Structure-Function relationship

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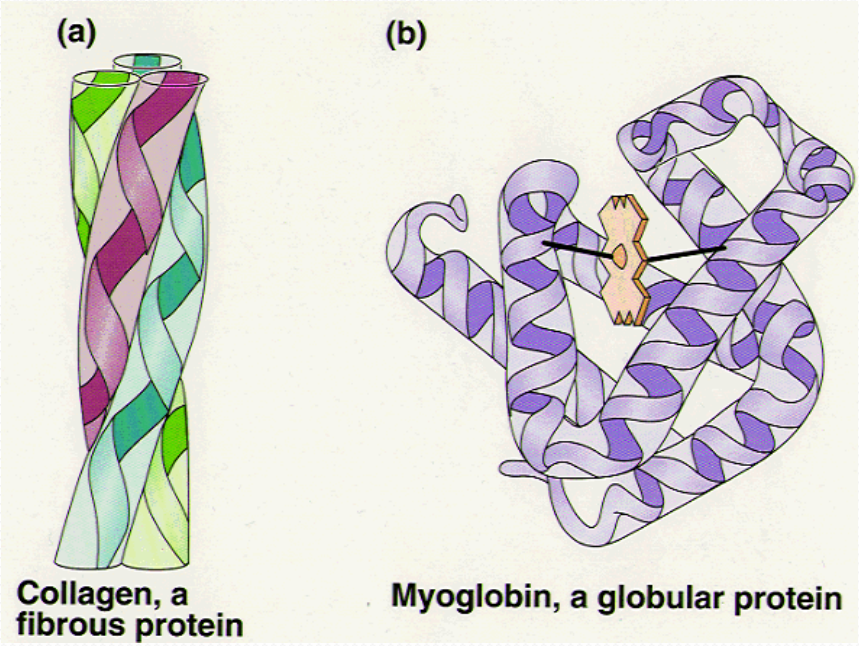
Types of proteins

- Proteins can be divided into two groups according to structure:
  - Fibrous (fiber-like with a uniform secondary-structure only)
  - Globular (globe-like with three-dimensional compact structures)

Examples

- Fibrous proteins: collagens, elastins, & keratins

- Globular proteins: myoglobin, hemoglobin, & immunoglobulin



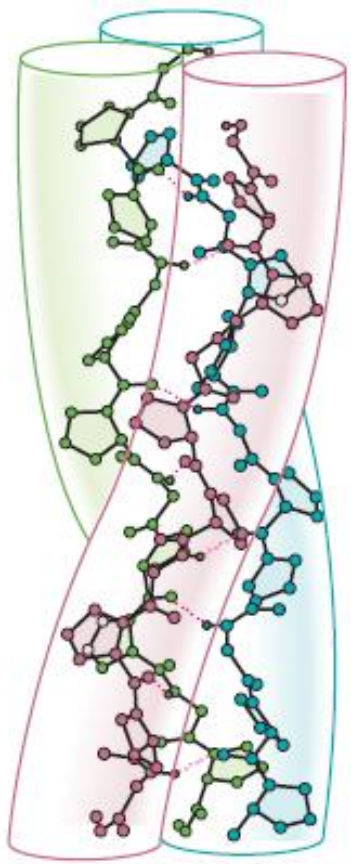
Fibrous Proteins

Collagen

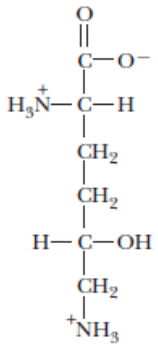
Elastin

Keratin

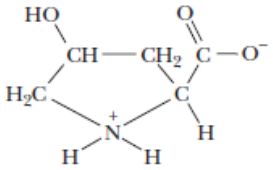
Collagen



- The most abundant protein in vertebrates
  - (25% of mammals protein)
- 25 different types (I, II, III, IV, … etc.)
- Found in all multicellular animals
- Organized in water-insoluble fibers
- Have a great strength
- Consists of 3 polypeptide chains wrapped around each other in a ropelike twist, or triple helix (tropocollagen)
- Has a repeating sequence of the amino acids;
  - Gly (33%) — X2(Pro 13%)—X3
  - Gly (33%) — X_2— X3 (Pro_{OH} 9%)
  - Hydroxy-lysine frequently occurs in collagen

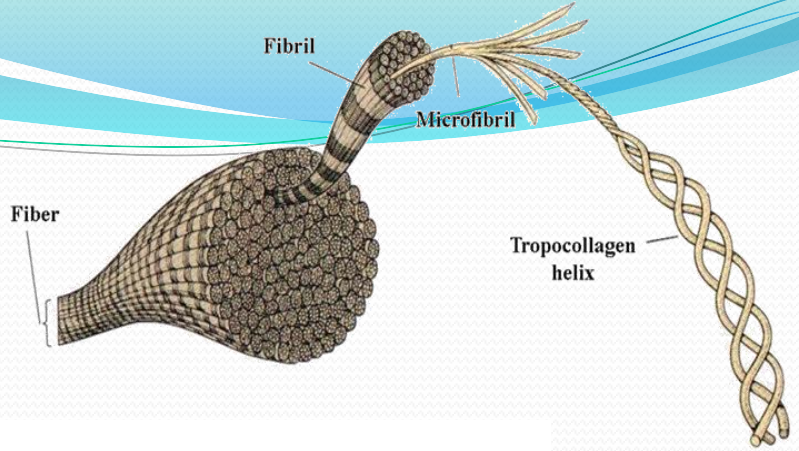


Hydroxylysine

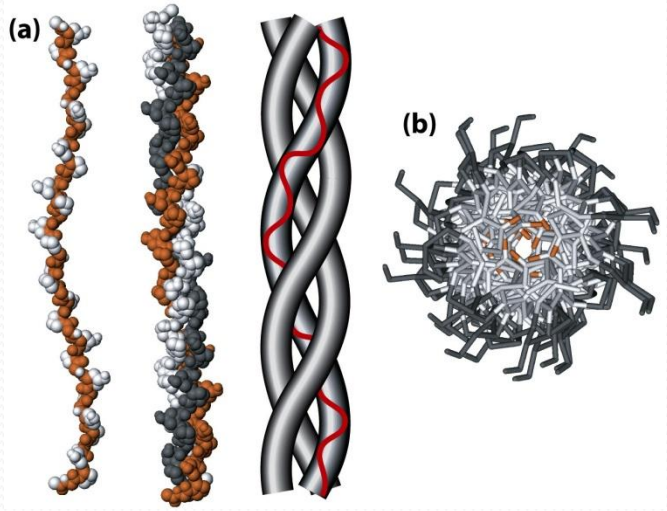


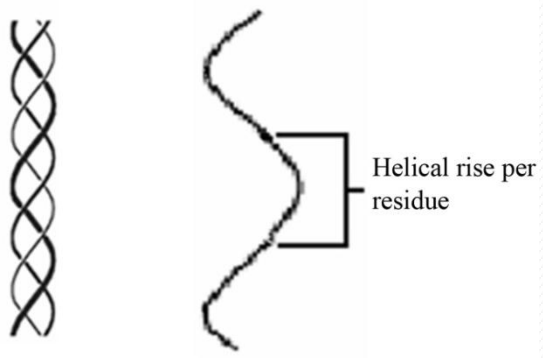
Hydroxyproline

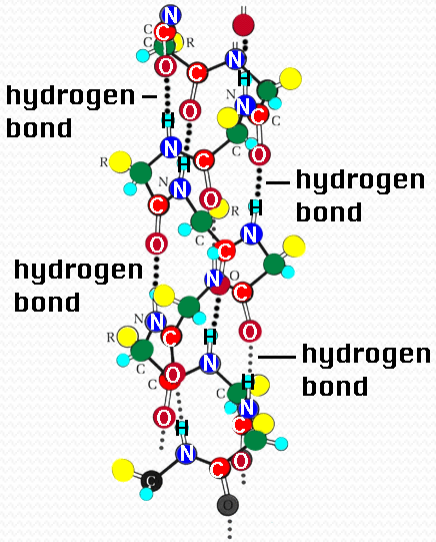
Collagen



- The triple helix (*tropocollagen)* is:
  - 300 nm long and 1.5 nm in diameter
  - left-handed, triple-stranded (α-chains), ropelike superhelix
  - Held together by H-bonding
  - Each strand have ≈ 800 amino acids (300 kDa)
- Compared to the α-helix (3.6 residues), the collagen helix is more extended with 3.3 residues per turn

