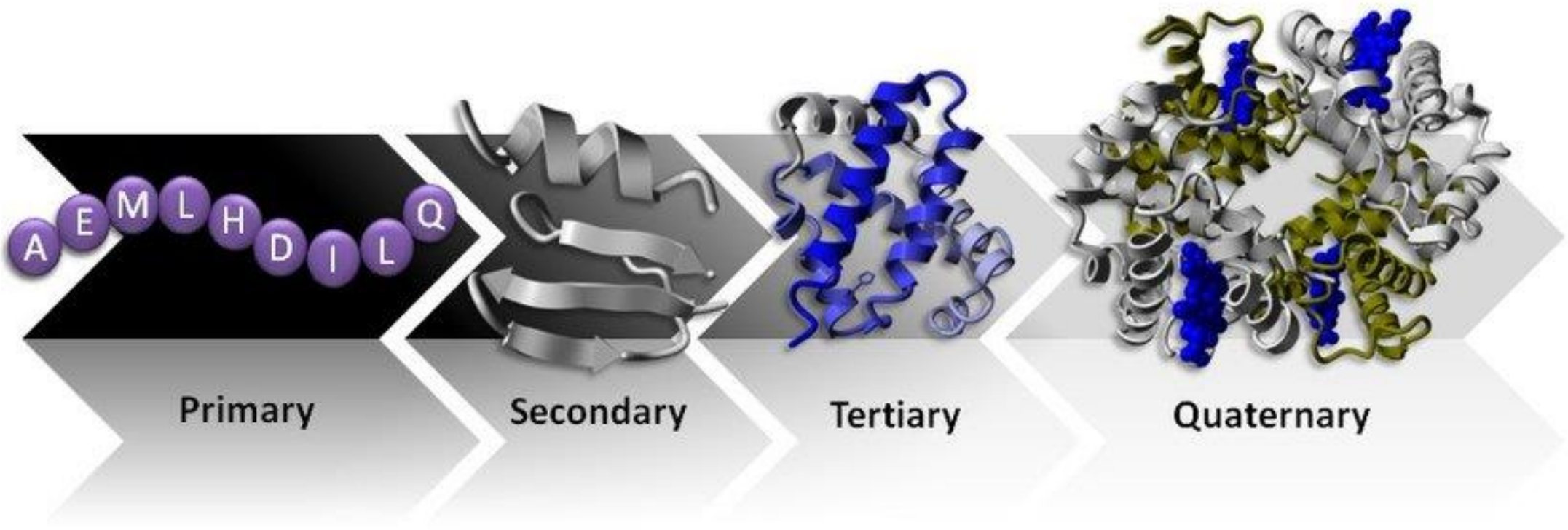
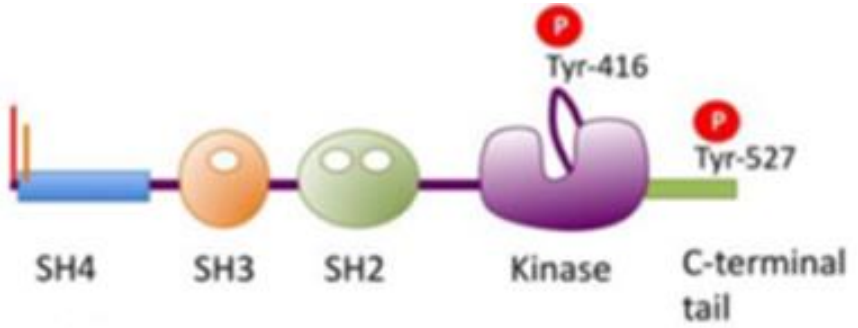
PROTEINS

The spatial arrangement of a protein involves multiple levels of organization



Protein Domains

The tertiary structure is composed of smaller folded units known as structural domains, which often exhibit a degree of conservation in their amino acid sequences as well as specific functions—such as catalytic centers, kinase domains, or SH2 and SH3 regions.



Catalytic center

Activation Loop

```
Lck  DLRAANILVSDTLSCKIADFGLARLIEDNEYTAREGAKFPIKWTAPEAINYGTFTIKSDV
Src  DLRAANILVGENLVCKVADFGLARLIEDNEYTARQGAKFPIKWTAPEAALYGRFTIKSDV
Fyn  DLRSANILVGNGLICKIADFGLARLIEDNEYTARQGAKFPIKWTAPEAALYGRFTIKSDV
Lyn  DLRAANVLVSESLMCKIADFGLARVIEDNEYTAREGAKFPIKWTAPEAINFGCFTIKSDV
Yes  DLRAANILVGENLVCKIADFGLARLIEDNEYTARQGAKFPIKWTAPEAALYGRFTIKSDV
Hck  DLRAANILVSASLVCKIADFGLARVIEDNEYTAREGAKFPIKWTAPEAINFGSFTIKSDV
     ***:**:**.  * **:*******:*********:**********  :* *******
```

N-terminal Unique domain

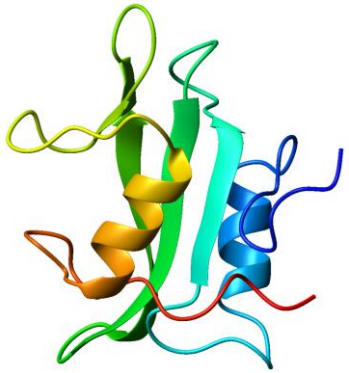
```
Lck  MGCGCSSHPEDDWMENIDV---------CENCHYPIVPLDGKGTLLIRNGSEVRDPLVTY
Src  MGSNKSKPKDASQRRRSLEPAENVH-----GAGGGAFPASQTPSKPA-SADGHRGPSAAF
Fyn  MGCVQCKDKEATKLTEERDGSLNQSS----GYRYGTDPT--PQHYPS-FGVTSIPNYNNF
Lyn  MGCIKSKGKDSLSDDGVDLKTQPV--RNTERTIYVRDPTSNKQQRPV-PESQLLPGQ-RF
Yes  MGCIKSKENKSPAIKYRPENTPEP--VSTSVSHYGAEPT--TVS-PC-PSSSAKGTAVNF
Hck  MGGRSSCEDPGCPRDEERAPRMGCMKSKFLQVGGNTFSKTETSASPHCPVYVPDPTSTIK
     **.  ..  .                          *                    :
```

A specific structural domain can be found in many different proteins, e.g., SH2, kinase structure

~40% of prokaryotic and ~65% of eukaryotic proteins consist of multiple distinct structural domains linked together by flexible segments of polypeptide chains (multidomain proteins).
The structural domains range in length from 25 to 500 amino acids

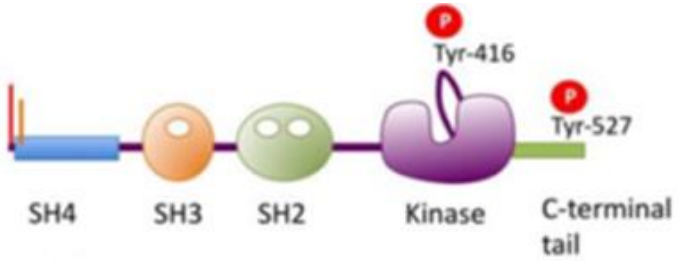
Many structural domains of eukaryotic proteins constitute independent proteins in prokaryotes, suggesting that multidomain proteins likely arose as a result of selective pressure during evolution to accommodate new functions.

SH2 domain



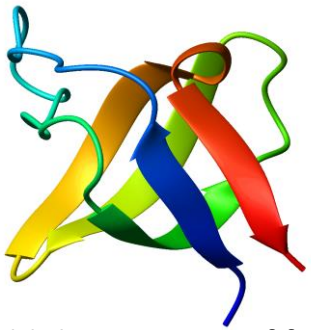
A large beta sheet (green) flanked by two alpha-helices (orange and blue).

Protein domains are distinct structural and functional units that give proteins specific abilities like binding, signaling, and catalysis.

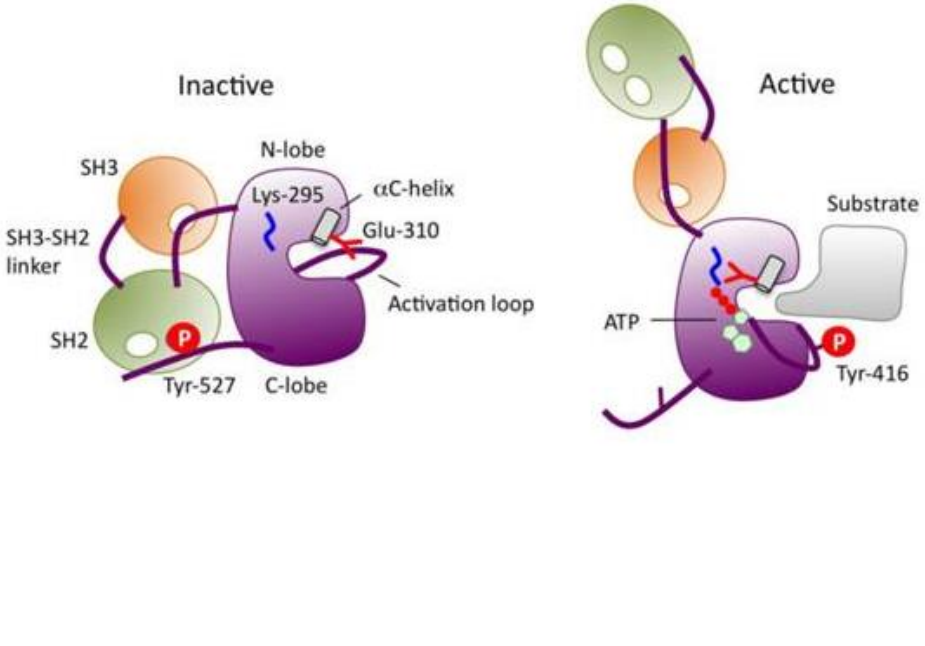


SH3 domain

An ancient fold found in eukaryotes as well as prokaryotes



β-barrel fold that consists of five or six β-strands arranged as two tightly packed anti-parallel β sheets. The linker regions may contain short helices.



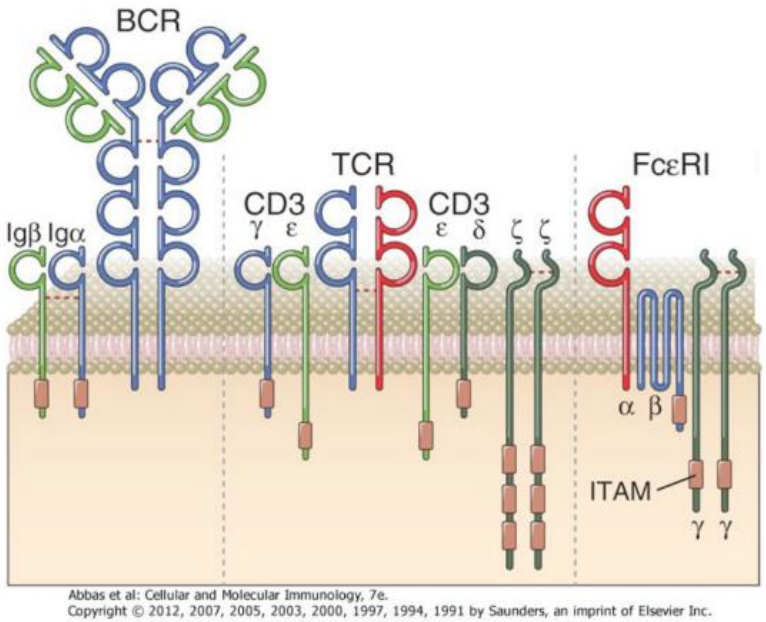
Protein motifs

A specific array of AA (short in length) that follow specific patterns of conservation and perform defined functions

pYEEI: A motif recognized by Src-family SH2 domains.

PxxP : the most well-known SH3 binding motif, where
P=proline and x=is any amino acid.

YxxL/I -6,8 YxxL/I: ITAMs (Immunoreceptor Tyrosine Activating Motifs): 2 YxxL/I repeats, separated by 6 or 8 Aas.
Y=Tyrosine, x=is any amino acid, (L/I): Leucine or Isoleucine.
The signal-initiating module of antigenic and Fc receptors



Abbas et al: Cellular and Molecular Immunology, 7e.
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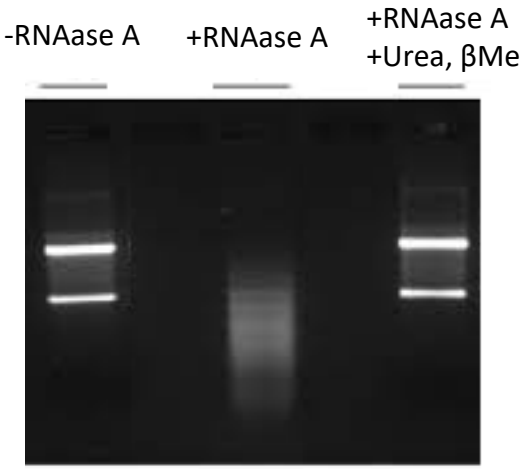
		Amino acid sequence
TCR	CD3γ	EQLYQPLKD–REYDQYSHL
	CD3δ	EQLYQPLRD–REDTQYSRL
	CD3ε	NPDYEPIRK–GQRDLYSGL
	CD3ζa	NQLYNELNL–GRREEYDVL
	CD3ζb	EGVYNALQKDKMAEAYSEI
	CD3ζc	DGLYQGLST–ATKDTYDAL
BCR	Igα	ENLYEGLNL–DDCSMYEDI
	Igβ	DHTYEGLNI–DQTATYEDI
FcR	FcRγ	DAVYTGLNT–RSQETYETL
	FcεRIβ	DRLYEELNH–VYSPIYSEL
NKR	DAP12	ESPYQELQG–QRPEVYSDL
Virus	BLV gp30	DSDYQALLP–SAPEIYSHL
	EBV LMP2A	HSDYQPLGT–QDQSLYLGL
	consensus	Dxx Yxx Lxx–xxxxx Yxx L/I E I I

Christian Anfinsen
Nobel Prize in Chemistry 1972

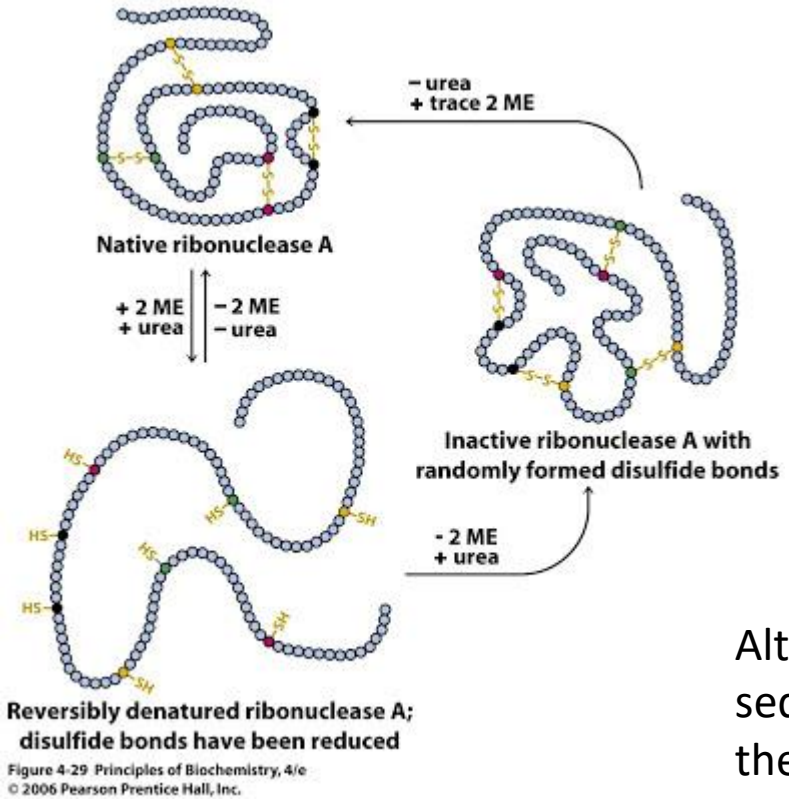


Anfinsen's Dogma: The amino acid sequence determines the three-dimensional structure and, therefore, the biological activity of the protein—the relationship between structure and function

Loss of the enzymatic activity of Ribonuclease A (RNase A) in a urea/β-ME solution, and its recovery upon removal of the chaotropic agents



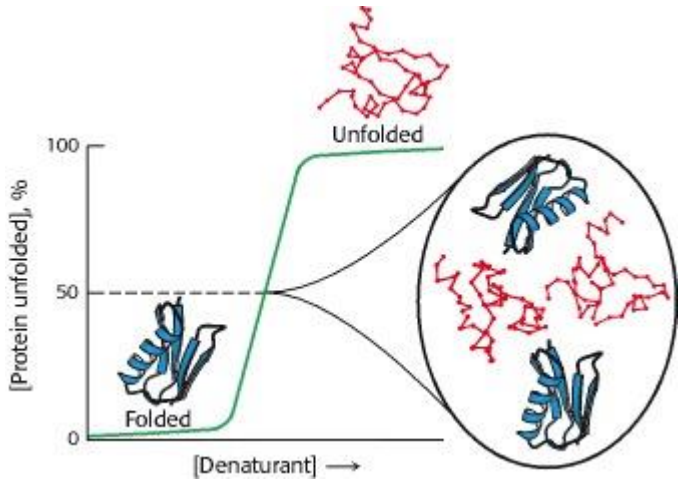
*Compounds such as **urea** (which breaks noncovalent bonds) and **β-mercaptoethanol** (which breaks disulfide bonds) cause a loss of tertiary structure (**protein denaturation**) and are called denaturing or chaotropic agents.*



Removal of β-ME and subsequently of urea: The protein refolded but regained only 1% of its enzymatic activity

Although a polypeptide chain **potentially has multiple "folding options"**, any given AA sequence will fold into one specific and biologically active 3D conformation, bypassing the formation of alternative, non-functional structures.

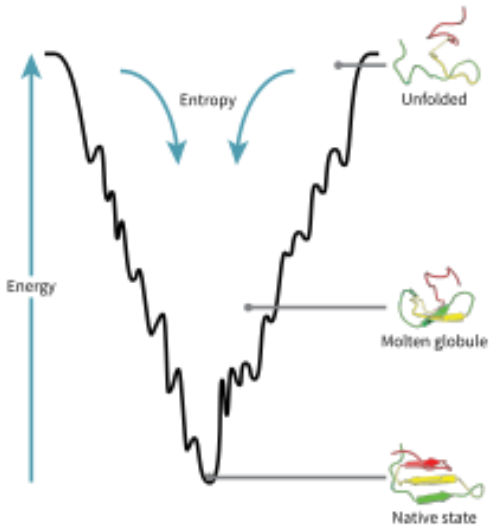
Why commit to a single configuration when there are thousands of possible combinations?



https://www.ncbi.nlm.nih.gov/books/NBK22342/bin/ch3f57.gif

The process of folding is based on the gradual stabilization of intermediate structures rather than on the quest for the “perfect structure” and the rejection of all incorrect ones.

Folding is driven by both hydrophobicity and thermodynamics, so as to maximize interactions such as hydrogen bonds and salt bridges, while at the same time protecting the hydrophobic residues from the cell’s aqueous environment (in the typical case of a soluble protein).



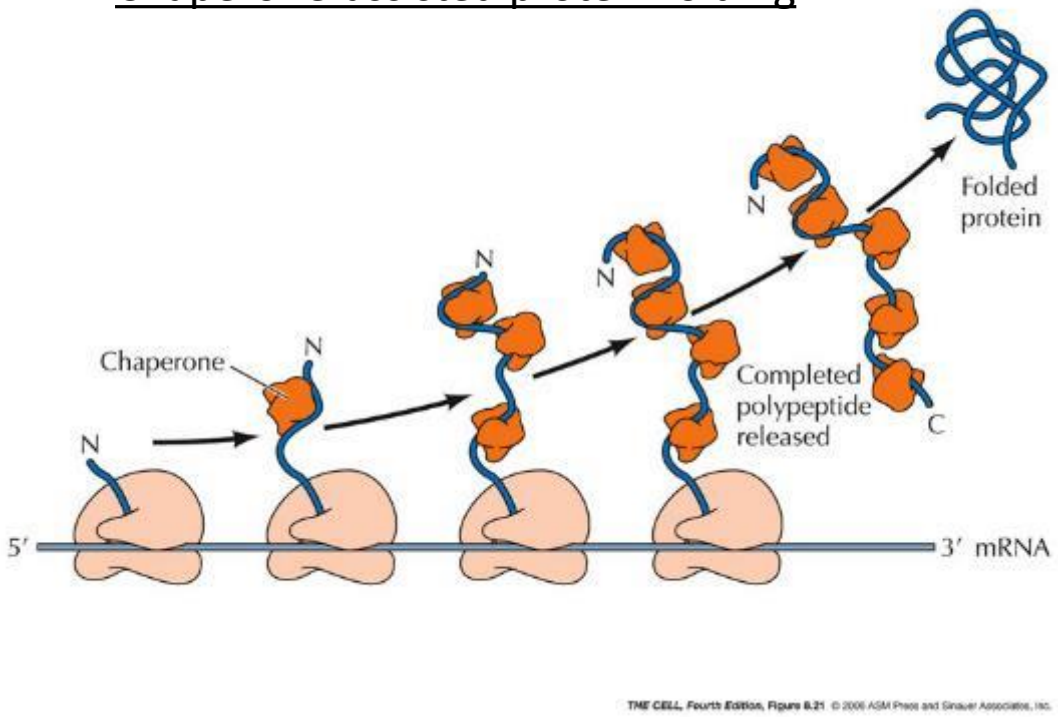
https://upload.wikimedia.org/wikipedia/commons/thumb/9/91/Folding_funnel_schematic.svg/220px-Folding_funnel_schematic.svg.png

This process is driven by a **decrease in free energy,** which is achieved only when the various conformations are converted to the protein's stable, native conformation.

Protein folding involves the transition from a mixture of unfolded molecules with high disorder to a solution of folded proteins with greater order and, therefore, lower entropy.

Chaperones: Cells possess specialized proteins that work together to fold proteins

Chaperone-assisted protein folding



THE CELL, Fourth Edition, Figure 6.21 © 2006 ASM Press and Sinauer Associates, Inc.

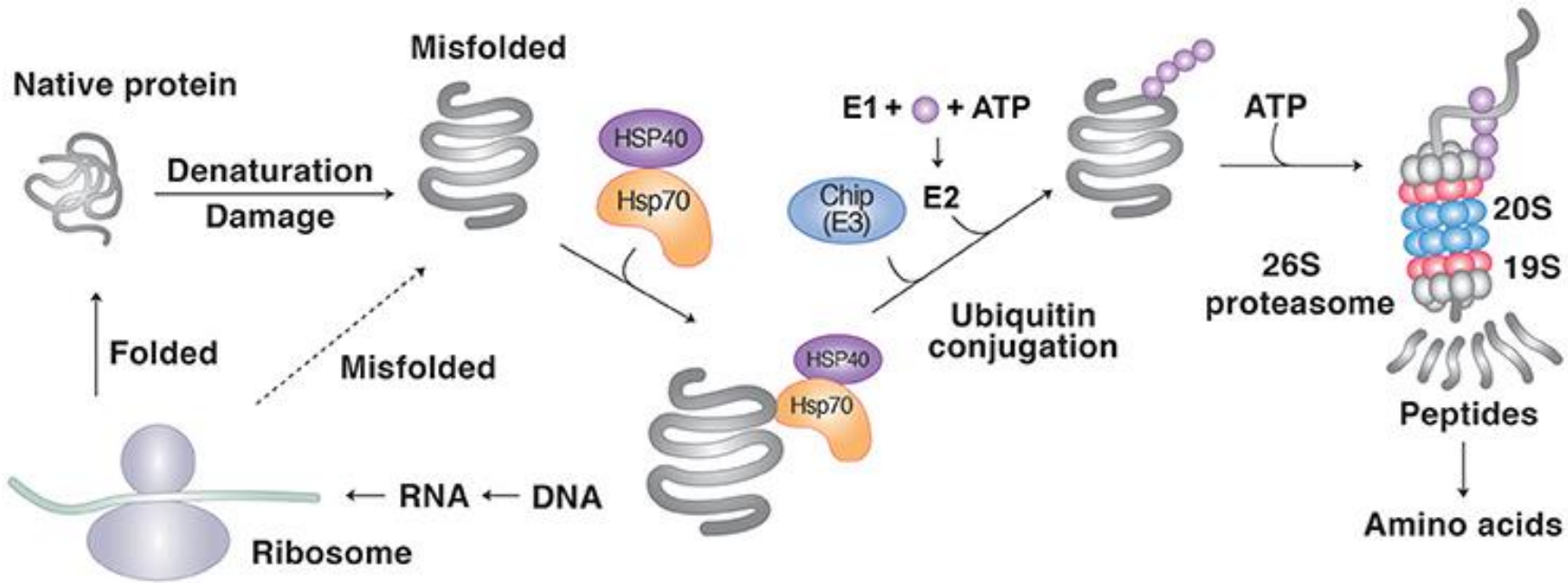
The intracellular environment is crowded, filled with many different macromolecules capable of interacting with a partially folded protein and interfering with its normal folding.

Chaperones surround a polypeptide chain, isolating it until the folding process is complete.

Although hundreds of bonds hold proteins together, most of these bonds are non-covalent and relatively weak. Consequently, folded proteins are fragile structures that can easily be denatured or unfolded.

An increase in body temperature of just a few degrees can significantly increase the rate of denaturation. When this happens, repair of existing proteins, using chaperones, is much more efficient than synthesizing new ones. Interestingly, cells synthesize or upregulate the expression of certain chaperones in response to "heat shock."

Chaperone-mediated degradation of misfolded proteins



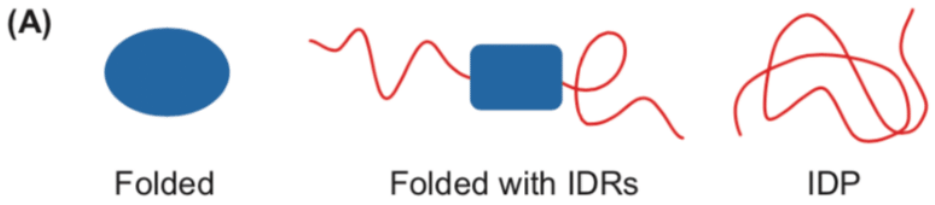
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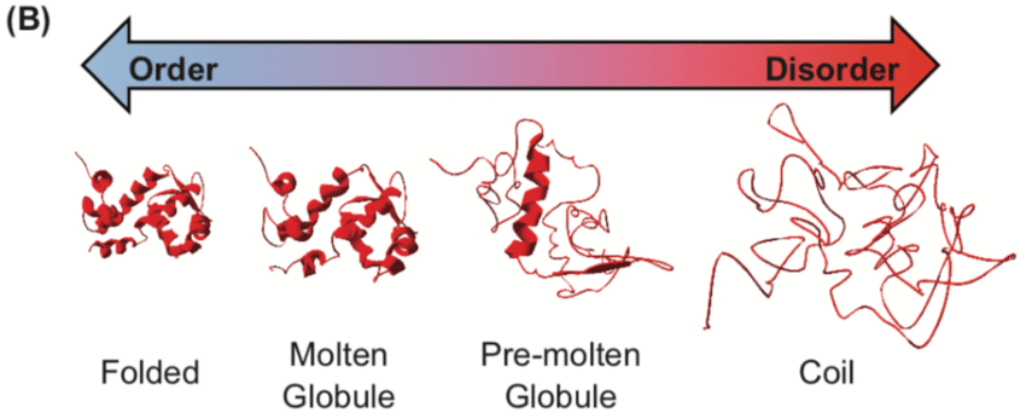
Chaperones also play a role in the removal of harmful or defective proteins by promoting interaction with ubiquitin (ubiquitin E3 ligase) and the subsequent marking of these proteins for proteolysis.

