Globular proteins

Myoglobin and hemoglobin

Summer semester, 2023

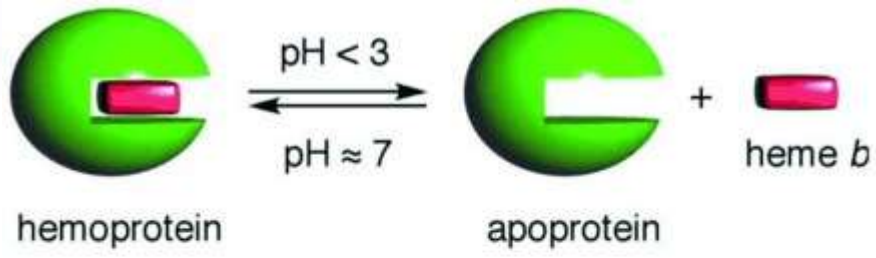
Functions of myoglobin and hemoglobin

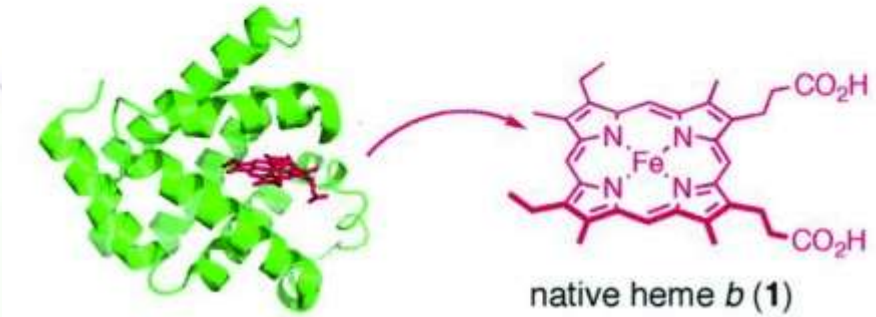
- Myoglobin functions in storing O2 in muscles. During periods of oxygen deprivation, oxymyoglobin releases its bound oxygen.

- Hemoglobin:
  - transport of O_2 and CO_2
  - blood buffering

Hemoproteins

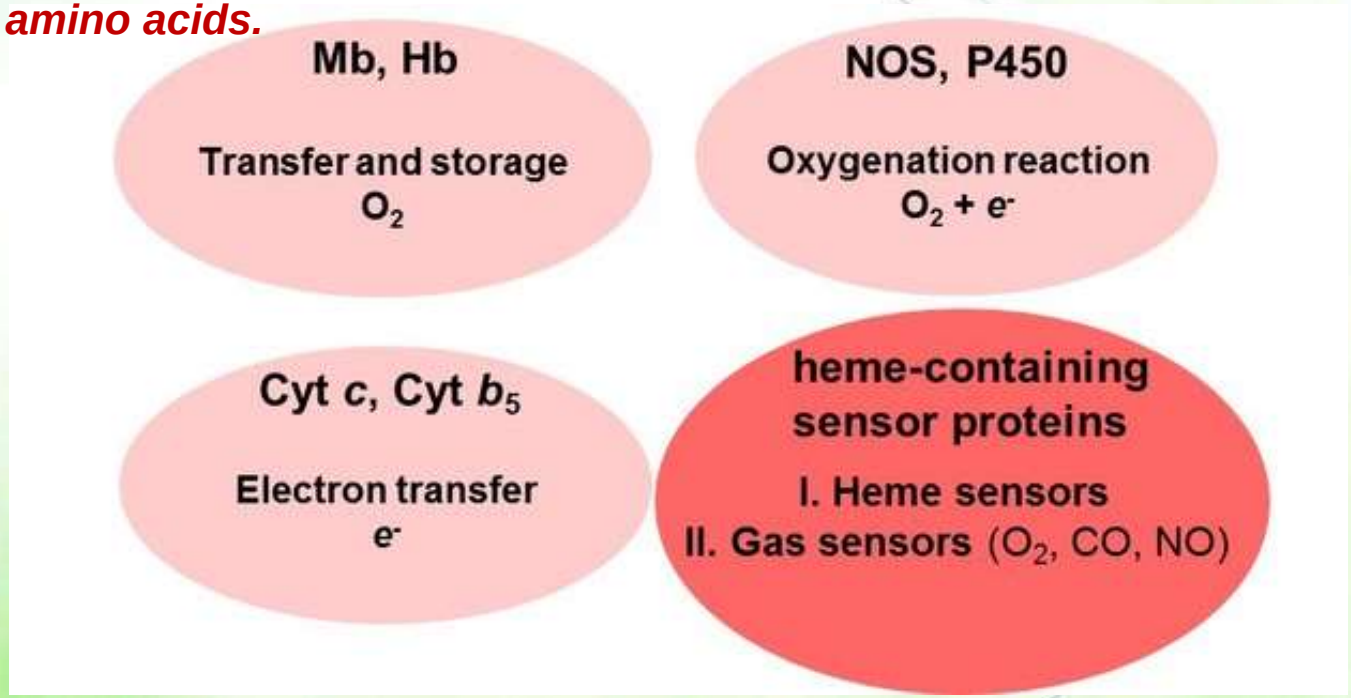
- Many proteins have heme as a prosthetic group called hemoproteins.





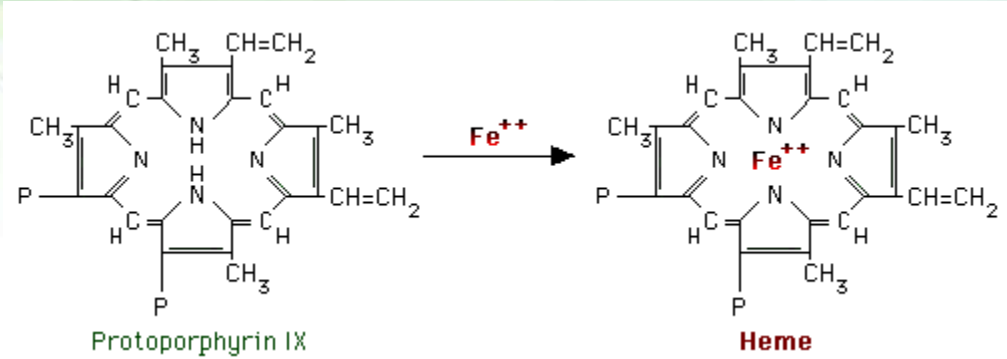
The protein environment dictates the function of the heme.

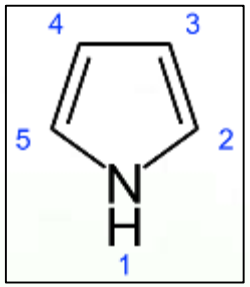
A prosthetic group is a tightly bound, specific non-polypeptide unit required for the biological function of some proteins. The prosthetic group may be organic (such as a vitamin, sugar, or lipid) or inorganic (such as a metal ion), but is not composed of amino acids.

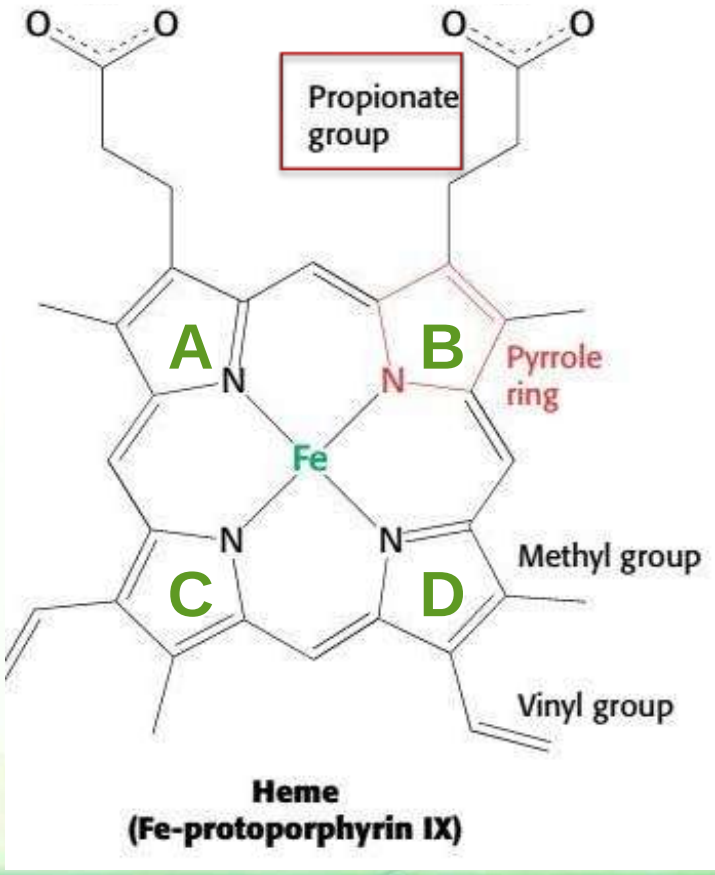


Heme structure

- It is a complex of protoporphyrin IX + Iron (Fe^{2+}).
- The porphyrin is planar and consists of four rings (designated A-D) called pyrrole rings.
- Each pyrrole can bind two substituents.
- Two rings have a propionate group each.
- *Note: the molecule is hydrophobic.*
- Fe has six coordinates of binding.