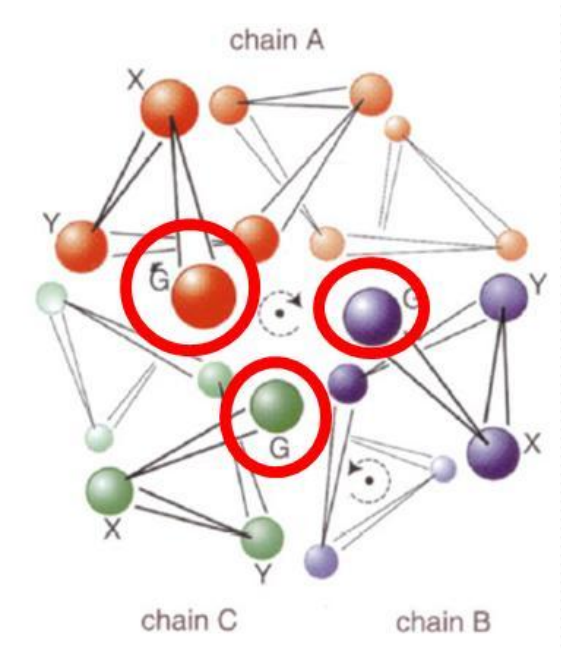


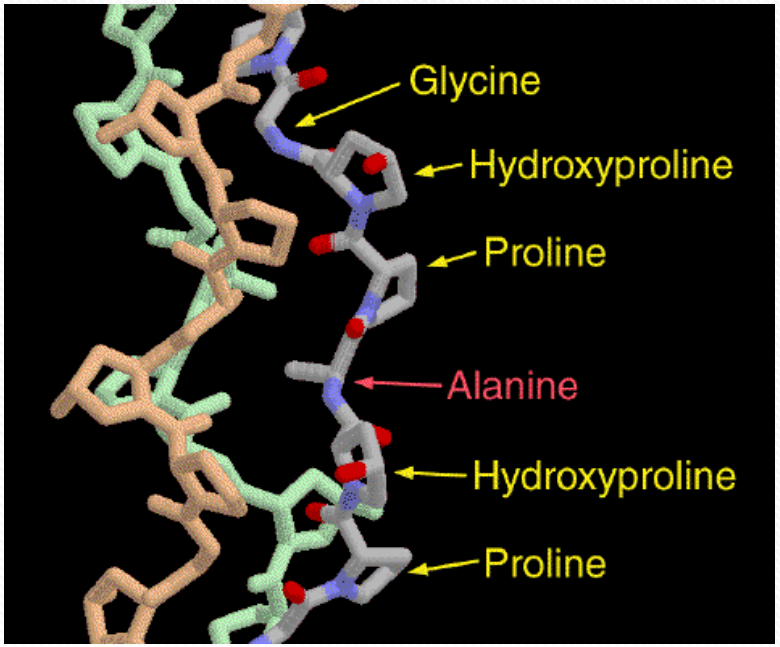




Functional purposes of amino acids

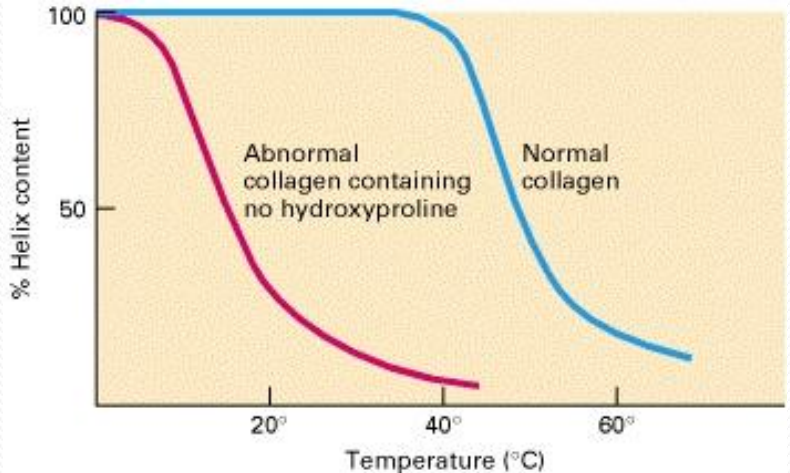
- Glycine: allows the three helical α-chains to pack tightly
- Proline: creates the kinks & stabilizes the helical conformation

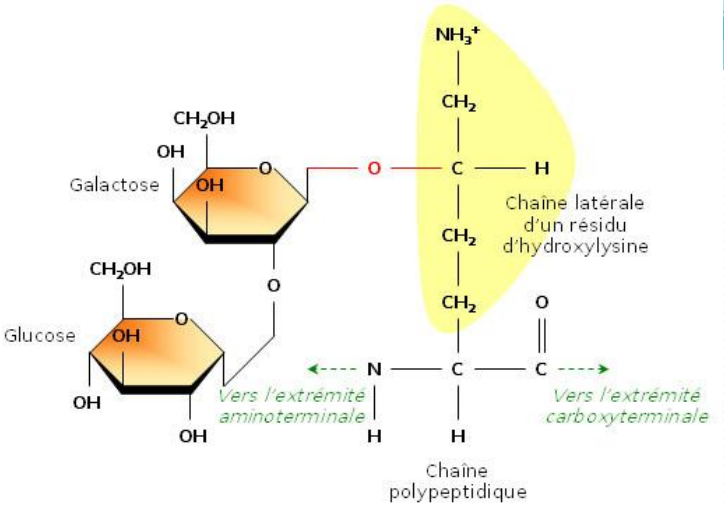


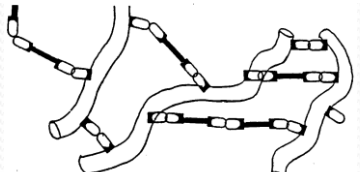


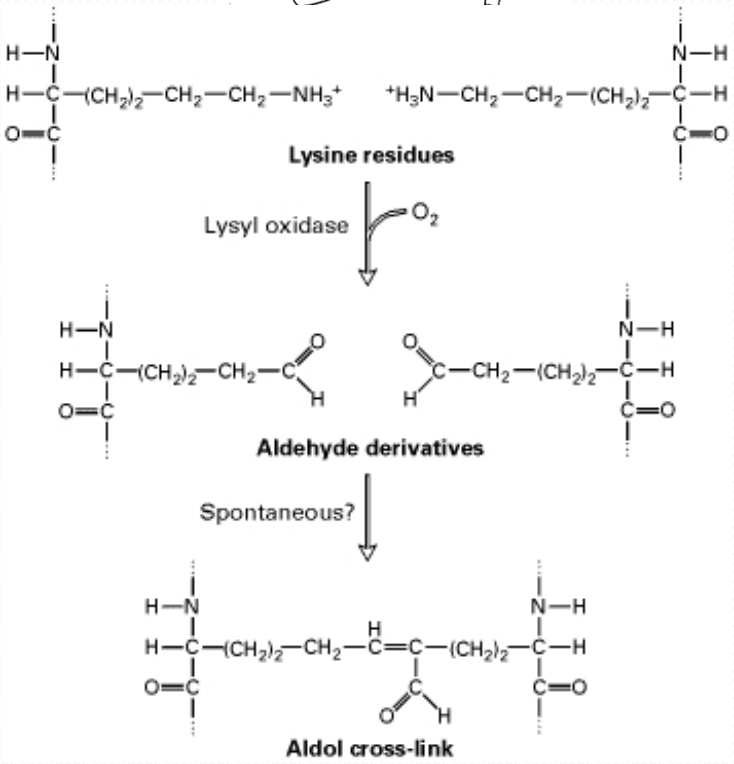
Functional purposes of amino acids

- Hydroxylysine:
  - Attachment sites of polysaccharides
  - Oxidation: to aldehyde followed by Covalent aldol cross-links form between hydroxylysine residues and lysine or another oxidized lysine
- Hydroxyproline:
  - Hydrogen bonding, Helical formation