Intrinsically Disordered Proteins/Protein Regions (IDPs/IDPRs)

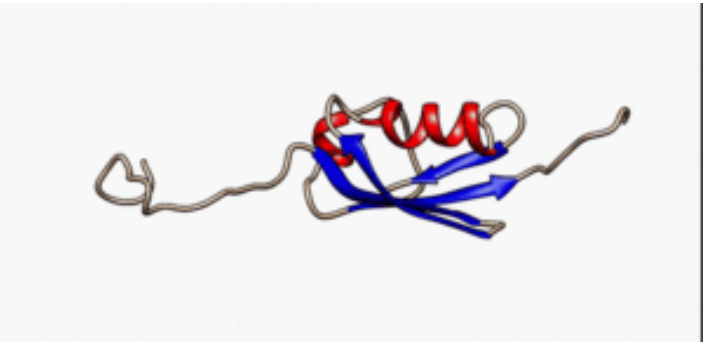
- Proteins that do not have a stable or precisely defined 3D structure.
- IDPs range from completely unstructured to partially structured and include the following conformations:
- random coil, molten globule-like aggregates, or flexible linkers within multidomain proteins
- They are sometimes considered a separate category of proteins along with globular, fibrous, and membrane proteins.

~50% of eukaryotic proteins contain at least one IDPR.

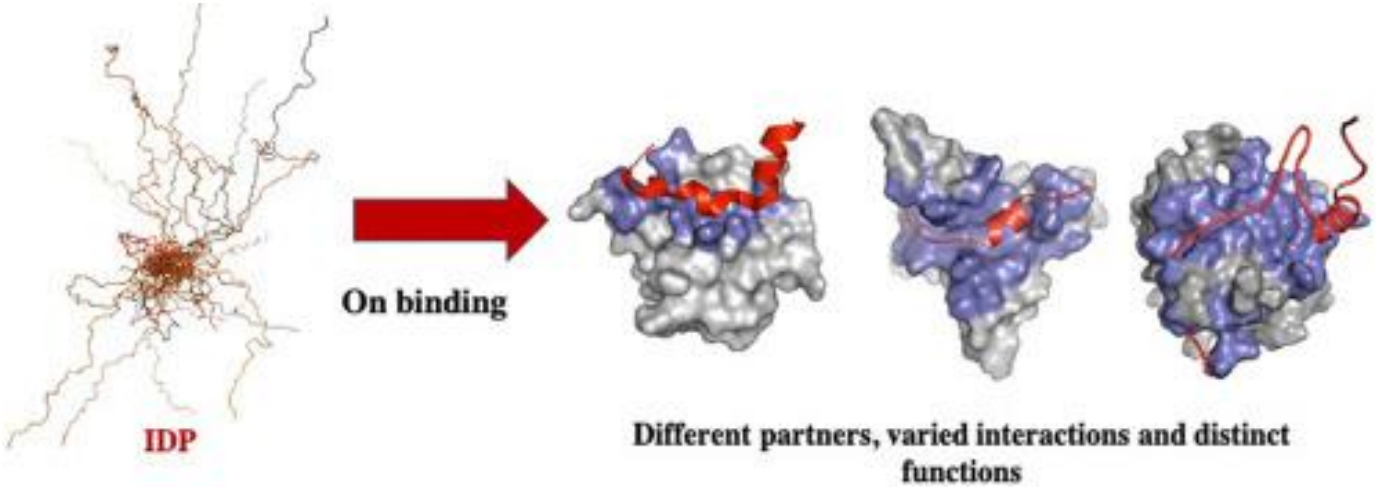


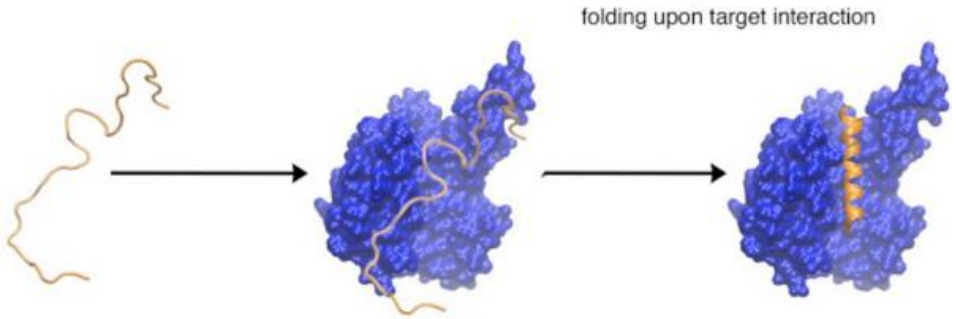


(B) The structures populated by IDP(R)s sit on a continuum between stably folded (left) and random coil (right), ranging from collapsed molten globule-like structures, in which a significant amount of secondary structure is present through to expanded coil-like structures with little or no secondary structure.



Conformational flexibility in SUMO-1 protein. The central part shows relatively ordered structure. Conversely, the N- and C-terminal regions (left and right, respectively) show 'intrinsic disorder', although a short helical region persists in the N-terminal tail.





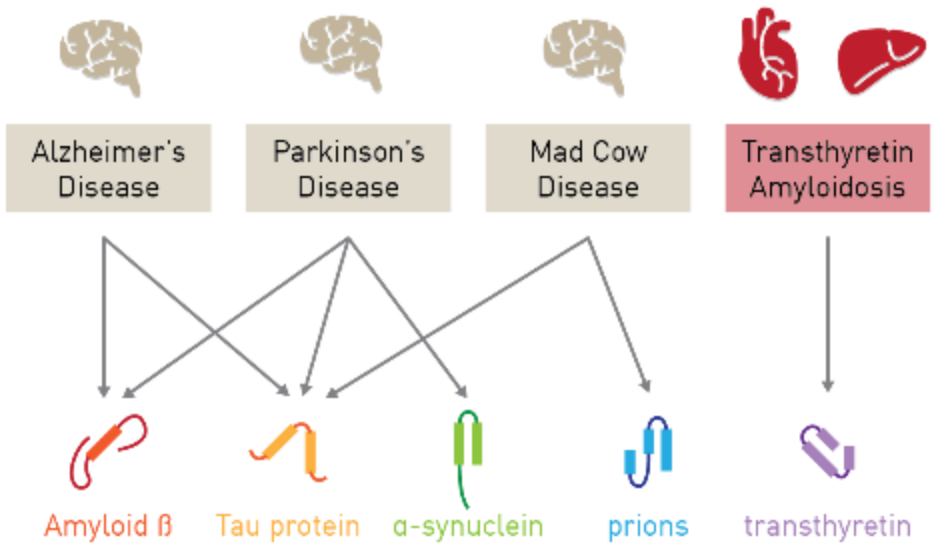
IDPs/IDRs can form complexes otherwise impossible, such as wrapping around the binding partner, hence providing the plasticity needed for achieving assemblies with specific functions.

IDRs can exhibit promiscuity, using the same region in the sequence to bind multiple partners, and act as hubs in protein-protein interaction networks (an essential part of the cell signalling network).

Some IDPs adopt a stable structure after binding to another macromolecule. This molecular adaptability means that a protein can have many different structures and interactions, performing various biological functions.

They usually participate in signaling and regulatory cellular functions (often being implicated in neoplasms and neurodegenerative diseases).

Amyloidoses: Group of diseases associated with protein misfolding



http://neurophage.com/wp-content/uploads/2014/07/Protein-Misfolding-Disease-Image-1.png

All these diseases are characterized by the **deposition of protein aggregates known as fibrils or amyloid plaques**.

Amyloid fibrils are formed from normally soluble proteins that aggregate to form insoluble fibers resistant to proteolysis.

Each disease is characterized by a specific protein or peptide that aggregates. Well-known examples of amyloid diseases include Alzheimer's disease, Parkinson's disease, and spongiform encephalopathies.

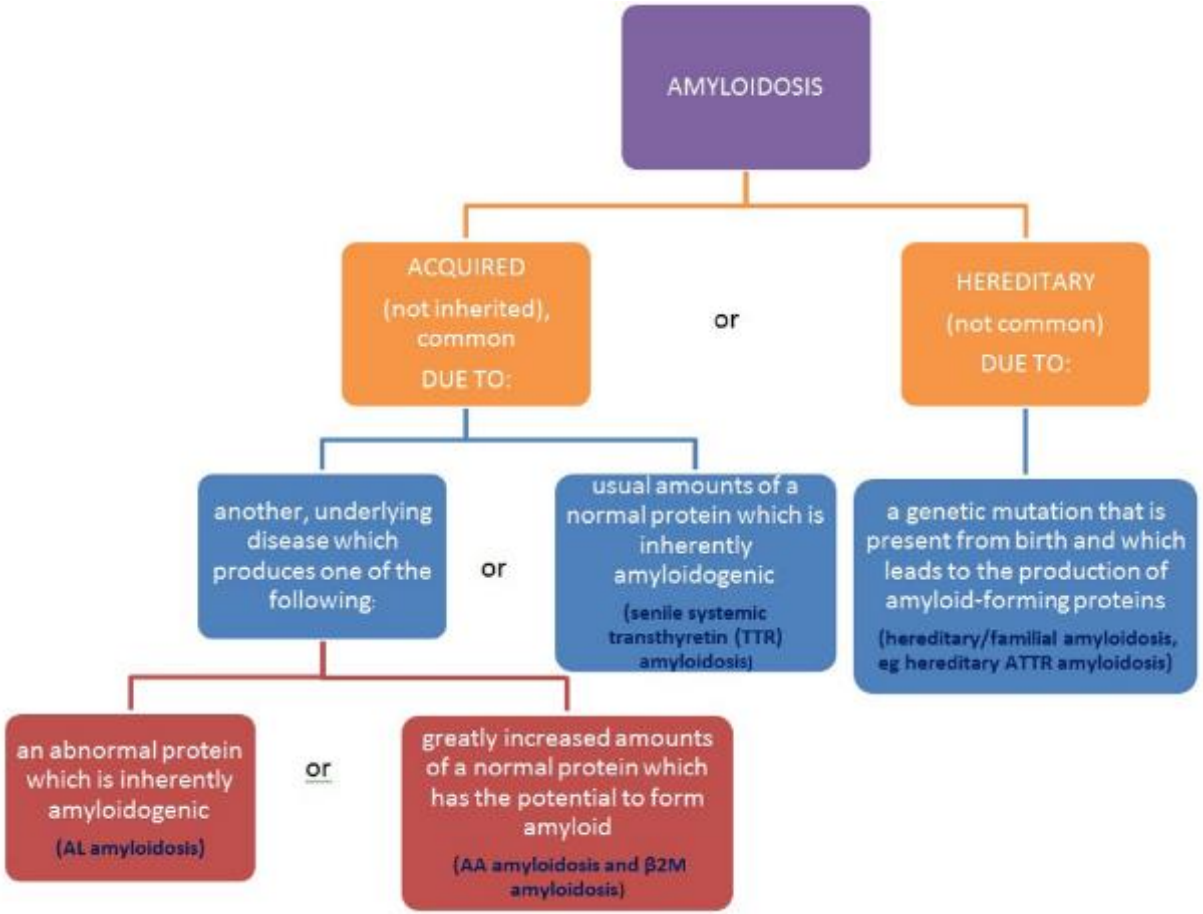
To date, 37 peptides or proteins have been found to form amyloid deposits in pathological conditions. Most of these are secreted, and the resulting deposits are located in the extracellular space.

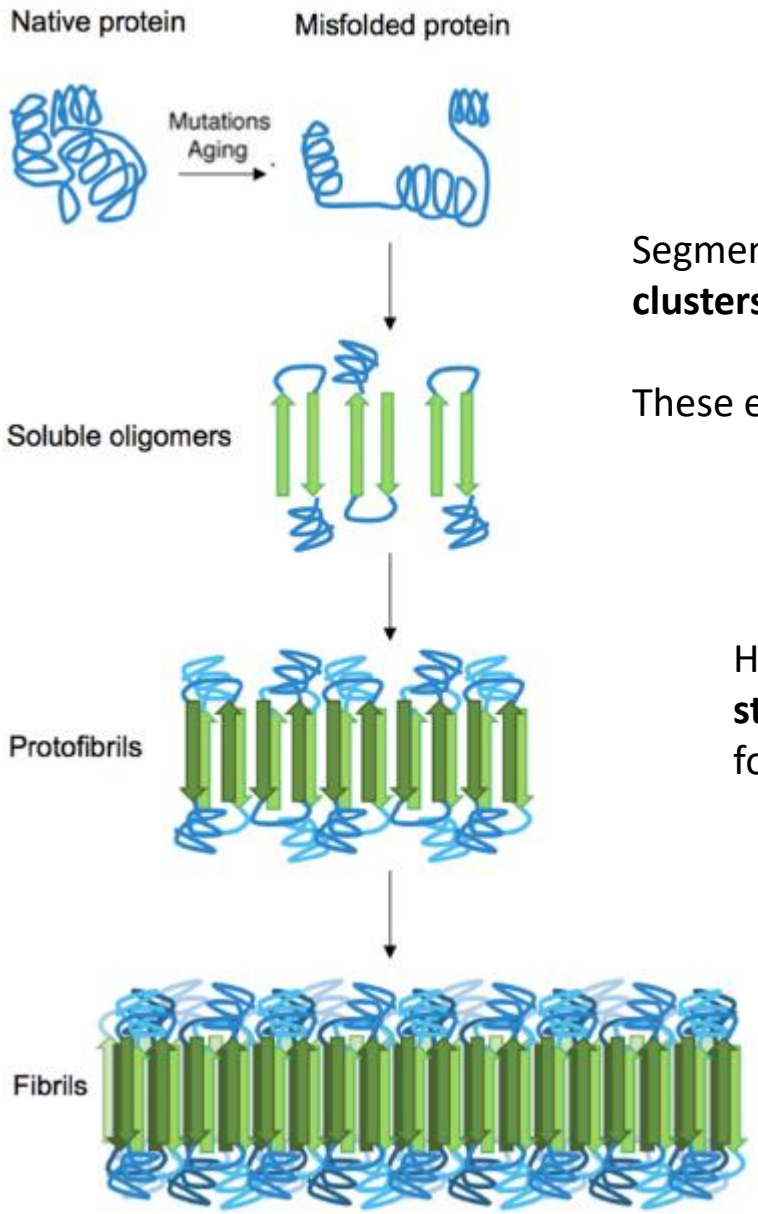
Of the known proteins associated with amyloidoses, 14 possess well-defined 3D structures.

13 are intrinsically disordered proteins (IDPs), 9 have an unknown structure, and 1 contains a combination of globular and disordered regions.

One-third of diseases are **hereditary** (autosomal dominant disorders) and manifest at a young age. Mutations are typically located in the regions of the polypeptide chain that are involved in the formation of aggregates and are pathogenic.

Half of the amyloidoses are largely **acquired** and <u>appear in advanced age</u>, suggesting that the phenomenon of protein aggregation stems primarily from the **progressive loss of regulatory control over folding associated with aging**.





Segments of the protein (potentially having a different structure, e.g., α-helices) **convert into β-sheets, and clusters comprising** a relatively small number of molecules are formed.

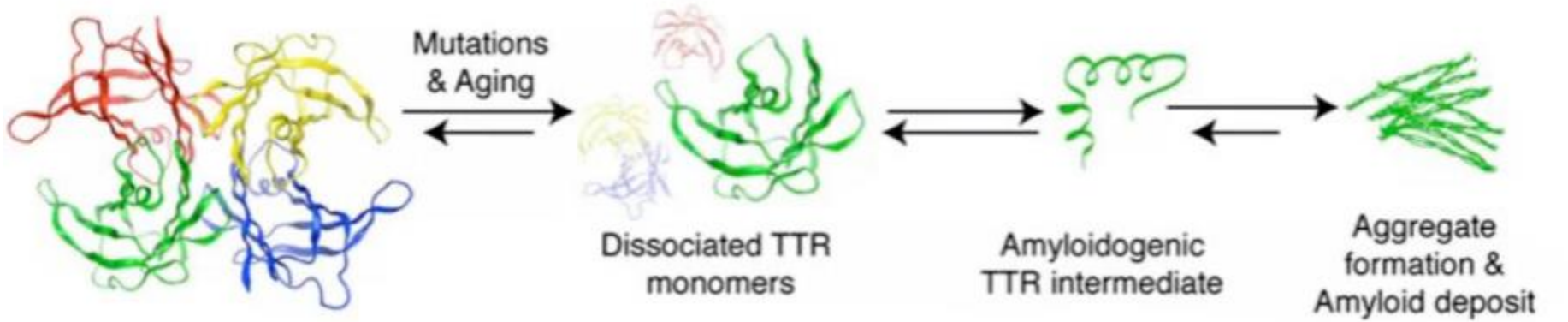
These early aggregates are usually unstable and can simply dissociate to regenerate soluble species.

However, if aggregation proceeds, the oligomers can undergo internal reorganization to form **more stable species with a β-sheet structure**—a process often accompanied by an increase in size and the formation of compact structures.

These β-structured oligomers grow further through **self-association or the addition of monomers**. Dramatic structural rearrangements lead to the formation of well-defined, compact fibrils with a β-sheet structure extending perpendicular to the fibril axis.

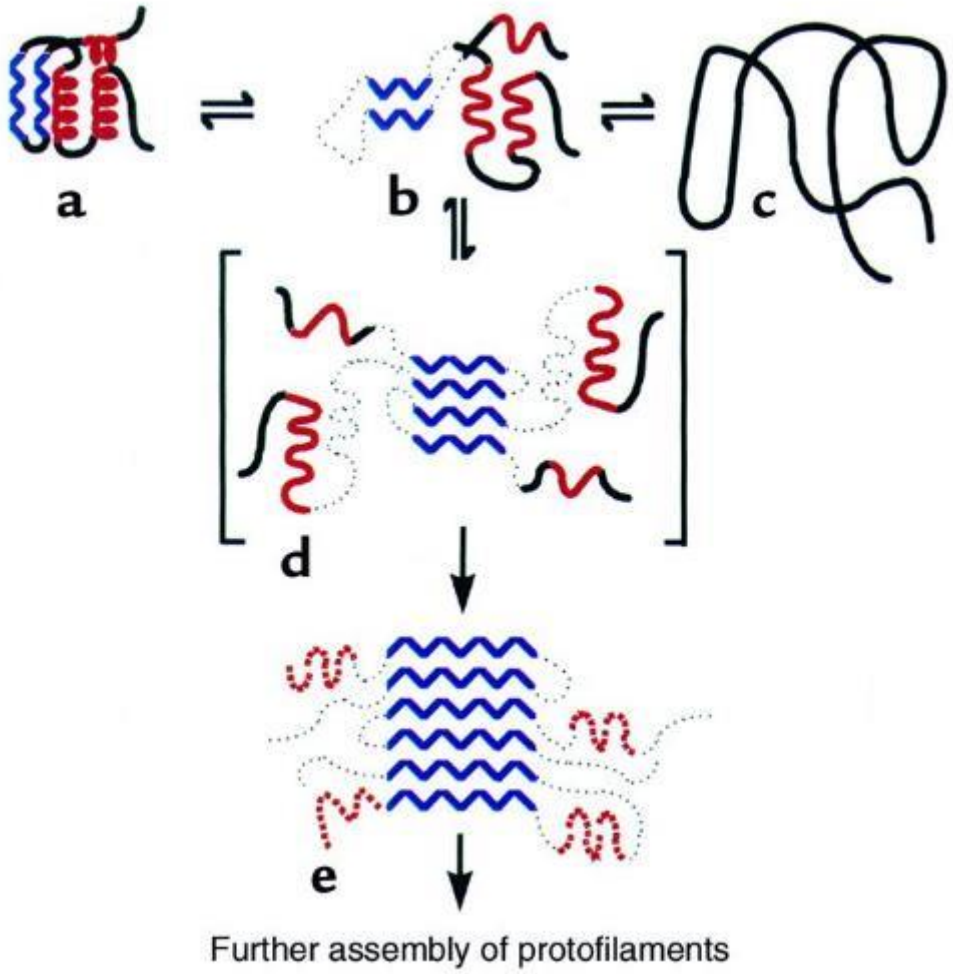
https://ars.els-cdn.com/content/image/1-s2.0-S1359511313004728-gr8.jpg

More than **100 mutations** associated with hereditary forms of transthyretin amyloidosis (familial amyloid polyneuropathy) have been identified; these mutations lead to the **destabilization of the protein's native tetrameric form**, resulting in elevated levels of the amyloidogenic monomer.



https://i1.wp.com/alhamadshehlab.org/wp-content/uploads/2015/09/lab1.png?w=1140

Transthyretin (TTR or TBPA) is a transport protein in the serum and cerebrospinal fluid that carries the thyroid hormone thyroxine (T4) and retinol-binding protein bound to retinol.



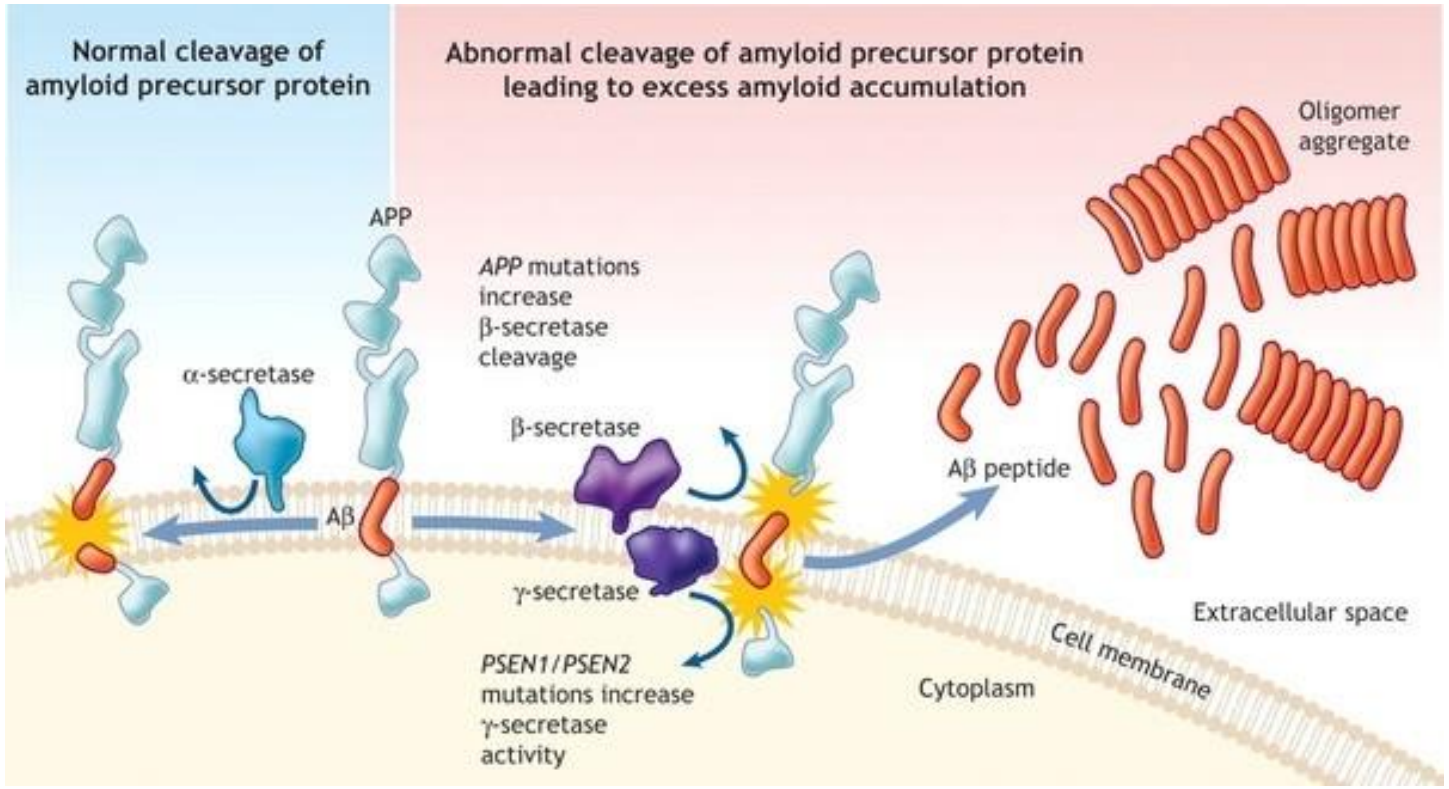
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To date**,** have been identified **eight pathogenic mutations in lysozyme** that **destabilize the formation of the 3D structure,** leading to an **incompletely folded protein.** The formation of partially folded structures facilitates aggregation, as hydrophobic segments of the polypeptide—normally buried in the interior—**become exposed and available for intermolecular interactions.**

Mechanism proposed for lysozyme amyloidosis. An amyloidogenic intermediate state (b) formed from destabilization of the native structure (a) is proposed to self-associate through β-strand elements (blue) to initiate fibril formation (d, e), rather than forming non-native monomer (c).

Other mutations render the proteins susceptible to proteolytic cleavage, generating amyloidogenic fragments.

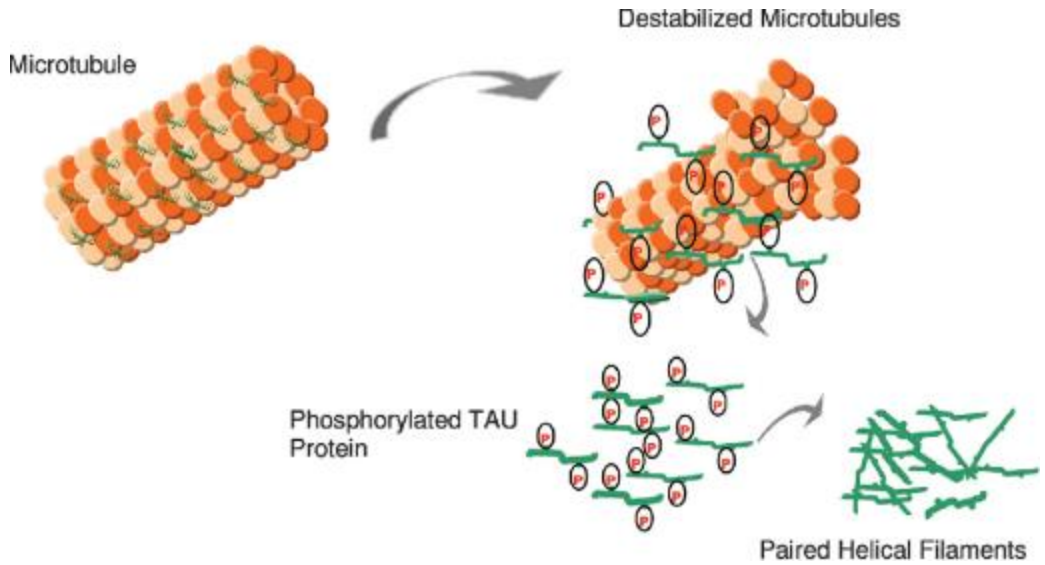
Amyloid Precursor Protein (APP) is considered to play a regulatory role in the formation of neuronal synapses. It is a transmembrane precursor protein cleaved by specific proteases (secretases) to generate peptides with various biological functions (such as transcriptional activation and apoptosis).



APP is best known as the precursor molecule whose proteolysis yields beta-amyloid (Aβ)—a polypeptide of 37–49 amino acids—the fibrillar form of which is the main component of amyloid plaques found in the brains of patients with Alzheimer's disease.

https://qph.fs.quoracdn.net/main-qimg-7be72392fe0c68aee9f0a5acef7666fb-c

The Tau protein (**t**ubulin-**a**ssociated **u**nit) has been implicated in the pathogenesis of neurodegenerative diseases such as Alzheimer's and Parkinson's; it belongs to the class of intrinsically disordered proteins (IDPs), and its interaction with filamentous actin promotes its folding into a three-dimensional conformation rich in alpha-helices.



https://ai2-s2-public.s3.amazonaws.com/figures/2017-08-08/d3d5483d65da899573b5885a84d3d441f4c53313/4-Figure2-1.png

Mutations that cause a loss of tau binding to microtubules result in the unfolding of the protein into a disordered, unstructured state prone to the formation of amyloid fibrils.
Furthermore, mutations also increase the aggregation rate of the resulting unfolded protein.

Protein Infection Agents- Prions

Prions are **infectious protein particles** formed by the abnormal folding and subsequent aggregation of proteins. It is not known what causes the misfolding of the normal protein and its conversion into infectious prions.

Prions are considered a subclass of amyloids in which protein aggregation becomes **self-propagating and infectious**.

Prion-related **amyloidoses** are **transmissible**.

