



# Globular proteins

## Myoglobin and hemoglobin

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# Functions of myoglobin and hemoglobin



- Myoglobin is storage of  $O_2$  in muscles. During periods of oxygen deprivation, oxymyoglobin releases its bound oxygen.
- Hemoglobin:
  - 1) transport of  $O_2$  and  $CO_2$
  - 2) blood buffering

# Hemoproteins

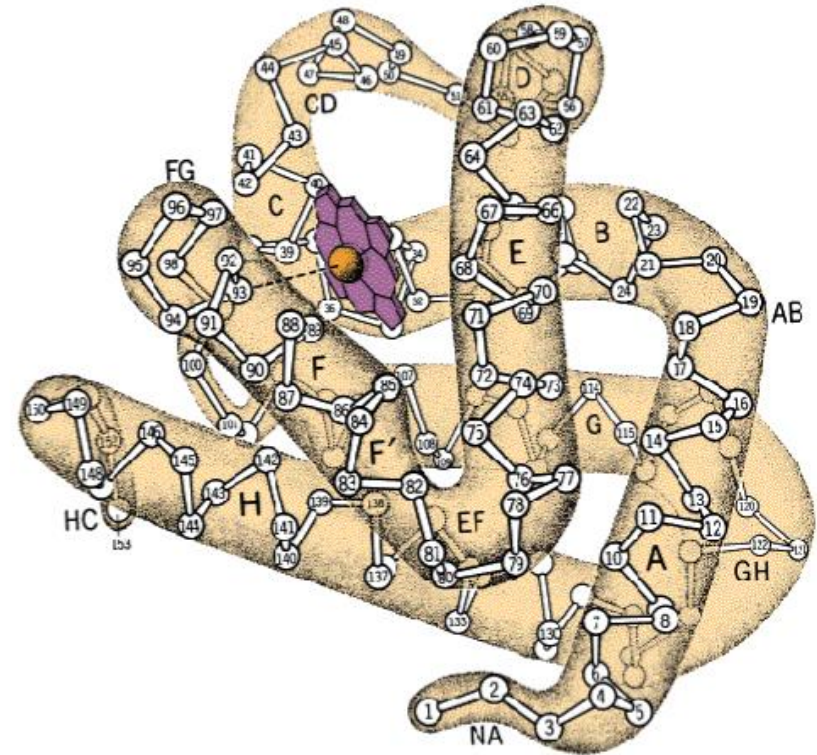


- Both myoglobin and hemoglobin are hemoproteins (a group of specialized proteins containing heme as a tightly bound non-protein group known as a prosthetic group).
- The protein environment dictates the function of the heme.

# Structure of myoglobin



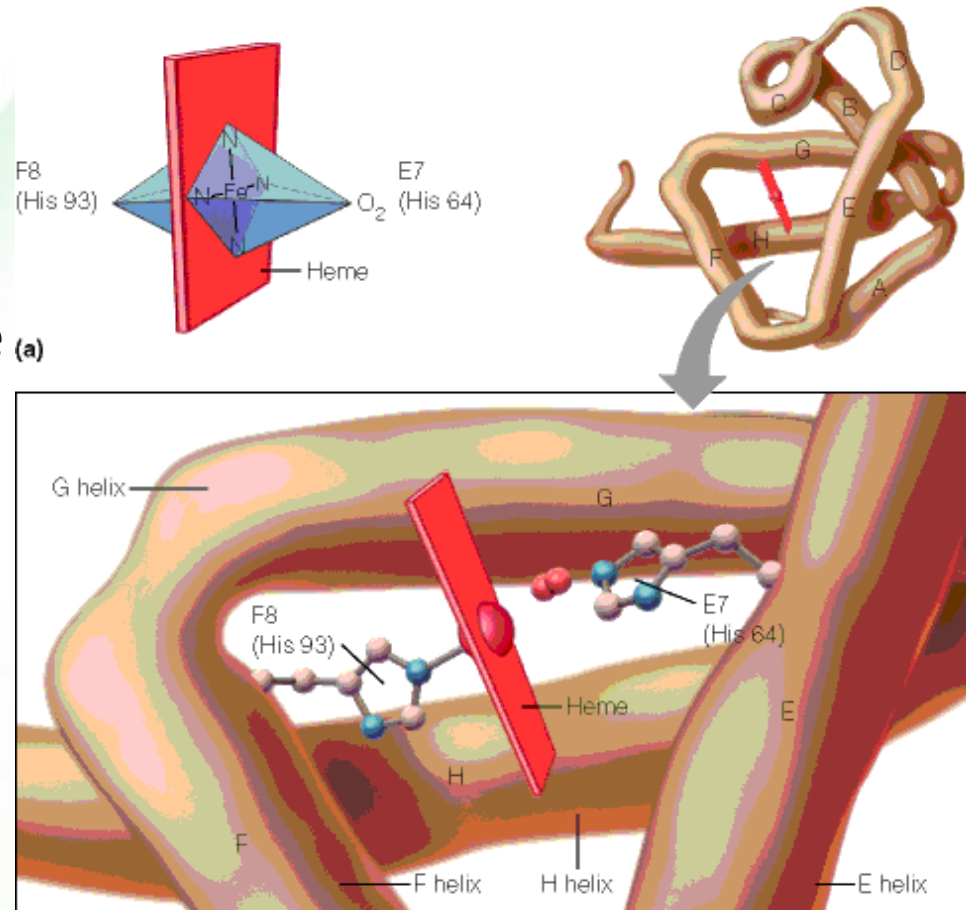
- Myoglobin is a monomeric protein that is mainly found in muscle tissue.
- It includes a prosthetic group, the **heme group**
- It can be present in two forms:
  - oxymyoglobin (oxygen-bound)
  - deoxymyoglobin (oxygen-free)
- The tertiary structure of myoglobin has 8  $\alpha$ -helices, designated A through H, that are connected by short non-helical regions.