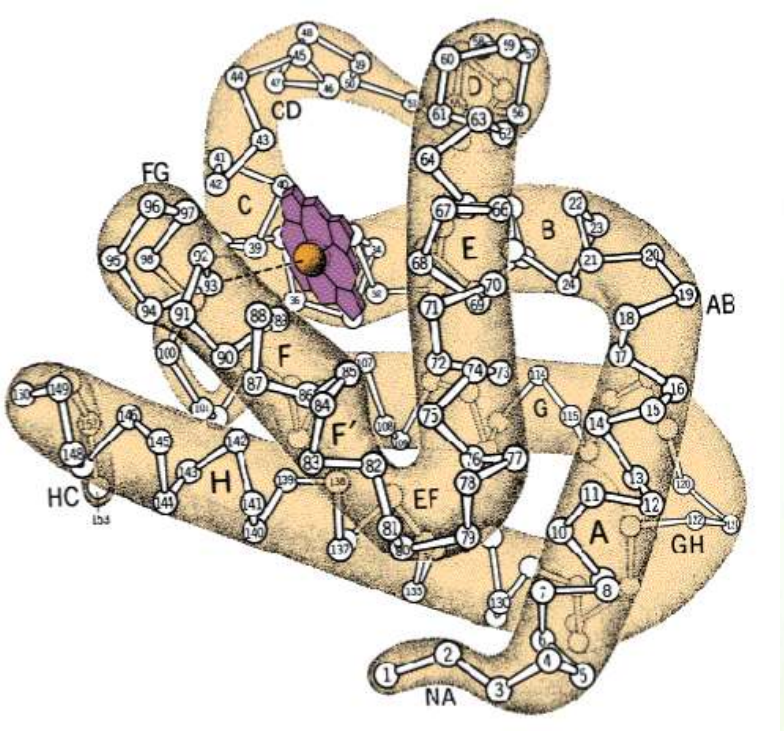






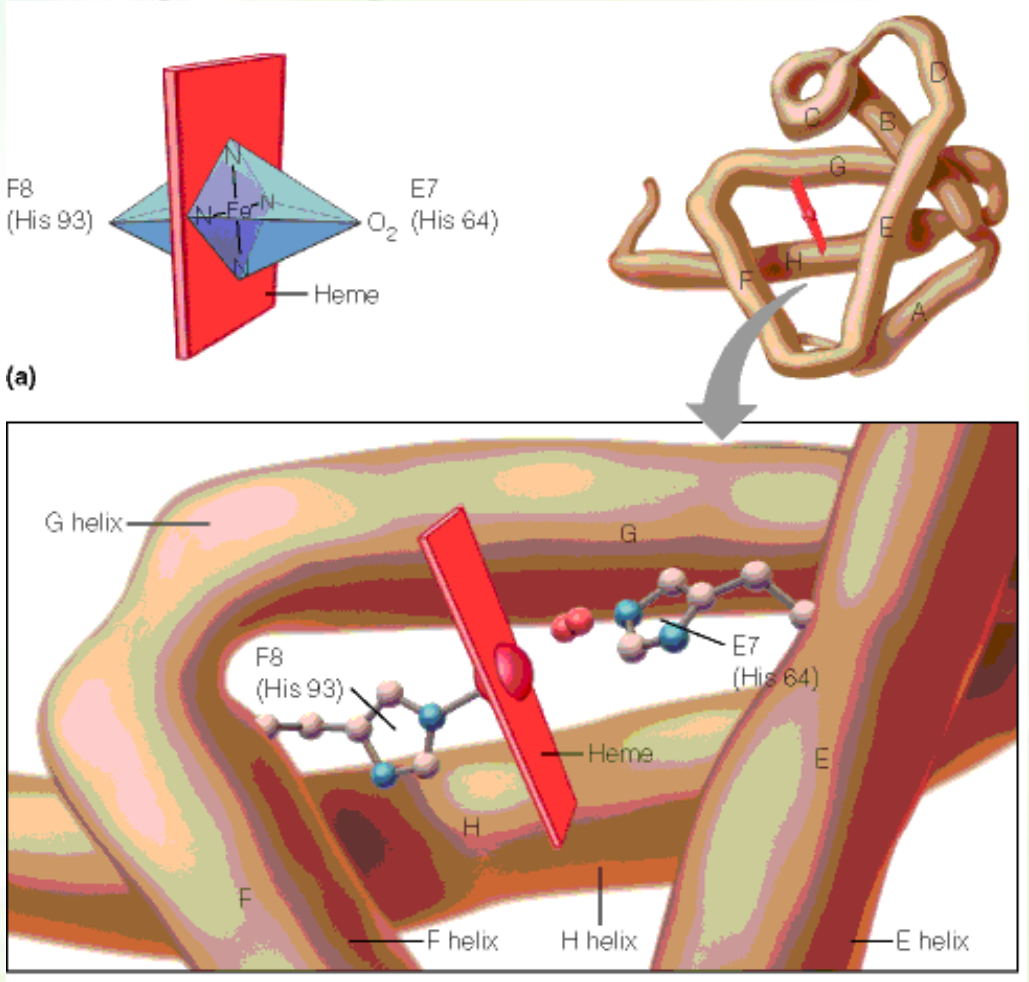
Structure of myoglobin

- Myoglobin is a monomeric protein that is mainly found in muscle tissue.
- The tertiary structure of myoglobin 8 α-helices, designated A through H, that are connected by short non-helical regions.
- The eight α-helices are connected by short coils, a structure that is known as the globin fold, which is a hydrophobic O_2-binding pocket.
- It contains heme as a prosthetic group internally.
- Myoglobin can be present in two forms:
  - oxymyoglobin (oxygen-bound)
  - deoxymyoglobin (oxygen-free)



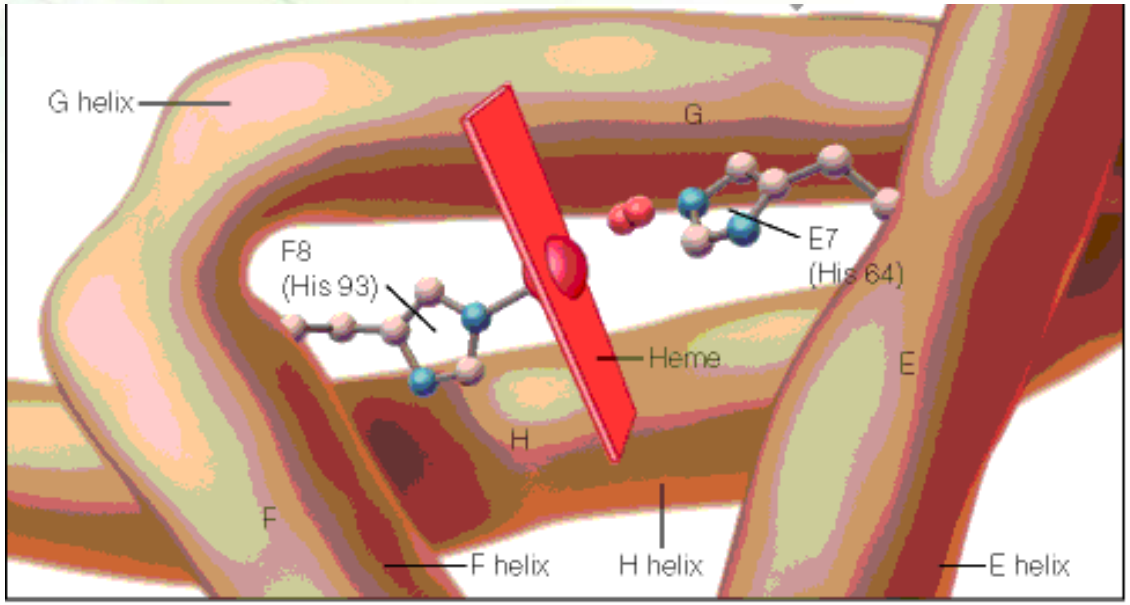
Arrangement of amino acids

- Like other globular proteins, the hydrophilic amino acids are generally on the surface, while hydrophobic amino acids are predominantly internal.
  - Except for two histidine residues in helices E and F (known as E7 and F8)
- F8 His is designated as proximal His, whereas E7 His is known as distal His.



Iron

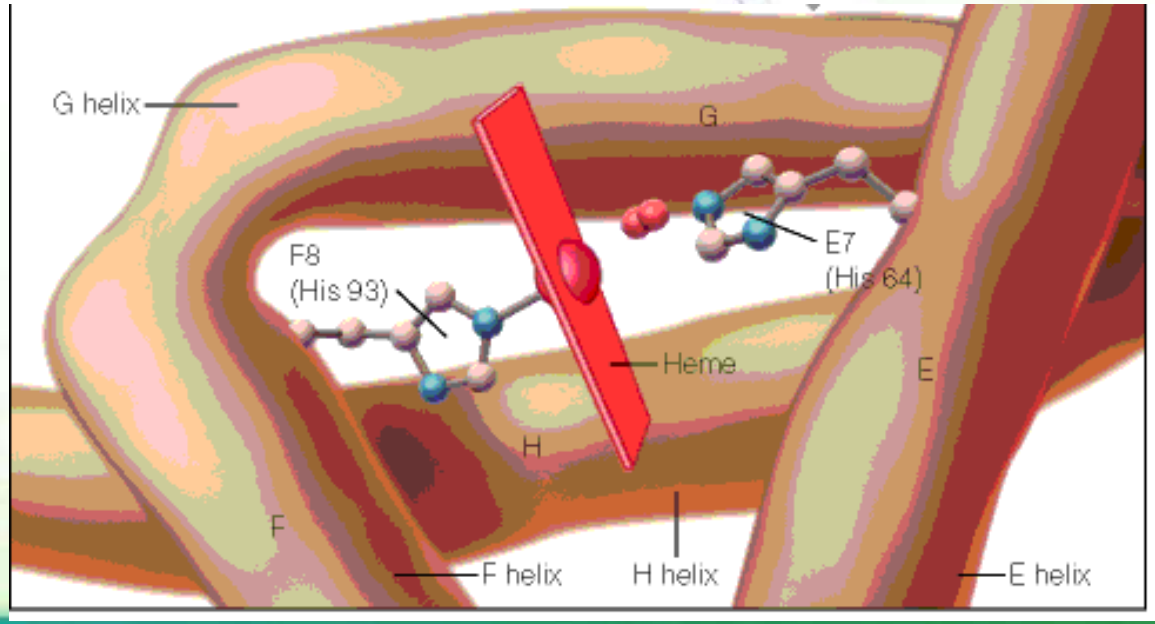
- Iron can bind in the center of the four rings.
- Fe is in the ferrous state (Fe^{2+}) and can form 6 bonds:
  - 4 bonds with the nitrogen of the rings,
  - One bond (known as the fifth coordinate) with the nitrogen of the proximal His.
  - A last one with O_2 (the sixth coordinate) when O_2 is there