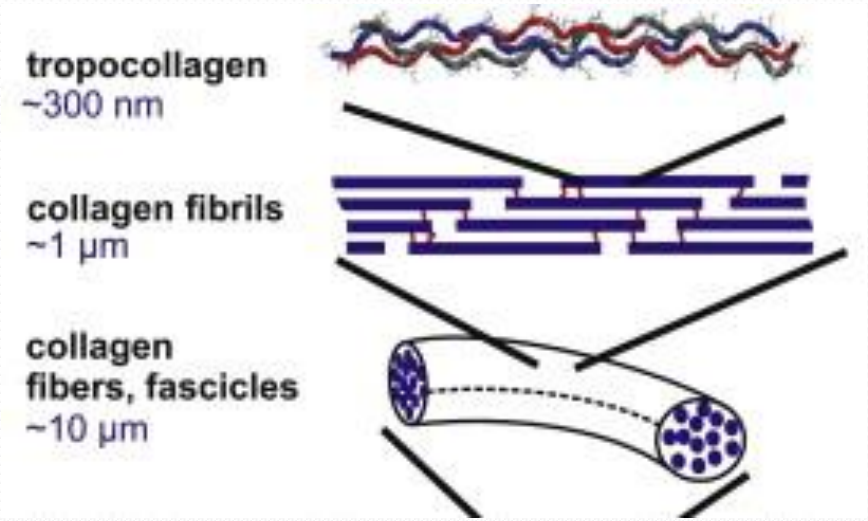


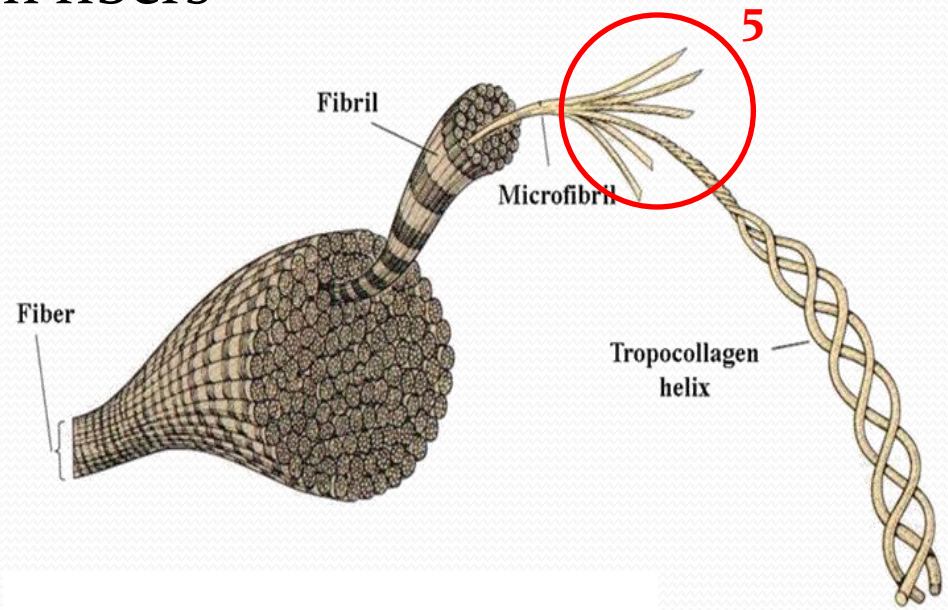




Formation of collagen fibers

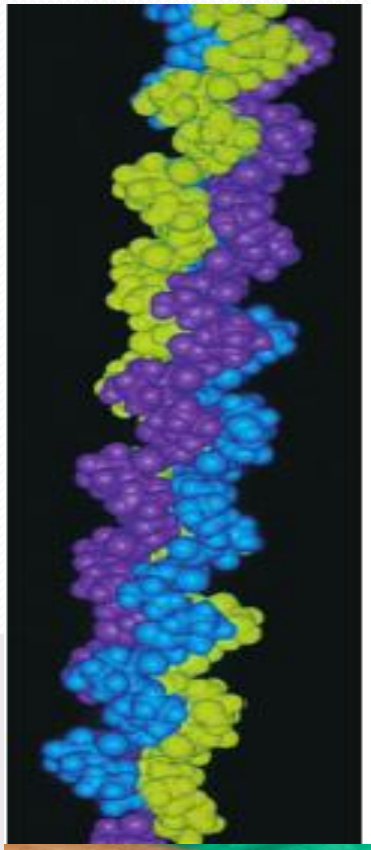
- Tropocollagen (5 of them) polymerize into a microfibril, which are connected with each other via aldehyde links
- Microfibrils align with each other forming larger collagen fibrils, which are strengthened by the formation of covalent cross-links between lysine residues
- Fibrils assemble into collagen fibers

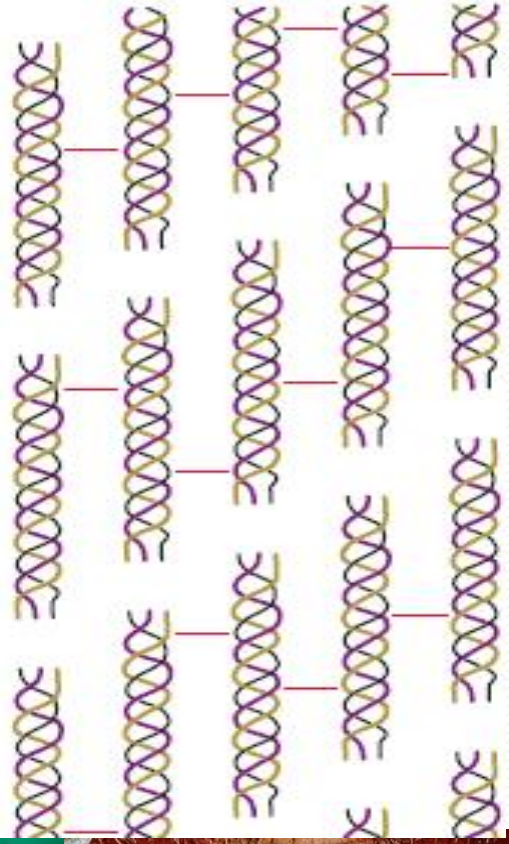


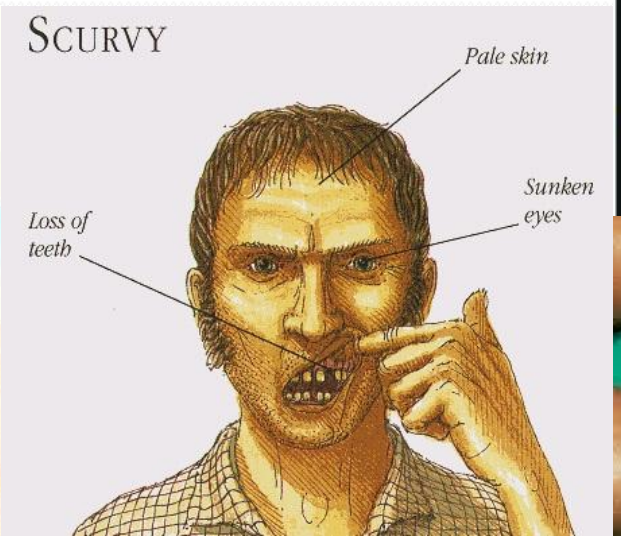


Collagen; Diseases

- Cross-linked intra- & inter-molecularly
- Cross-linking amounts varies according to tissue & increases with age (meat)
- Deficiency of cross-linking (Scurvy & osteogenesis imperfecta)

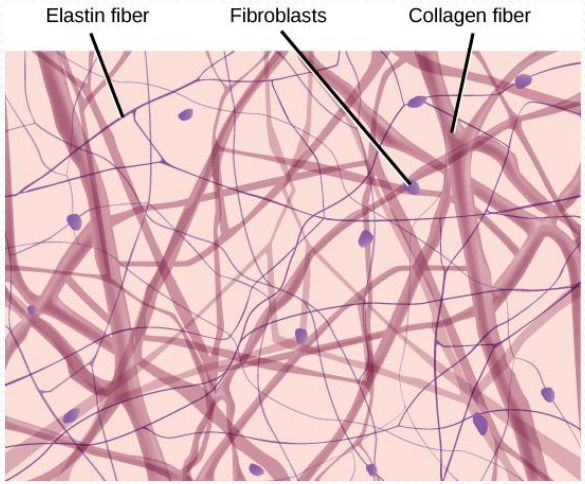


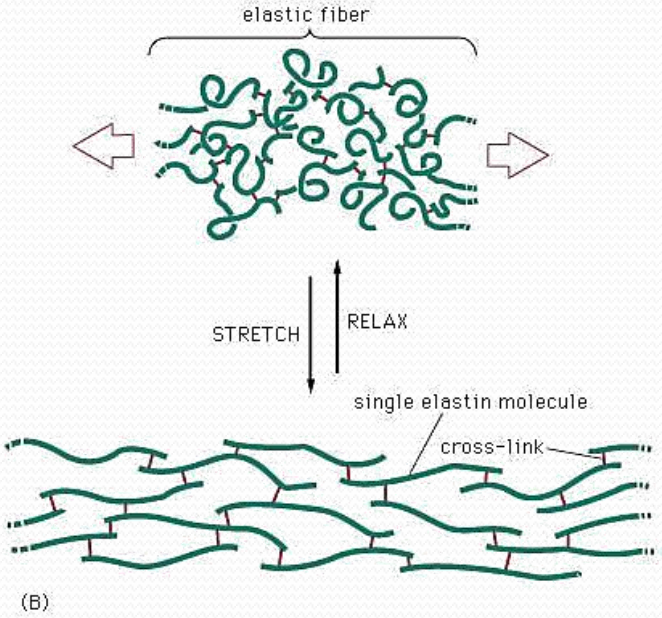




Elastin

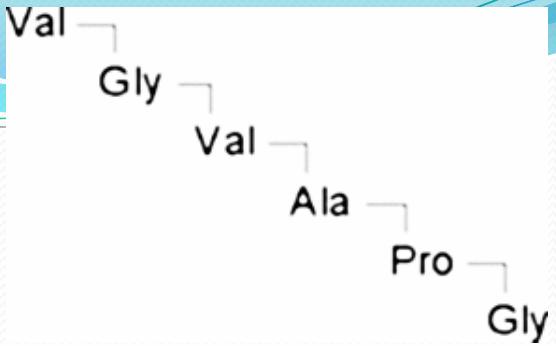
- Skin, blood vessels, and lungs
- Resilience vs. flexibility
- Long, inelastic <u>collagen fibrils are interwoven</u> with the elastic fibers to limit the extent of stretching and prevent the tissue from tearing
- It is <u>not glycosylated</u>