<u>Like other infectious agents (bacteria, viruses), prions can spread from one organism to another.</u>

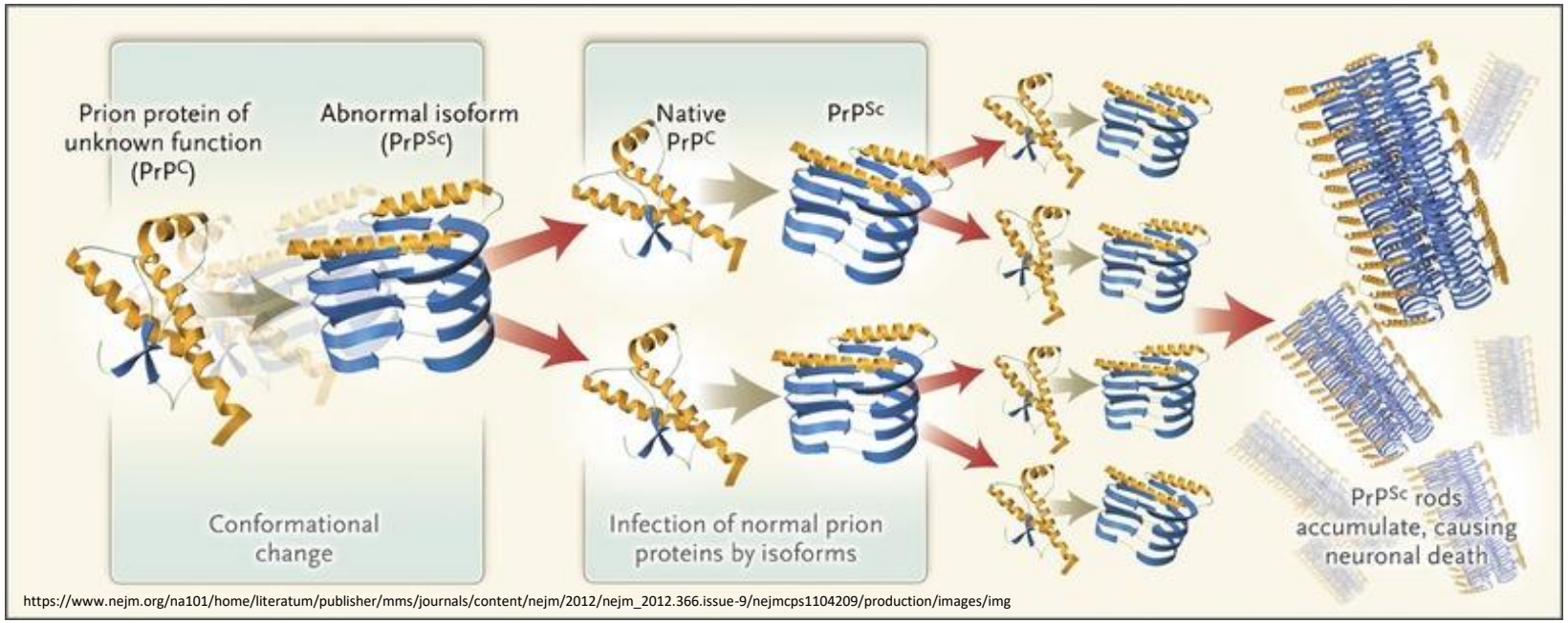
Ingestion through food is the most common natural mode of transmission.
Ritualistic cannibalism has been identified as the cause of the transmission of a prion disease known as Kuru in Papua New Guinea, and a small number of people are believed to have contracted Creutzfeldt-Jakob disease from consuming cattle infected with the bovine form of the disease.

People have also been infected through blood transfusions, injections of human hormones, and surgery involving contaminated instruments.



Unlike viruses and all living organisms, and although prions lack the standard information-storage molecules (DNA, RNA), they can replicate and transmit biological information.

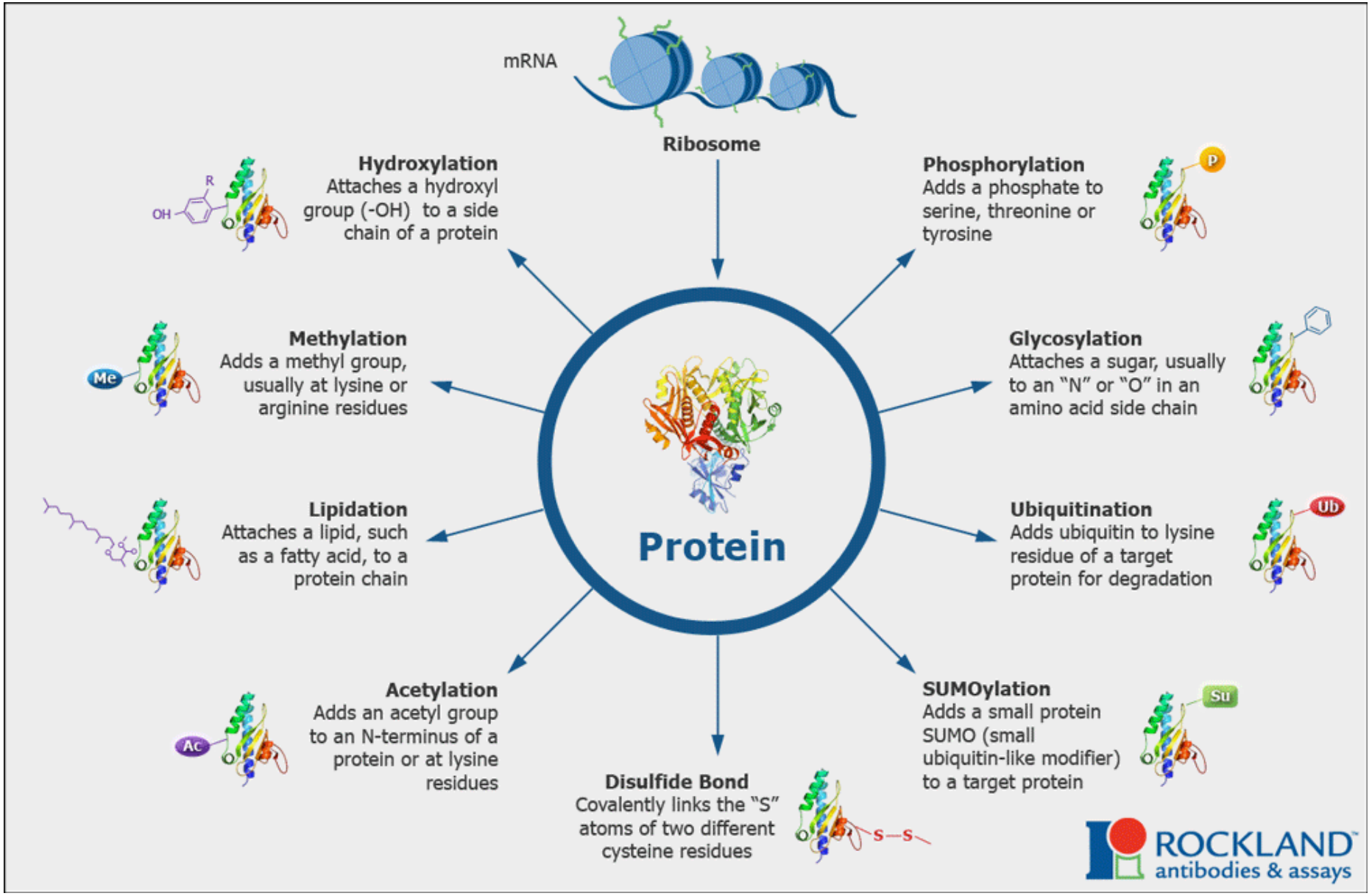
The particles that spread scrapie in sheep, spongiform encephalopathy in cows, and Creutzfeldt-Jakob disease in humans consist of extremely stable crystals of a normally harmless, alpha-helix-rich protein (PrPC) found in the brains of sheep.



https://www.nejm.org/na101/home/literatum/publisher/mms/journals/content/nejm/2012/nejm_2012.366.issue-9/nejmcps1104209/production/images/img

The progression of the disease involves **the interaction of endogenous PrP^C with the prion form, PrP^{SC}**(which is typically acquired through diet). This interaction promotes conformational changes in PrPC, converting it into the β-sheet-rich PrPSc. The process continues until nearly all of this protein within a cell or tissue has been converted into prions. In the case of scrapie and other mammalian diseases, the consequence of this self-amplifying cycle is the accumulation of toxic protein aggregates that destroy neurons.

Post translational modifications



Most proteins undergo some form of post-translational modification after synthesis.

TABLE 3–4 Conjugated Proteins

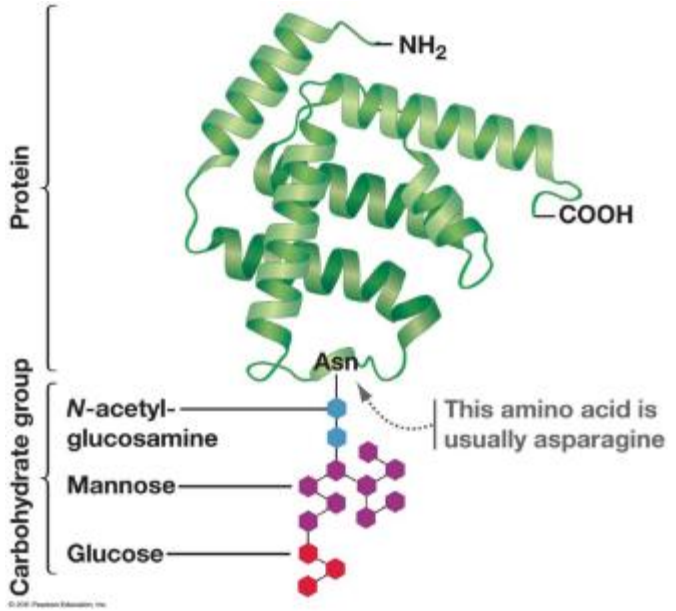
Class	Prosthetic group	Example
Lipoproteins	Lipids	β_1-Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Metalloproteins	Iron	Ferritin
	Zinc	Alcohol dehydrogenase
	Calcium	Calmodulin
	Molybdenum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

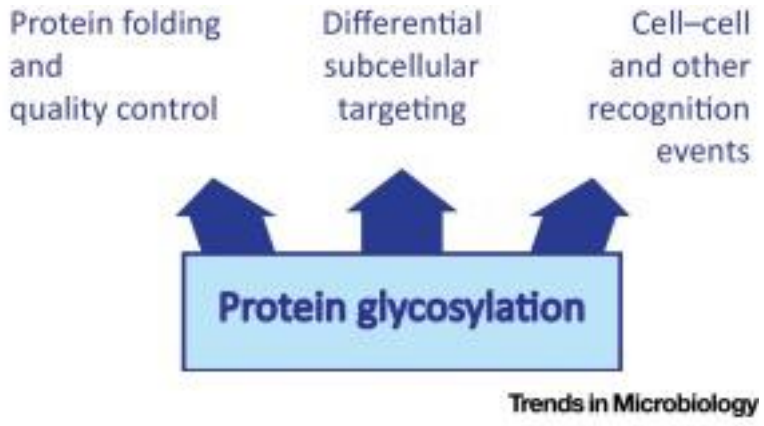
Table 3-4
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W. H. Freeman and Company

Glycosylation

The attachment of sugars to proteins (Ser, Thr, and Asn residues) is a post-translational modification that provides greater proteome diversity.

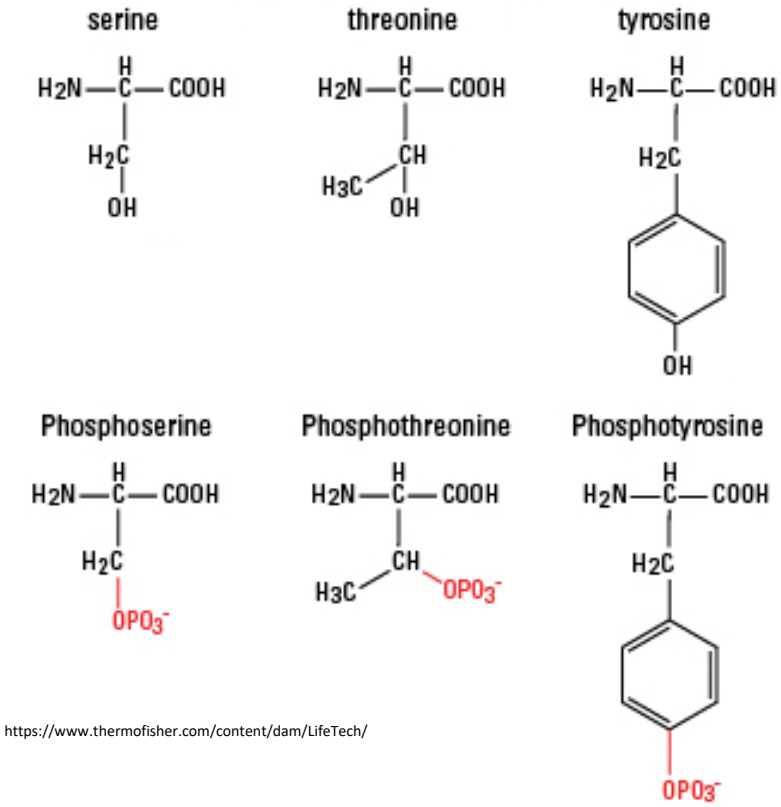
Glycosylation is critical for a wide range of biological processes, including cell adhesion, interaction with the extracellular matrix, and protein-ligand interactions.



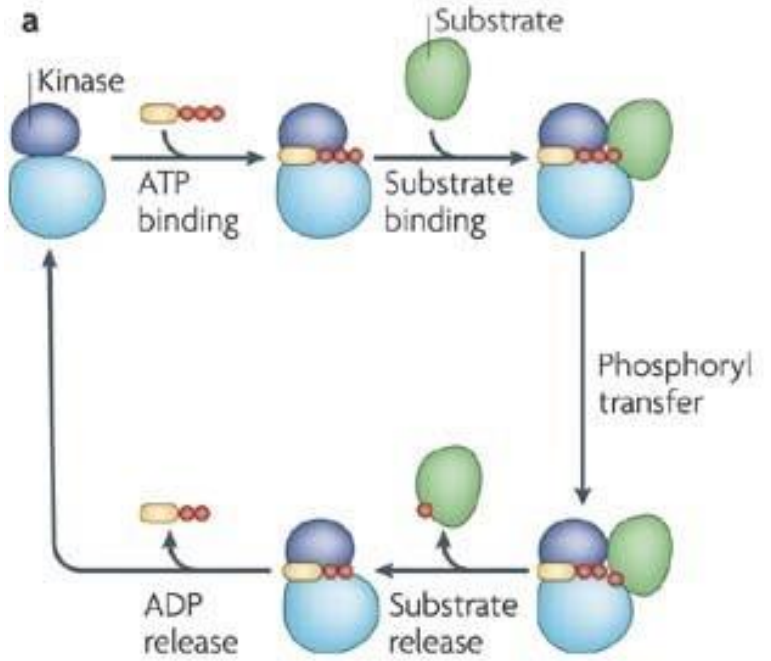


Phosphorylation

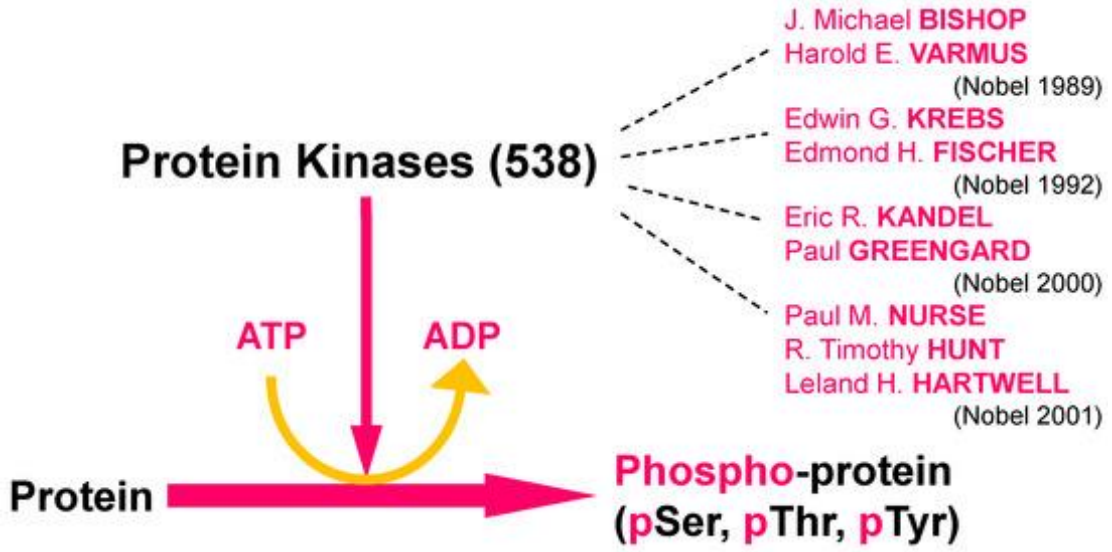
Reversible process, significant for the biological importance of proteins.



https://www.thermofisher.com/content/dam/LifeTech/

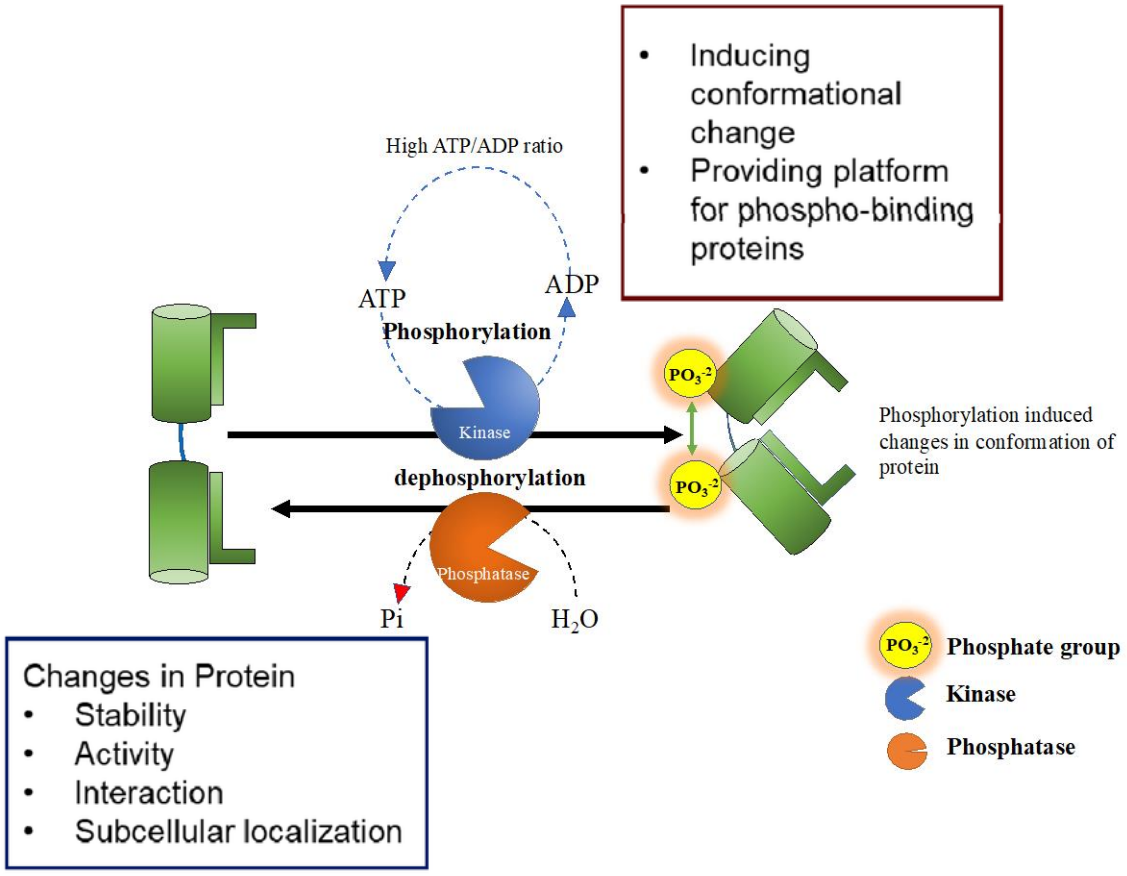


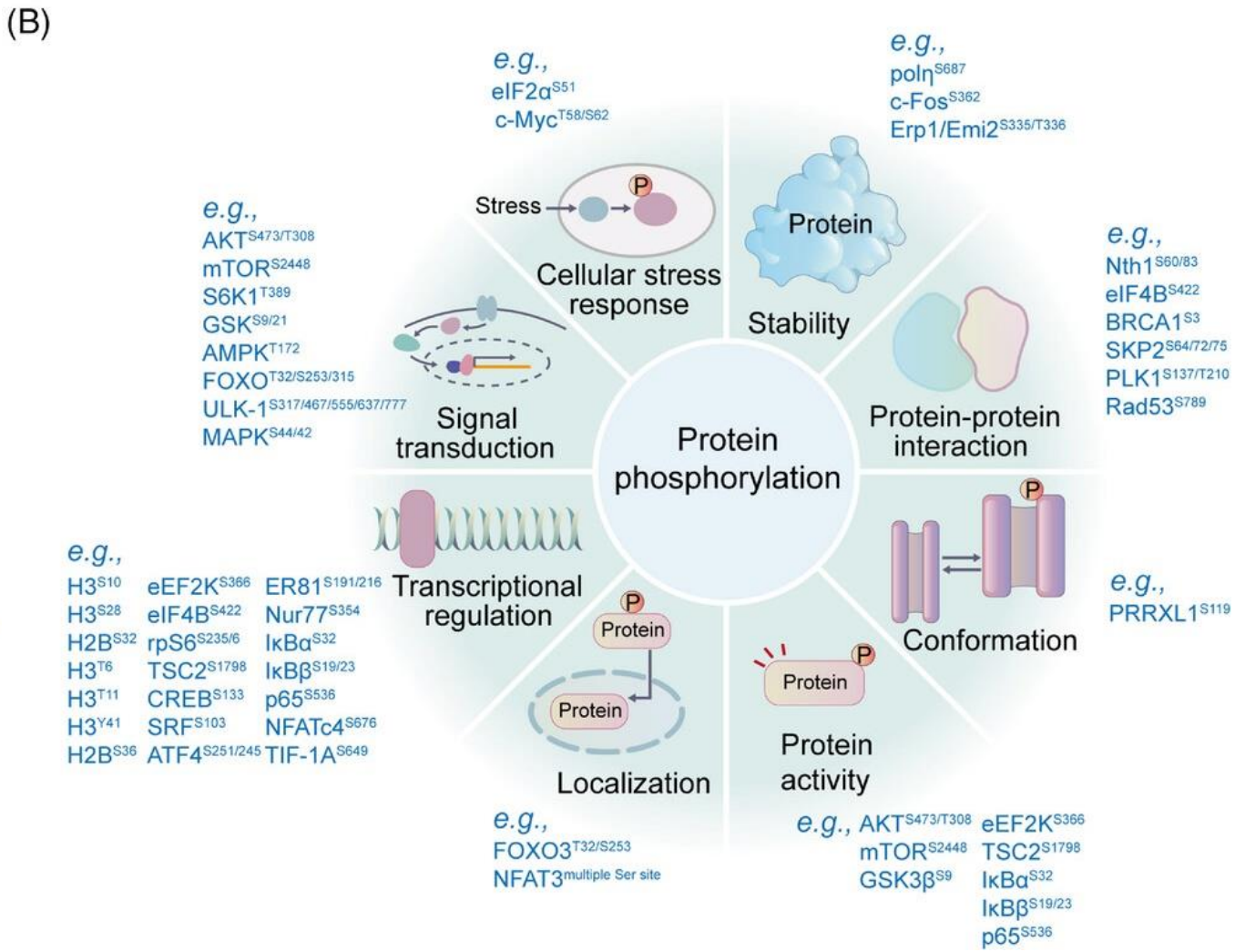
The human kinome comprises 538 protein kinases.



Protein kinases catalyze the transfer of the γ-phosphate of ATP to the hydroxyl substituents of serine, threonine, or tyrosine residues, altering the physiological properties of their protein substrates.

Protein phosphatases are reversing the action of protein kinases (remove phosphate groups from phosphorylated proteins), acting as a molecular switch to turn cellular signals on or off

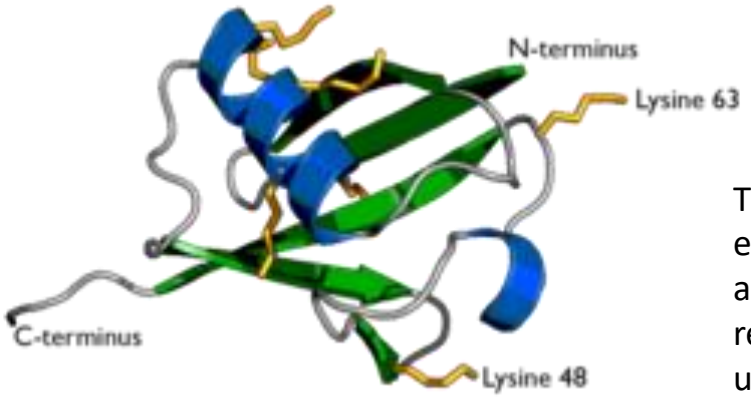




https://doi.org/10.1002/mco2.261

Ubiquitination

Ubiquitin: A small, globular (~76 amino acids, 8.5 kDa), highly conserved protein found in all eukaryotic organisms.
>95% homology across species (differing by only 2–3 amino acids between humans and yeast).



Ubiquitin β-grasp fold. The seven lysine sidechains are shown in yellow/orange.

β-grasp fold: a central alpha helix flanked by a five-stranded antiparallel beta sheet.

The structure is extremely stable, allowing ubiquitin to remain intact even under harsh conditions (e.g., pH or temperature fluctuations).

In humans, ubiquitin is encoded by four genes. All of them encode the exact same 76-amino-acid protein sequence but differ in their organization and expression.

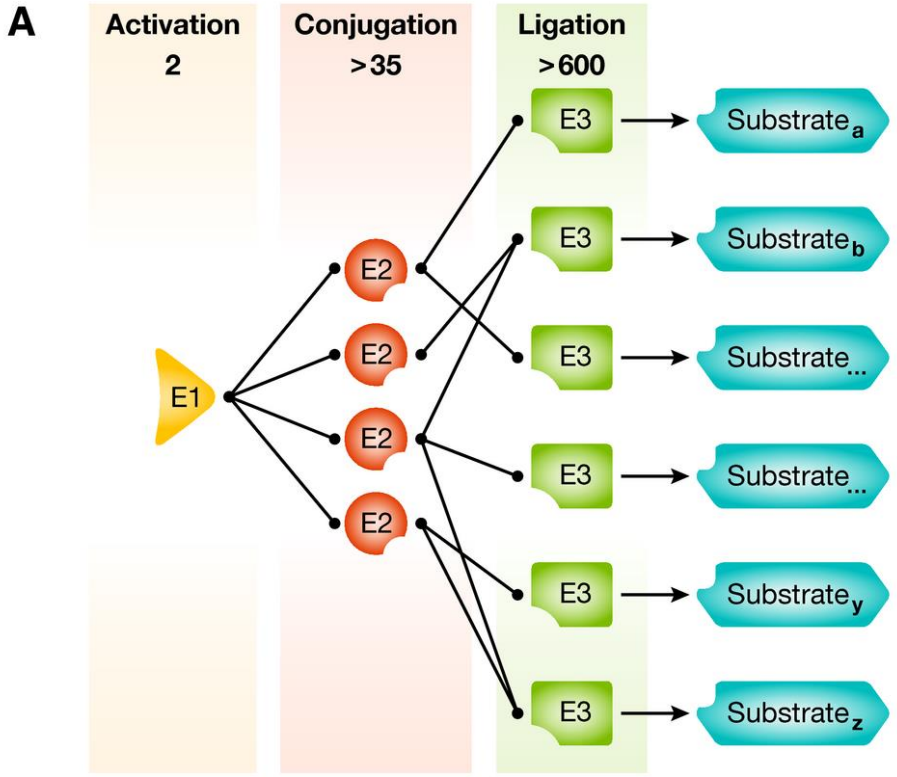
- **UBB**

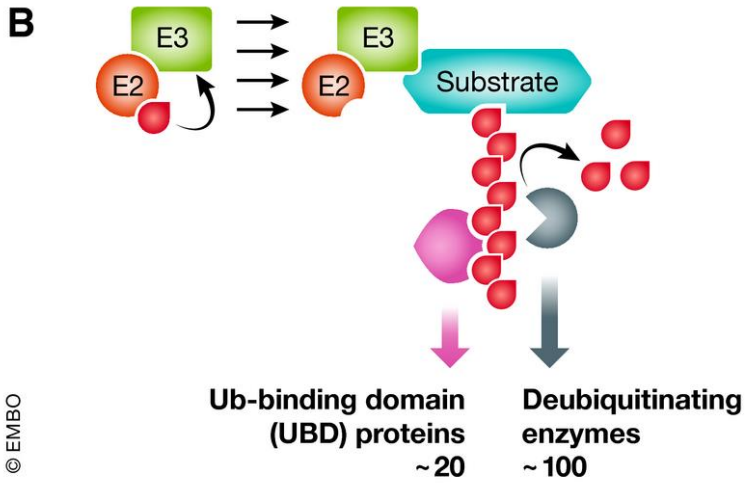
It encodes a polyubiquitin precursor chain—specifically, approximately 3–4 tandem repeats of the ubiquitin sequence.
Following translation, intracellular proteases cleave the polypeptide chain into ubiquitin monomers.