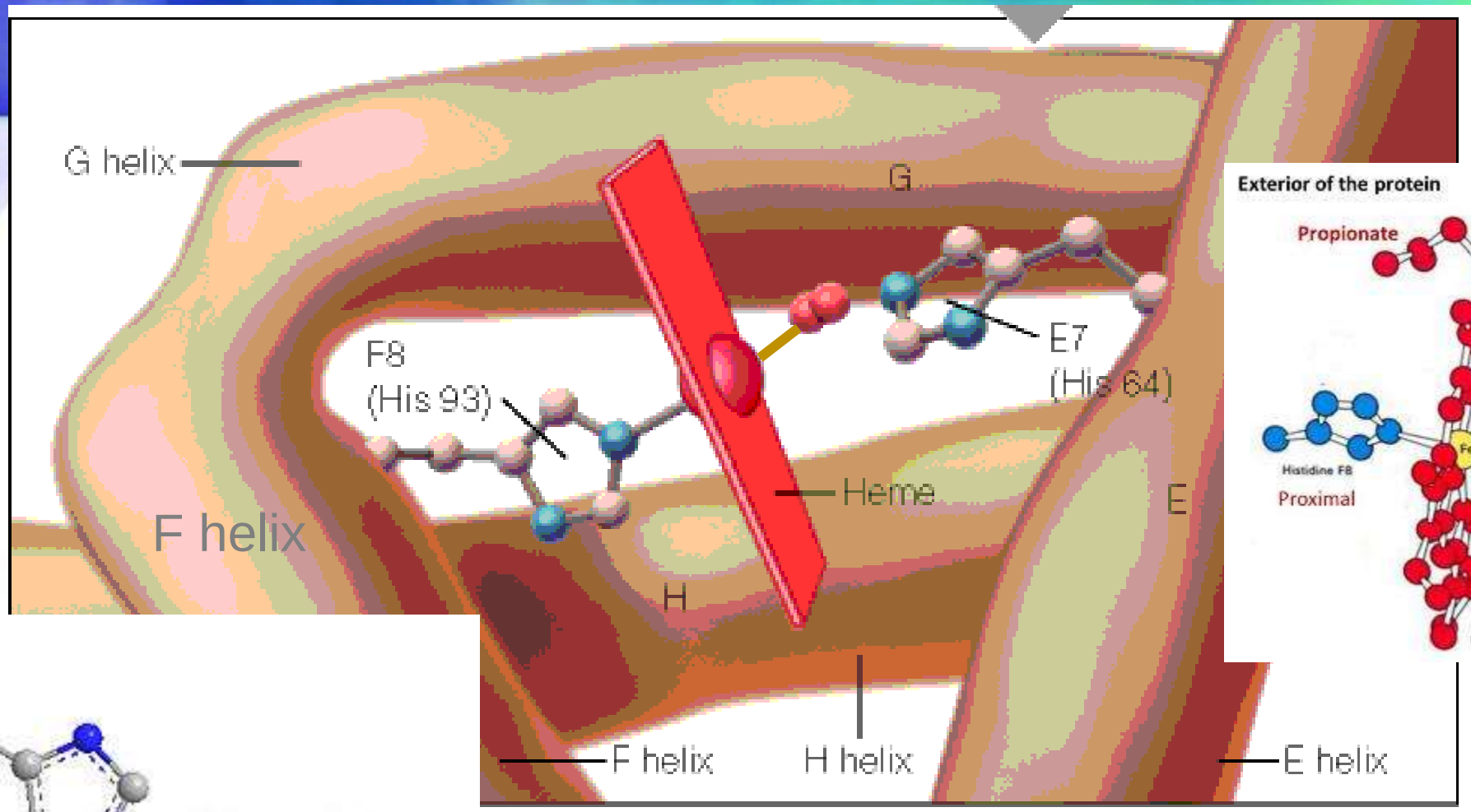


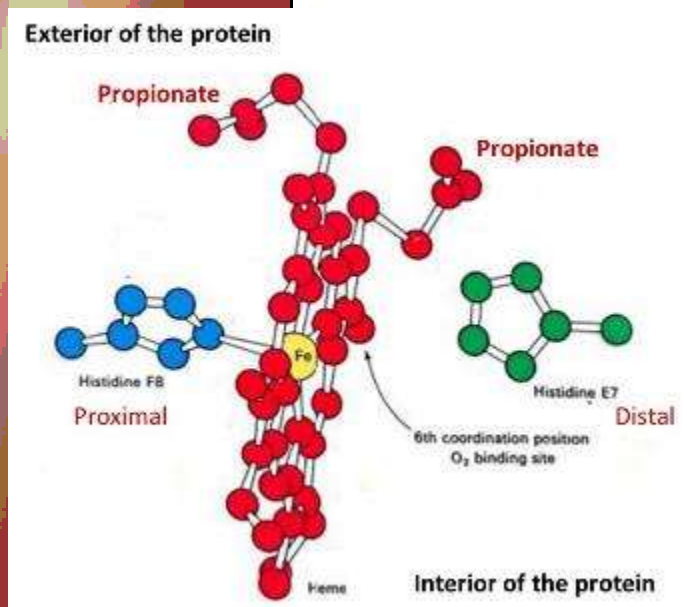
- Oxidation of iron to the Fe^{3+}, ferric, state makes the molecule incapable of normal O_2 binding.
- Upon absorption of light, heme gives a deep red color.

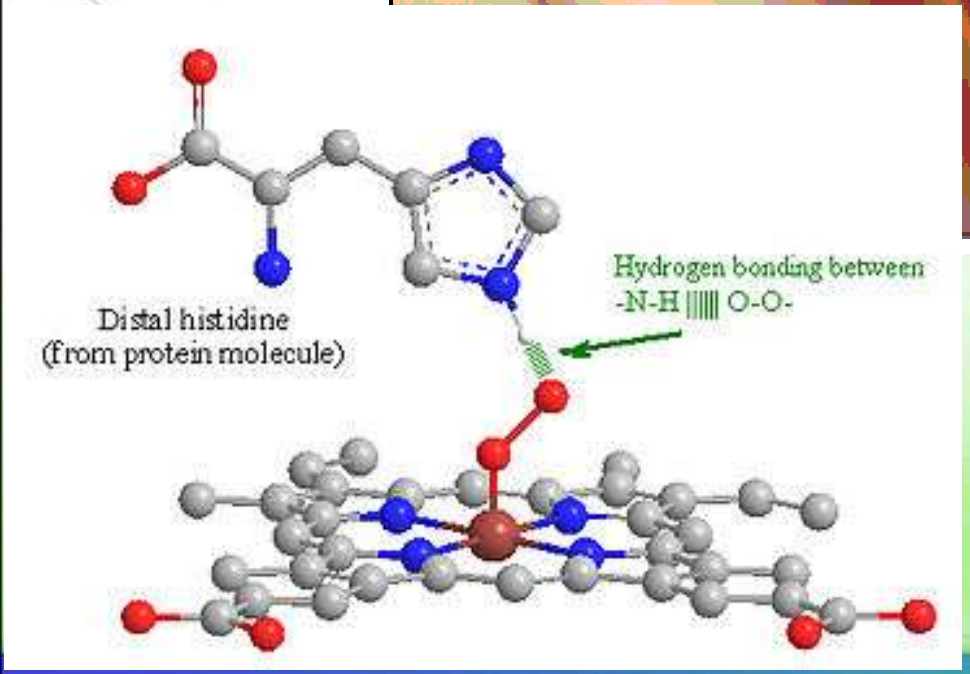
Structure-function relationship

- The planar heme group fits into a hydrophobic pocket of the protein and the myoglobin-heme interaction is stabilized by hydrophobic attractions.
- The heme group stabilizes the tertiary structure of myoglobin.
- The hydrophobic interior of myoglobin (or hemoglobin) prevents the oxidation of iron, and so when O_2 is released, the iron remains in the Fe(II) state and can bind to another O_2.
- The distal histidine acts as a gate that opens and closes as O_2 enters the hydrophobic pocket to bind to the heme.
- It also stabilizes the interaction with oxygen.



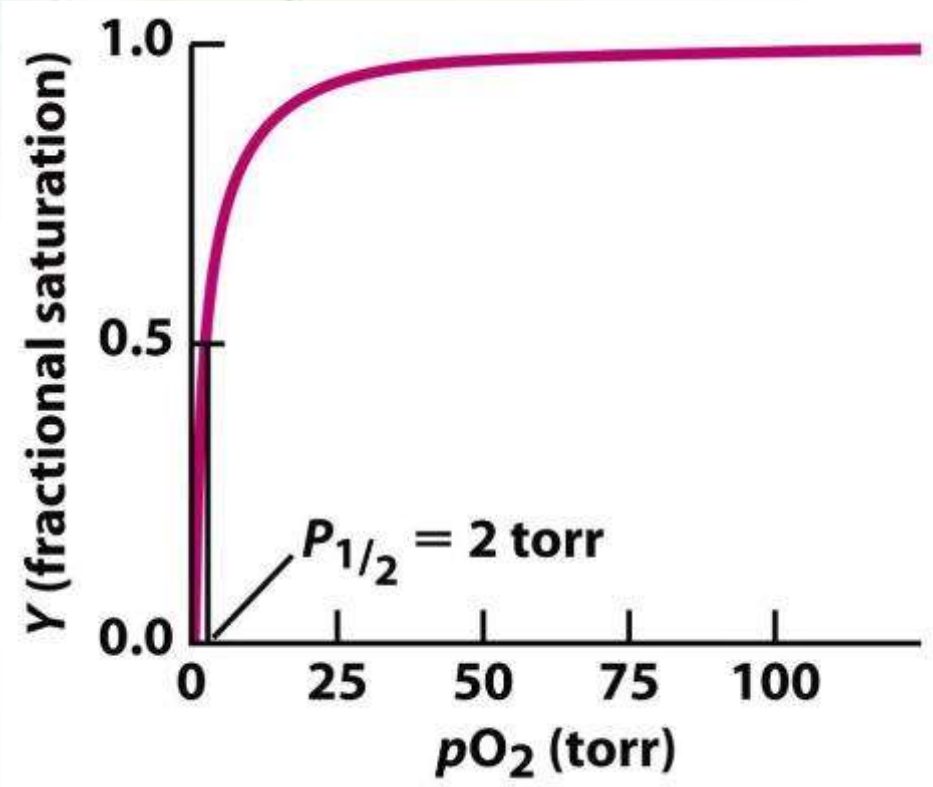

G helix
G
E7
(His 64)
F8
(His 93)
Heme
F helix
H
E
F helix
H helix
E helix


Exterior of the protein
Propionate
Propionate
Histidine F8
Proximal
Histidine E7
Distal
6th coordination position
O_2 binding site
Heme
Interior of the protein


Distal histidine
(from protein molecule)
Hydrogen bonding between
-N-H ||||| O-O-

Oxygen binding to myoglobin

- Myoglobin binds O_2 with high affinity.
- The P50 (oxygen partial pressure required for 50% of all myoglobin molecules) for myoglobin ~2.8 torrs (or mm Hg).
- Given that O_2 pressure in tissues is normally 20-40 mm Hg, it is almost fully saturated with oxygen at normal conditions.