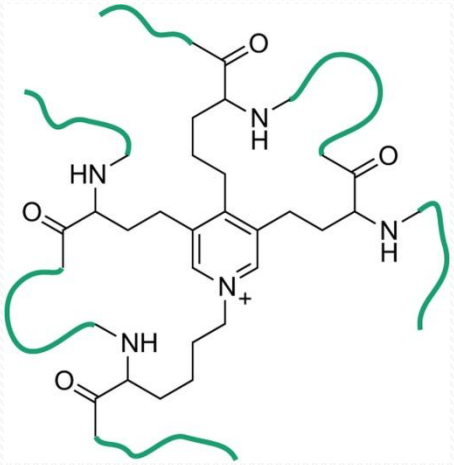


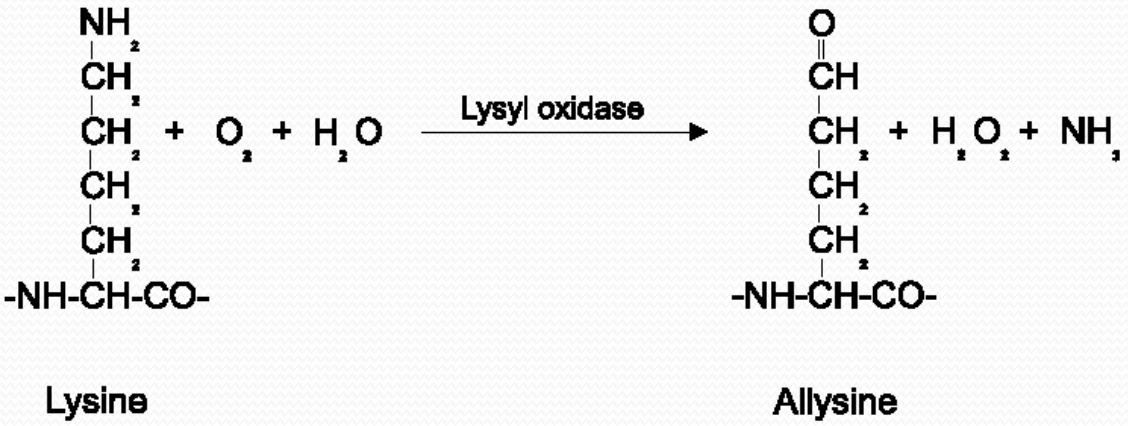


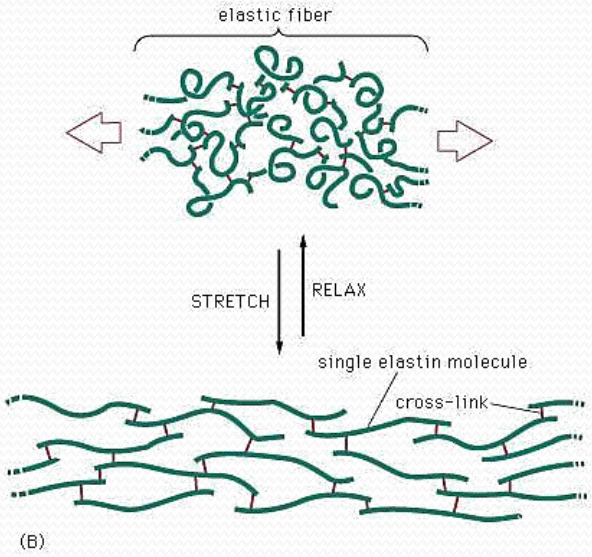
Elastin

- Rich in hydrophobic amino acids (ex. Gly, Val & Pro); mobile hydrophobic regions bonded by crosslinks between Lys
- Elastic fibers in arteries are composed mainly of elastin (≈70%)
- Tropoelastin → Elastin (Lysyl oxidase)
- Three allysyl side chains plus one unaltered lysyl side chain



