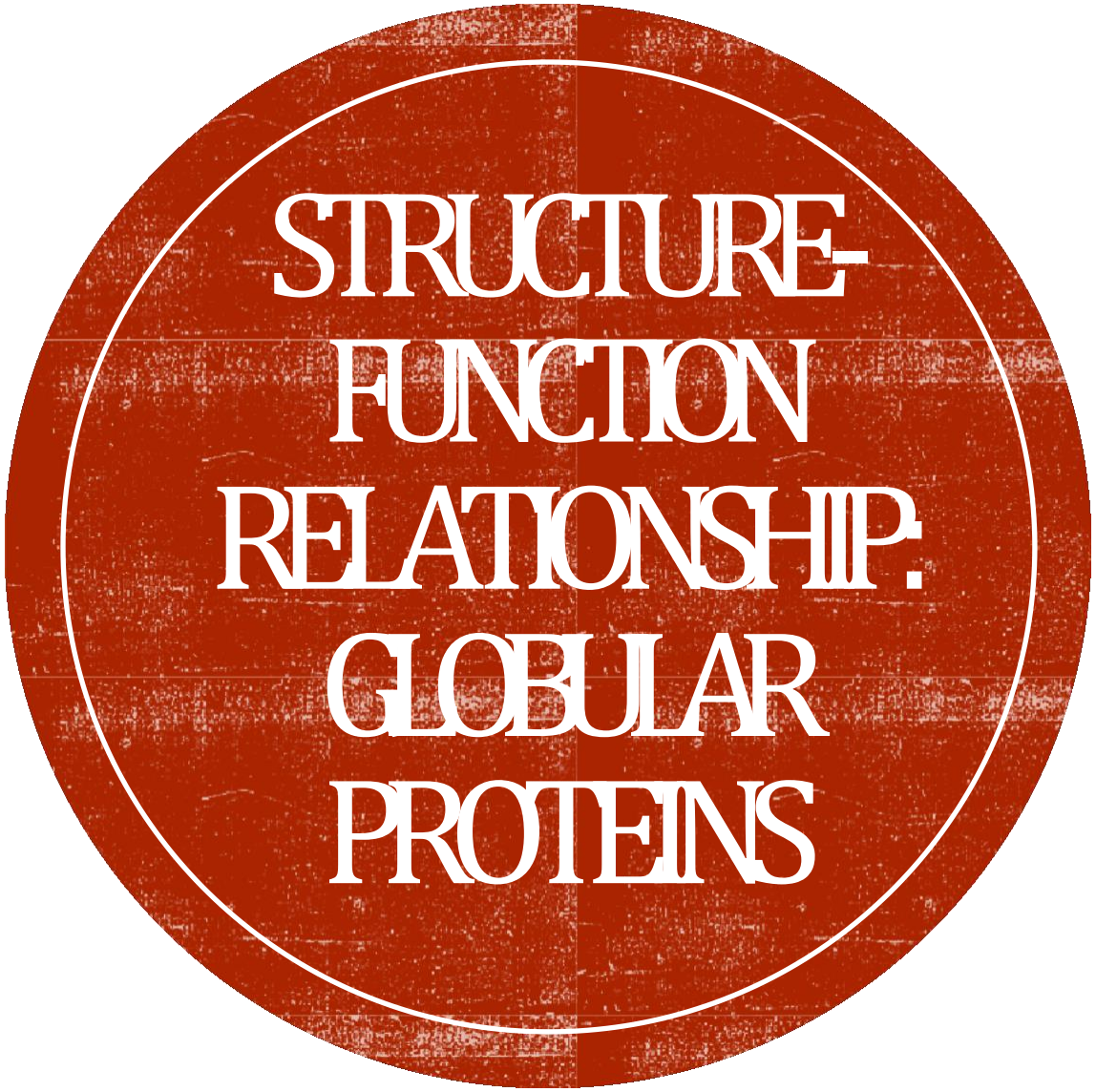
بسم الله الرحمن الرحيم

BioChemistry | FINAL 4

Globular Proteins pt.1

Written by : Mousa Al-Neimat
Nadeen Abedalwahab

Reviewed by : Rayan Theeb



Myoglobin and hemoglobin

FUNCTIONS OF MYOGLOBIN AND HEMOGLOBIN

Hemoglobin and Myoglobin have very similar structures and this explains why they have the same function.

- Myoglobin is storage of O2. During periods of oxygen deprivation, oxymyoglobin releases its bound oxygen.

Hemoproteins -> proteins that have **heme group** in their structure.

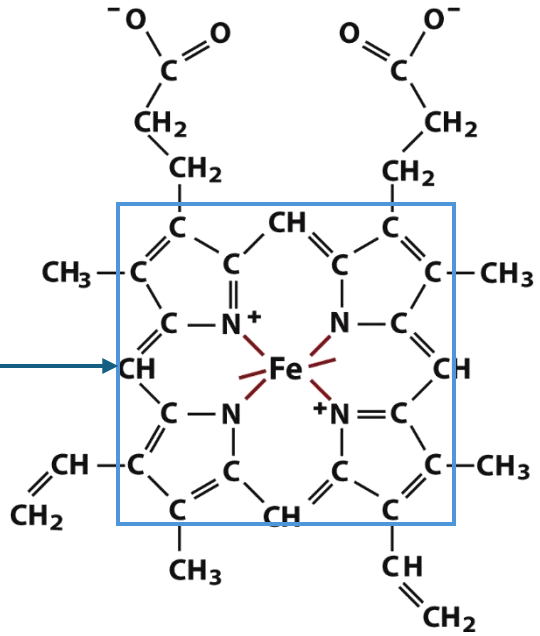
That's why it's called hemoglobin.

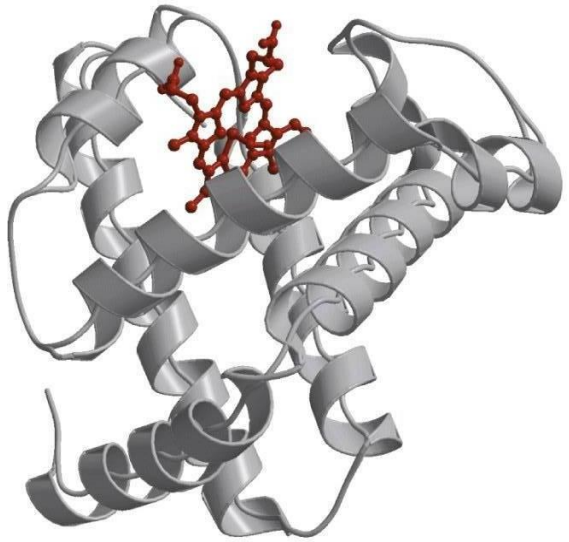
- Hemoglobin:
  - → Transport of O2 and CO2.
  - → Blood buffering.

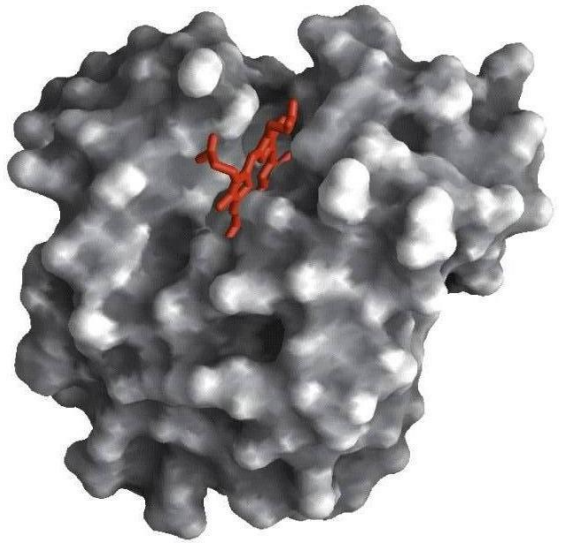
HEMOPROTEINS

- 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.

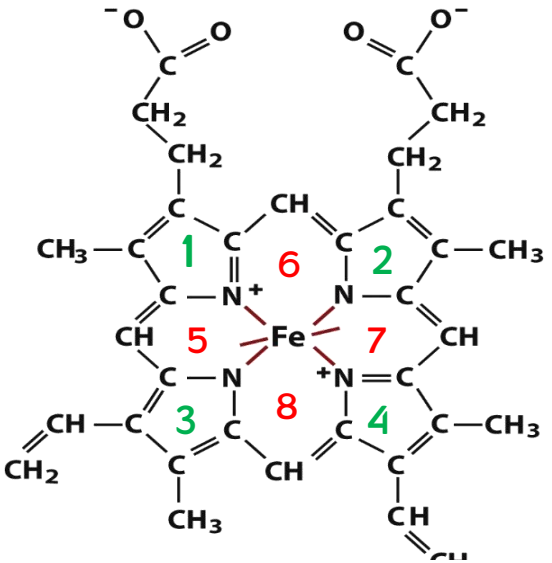
Heme ring
is a large Macro
Cyclic ring.



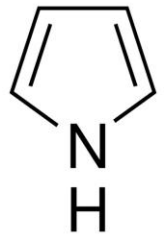




This structure is a **heme ring** which is a **porphyrin ring** that contains an iron in the middle bounded with a coordination covalent bond (not mentioned good to know) to the 4 nitrogens of the 4 small rings that form the porphyrin ring.



Some people get confused and think the porphyrin has eight rings. In fact, it has only four small rings (pyrrole rings). The other four shapes are open, so they are not real rings.



Pyrrole ring

We can synthesize porphyrin ring in our body. It is first synthesised as an open chain and then converted into ring.

Fe can form up to **6 bonds** (**4 on the plane** and **one to the up** and the other one **down**) [Fe on the porphyrin binds to 4 pyrrole rings (on the plane) and with proximal histidine and Oxygen(above and below Fe) (don't care about proximal histidine and oxygen, you will understand as we are moving through the slides)].

HEMOPROTEINS

- The protein environment dictates the function of the heme.

Cytochromes

Protein that contains heme group and work as electron carrier.

Cytochromes

- **An electron carrier (alternately oxidized & reduced)**

The 2 ionization states of iron (Fe+3[ferric] and Fe+2 [ferrous]) allow it to work as an electron carrier since it can bind to an electron (become Fe+2) or release it (become Fe+3).

Catalytic enzymes

The presence of heme group allows it to work as an enzyme.

Catalase Heme protein binds to another substance, making a reaction and converting it into a new substance.