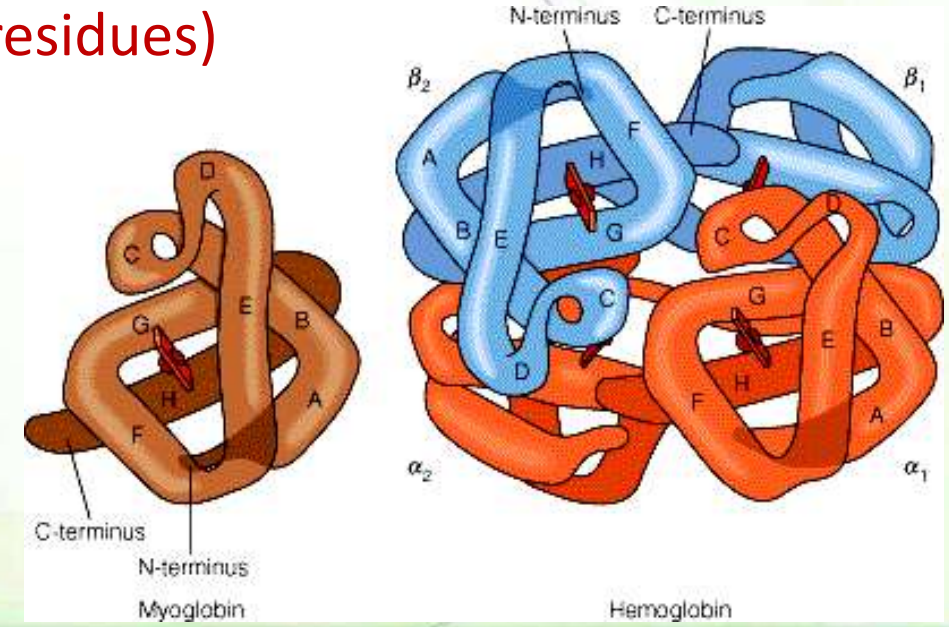


The binding of **O2** to myoglobin follows a hyperbolic saturation curve.

Hemoglobin

Hemoglobin structure

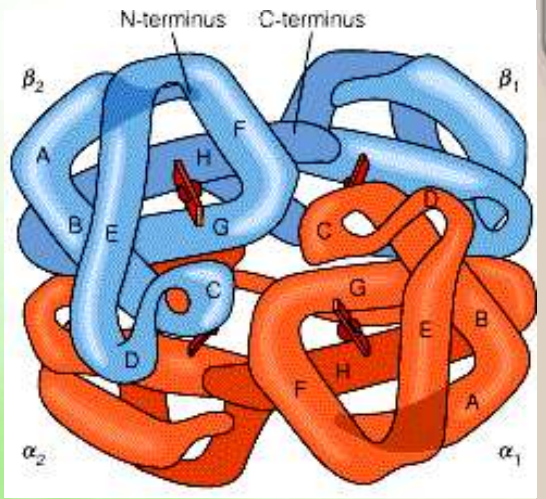
- Hemoglobin is a tetrameric hemeprotein (four globin protein chains with each bound to heme).
- In adults, the four globin proteins are of two different types known as α and β, so a hemoglobin protein is termed α2β2 globin protein.
- The α and β chains contain multiple α-helices
  - α contains 7 α-helices (141 aa residues)
  - Like myoglobin, β contains 8 α-helices (146 aa residues)

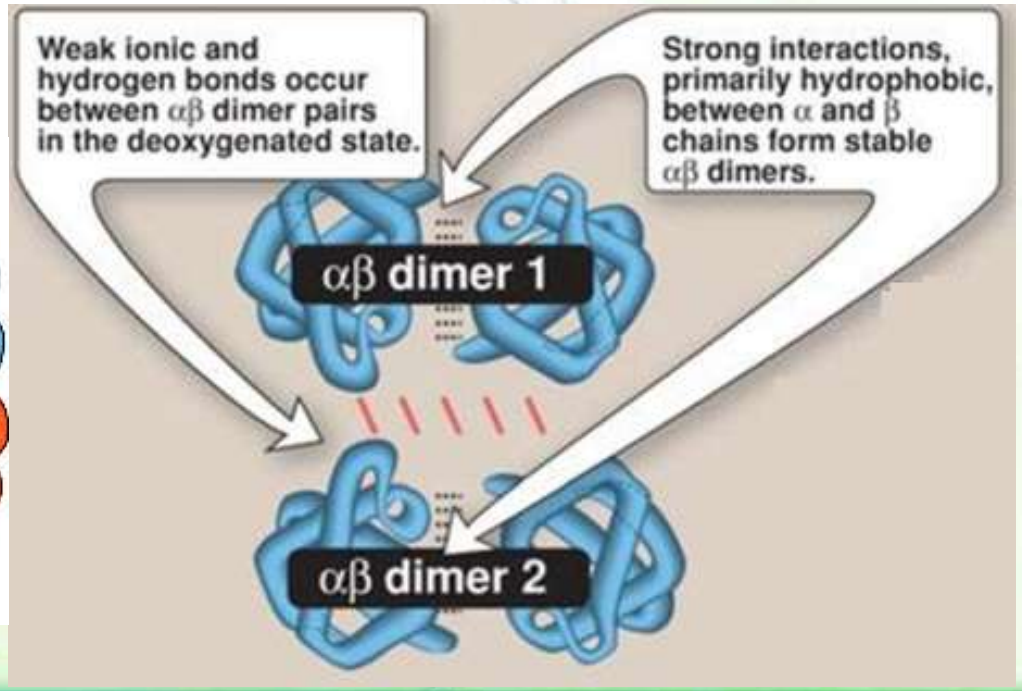


How are the subunits bound?

- A dimer of dimers (I made up this term) OR two αβ-protomers
  - $(\alpha\text{-}\beta)_2$
- The chains interact with each other via hydrophobic interactions.
  - Therefore, hydrophobic amino acids can also be present on the surface.