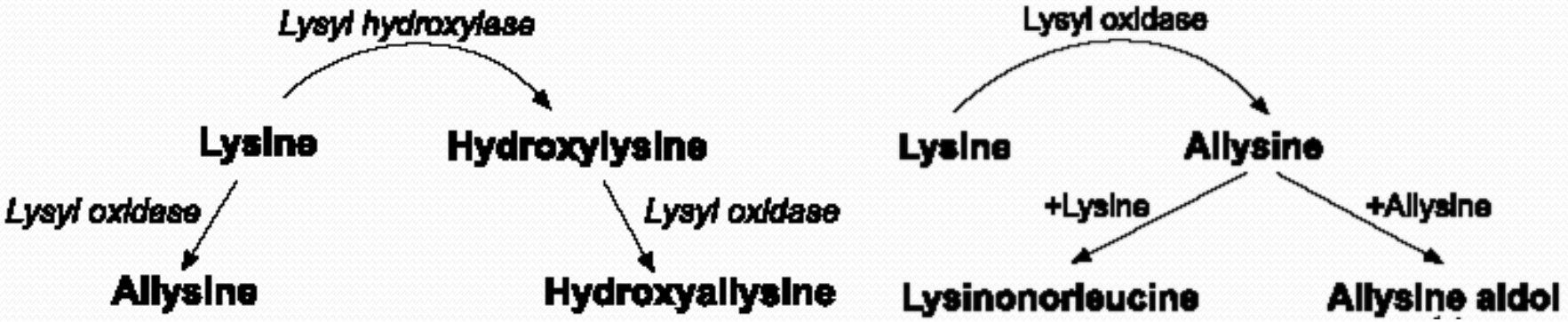




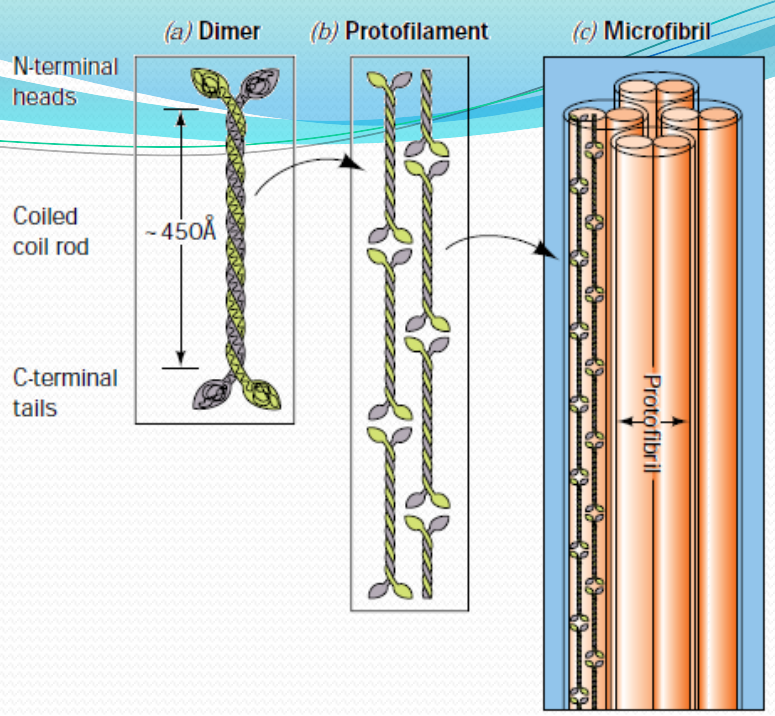


Elastin & hydroxylysine

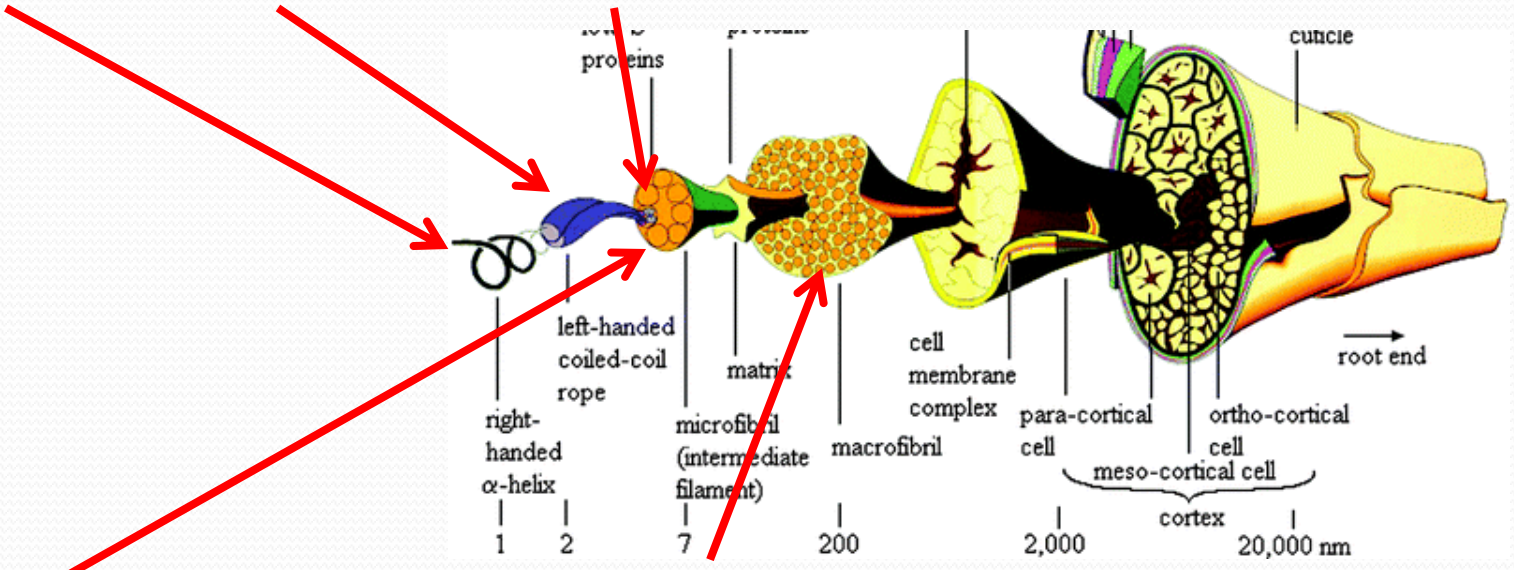
- Collagen contain lysine that can be hydroxylated by lysyl-hydroxylase to form hydroxyl-lysine or by lysyl-oxidase to form Allysine
- Cross-linking of elastin occurs through the enzyme lysyl-oxidase producing the Allysine, the pathway for oxidation through lysyl-hydroxylase does not occur in elastin



Keratin



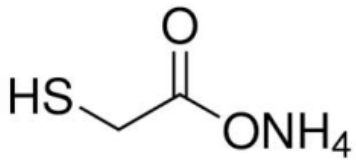
- Principal component of epidermis & related appendages (hair, horn, nails, & feathers)
- α (mammals) or β (birds & reptiles)
- Mammals: ≈30 types, tissue-specific
- Structure:
  - α-helix (1), Coiled coil (2), Protofibril (4),



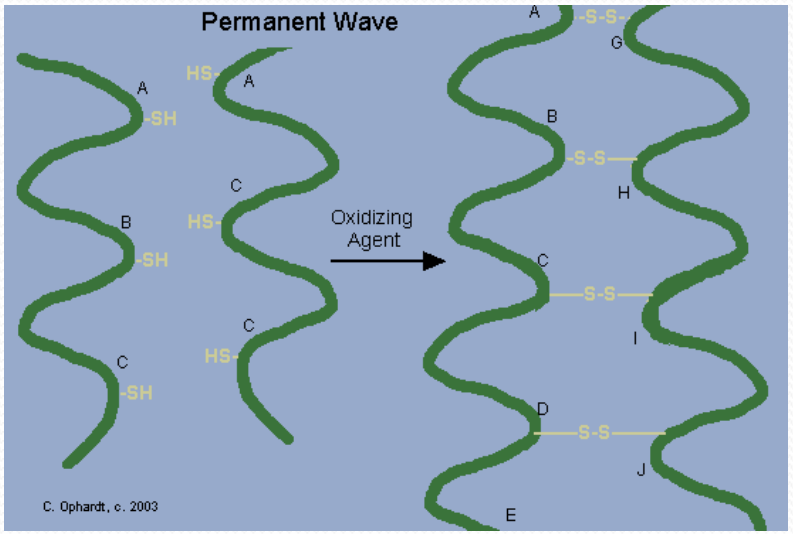
  - Microfibril (28-32) (7-8 proto), Macrofibril (1000_s) (100_s micro)

Keratin

- Unusual content of Cys
- Classified as “hard” or “soft” according to S content (Cys)
- How is a perm done?



  - A basic reducing substance (usually ammonium thioglycolate) is added to reduce and rupture some of the disulfide cross-links
  - Temporary Wave (affect H-bonding)
  - Vs. permanent wave (affect H & S-S bonding)