- **Catalyzes the breakdown of O_2 peroxide**

Binding proteins

Hemoglobin & Myoglobin (conjugated)

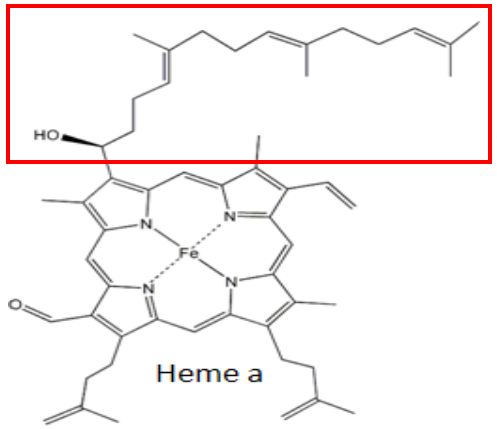
- **Reversibly bind O_2** [Binding and releasing of Oxygen as we said before in the previous lectures].

Heme group outside the protein is called 4 ligated heme, In proteins:
If the hemoprotein is involved in electron transport and → The heme group is 6 coordinate (ligated).
If the hemoprotein is involved in catalyst (enzyme) or protein binding→ The heme group is 5 coordinate (ligated).

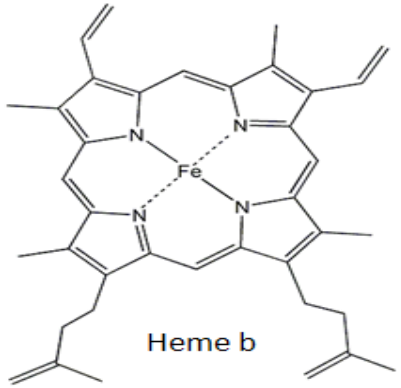
STRUCTURES OF PORPHYRIN & HEME

There are so many types of heme groups depending on the side chains binding to the Heme

Hydrocarbon chain with hydroxyl group

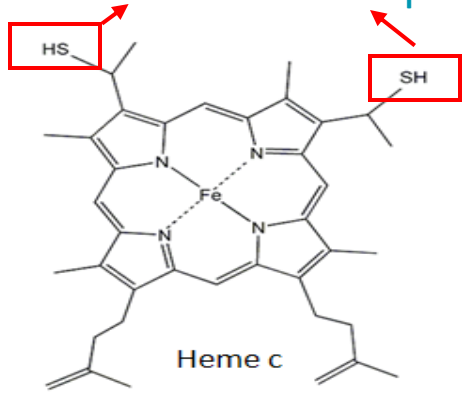


Heme a

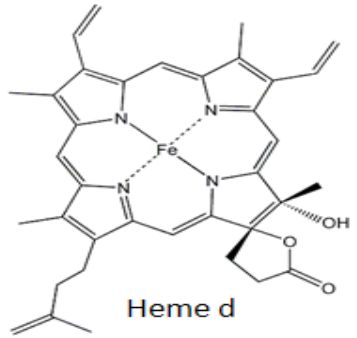


Heme b

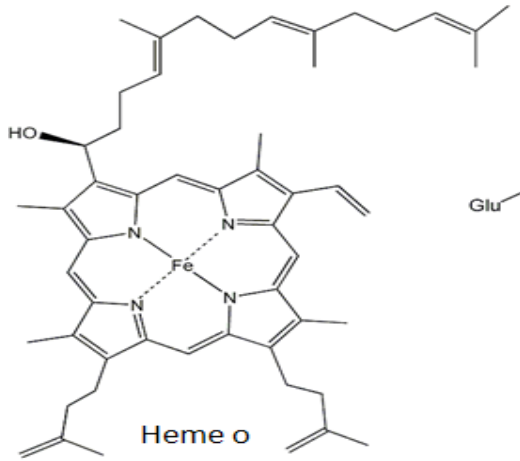
Covalent interaction with proteins.



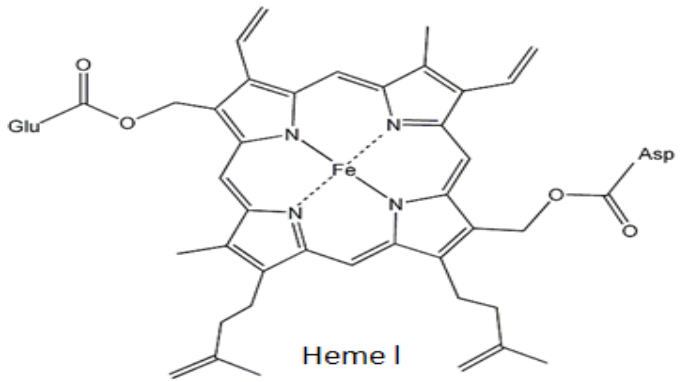
Heme c



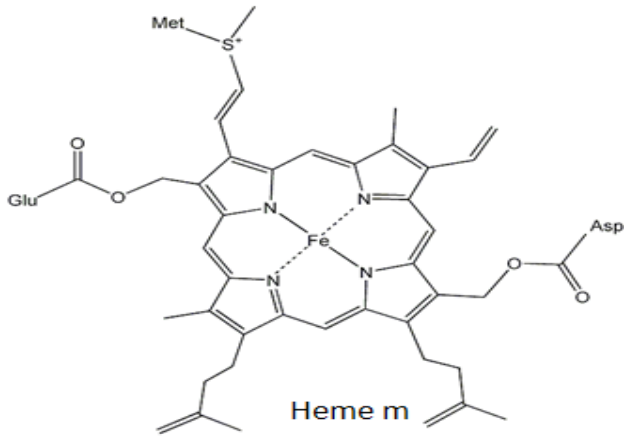
Heme d



Heme o



Heme l



Heme m

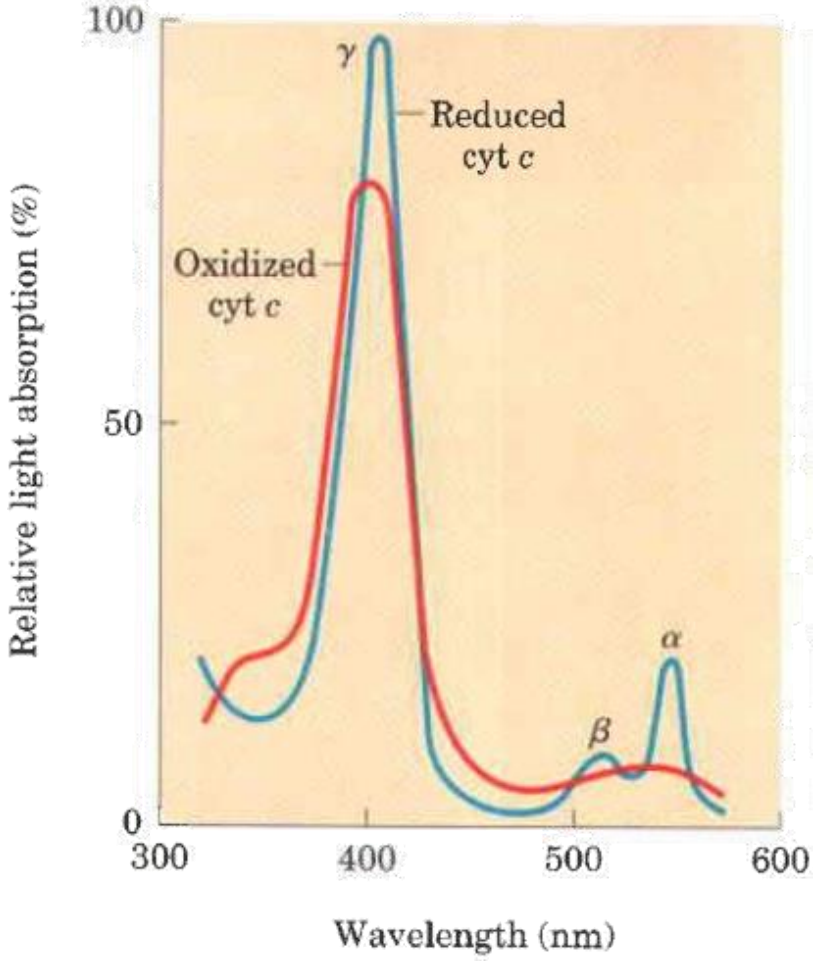
- **Pyrrole, Porphyrin, Fe, Heme, Ligation**

(Cytochrome B contains Heme B And Cytochrome C contains Heme C
Cytochrome aa3 contains two types of heme a and like this).

Most abundant Heme protein in our bodies → Hemoglobin → Heme B → which means Heme b is the most abundant hemoprotein in our bodies.

Simply we can recognise these types depending on structure, but if we don't know the structure can we differentiate between them in other way ?
Read next slide ☺