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

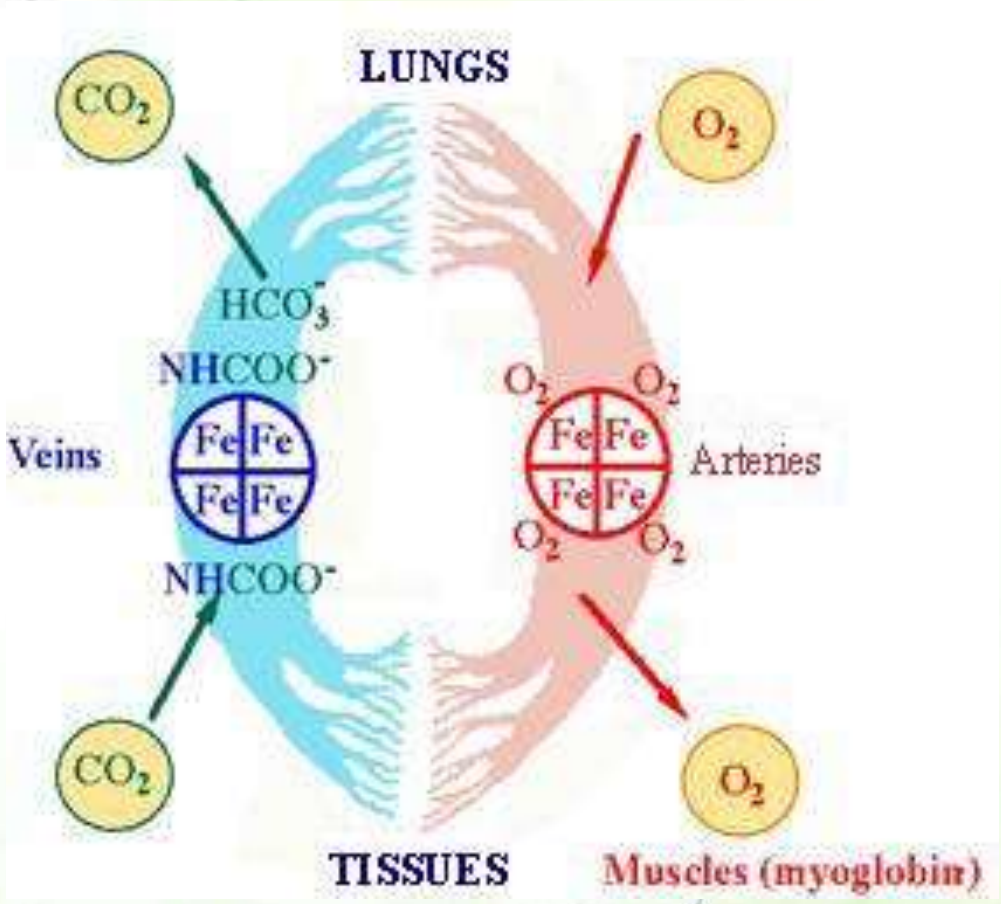




Oxygen binding to hemoglobin

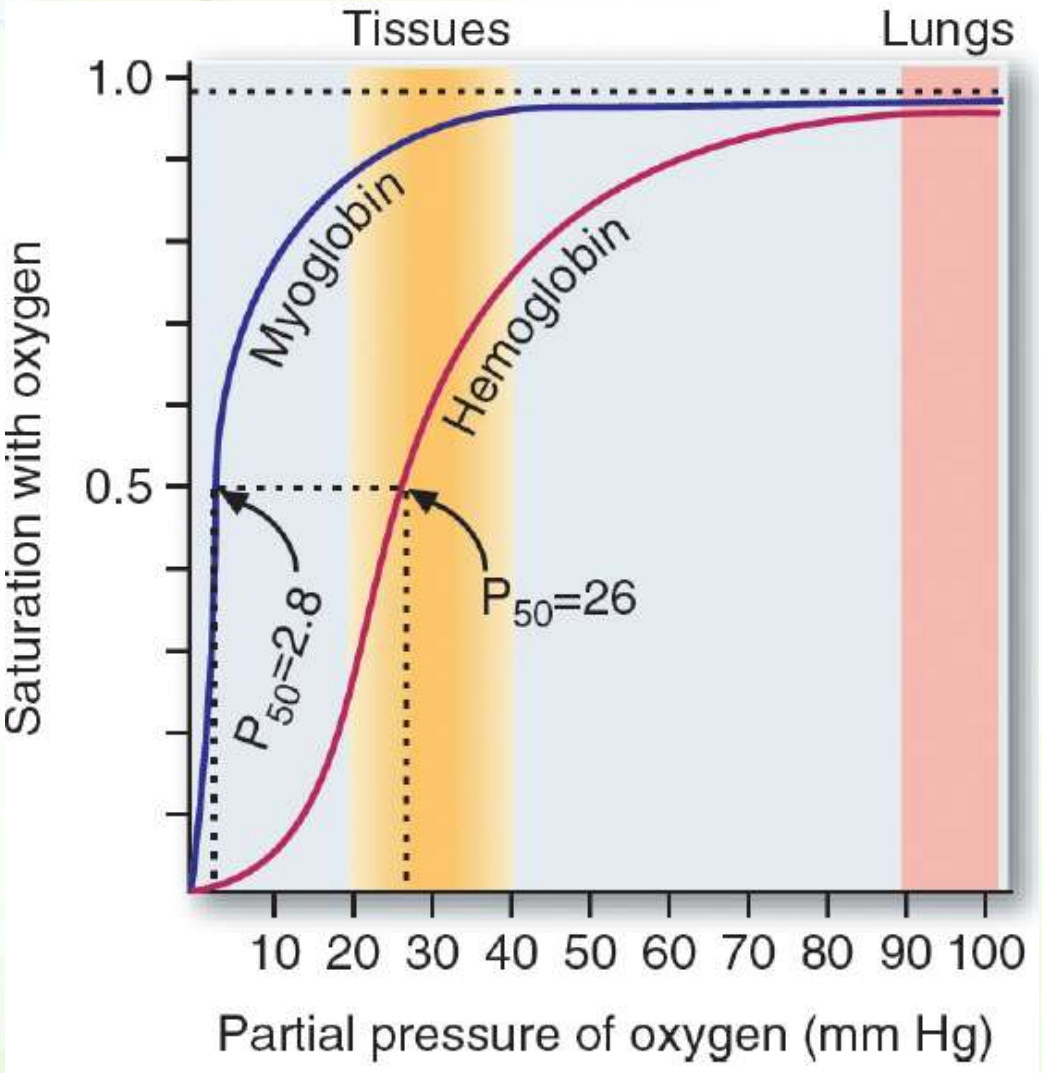
- Hemoglobin must bind oxygen efficiently and become saturated at the high oxygen pressure found in the lungs (approximately 100 mm Hg).
- Then, it must release oxygen and become unsaturated in tissues where the oxygen pressure is low (about 30 mm Hg).

Do you expect hemoglobin to have a high or low affinity for oxygen?

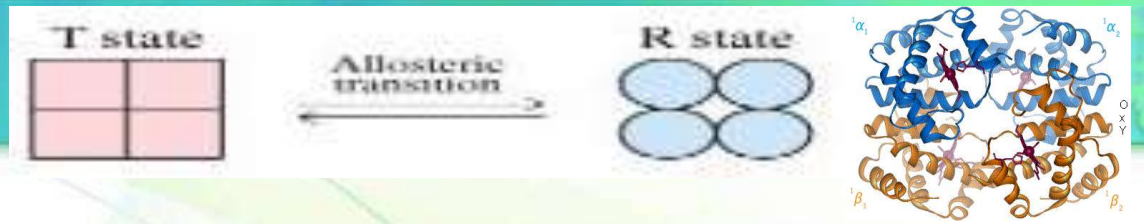


The saturation curve

- The saturation curve of hemoglobin binding to O_2 has a sigmoidal shape.
  - A sigmoidal curve indicates that the protein has different structures.
- 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.
  - *Relate the value of p50 to affinity*

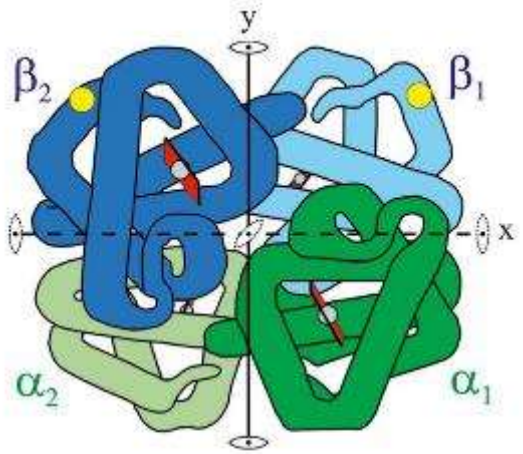


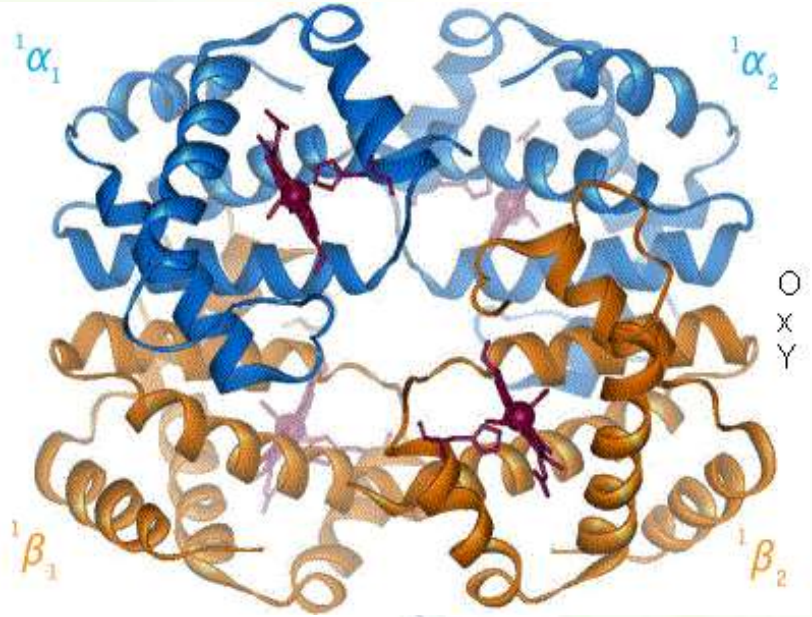
Hemoglobin is allosteric

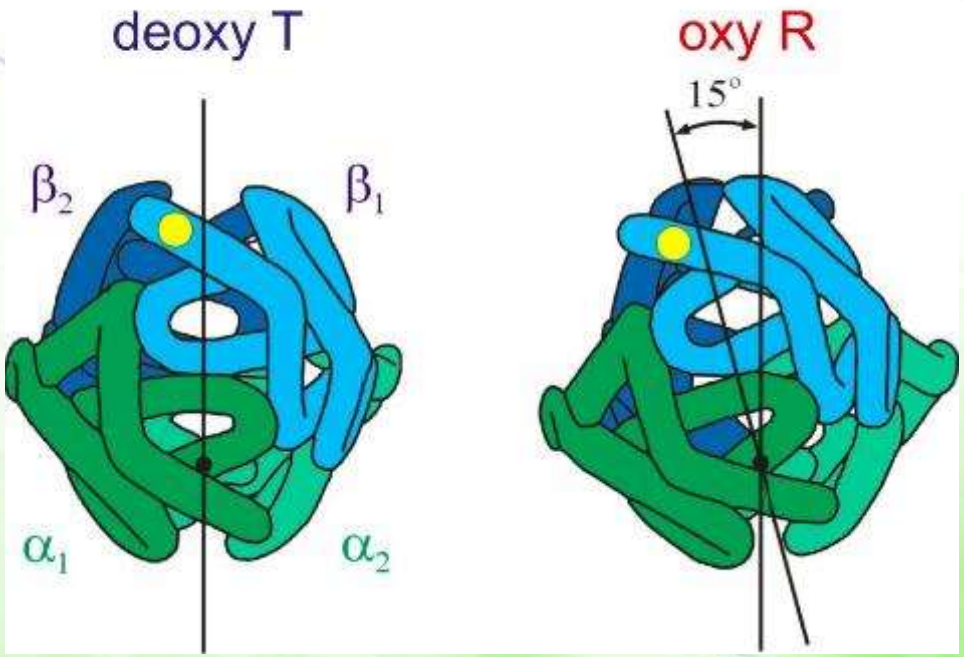


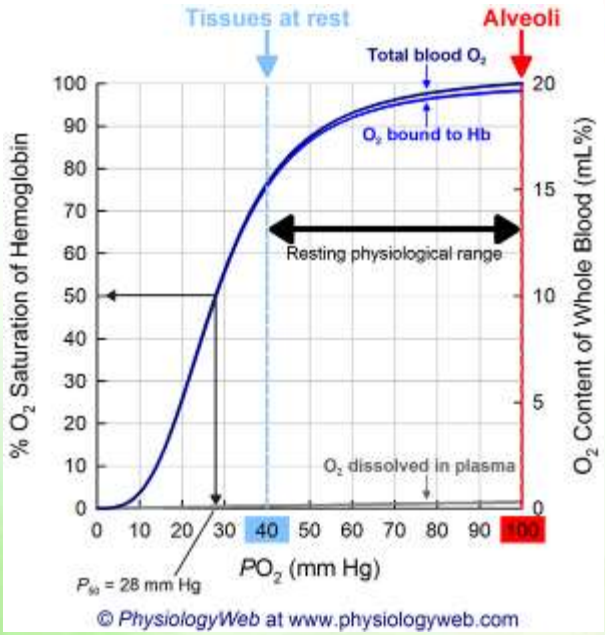
- Hemoglobin is an allosteric protein (from Greek "allos" = "other", and "stereos" = "shape").
  - An allosteric protein: a multi-subunit protein where binding of a molecule (ligand) to one part of the protein affects binding of a similar or a different ligand to another part of the protein by changing its structure slightly.
- Hemoglobin exists in two allosteric forms, T-state and R-state
  - The T-state is also known as the "taut" or "tense" state and it has a low binding affinity to oxygen.
  - The R-state is known as the "relaxed" state, and it has 500 times higher affinity to oxygen than the T conformation.
- Binding of O_2 causes conformational changes in hemoglobin, converting it from the low-affinity T-state to the high-affinity R-state .