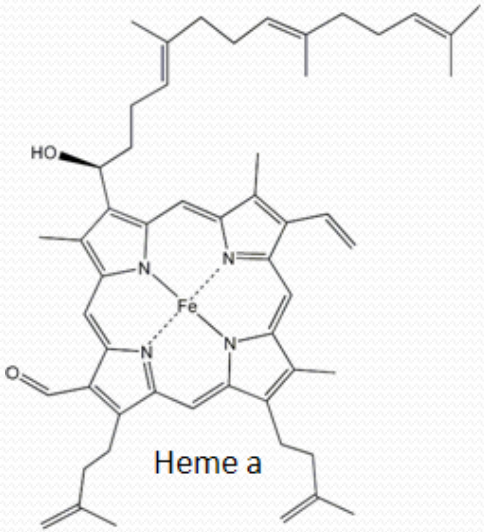


Globular Proteins

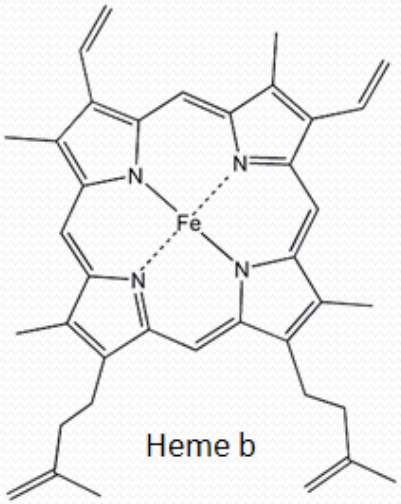
Myoglobin

Hemoglobin

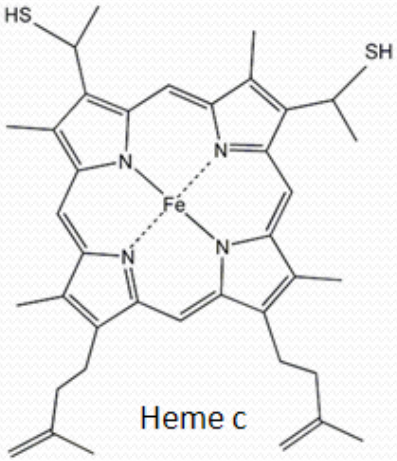
The heme



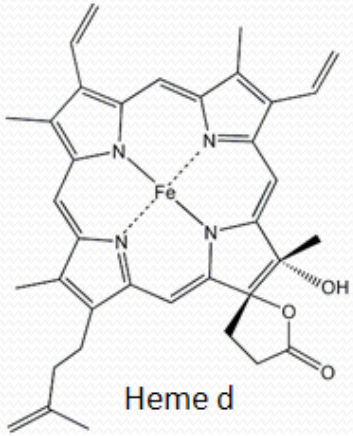
Heme a



Heme b

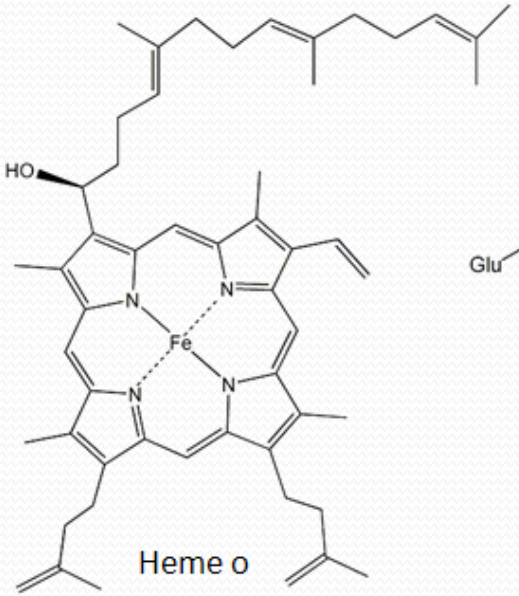


Heme c

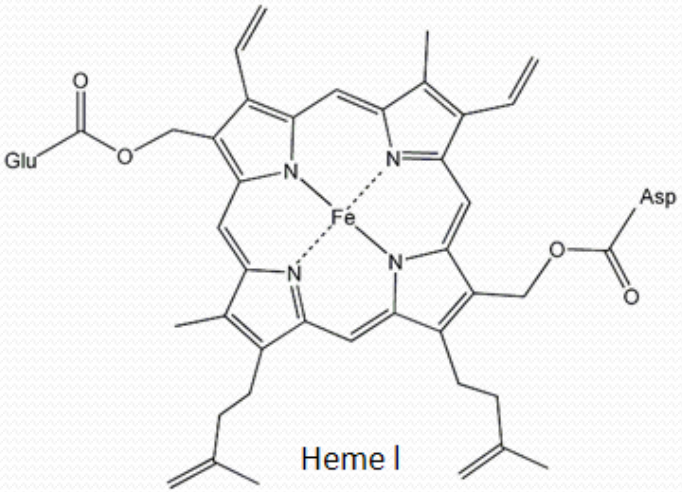


Heme d

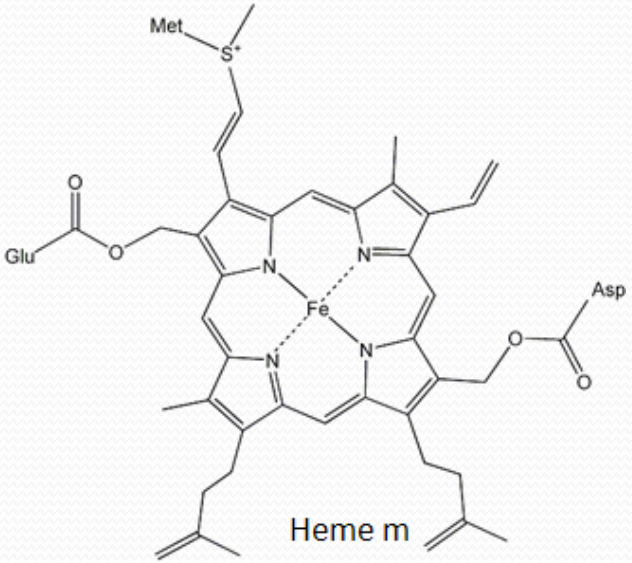
Prosthetic, Flat, Porphyrin, Pyrrole, Fe



Heme o

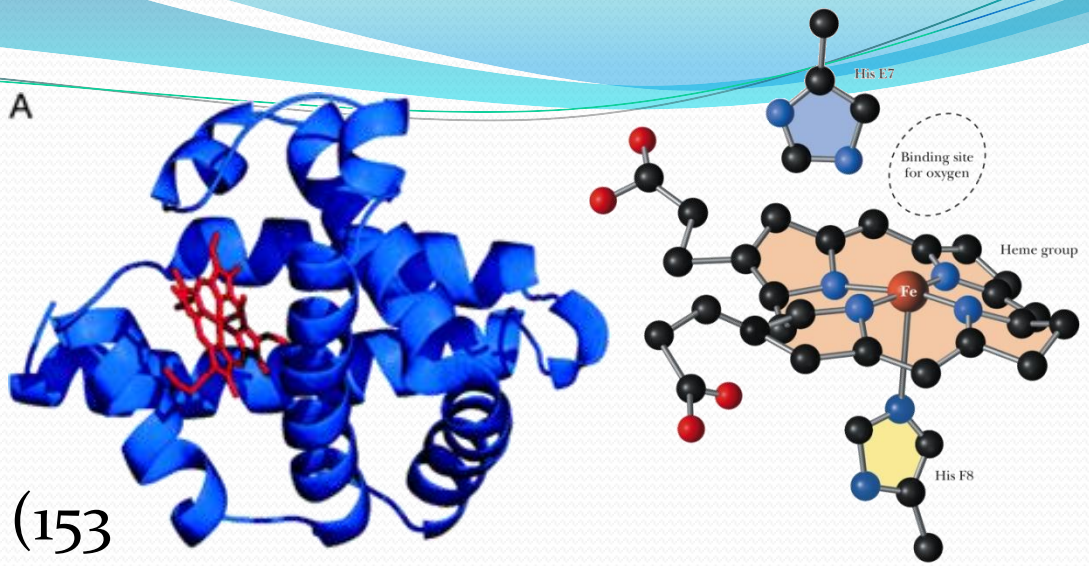


Heme l

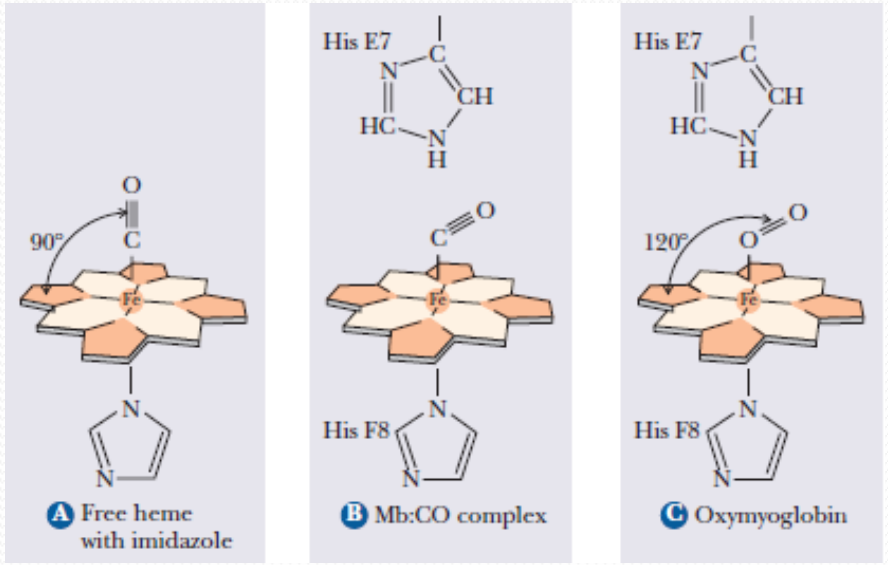


Heme m

Myoglobin



- The first to be determined structurally
- A single polypeptide chain (153 a.a)
- A single heme in a hydrophobic pocket
- 8 α-helices (A-H); no β-sheets
- Most polar (exterior), Nonpolar (interior). Two His residues: E7 (distal), F8 (proximal)
- Oxy vs. deoxymyoglobin
- Fe(II) coordination (redox)

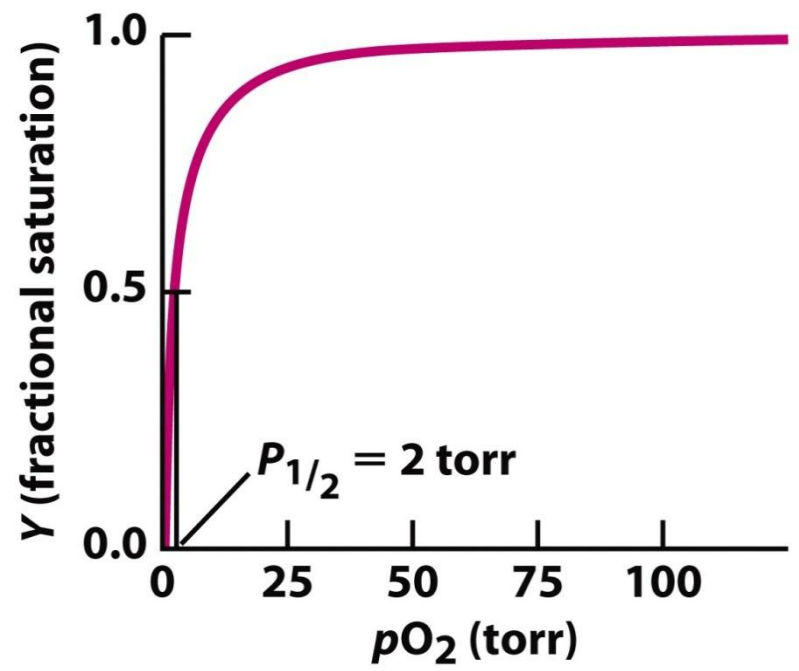


Structure-function relationship

- The heme fits into a hydrophobic pocket & myoglobin-heme interaction is stabilized by hydrophobic interactions
- The heme group stabilizes its' tertiary structure
- The distal histidine acts as a gate that opens and closes as O_2 enters the hydrophobic pocket
- The hydrophobic interior of myoglobin (or hemoglobin) prevents the oxidation of iron