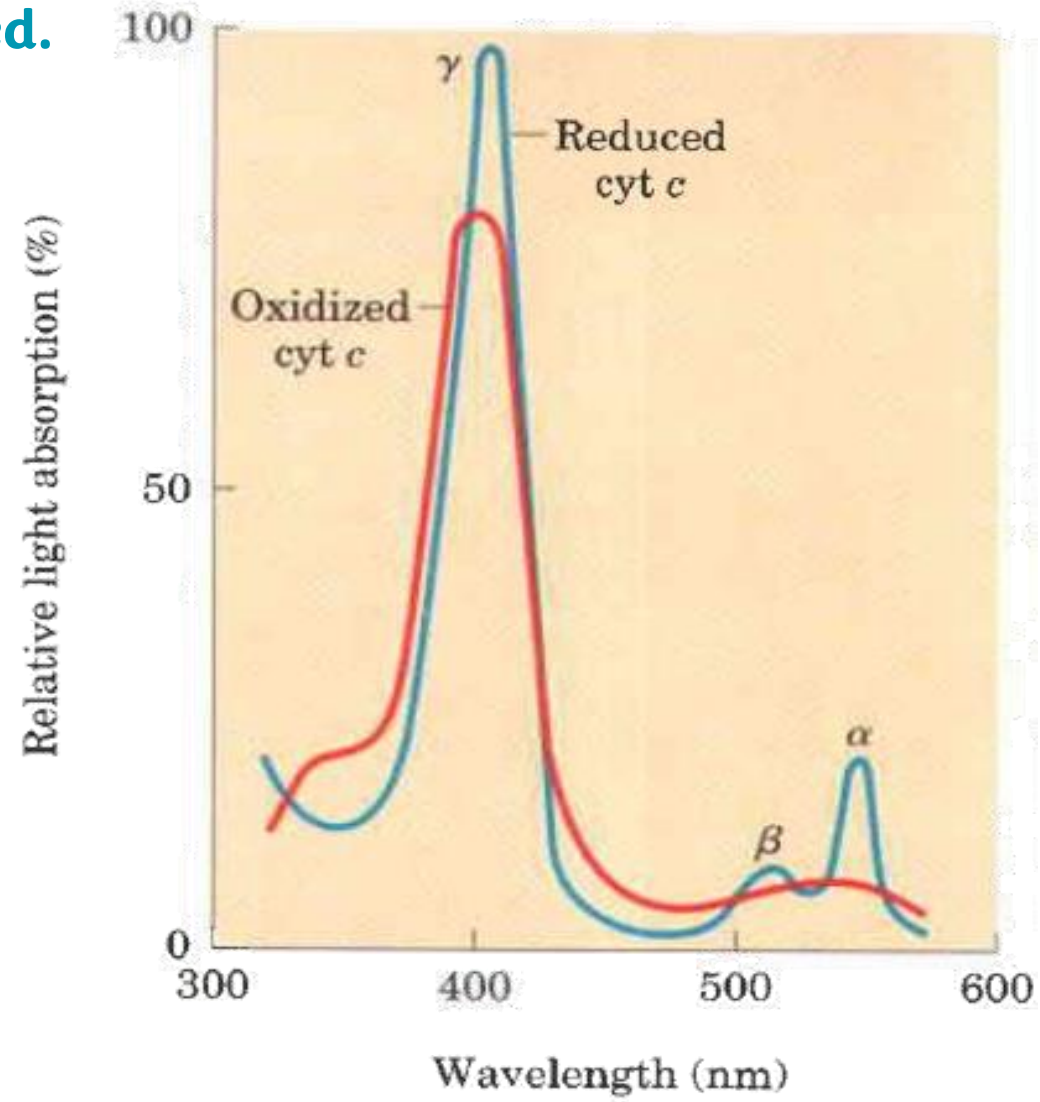
HEME – SPECTROSCOPIC FEATURES



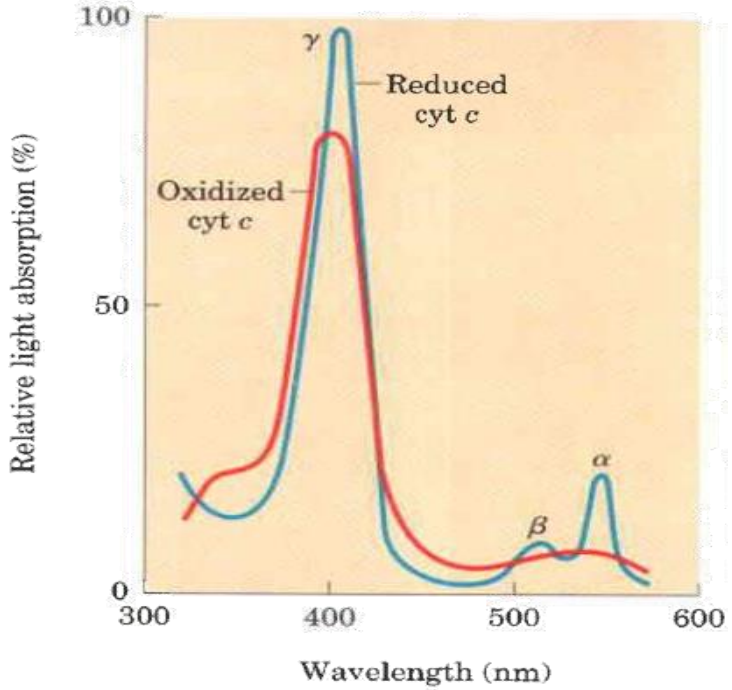
- Characteristic strong absorption of visible light (Fe-containing heme prosthetic groups).
- Classification based on light absorption & Mode of binding (a, b, c).
- Light absorption: Each cytochrome in its reduced (F^{+2}) state has 3 absorption bands in the visible range.
- α band : near 600 nm in type a; near 560 nm in type b, & near 550 nm in type c.
- Heme can carry one electron.

- Heme group could be **oxidized** and could be **reduced.**
- **Oxidized heme group** has only one peak around **400nm**
- **Reduced heme group** will give us 3 peaks each peak around a specific wavelength representing different heme type.
  Named from higher wave length to shortest wave length α, β and γ.

- We differentiate between them spectroscopically (the percentage of the light absorbed at different wave length by heme group depending on its type).
- Every type has its own distinct peak at which it absorbs light with specific length most effectively and by this characteristic we can recognise what type of heme does the protein has.



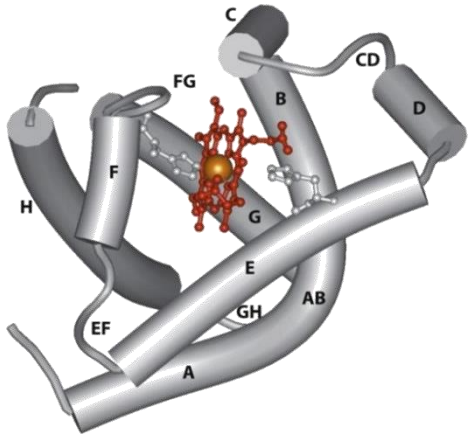
- Imagine you have a solution containing proteins with different types of heme groups. If you shine light starting from 300 nm onto the solution and measure the percentage of light absorbed by the heme groups, you would observe that at 400 nm, 98% of the light is absorbed, forming a peak (meaning absorption is lower on both sides of this wavelength). This indicates the presence of a specific type of heme that absorbed this light. Another peak appears at 530 nm, showing that light at this wavelength is absorbed by another protein containing a different type of heme group.

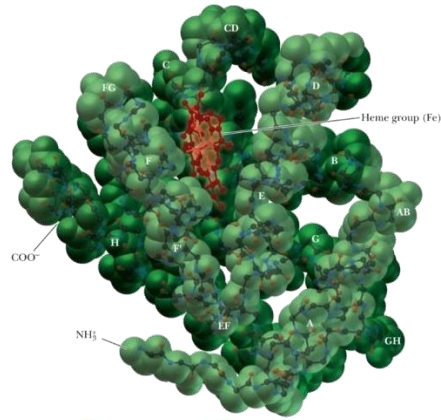


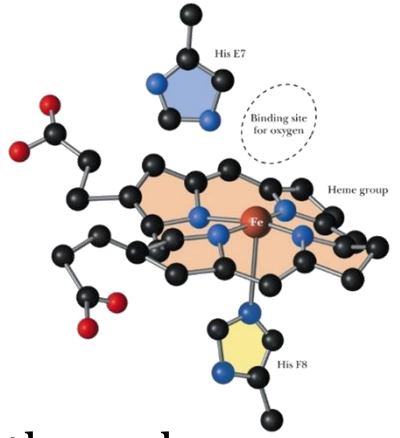
- If all the heme groups are in the oxidized state, it becomes harder to distinguish their types spectroscopically, because the characteristic α and β bands are less pronounced or even shifted compared to the reduced state.
- Spectroscopic classification of heme types mainly relies on the reduced form, where the α band position is clearly visible and type-specific.

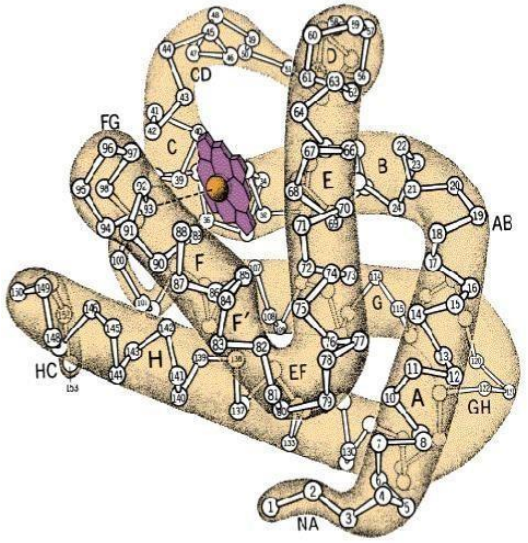
- In the oxidized form, peaks can overlap or have lower intensity, making it difficult to identify the type accurately without additional techniques (like redox cycling the sample to reduce the heme first).

STRUCTURE & FUNCTION OF MYOGLOBIN









- Myoglobin is a monomeric protein (153 aa)
- 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 8 a-helices, designated A through H, that are connected by short non-helical regions.

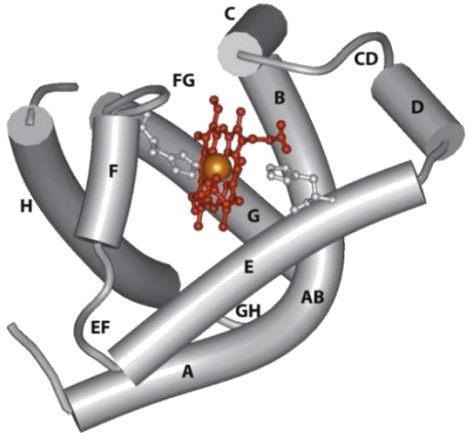
STRUCTURE & FUNCTION OF MYOGLOBIN

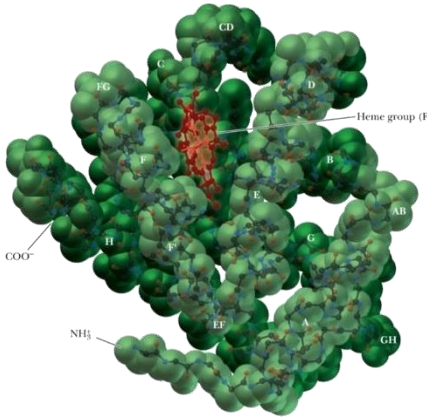
Globular proteins in contrast to fibrous ones are composed of repeated units of different 2nd structure types and that prevent them from being compressed so they still have a space inside them that is filled with water and this makes globular proteins soluble.

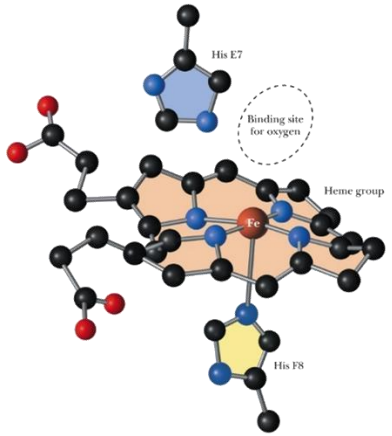
Hemoglobin and myoglobin are exception for this rule, they are globular proteins and soluble in water composed only of one type of 2nd structure (which is α-helix) with short sequences of amino acid connecting them,

Heme group is located in the hemoglobin and myoglobin in a pocket called hydrophobic pocket because it isn't covalently bounded like heme c (it stabilises the heme in place).