- **UBC**

It is similar to UBB but typically contains more ubiquitin repeats (up to nine).
Its expression is induced by stress (e.g., heat shock, oxidative stress, inflammation).
It is considered an "inducible" ubiquitin gene and helps maintain protein homeostasis when the demand for protein degradation increases.

- **UBA52** It encodes a fusion of ubiquitin with ribosomal protein L40 (RPL40). It is translated into a ubiquitin-L40 fusion protein, which is subsequently cleaved enzymatically to release ubiquitin and the ribosomal protein.
- **UBA80 / RPS27A.** Similar to UBA52, it encodes a ubiquitin-ribosomal protein S27a fusion. It is also cleaved into two separate functional proteins.





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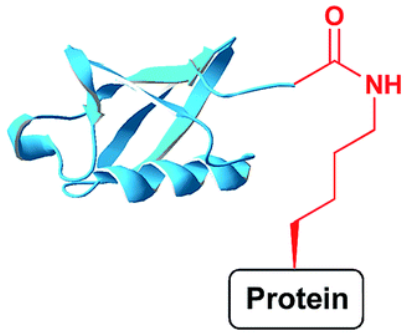
Ubiquitin acts as a molecular "tag" that attaches to target proteins via a covalent bond between a C-terminal glycine (Gly76) of ubiquitin and lysine residues on the target protein.

This process is called ubiquitination and proceeds through three enzymatic steps:

E1 – Activating enzyme:
Activates ubiquitin using ATP, forming a thioester intermediate with a cysteine residue of E1.

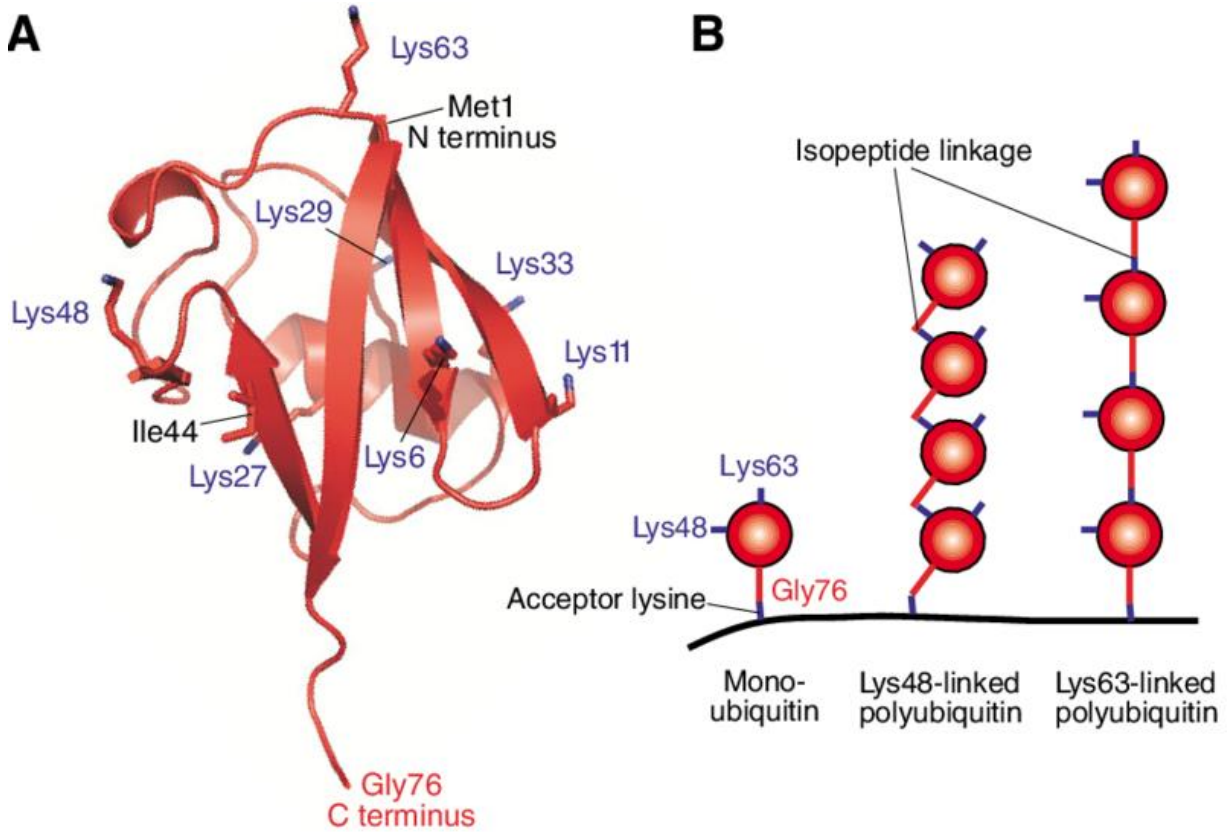
E2 – Conjugating enzyme:
Receives the activated ubiquitin from E1 and holds it via a thioester bond.

E3 – Ubiquitin ligase, provides substrate specificity:
Specifically recognizes the target protein and catalyzes the transfer of ubiquitin from E2 to a lysine residue on the target protein.



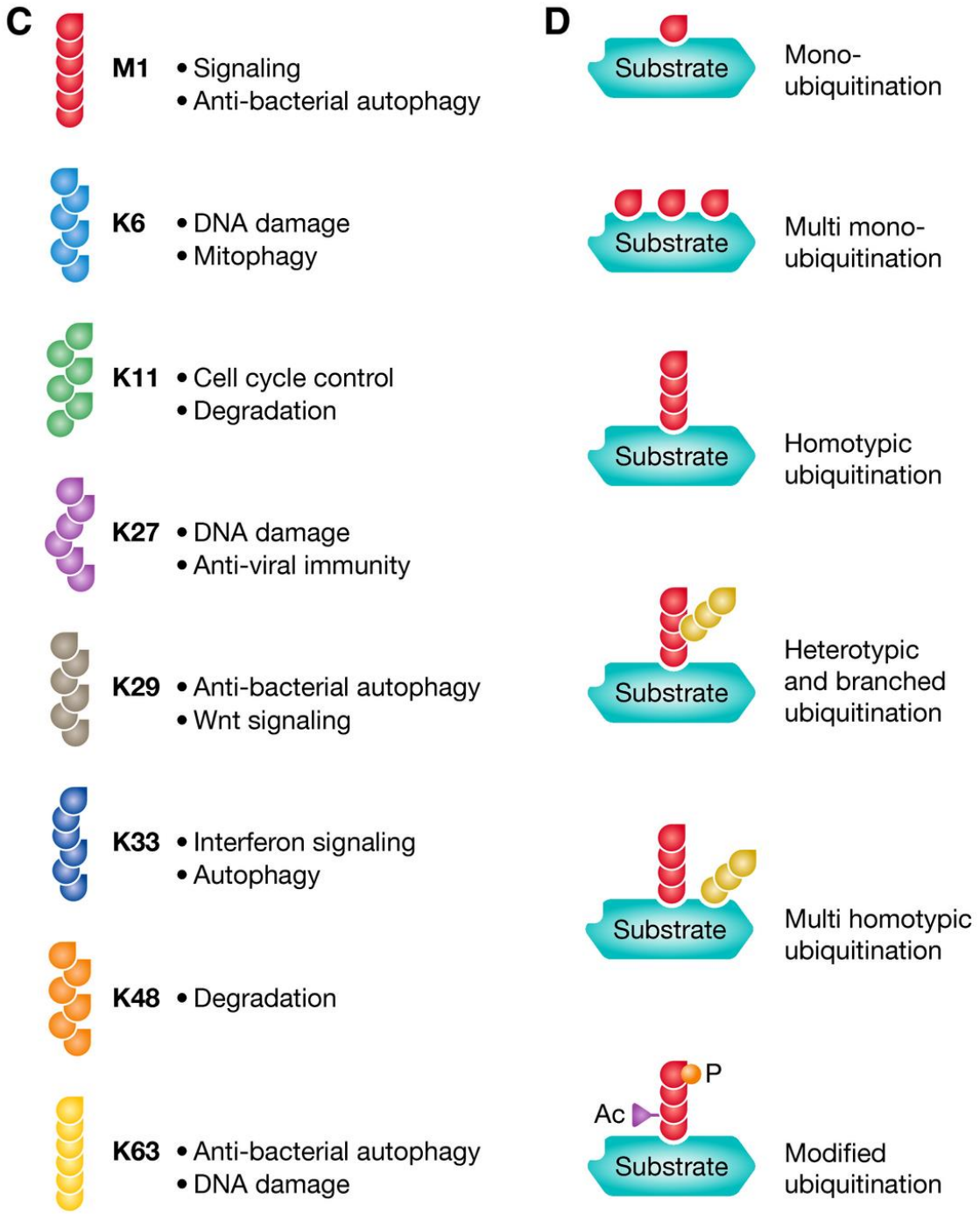
Isopeptide-linked ubiquitinated protein

***Isopeptide bonds**: Form between the α-carboxyl group of Gly76 of ubiquitin and the amino group of a lysine side chain on a target protein.*



When ubiquitin attaches to a substrate, it can subsequently conjugate with itself.

Internal lysine residues at positions 6, 11, 27, 29, 33, 48, and 63 of the ubiquitin molecule act as attachment sites for other ubiquitin molecules → formation of different types of polyubiquitin chains.

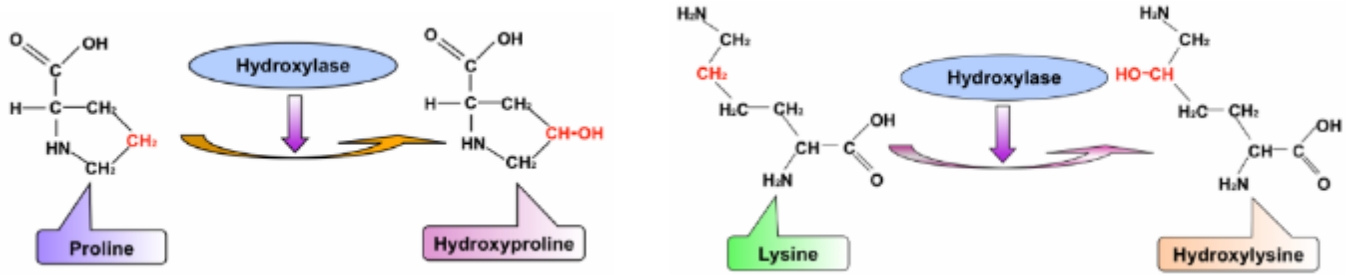


Depending on the motif and the number of attached ubiquitin molecules, the tag signals different cellular fates:

E.g., Polyubiquitination via Lys48 → targeting of the protein for degradation by the proteasome.

Monoubiquitination or polyubiquitination via other lysines (e.g., Lys63) → regulation of mechanisms such as endocytosis, signal transduction, DNA repair, etc.

Hydroxylation

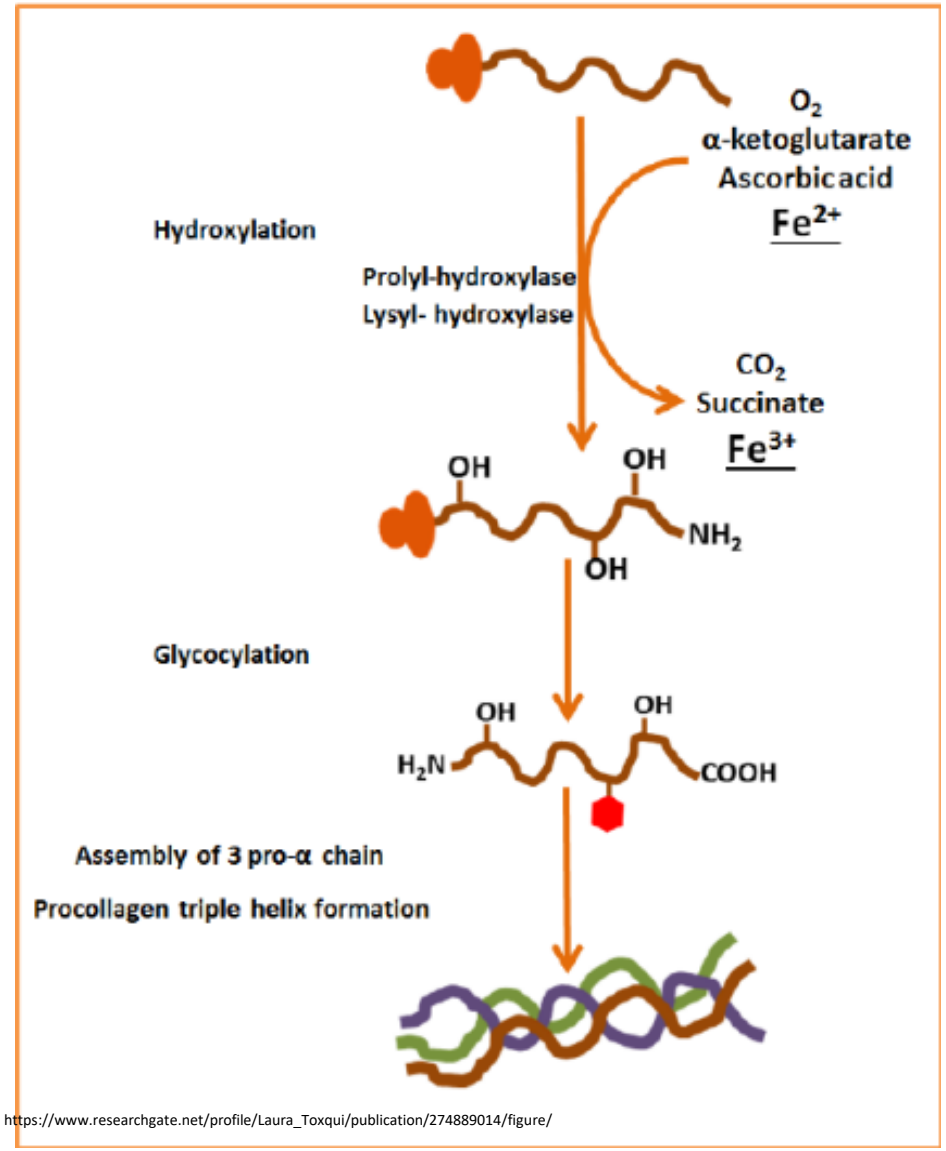


https://www.researchgate.net/profile/Xiao_Jian_Shao/publication/

Collagen has a **triple-helix structure**.
Initially, a three-dimensional, thread-like structure—composed primarily of the amino acids **glycine and proline**—is assembled; this is known as procollagen (a precursor molecule).

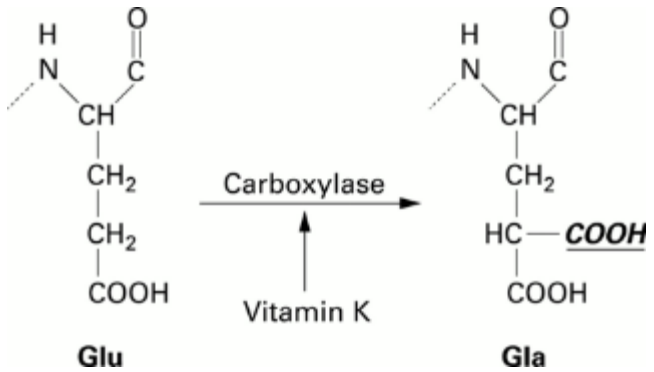
Procollagen is modified through the addition of hydroxyl (OH) groups to proline and lysine residues. **This step is crucial for stabilizing the collagen's triple-helix structure.**

Since the hydroxylase enzymes that catalyze these reactions require **vitamin C** as a cofactor, a prolonged deficiency of this vitamin results in impaired collagen synthesis and scurvy.



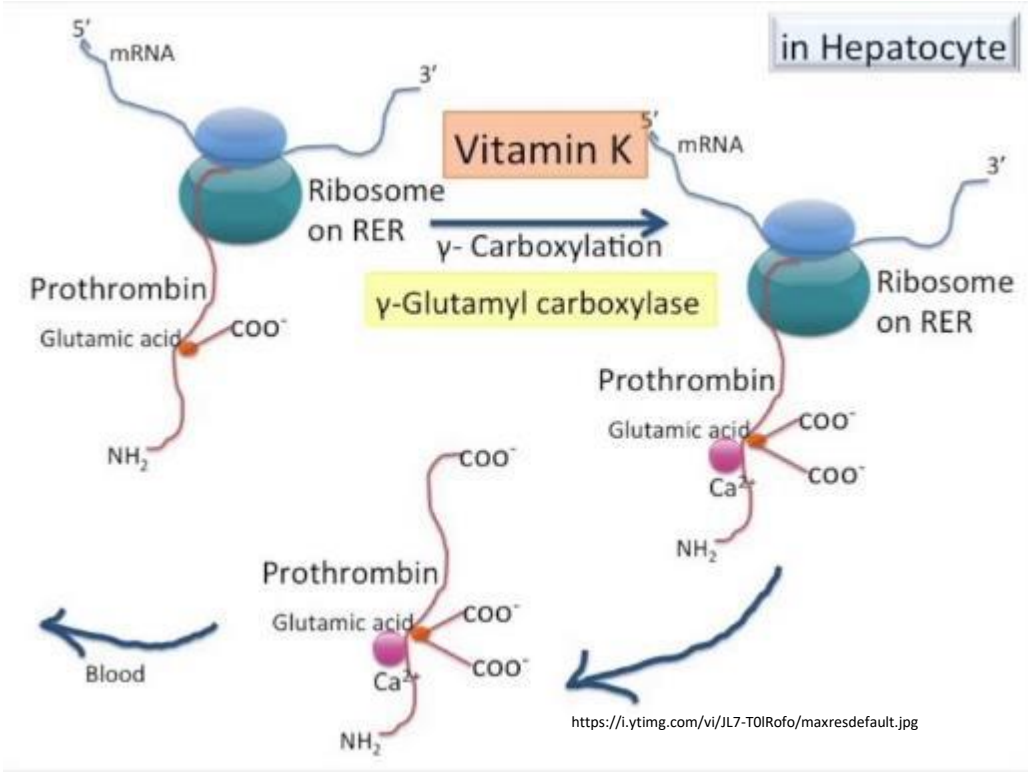
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Carboxylation



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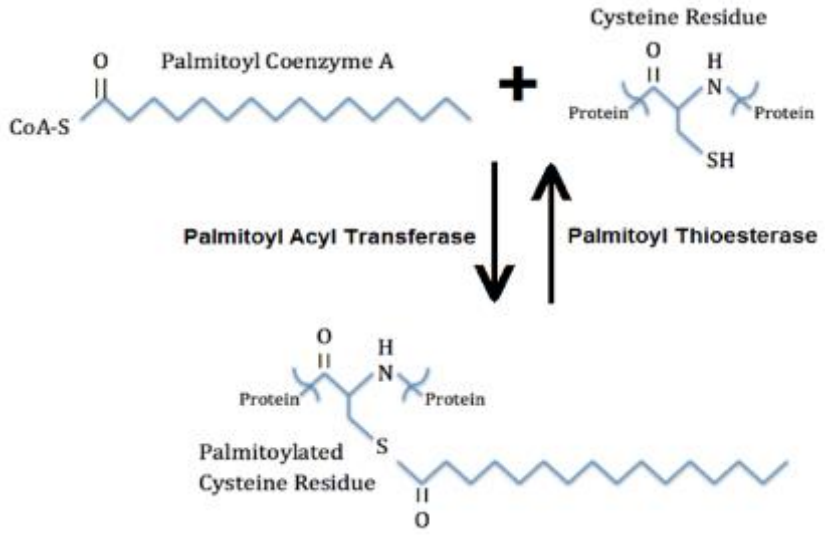
Vitamin K is required for blood clotting; it is an essential cofactor for the **enzyme γ-glutamyl carboxylase**, which mediates the carboxylation of glutamic acid residues.



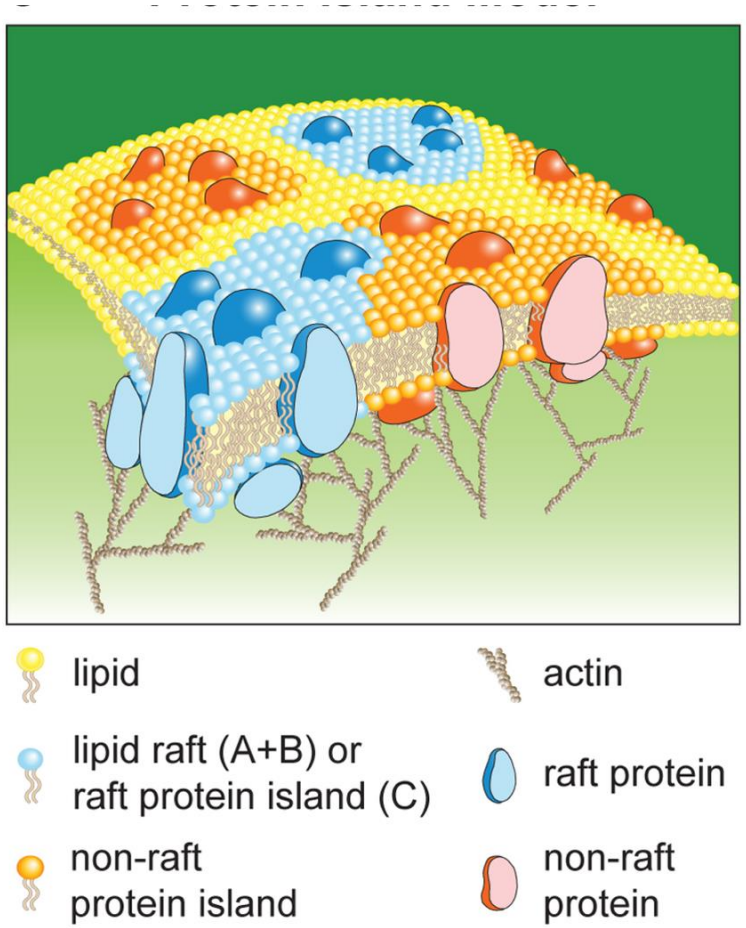
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These γ-carboxyglutamic acid (Gla) residues are located on the prothrombin molecule fragment. The Gla region is essential for the calcium-dependent binding of phospholipids to prothrombin, a process necessary for the conversion of prothrombin into biologically active α-thrombin.

Protein Lipidation



https://upload.wikimedia.org/wikipedia/commons/7/7d/Palmitoylation.png

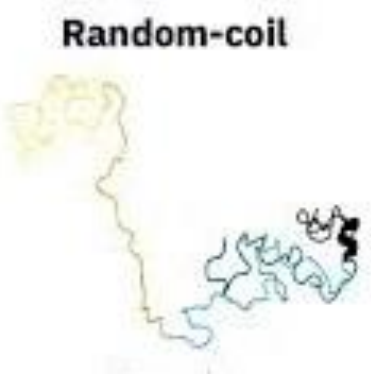


Protein lipidation—including cysteine prenylation, N-terminal glycine myristoylation, cysteine palmitoylation, and fatty acylation of serine and lysine—occurs in many proteins in eukaryotic cells and regulates numerous biological functions, such as association with cell membranes, protein secretion, signal transduction, and apoptosis.

Palmitoylation is a reversible process involving the attachment of palmitic acid to cysteine—and less frequently to serine and threonine—residues of proteins, playing a significant role in protein compartmentalization and subcellular redistribution.

Based on their shape, proteins are classified into **fibrous** and **globular**.

Polypeptides form elongated, fibrous chains.
The chains are typically linked by disulfide bridges, making the proteins very stable and strong.
They are generally insoluble.
Fibrous proteins serve structural functions:
e.g., keratin in skin and hair
collagen—found in bones, cartilage, tendons, and ligaments.



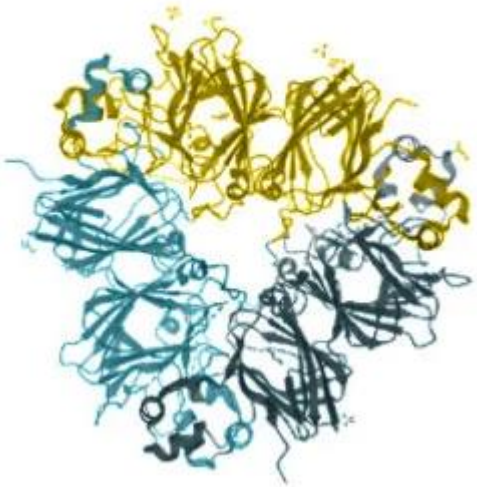
The molecule forms a coiled shape (globule).
The hydrophobic groups are positioned in the interior of the molecule, away from the water.
Only hydrophilic groups are exposed on the outside of the molecule, making globular proteins soluble.