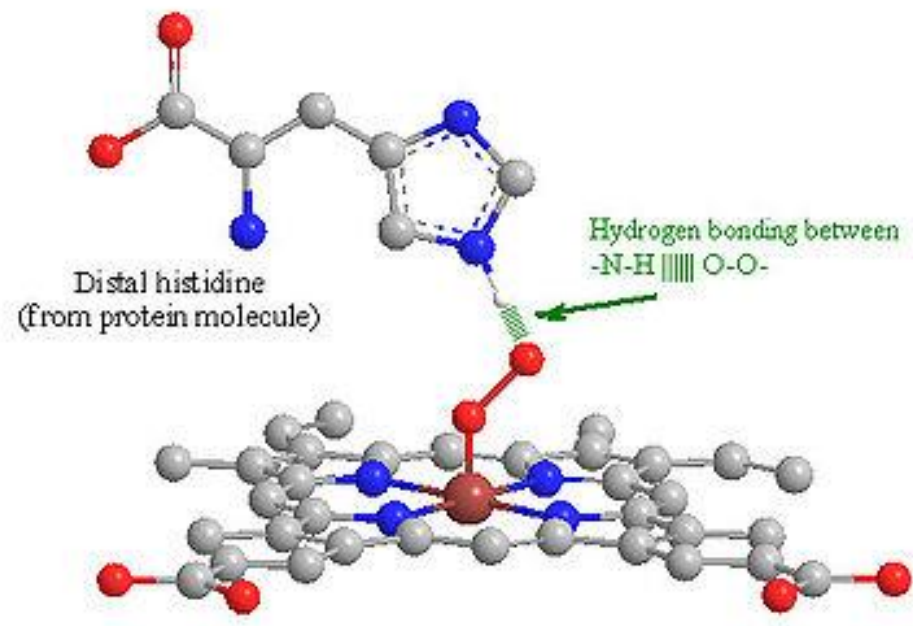
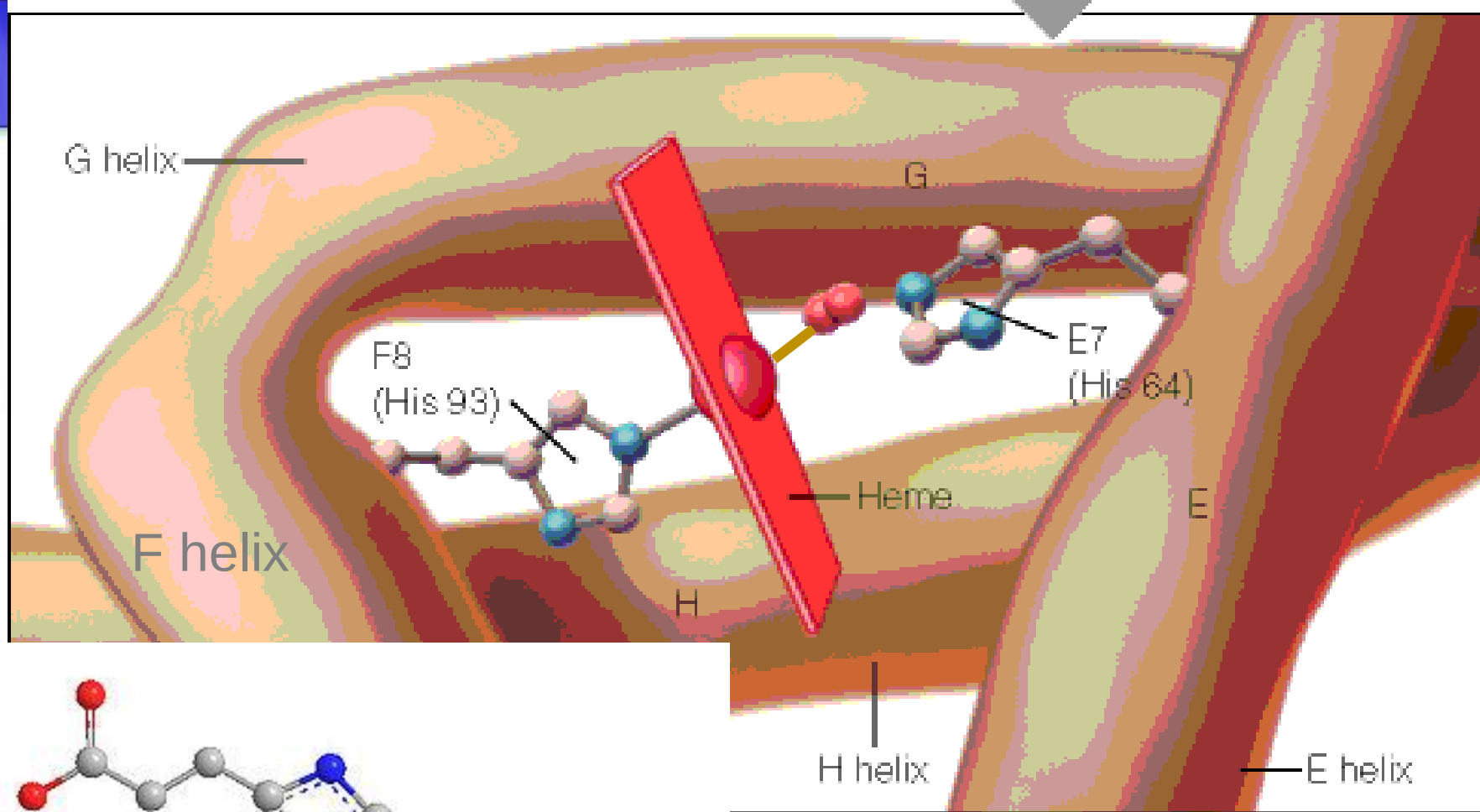


# Arrangement of amino acids



- Like other globular protein, amino acid R-groups exposed on the surface of the molecule are generally hydrophilic, while those in the interior are predominantly hydrophobic.
- 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