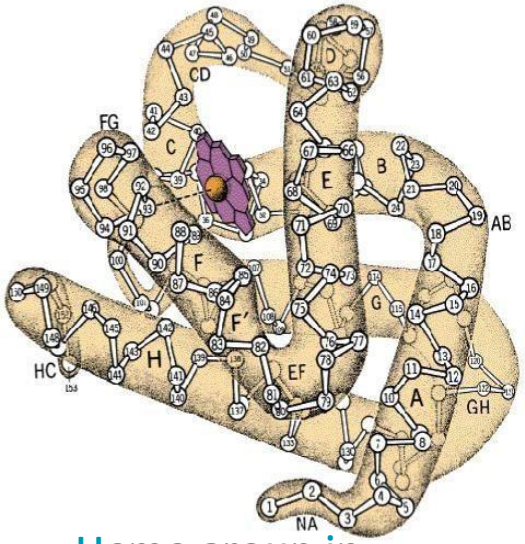
Also, the hydrophobic pocket preserve the iron in it's reduced state so it still able to bind to oxygen.







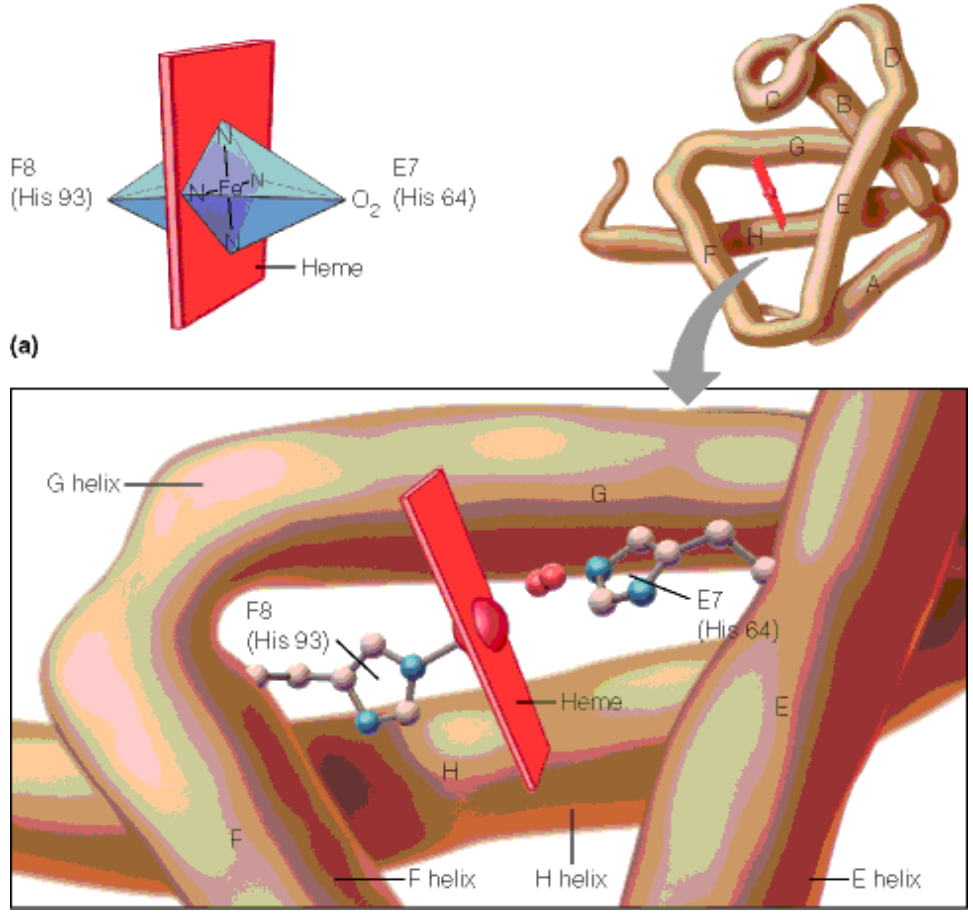
Heme group mostly hydrophobic in nature (composed of hydrogen and carbon)



Heme group in hemoglobin and myoglobin work as a binding protein which means it is 5 ligated.

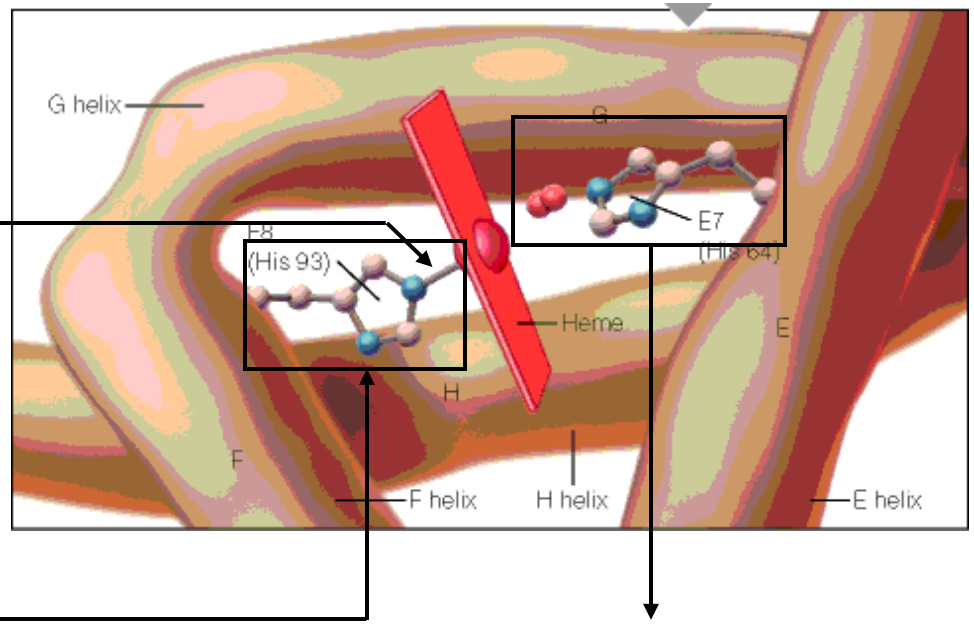
ARRANGEMENT OF AMINO ACIDS

- 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.



ARRANGEMENT OF AMINO ACIDS

Side chain of proximal histidine binds to the iron of the heme group (covalently) disrupting the planar shape of the heme group (converted into dome like shape resulting from the withdrawal of the iron atom by histidine's side chain).



Side chain of amino acid (Histidine), Called proximal histidine because it is closer to the heme group than the other one (distal histidine).

Distal histidine is not bounded to the iron of the heme group but it makes a hydrogen bond with the Oxygen when the oxygen come and bind to the iron of the heme group.

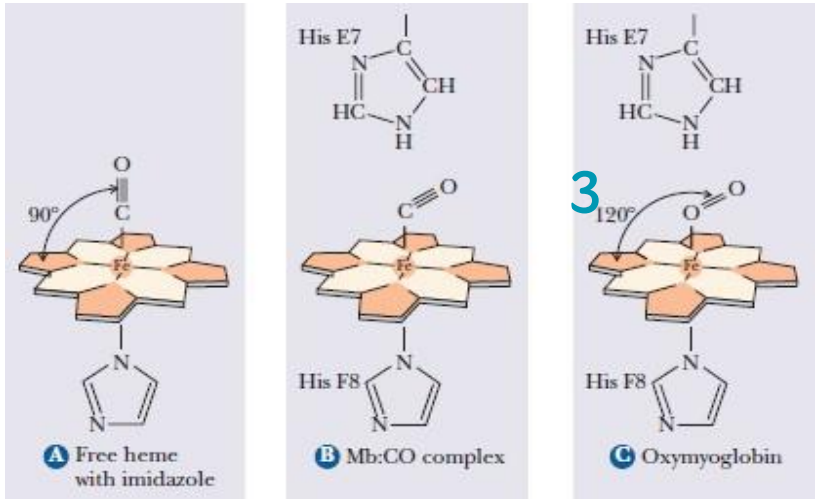
We mentioned previously that the binding of proximal histidine to the iron of the heme group converts it from a planar to a dome-like shape. When distal oxygen binds to the iron, the distal histidine binds with oxygen through a hydrogen bond, which withdraws the iron in the opposite direction from the initial withdrawal caused by the proximal histidine, returning it to the planar shape.

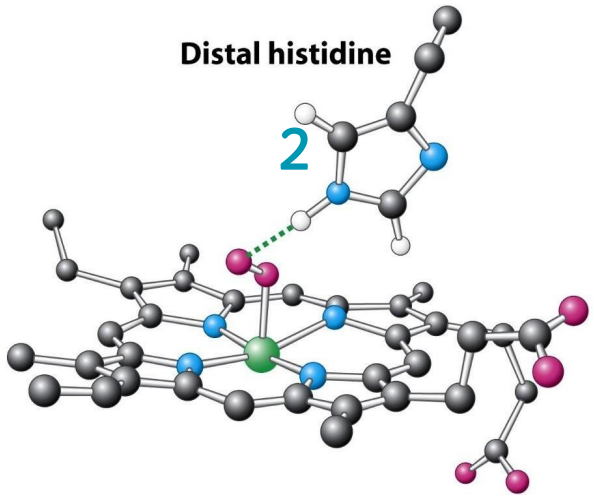
What is the functions of the distal histidine?

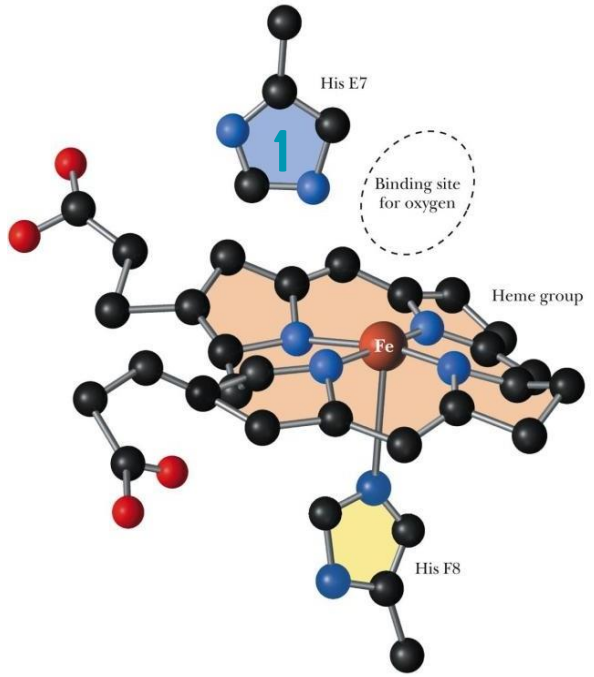
1) It acts as a gate : opens allowing oxygen to pass and bind to iron and close after it enter.