Fibrous

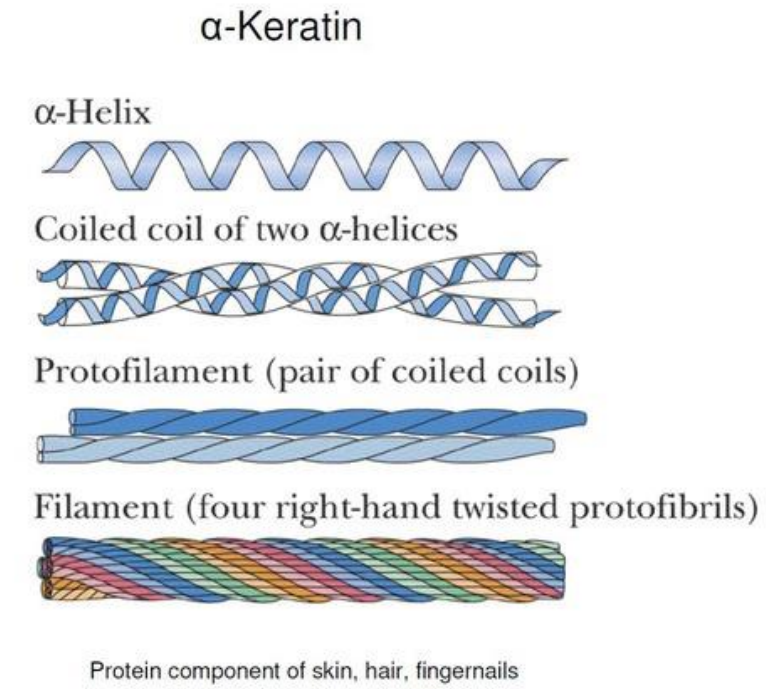


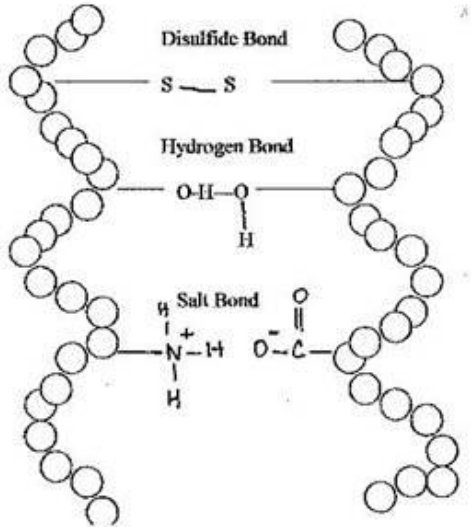
	Fibrous	Globular
Shape	Long and narrow	Round / spherical
Purpose	Structural	Functional
Acid Sequence	Repetitive amino acid sequence	Irregular amino acid sequence
Durability	Less sensitive to changes in pH, temperature, etc.	More sensitive to changes in pH, temperature, etc.
Examples	Collagen, myosin, fibrin, actin, keratin, elastin	Enzymes, haemoglobin, insulin, immunoglobulin
Solubility	(Generally) insoluble in water	(Generally) soluble in water

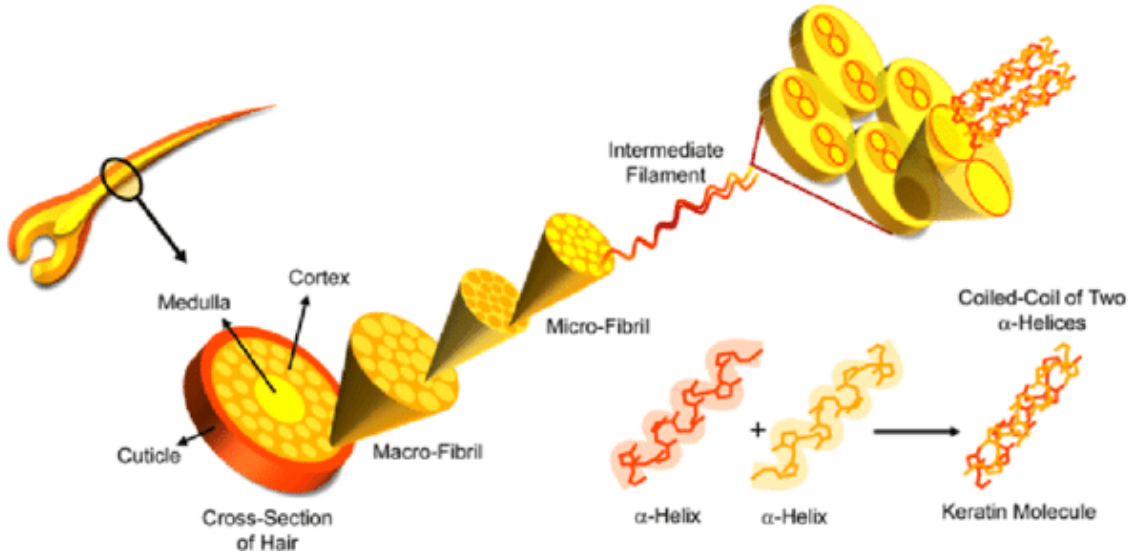
Globular



α-Keratins

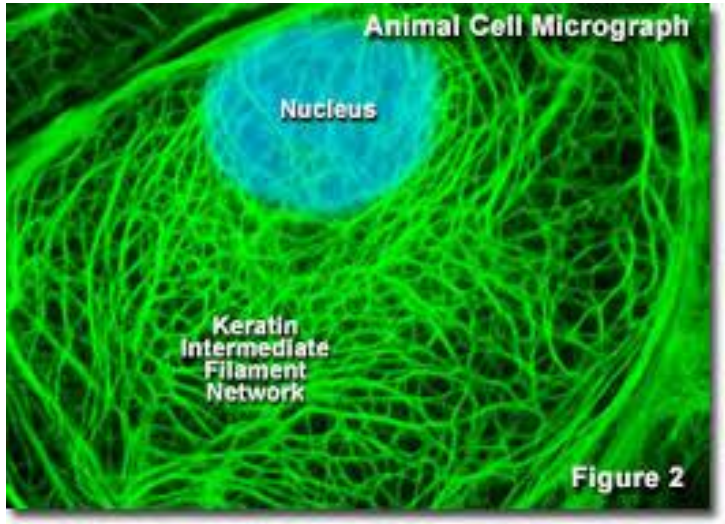


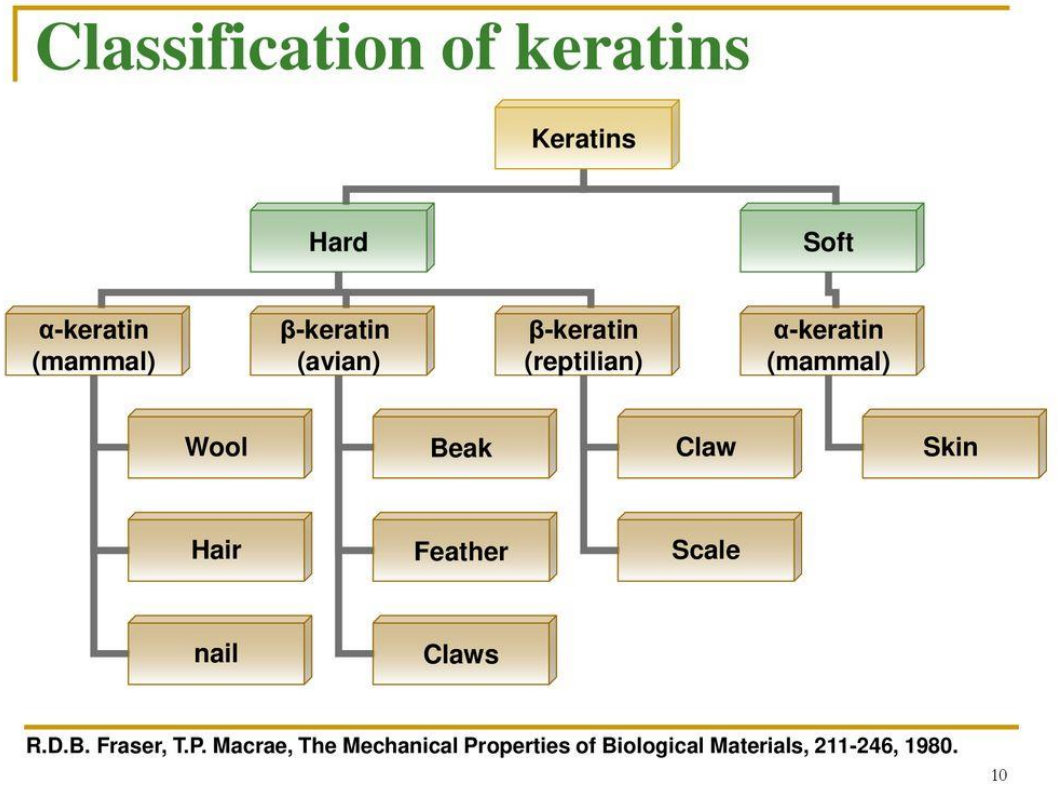




- Polypeptide chain with an alpha-helical conformation
- **High content of alanine, leucine, arginine, and cysteine**
- Two alpha-helices intertwine to form a superhelix known as a coiled-coil
- The two helices in the coiled-coil are linked by ionic interactions, van der Waals forces, hydrogen bonds, and disulfide bonds
- Subsequently, the dimers align, their ends connect to the ends of other dimers, and two of these resulting chains link via disulfide bonds to form a protofilament
- Two protofilaments associate to form a protofibril, and four protofibrils polymerize to form the intermediate filament (IF).

α-Keratins are key components of the structure of hair and nails, as well as the cytoskeleton (intermediate filaments).





Keratins can be divided into soft and hard keratins depending on their cysteine content. Soft keratin occurs in skin and contain approximately 2% cysteine, whereas hard keratin is present in hair and contains approximately 14% cysteine.

Hard α-keratins, such as those found in nails, have a higher cysteine content in their primary structure. This results in an increased number of disulfide bonds that stabilize the keratin structure, making it more durable.

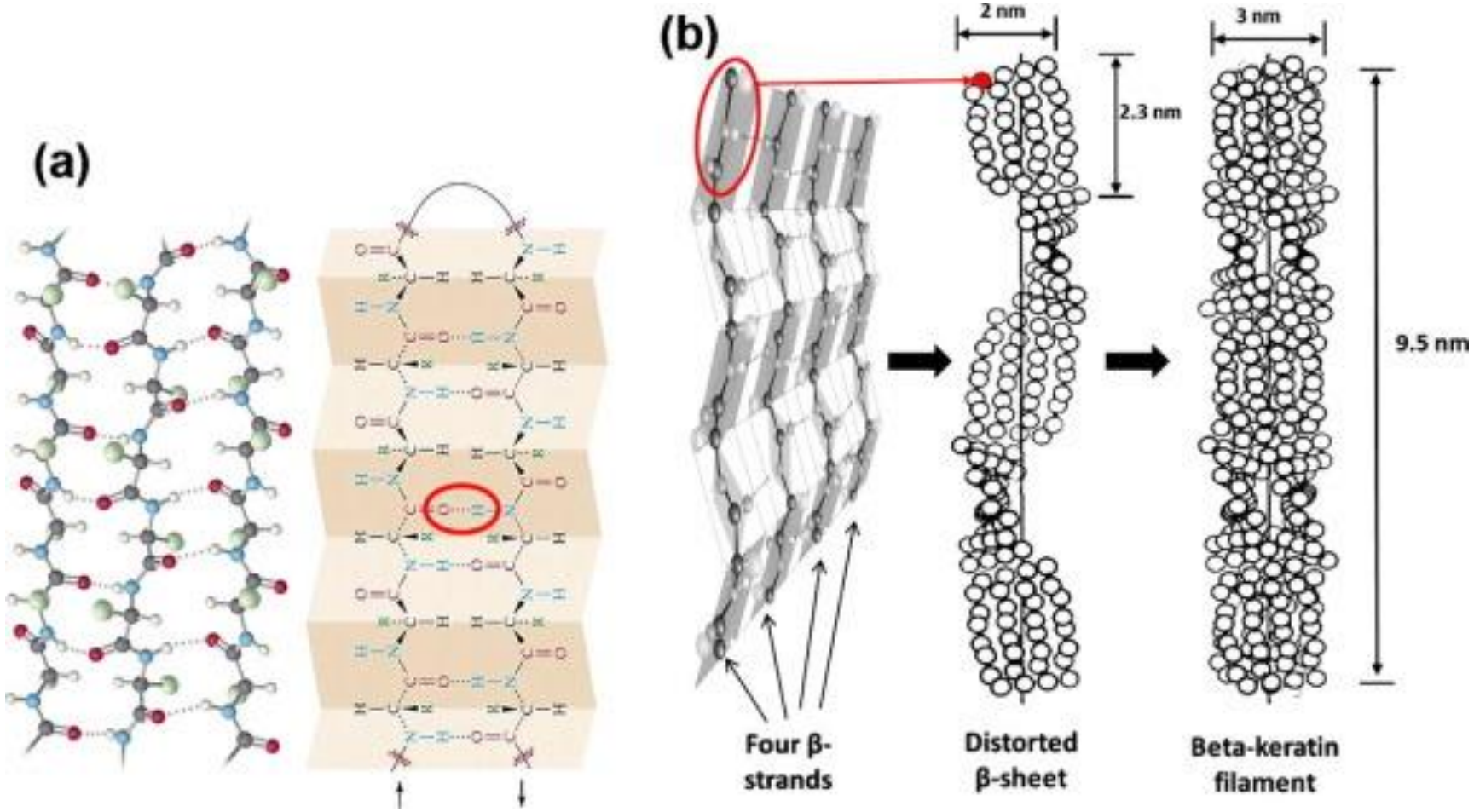
Soft α-keratins, such as those found in the skin, contain a comparatively smaller number of disulfide bonds, making their structure more flexible.

β-Keratins

B-keratins are found exclusively in reptiles and birds. Fibroins (β-keratin type) are making up silk and spider webs

their secondary structure is β-sheets.

β-keratin is harder than α-keratin. Structurally alpha-keratin have alpha-helical coiled coil structure while beta-keratin have twisted beta sheet structure.
β-keratins are found exclusively in reptiles and birds.



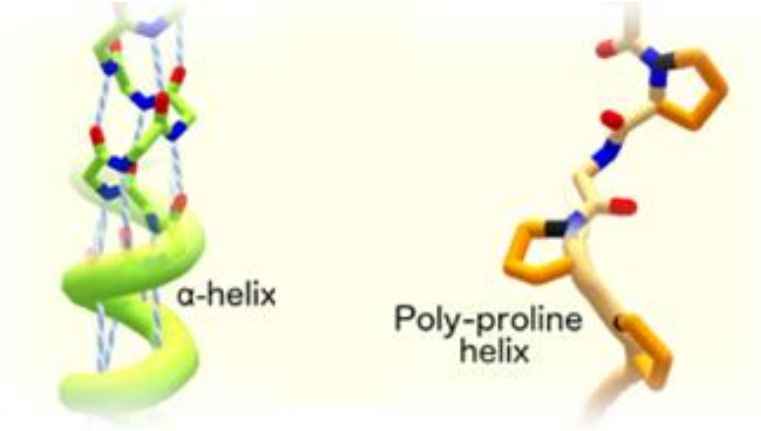
Structure of the beta-keratin filaments: (a) ball-and-stick model of the polypeptide chain, and illustration of the pleated beta-sheet; (b) schematic drawing of the formation of beta-keratin filament: one polypeptide chain folds to form four β-strands which twist to form the distorted β-sheet. Two sheets assemble to form a beta-keratin filament.

Collagens

•In vertebrates it is the component of **connective tissue** which forms:

- Tendons
- Cartilage
- Ligaments
- Bones
- Teeth
- Skin
- Walls of blood vessels
- Cornea of the eye

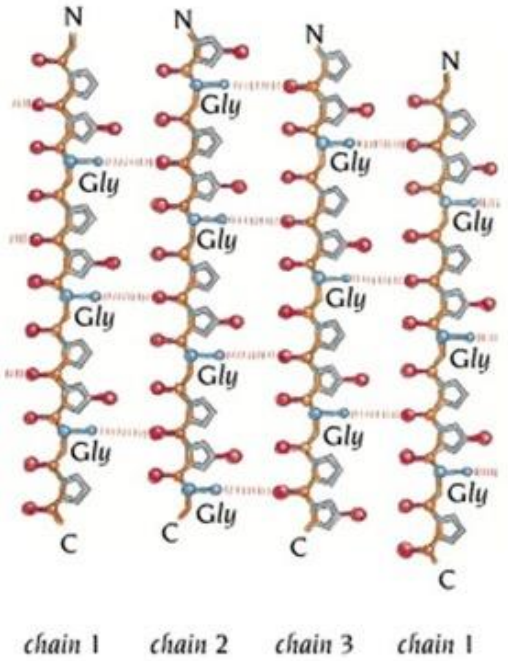
- Each polypeptide chain contains approximately 1,000 amino acids, **with glycine, proline, and hydroxyproline** being the most common.
- It has a helical conformation (though not an α-helix, as the chain is not as tightly coiled).



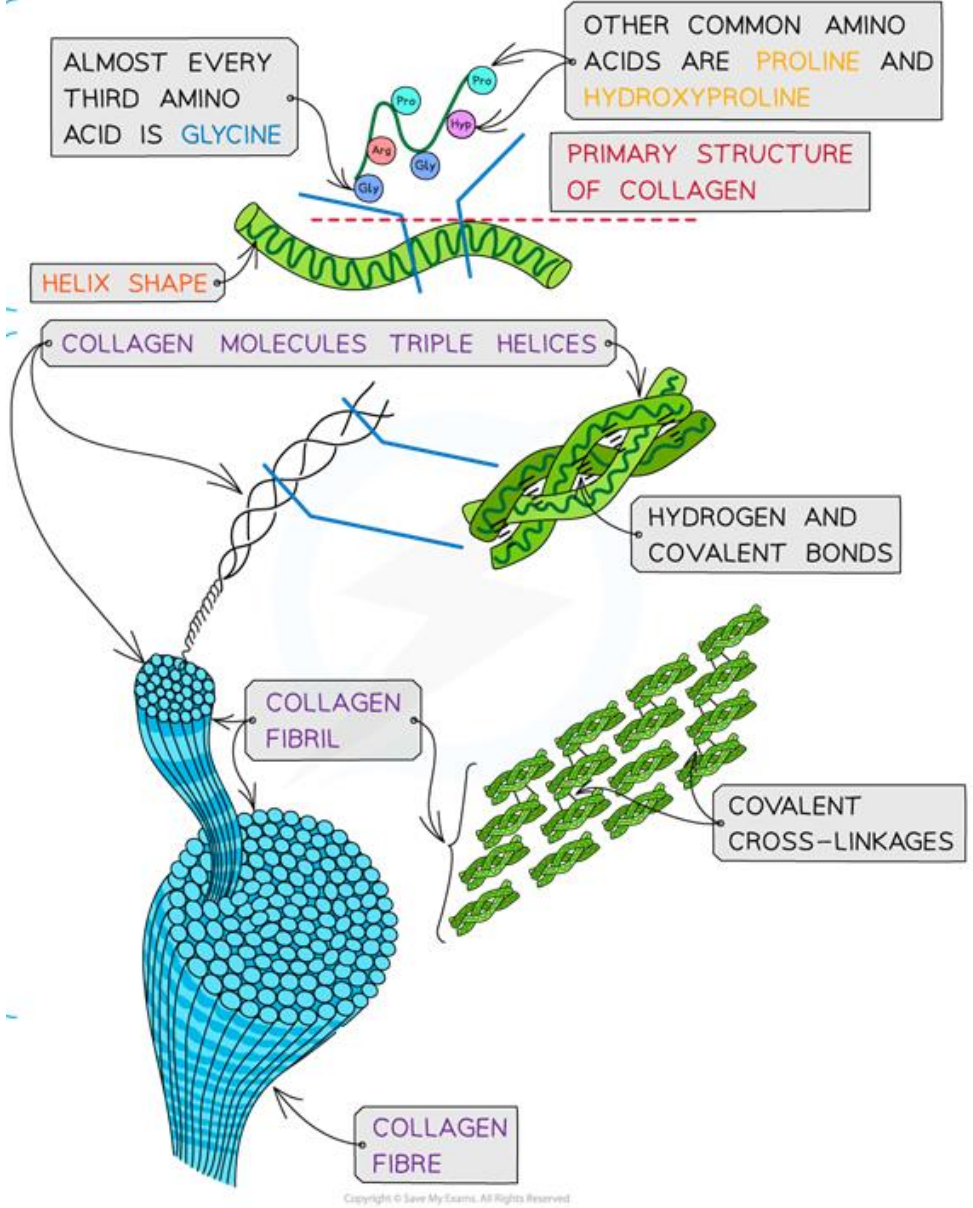
Collagen forms a triple-helix



Collagen is formed from three polypeptide chains held tightly together by hydrogen bonds, forming a triple helix (known as tropocollagen).



- In the regular collagen triple helix, the three chains are held close together by direct interchain hydrogen bonds between proline C=O groups and glycine NH groups.



Collagen is a fibrous structural protein that is formed by triple helices collagen molecules arranging into collagen fibrils and finally into collagen fibers which have high tensile strength

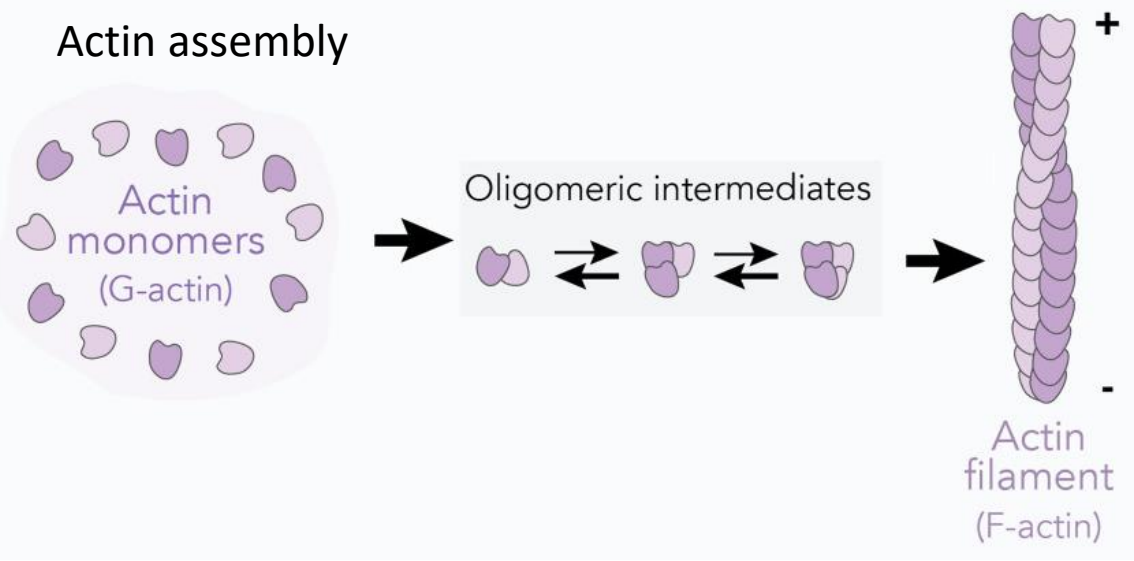
- In addition to the hydrogen bonds formed between the three chains, covalent bonds are also present.
- Covalent bonds also form cross-links between the R-groups of amino acids in interacting triple helices when they are aligned parallel to one another.
- These cross-links hold the collagen molecules together to form fibrils.
- When multiple fibrils are assembled together, they form collagen fibers.

The presence of numerous hydrogen bonds in the triple-helix structure of collagen results in high elasticity. This allows collagen to withstand significant tensile forces without stretching or breaking.

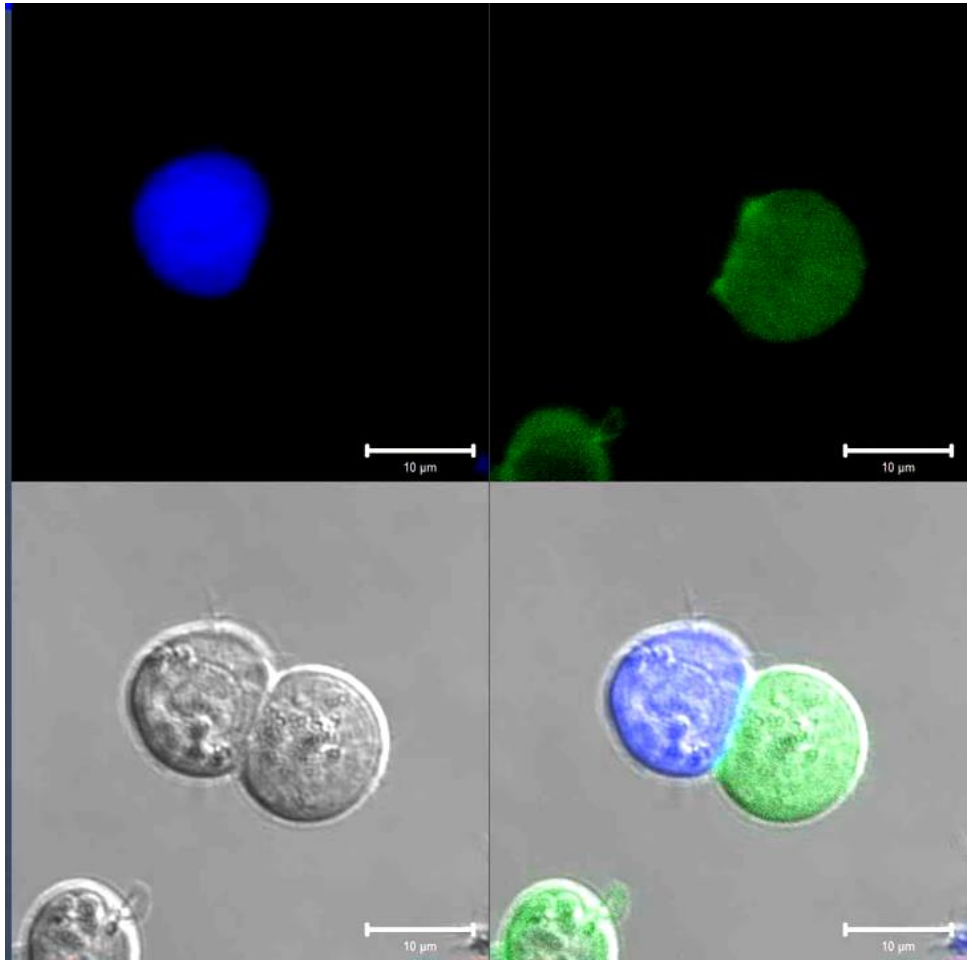
G ACTIN	F ACTIN
Globular monomeric form of actin	Fibrous actin polymerized in the form of a double-helix
Globular	Filamentous
Soluble protein	Insoluble
Forms the actin filament	Forms cytoskeleton and contractile apparatus



Actin assembly



Immunological Synapse

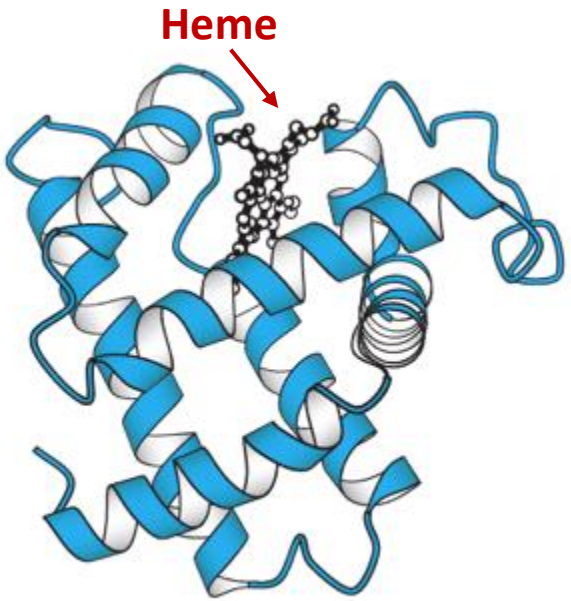


Immunological synapse between Jurkat T cell expressing GFP-actin (green) and Raji B cell stained with CMAC (blue). Synapse formation was induced by Staphylococcal enterotoxin E superantigen.

Globins

A family of soluble **metalloproteins** found in all organisms (plants, invertebrates, vertebrates, microorganisms). Hemoglobin (Hb) and myoglobin (Mb) are the best-known members.

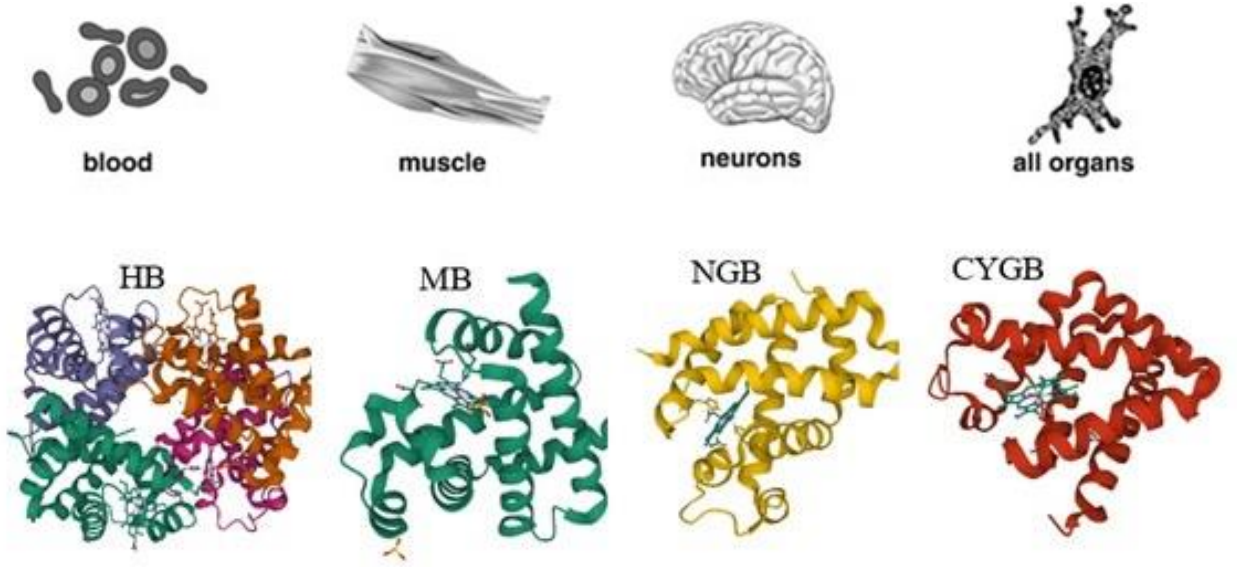
In mammals, the secondary structure is dominated by **α-helices** connected by short non-helical segments, organized into a globular, tightly packed 3D conformation (globin fold).



Μυοσφαιρίνη

Polar amino acids are found almost exclusively on the outer surface, contributing to the very high solubility of the globins.