Structural change of hemoglobin



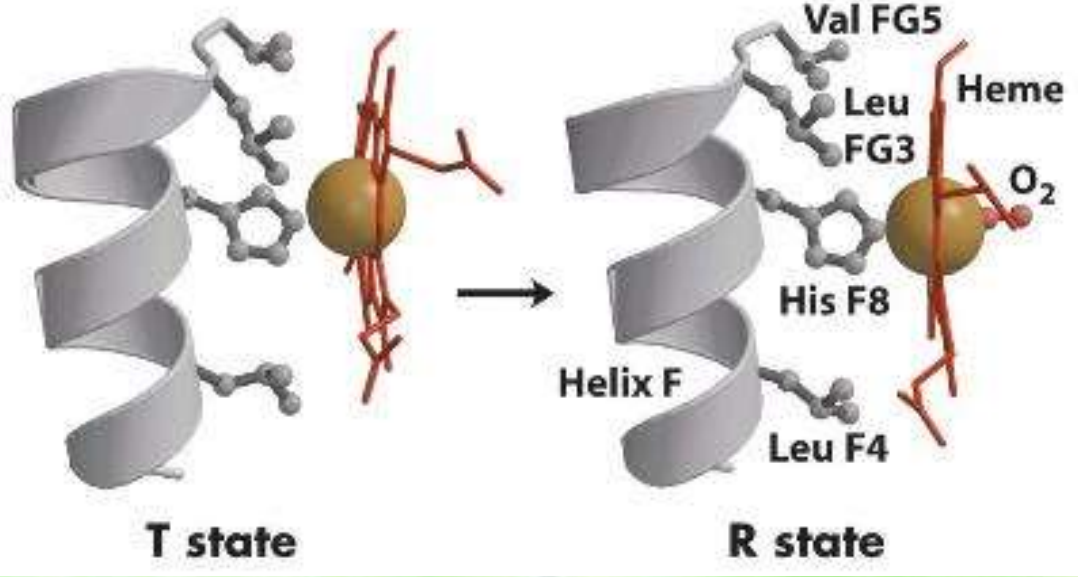


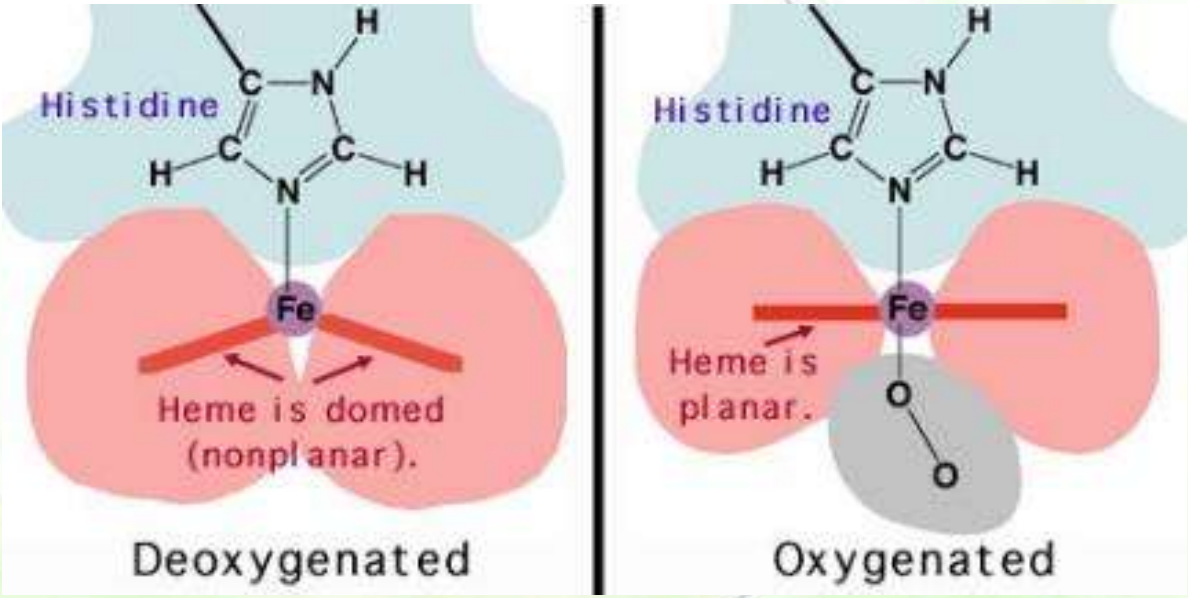




How does the structure change? (1)

- When heme is free of oxygen, it has a domed structure and iron is outside the plane of the heme group.
  - Because the hydrophobic heme is repelled by the proximal His.
- When oxygen binds to an iron atom, heme adopts a planar structure and the iron moves into the plane of the heme pulling proximal histidine (F8)

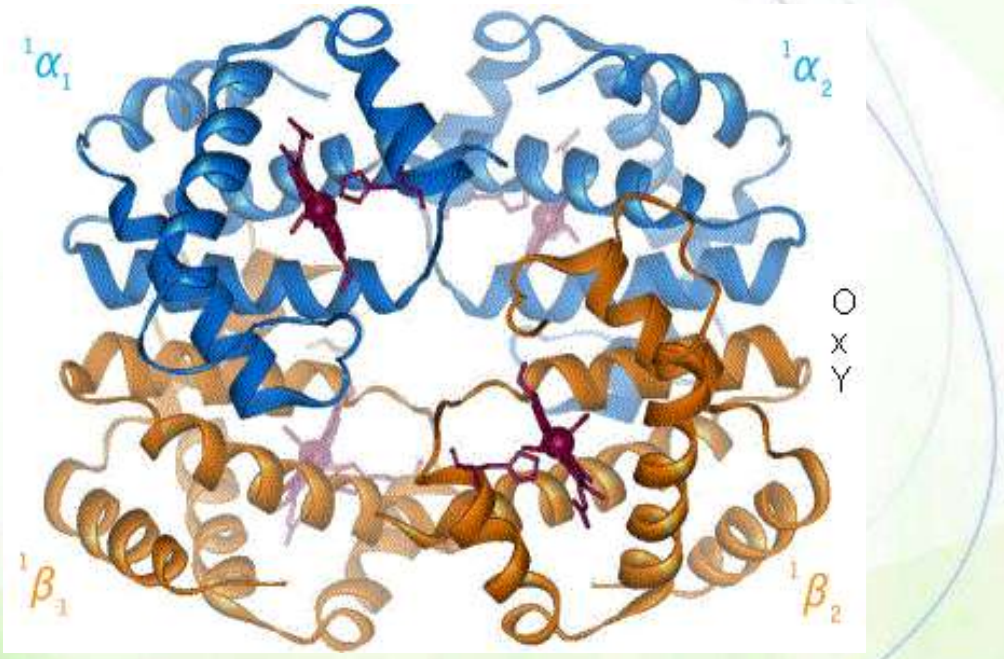




How does the structure change? (2)

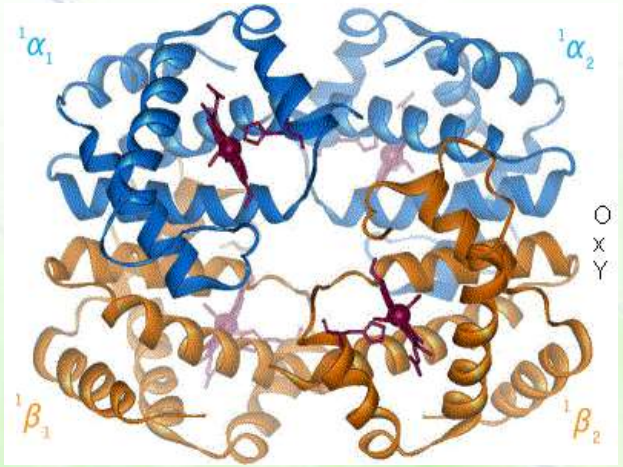
- This movement triggers
  - changes in tertiary structure of individual hemoglobin subunits
  - breakage of the electrostatic bonds at the other oxygen-free hemoglobin chains.