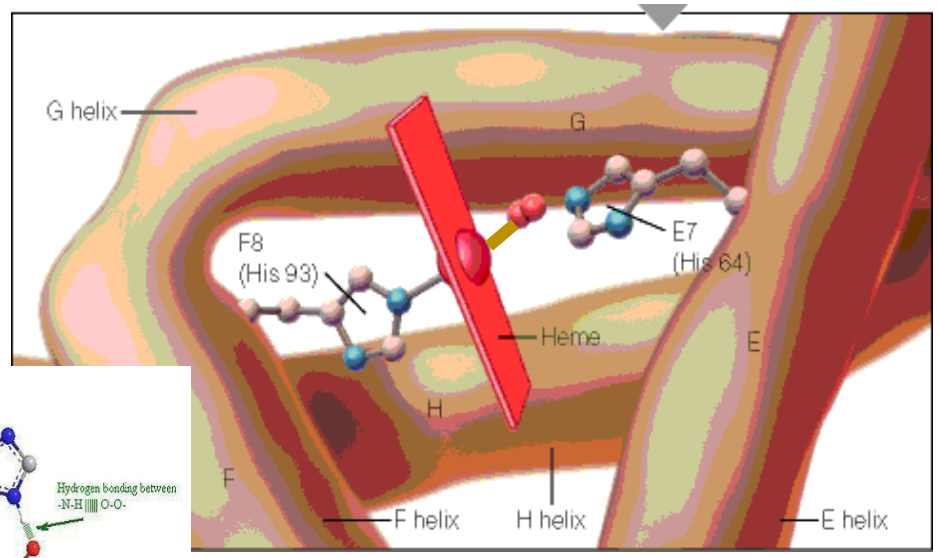
2) It makes hydrogen bond with the oxygen stabilising oxygen binding.

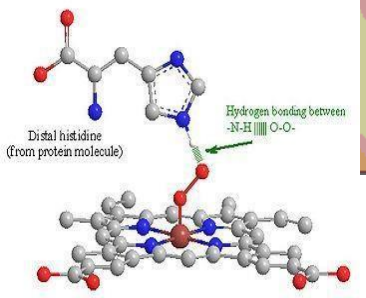
3) Tilts the bond between iron and oxygen making it less stable. Make it 120 degrees which is less stable than being perpendicular at the plane of the heme group, and this allows it to be released easily.

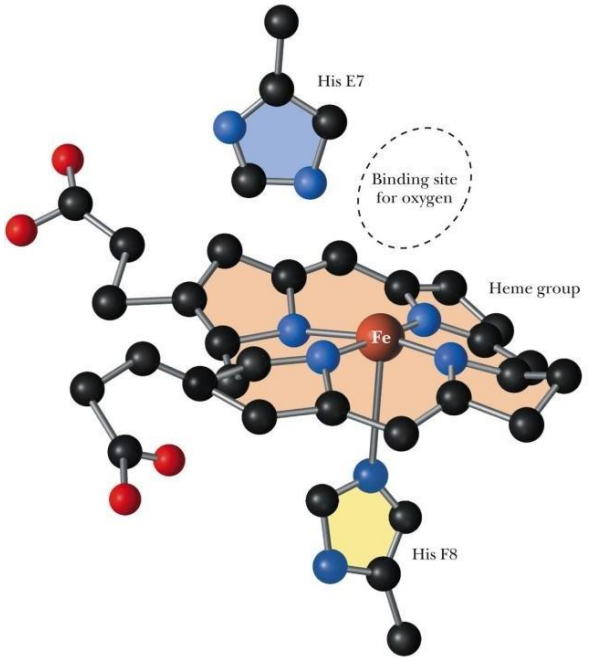




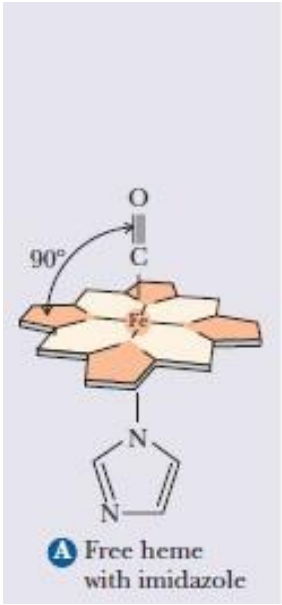


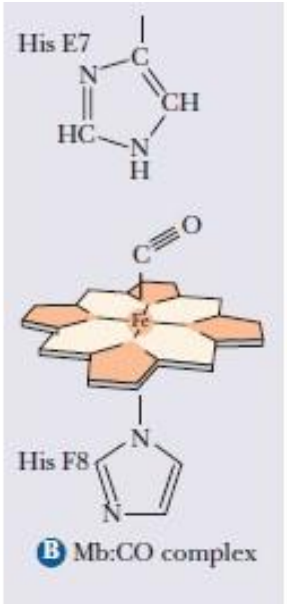


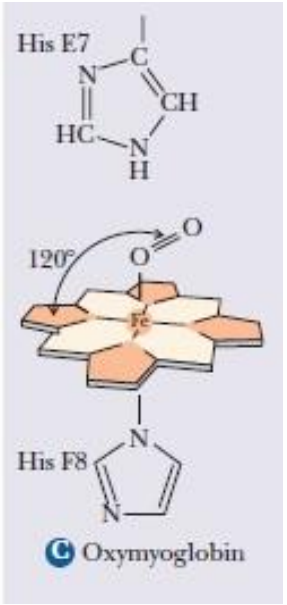




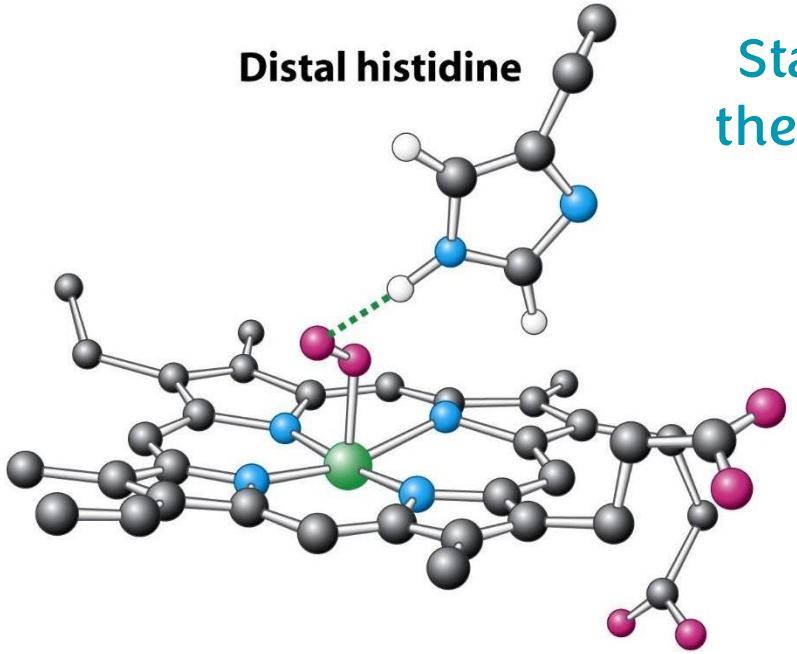
Acts as a gate







Tilting the oxygen iron bond.



Stabilises the oxygen

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 O2 enters the hydrophobic pocket to bind to the heme.
- The **hydrophobic interior** of myoglobin (or hemoglobin) **prevents the oxidation of iron**, and so when O2 is released, the iron remains in the Fe(II) state and can bind another O2.

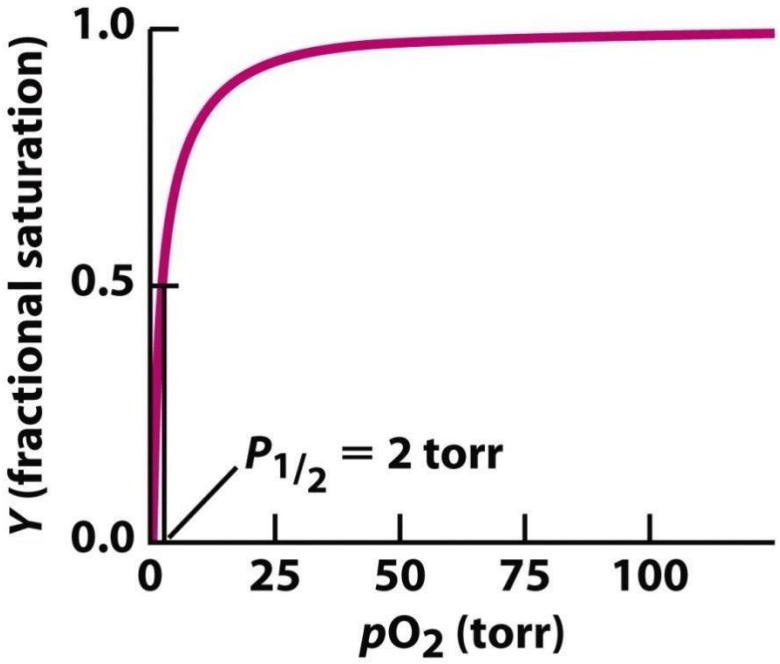
Hemoglobin is a tetrameric protein made of 4 subunits (2 alpha and 2 beta)

OXYGEN BINDING TO MYOGLOBIN

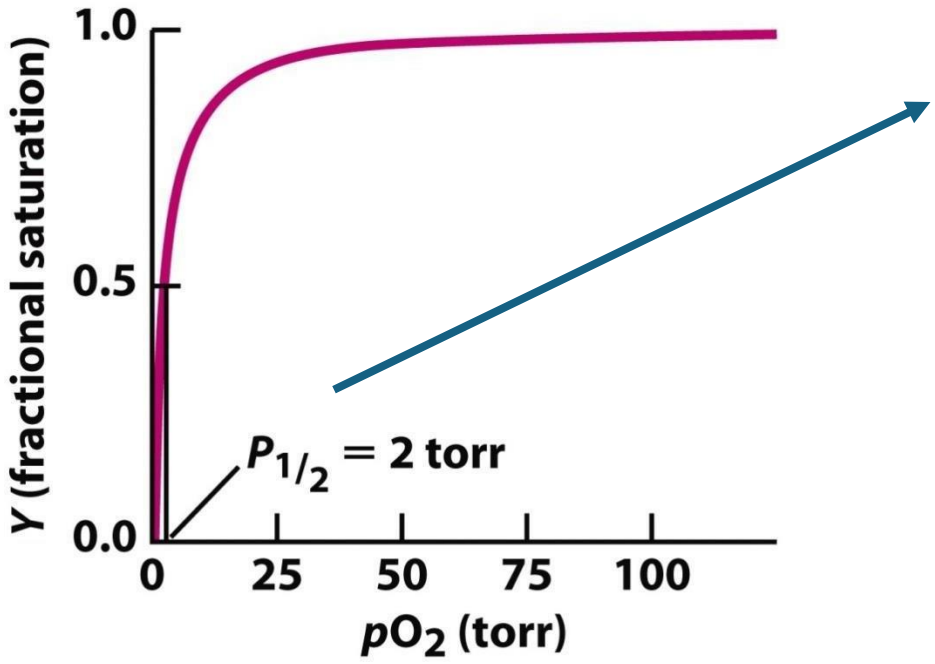
Myoglobin binds O2 with high affinity.