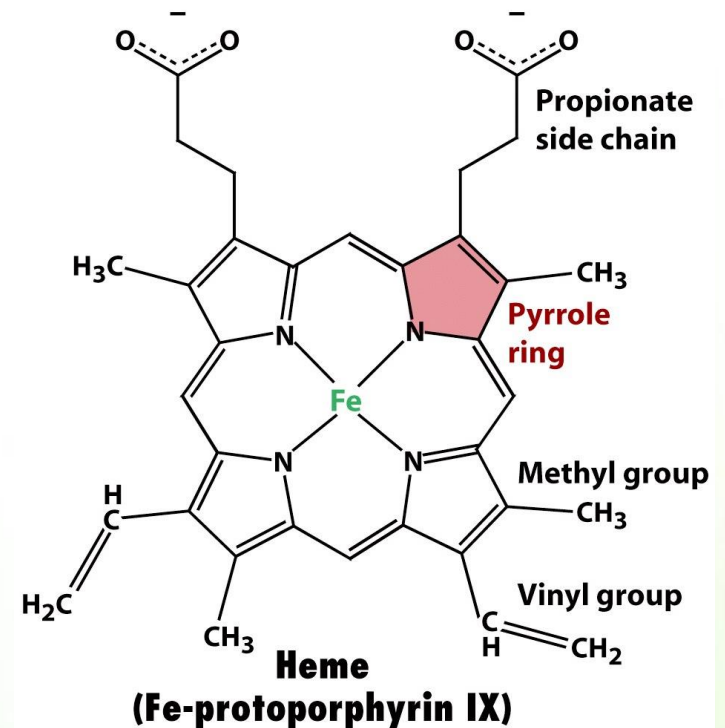




# Heme group

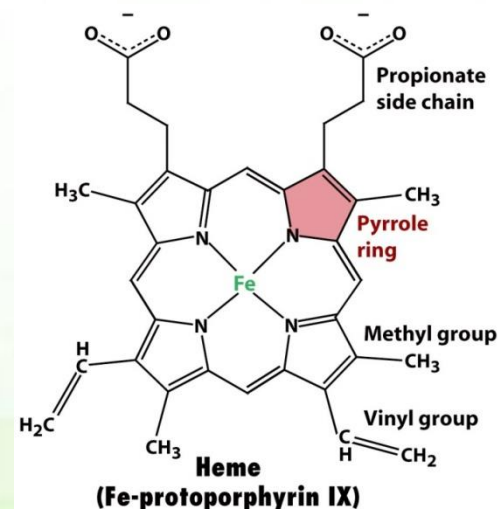
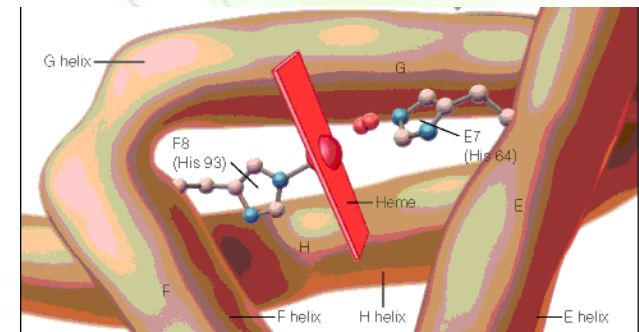
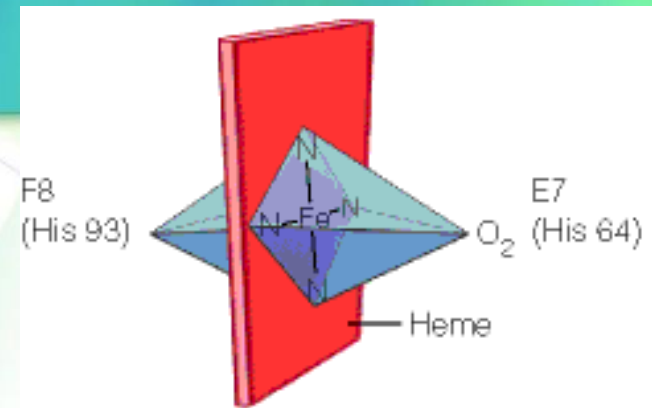


- Both proteins contain a heme group.
- It is a prosthetic group (a non-protein group covalently attached to a protein).
- Heme is a flat molecule that has four cyclic groups known as pyrrole rings.



# Iron

- Iron can bind in the center of the four rings.
- Fe is in the ferrous state ( $\text{Fe}^{2+}$ ) can form 6 bonds:
  - 4 with the nitrogen of the rings,
  - One (known as the fifth coordinate) with the nitrogen of a histidine imidazole (known as proximal His).
  - One with  $\text{O}_2$  (the sixth coordinate)
- Oxidation of iron to the  $\text{Fe}^{3+}$ , ferric, state makes the molecule incapable of normal  $\text{O}_2$  binding
- Upon absorption of light, heme gives a deep red color.

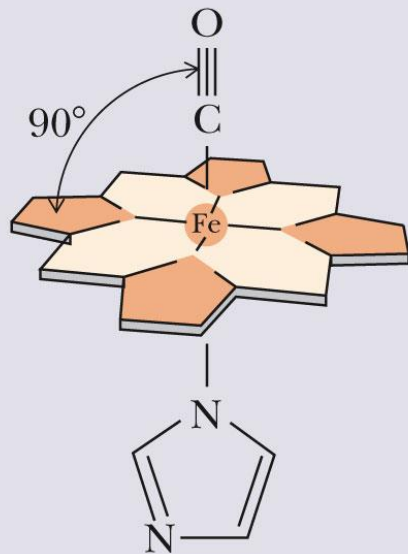


# Structure-function relationship

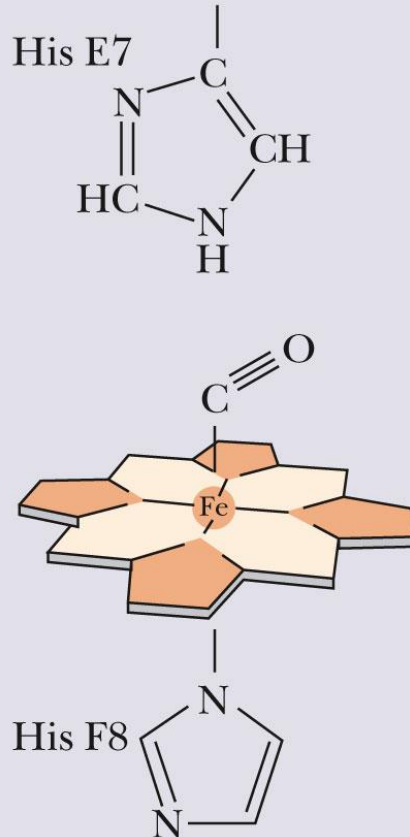


- The planar heme group fits into a hydrophobic pocket of the protein and the myoglobin-heme interaction is stabilized by hydrophobic attractions.
- The heme group stabilizes the tertiary structure of myoglobin.
- The distal histidine acts as a gate that opens and closes as  $O_2$  enters the hydrophobic pocket to bind to the heme.
- 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 another  $O_2$ .