The hydrophobic amino acids in the interior are arranged to stabilize a fold that forms a pocket housing the heme prosthetic group.



NGB and CYGB reversibly bind O_2 with high affinities (comparable with that of MB).
Believed to have a function in providing O_2 to high-energy demanding cells when required, and also in protecting oxygen-sensitive cells from disruptions in O_2 availability

Elevated levels of CYGB and NGB in marine mammals are considered one of the mechanisms protecting O2-sensitive tissues from low PO2.

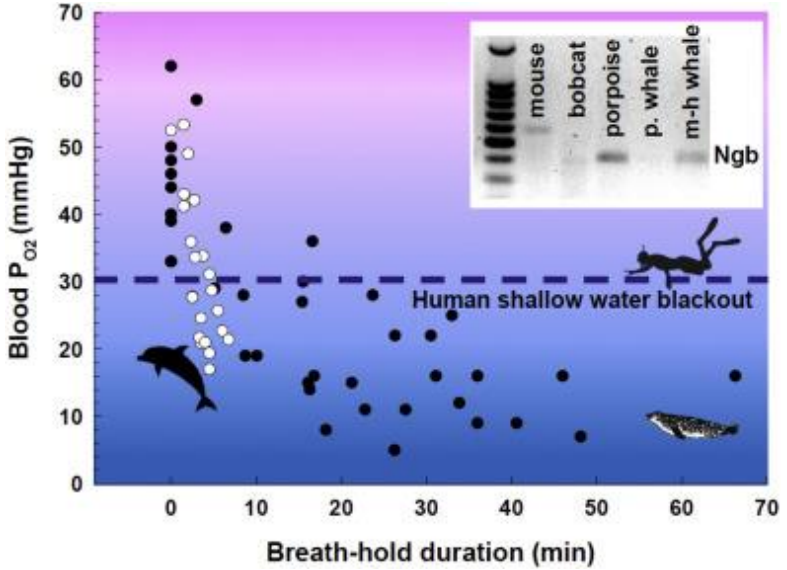
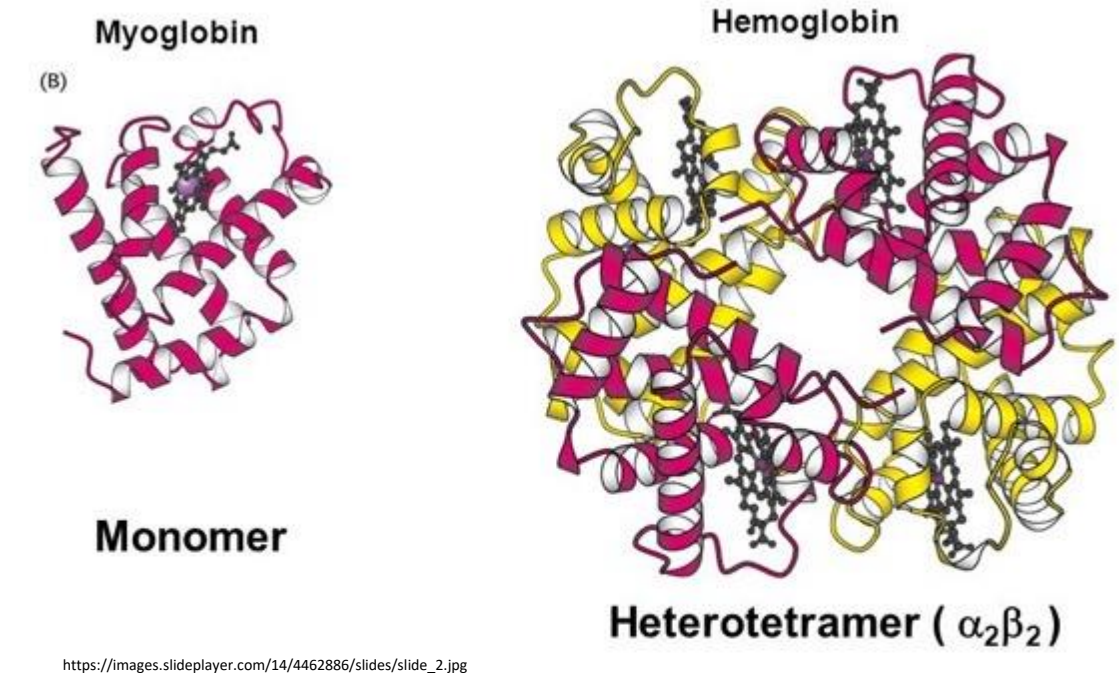


Fig. The effect of breath-hold duration on the partial pressure of blood oxygen in marine mammals. Sedentary bottlenose dolphins (n = 3, open circle) and Weddell seals freely diving beneath the Antarctic sea ice (n = 4, closed circle) are compared. Each point represents a single, mixed venous blood sample obtained within 2 min of surfacing. The dashed horizontal line denotes the circulating P_{O2} level associated with unconsciousness in humans. The inset shows a representative mRNA expression analysis for the cerebral cortex of five mammalian species

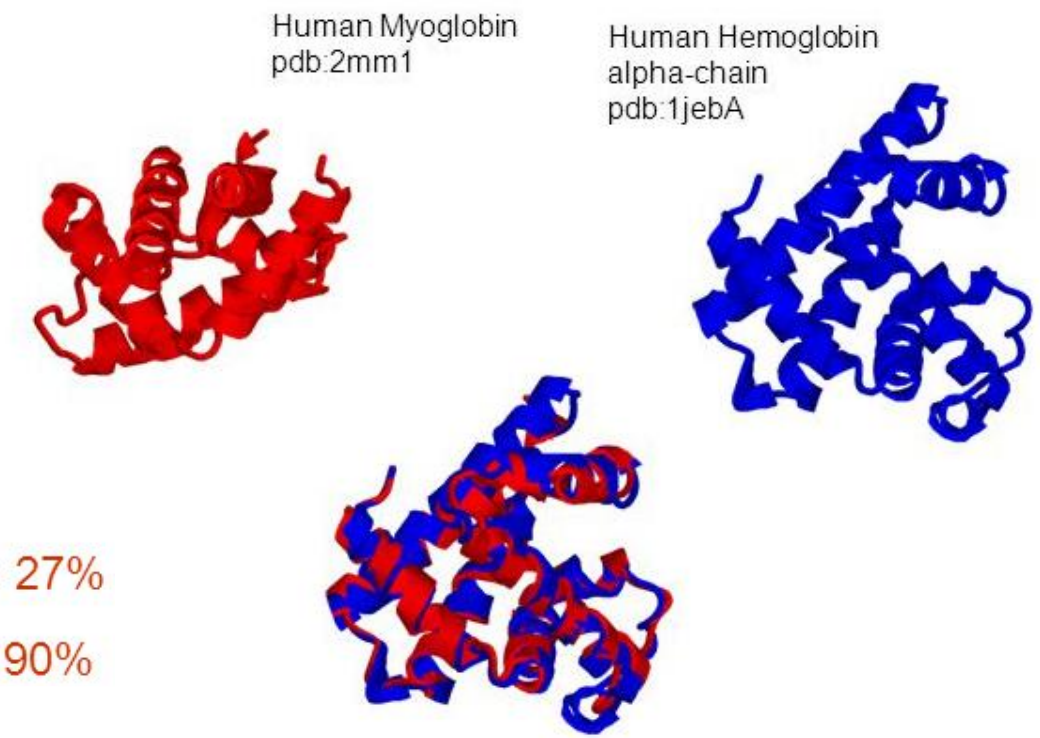
Myoglobin is a **monomeric** protein, whereas adult hemoglobin has a quaternary structure [α(2)β(2)].

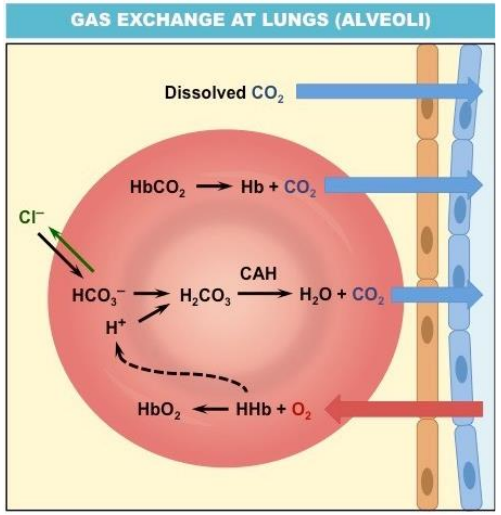


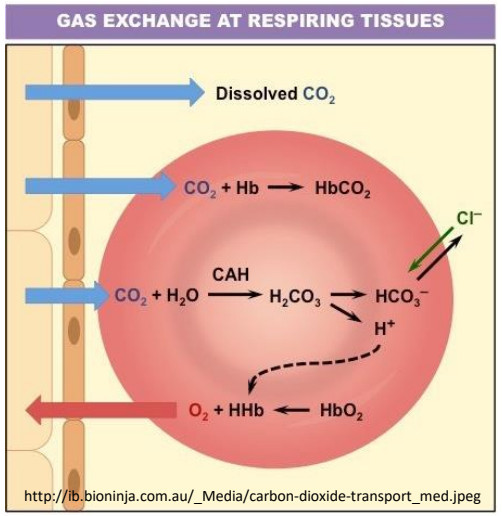
https://images.slideplayer.com/14/4462886/slides/slide_2.jpg

Similar tertiary structure, different quaternary structure.
The quaternary structure of Hemoglobin confers allosteric properties.

Myoglobin is structurally very similar to the **alpha-subunit** of hemoglobin.







Hemoglobin (Hb): Transporter of O2 and CO2 between the lungs and peripheral tissues.

In mammals, it constitutes approximately 35% of the total content of red blood cells (including water).

In the lungs, hemoglobin (Hb) binds molecular oxygen—forming oxyhemoglobin (HbO2)—while simultaneously releasing the CO2 it had acquired from the tissues.

n the tissues, hemoglobin releases O2 and takes up CO2, which it transports to the lungs—carbaminohemoglobin ($HbCO_2$).

Myoglobin (Mb): Stores O2 in the cytoplasm and releases it to the mitochondrion when required.

It is found in the cytoplasm of skeletal, cardiac, and some smooth muscle cells; it binds to the O_2 released from Hb in the capillaries, which then diffuses into the muscle cells.

Heme



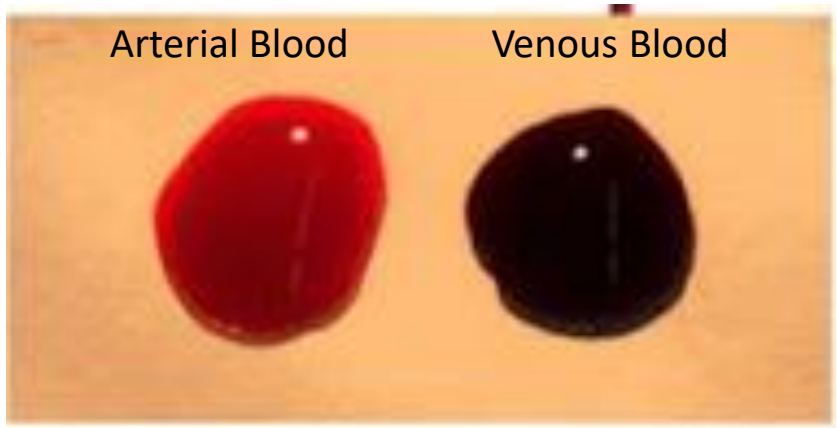
O2 binding occurs via the heme prosthetic group.

Heme is a heterocyclic compound (protoporphyrin IX) with an **Fe ion bound at its center**, which serves as the O2 binding site.

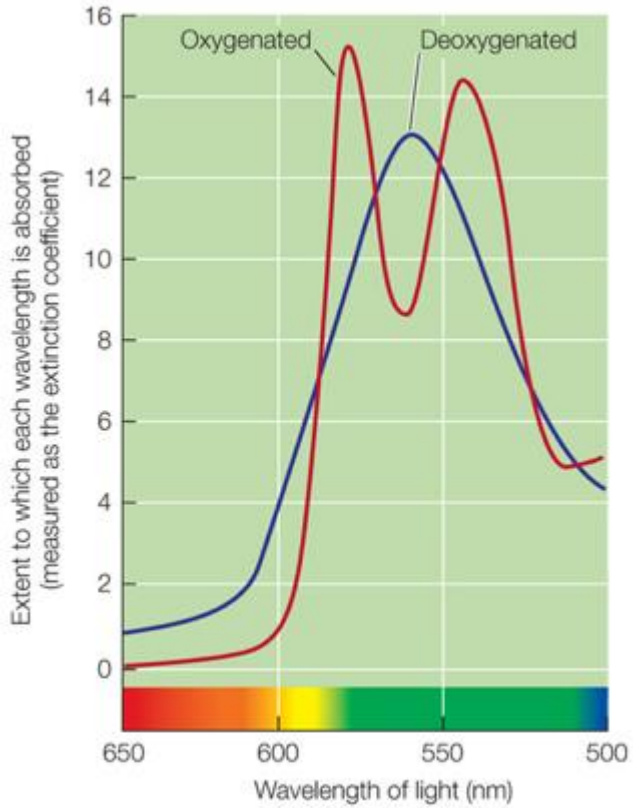
The Fe ion is coordinated to the four nitrogen atoms at the center of the pyrrole ring.



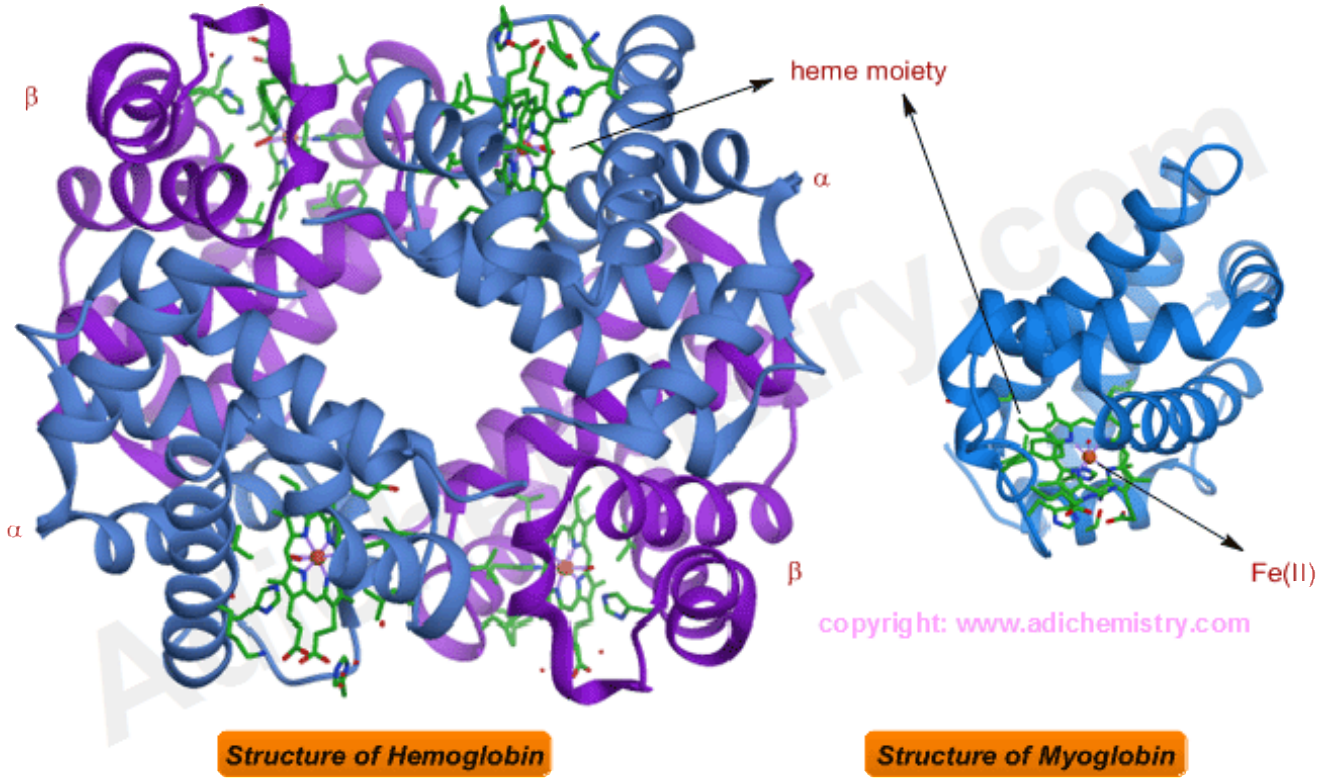
Heme is a red pigment and is responsible for the red color of blood and muscles.



The geometry of the heme group—and, by extension, of hemoglobin—changes depending on whether O_2 is bound to the iron ion. The two configurations have different light-absorption properties.



$HbCO_2$ has a deeper reddish-purple color than HbO2; for this reason, venous blood is darker than arterial blood, which is rich in oxyhemoglobin.



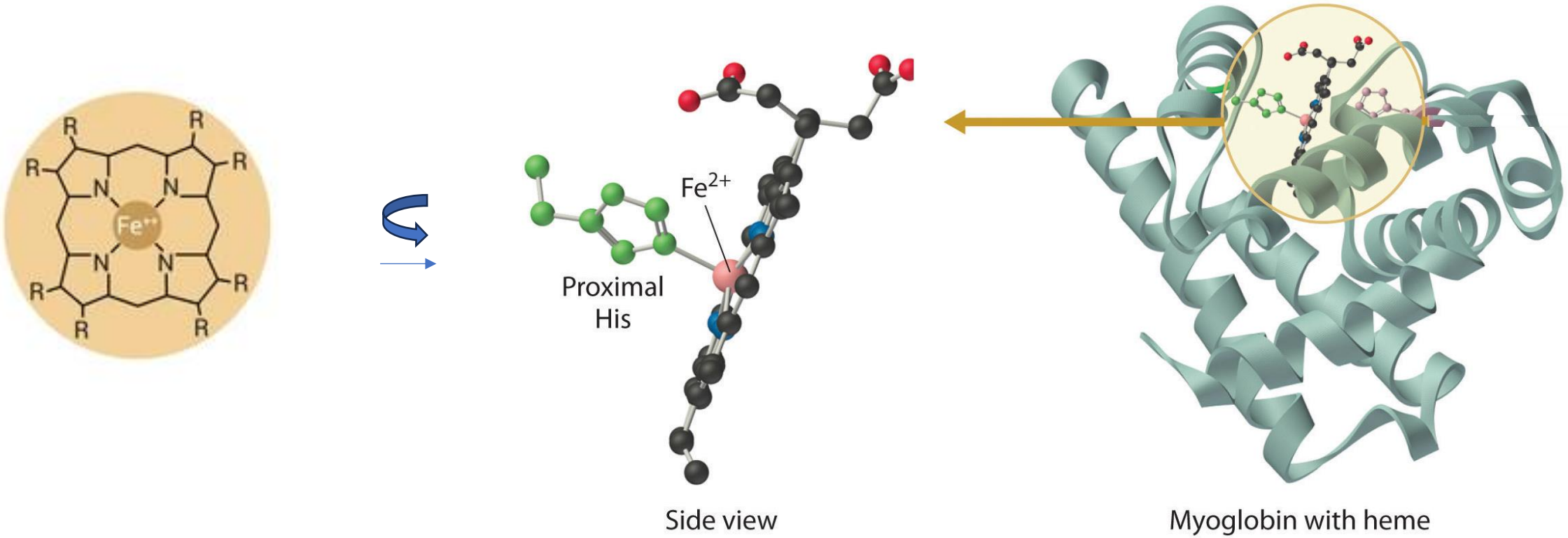
- Each myoglobin molecule contains a single heme prosthetic group.
- In adults, the most common type of hemoglobin is Hemoglobin A, a tetramer consisting of two identical α chains and two identical β chains (α2β2). The α and β subunits are structurally similar and are approximately the same size. Each chain contains 1 molecule of heme (4 in total). In a saturated state, Hb can bind up to 4 O_2 molecules.

CO_2 binds to Hb at the **free amino groups of the amino acid residues in its protein component**.

The quaternary structure of hemoglobin allows for significant allosteric interactions between the subunits, a property that plays a key role in the efficient transport of O_2 and which monomeric myoglobin lacks.

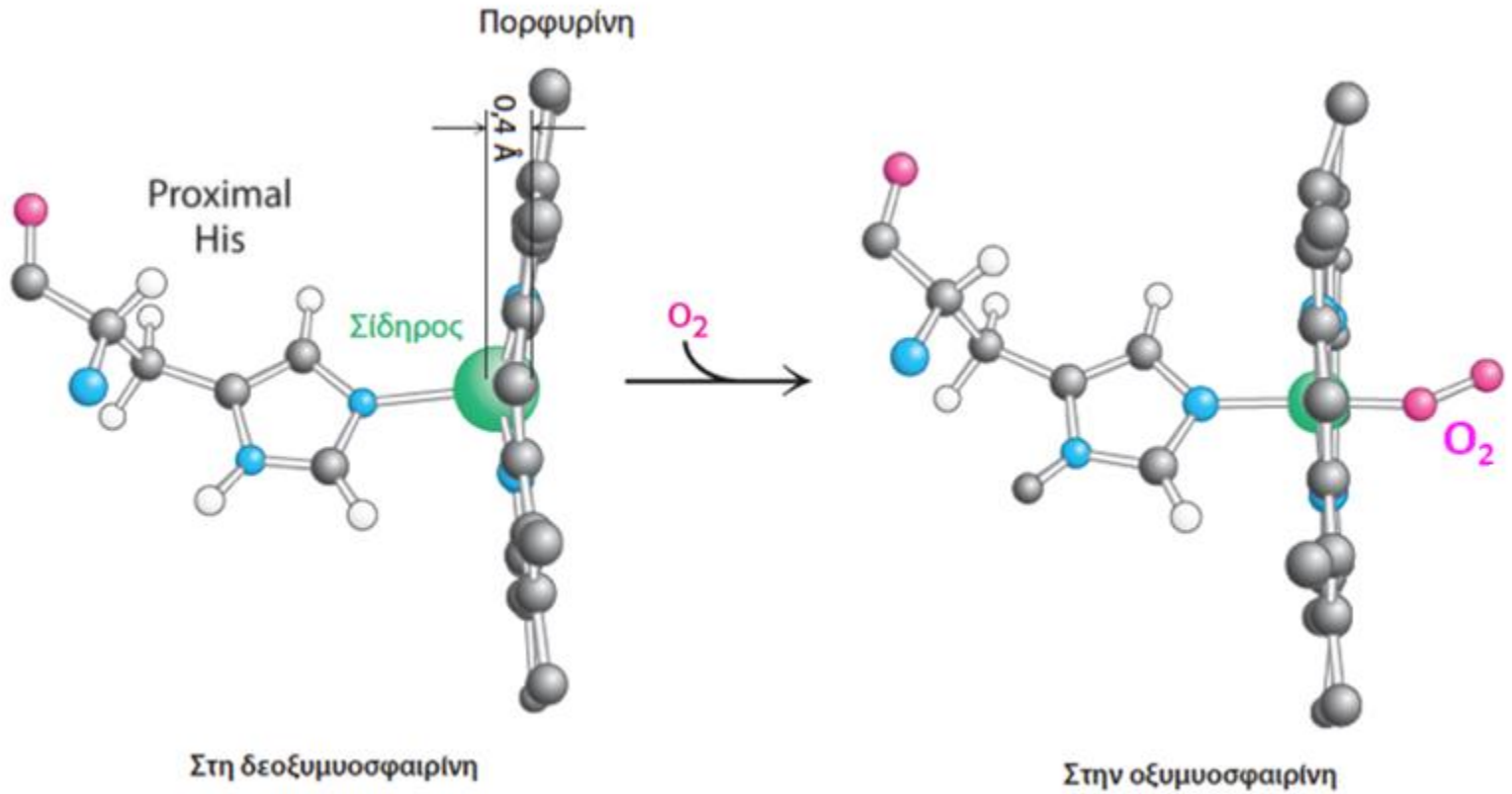
Heme is linked to the polypeptide chain by a covalent bond between the iron ion and the side chain of a histidine residue (via the nitrogen atoms of the imidazole ring).

- Proximal histidine (residue 87 in the alpha chain and 92 in the beta chain)



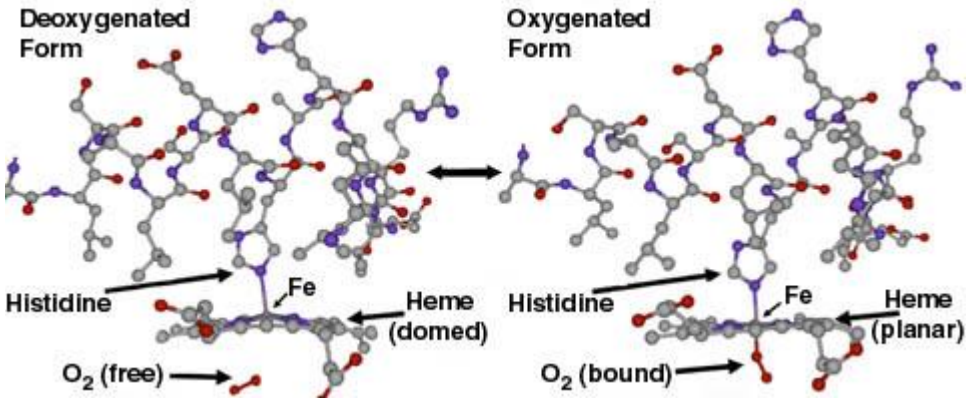
https://images.flatworldknowledge.com/averillfwk/averillfwk-fig23_023.jpg

The Fe ion is slightly larger than the available space within the well-defined cavity of the pyrrole ring and sits approximately 0.4 Å out of the heme plane. The binding of an oxygen molecule redistributes the electrons within the Fe ion, reducing its size and causing it to move into the plane of the ring.



Tense (T)

Relaxed (R)



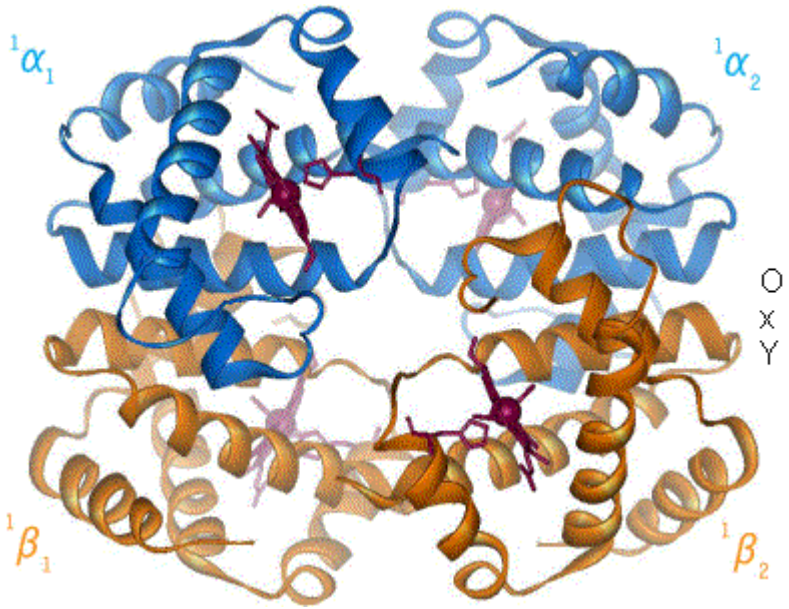
The binding of O2 to a Fe ion causes it to move toward the center of the porphyrin ring plane, **pulling with it the α-helix containing the "proximal histidine."**

As a result, the entire F-helix shifts by approximately 1 Å.

Although minor, these changes are accompanied by **the breaking of existing bonds at the heterodimer** interfaces and the formation of new ones, shifting the protein's overall quaternary structure into a "relaxed" state known as the **R (Relaxed) state**.

This conformational change facilitates O2 binding to the remaining three Hb subunits.

