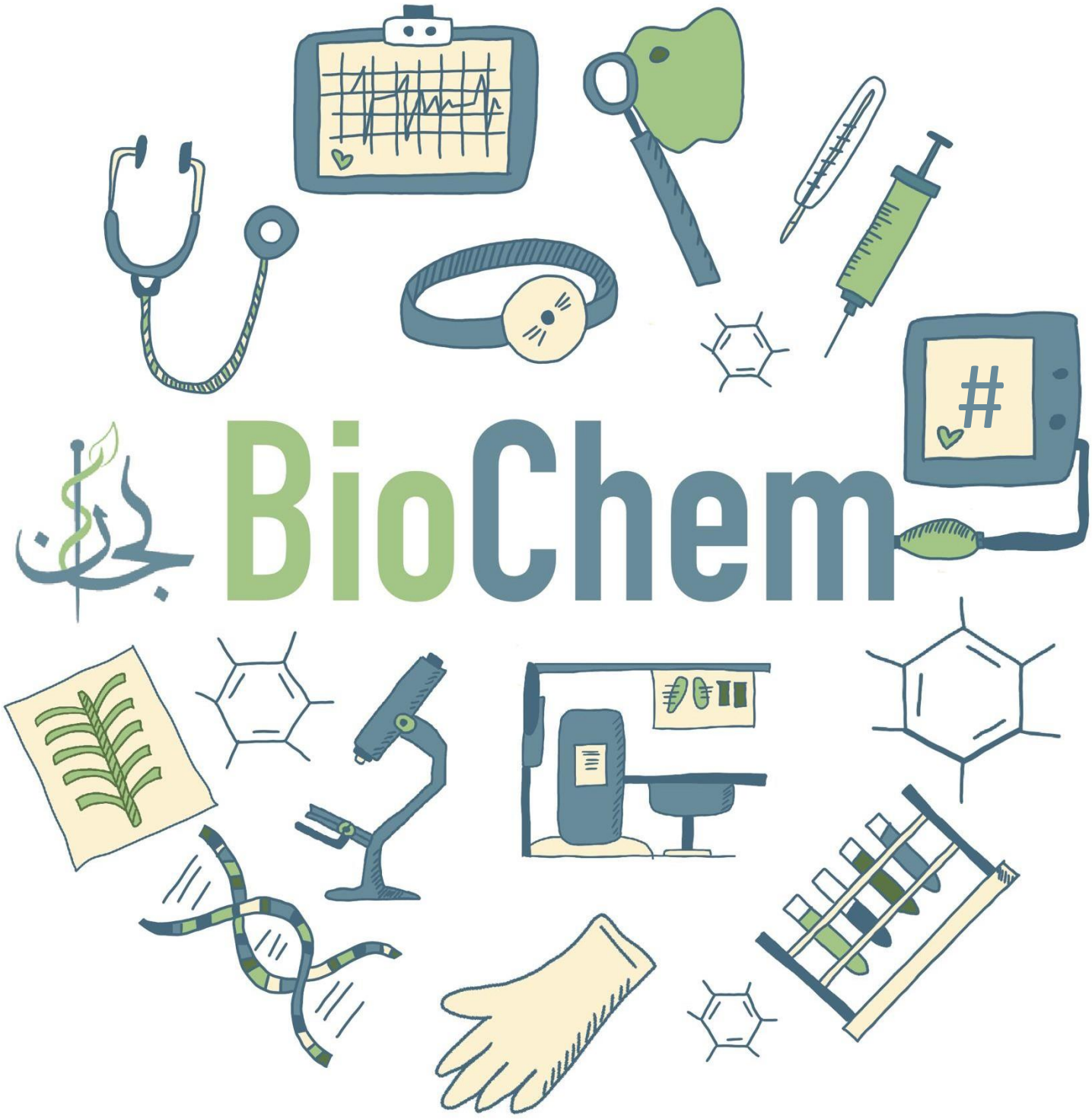

BioChem

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Globular proteins

Major examples of Globular proteins are myoglobin and hemoglobin .

Myoglobin : "storage protein" exists in muscles.

Muscles need O2 for metabolism and generation of ATP. In case of hypoxia (deprivation) in muscles (low O2 levels), oxymyoglobin releases O2 and muscle uses that O2 .

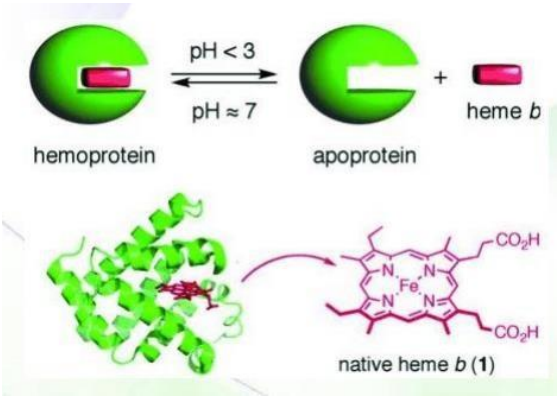
Hemoglobin : "transport protein**"** exists in abundance in every blood cell. It has 2 main functions :

- Transport O2 from lungs to the peripheral tissues, and on the way back; hemoglobin transports CO2 to lungs, and exhalation of CO2 occurs.
- Blood buffering by maintaining blood pH because it's present in large quantities in blood.