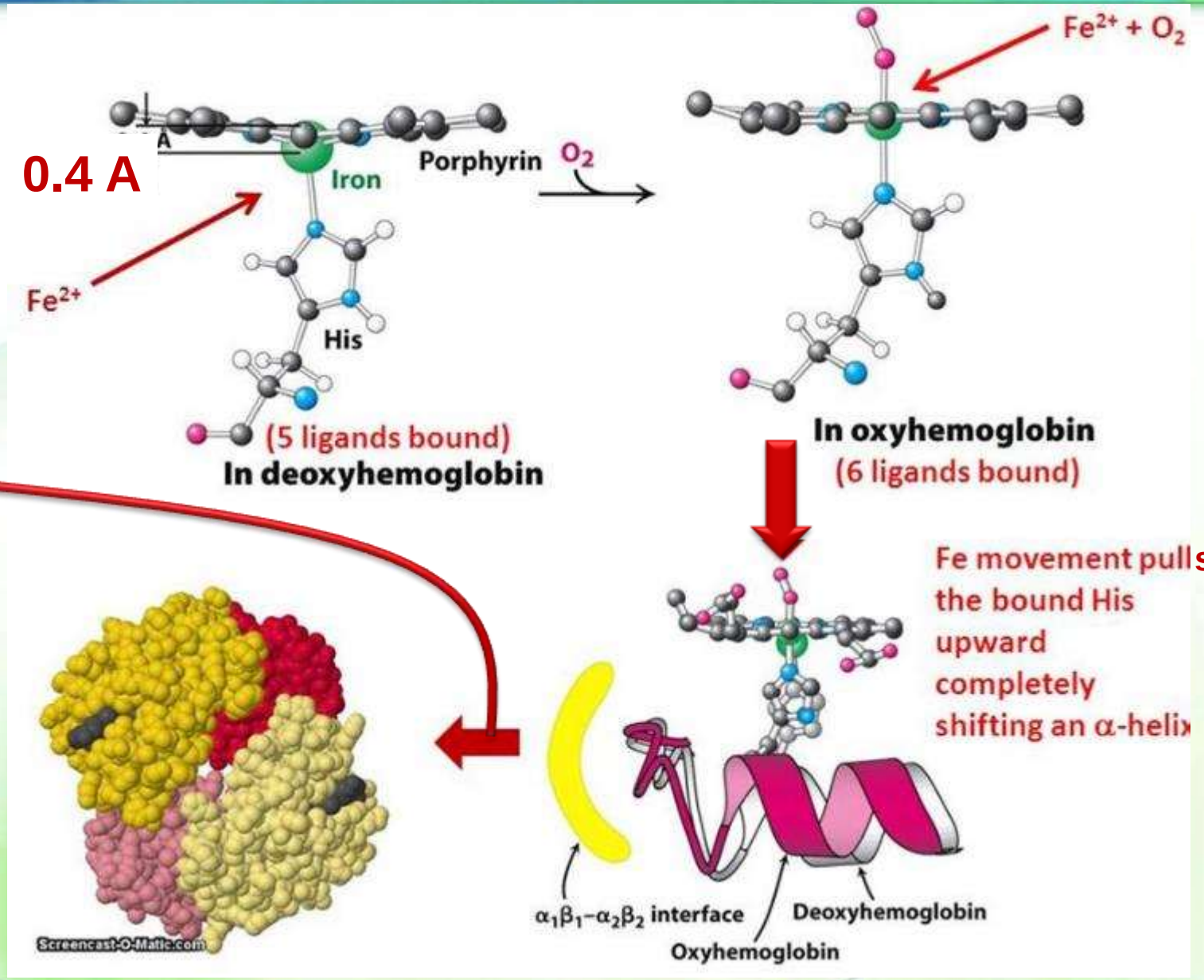
In myoglobin, movement of the helix does not affect the function of the protein.



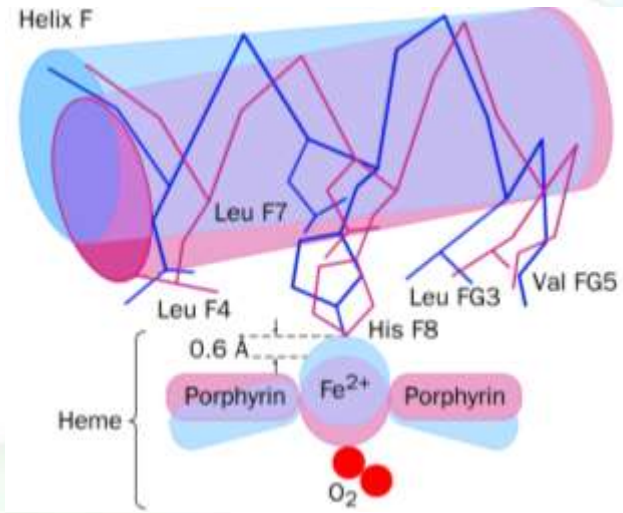
Structural amplification change

- **Changes in tertiary structure of individual hemoglobin subunits**
- **Breakage of the electrostatic bonds at the other oxygen-free hemoglobin chains.**



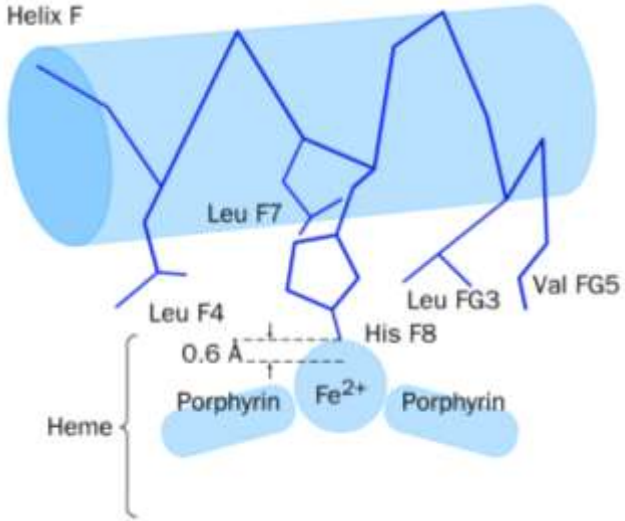


Another look at it

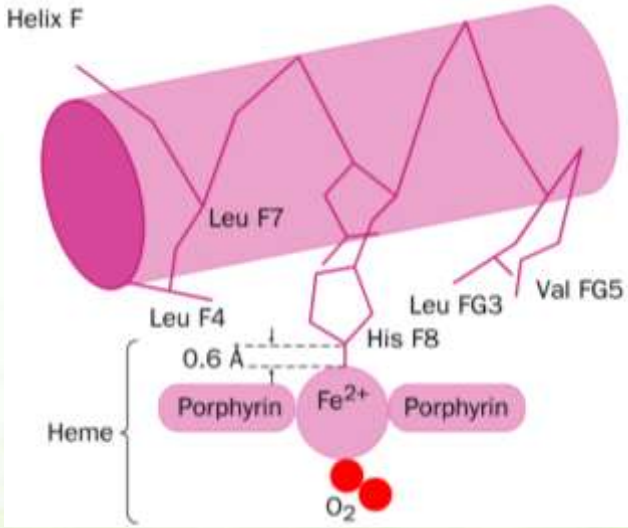


Movements of the hemoglobin's heme and F helix during the T → R transition.

Fig. 7-9 diagrams how the binding of O_2 to one hemoglobin site induces conformation changes that influence the O_2 -binding affinity of the other sites.

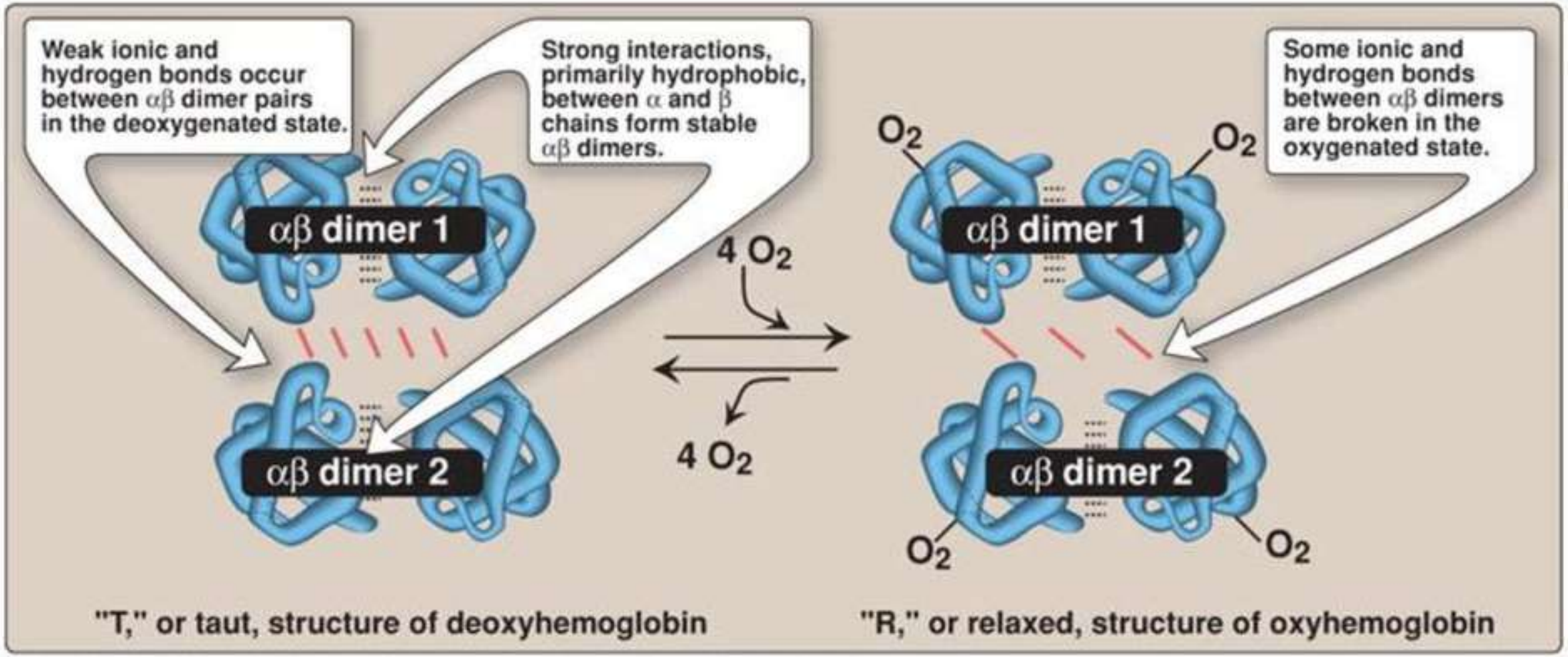


In the absence of bound O_2 ,the Fe(II) lacks a sixth ligand, and resides about 0.6 Angstrom out of the plane of the heme toward its His ligand (the proximal His).



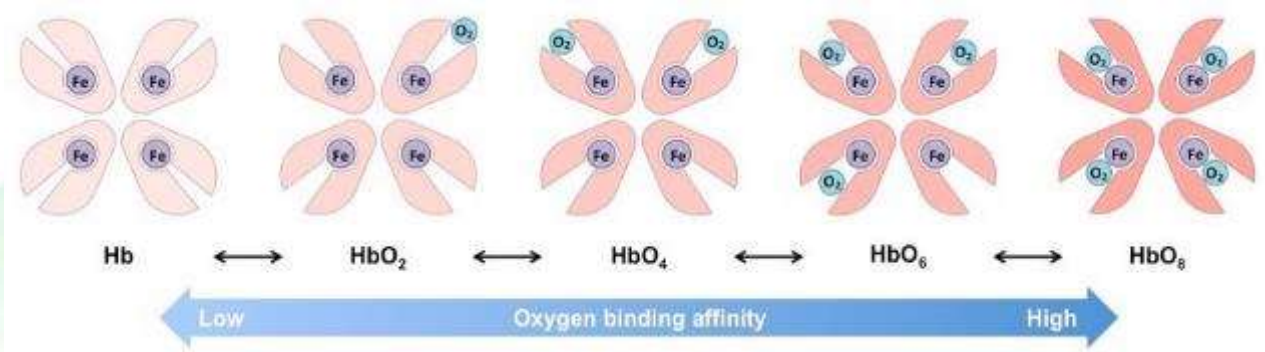
Upon binding O_2 ,the Fe(II) is pulled towards the O_2 into the plane of the heme. This also pulls the attached proximal His towards the heme. Since the proximal His is part of the F helix, this entire helix is also pulled toward the heme. These conformational changes induce a rearrangement of the alpha and beta subunits in the hemoglobin tetramer.