The P50 (oxygen partial pressure required for 50% of all myoglobin molecules) for myoglobin ~2.8 torrs or mm Hg.

Given that O2 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



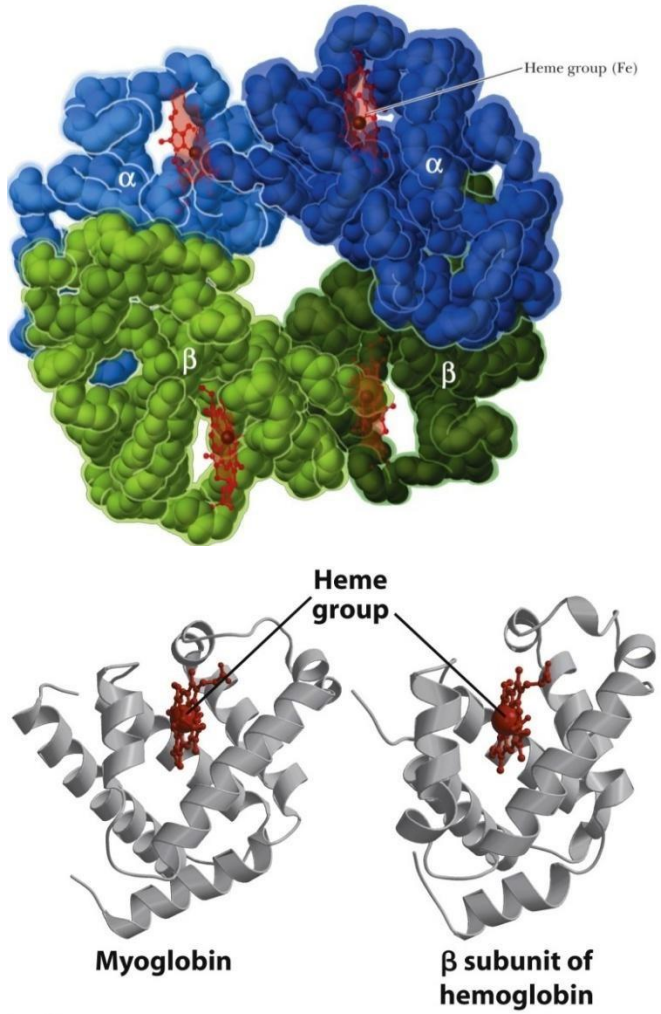
What is meant by this point?

This is the P50 value the partial pressure of oxygen at which myoglobin is 50% saturated. It reflects the oxygen-binding affinity of myoglobin.

HEMOGLOBIN

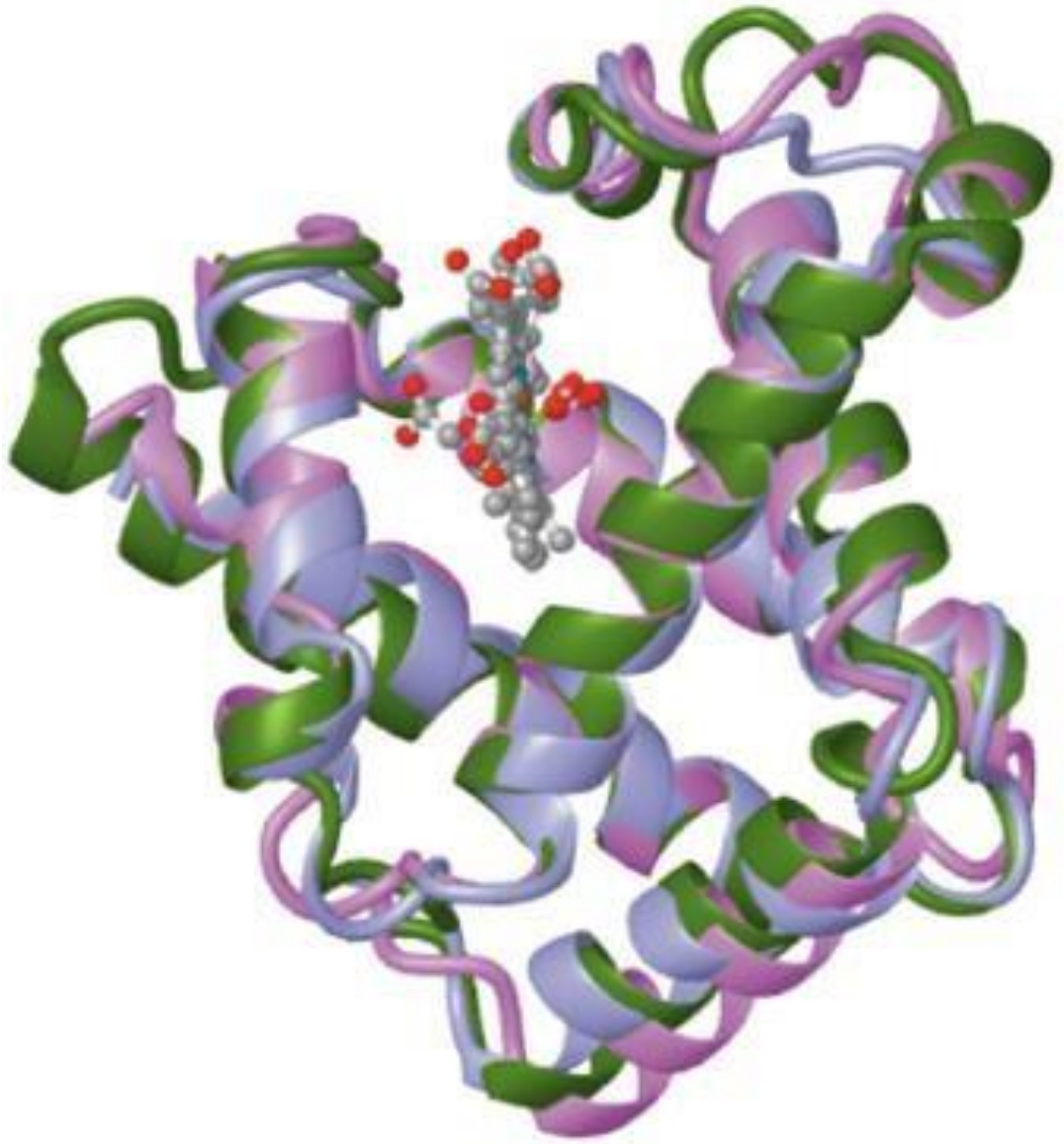
STRUCTURE & FUNCTION OF HEMOGLOBIN

- Why do we need it? RBC/ml: (5×10^9); Hemoglobin/RBC: (2.8×10^8); O_2/hemoglobin: (4); O_2/100ml: (5×10^9)(2.8×10^8)(4)(100) = (5.6×10^{20})
- Hemoglobin A: a tetramer $\alpha 2 \beta 2$: α-chains (141 a.a, 7 helices) & β-chains (146 a.a, 8 helices)
- Non-covalent hydrophobic interactions
- Ionic and hydrogen bonds also occur
- Each subunit is similar to myoglobin
- 1 heme group in each ($4O_2$). Can transport H^+ and CO_2
- Hemoglobin is an allosteric protein



The doctor focused on these numbers!!

RBCs per ml: 5×10^9
Hemoglobin molecules per RBC: 2.8×10^8
O_2 molecules per hemoglobin: 4
This gives us 5.6×10^{20} oxygen molecules per 100ml of blood – an enormous carrying capacity that would be impossible with oxygen dissolved in plasma alone.

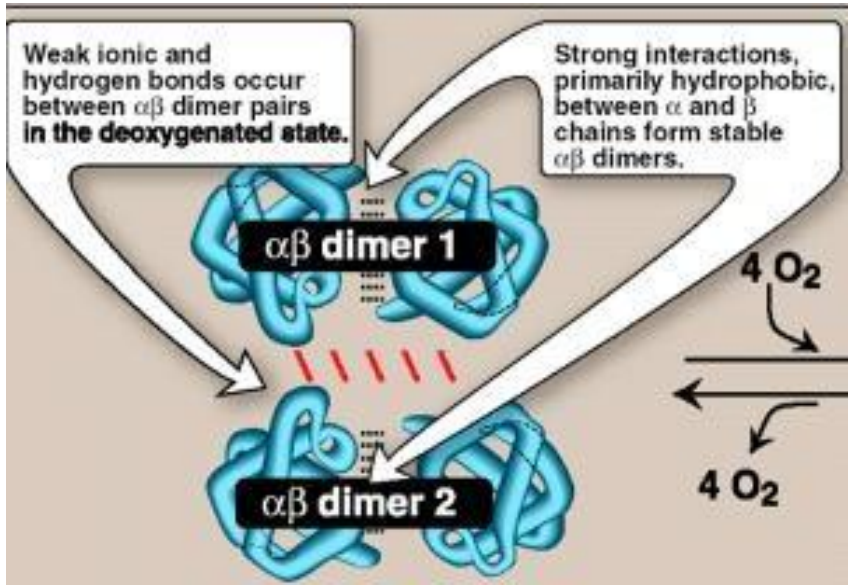


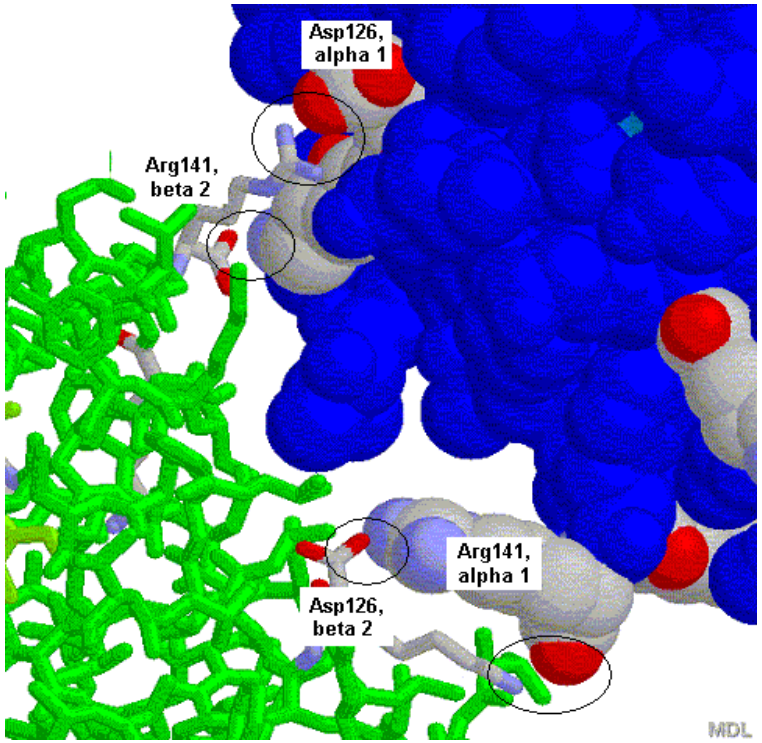
3D STRUCTURE OVERLAP MYOGLOBIN, α-GLOBIN AND β-GLOBIN