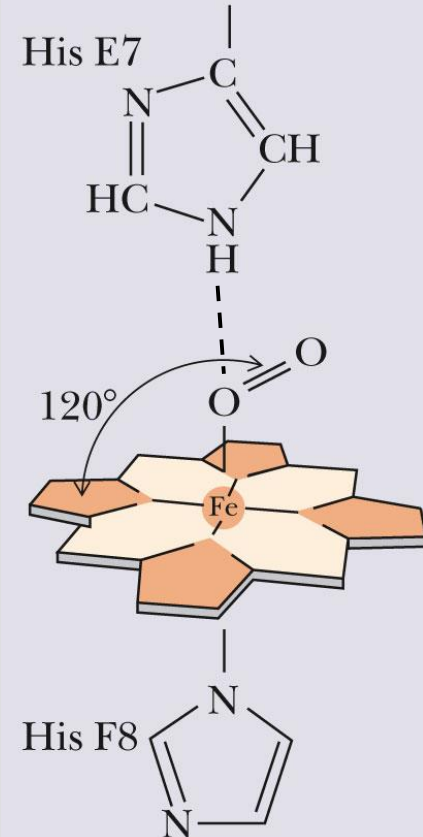
# Another significance of distal histidine



**A** Free heme with imidazole



**B** Mb:CO complex

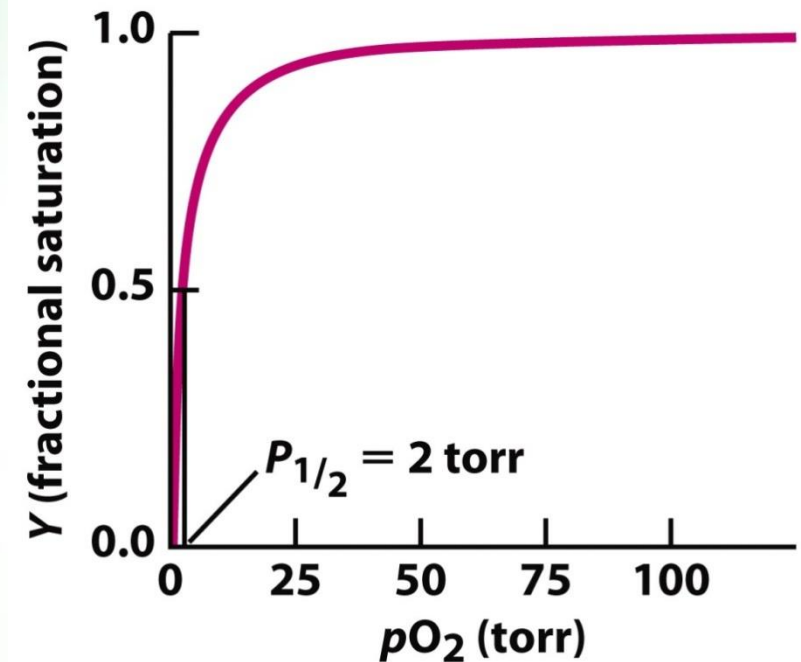


**C** Oxy-myoglobin

# Oxygen binding to myoglobin



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



The binding of  $O_2$  to myoglobin follows a hyperbolic saturation curve.