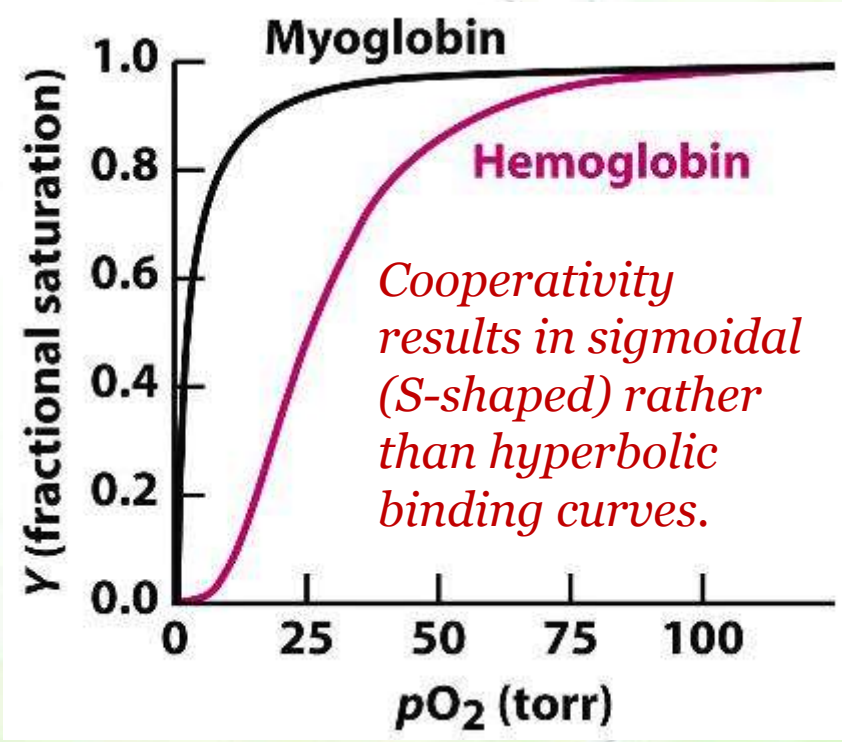
Electrostatic interactions are broken



Binding is cooperative

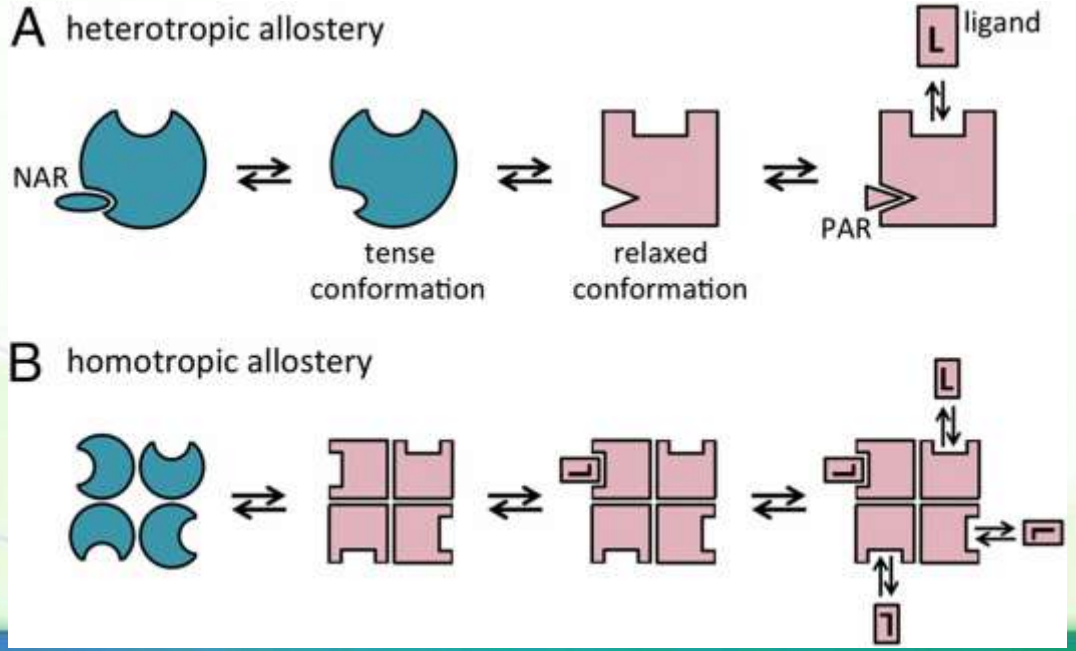
- Conformational changes lead to cooperativity among binding sites.
- Binding of the first O_2 breaks some salt bridges with the other chains increasing the affinity of the binding of a second molecule.
- Binding of the second O_2 molecule breaks more salt bridges increasing the affinity towards binding of a third O_2 even more, and so on.
- Binding is cooperative.
- Oxygen is a homotropic effector (the allosteric modulator is the substrate itself).





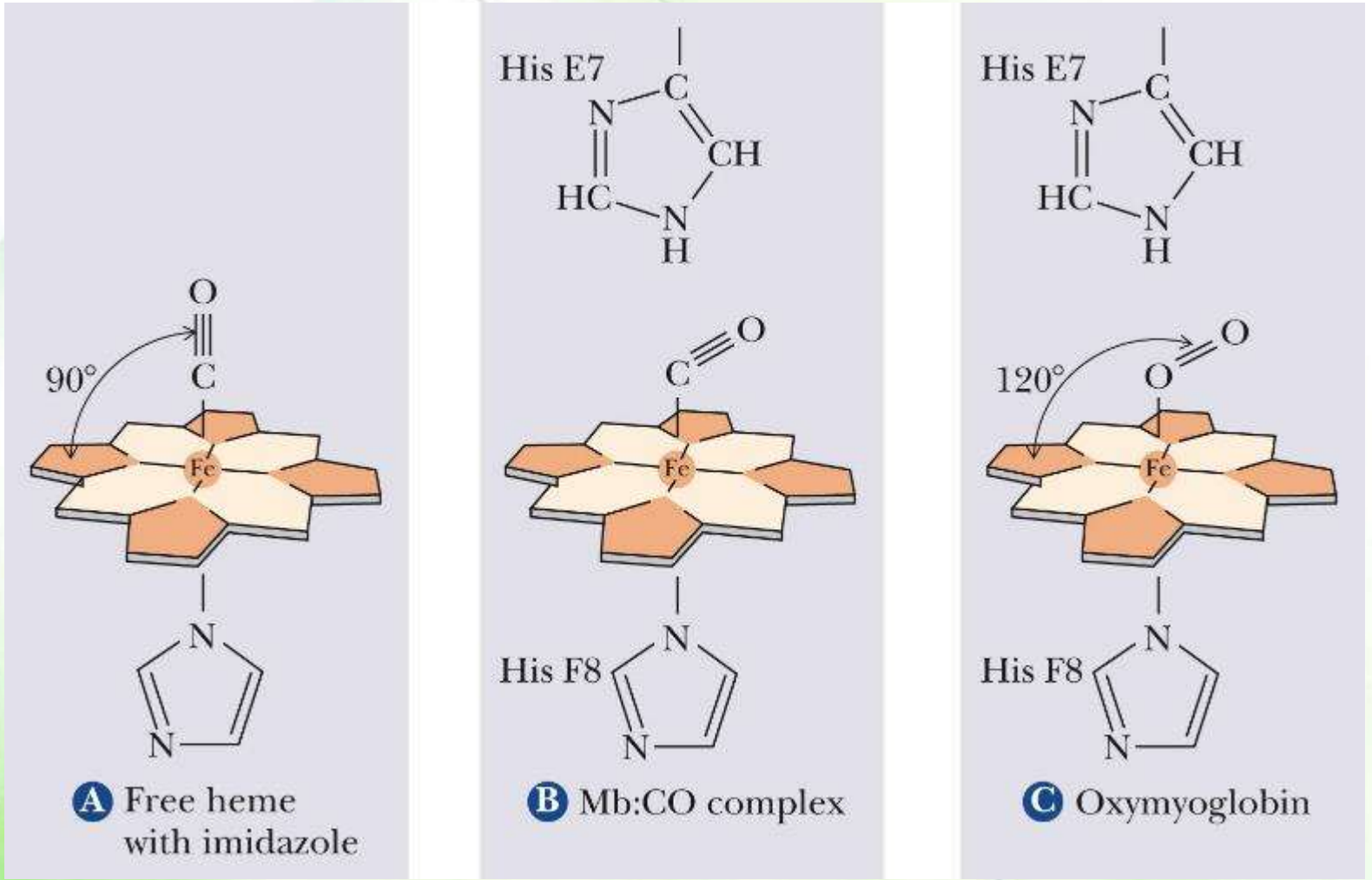
Some terminologies

- Homotropic allosteric regulator/effector: effector and ligand regulated by the effector are the same molecule (e.g., O_2 binding affects subsequent O_2 binding).
- Heterotropic allosteric regulator: effector and ligand are different molecules (e.g., H^+ or BPG binding affects O_2 binding).
- Positive allosteric interaction: effector binding increases affinity for ligand.
- Negative allosteric interaction: effector binding decreases affinity for ligand.



Another significance of distal histidine

- CO prefers straight bonding, but O_2 prefers bent bonding.
- CO binds to free heme with higher affinity (thousands folds more) than O_2.
- The affinity of CO to myoglobin-bound heme is only 250 times more than O_2.
- Yet, CO occupies 1% of hemoglobin, but 99% if distal His does not exist.



Accidents