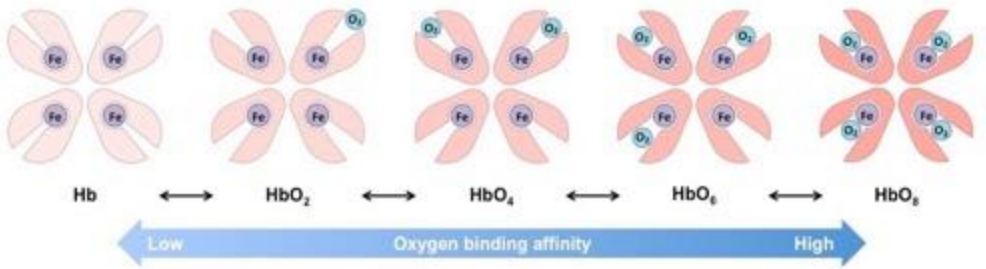
Cooperative oxygen binding.



In the absence of bound O2 (deoxygenated hemoglobin), the stereoconfiguration of Hb is termed **T (Tense);** in this state, it exhibits reduced affinity for O_2, **thereby facilitating its release from Hb.**

In the T state, the interactions between the α/β heterodimers are stronger; in the R state, these non-covalent bonds are collectively weaker.

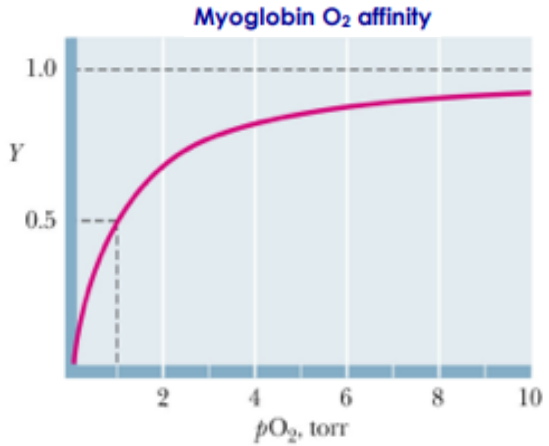
The transition between the two conformations is accompanied by the breaking of existing bonds and the formation of new ones at the heterodimer interfaces.



Factors favoring the R or T state

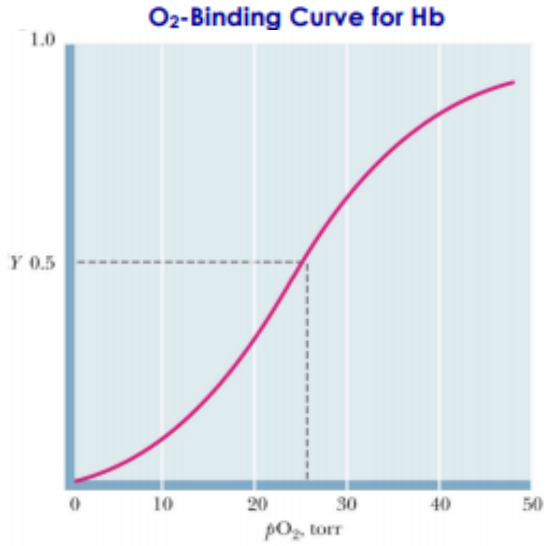
Hb must be efficient at O_2 uptake and CO_2 release in the lungs, and at CO_2 uptake and O_2 release in the tissues. The R state should be favored in the lungs, and the T state in the tissues. Hb has evolved to respond to environmental cues that signal the need for oxygen delivery.

A) Regarding the system's partial pressure: at high O2 partial pressures (such as in the pulmonary alveoli), the R state is favored. Conversely, at low partial pressures, the T state is preferred.



The cooperative nature of O_2 binding is aptly illustrated by the Hb saturation curve

The Mb binding curve shows a simple chemical equilibrium, and its slope indicates that it binds O_2 with greater affinity than Hb, and therefore releases O_2 at a much lower PO_2



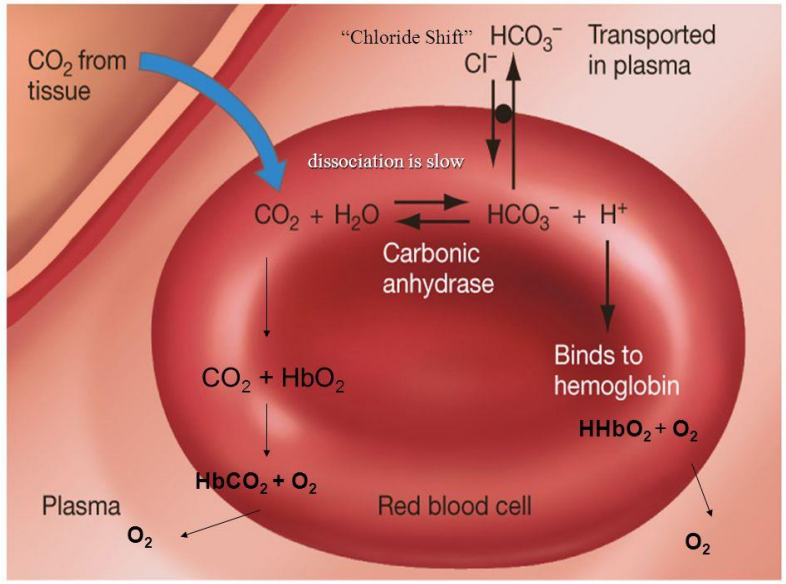
The sigmoid curve suggests that O_2 binding at one site on the tetramer increases the likelihood of O_2 binding at the remaining vacant sites. Similarly, the release of O_2 from one heme facilitates its release from the others as well.

In the lungs, where the partial pressure of O_2 is ~100 torr, nearly all Hb is saturated. As it travels to the tissues, where pO_2 is ~20 torr, the saturation level drops to ~34% (utilizing ~66% of the potential O_2 binding sites) . If Mb were used for O_2 transport, it would remain 91% saturated in the tissues, releasing very little O_2

B) High CO2 concentration and low pH indicate the T state (Bohr effect).The Bohr effect explains how high CO_2 levels promote the release of O_2 from Hb

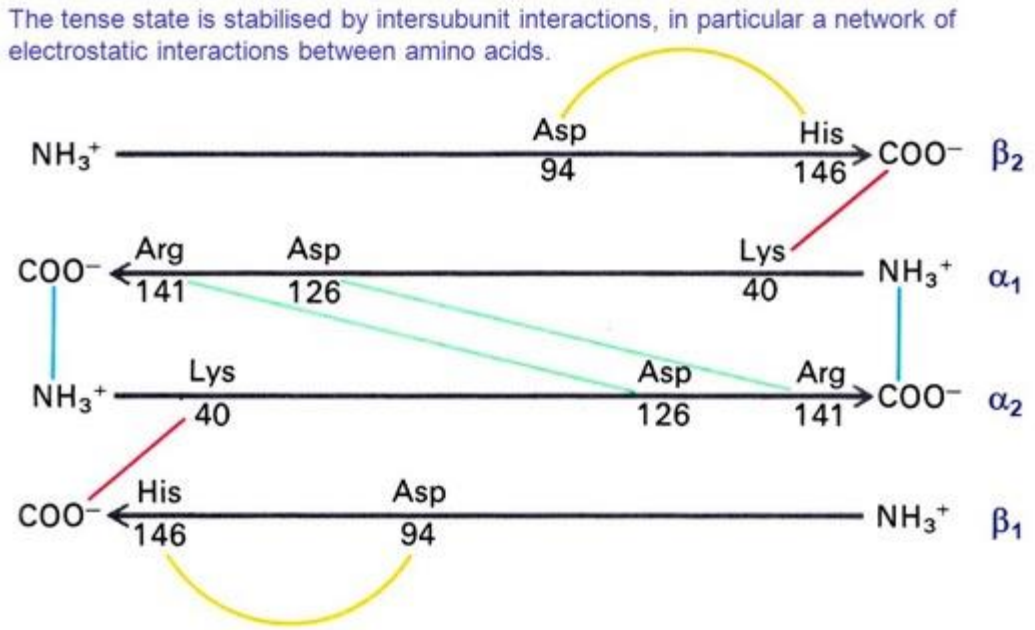
Tissues with high metabolic activity (e.g., contracting muscles) produce large amounts of CO_2.

CO_2 reacts with water via the enzyme carbonic anhydrase to form carbonic acid, which dissociates into carbonic acid salt and protons:

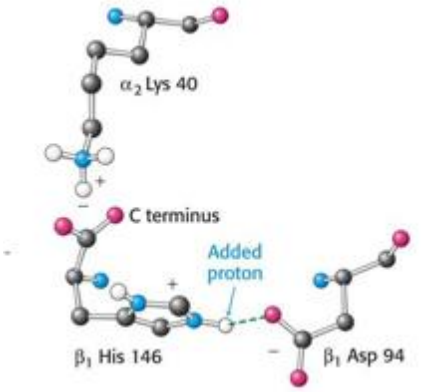


$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$ ➡ Protonation of βHis146 ➡ T state is favored = Oxygen supply to tissues

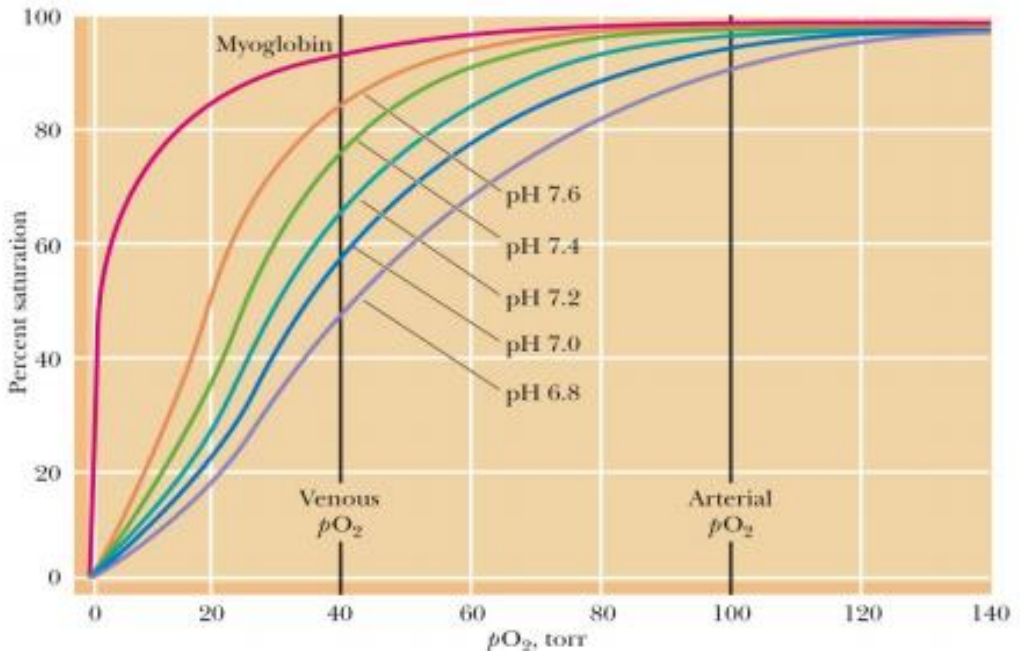
Numerous interactions within the Hb tetramer "lock in" the T state.
Among these, the the C-terminal βHis146 forms a salt bridge with α1Lys40, and this interaction allows the formation of an additional salt bridge between βHis146 and βAsp94 (aspartic acid 94)

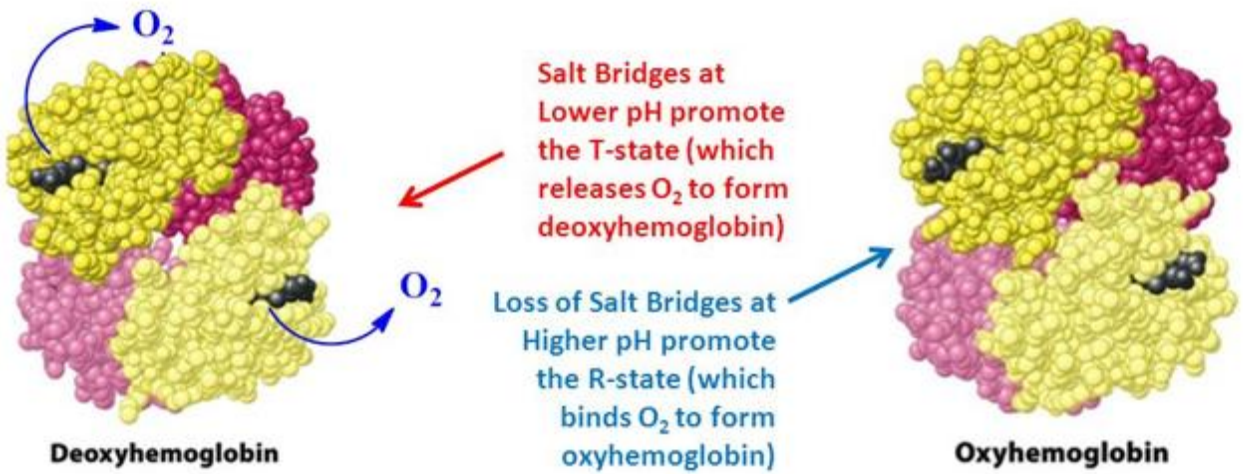


<u>Prerequisite: The hydrazine ring must be protonated</u>

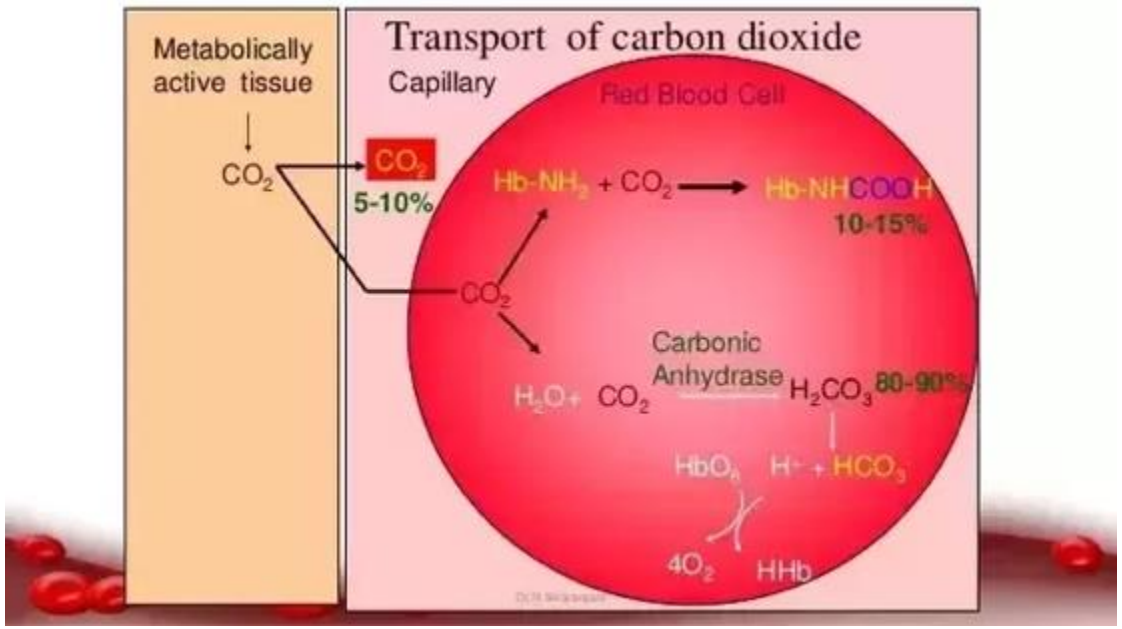


$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$ → Decrease in pH → Condition T is favored = Oxygen supply to tissues



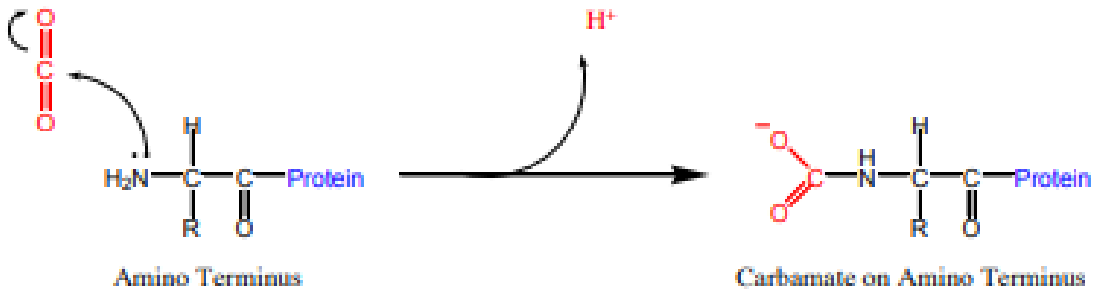


Direct interaction between CO_2 and Hb also contributes to the release of O_2

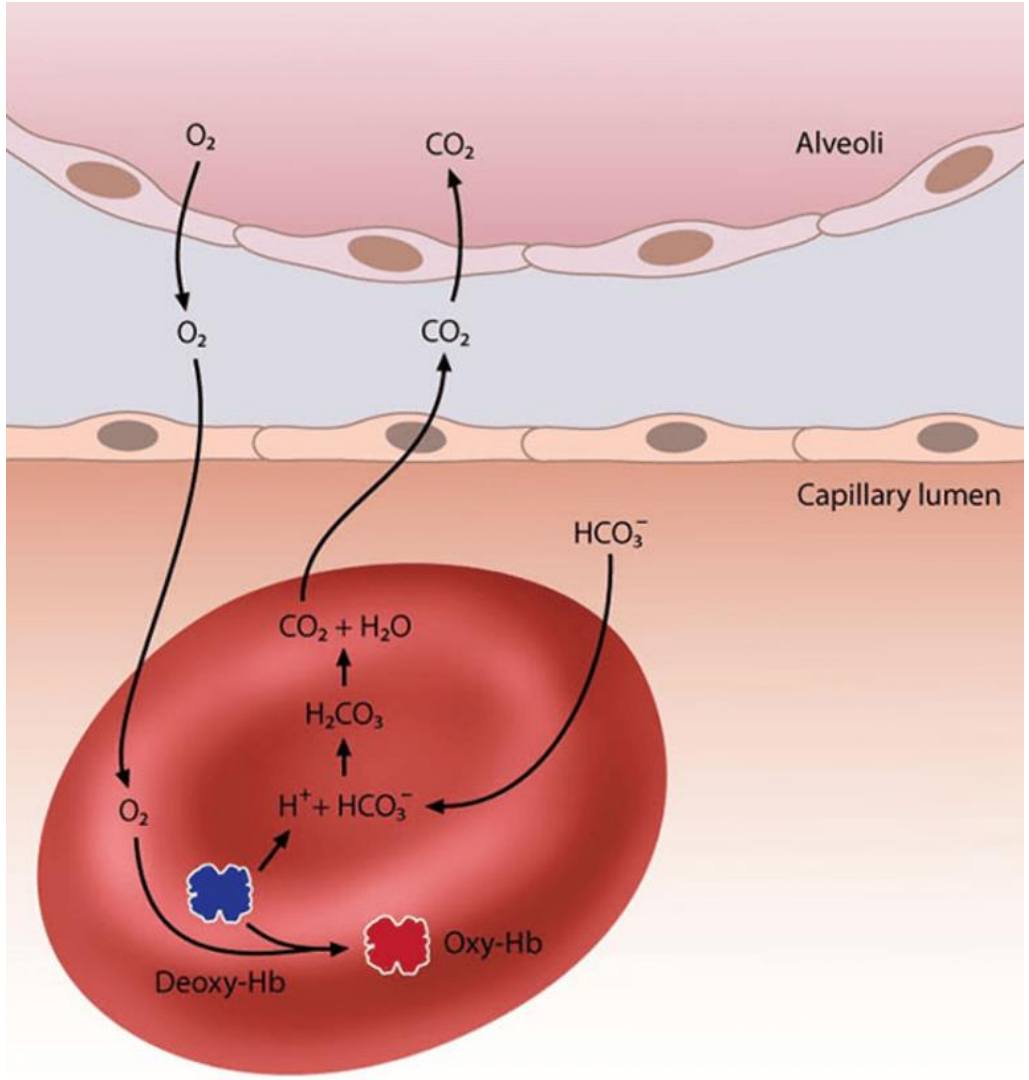


CO_2 binds free N-terminal amino group as well as amino groups on the side chains of arginine and lysine. This converts the neutral nitrogen into a negatively charged **carbamate group** (-NH-COO^-).

These newly formed negative charges line up to form additional **salt bridges** (ionic bonds) with nearby positive amino acids and further stabilize the T state



Haldane Effect



Lungs:

High pO2: Transition to the R state (proximal His)

Hb deprotonation (mechanical forces break the protonated-His-mediated bonds and protons are released)

Carbonic Anhydrase is a bidirectional enzyme

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

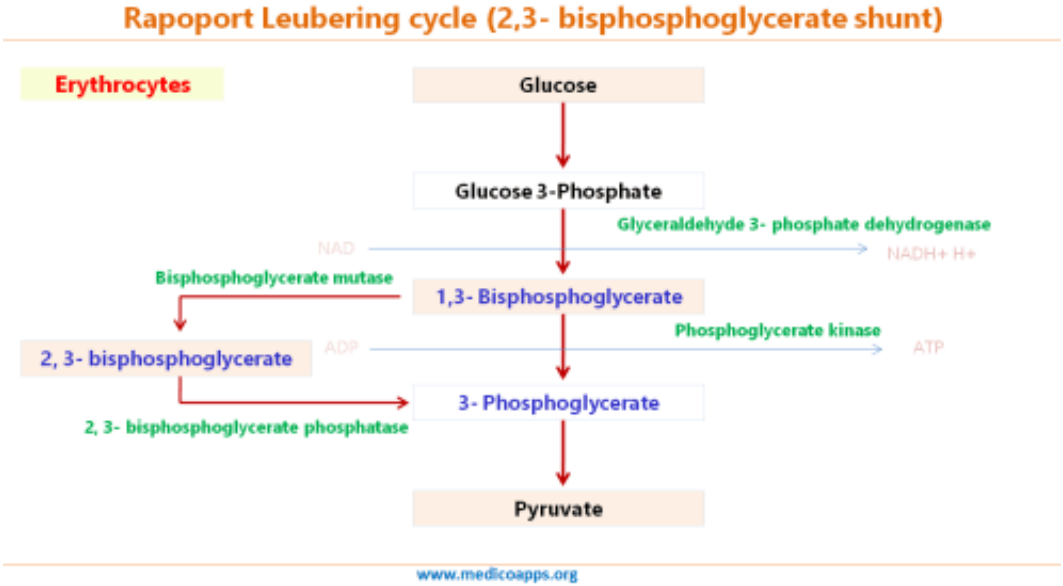
Higher pH (loss of ionic interactions favoring T state)

R state-related conformational rearrangements pull carbamates away from their positively charged partners, leaving the now unstable carbamates to be converted back to CO_2.

Thus, CO_2 affects the affinity of Hb for O_2, and O_2 affects the binding of Hb to CO_2.

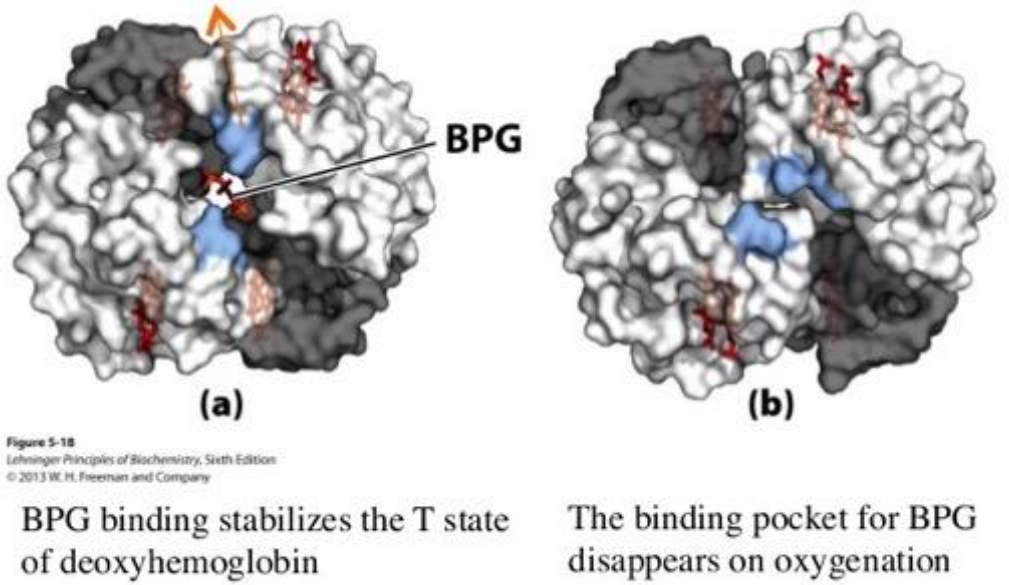
BOHR EFFECT	HALDANE EFFECT
Decrease in the oxygen affinity of a respiratory pigment such as hemoglobin in response to decreased blood pH resulting from increased carbon dioxide concentration in the blood	Decrease in the carbon dioxide affinity of hemoglobin in response to increased blood pH resulting from increased oxygen concentration in the blood
First described by Christian Bohr	First described by John Scott Haldane
Occurs at the metabolizing tissue	Occurs in the lungs
Describes the release of oxygen	Describes the release of carbon dioxide
Effective under low blood pH	Effective under high blood pH
Caused by the take up of carbon dioxide at the metabolizing tissue	Caused by the uptake of oxygen in the lungs
Facilitates the release of oxygen at the metabolizing tissue	Facilitates the binding of oxygen to the hemoglobin

2,3-diphosphoglycerate (2,3-BPG or 2,3-DPG). A metabolic product of a one-step diversion in the glycolysis pathway in red blood cells (the Luebering–Rapoport pathway). **The T state is very unstable; the role of 2,3-BPG is to stabilize it until the partial pressure of O_2 is sufficiently high (in the lungs) to allow the transition to the R state.**



2,3-BPG binds to the center of the tetramer of deoxygenated Hb (at a site present only in the T form). During the transition from the T to the R state, this pocket collapses and 2,3-BPG is released.