Oxygen binding to myoglobin

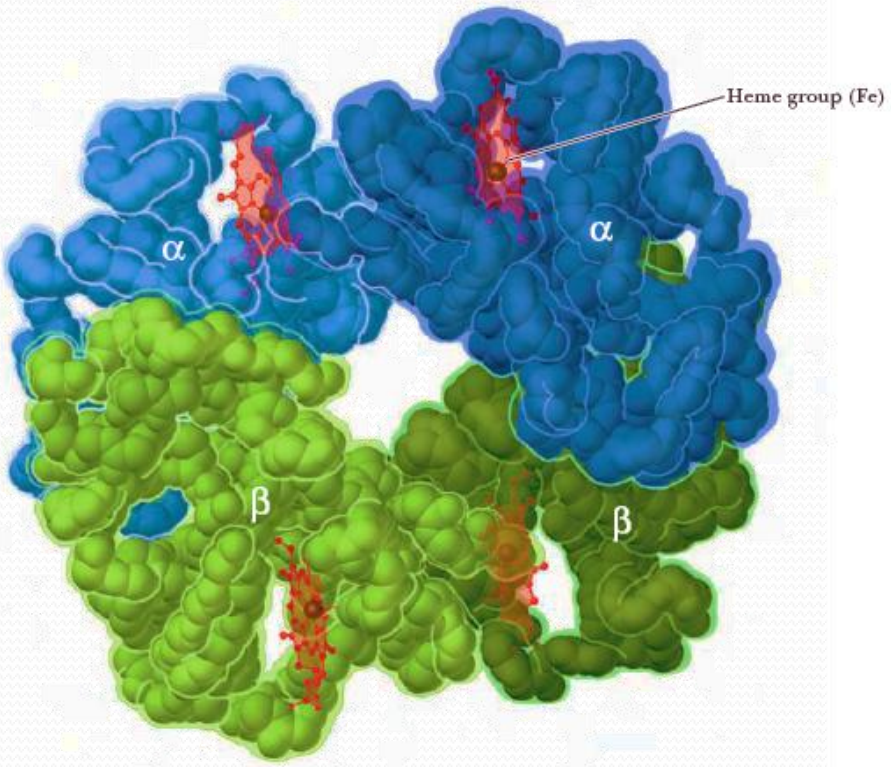
- High affinity
- The P50: ~2 torrs or mm Hg
- Tissues O_2 pressure: ~20 mm Hg (saturation)



The binding of O_2 to myoglobin follows a hyperbolic saturation curve

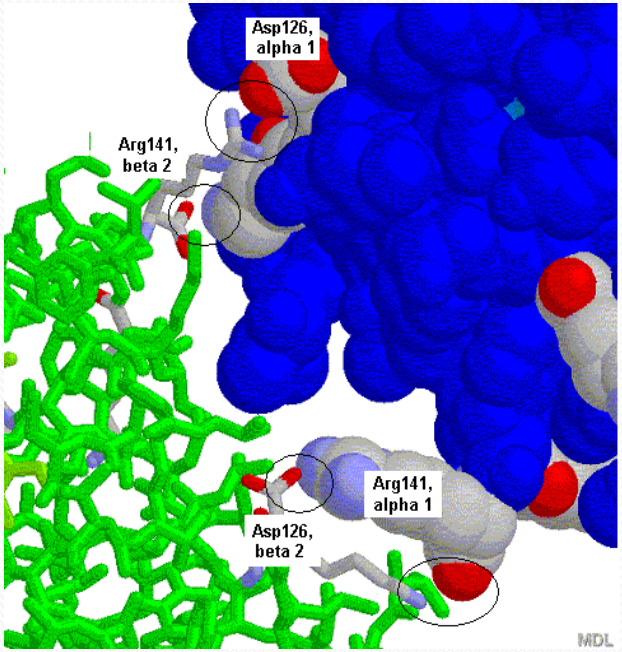
Hemoglobin

- A tetramer α2β2:
  - α-chains (141 a.a) - 7 α-helices
  - β-chains (146 a.a) - 8 α-helices
- 1 heme group in each (4O2)
- Myoglobin (storage) vs. hemoglobin (transport): positive cooperativity & saturation percentage
- lungs (100 torr), capillaries (20 torr)

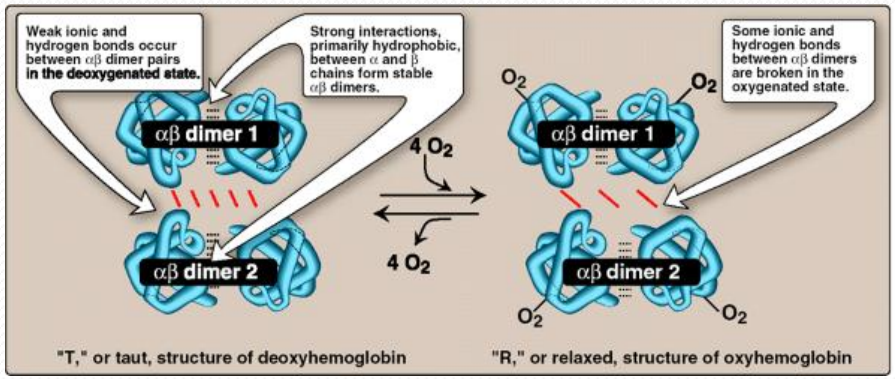


Chain interaction

- The chains interact with each other via hydrophobic interactions
  - Therefore, hydrophobic amino acids are not only present in the interior of the protein chains, but also on the surface

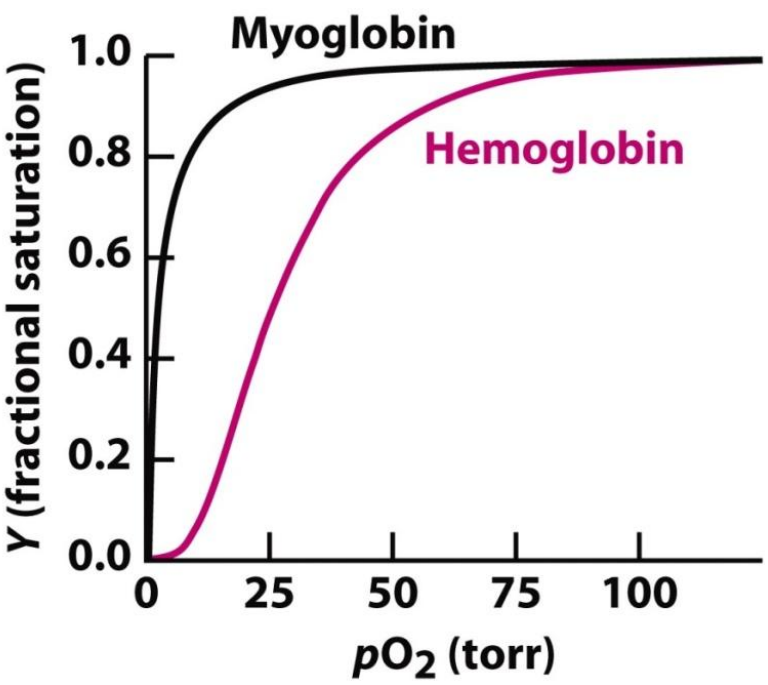


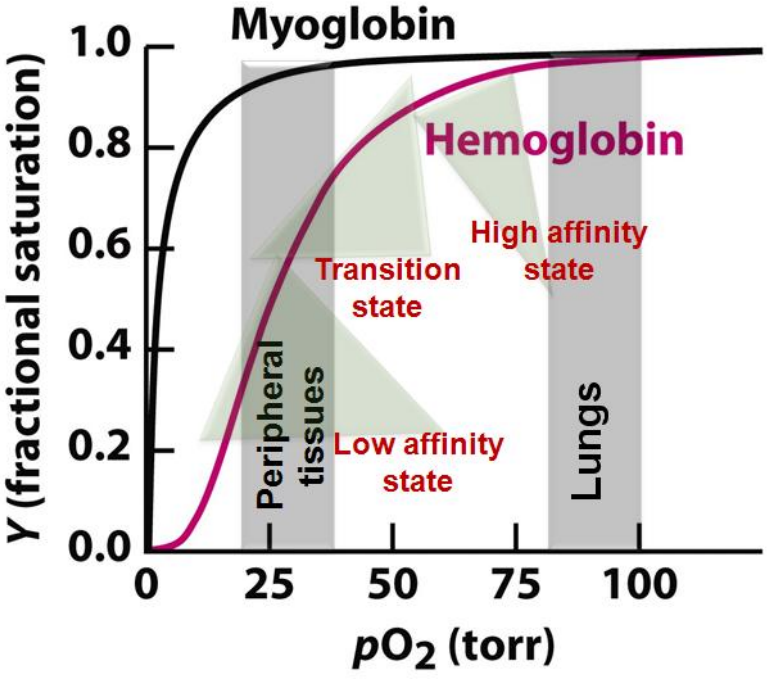
- Electrostatic interactions (salt bridges) and hydrogen bonds also exist between the two different chains