- α-Globin (blue)
- β-Globin (violet)
- Myoglobin (green)

STRUCTURE & FUNCTION OF HEMOGLOBIN 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.

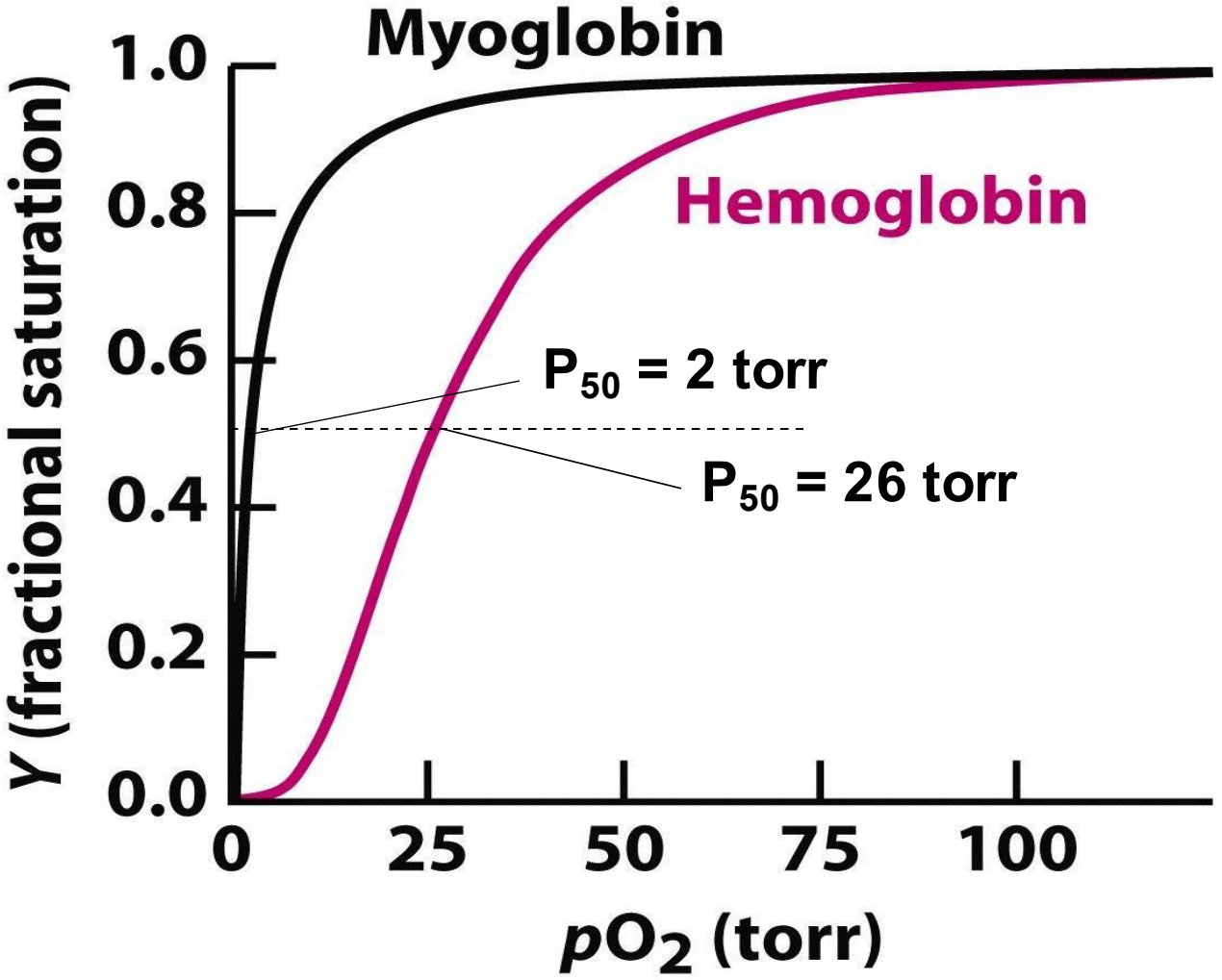




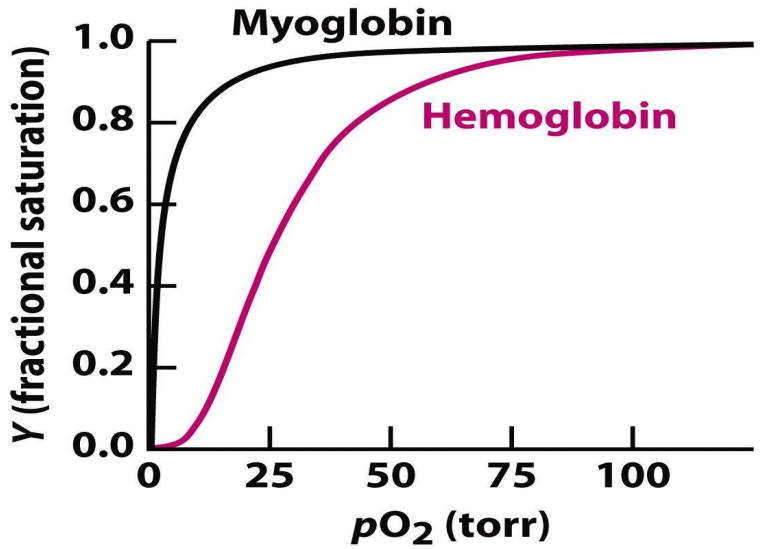
OXYGEN BINDING

HEMOGLOBIN

- How is the function related to pressure?
  - Lungs vs. tissues (100 vs. 20 mmHg)
- Cooperativity and sigmoidal plot
- At 100 mm Hg, hemoglobin is 95-98% saturated (oxyhemoglobin)



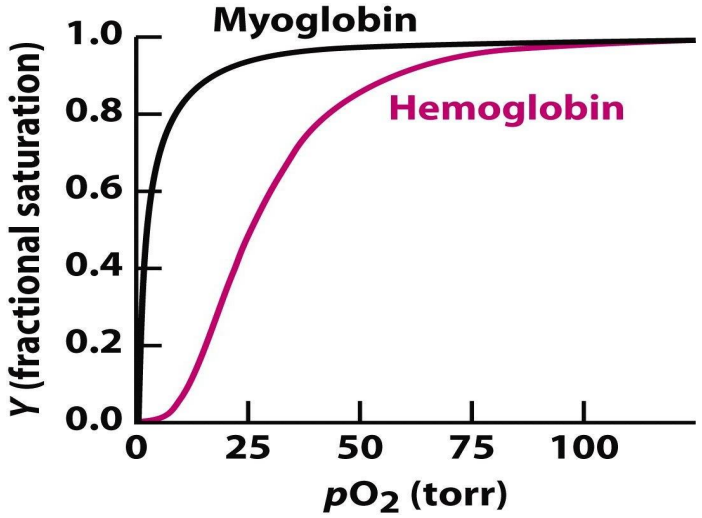
The oxygen-binding curves (also called oxygen-dissociation curves) show how hemoglobin and myoglobin bind oxygen at different partial pressures. These curves are fundamental to understanding oxygen transport and storage in the body :



Saturation vs. pressure graph
X-axis: oxygen partial pressure (pO_2, in mmHg or torr).
Y-axis: oxygen saturation (%).
Hemoglobin shows a sigmoidal curve (S-shape)
Myoglobin shows a hyperbolic curve because it binds oxygen with high affinity