Without 2,3-BPG, Hb would release only 8% of its load into the tissues

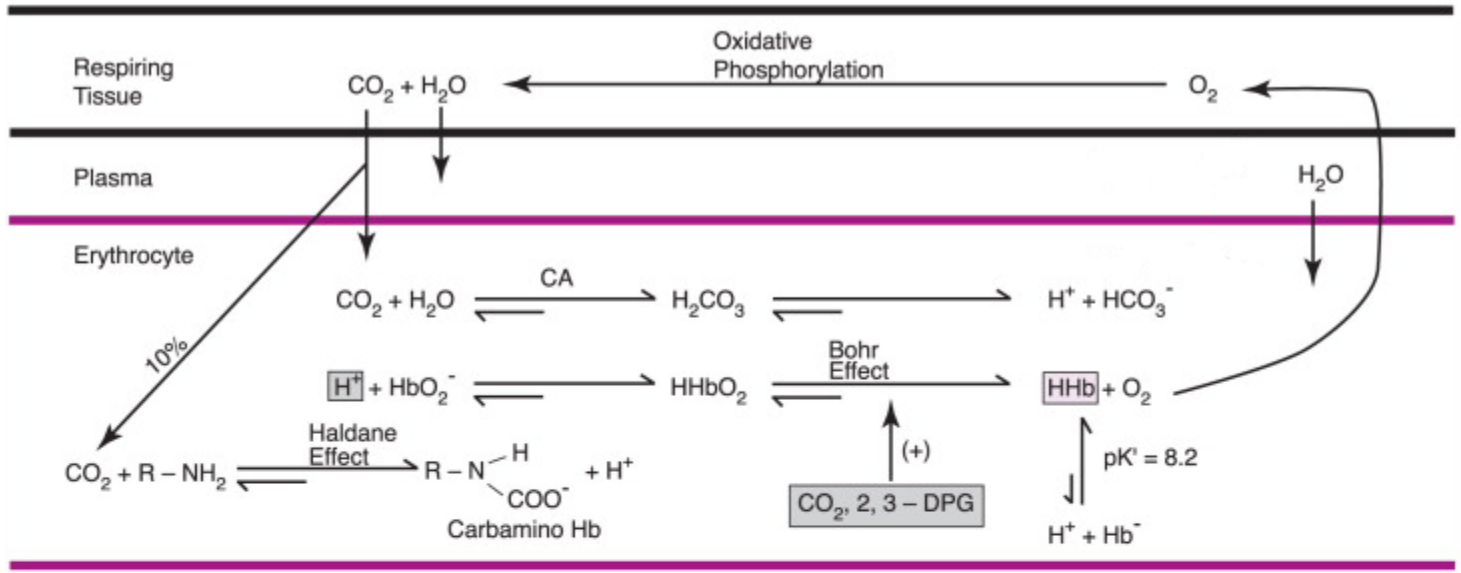


BPG binding stabilizes the T state of deoxyhemoglobin

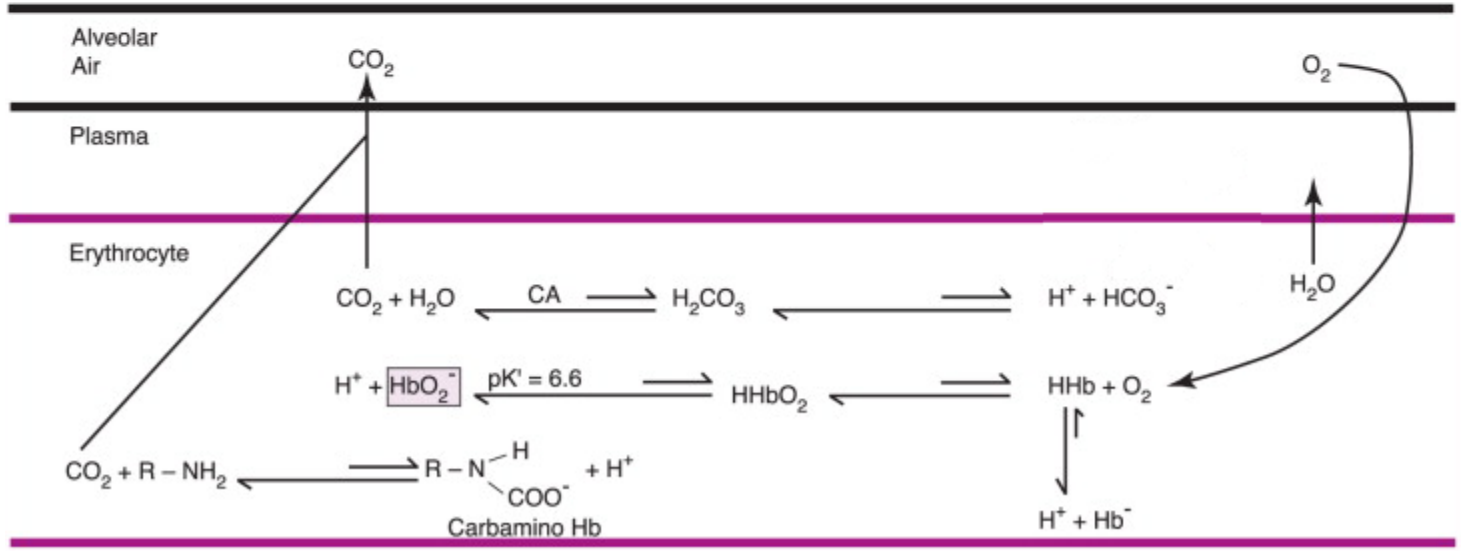
The binding pocket for BPG disappears on oxygenation

H^+, CO_2 and 2,3 BPG are allosteric ligands; that is, they bind to sites distinct from those where O2 binds and modify the protein.

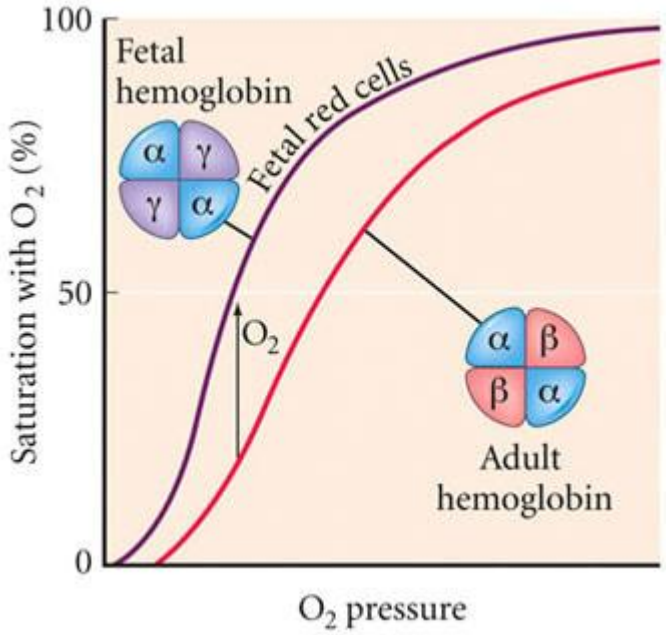
Events in the Erythrocyte when Arterial Blood is Delivered to Respiring Tissues



Events in the Erythrocyte when Deoxygenated Blood is Delivered to the Lungs



Fetal Hemoglobin (HbF)



A variant form of hemoglobin, a tetramer composed of 2 alpha and 2 gamma chains (α2γ2). It is the primary oxygen-carrying protein in the human fetus during the last 7 months of development and is replaced by α2β2 Hb by the 6th month of life.

After birth, the gene encoding the γ chain is silenced and the gene encoding the β chain is activated, so that adults primarily produce HbA (α2β2). Nevertheless, low levels of HbF continue to be produced in adulthood and are distributed heterogeneously among certain red blood cells (F cells) that express increased amounts of HbF.

HbF is 72% identical to Hb. **The substitution of a histidine residue with a serine at the 2,3-BPG binding site reduces HbF's affinity for it**. Consequently, HbF exhibits a higher affinity for O_2, allowing O_2 to be transported efficiently from maternal to fetal red blood cells in an environment of low partial pressure of O_2.

In adults, the production of fetal hemoglobin can be pharmacologically reactivated and is used in the treatment of diseases such as sickle cell disease.

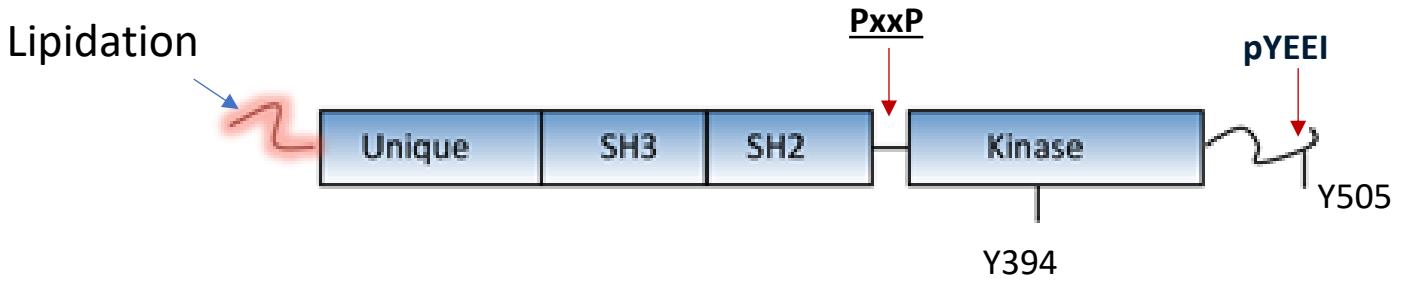
Carbon Monoxide (CO) Poisoning

CO: combustion product (car exhaust, LPG appliances, firewood)
Responsible for 2,500 deaths annually in the US (~2,000 of these are suicides)

It binds to Hb at the same site as O_2 and with ~200 times greater affinity. Even if the partial pressure of CO in the blood is low, it will displace O_2 from Hb, depriving the tissues of oxygen.
Binding of CO to even a single site on the tetramer pushes Hb into the R state, further preventing the release of O_2 to the tissues

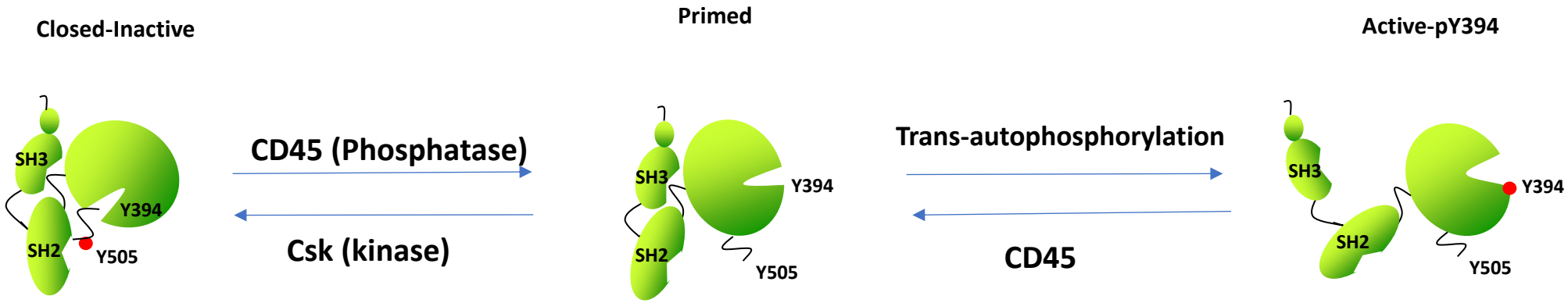
Treatment: administration of 100% O_2 (concentration higher than atmospheric—hyperbaric oxygen therapy)
To raise the partial pressure of O_2 high enough to increase the rate at which CO is replaced by O_2 in Hb

Intramolecular interaction can regulate enzymatic activity



pYEEI: A motif recognized by Src-family SH2 domains.

PxxP : the most well-known SH3 binding motif, where P=proline and x=is any amino acid.

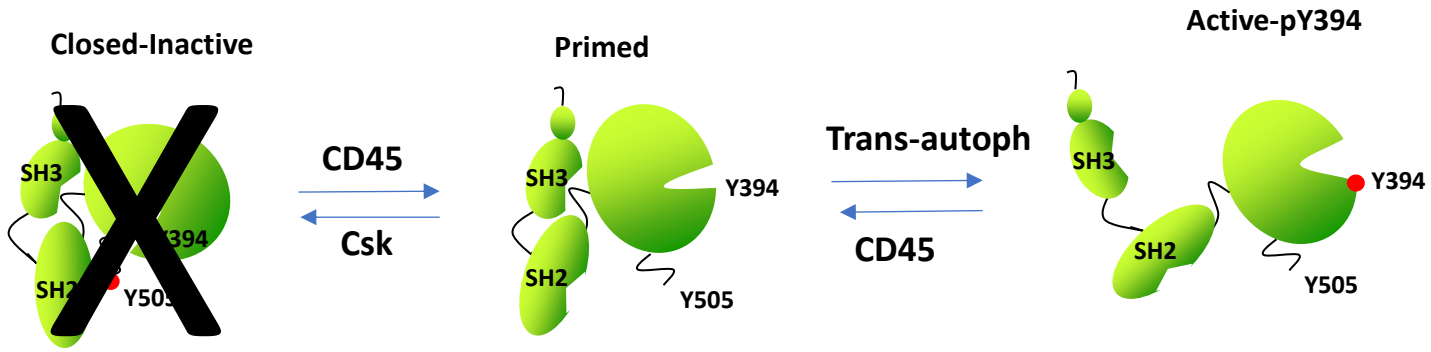


A proto-oncogene can become an oncogene by a relatively small modification of its original sequence.



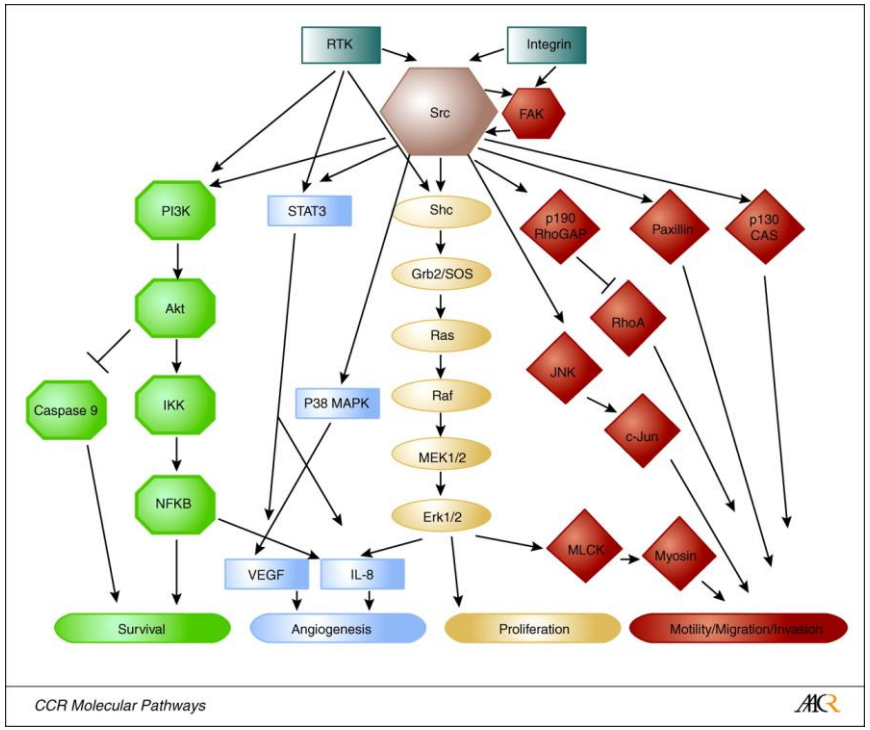
[Francis Peyton Rous](#) first proposed that viruses can cause cancer. He proved it in 1911 and was later awarded the Nobel prize in 1966

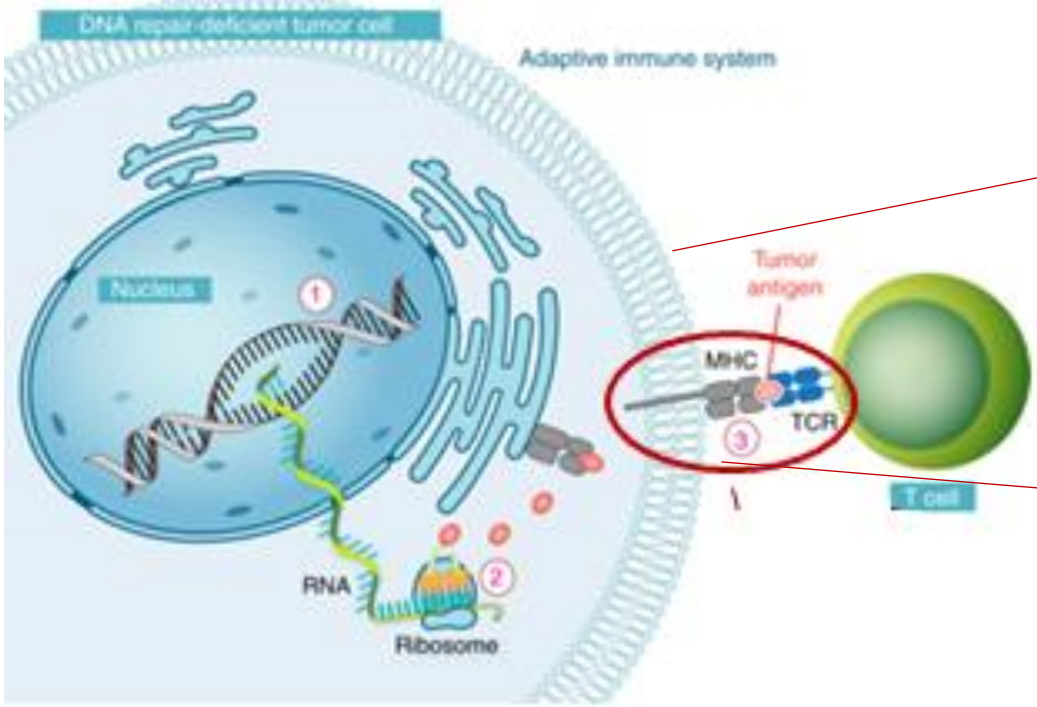


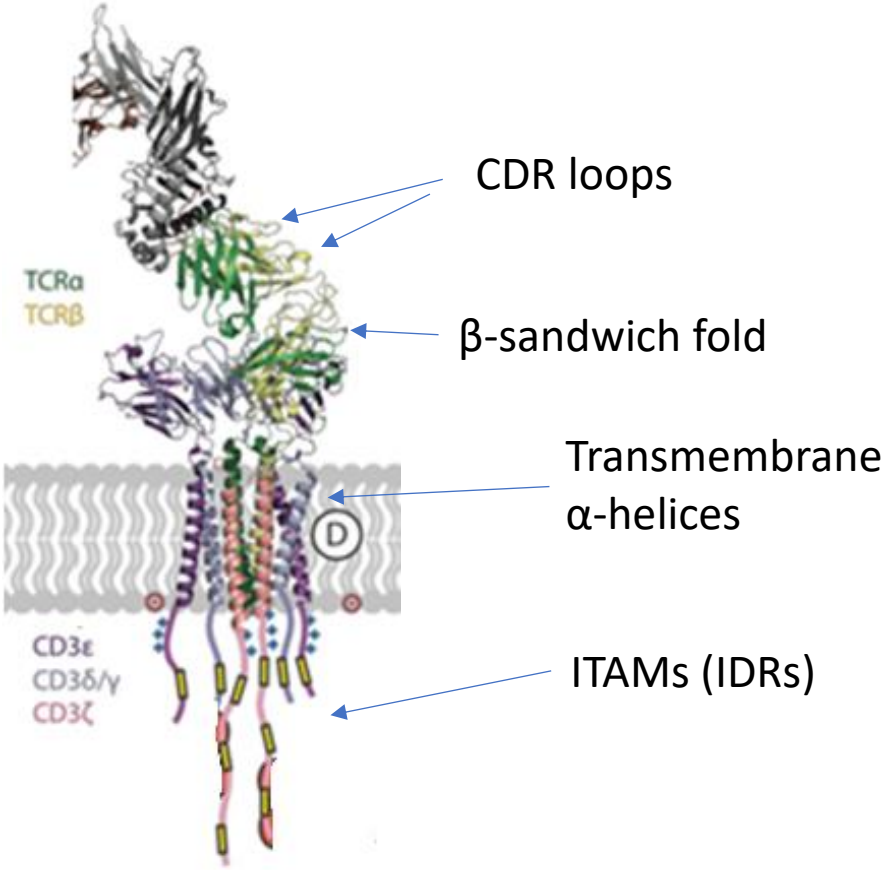


The protein tyrosine kinase v-Src , the transforming product of Rous sarcoma virus, is the first identified oncogene.

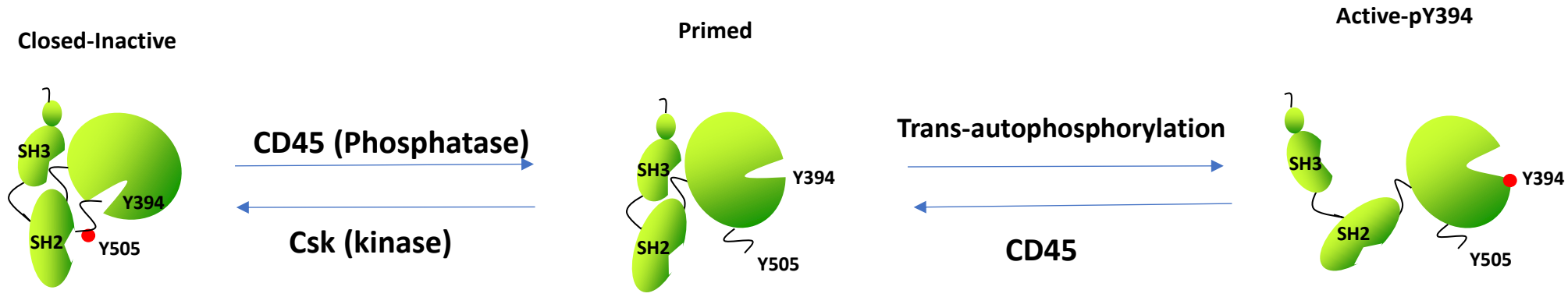
v-Src lacks the C-terminal inhibitory phosphorylation site (tyrosine-527), which results in loss of binding to the SH2 domain and stabilizes the `closed' or inactive conformation of the molecule





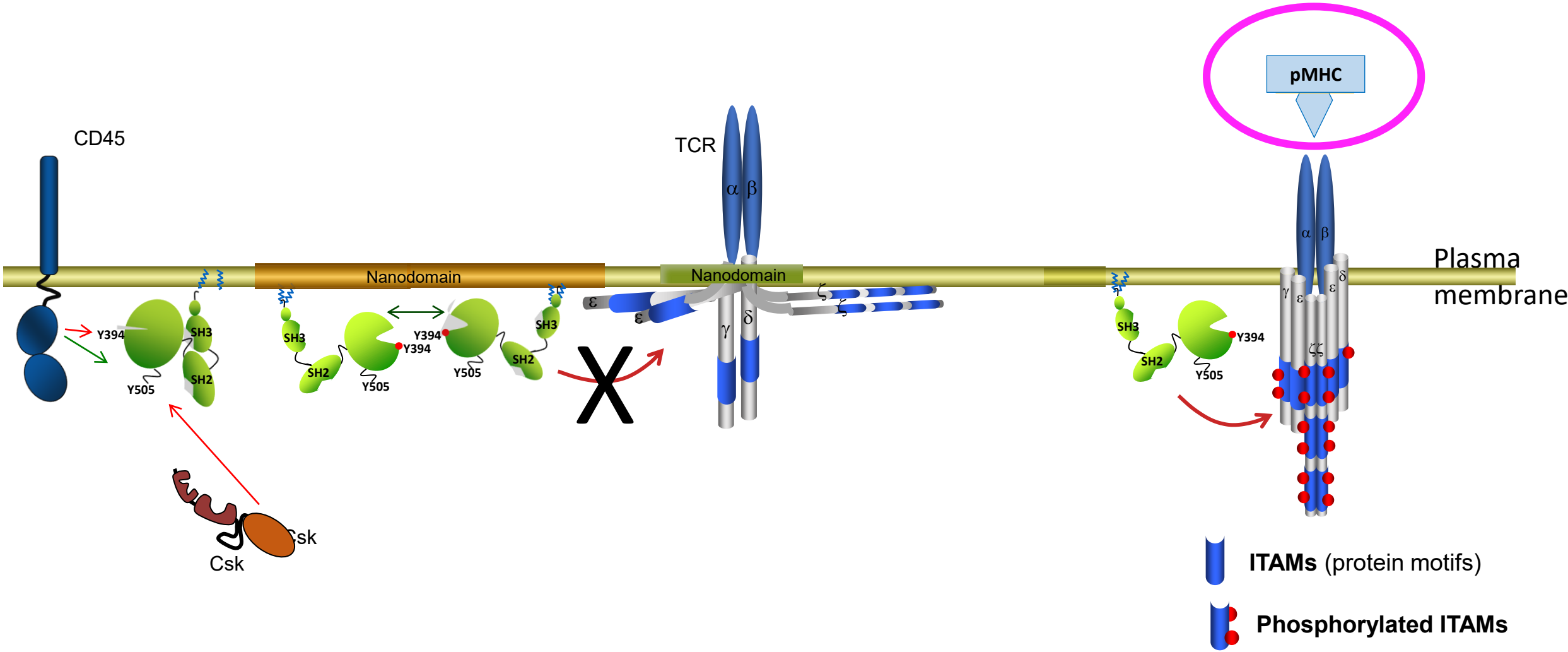


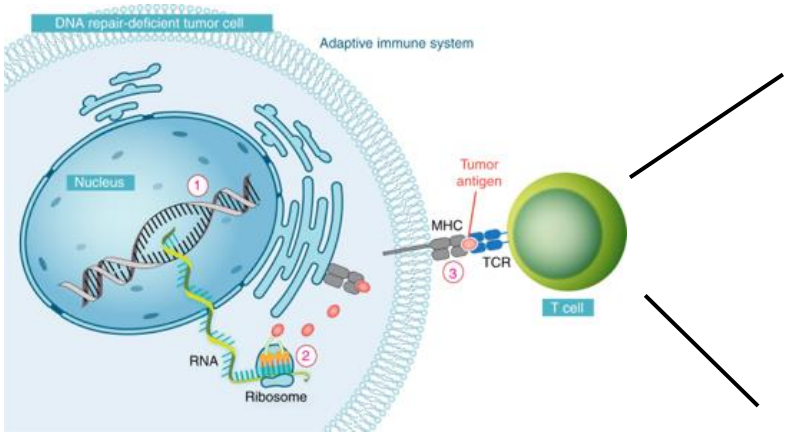
Simultaneous co-existence of all different conformations, how?

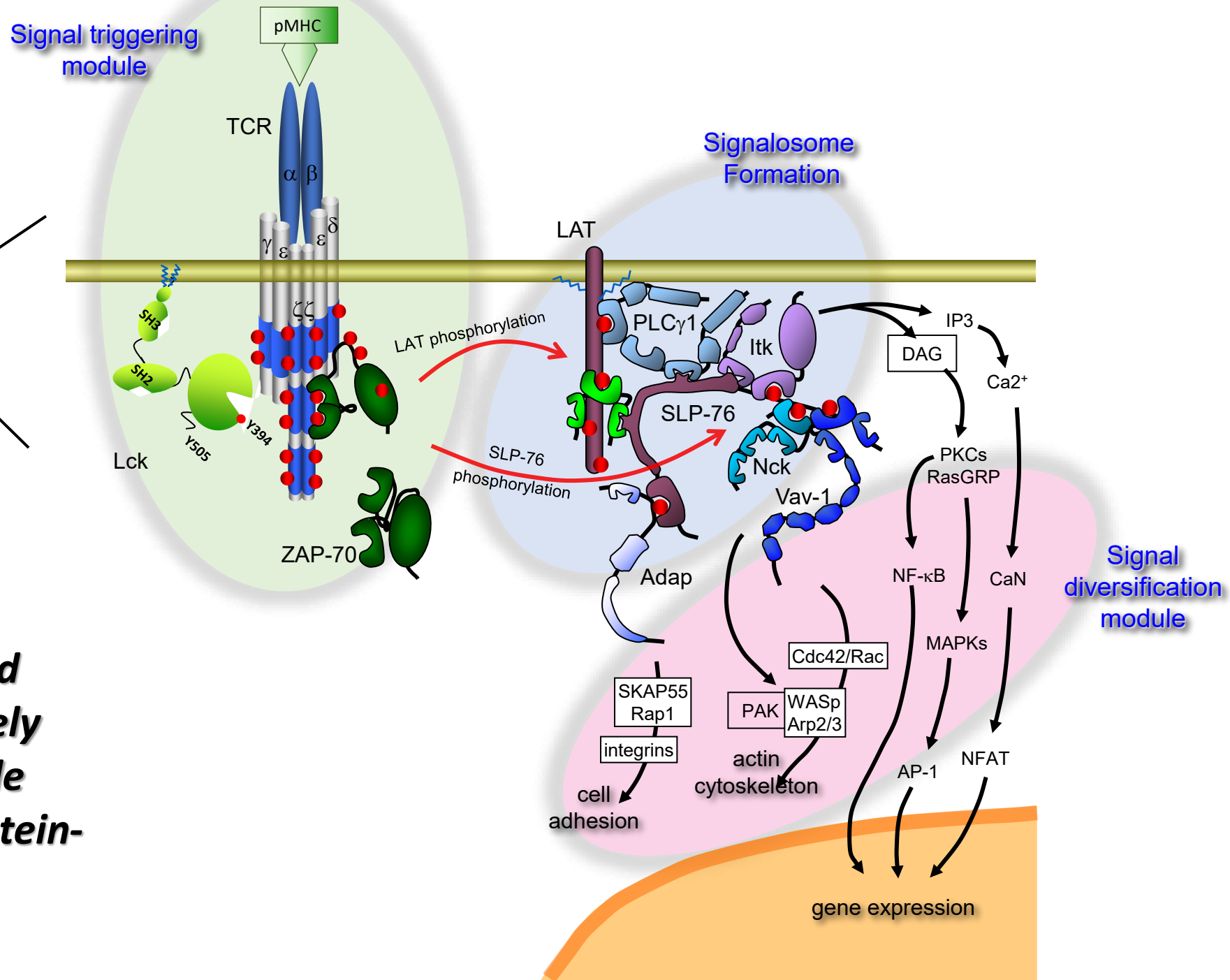


How unauthorized substrate phosphorylation (and subsequent cell activation) is prevented?

- **Membrane localization/compartmentalization enables simultaneous existence of different activation states and also proximity to the substrate.**
- **ITAM binding to membrane phospholipids mask tyrosine targets**







Cellular responses and functions are extensively regulated by reversible phosphorylation and protein-protein interactions