The saturation curve

- The saturation curve of hemoglobin binding to O_2 has a sigmoidal shape
- At 100 mm Hg, hemoglobin is 95-98% saturated (oxyhemoglobin)
- As the oxygen pressure falls, oxygen is released to the cells
- In contrast to a low p50 for myoglobin, the p50 of hemoglobin is approximately 26 mm





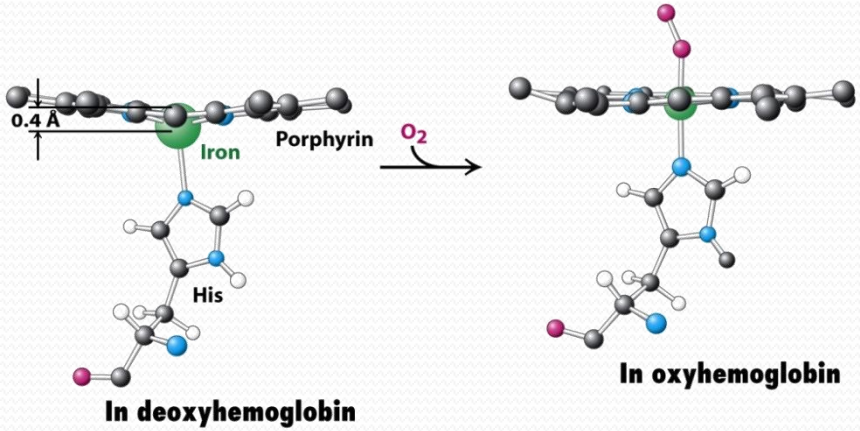
Hemoglobin is allosteric – Myoglobin not

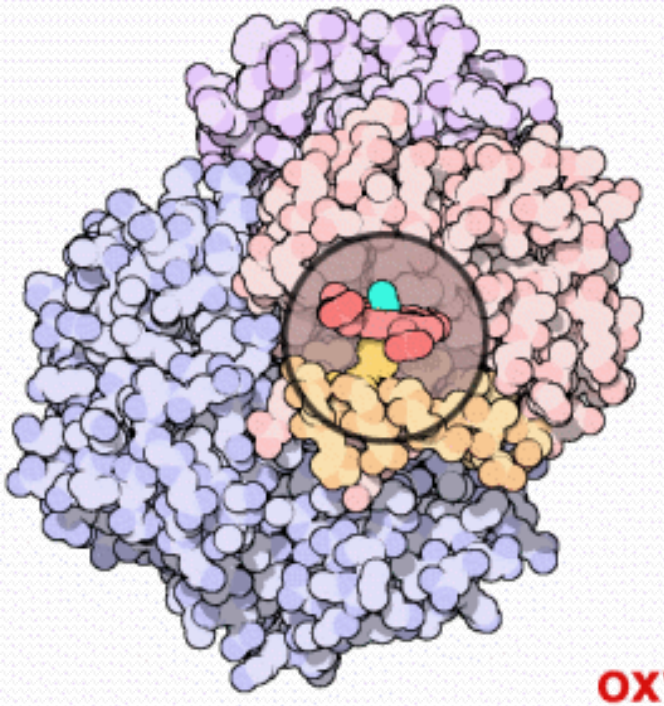
- Hemoglobin is an allosteric protein (from Greek "allos" = "other", and "stereos" = "shape").
  - An allosteric protein: a protein where binding of a molecule (ligand) to one part of the protein affects binding of a similar or a different ligand to another part of the protein
- Hemoglobin exists in two forms (myoglobin only 1 state):
  - T-state: "taut" or "tense"; low-binding affinity to oxygen
  - R-state: "relaxed"; 500 times higher affinity to oxygen

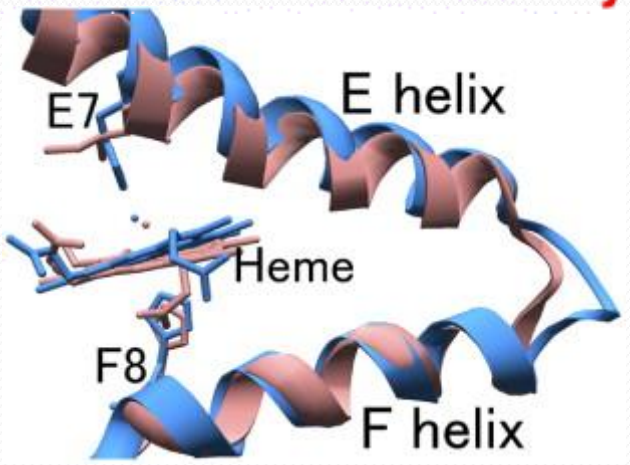
- Binding of O_2 causes conformational changes in hemoglobin, converting it from the low affinity T-state to the high affinity R-state

How does the structure change? (1)

- Dome (deoxy) – Fe out of the plane
- Planar (Oxy) – Fe into the plane → pulling proximal His (F8)



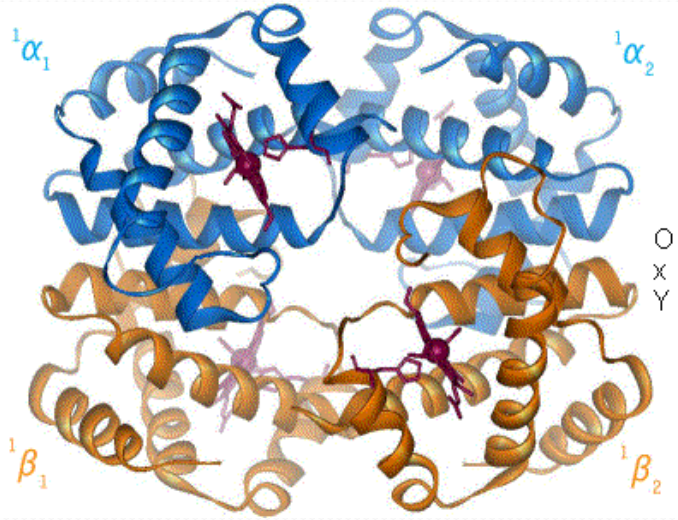


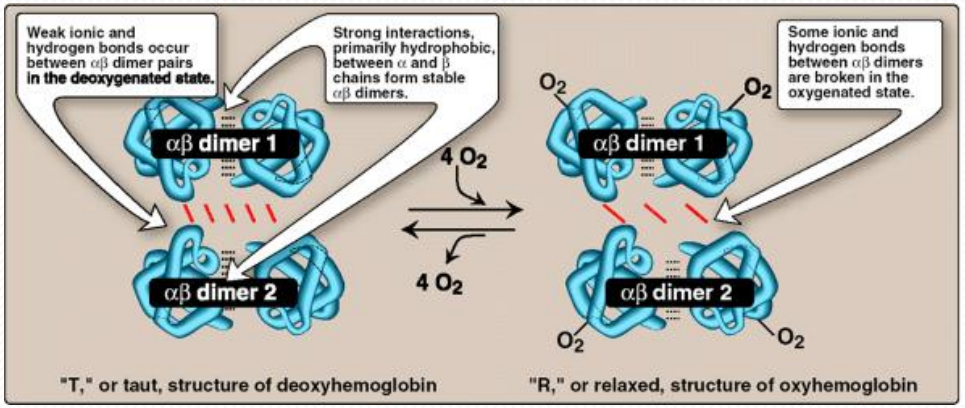


Myoglobin: movement of the helix doesn't affect the function

How does the structure change? (2)

- This movement triggers
  - Changes in tertiary structure of individual hemoglobin subunits and
  - Breakage of the electrostatic bonds at the other oxygen-free hemoglobin chains





The saturation curve is sigmoidal because…

- Conformational changes lead to cooperativity among binding sites
- Binding of the first O_2 breaks some salt bridges with the other chains increasing the affinity of the binding of a second molecule
- & so on