بسم الله الرحمن الرحيم

BIOCHEMISTRY

Lecture 19

Globular proteins

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

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Myoglobin and hemoglobin

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Some notes about the previous lecture:

- **The forces that determine the protein structures are:**
- **non covalent interactions such as electrostatic , hydrogen bonds, van der waal and hydrophobic interactions**

Hydrophobic interactions are really important because they are crucial in determining the tertiary protein structure which is the 3D structure

- Fibrous proteins are fibrous and elongated they are mainly structural proteins which means that their function is to support structures and organs
- Collagen is a hydrophobic protein to make the fibers and amino acids cluster so when it's stretched it is packed to each other to give support and strength to the structures
- Elastin is also hydrophobic while both elastin and collagen have hydrophilic regions
- keratin is abundant in hair and nails and what makes it stronger is cysteine disulfide bonds and nails are stronger because they have more disulfide

Functions of myoglobin and hemoglobin

Mayo = muscle

globin ;They are a class of proteins that have their own structural features

So this protein (**myoglobin**) exists in muscles tissue

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

Which is Hypoxia

Hemoglobin:

transport of O_2 and CO_2

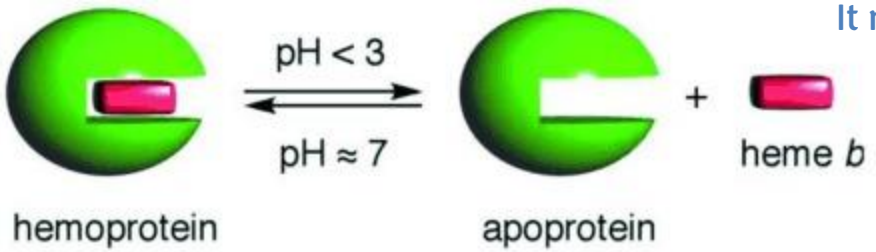
blood buffering

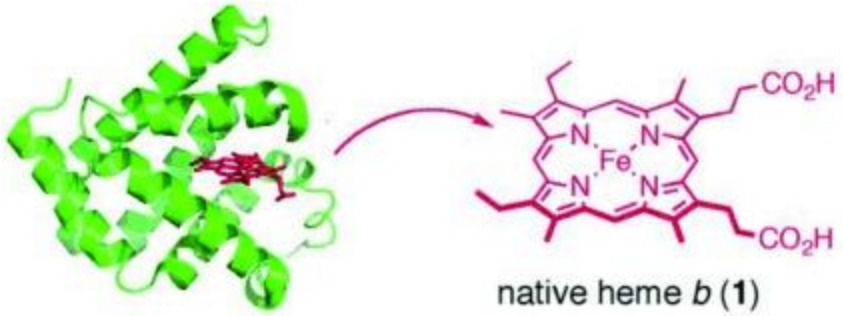
Hemoglobin and myoglobin both bind to oxygen but hemoglobin main function is to transport it from lungs to tissues and its secondary function is to transport CO2 from tissues to the lungs so it can be exhaled

Hemoproteins The class of proteins that hemoglobin and myoglobin belongs to

They proteins that has a heme group attached to it

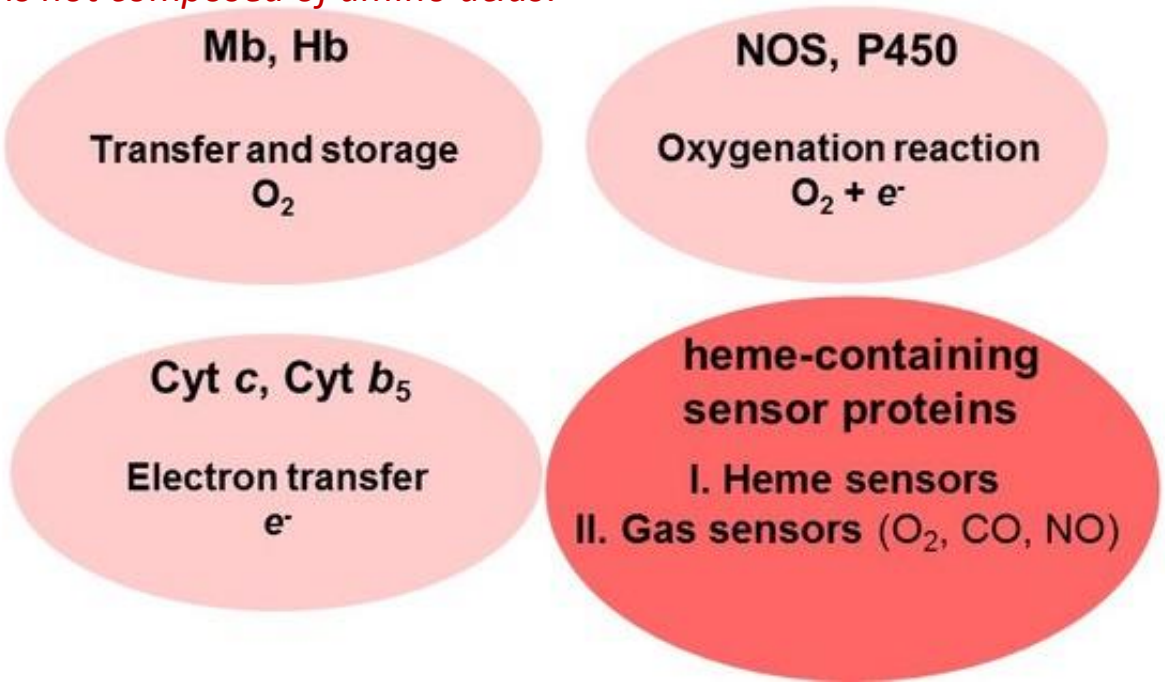
Many proteins have heme as a prosthetic group called hemoproteins.





It means The extra thing and they bind to them covalently

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.*



The protein environment dictates the function of the heme.

About the previous slide

- Both hemoglobin and myoglobin are **holoproteins** which are the proteins that has a non protein group attached to it which is the heme group and if we removed it we will have a **apoprotein**
- Apoproteins are the protein components of lipoproteins that we took in the mid exam
- We have a class of enzymes that will be studied in the next course that are called cytochrome p450 and p means pigment and 450 as it absorbs light at a wave length 450 nm they do some reactions in different tissues mainly in liver cells
- Those hemoproteins bind to an electron or oxygen and they transfer these electrons to generate energy again and we have some hemeproteins that are sensors (gas sensors) for oxygen, carbon monoxide and so on

- The **amino acids** that surrounds the heme molecule are the ones that determine their function **Any different in amino acid → different function**
- Heme group is **prosthetic groups** that attache to the proteins covalently

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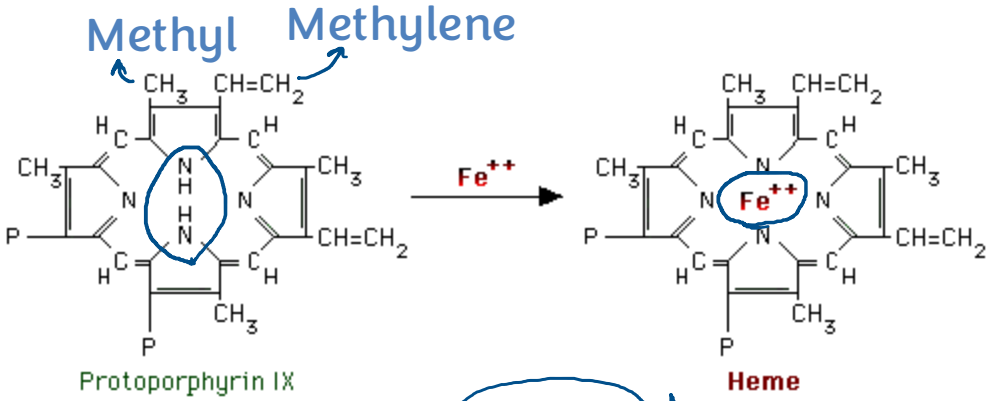
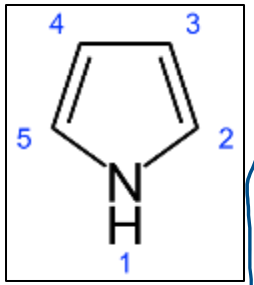
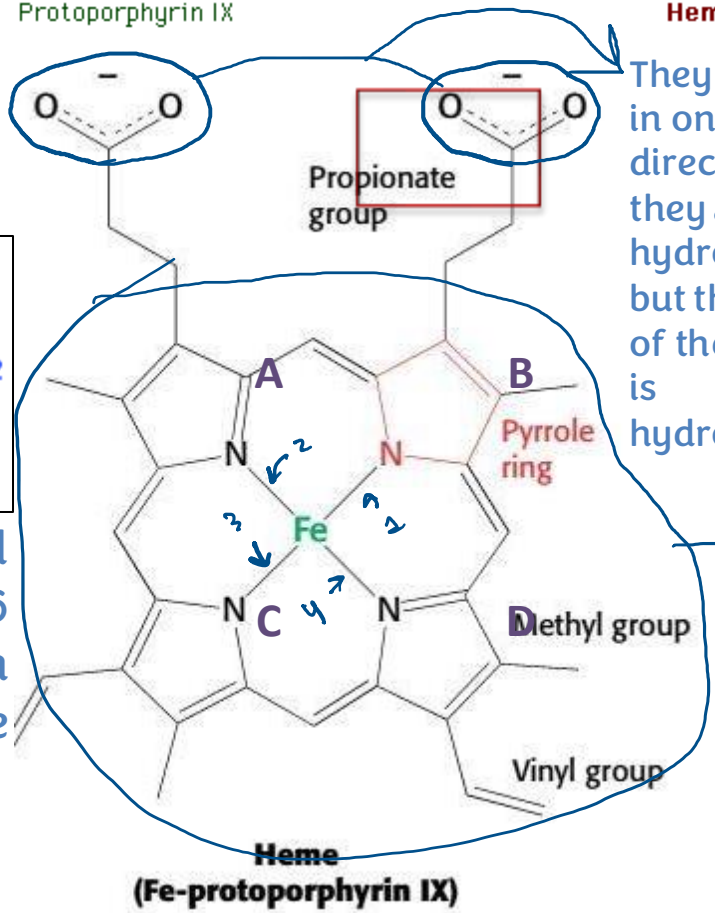
Heme structure

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.







They extend in one direction and they are hydrophilic but the rest of the heme is hydrophobic

Iron is in the middle and has the ability to form 6 covalent bonds 4 of them is with the heme

This molecule is composed from four rings structures that are called pyrrole rings

Structure of myoglobin

It's composed of one polypeptide chain

Myoglobin has hydrophilic amino acids that is to the outside and hydrophobic to the center where they cluster inside the protein and they surround the heme molecule as it's a hydrophobic molecule

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.

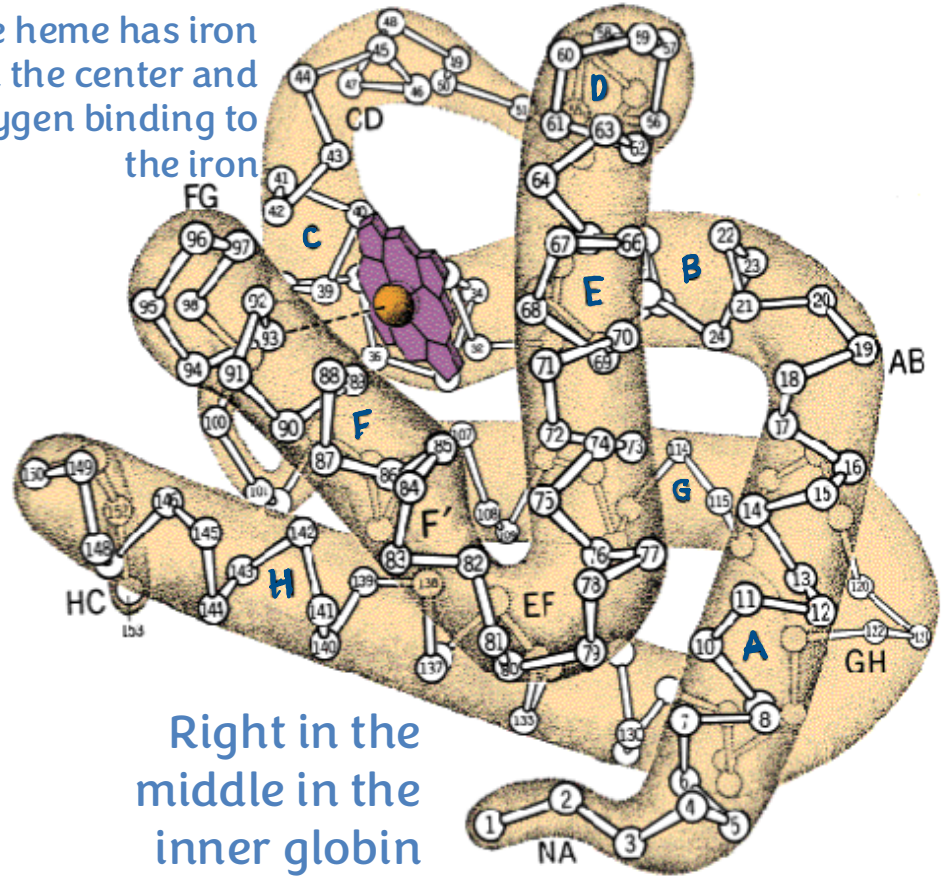
The α-helices are connected by short coils, a structure that is known as the globin fold, which is a hydrophobic O_2-binding pocket.

It contains heme as a prosthetic group internally.

Myoglobin can be present in two forms:

- oxymyoglobin (oxygen-bound)
- deoxymyoglobin (oxygen-free)

The heme has iron in the center and oxygen binding to the iron



Right in the middle in the inner globin fold we have the heme molecule

Dr.mamoun said that there is different definitions of the fold but are required to know that the globin fold is oxygen binding bocket

Arrangement of amino acids

Whenever we see a charged amino acids in the center we know that they have a special function

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.

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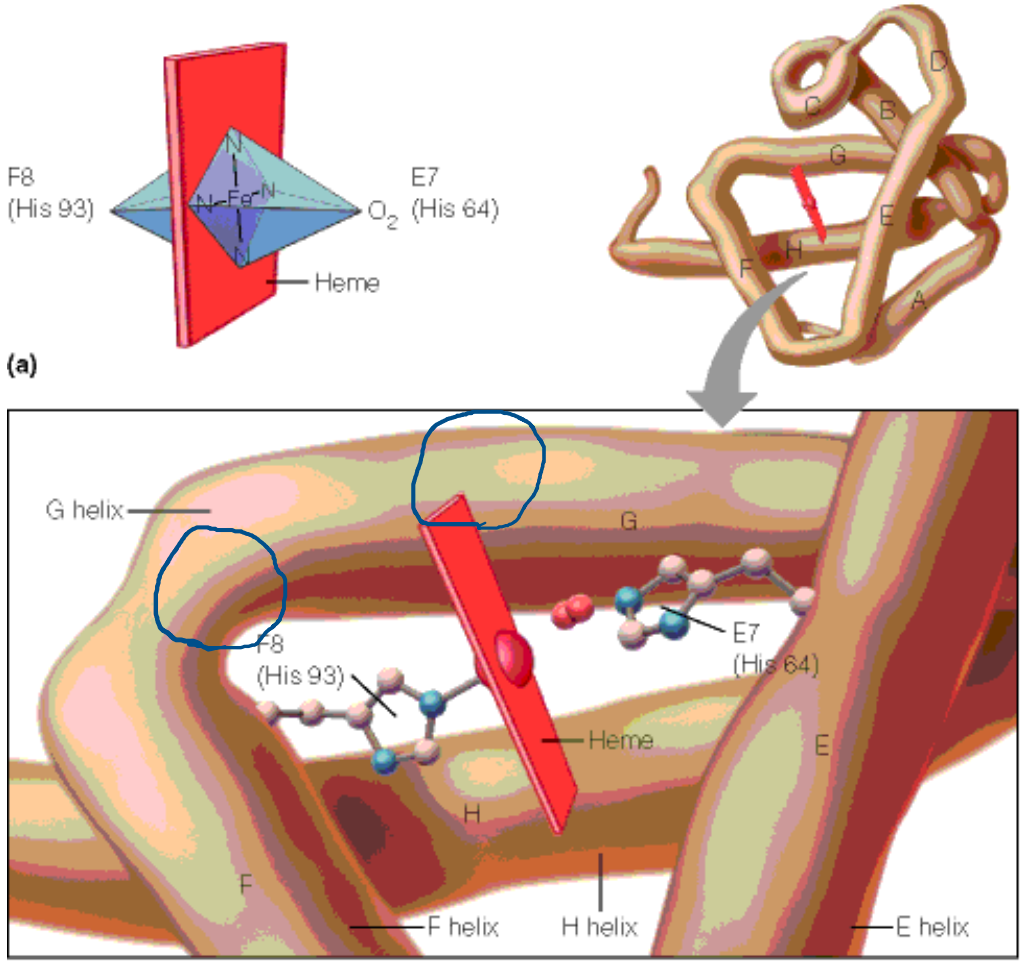
E7 means that it's on helix E and it's amino acid number 7

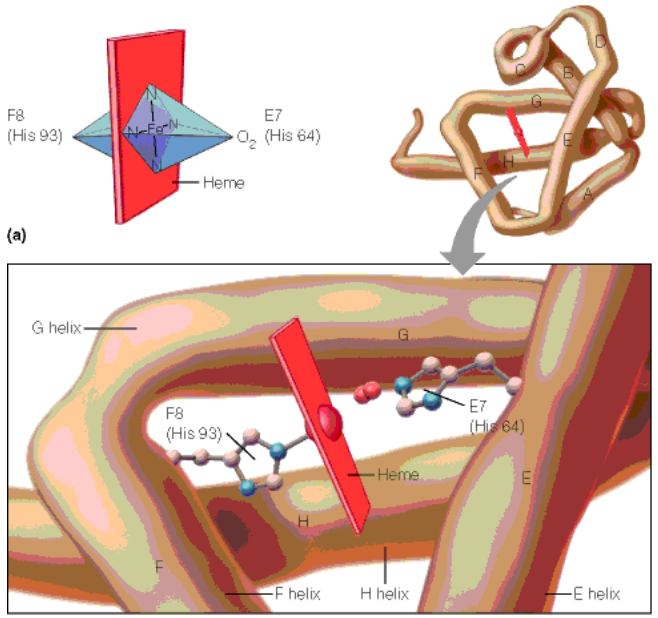
As we said before iron bind 4 bonds with the heme and the 2 left are : **one** with the proximal His which is covalently bound to it and the **other** with oxygen at the sixths coordinate then we call it oxygenated myoglobin



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



When oxygen is released iron is oxidized forming ferric Fe3+ but this doesn't happen because it's surrounded by hydrophobic amino acids that have the ability to prevent the oxidation of iron because we must have iron in the ferrous state to bind to oxygen which is the reduced state

- 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.

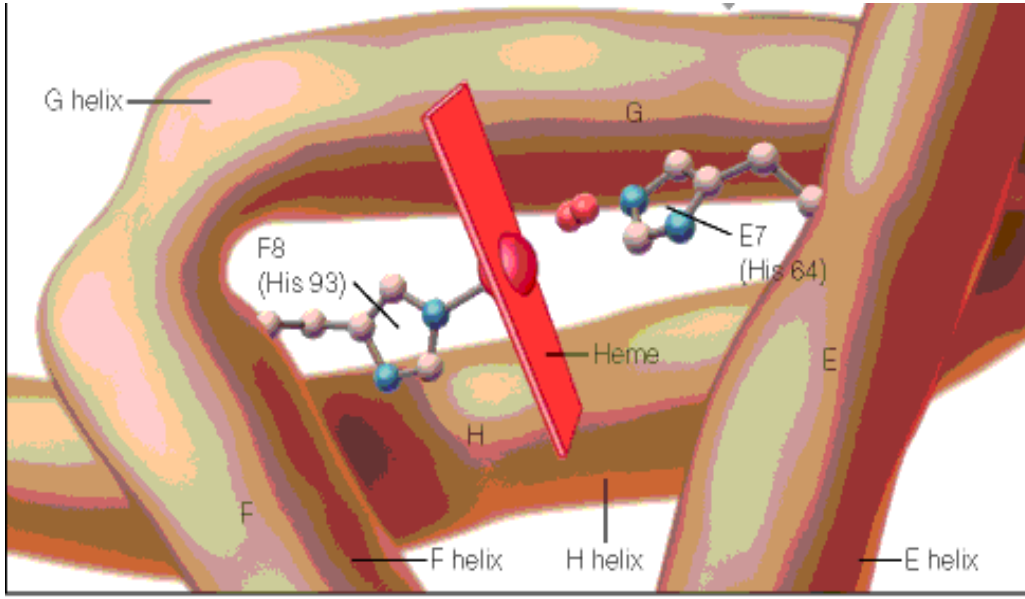
This is why muscles are red

Structure-function relationship

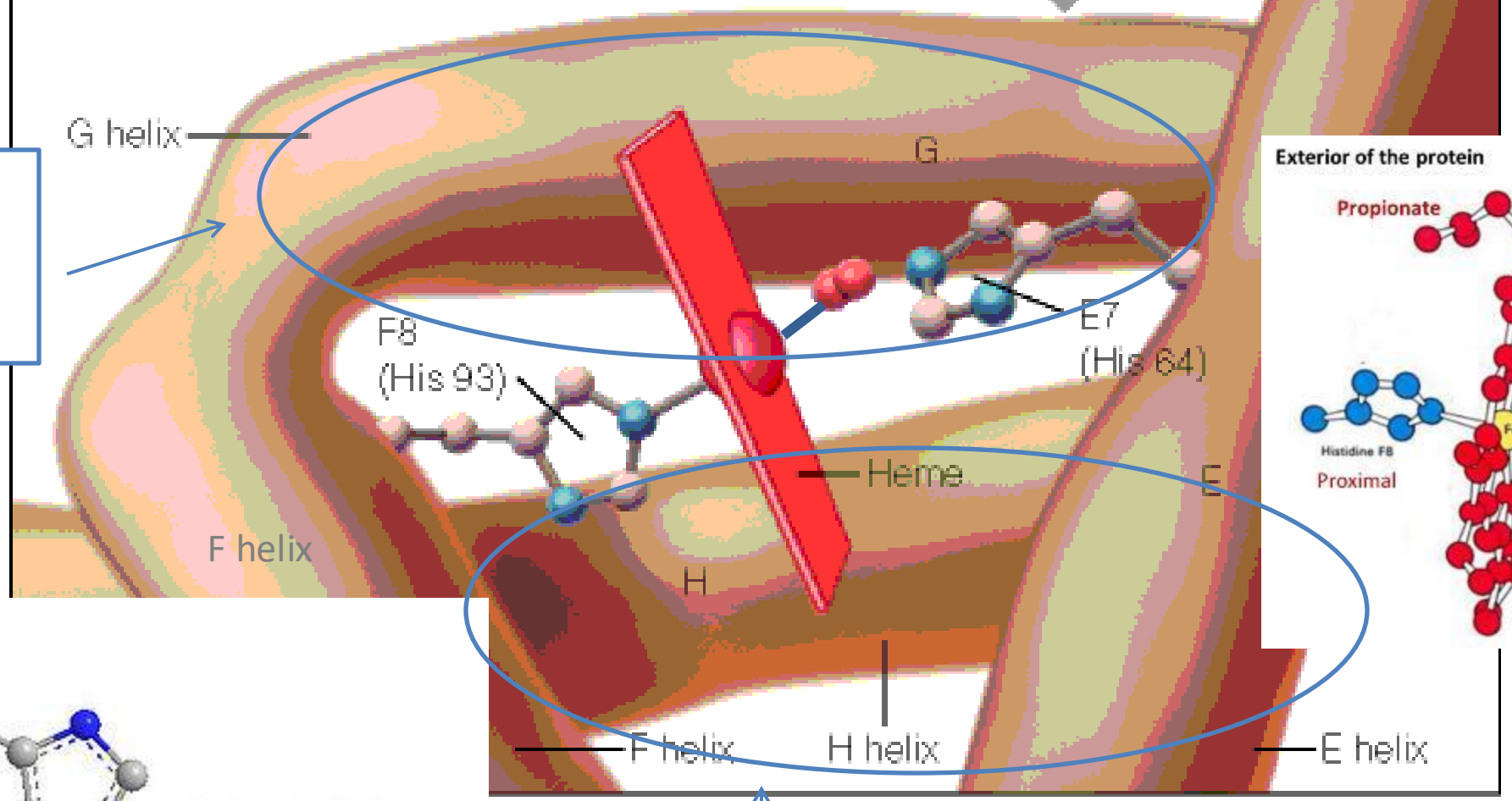
The planar heme group fits into a hydrophobic pocket of the protein and the myoglobin-heme interaction is stabilized by hydrophobic attractions.
The heme group stabilizes the tertiary structure of myoglobin.
The hydrophobic interior of myoglobin (or hemoglobin) prevents the oxidation of iron, and so when O_2 is released, the iron remains in the Fe(II) state and can bind to another O_2.

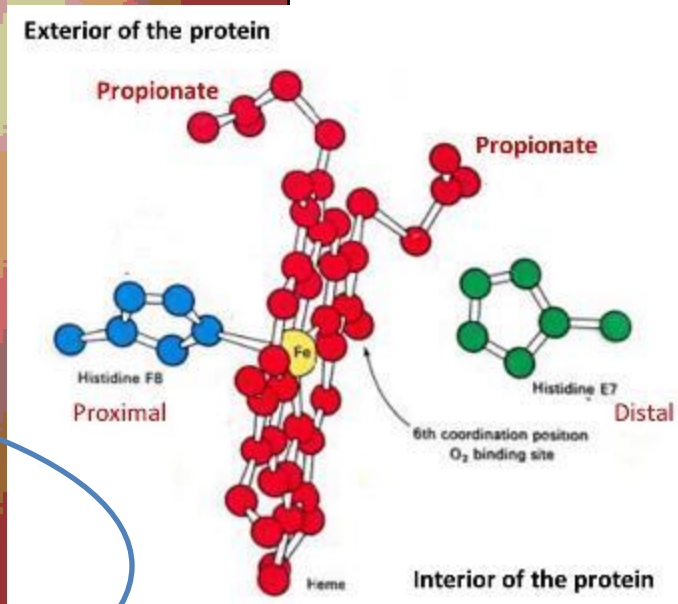
The covalent bond between iron and the heme with the proximal histidine stabilizes the myoglobin

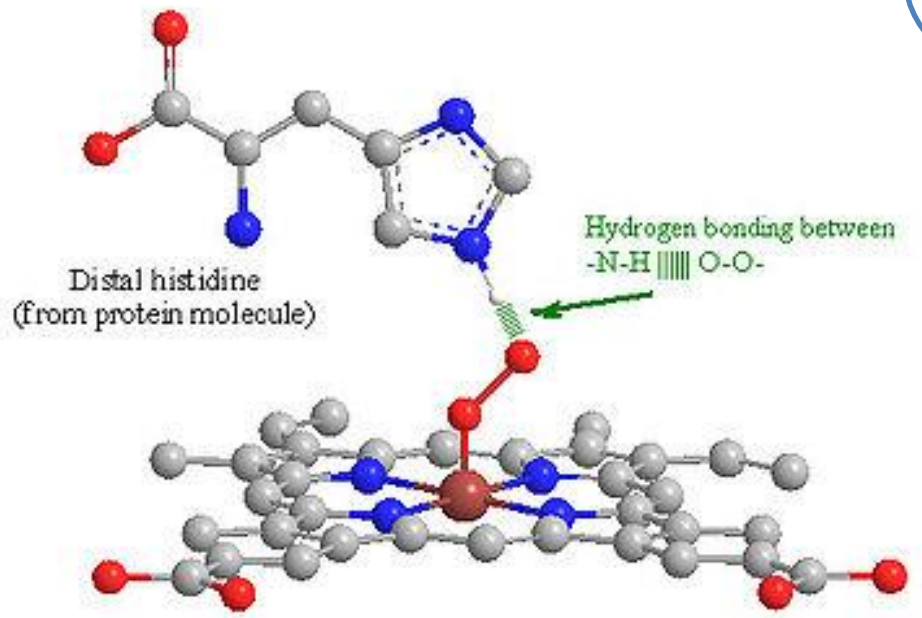
- The distal histidine acts as a gate that opens and closes as O_2 enters the hydrophobic pocket to bind to the heme. Doesn't allow anything else to pass because if it allowed anything to pass it will interact with the iron
- It also stabilizes the interaction with oxygen.



Propionate interact with amino acids on surface.







Hydrophobic here

Distal histidine (positively charged) is the gate keeper that allows only oxygen binding because anything can bind with iron easily
Distal histidine forms a hydrogen bond with oxygen stabilising the interaction with myoglobin protein (favorable interaction)
If any other molecule came, the interaction will be unfavorable hence no interaction will happen.

Heme molecule is:

1. Flat
2. The iron is at the center
3. Hydrophobic molecule except for two groups (propionate are charged hydrophilic and they interact with the hydrophilic amino acids on the surface of the molecule further stabilising the interaction between heme & myoglobin.)

Oxygen binding to myoglobin

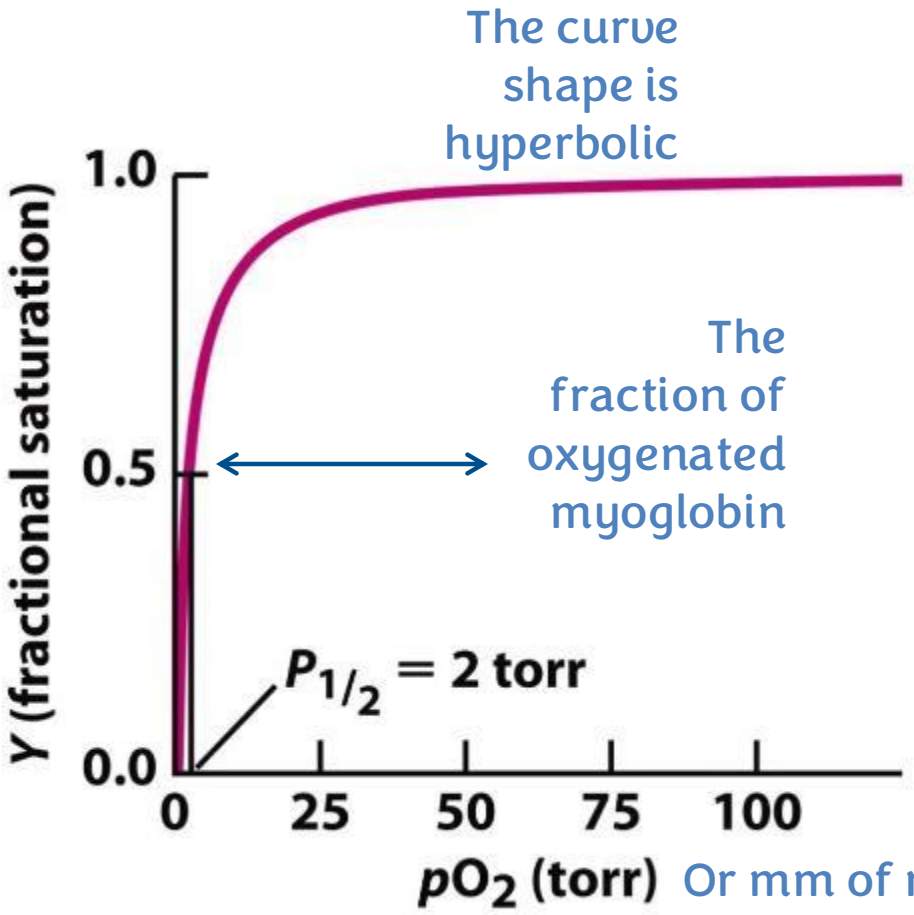
Strong interaction to prevent any release of oxygen in the tissues unless it's hypoxia

-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).

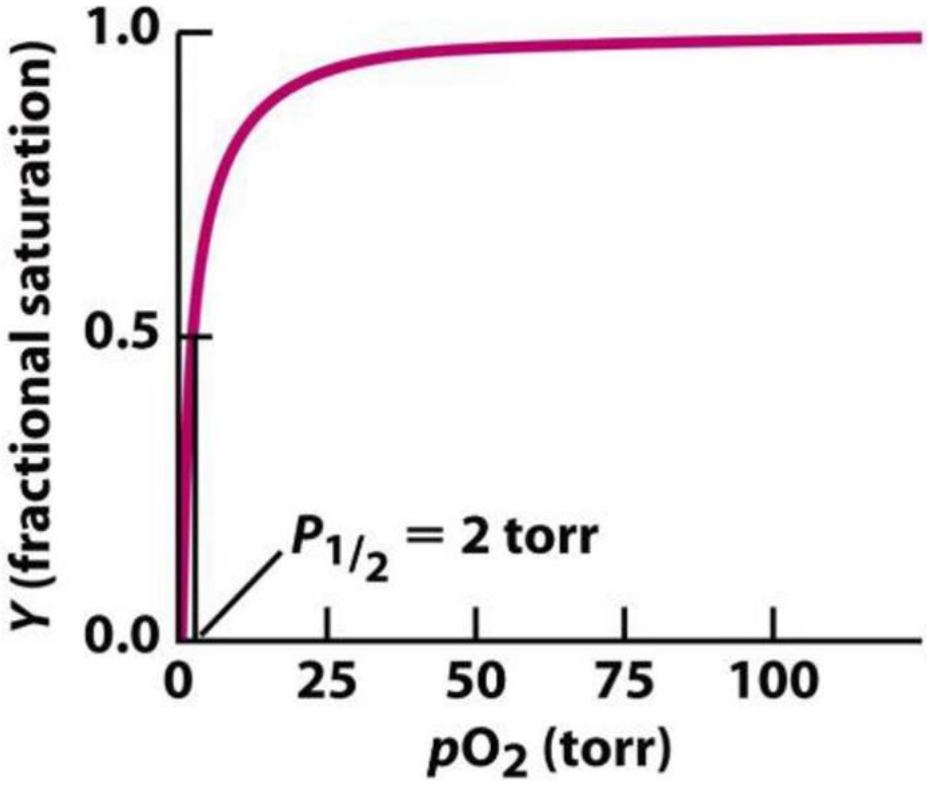
-Given that O_2 pressure in tissues is normally 20-40 mm Hg, it is almost fully saturated with oxygen at normal conditions.