Holoproteins: proteins that are connected to a non-protein group (prosthetic group). If the non-protein group is removed, then the protein is called **Apoprotein**.

Example: Lipoprotein is a Holoprotein, and if we removed the lipid part, then the protein is known as Apolipoprotein.

**Myoglobin & Hemoglobin are Holoproteins. Specifically they belong to a large group of proteins known as Hemoproteins (have heme group associated with them) .



There are many examples of hemoproteins such as :

1. Mb, Hb Transfer and storage O2
2. Cyt c, Cyt b5 Electron transfer e (in electron transport chain)

3. NOS,P450 :Oxygenation reaction O2 + e- (detoxification reaction of xenobiotics)

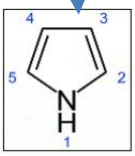
4. heme-containing sensor proteins : 1. heme sensors 2. Gas sensors (O2, CO, NO)

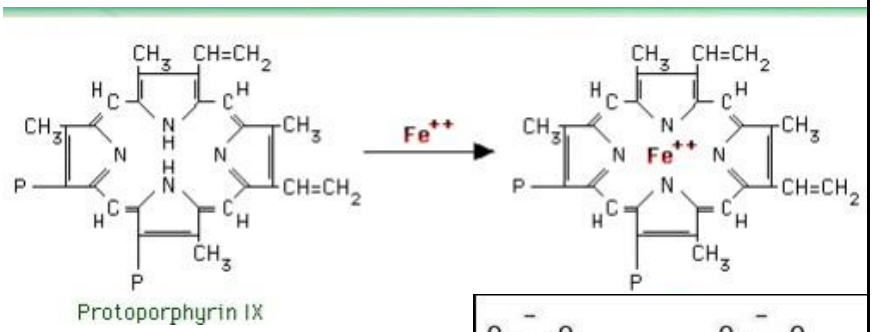
Heme group is also known as **prosthetic group** which is non-protein group, that is tightly bound (covalently) to a protein, and not composed of amino acids. It can be organic (vitamin, sugar, heme, lipid, etc.), or inorganic (metal ions) .

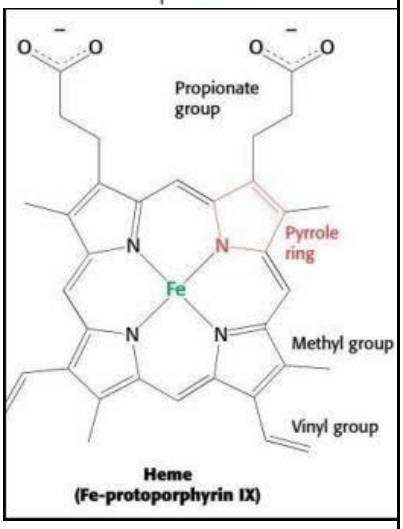
❖ Heme structure

Precursor of heme → protoporphyrin IX associated with Iron in ferrous state (Fe+2).

- Heme is composed of 4 pyrrole rings, designated as A,B,C and D.







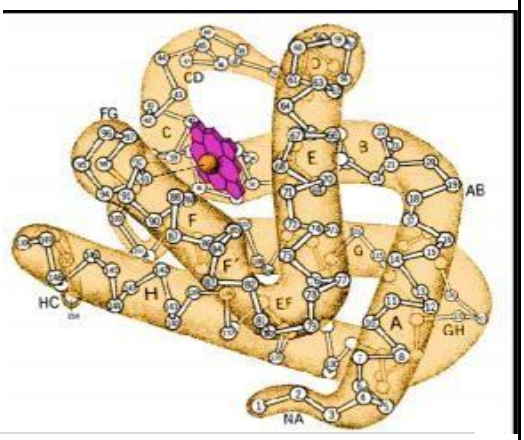
- In the center of heme we have Fe+2 bound to the 4 rings by covalent bonds. Fe+2 can form 6 covalent bonds, 4 of them are with 4 pyrrole rings, so we have 2 bonds left (we will talk about them soon).

- Heme molecule has many side chains; such as vinyl, methyl & 2 propionate groups (propionate groups are negatively charged and they interact with the hydrophilic amino acids in the surface), but the overall heme molecule is hydrophobic & planer (flat).

- Structure of Myogoblin

I. Myoglobin is a monomeric protein that is mainly found in in muscle tissue. Monomeric: composed of 1 single polypeptide chain.

II. In the center, there is heme group bound to it.



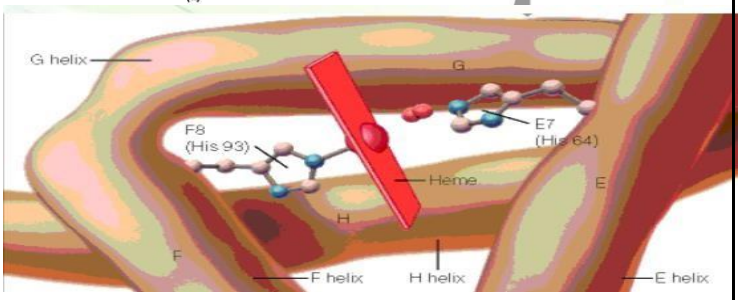
III. It can be in two forms:

1. Oxymyglobin (oxygen- bound)
2. Deoxymyoglobin (oxygen-free)

IV. ■ Composed of 8 α helices designated from A (starts at N terminus) to H (at C terminus).

V. ■ We have turns (short non-helical regions) between α helices.

• Arrangement of amino acids

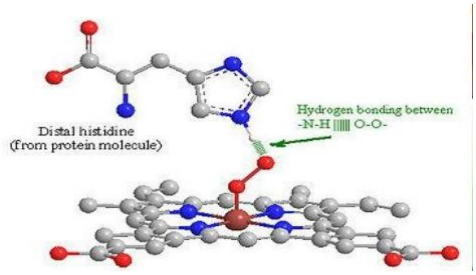


Amino acids at surface of the molecule are Hydrophilic (polar) & Amino acids in the interior (center) of the molecule: predominately hydrophobic (non-polar) .

But there are two exceptions 2 Histidine residues are hydrophilic and exist at the center (core) of the molecule, in helices E & F, known as (E7 and F8)

● F8 (proximal histidine): it's closer to the heme so it binds to iron and makes the 5th bond of iron covalently (known as the fifth coordinate) , the 6th bond that iron makes is with O2

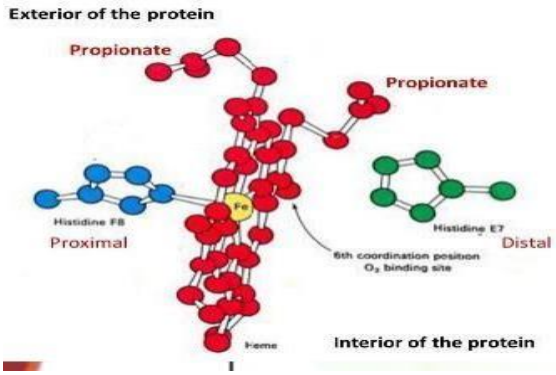
● E7 (distal histidine): ● it's close enough to O2 bound to heme, so it stabilizes the interaction between iron and O2, by forming a Hydrogen bond with O2 , overlooks the position where O2 binds to and it acts as a gate that opens and allows O2 to get in, and prevent other molecules to get in.



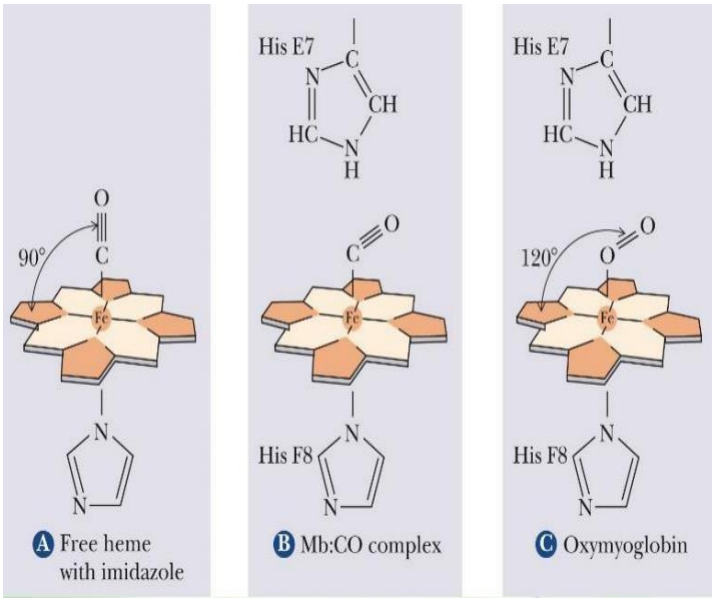
Heme molecule has 2 polar propionate groups on the side chain, and heme location is in the middle of myoglobin, and the middle of myoglobin is hydrophobic so we are having a polar (propionate) group inside myoglobin hydrophobic pocket (region).

** Upon absorption of light, heme gives a deep red color.

Propionate groups extend from heme molecule to the outer hydrophilic surface of myoglobin, and interact with amino acids there .



- How heme group is stabilized inside the hydrophobic pocket of myoglobin? By interactions between heme and hydrophobic amino acids.
- Also heme group itself stabilizes the tertiary structure of myoglobin.
- Oxidation of iron to the Fe+3, ferric state makes the molecule incapable of normal O2 binding.
- The hydrophobic interior of myoglobin (or hemoglobin) prevents the oxidation of iron, and so when O2 is released, the iron remains in the Fe(ll) state and can bind another O2.



- CO has much higher affinity towards heme. It binds to heme many orders of magnitude (thousands of times) compared to O2.
- Heme is part of myoglobin molecule, and because it overlooks the distal histidine, there is repulsion created between CO & distal histidine, so CO binds at angle (NOT preferred to CO), CO prefers straight bonding.
- Binding at angle weakness the affinity between CO & heme molecule. Due to this weakness; CO can bind to hemoglobin only 250 times more than O2.
- We have a little amount of CO in our bodies, so O2 wins the competition and binds to heme molecule.
- O2 prefers to be bent at interaction with iron. (remember CO doesn't)
- CO occupies 1% of hemoglobin, but it will occupy 99% if distal His does not exist.

☠ Accidents ☠

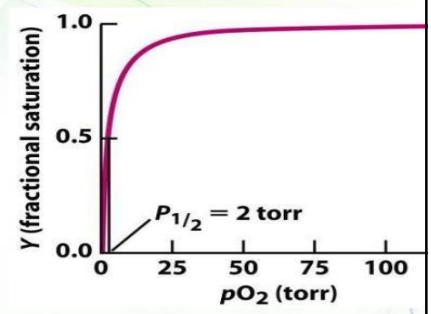
Heaters produce a lot of CO, some people sleep while keeping heaters on, and they get poisoned as a result of CO produced.

In US ,people keep their cars in closed garages specially in winter, at morning when those people turning their cars on for couple of minutes before getting into the car, car produces CO in the closed garage, and they get poisoned by CO.

People who want to commit suicide, they connect a hose from exhaust of the car to inside of it, and they close the car, and sit in it waiting to die.

♋ Oxygen binding to myoglobin ♋

- Myoglobin binds O2 with high affinity and releases it in the case of emergency (hypoxia). Due to this high affinity, O2 will not be released from myoglobin unless the amount of O2 is very low.



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

The binding of O2 to myoglobin curve is hyperbolic .

- Almost 100% of myoglobin is saturated when you have only 25 torr of O2 in tissues.

The x-axis of the curve shows the amount of O2 in torr (mmHg).

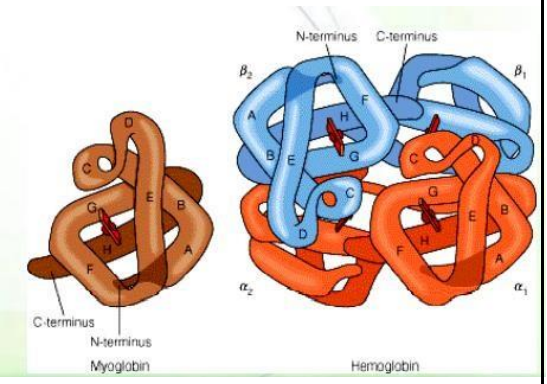
The y-axis shows fractional saturation of myoglobin bound to O2.

We care too much about the 50% mark:

- It's a very important mark, and gives you an idea about things like affinity of binding(strength of interaction).
- What is the p50? It's the pressure of O2 where 50% of myoglobin is saturated and bound to O2.
- The p50 of myoglobin is 2 torr. It's an indication about very high affinity ,it means we need a very little amount of O2 to saturate a myoglobin molecule. (The lower the p50 , the higher the affinity)
- Given that O2 pressure in tissues is normally 20 torr, it is almost fully saturated with oxygen at normal conditions.

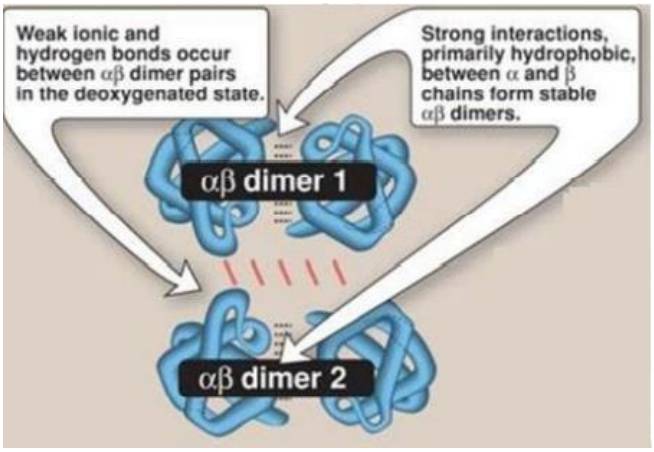
Hemoglobin structure :

- Hemoglobin is tetrameric hemeprotein (four protein chains known as globins with each bound to heme) remember the myoglobin is monomeric .
- In adults, the four globin proteins are of two different types known as (α globin protein) and (β globin protein), so because of having 2 α chains and 2 β chains; hemoglobin protein is an α2β2 globin protein,some other proteins they can be composed of alpha,beta & gamma but those chains are different than chains in hemoglobin they are designated for Hb.



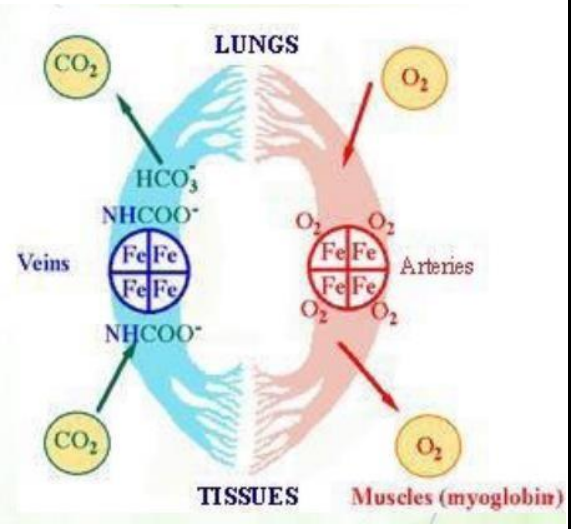
Each one of these 4 chains contains a heme molecule, so we have 4 heme molecules in the hemoglobin.

- The α and β chains contain multiple α-helices, where α contains 7 α-helices, and β contains 8 α-helices.
- β globin chain is similar in structure to myoglobin molecule.
- Hemoglobin is considered as a dimer of dimers.
- (αβ) dimer interacts with other (αβ) dimer via hydrogen bonds and electrostatic interactions(salt bridges).



- The alpha chain is composed of 141 aa residues &The beta chain is composed of 146 aa residues.
- Within the same (αβ) dimer, α and β polypeptide chains are linked to each other via hydrophobic interactions.
- Previously, we said that the hydrophobic amino acids are located at the core (center) of hemoglobin. Could we find a hydrophobic amino acids at the surface of hemoglobin?! hydrophobic amino acids are not only present in the interior of the protein chains, but also on the surface, to make the hydrophobic interactions between chains.

Oxygen binding to hemoglobin

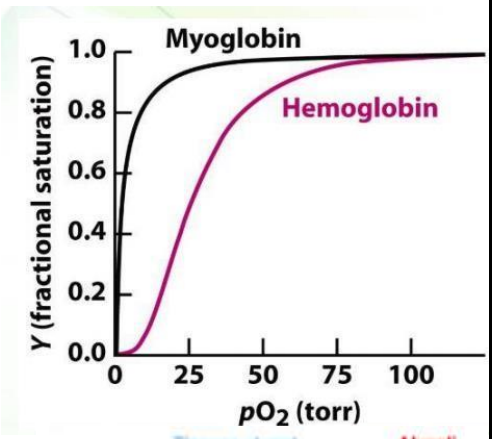


Does hemoglobin have a high or low affinity to O2?

● If it has high affinity for O2 → it will bind very efficiently to O2 in lungs, but it wouldn't go to tissues or even release the O2 in tissues.

● If it has low affinity for O2 → hemoglobin will not be saturated at lungs, and when it goes to tissues, there isn't enough O2 to be released.

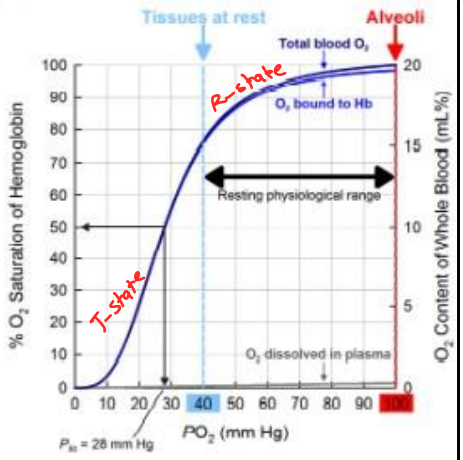
The answer is → hemoglobin has both high and low affinity for O2 as we will see now:



▮ 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 becomes unsaturated in tissues where the oxygen pressure is low (about 30 mm Hg).

▮ The saturation curve of hemoglobin binding to O2 has a <u>sigmoidal shape.</u>



▮ Sigmoidal curve indicates that protein has 2 states (structures):first one low O2 pressure → low affinity state (in tissues), and the second high O2 pressure → high affinity state (in lungs). As the pO2 falls, O2 is released to the cells.

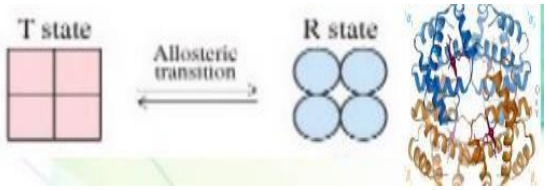
● At 100 mm Hg, hemoglobin is 95-98% saturated (oxyhemoglobin). In contrast of the p50 of myoglobin, the p50 of hemoglobin is ≈ 26 mmHg , intissue the pressure is around 30-40 mmHg.

NOW the question is, how is it possible for hemoglobin to have to states?

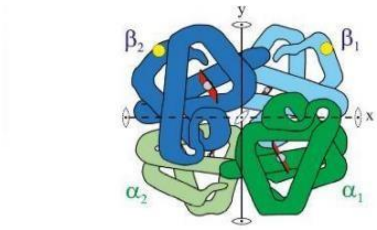
▮ Hemoglobin has two structures (considered as allosteric protein).

● an allosteric protein: is the protein that has different structures shifting between them ,,allo= other ,, stereous= shape. Allosteric proteins should have quaternary structure, that's why myoglobin isn't considered allosteric.

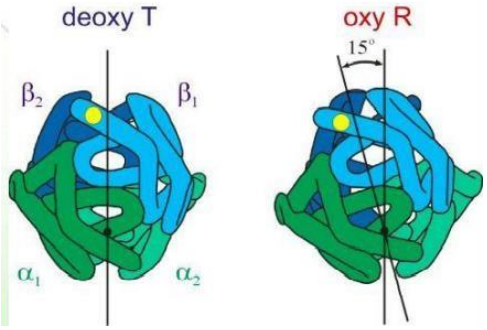
▮ An allosteric protein: a protein where binding of a molecule (ligand) to one part of the protein affects binding of a similar or a different ligand to another part of the protein.



▮ Hemoglobin exists in two states: 1● T-state: Known as "taut", "tense" or "tight" state , Low O2 pressure, Low binding affinity to O2, In tissues, Deoxygenated.



2● R-state: Known as "relaxed" state, High O2 pressure, High binding affinity to O2 500 times higher than T-state , In lungs , Oxygenated.



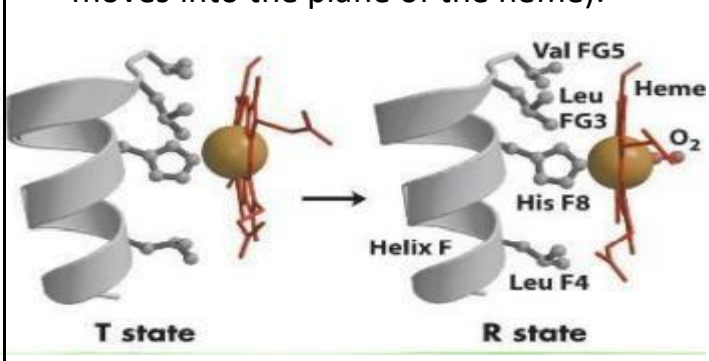
- ❖ Binding of O2 causes conformational changes in hemoglobin, converting it from the low affinity T-state to the high affinity R-state.

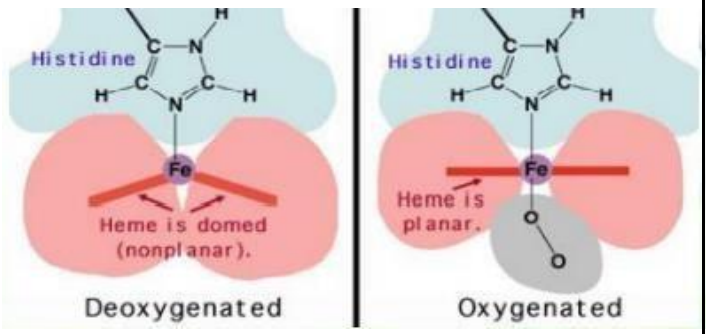
Conformational differences between T & R states are little, only by 15°, but it gives a big functional change in hemoglobin.

How the switch between T & R states occurs ?

When heme is free of oxygen, heme molecule has a domed structure, and it's not straight, and iron is outside the plane of the heme group.

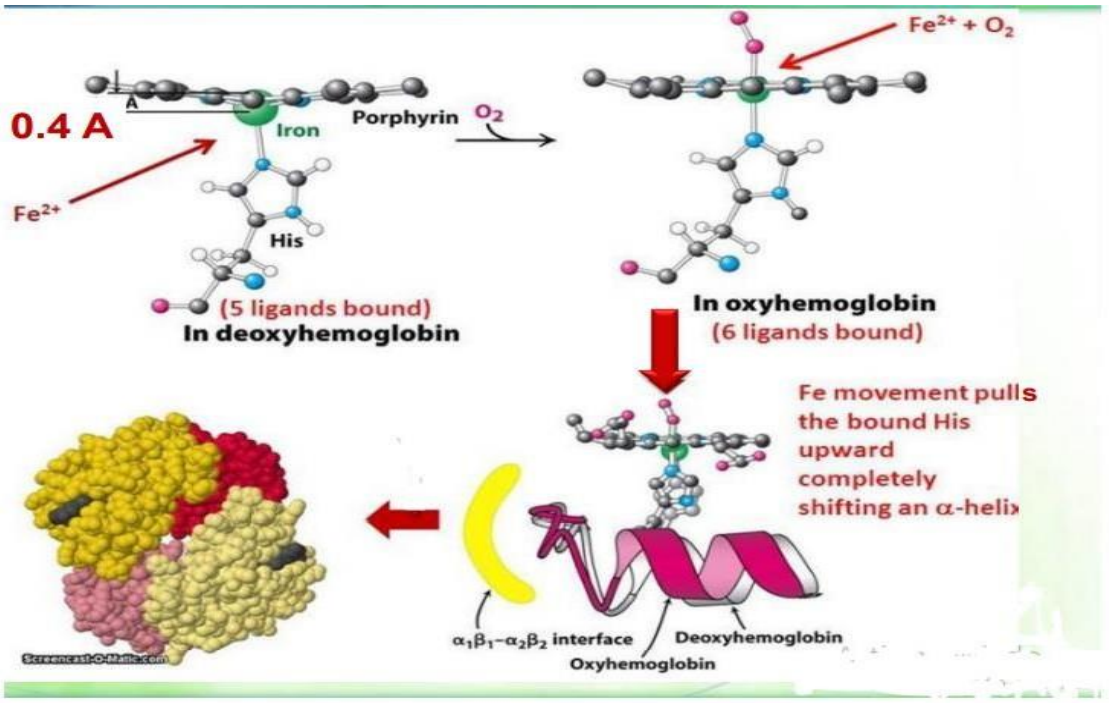
▮ When oxygen binds to an iron atom, heme adopts a planar structure (becomes straight), and the proximal histidine (F8) is pulled along with the iron atom (which moves into the plane of the heme).



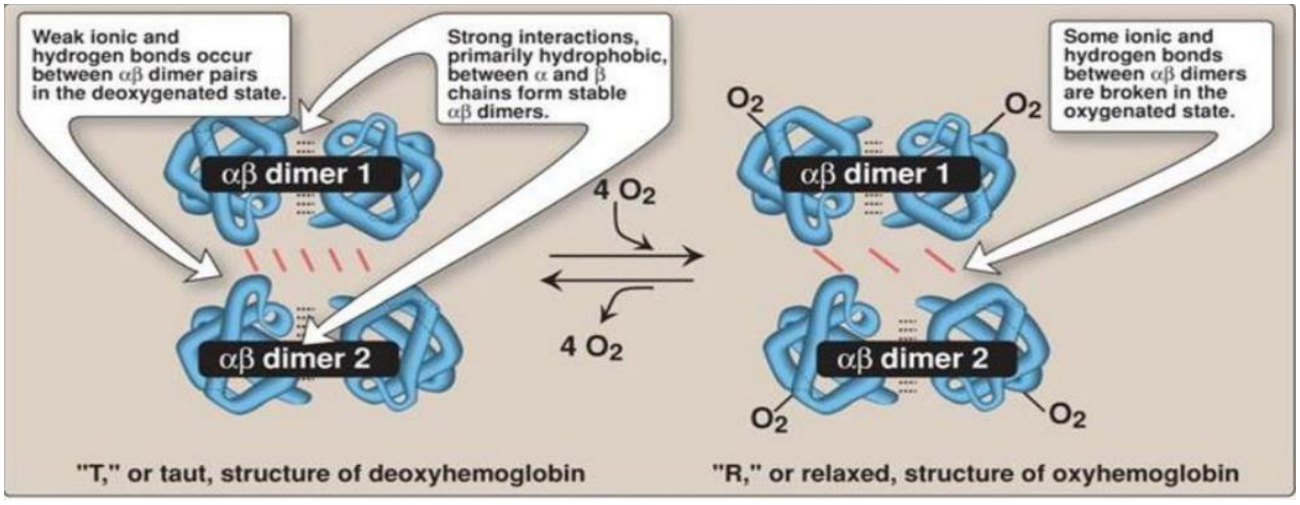


- This movement in the structure of heme molecule changes the secondary structure of alpha helices which contain proximal histidine that triggers:
- changes in tertiary structure → changes in individual hemoglobin subunits(polypeptides).
- changes in quaternary structure → by breakage of electrostatic bonds between molecules (at the other oxygen-free hemoglobin chains). The molecule becomes relaxed.

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



Electrostatic interactions are broken

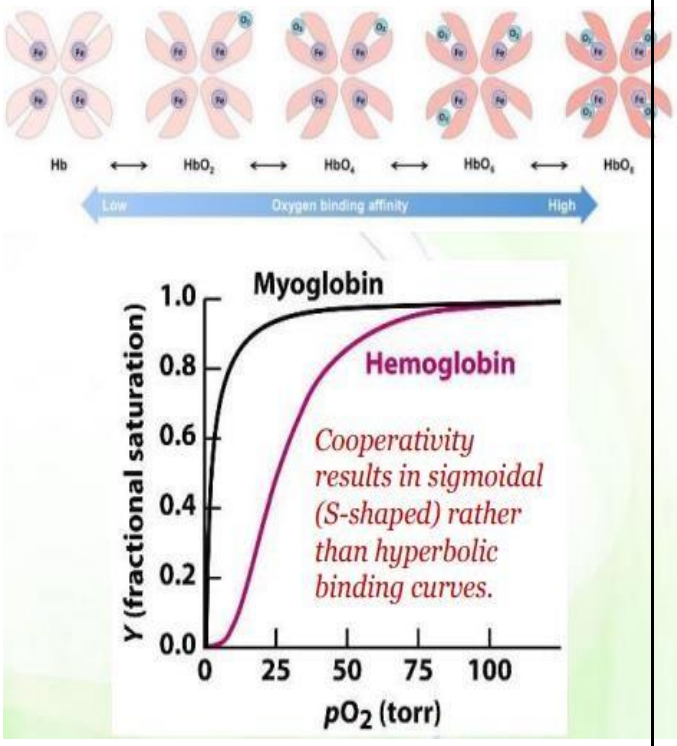


Binding is cooperative

Remember Hemoglobin saturation curve is sigmoidal, and sigmoidal means that: molecule changes in structure and binding is cooperative.

- ❖ Conformational changes lead to cooperativity among binding sites.

I.e Binding of the first O2 breaks some salt bridges with the other chains increasing the affinity of the binding of a second molecule.



- ● Binding of the second O2 molecule, breaks more salt bridges increasing the affinity towards binding of athird O2 even more, and so on. (positive effect)

- ❖ Oxygen is a homotropic effector (the allosteric modulator is the substrate itself).

To sum up the characteristics of allosteric proteins:

1. Sigmoidal shape of activity
2. They have different structures
3. Their binding is cooperative.

Hemoglobin is an allosteric protein. There are some factors that regulate and affect the allosteric proteins these factors are called: Allosteric effectors/regulators.

- ∎ These allosteric effectors/regulators can be either homotropic or heterotropic.

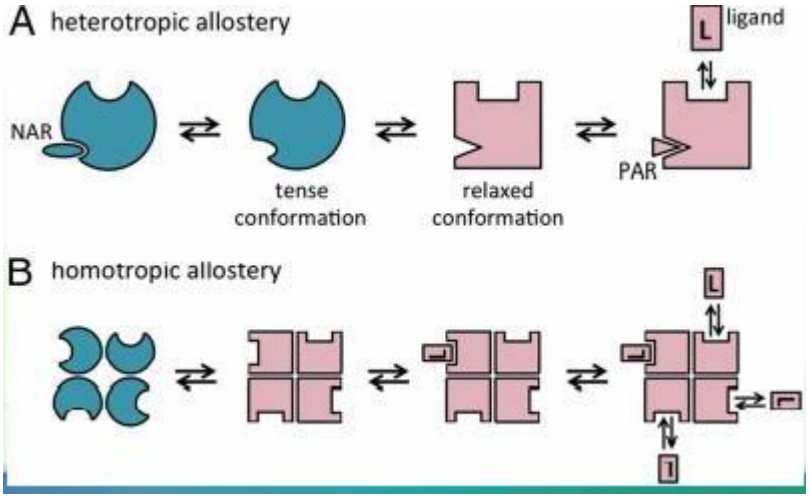
- ● if the regulator and the ligand are the same → homotropic regulator.

Example : O2 binding affets subsequent O2 binding . It stimulates and makes the binding of the second molecule easier.

- ● if the regulator is different from the ligand → heterotropic allosteric regulator. e.g., H+ or BPG binding affects O2 binding. (will be discussed in next lectures)

▮ Also allosteric regulators can have either positive or negative effects:

● Positive allosteric interaction → increases affinity for ligand (makes the binding easier).
● Negative allosteric interaction → decreases affinity for ligand (makes the binding harder).



SHORT QUIZ :

1. Electrostatic bonds broke down and reduced in number. This is considered as quaternary structure changing. The hemoglobin become:
A. In T state
B. Relaxed and oxygenated
C. Deoxygenated
D.A + C

2. Which of the following best describes the quaternary structure of Hb A?
A. Hb is a tetramer consisting of four identical subunits
B. Hb is a tetramer consisting of four non-identical subunits
C. Hb is a tetramer consisting of 2 pairs of identical but non-homologous subunits
D. Hb is a tetramer consisting of 2 pairs of identical and homologous subunits.

3. Which of the following is a hemoprotein ?
A. myoglobin
B. hemoglobin
C. mitochondrial cytochrome c
D. p450
E. all the above

4. when comparing myoglobin and hemo globin we can say:
A. myoglobin doesn't have a quaternary structure
B. they both have an allosteric function
C. hemoglobin changes its tertiary structure in response to O2 binding while myoglobin doesn't
D.their sigmoid binding graphs show their positive mechanisms
E. more than one of the above is correct

5. What are the two critical amino acids near the heme group in both myoglobin and hemoglobin?
A.Glycines
B. Histidines
C.Glycines and histidines
D.arginines

Answers : 1.B 2. C 3.E 4. E 5.B