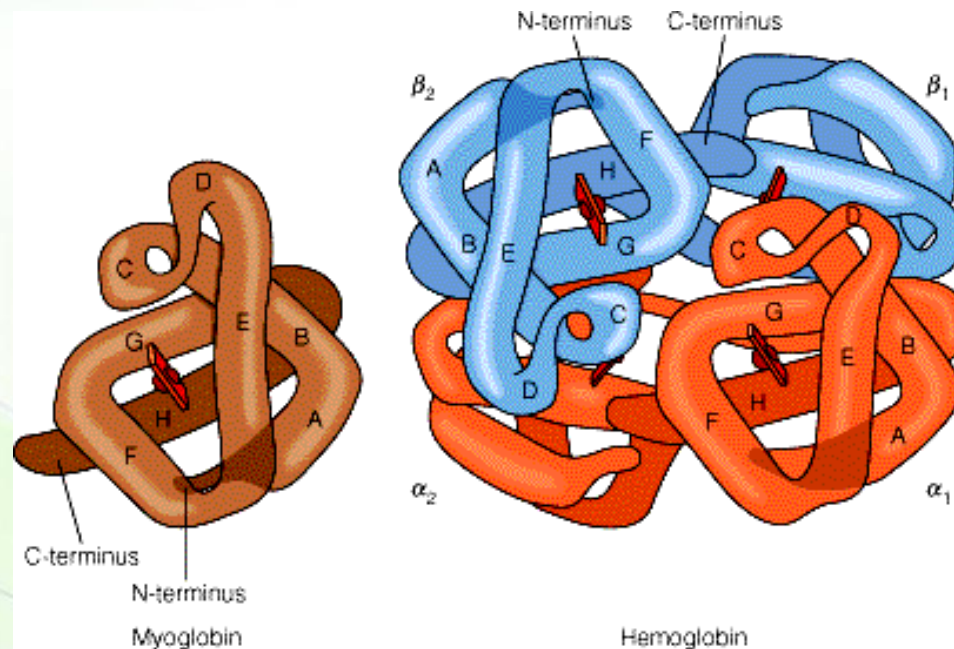


# ***Hemoglobin***

# Hemoglobin structure



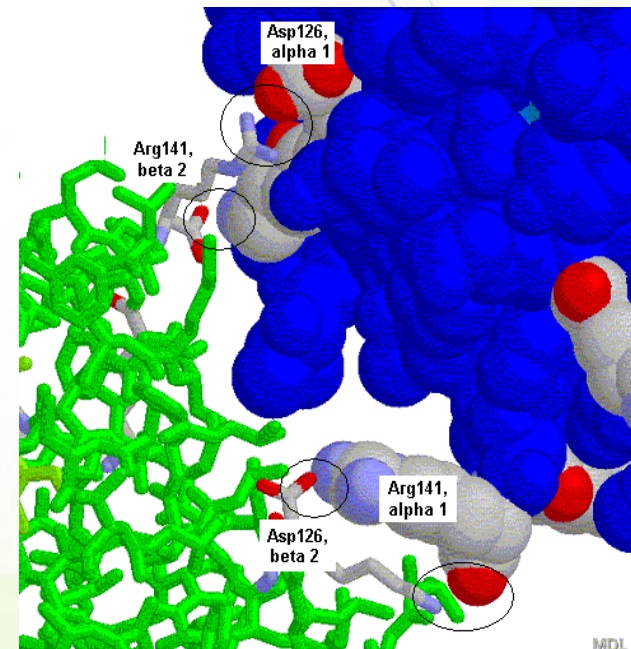
- Hemoglobin is tetrameric hemeprotein (four protein chains known as globins with each bound to heme).
- In adults, the four globin proteins are of two different types known as  $\alpha$  and  $\beta$ , so a hemoglobin protein is an  $\alpha_2\beta_2$  globin protein.
- The  $\alpha$  and  $\beta$  chains contain multiple  $\alpha$ -helices where  $\alpha$  contains 7  $\alpha$ -helices and  $\beta$  contains 8  $\alpha$ -helices (similar to myoglobin).



# Chain interaction



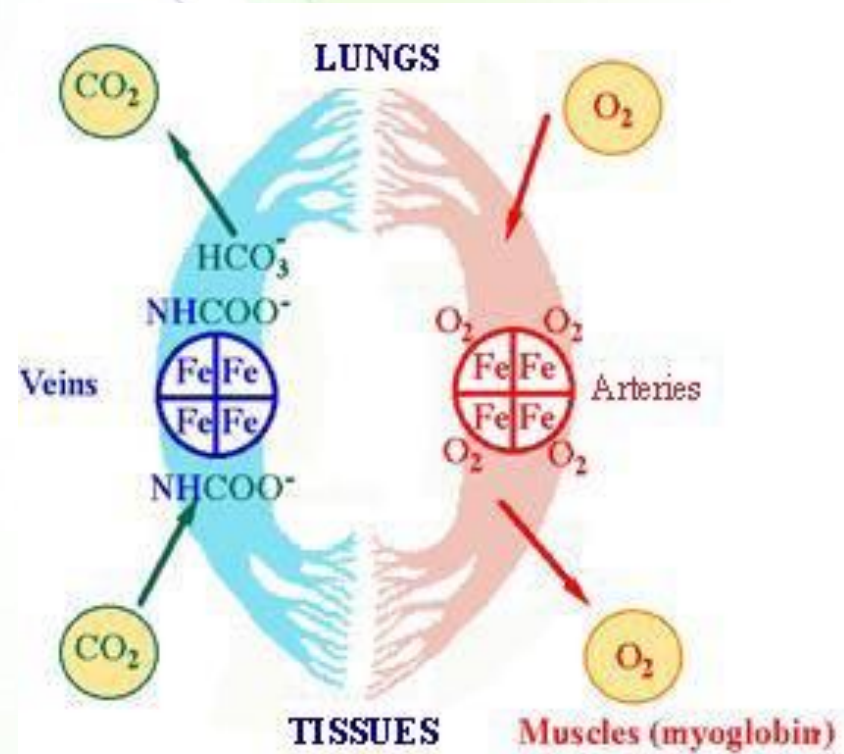
- The chains interact with each other via hydrophobic interactions.
  - Therefore, hydrophobic amino acids are not only present in the interior of the protein chains, but also on the surface.
- Electrostatic interactions (salt bridges) and hydrogen bonds also exist between the two different chains.



# Oxygen binding to hemoglobin



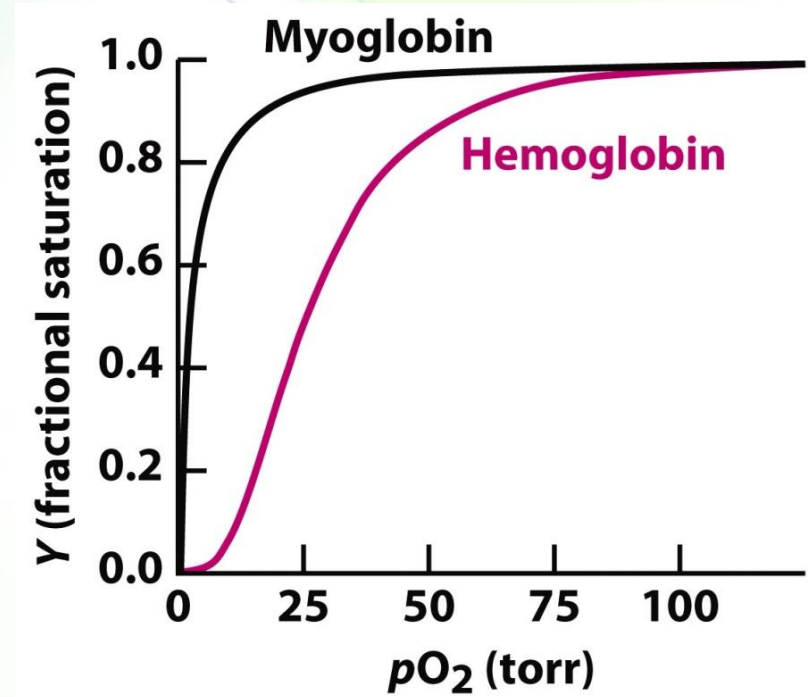
- Hemoglobin must bind oxygen efficiently and become saturated at the high oxygen pressure found in lungs (approximately 100 mm Hg).
- Then, it releases oxygen and become unsaturated in tissues where the oxygen pressure is low (about 30 mm Hg).