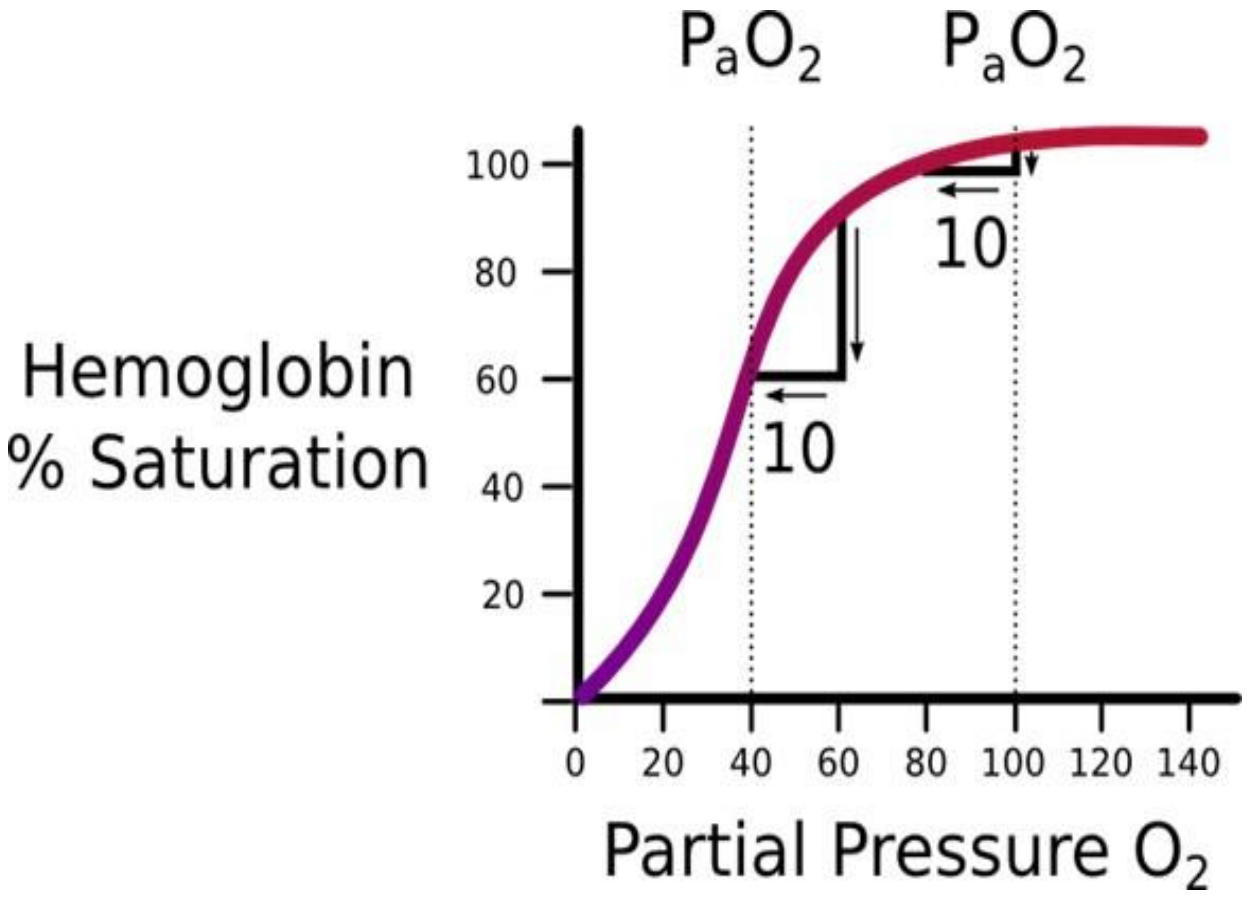
Myoglobin
Structure: Single polypeptide chain with one heme group
Oxygen affinity: Very high affinity for oxygen
Hemoglobin
Structure: Four polypeptide chains (2α, 2β) with four heme groups
Oxygen affinity: Lower affinity than myoglobin, but cooperative binding

The fundamental difference between these curves reflects their biological roles: myoglobin's hyperbolic curve with high oxygen affinity makes it an excellent oxygen storage molecule, while hemoglobin's sigmoidal curve with cooperative binding and moderate affinity makes it perfect for oxygen transport between lungs and tissues. Together, they form an integrated system for oxygen delivery and utilization in the body.



Myoglobin's curve is higher because it binds oxygen more tightly than hemoglobin, making it great for storage, not transport.

When the oxygen pressure (pO_2) is less than P50, the protein releases oxygen. When the pressure is greater than P50, the protein binds oxygen.
This is true for both hemoglobin and myoglobin, but their P50 values are different due to their different affinities.

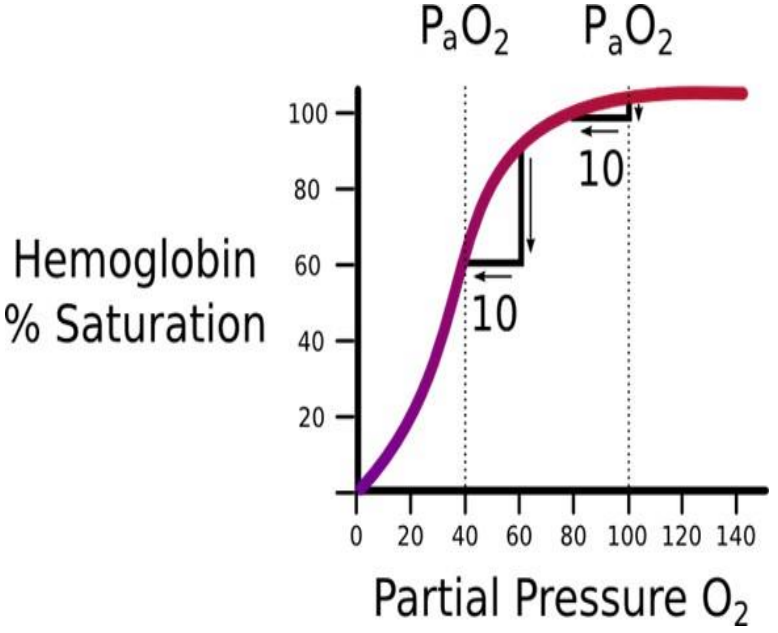


IT IS A PROTECTIVE MECHANISM

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

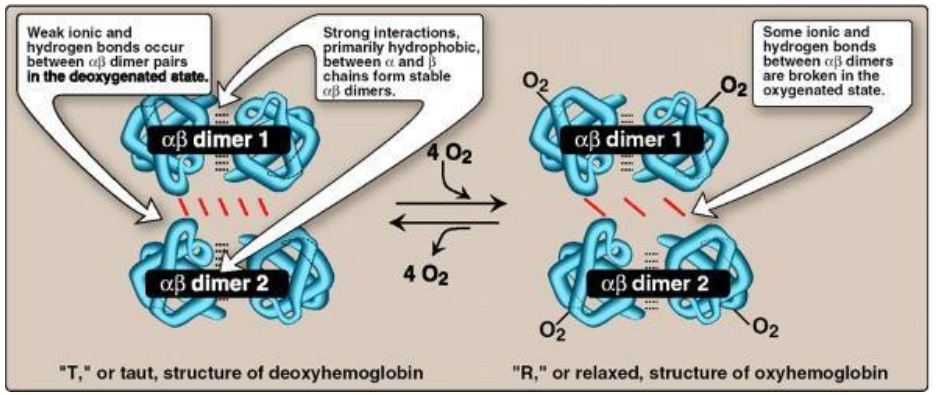
Homoglobin has an allosteric behaviour which is the effect of one subunit on the other subunits.

This demonstrates that even with a significant drop in oxygen partial pressure (from 100 to 60 mmHg), hemoglobin saturation only drops by about 10%. This protective feature ensures adequate oxygen delivery even when oxygen availability decreases, such as at high altitudes where atmospheric pressure and oxygen partial pressure are reduced.
The steep middle portion of the curve (around 20-60 mmHg) allows for efficient oxygen unloading in tissues



DEOXYHEMOGLOBIN & OXYHEMOGLOBIN

- T form
  - ✓Taut (tense)
  - ✓Deoxy
  - ✓The two dimers movement is constrained (ionic & H-bonds)
  - ✓Low-oxygen-affinity



- R form:
  - ✓Relaxed, Oxy
  - ✓Breakage of some ionic & H-bonds
  - ✓More freedom of movement
  - ✓High-oxygen-affinity (500 times higher affinity)

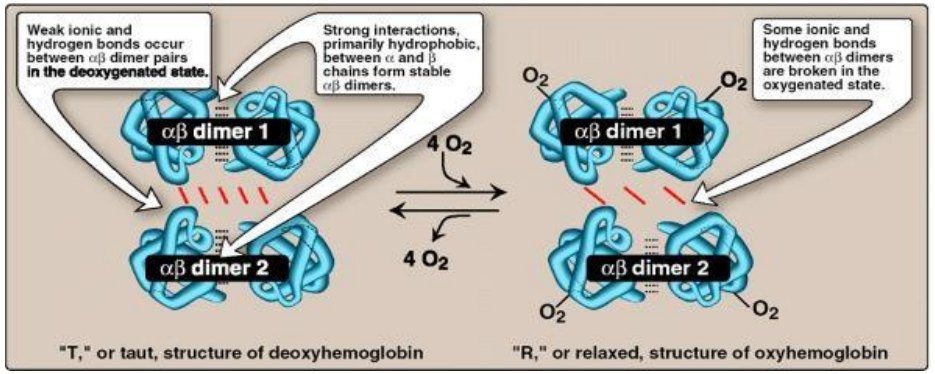
When oxygen binds to one subunit, it triggers the transition from T to R state, which increases the oxygen affinity of the remaining subunits

T conformation with the four subunits (two α and two β) arranged in a more compact, rigid structure

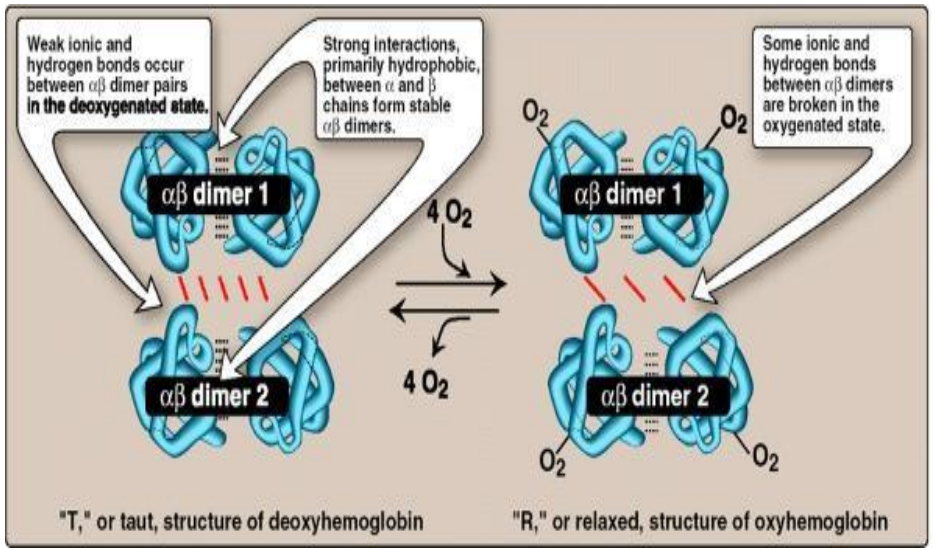
The subunits are held tightly together by weak ionic bonds and hydrogen bonds

The heme groups are positioned in a way that has low oxygen affinity

The overall structure appears more constrained with limited flexibility between the dimers



the relaxed conformation where the same four subunits have shifted positions shows a more open, flexible arrangement after some ionic and hydrogen bonds have been broken the structural change allows for greater freedom of movement between the α1β1 and α2β2 dimers the heme groups are now positioned for high oxygen affinity (500x higher than T form)



For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	Slide 6; Cytochromes Slide 14; Right-down note	Fe+3[ferrous] and Fe+2 [ferric] Distal histidine is bounded to the iron	Fe+3[ferric] and Fe+2 [ferrous] Distal histidine is not bounded to the iron
V1 → V2			

رسالة من الفريق العلمي: