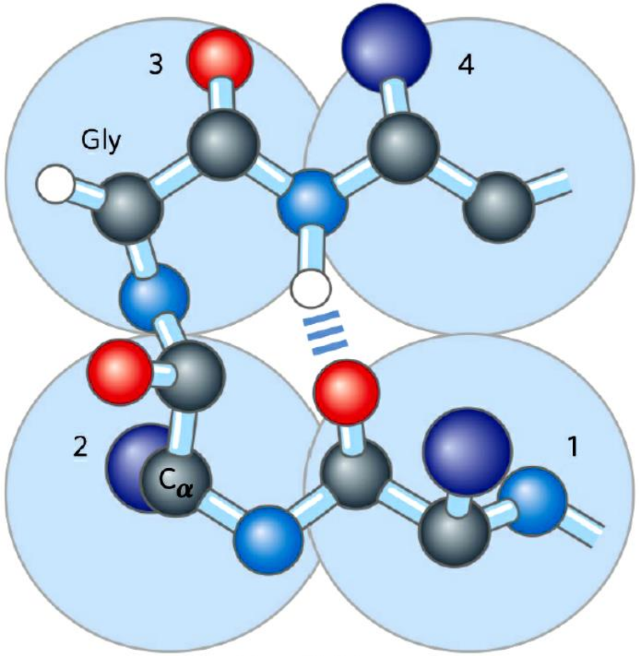


β Turns

- The structure is a 180° turn involving four amino acid residues, with the carbonyl oxygen linked through a hydrogen bond with the fourth hydrogen in amino group.
- The peptide bonds of the central two residues do not participate in any inter-residue hydrogen bonding.
- **Gly and Pro** often occur in β turns, the former because it is small and flexible, the latter because peptide bonds involving the rigid cyclic imino nitrogen of proline is particularly amenable to a tight turn.
- β- turns are often found near the surface of a protein, where the peptide groups of the central two amino acid residues in the turn can form a hydrogen-bond with water.



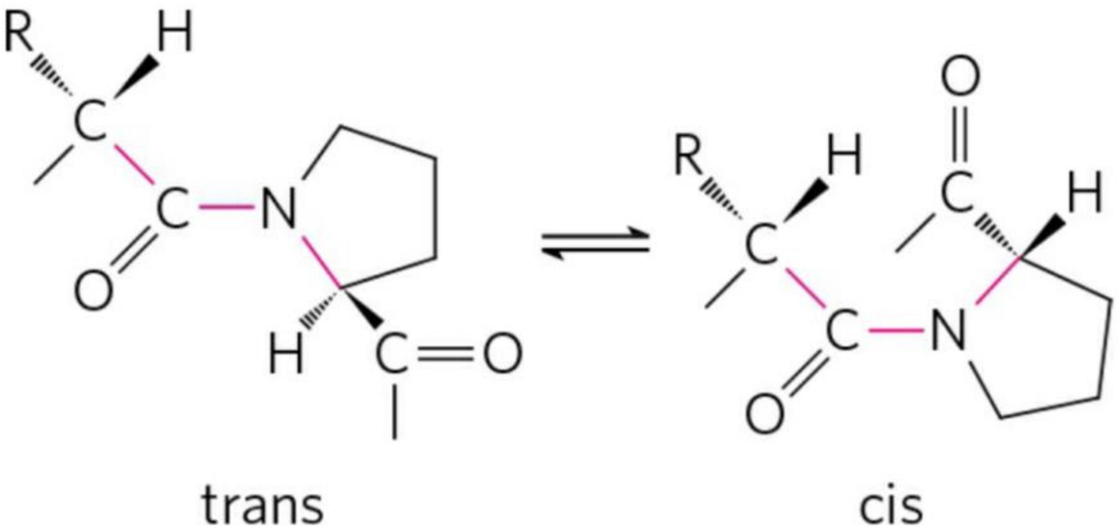
Type I β turn



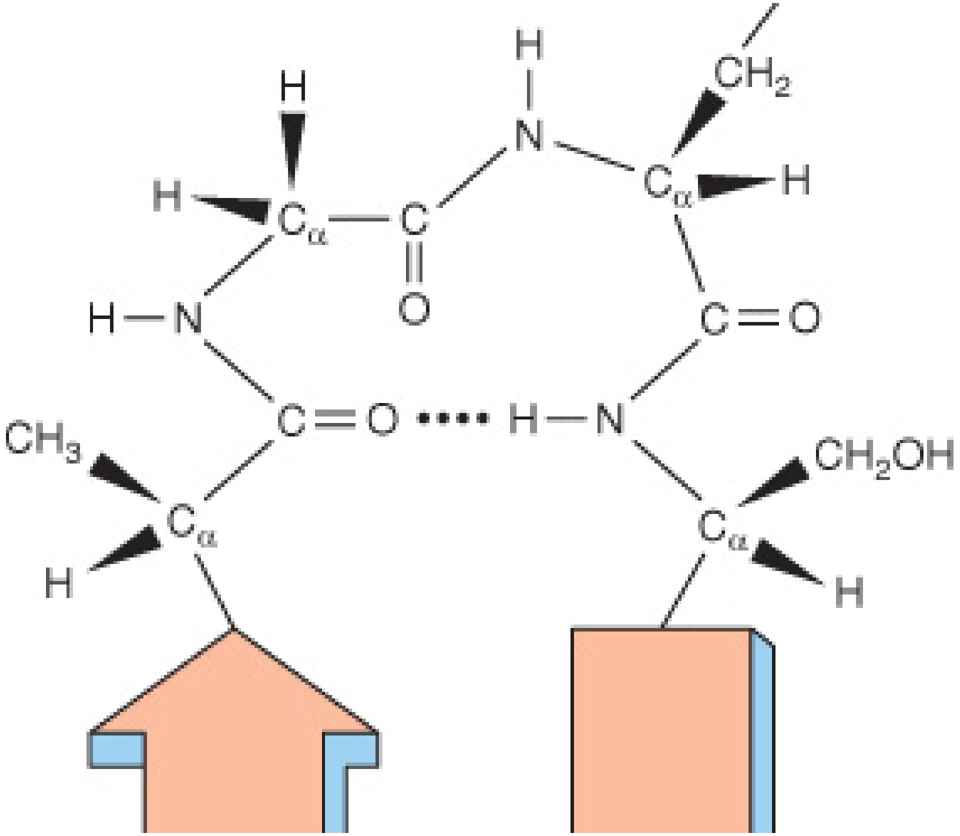
Type II β turn

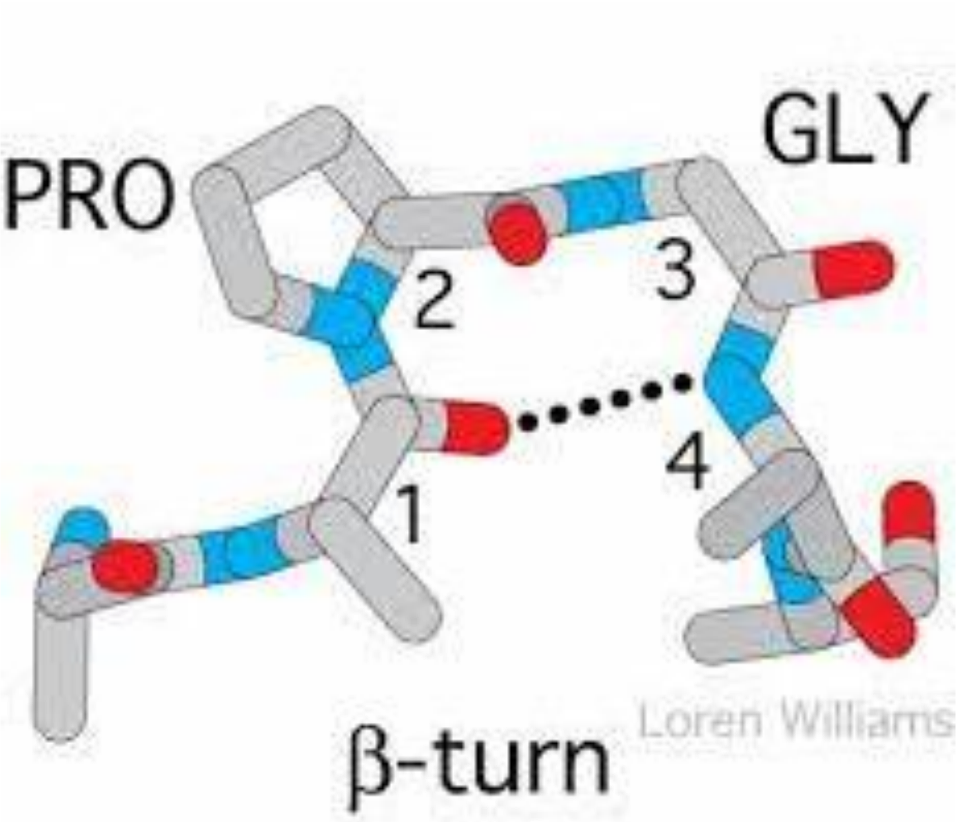
Trans and cis isomers of a peptide bond involving the imino nitrogen of proline

- Of the peptide bonds between aminoacid residues, more than 90% **are in the trans configuration**.
- For peptide bonds involving the imino nitrogen of proline, however, about 6% are in the cis configuration; many of these occur at β turns.

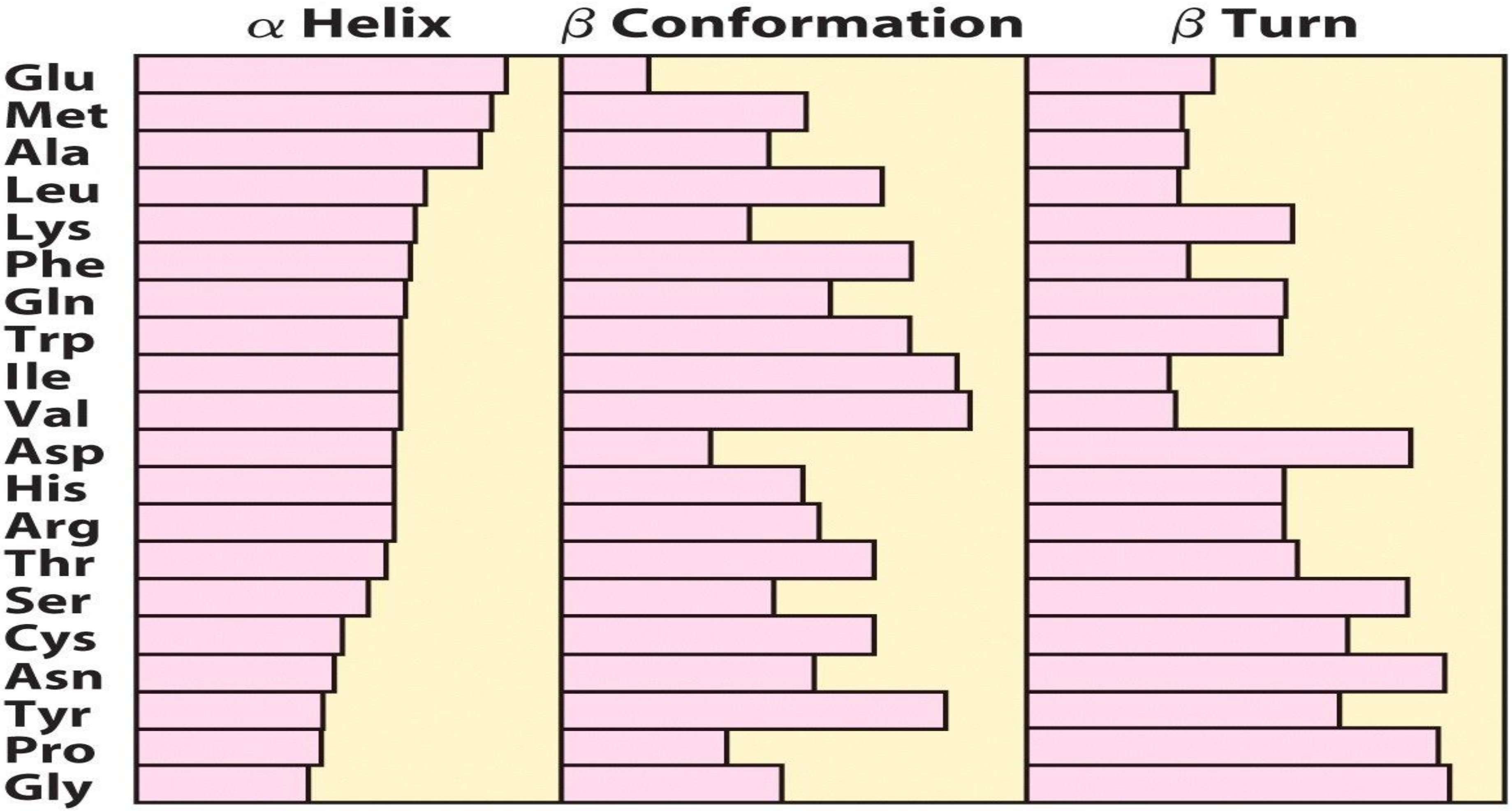


Proline isomers


H
H
H
Cα
C
O
N
H
CH2
Cα
H
H—N
C=O
C=O ···· H—N
CH3
Cα
H
CH2OH
Cα
H

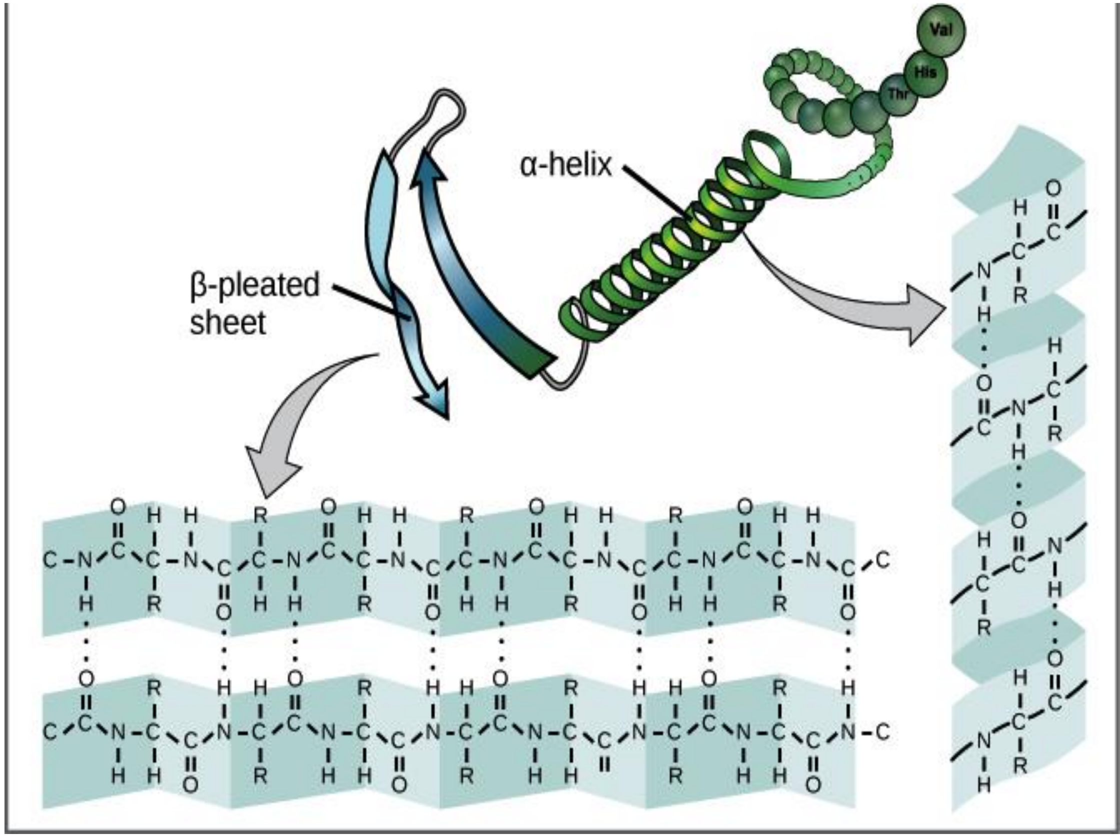

PRO
GLY
1
2
3
4
β-turn
Loren Williams

Secondary Structure of Proteins

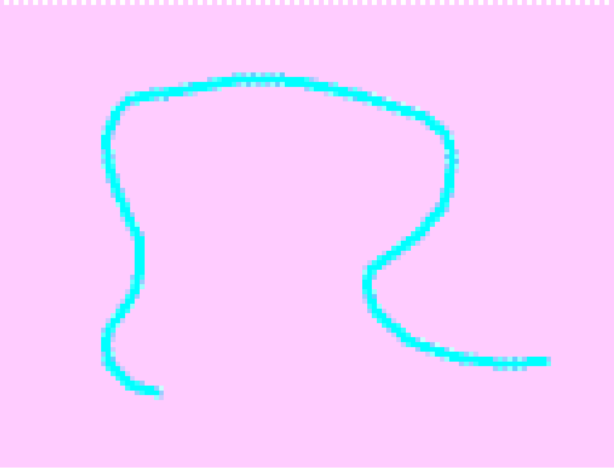


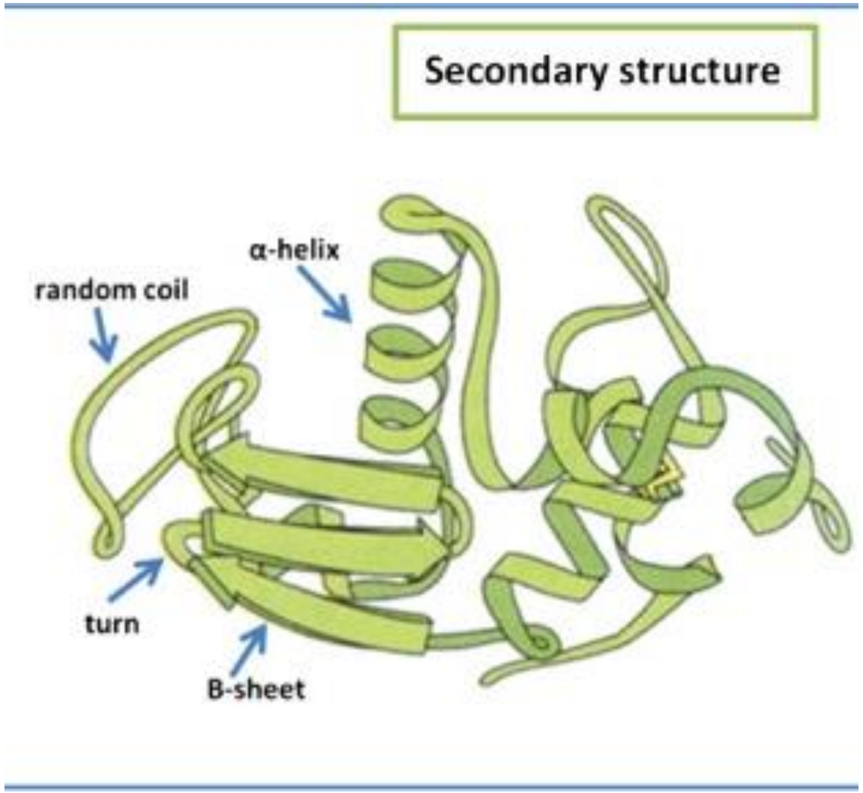
3D secondary structure of some proteins

The individual filaments of β-sheets and α-helices can be connected with β-turns

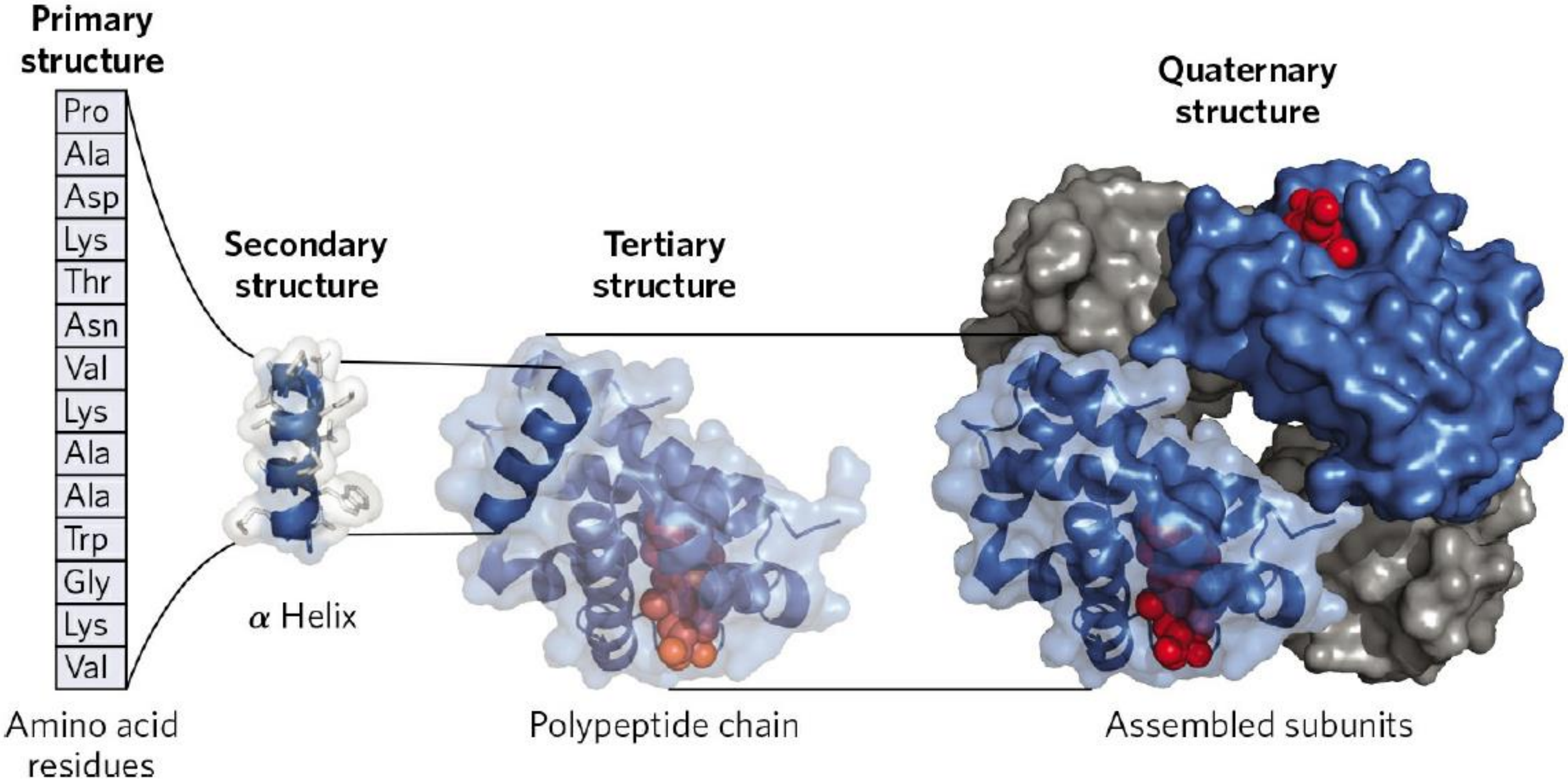


Random coil

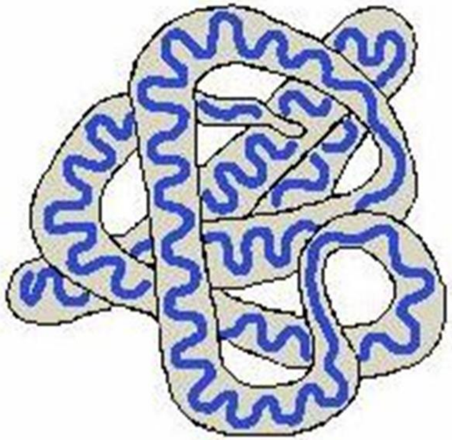




- Non-repetitive secondary structure (4-20 aa).
- Not hydrogen bonding pattern
- Terminal arms - both at the N-terminus and the C-terminus of the protein;
- Loops - Loops are unstructured regions found between regular secondary structure elements.


Primary structure
Pro
Ala
Asp
Lys
Thr
Asn
Val
Lys
Ala
Ala
Trp
Gly
Lys
Val
Amino acid residues
Secondary structure
α Helix
Tertiary structure
Polypeptide chain
Quaternary structure
Assembled subunits

Tertiary structure
Part I



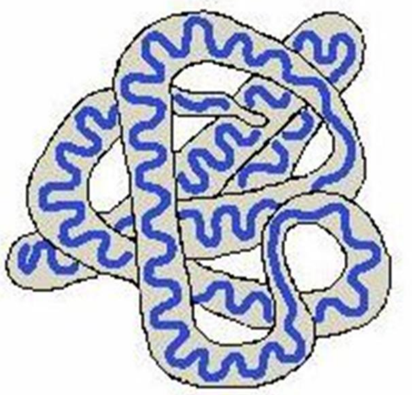
- Whereas the term “secondary structure” refers to the spatial arrangement of amino acid residues that are close (not adjacents) in a segment of a polypeptide, **tertiary structure includes *longer-range* aspects of amino acid sequence.**

- Amino acids that are far apart in the polypeptide sequence and are in different types of secondary structure may interact within the completely folded structure of a protein.

- The location of turns and loops (including *β* turns) in the polypeptide chain and the direction and angle of these bends are determined by the number and location of **specific bend-producing residues (such as Pro, Thr, Ser, Gly and Cys).**

- Interacting segments of polypeptide chains are held in their characteristic tertiary positions by several kinds of **weak interactions (and by disulfide bridges) between the segments.**

Tertiary structure
Part II



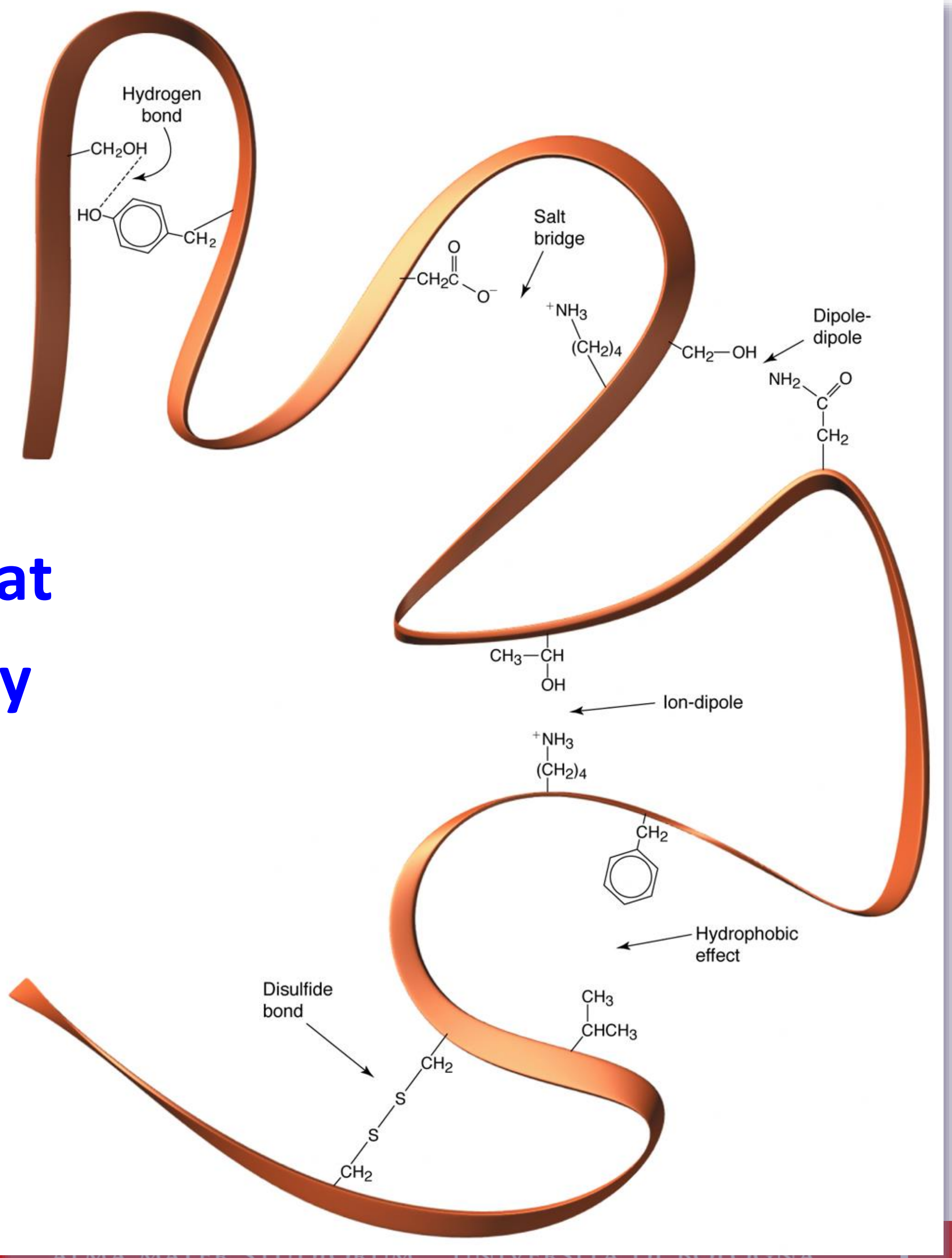
- ✓ The structure is stabilized by **electrostatic attractions** between oppositely charged ionic groups, by weak **van der Waals forces, by hydrogen bonding, hydrophobic interactions and, in some proteins, by disulphide (-S-S-) bridges** formed by the oxidation of spatially adjacent thiol groups (-SH) of cysteine residues.

- ✓ The three-dimensional folding of polypeptide chains is such that **the interior consists predominantly of non-polar, hydrophobic amino acid residues such as valine, leucine and phenylalanine. In this way they can stable stay in the aqueous environment.**

- ✓ The specific folding of a protein is only thermodynamically stable within a restricted range of environmental parameters (*i.e*. the right temperature, pH and ionic strength). Outside of this range, the protein could unfold and lose its activity.

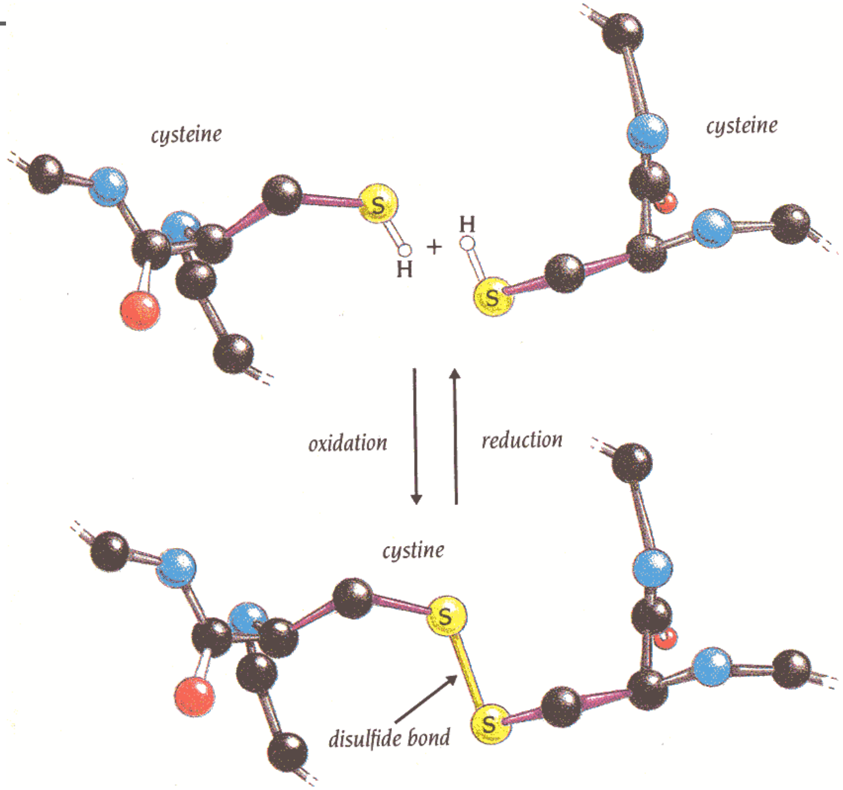
Tertiary Structure

The interactions that stabilize the tertiary structure



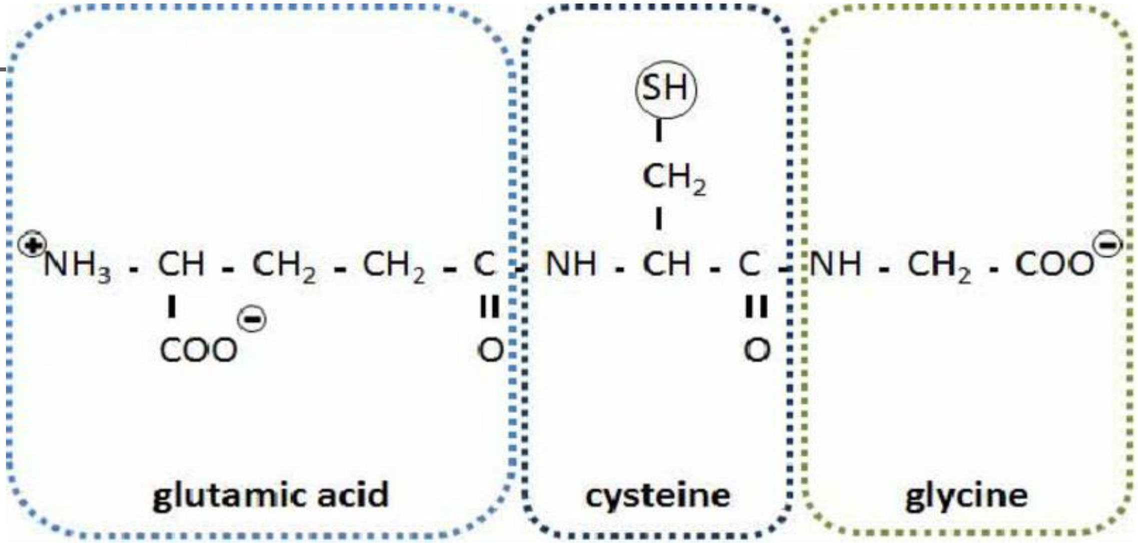
Disulfide Bonds

- **Two cysteines in close proximity can form a covalent bond (disulfide bridge)**
- **It significantly stabilizes the tertiary structure.**

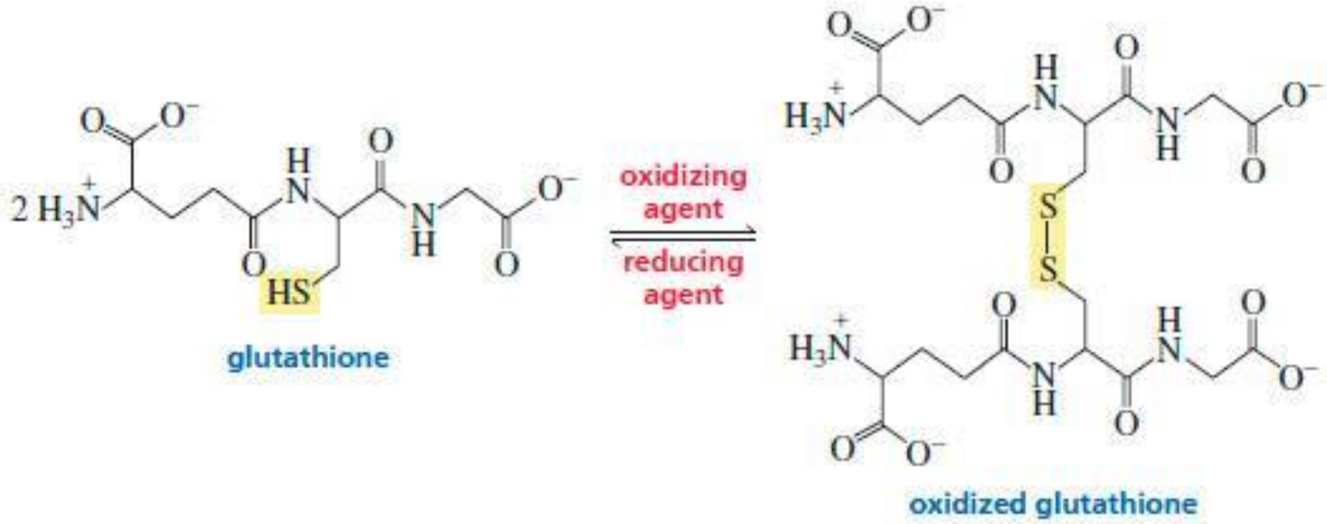


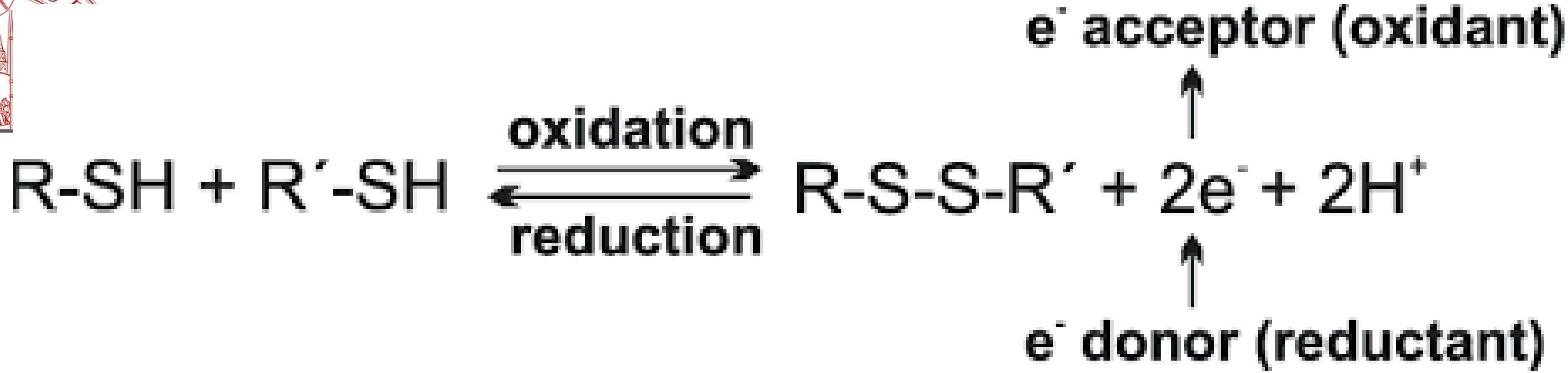
Many proteins do not have disulfide bridges. The environment within most cells is highly reducing due to high concentrations of reducing agents such as **glutathione, and most thiols will thus remain in the reduced state**. Outside the cell, the environment is often more oxidizing, and disulfide formation is more likely to occur. In eukaryotes, disulfide bridges are found primarily in excreted, extracellular proteins (for example, the hormone insulin).

GLUTATHIONE



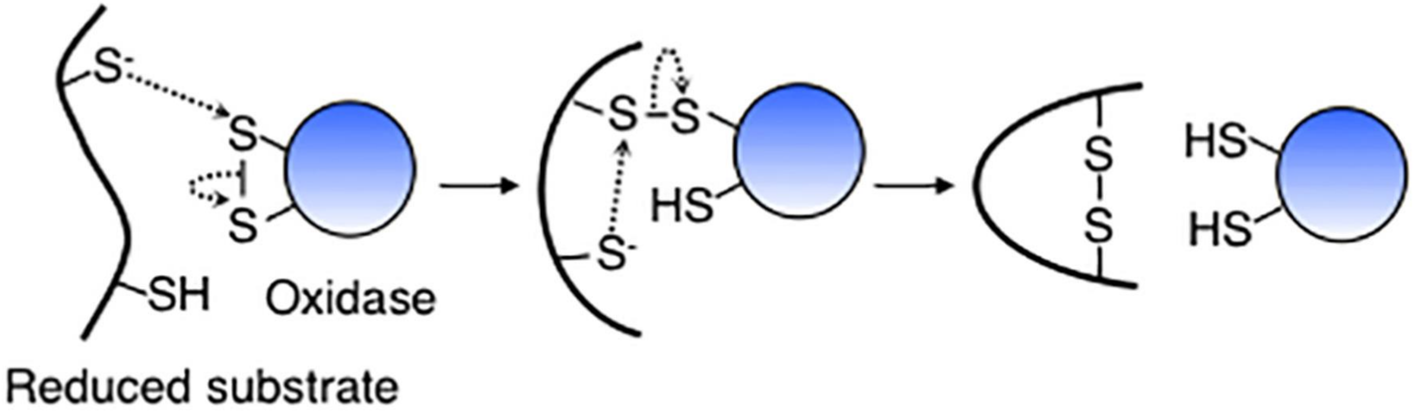
$$^{\oplus}NH_3 - \underset{\substack{| \\ COO^{\ominus}}}{CH} - CH_2 - CH_2 - \underset{\substack{\| \\ O}}{C} - NH - \overset{\substack{SH \\ | \\ CH_2 \\ |}}{CH} - \underset{\substack{\| \\ O}}{C} - NH - CH_2 - COO^{\ominus}$$



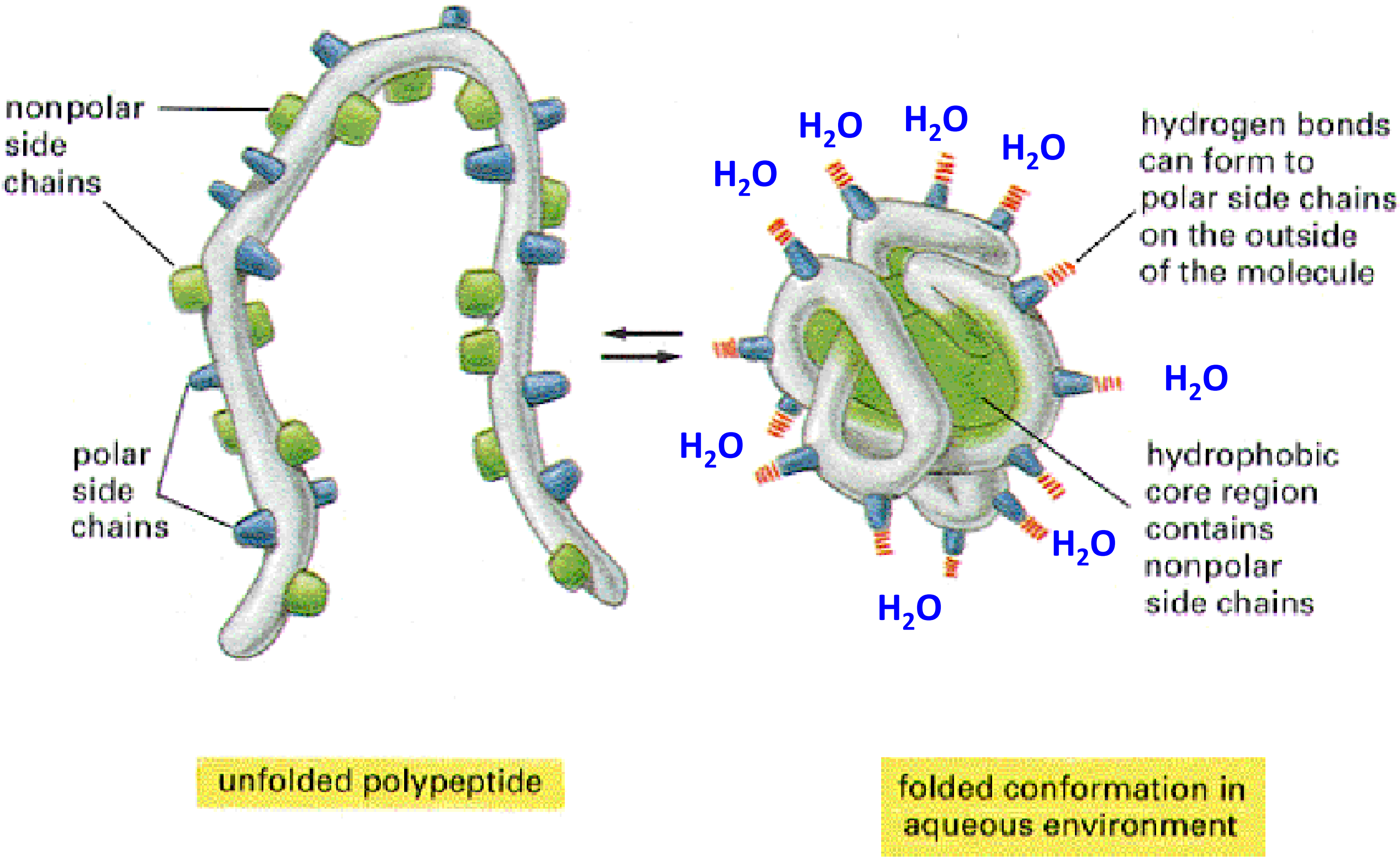


$$\mathrm{R\text{-}SH + R'\text{-}SH \xrightleftharpoons[\text{reduction}]{\text{oxidation}} R\text{-}S\text{-}S\text{-}R' + 2e^{-} + 2H^{+}}$$

Formation of a disulfide bond



The hydrophobic effect and weak interactions stabilize the tertiary structure of the proteins



Fibrous and globular proteins

- Fibrous proteins usually consist of a single type of secondary structure, and their tertiary and quaternary structures are relatively simple.
- Functionally, fibrous proteins provide support, shape, and external protection to vertebrates (α-keratin and collagen).
- All fibrous proteins are insoluble in water, a property conferred by a high concentration of hydrophobic amino acid residues both in the interior of the protein and on its surface. These hydrophobic surfaces are largely buried, as many similar polypeptide chains are packed together to form elaborate supramolecular complexes.

- In a globular protein, different segments of the polypeptide chain (or multiple polypeptide chains) fold back on each other, generating a more compact globular shape than in the fibrous proteins.
- Globular proteins often contain several types of secondary structures.
- Functionally, most enzymes, antibodies, carrier proteins and regulatory proteins are globular proteins.
- The folding also provides the structural diversity necessary for proteins to carry out a wide array of biological functions.

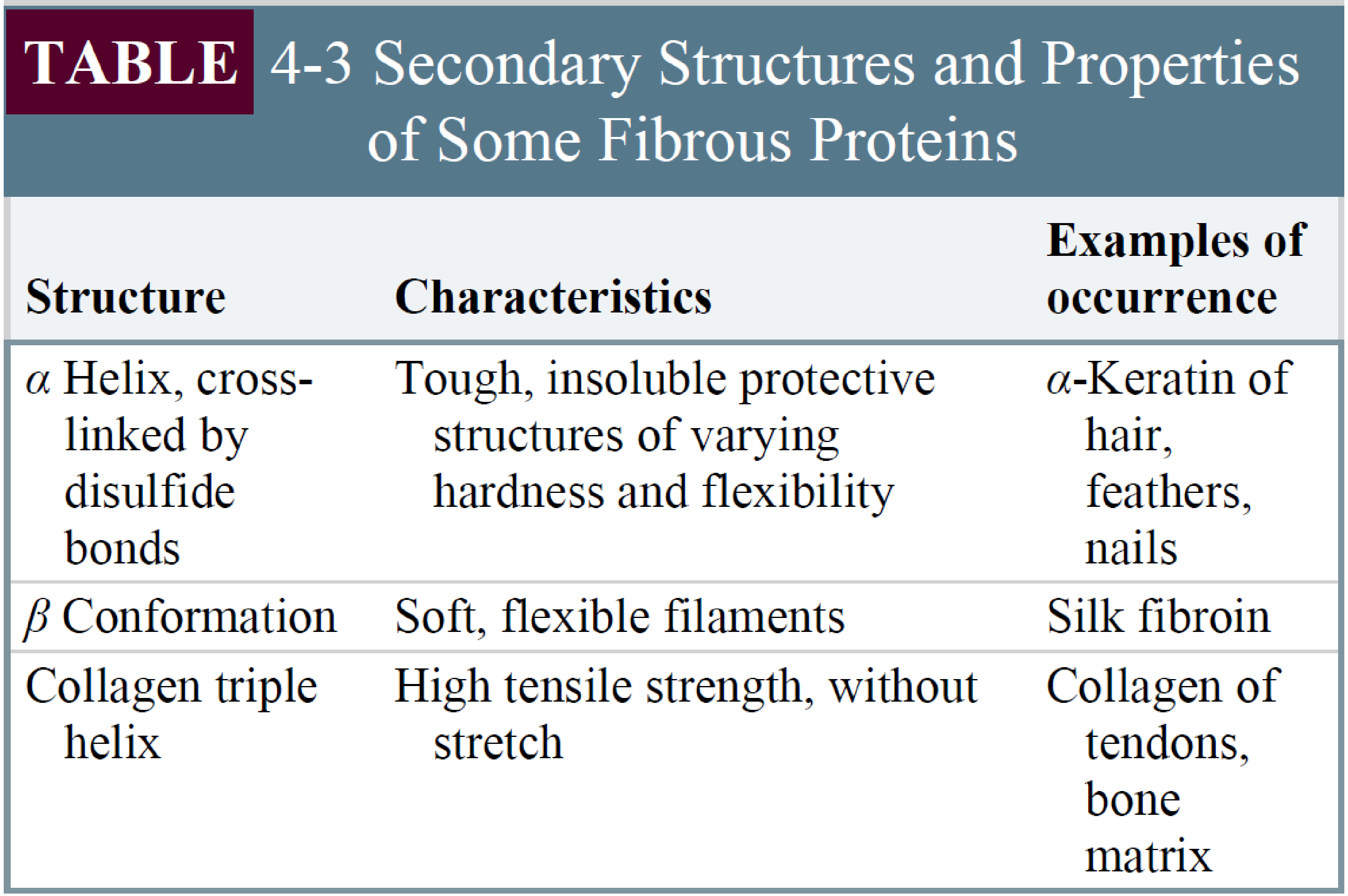


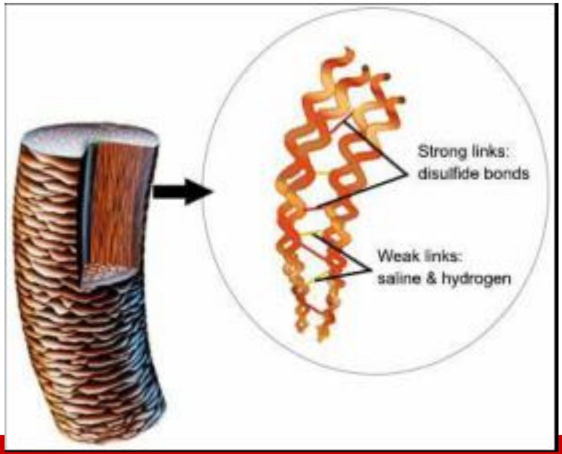
TABLE 4-3 Secondary Structures and Properties of Some Fibrous Proteins

Structure	Characteristics	Examples of occurrence
α Helix, cross-linked by disulfide bonds	Tough, insoluble protective structures of varying hardness and flexibility	α-Keratin of hair, feathers, nails
β Conformation	Soft, flexible filaments	Silk fibroin
Collagen triple helix	High tensile strength, without stretch	Collagen of tendons, bone matrix

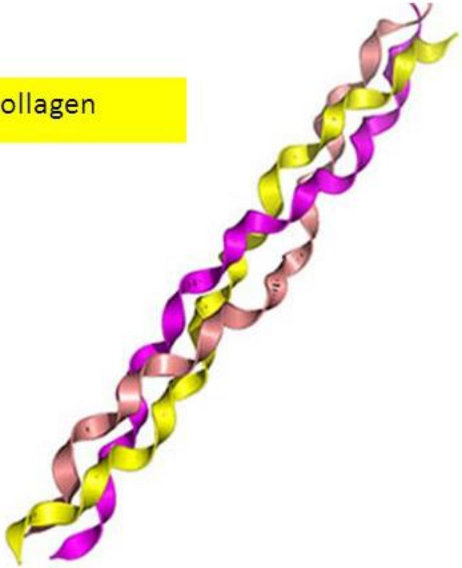
FIBROUS PROTEINS

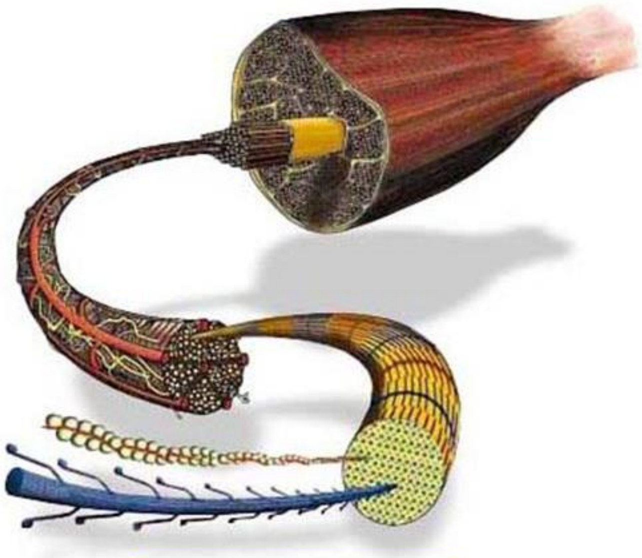
Fibrous proteins **have a high tensile strength and mechanical stability. Their function is to provide *structural support* to tissues. Collagen, for example, gives connective strength to skin, bones, teeth and tendons. Keratin is the major component of hair and nails.**

keratin

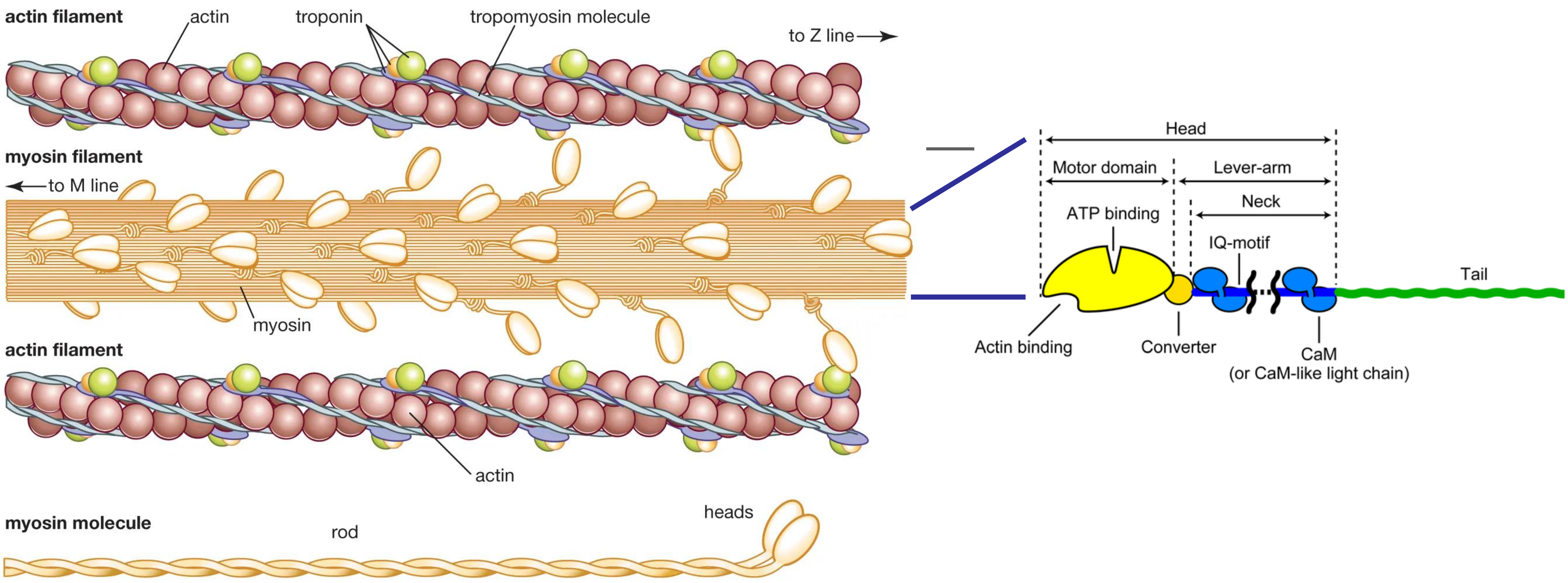


collagen





Myosin and actin



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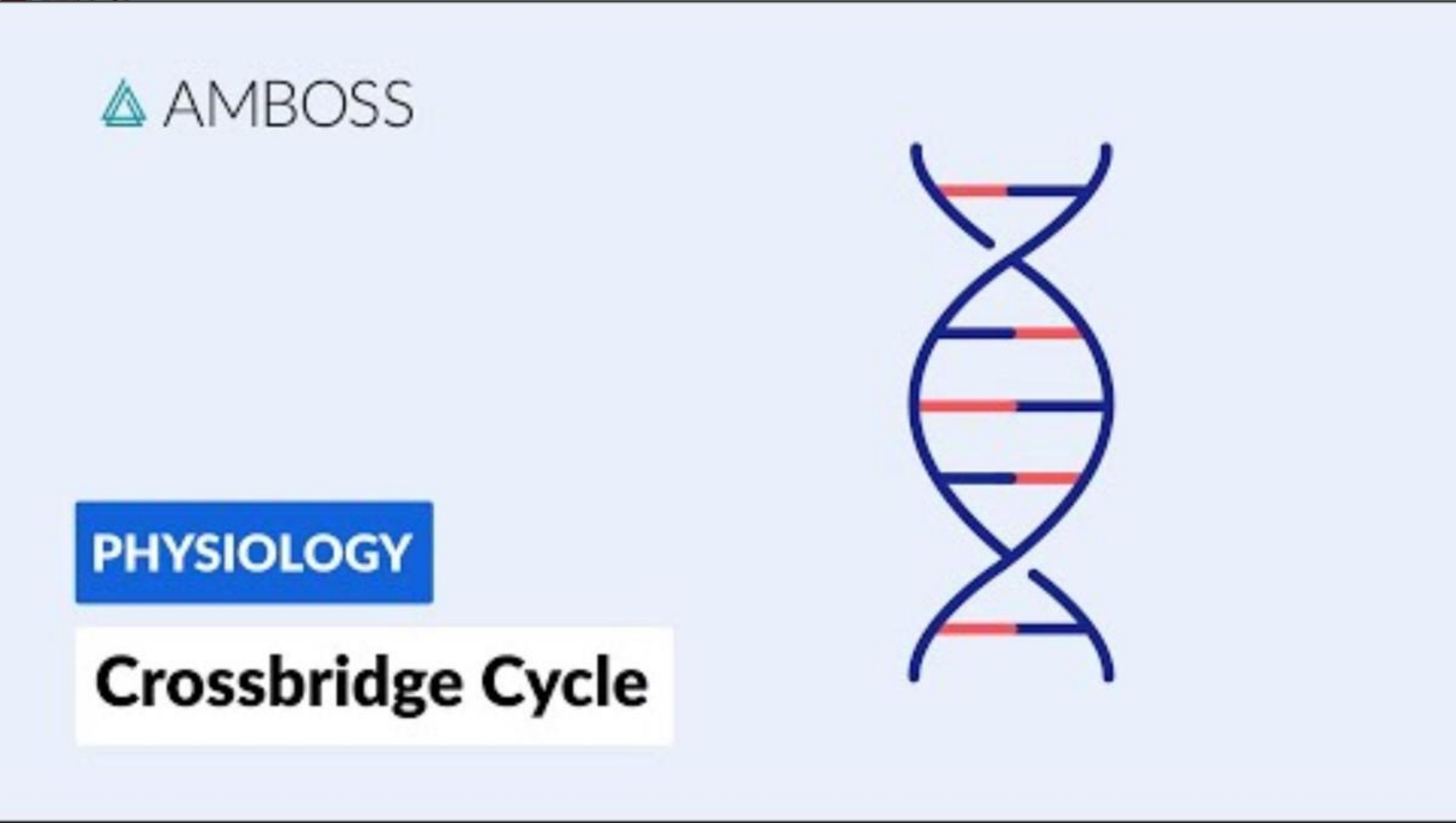
Actin is globular in its monomeric form (G-actin) and becomes fibrous when polymerized into filaments (F-actin). G-actin serves as the basic building block for actin filaments. It can bind ATP or ADP, which influences its ability to polymerize.

Myosin is composed by:

- Head: Motor activity (ATPase and actin binding), the globular region of the molecule.
- Neck: Acts as a mechanical amplifier.
- Tail: Provides structural support, allows for dimerization, and mediates specific interactions.

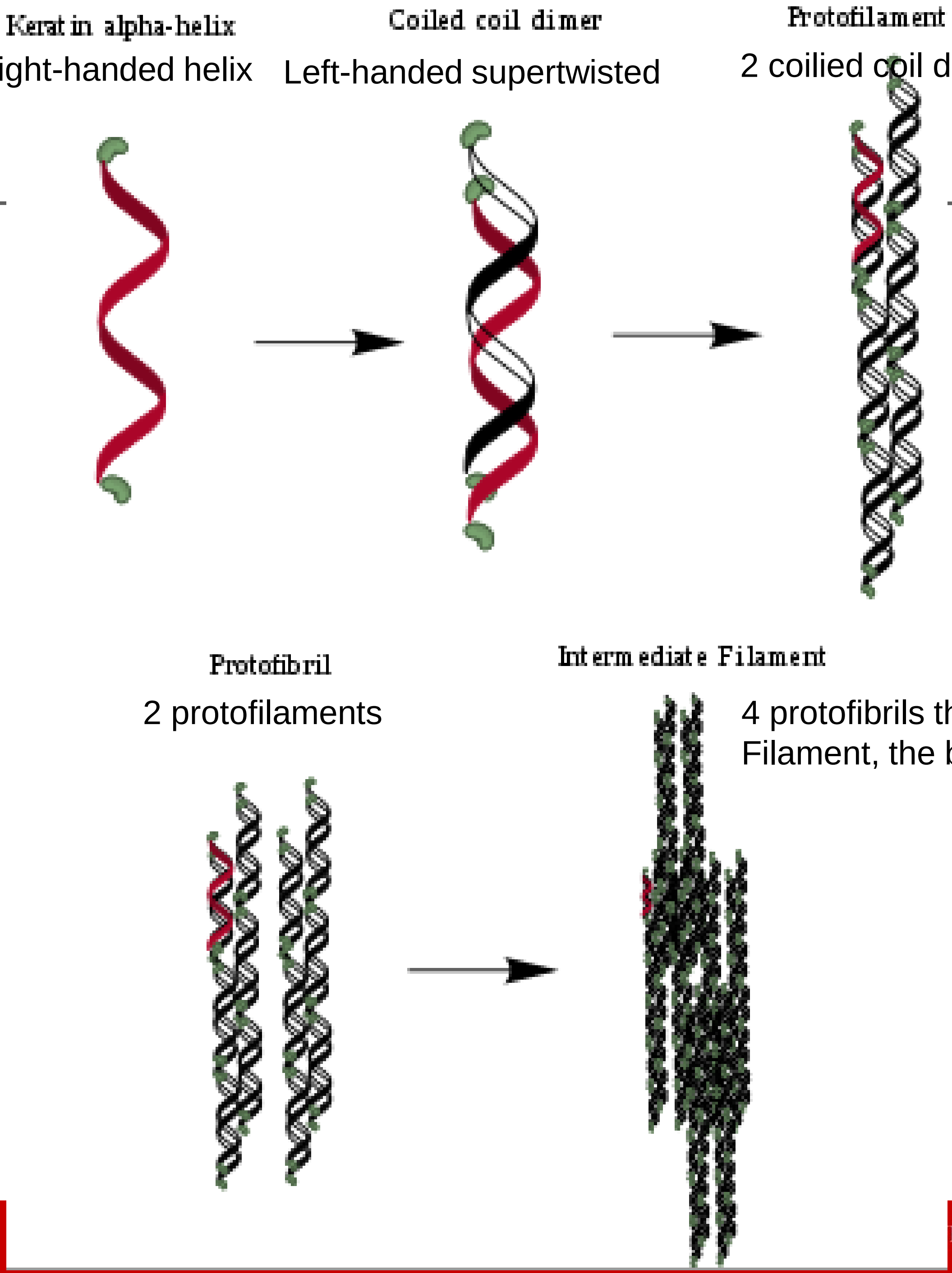


PHYSIOLOGY

Crossbridge Cycle

α-KERATINS

- Large family of proteins in which the **α-helix** secondary structure prevails.
- In mammals they represent almost all of the dry weight of hair, wool, skin, nails, horns, hooves.
- They are elongated, insoluble, not very reactive, rich in hydrophobic amino acids, consisting of long filaments wrapped in bundles.
- Very robust, suitable to perform coating, protective, connection or support functions; their resistance is greatly increased by the presence of disulfide bridges.
- Proteins of hair and wool, having few disulfide bridges, are very flexible, while those of horns, nails and hooves have more disulfide bridges and are much more compact and hard.



right-handed helix

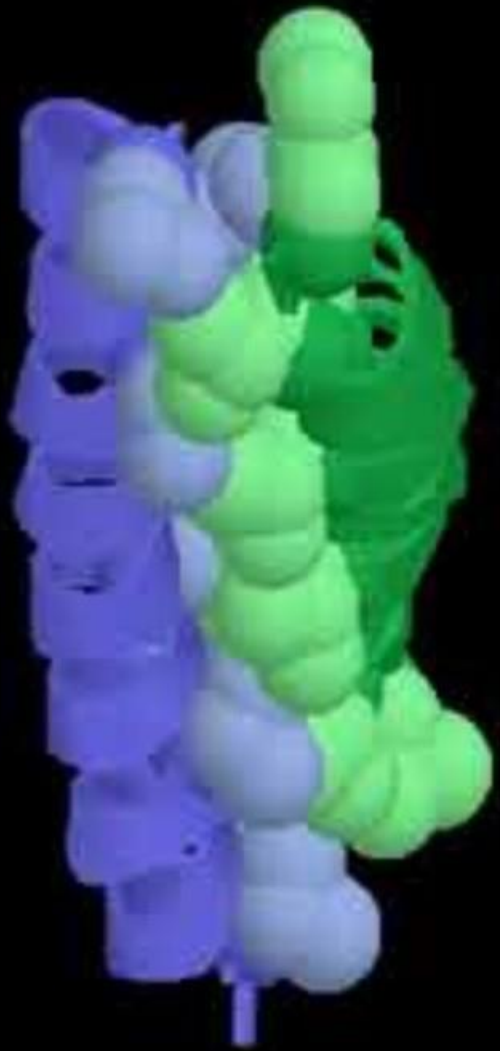
Left-handed supertwisted

2 coilied coil dimers mainly linked with SS bridges

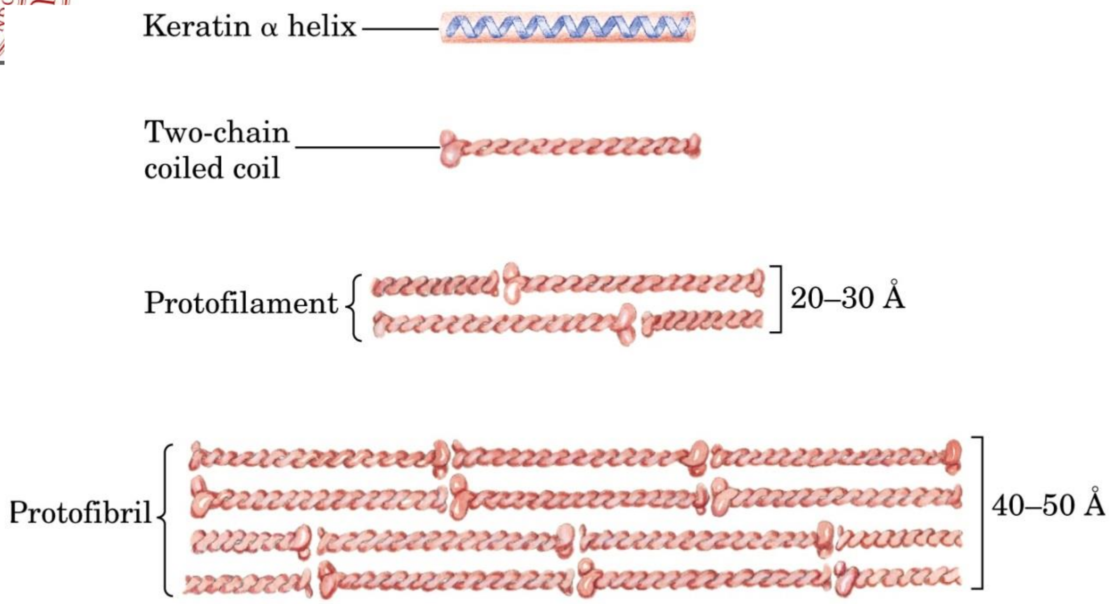
2 protofilaments

4 protofibrils that polymerize in Intermediate Filament, the basic subunit of α-keratins

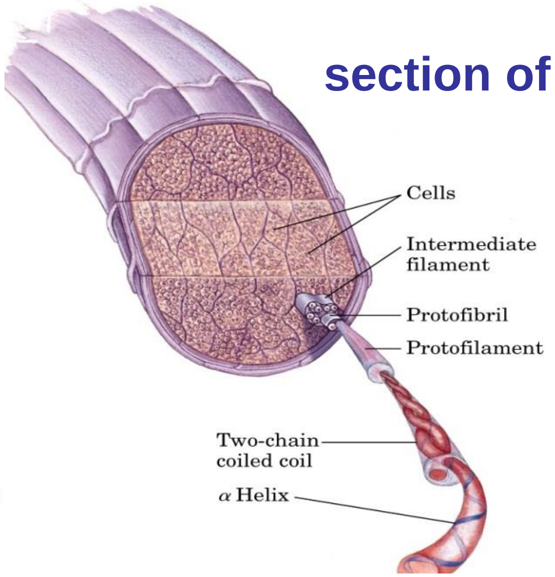
https://www.youtube.com/watch?v=4l1Hjf6DBW0

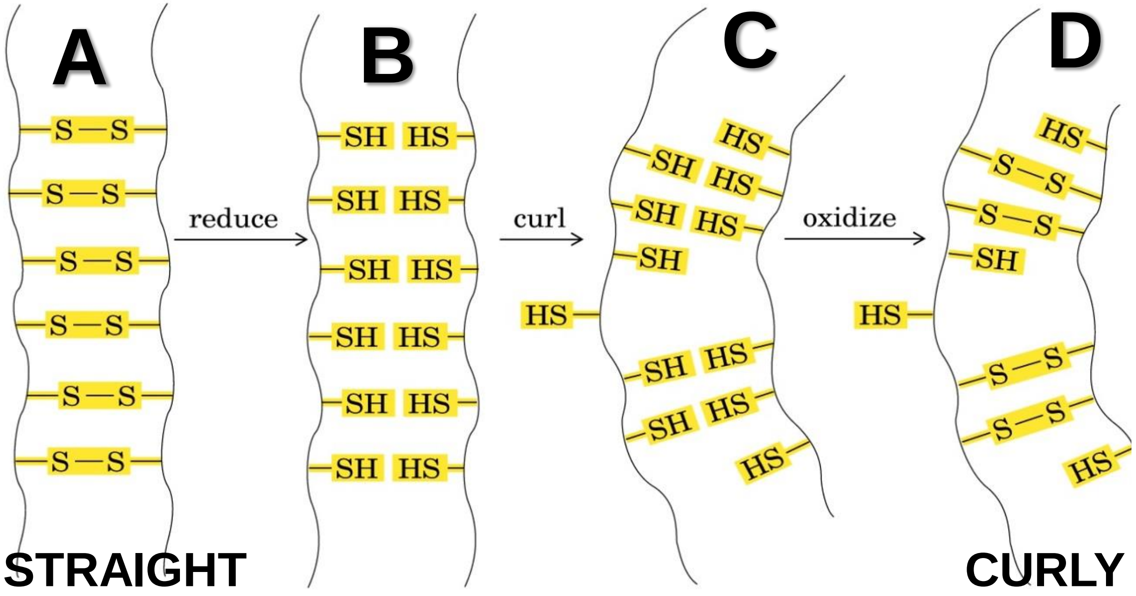


α-KERATINS OF HAIR

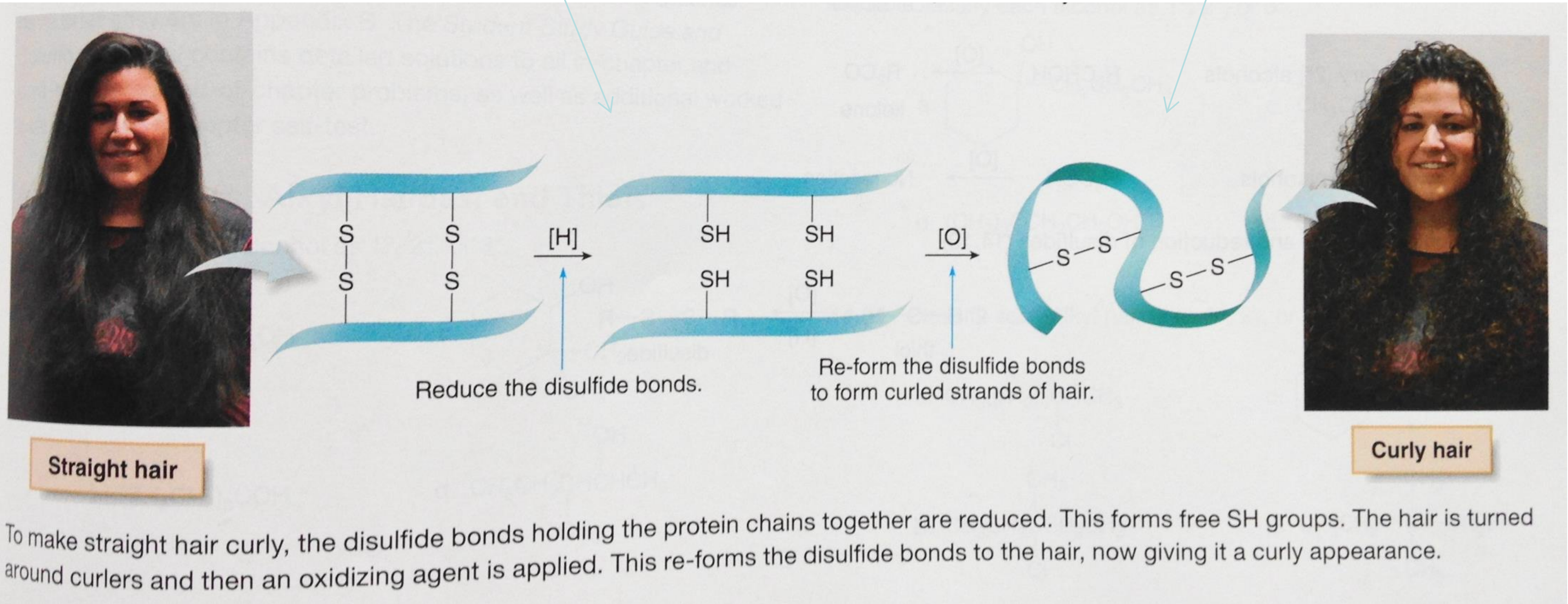


section of a hair





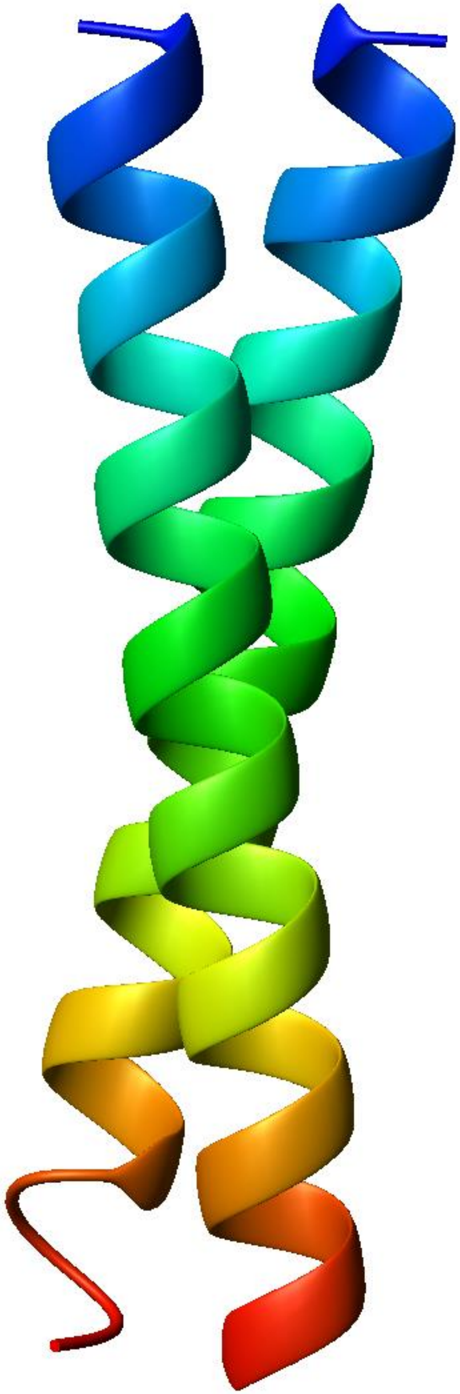
The permanent wave is the result of a series of chemical reactions. The initial structure of the hair keratin (A) is held together by disulfide bridges. By reduction (B) -SH groups (Cys) are formed which, after the hair has been folded onto a support (C), are oxidized again, reforming disulphide bridges in different positions from the initial ones.



To make straight hair curly, the disulfide bonds holding the protein chains together are reduced. This forms free SH groups. The hair is turned around curlers and then an oxidizing agent is applied. This re-forms the disulfide bonds to the hair, now giving it a curly appearance.

THE COILED COIL: A SUPERSECONDARY STRUCTURE

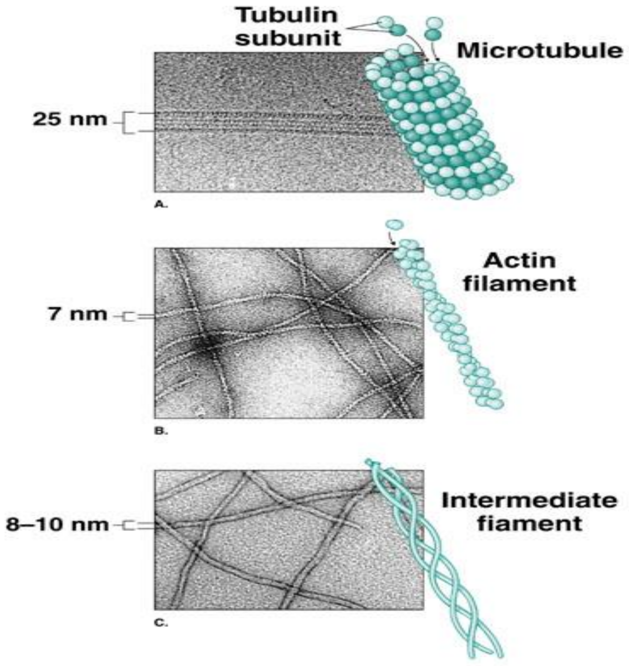
A coiled coil is a structural motif in proteins in which 2–7 α-helices are coiled together like the strands of a rope (dimers and trimers are the most common types). Many coiled coil-type proteins are involved in important biological functions such as the regulation of gene expression, *e.g.* some transcription factors.



α-keratins
Hard vs. Soft

Hard α-keratins, such as those found in nails and horns, have a higher cysteine content in their primary structure. This causes an increase in disulfide bonds that are able to stabilize the keratin structure, allowing it to resist a higher level of force before fracture. On the other hand, soft α-keratins, such as ones found in the skin and hair, contain a comparatively smaller amount of disulfide bonds, making their structure more flexible.

Tobin/Dusheck, Asking About Life, 2/e
Figure 4.16



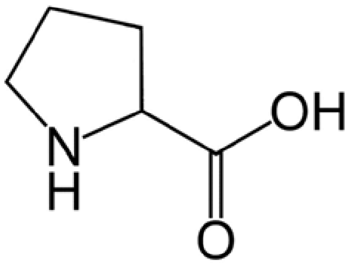
Collagen

- The most abundant protein in the body, making 25%-35% of all the whole-body proteins.
- Collagen contributes to the stability of tissues and organs. It maintains their structural integrity and has great tensile strength.
- The main component of cartilage, ligaments, tendons, bone and skin.
- Plays an important role in tissue and organ development.

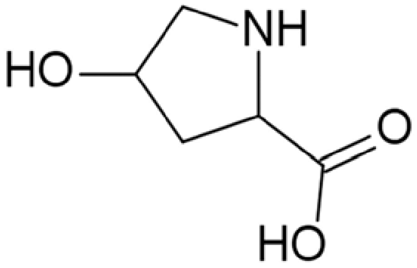
Fibrillar Collagen Structure

Collagen is an insoluble glycoprotein (protein + carbohydrate)

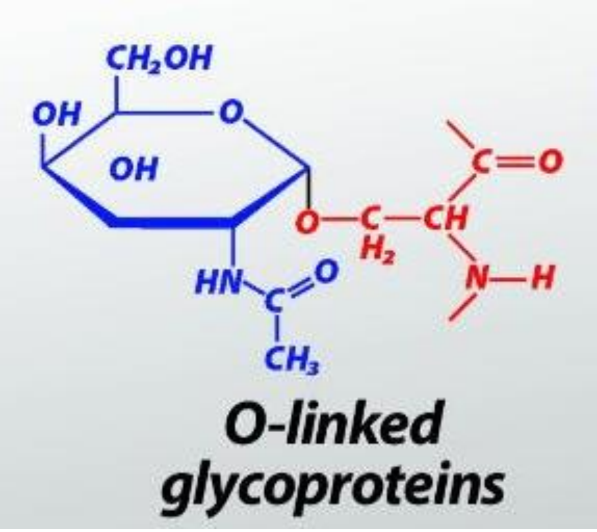
<u>Proline and hydroxyproline</u> constitute about 1/6 of the total sequence and provide the stiffness of the polypeptide chain.



Pro (Proline)



Hyp (Hydroxyproline)

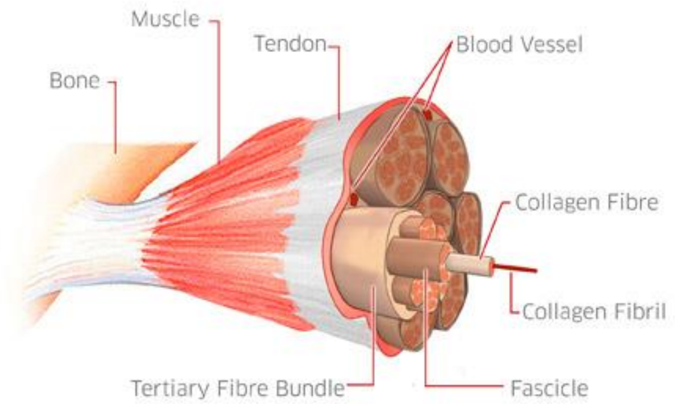


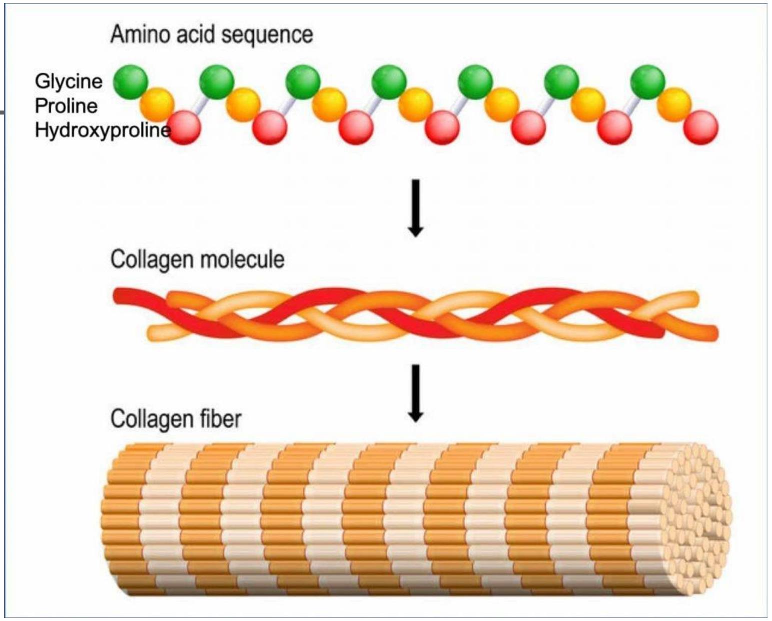
O-linked glycosilation : glucose, galactose

Remarkably, intact collagen has been discovered in soft tissue of the fossilized bones of a 68 million-year-old *Tyrannosaurus rex* fossil, <u>the oldest protein detected as of 2010.</u>

Shoulders MD and Raines RT Ann Rev Biochem 2010

COLLAGEN



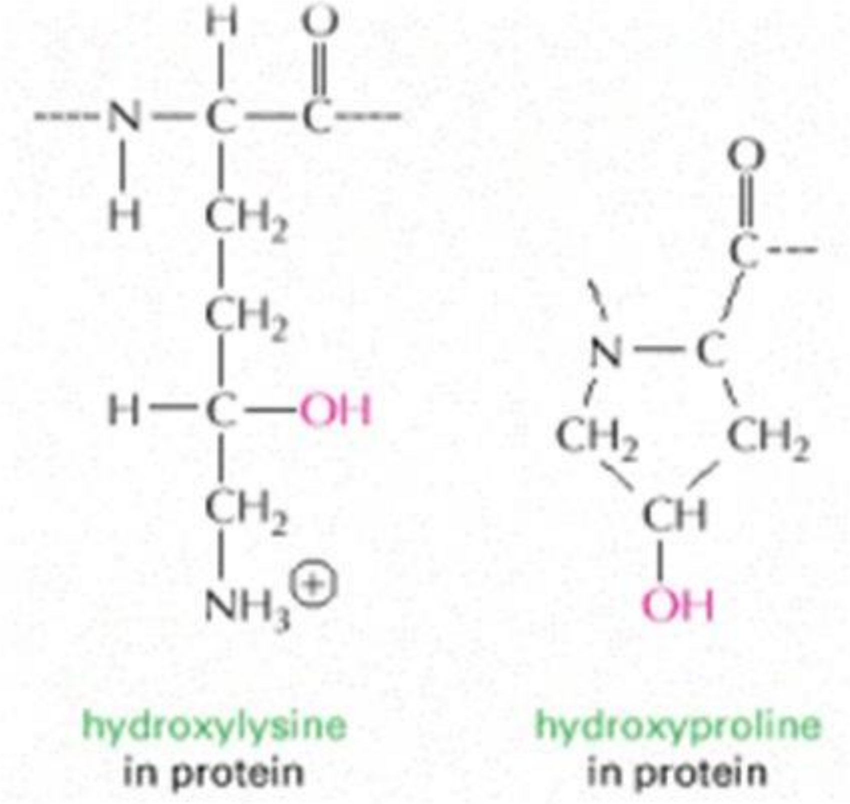


- Principal component of connective tissue such as tendons, cartilage, the organic matrix of bone, and the cornea of the eye.

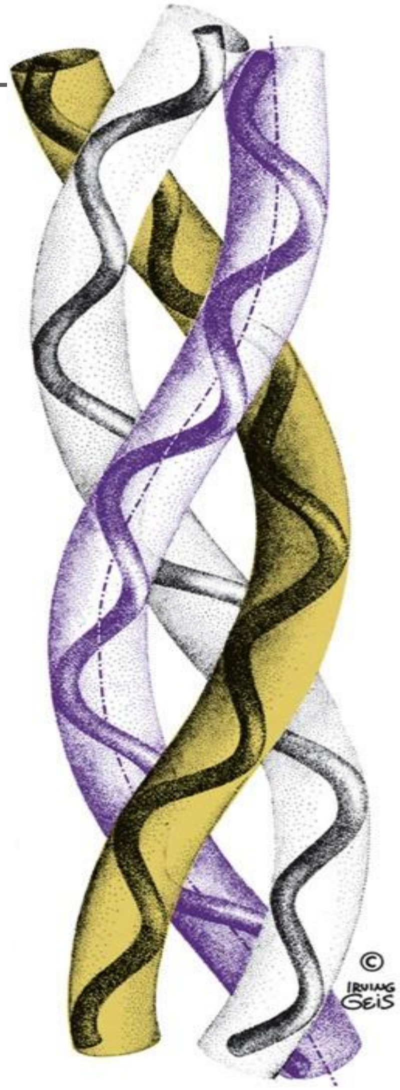
- The collagen helix is a unique secondary structure, quite distinct from the α-helix. It is left-handed and has three amino acid residues per turn.
- Collagen is also a coiled coil, but with distinct tertiary and quaternary structures: three separate polypeptides, called α chains (not to be confused with α helices), are supertwisted each other. The superhelical twisting is right-handed in collagen, opposite in sense to the left-handed coiled coil dimer of the α-keratins.
- The amino acid sequence in collagen is generally a repeating tripeptide unit, Gly–X–Y, where X and Y are often Pro or 4-Hypro (the most abundants) and HyLys.
- The tight wrapping of the α chains in the collagen triple helix provides high tensile strength.

Hydroxylysine

- Collagen is also composed of hydroxylysine, which serves as attachment sites of polysaccharides making collagen a glycoprotein



Collagen has a distinct tertiary and quaternary structure



Irving Geis/Geis Archives Trust. Copyright Howard Hughes Medical Institute. Reproduced with permission.

1.4 nm diameter

- **Collagen is**

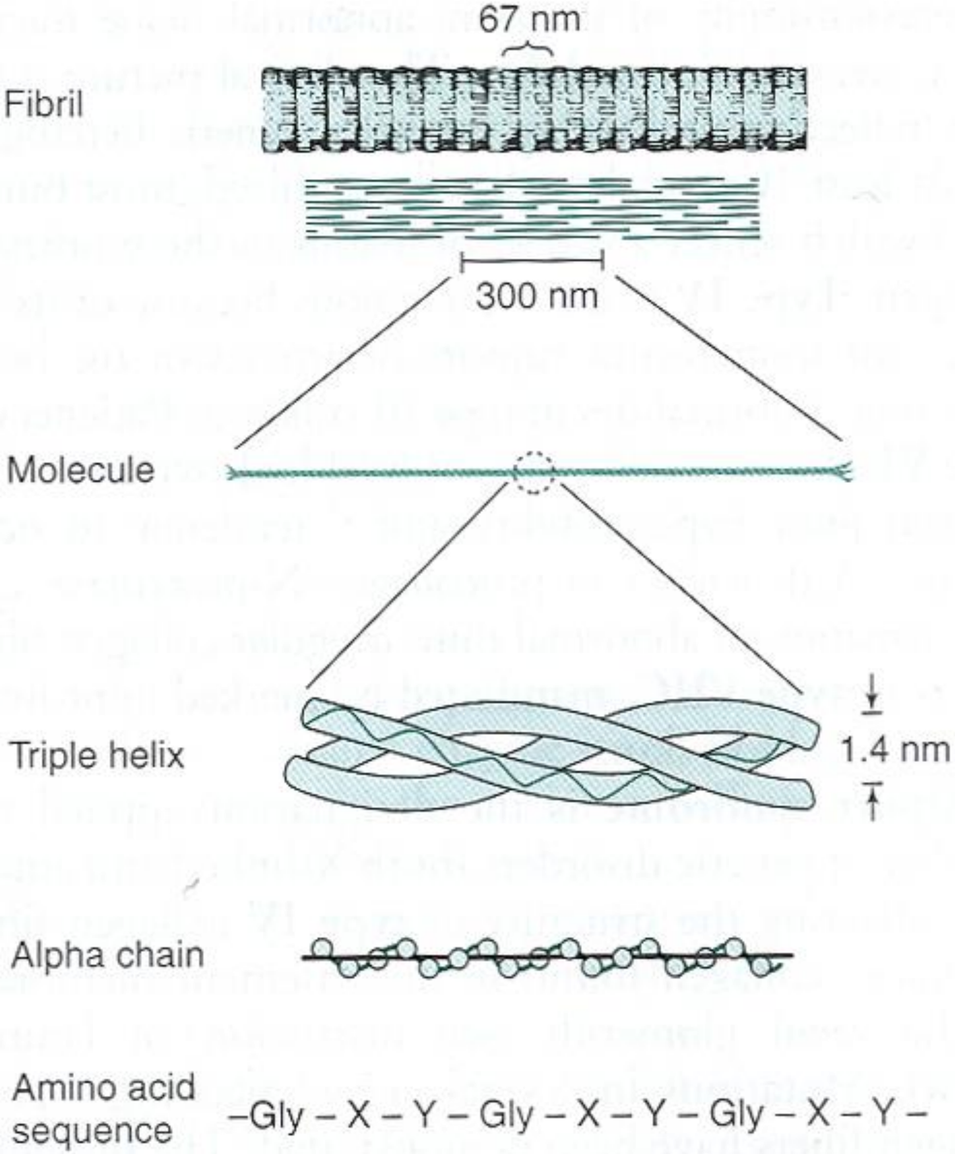
  35% Gly
  11% Ala
  21% Pro and Hyp
  (Hyp is 4-hydroxyproline)

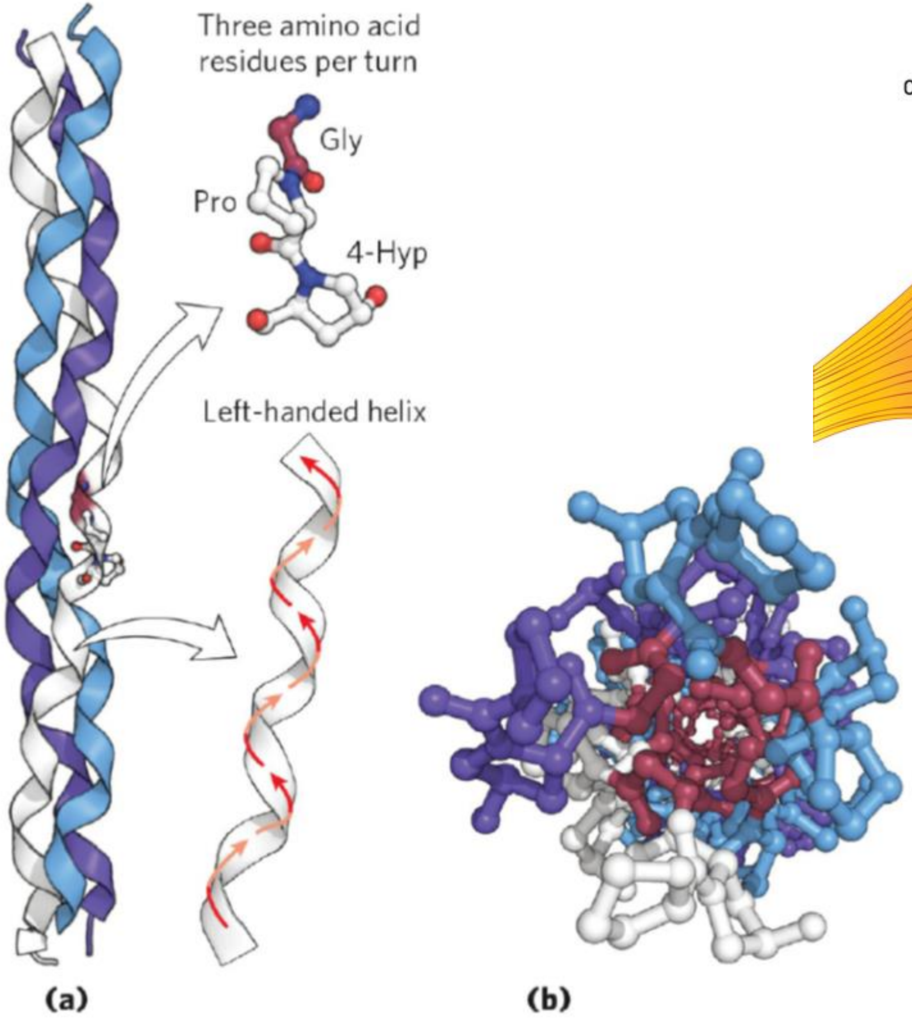
- **Repeating TRIPEPTIDE sequence Gly-X-Y, where X and Y are often Pro or Hyp (respectively)**

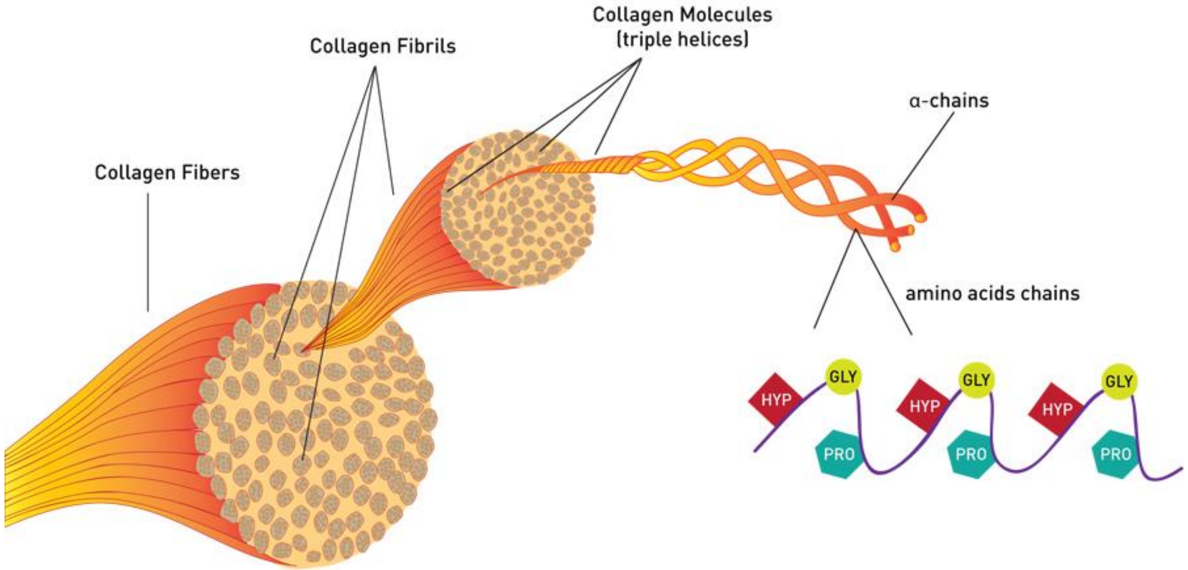
- **Unique secondary structure distinct from α-helix; it is left-handed and has three amino acid residues per turn.**

- **Forms a three-stranded right-handed superhelix**

Different levels of the structure of the collagen fibril




Three amino acid residues per turn
Gly
Pro
4-Hyp
Left-handed helix
(a)
(b)


Collagen Molecules (triple helices)
Collagen Fibrils
α-chains
Collagen Fibers
amino acids chains
HYP
GLY
PRO
HYP
GLY
PRO
HYP
GLY
PRO

SCURVEY

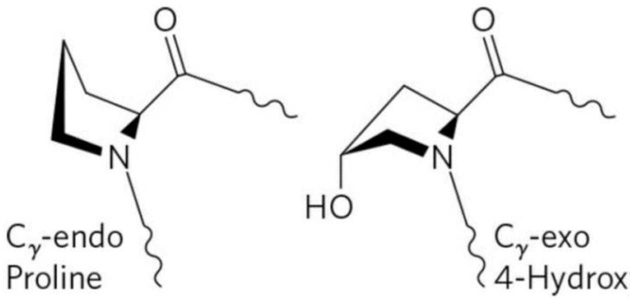
“The signal was this: when the nose began to bleed, then death was at hand.”

*—The Memoirs of the Lord of Joinville, ca. 1300**

- Scurvy is caused by lack of vitamin C, or ascorbic acid (ascorbate). Vitamin C is required for, among other things, the hydroxylation of proline and lysine in collagen; scurvy is a deficiency disease characterized by general degeneration of connective tissue.

- Collagen is constructed of the repeating tripeptide unit Gly–X–Y, where X and Y are generally Pro or 4-Hyp—the proline derivative (4R)-L-hydroxyproline, and Hydroxylysine which plays an essential role in the folding of collagen and in maintaining its structure.

- In the absence of vitamin C, the Pro or Lys cannot be hydroxylated. This leads to collagen instability and connective tissue problems seen in scurvy.



SCURVEY

- In case of ascorbic acid deficiency (so, lack of prolyl and lysyl hydroxylation), collagen fibers cannot be crosslinked → greatly decreasing tensile strength of assembled fiber.
- One resulting deficiency disease is scurvy.
- Patients with ascorbic acid deficiency also often show bruises on limbs as a result of subcutaneous extravasation of blood (capillary fragility)

The legs of a 46-year-old man with scurvy.

Globular proteins

- ✓ **The three-dimensional structure of a typical globular protein can be considered an assemblage of polypeptide segments in different secondary conformations, linked by connecting bends.**
- ✓ **Each globular protein is folded compactly, and in each case the hydrophobic amino acid side chains are oriented toward the interior (away from water) and the hydrophilic side chains are on the surface. The structures are also stabilized by a multitude of hydrogen bonds and some ionic interactions (salt bridges).**

***Enzymes**:* biochemical catalysts, which lower the activation energy greatly accelerating the reaction rate of biological reactions. An enzyme can only react with a substrate if the location of its functional groups and hydrogen bonds as well as its shape matches the active site of the enzyme. Ex. Ribonuclease, secreted by the pancreas to specifically digest ribonucleic acid.
Antibodies: can recognize intruders, *antigens*, and bind to them in a *key-lock mechanism*.
Proteins also function for ***transport* and *storage***. Ex. hemoglobin is responsible for the transport of oxygen in the blood stream, transferrin for the transport of iron, ferritin for the storage of iron in the liver.
Hormones: polypeptides can also act as *chemical messengers*. By interacting with a matching receptor, usually found in the cell membrane, they regulate a wide variety of tasks in metabolism. Ex. glucagon, insulin and somatostatin regulate the storage and release of glucose and fatty acids.

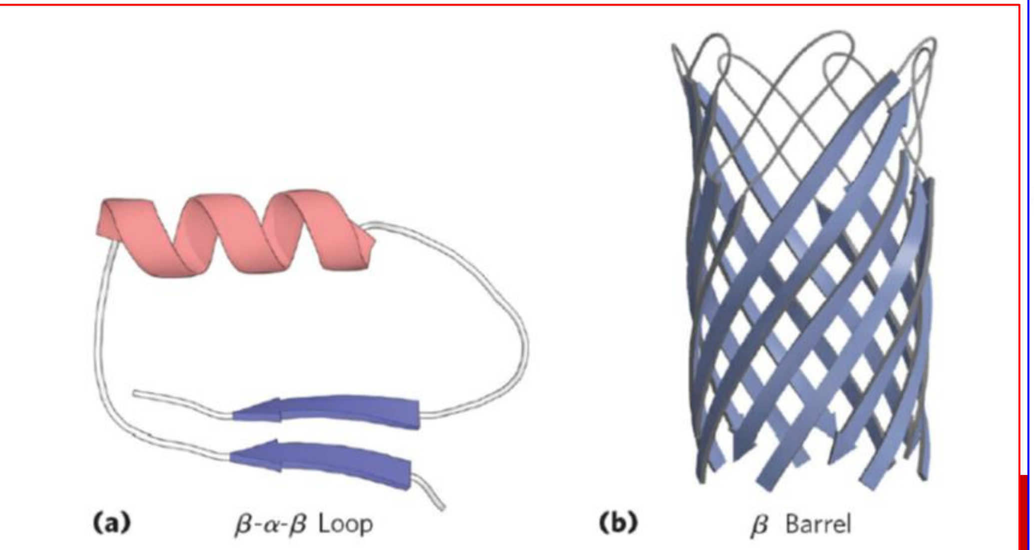
1962 Chemistry Prize – John Kendrew & Max Perutz

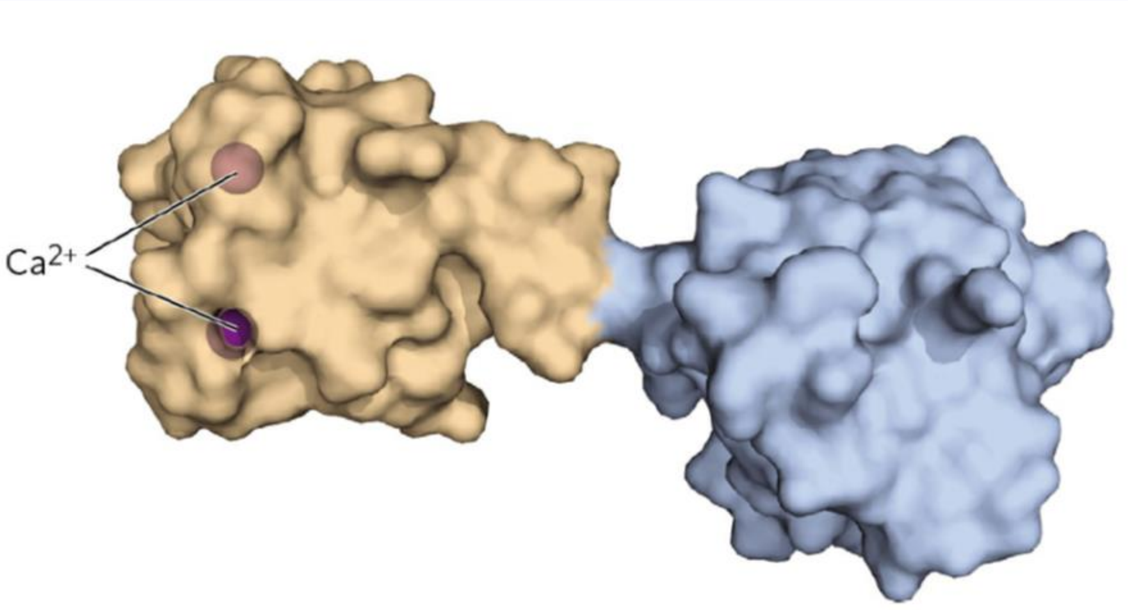
“for their studies of the structures of globular proteins”

Protein folding patterns

To understand a complete three-dimensional (tertiary) structure, we need to analyze its folding patterns.

- The first term is the **motif,** also called a **fold**, that is a recognizable folding pattern involving different secondary structures and the connection(s) between them.
- The second term is **domain**. A domain is a part of a polypeptide chain that is independently stable or could undergo movements as a single entity with respect to the entire protein. Polypeptides with more than a few hundred amino acid residues often fold into two or more domains, sometimes with different functions. Different domains often have distinct functions, such as the binding of small molecules or interaction with other proteins.


(a) β-α-β Loop **(b)** β Barrel



Quaternary Structure

- Some proteins contain multiple polypeptides (multimer)
- Each polypeptide chain is called subunit
- The polypeptides are held together by the same types of interactions that stabilize the tertiary structure

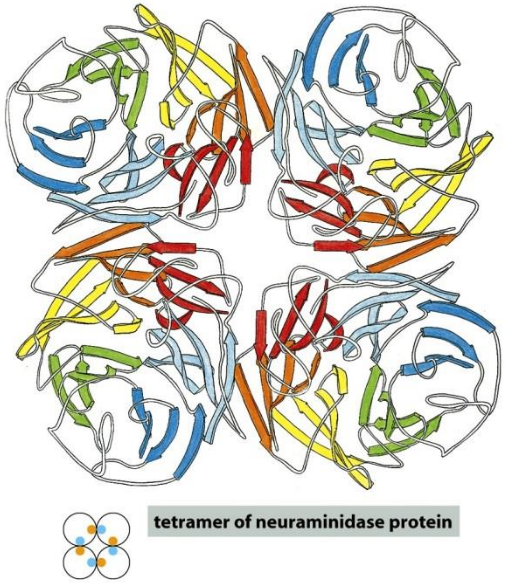
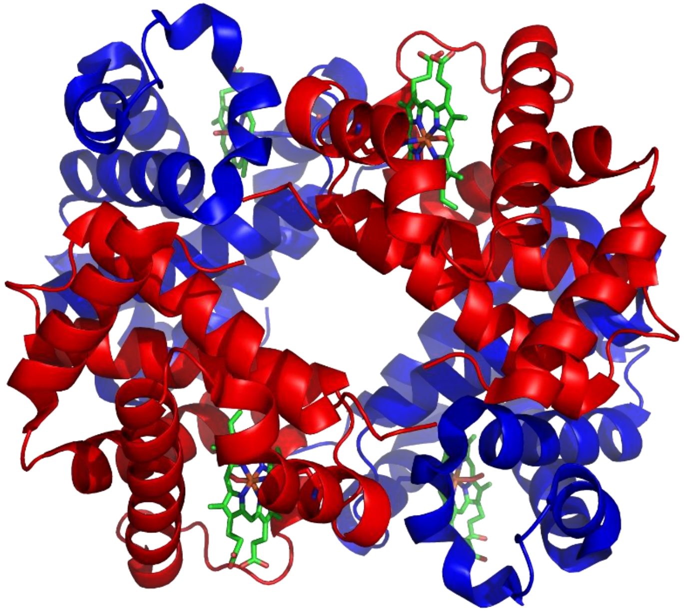


Figure 3-21 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Protein containing 4 identical polypeptide chains: Neuraminidase



Protein formed by 2 different subunits: Hemoglobin

Quaternary Structure

- ✓ Quaternary structure is restricted to **oligomeric proteins** (more than 1 subunit), which consist of the association of two or more polypeptide chains held together by electrostatic attractions, hydrogen bonding, van der Waals forces and occasionally disulfide bridges (within a chain as well as between the two chains).

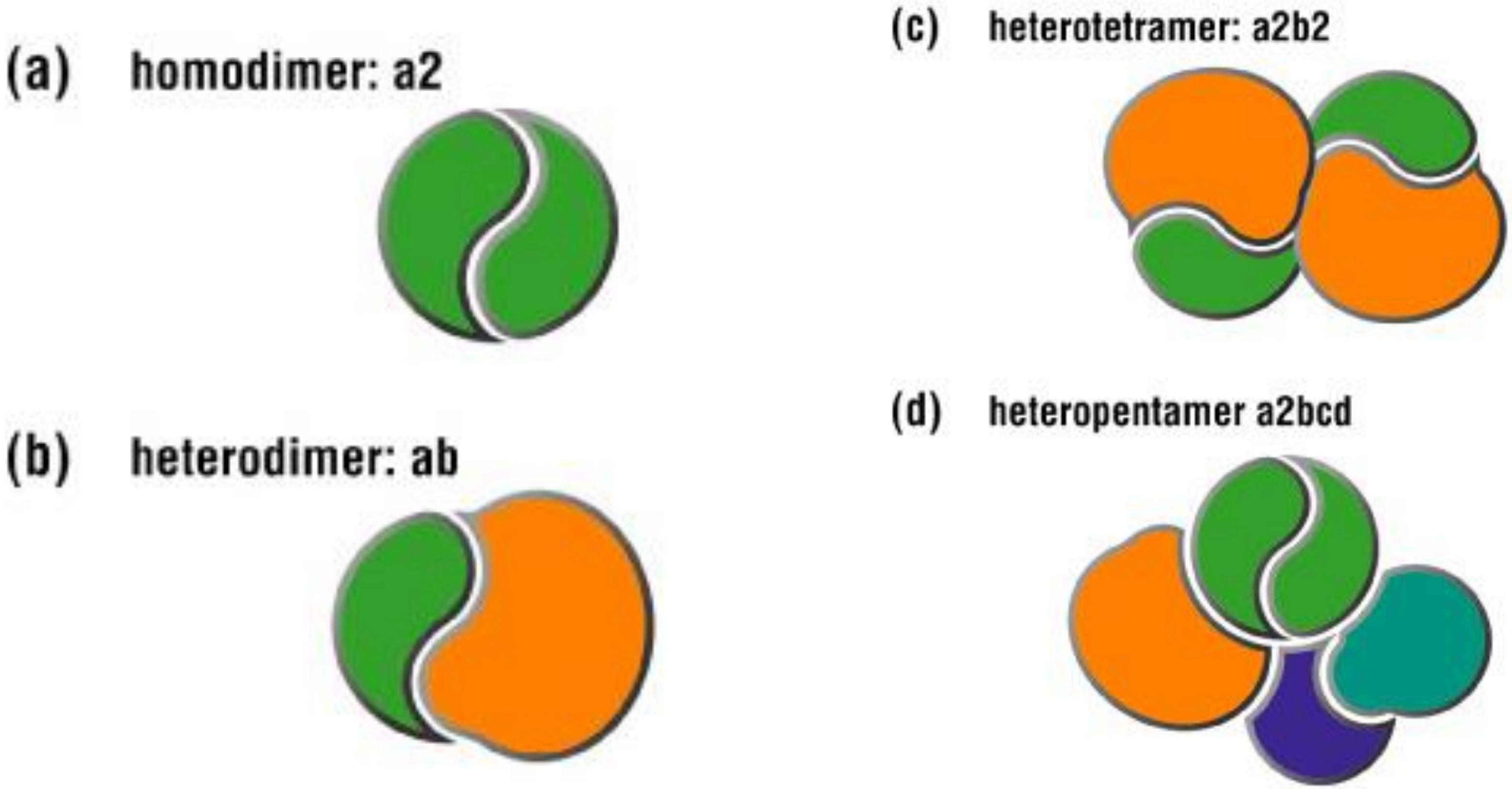
- ✓ The separate polypeptide chains forming a protein can be identical (homogenic protein), or different (heterogenic protein), as in the case of insulin and hemoglobin.

- ✓ Many multisubunit proteins have regulatory roles; the binding of small molecules may affect the interaction between subunits, causing large changes in the protein's activity in response to small changes in the substrate concentration or regulatory molecules.

- ✓ In other cases, separate subunits take on separate but related functions, such as catalysis and regulation.

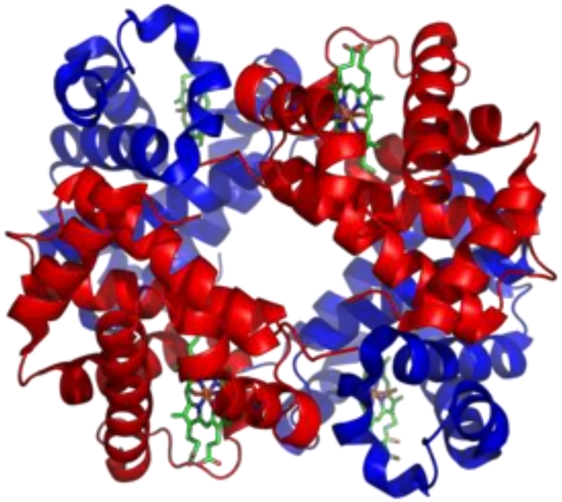
Oligomers: composed of more than one polypeptide chain or subunit

Most common



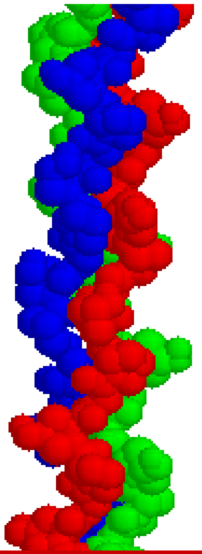
Quaternary structure

- **Globular:**
  - Folded in a globular shape
  - Same or different types of secondary structure
  - Example: hemoglobin



Hemoglobin

- **Fibrous:**
  - Polypeptides arranged in long strands or sheets
  - One type of secondary structure
  - Examples: intermediate filaments and collagen



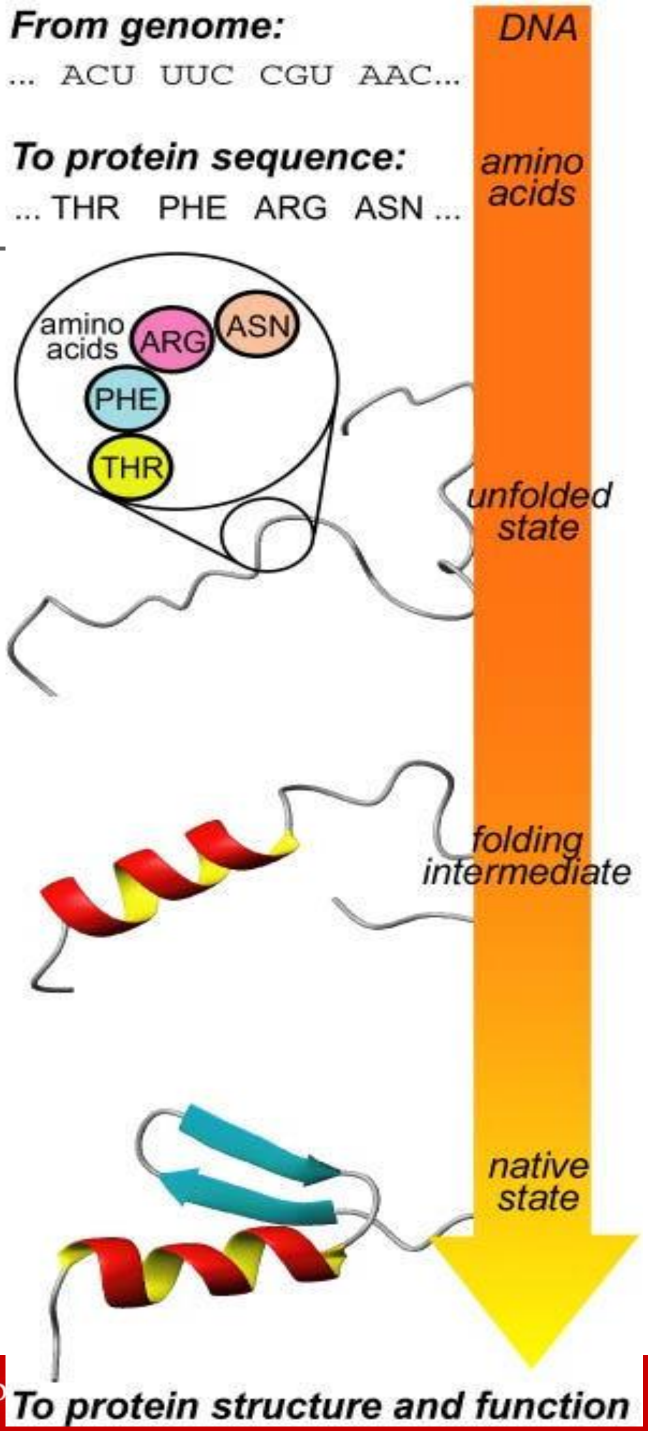
Collagen

What are the structural and functional advantages driving quaternary association?

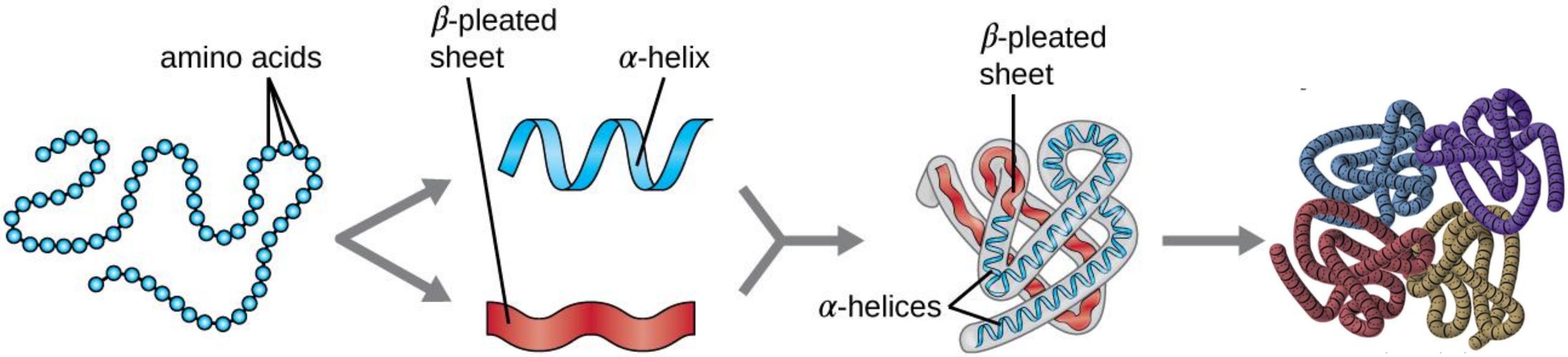
- Stability: reduction of surface to volume ratio
- Genetic economy and efficiency
- Bringing catalytic sites together
- Cooperativity

FOLDING

In order to carry out their function (for instance as enzymes or antibodies), proteins must take on a particular 3D shape. Thus, proteins are truly amazing machines: before they do their work, they assemble themselves! This self-assembly is called "FOLDING."



Summary of Structure



Primary Protein Structure
Sequence of a chain of amino acids

Secondary Protein Structure
Local folding of the polypeptide chain into helices or sheets

Tertiary Protein Structure
three-dimensional folding pattern of a protein due to side chain interactions

Quaternary Protein Structure
protein consisting of more than one amino acid chain

Protein Denaturation or unfolding

It is the loss of the three-dimensional structure sufficient to cause a protein's function loss. Denatured proteins exist in a set of partially folded states.

Because the secondary, tertiary and quaternary structures of proteins are stabilized by weak, non-covalent interactions, these structures are easily disrupted by chemical agents that disrupt these interactions, including:

- Changes in temperature
- Changes in *pH*
- Mechanical stress
- Detergents (soap)

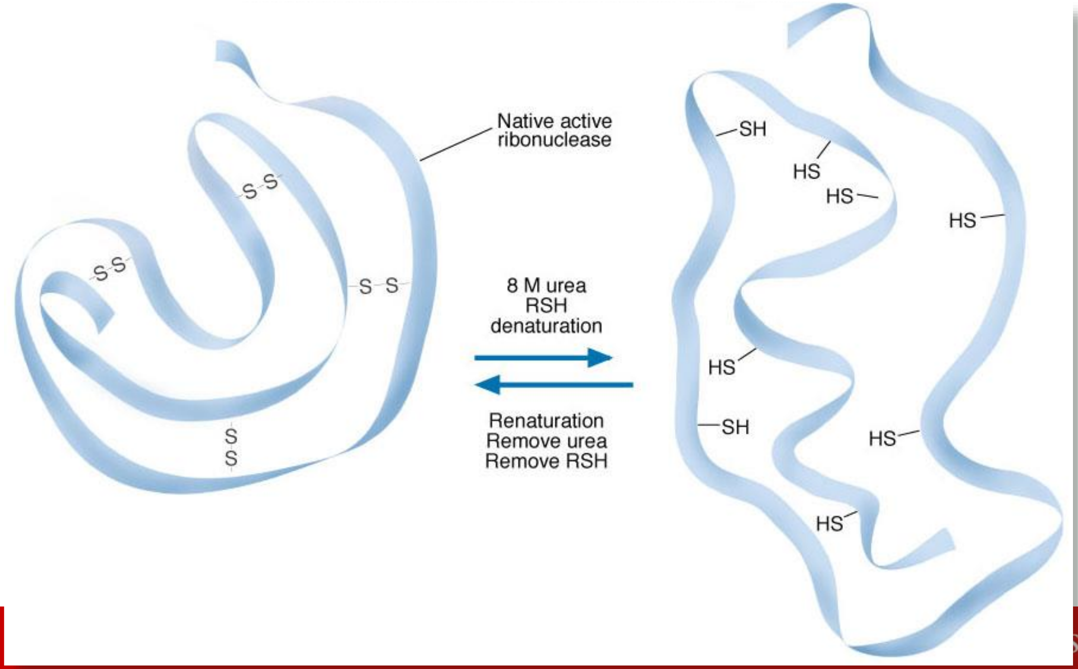
- These agents typically cause the protein to unfold
  - Only the primary structure remains
  - The protein loses it function

Protein Denaturation

Christian B. Anfinsen won a Nobel Prize (1972) for showing that protein denaturation can be sometimes reversed.

- **The experiment demonstrated that the information necessary to obtain the correctly folded protein structure is contained within the protein’s amino acid sequence (primary structure)**

Active Protein

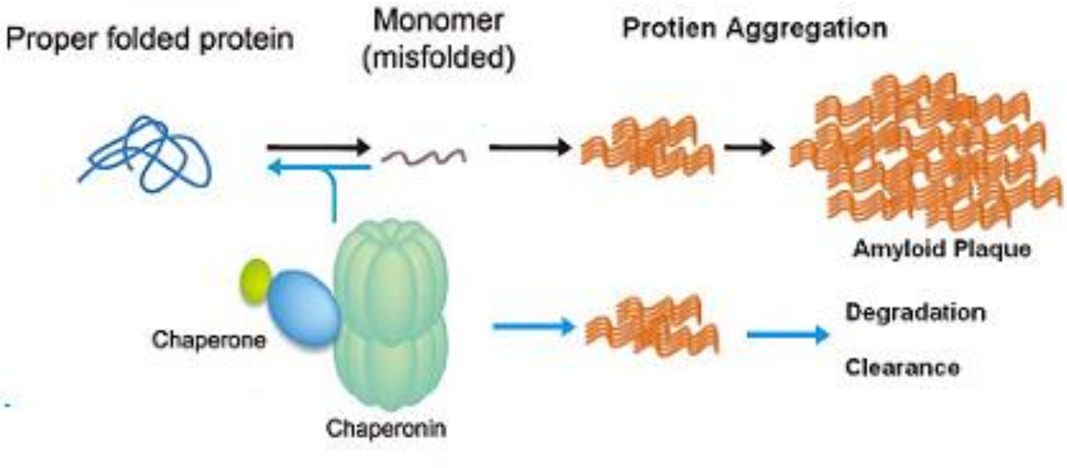


Inactive Protein

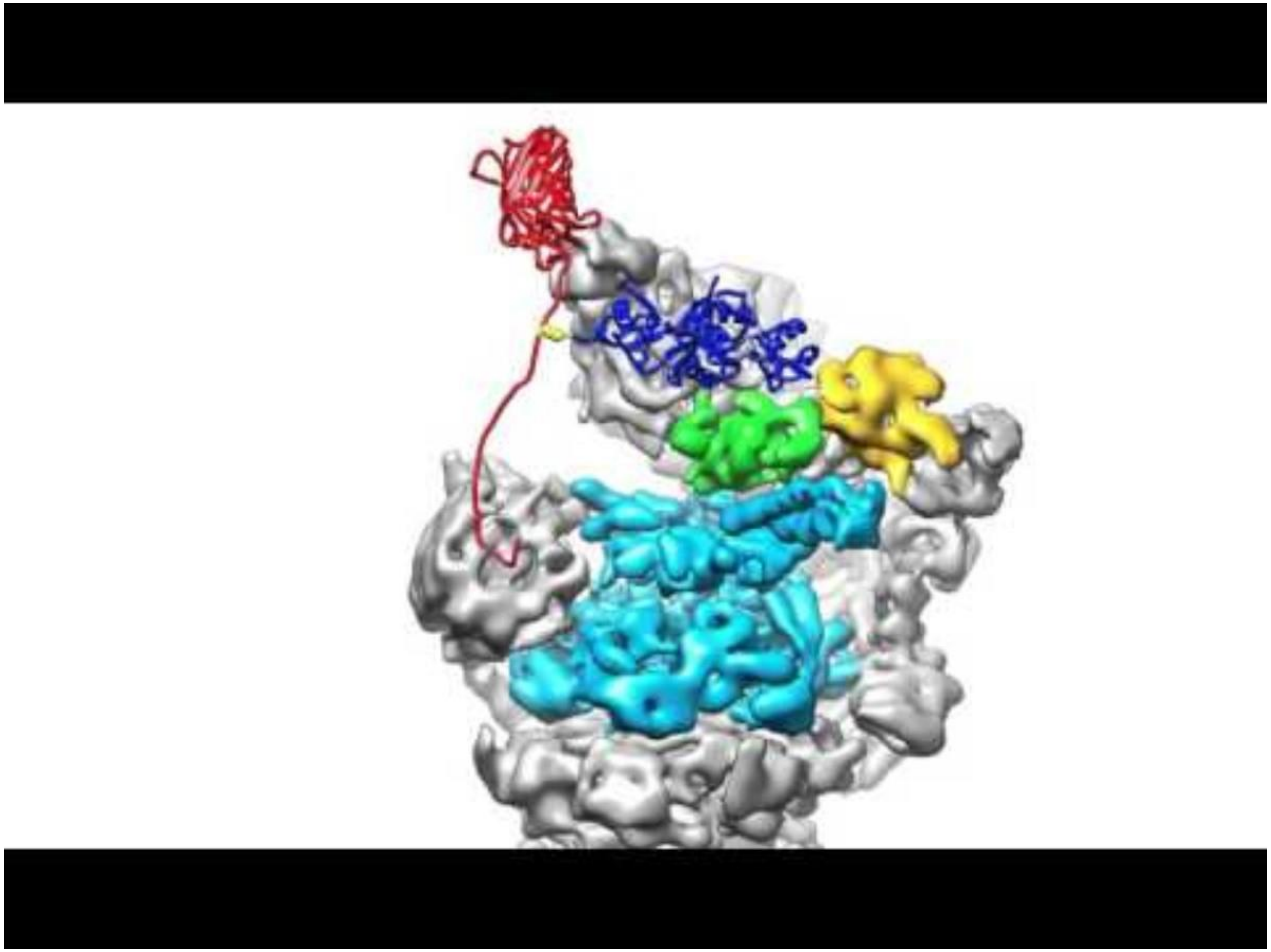
Proteostasis (Protein homeostasis)

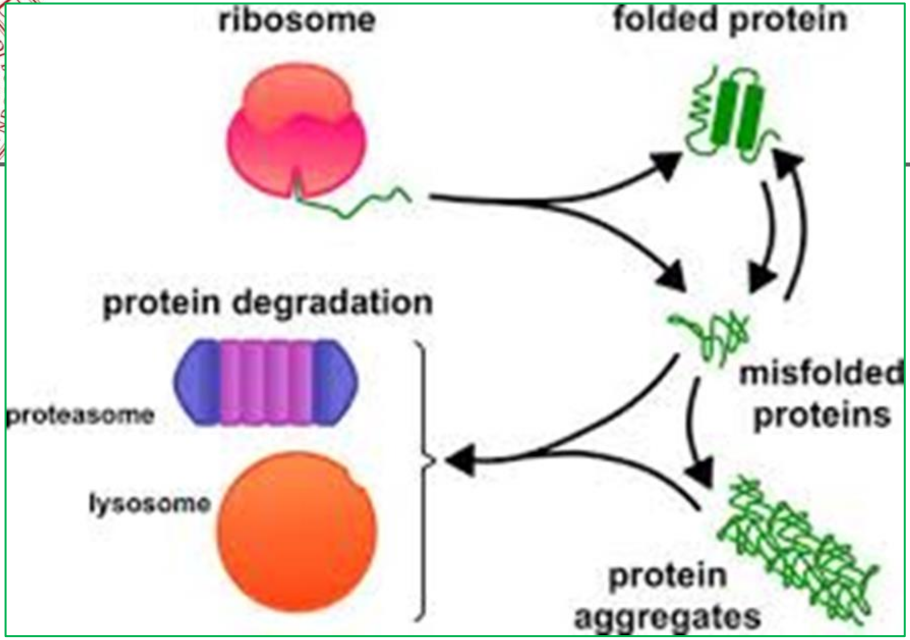
The maintenance of protein folding, and subsequent removal of misfolded proteins which is achieved mainly through:

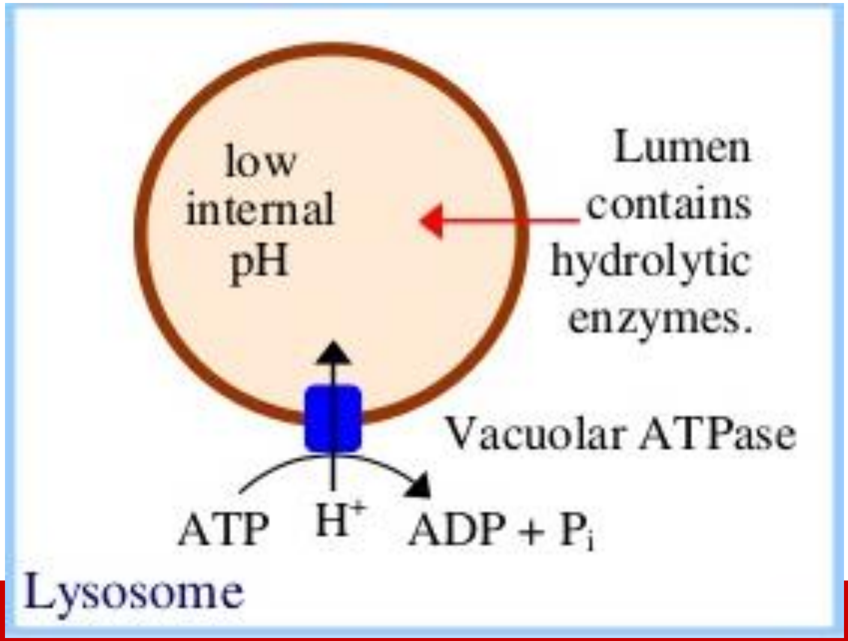
1. Heat Shock Proteins (HSPs: chaperones and chaperonins)
2. Endoplasmic Reticulum Associated Degradation (ERAD)
3. Ubiquitin Proteasome System (UPS)
4. Autophagy

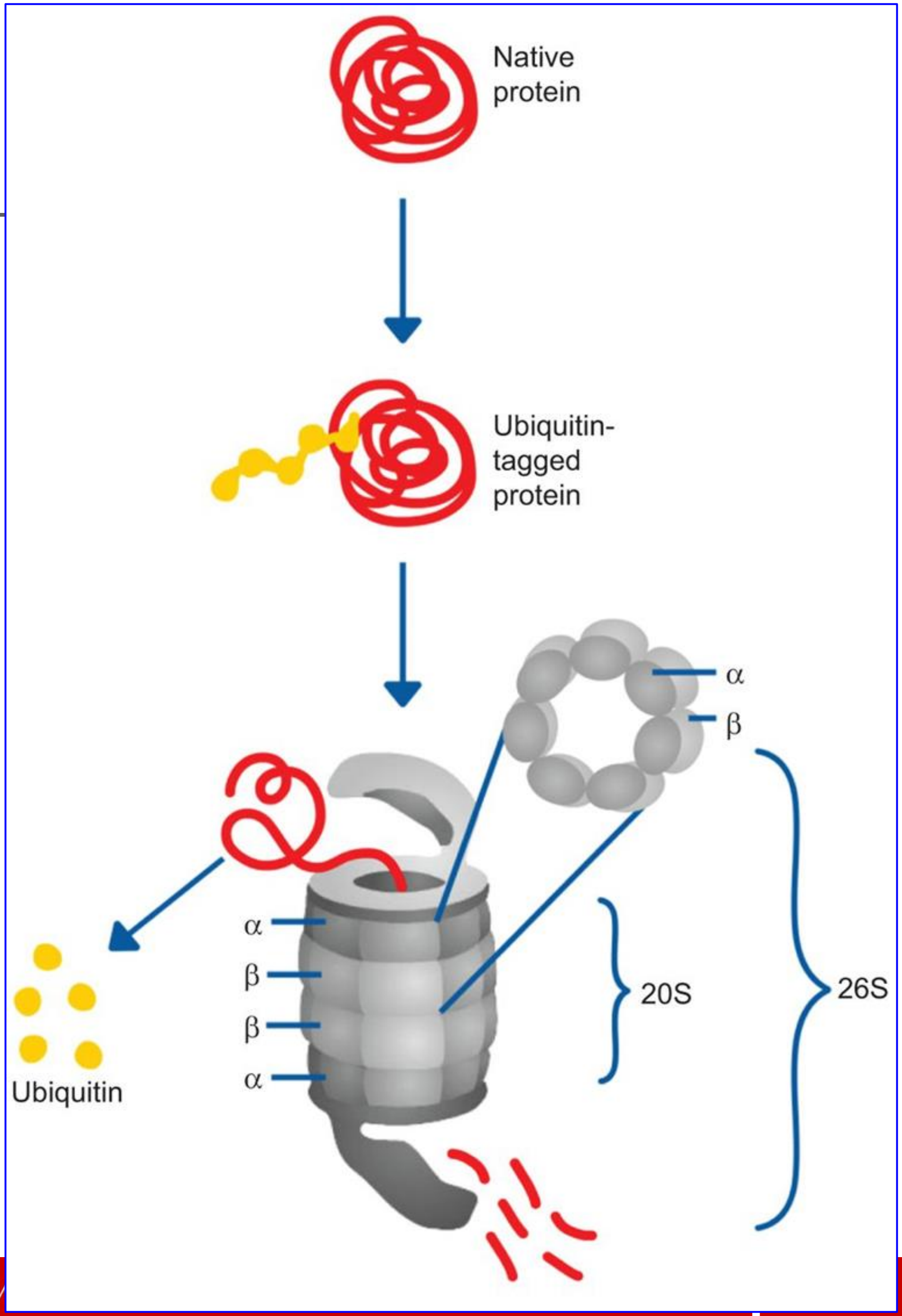


https://www.youtube.com/watch?v=-GwI-UrhpEo

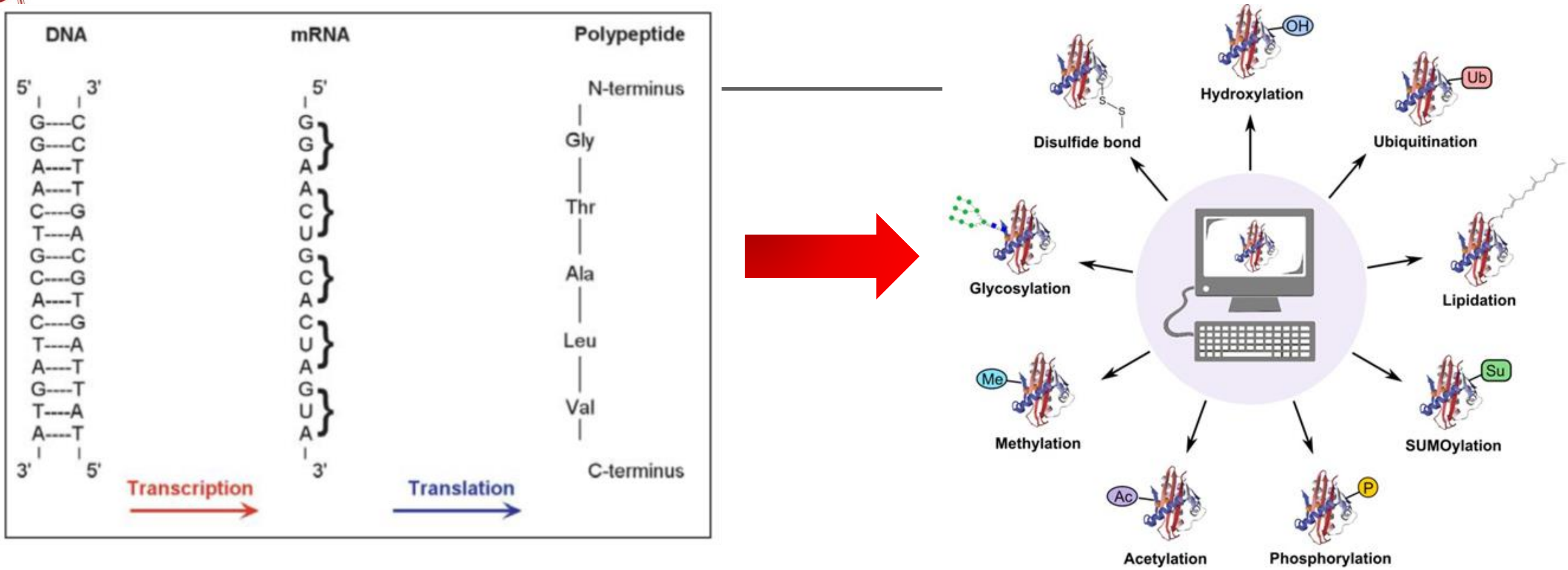



ribosome
folded protein
protein degradation
proteasome
lysosome
misfolded proteins
protein aggregates


low internal pH
Lumen contains hydrolytic enzymes.
Vacuolar ATPase
ATP
H+
ADP + Pi
Lysosome


Native protein
Ubiquitin-tagged protein
α
β
α
β
β
α
20S
26S
Ubiquitin

POST-TRANSLATIONAL MODIFICATIONS



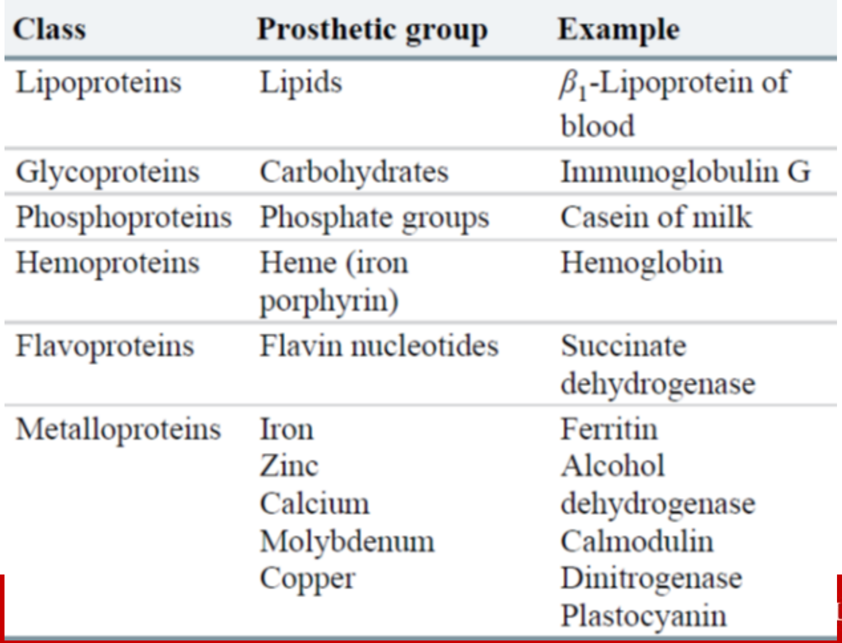
Many amino acids and proteins undergo modifications once they leave the ribosome, where one or more amino acid side chains are modified by the addition of a further chemical group. Post translational modifications lead to their final active, biological form of the protein.

Examples:

- ✓ the addition of chains of carbohydrates to form glycoproteins;
- ✓ the addition of a hydroxyl group to proline to produce hydroxyproline (found in the structure of collagen).

Some proteins contain prosthetic groups other than amino acids

- Many proteins, for example the enzymes ribonuclease A and chymotrypsin, contain only amino acid residues and no other chemical constituents; these are considered **simple proteins**.
- Some proteins contain permanently associated chemical components in addition to amino acids; these are called **conjugated proteins**. The non–amino acid part of a conjugated protein is usually called its **prosthetic group**. Conjugated proteins are classified on the basis of the chemical nature of their prosthetic groups.



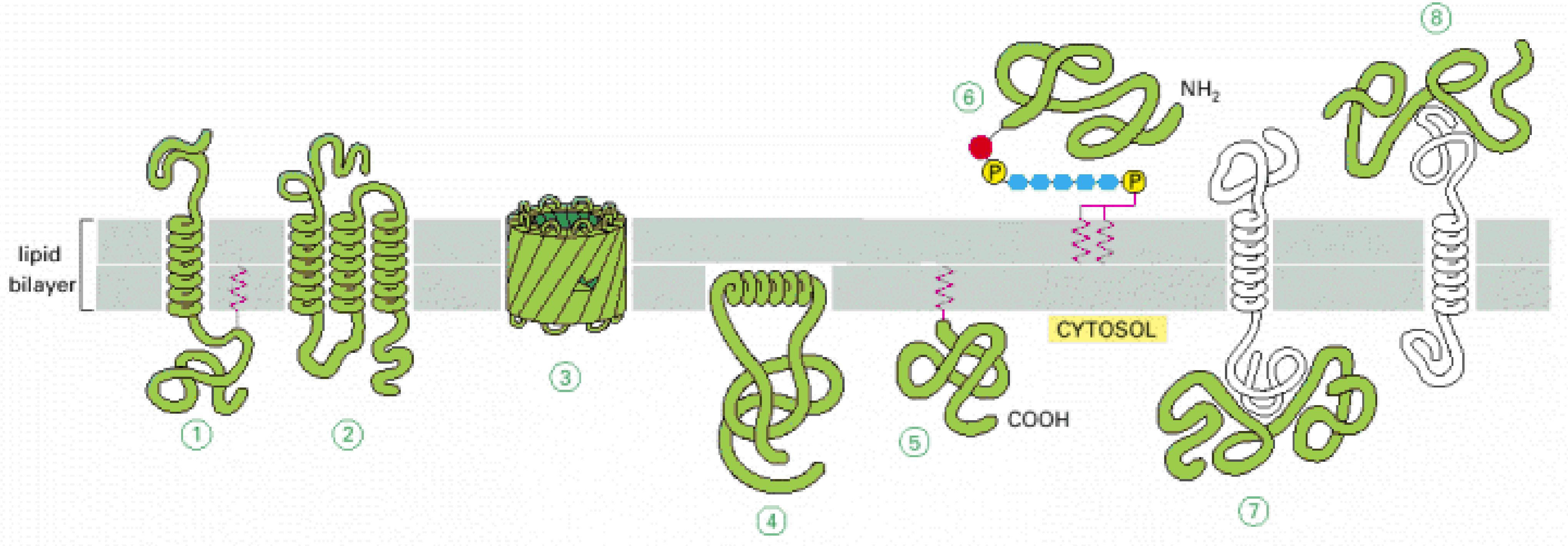
Class	Prosthetic group	Example
Lipoproteins	Lipids	β_1-Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
Metalloproteins	Iron Zinc Calcium Molybdenum Copper	Ferritin Alcohol dehydrogenase Calmodulin Dinitrogenase Plastocyanin

Flavin nucleotide is a stronger oxidizing agent and is particularly useful because it can take part in both one- and two-electron transfers.

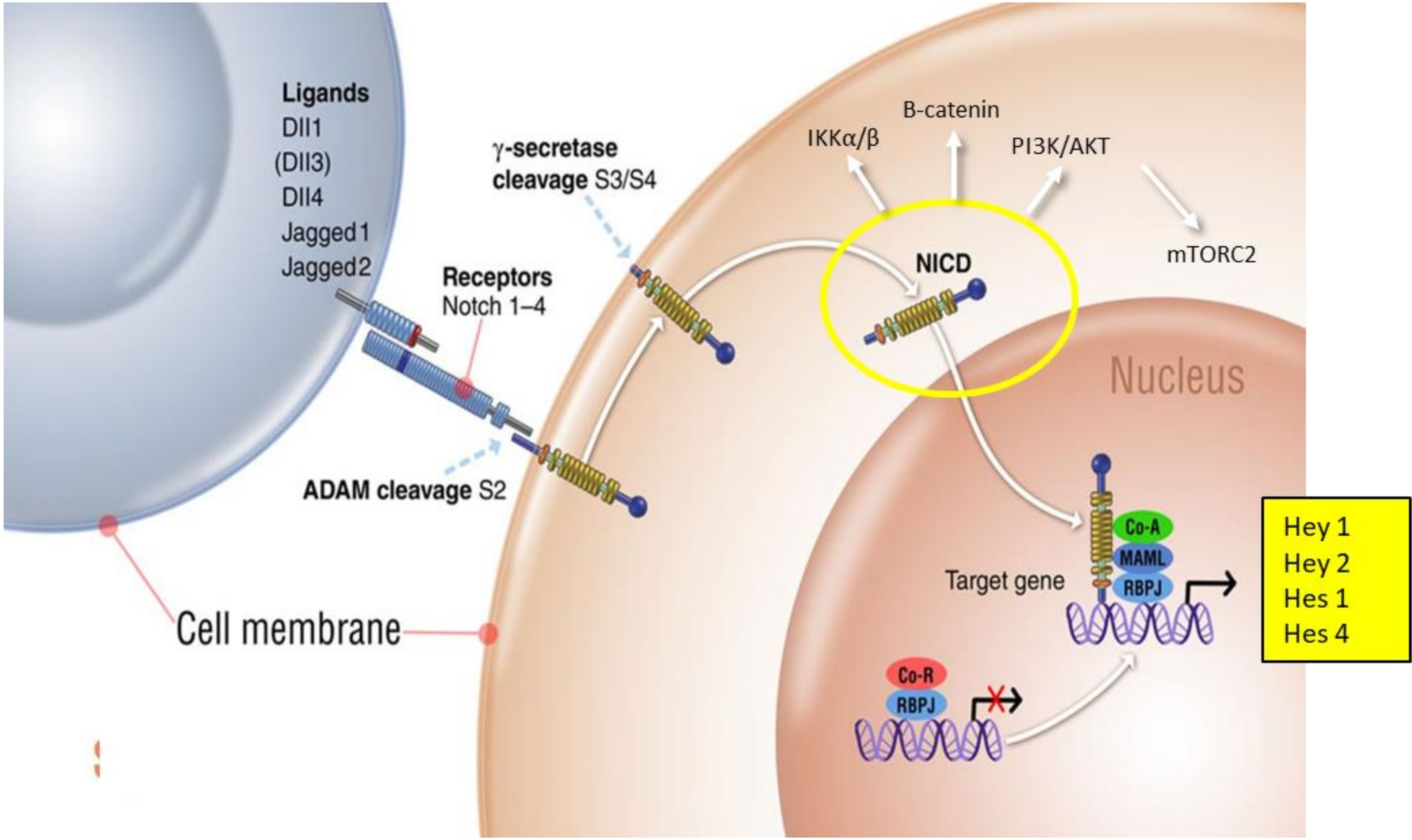
Classification of Proteins

- **Simple**: hydrolyze to amino acids only
- **Conjugated**: bound to a non-protein group, such as sugar, nucleic acid, or lipid
- **Fibrous**: long, stringy filaments, insoluble in water, function as structural components.
- **Globular**: folded into spherical shape, function as enzymes, hormones, or transport proteins.
- **Membrane proteins:** bound or associated with biological membranes

Membrane proteins associate with the lipid bilayer in different ways



The Notch Pathway



Kahoot!