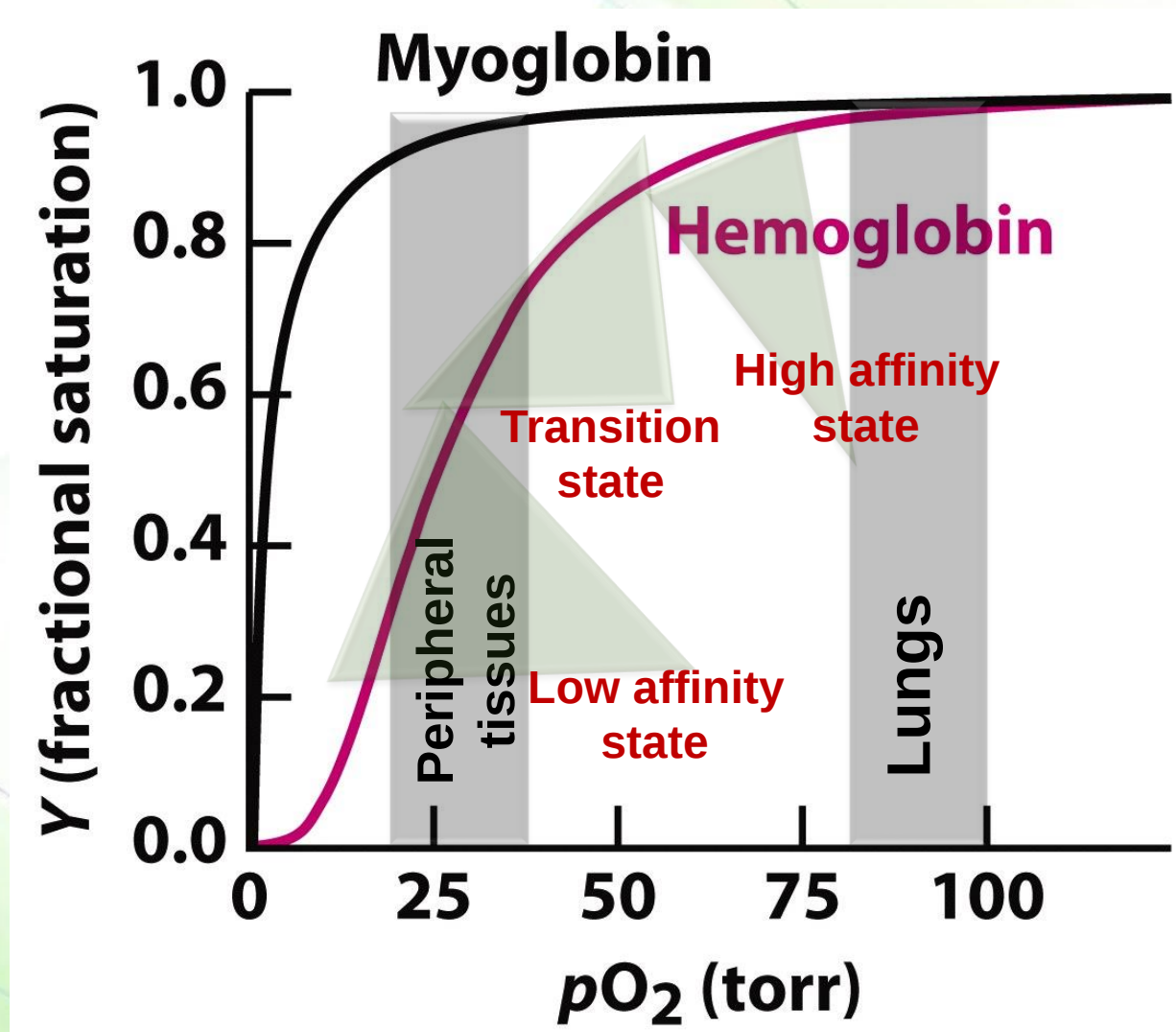
# The saturation curve



- 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  $p_{50}$  for myoglobin, the  $p_{50}$  of hemoglobin is approximately 26 mm Hg.



# The two structures of hemoglobin

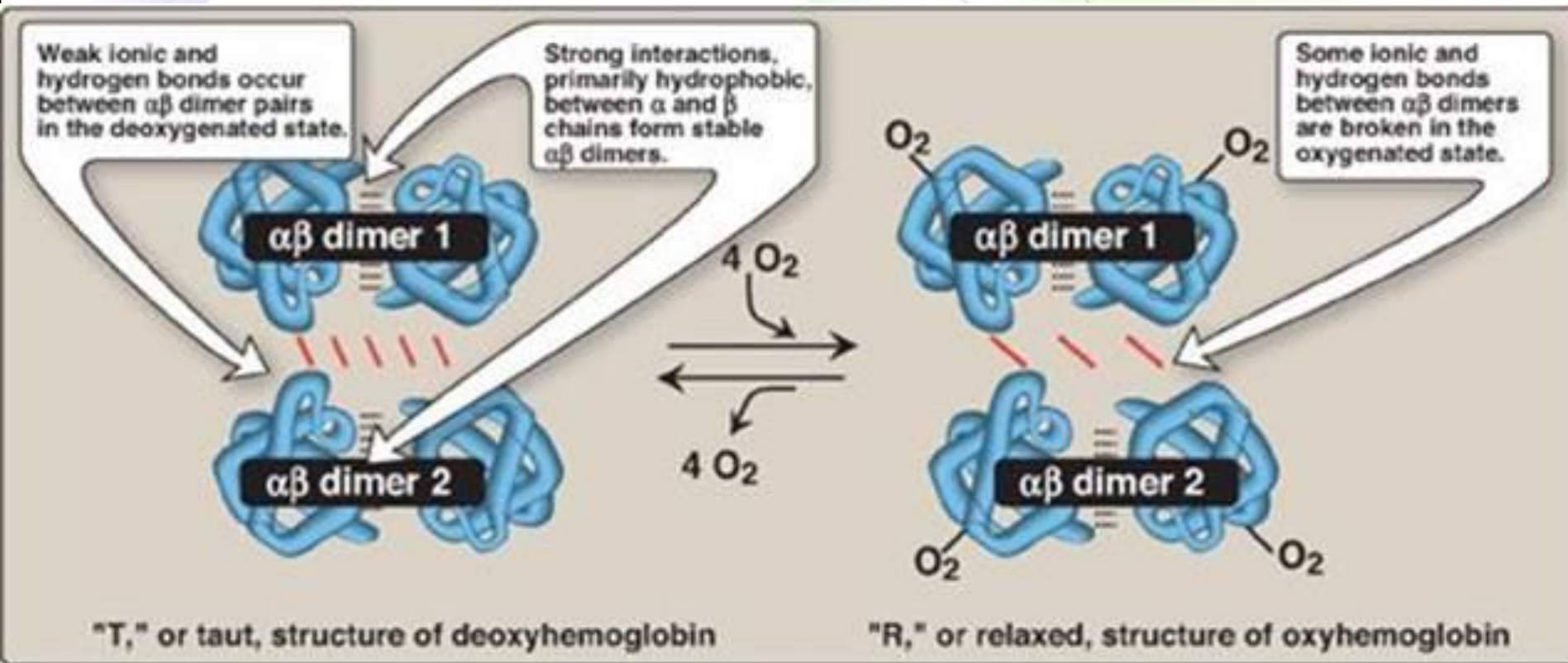


# Hemoglobin is allosteric



- 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, 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 as the T conformation .
- Binding of O<sub>2</sub> causes conformational changes in hemoglobin, converting it from the low affinity T-state to the high affinity R-state .

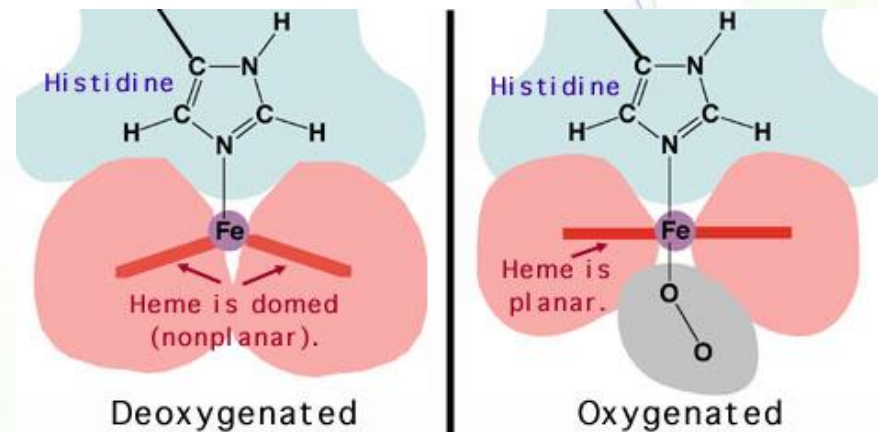
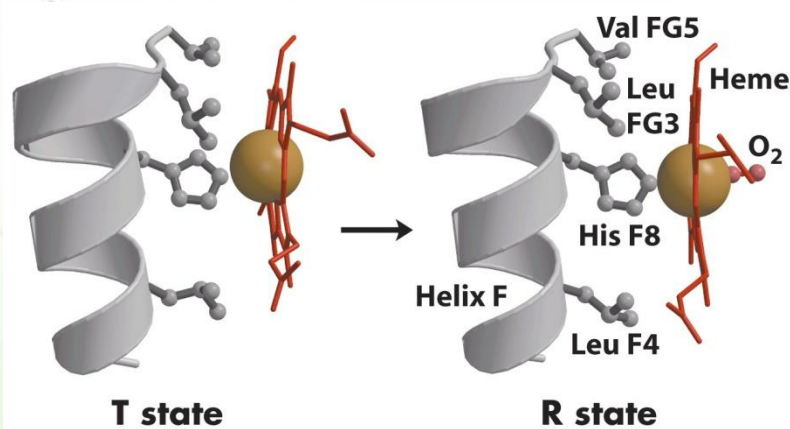
# Oxygen binding to Hemoglobin

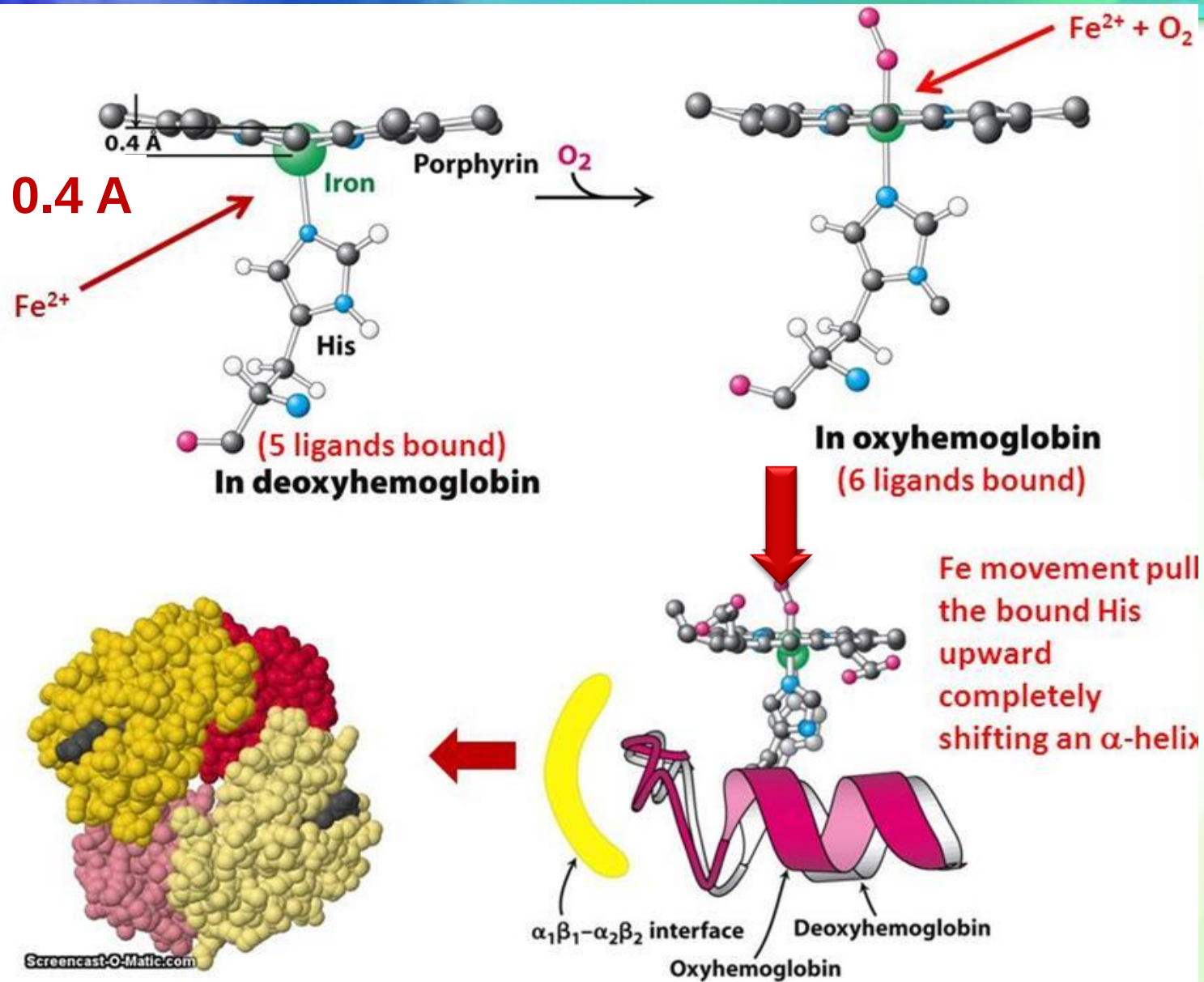


# How does the structure change? (1)



- When heme is free of oxygen, it has a domed structure and iron is outside the plane of the heme group.
- 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) along with it.



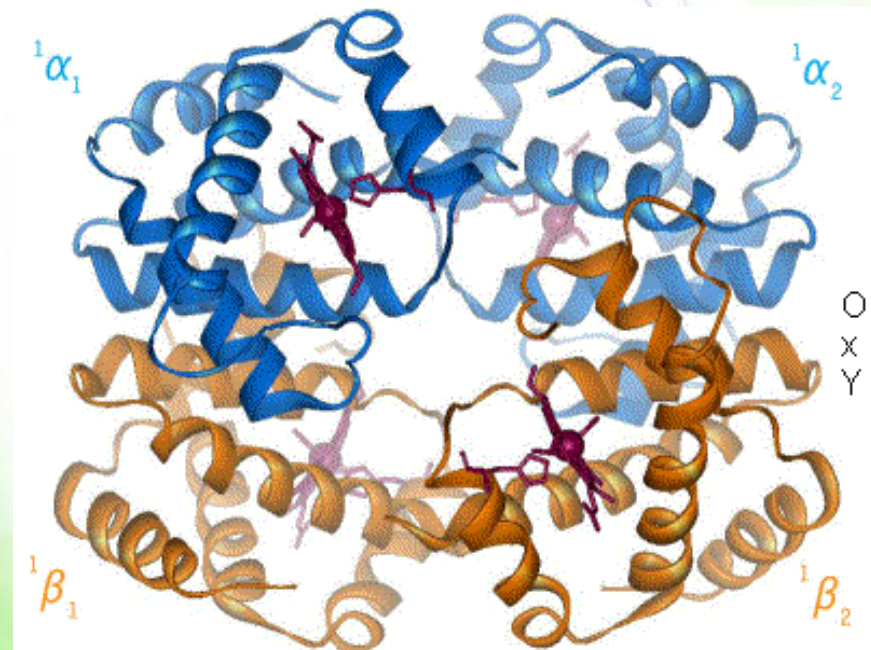


# How does the structure change? (2)



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

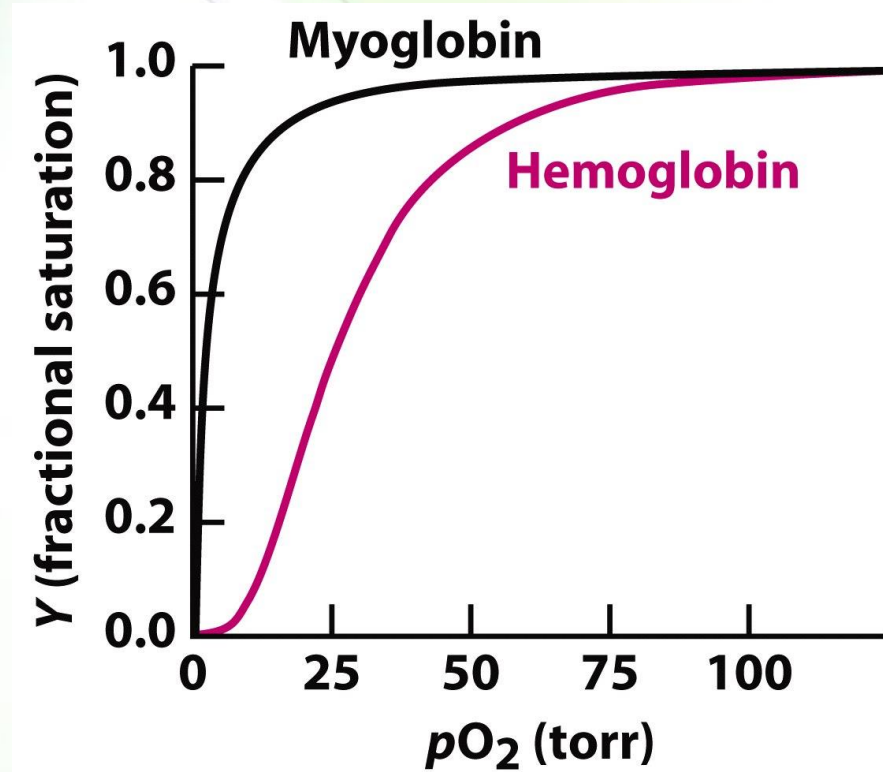
**In myoglobin, movement of the helix does not affect the function of the protein.**



# 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.
- 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.**



# It is a protective mechanism



- A sudden drop in pulmonary capillary oxygen tension does not affect hemoglobin saturation

- High altitudes

Hemoglobin  
% Saturation