The pressure of oxygen in lungs is approximately 100 torr and hemoglobin (or myoglobine the doctor said both of them are correct) molecules is almost 100% oxygenated in tissues the pressure is 30-40 torr and they are also 100% oxygenated



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

- But when we reach the hypoxic level we will have a sudden drop in the oxygen level so the myoglobin release oxygen
- How much is the p50 ? P50 is the pressure of oxygen when 50% of myoglobin is saturated and 50% is unsaturated
- Whenever the p50 decrease the strength of binding is higher meaning that a lower partial pressure of oxygen is needed to achieve half saturation thus the lower p50 corresponding to a higher affinity of myoglobin for oxygen



Hemoglobin

Hemoglobin structure

Quaternary structure unlike myoglobin that is made up of one polypeptide chain

2 alpha 2 beta

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.

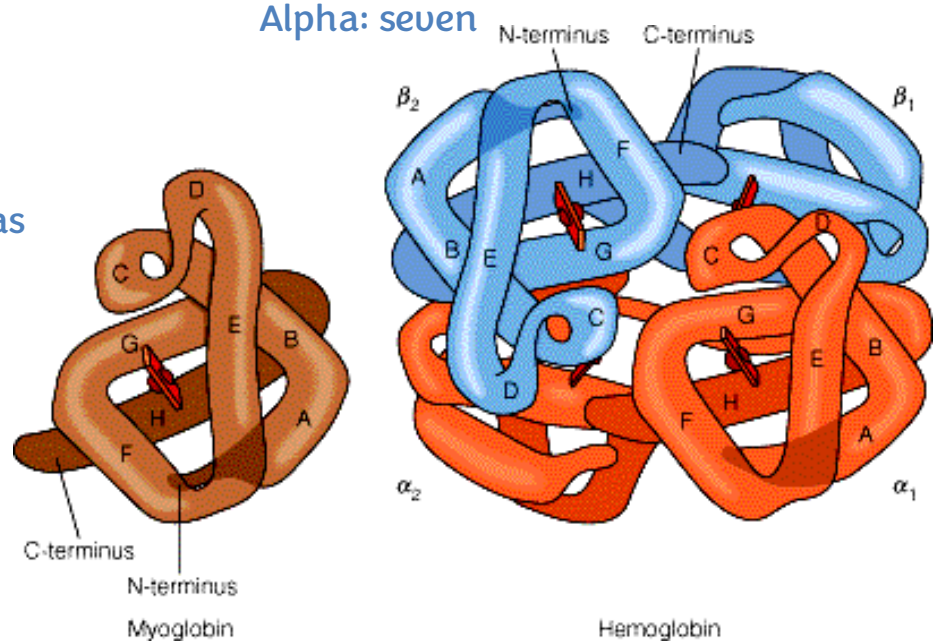
- α polypeptide = 141 amino acids (Val1 & Arg141)
- β polypeptide = 146 amino acids (His146)

The alpha and beta chains are like myoglobin made of alpha helices
Beta: eight
Alpha: seven

Hemoglobin: primary, secondary, tertiary, Quaternary structure
Myoglobin: primary, secondary, tertiary only.

Each polypeptide has one heme (four in hemoglobin; one in myoglobin
- Each heme can bind to one oxygen

Remember:
-Quaternary structure made of more than one peptide
-Secondary structure of myoglobin is eight alpha helices



How are the subunits bound?

The four polypeptide are associated together non-covalently

A dimer of dimers (I made up this term) OR two αβ-protomers

$(\alpha\text{-}\beta)_2$ Two Dimer alpha-beta come together to form a tetramer

The chains interact with each other via hydrophobic interactions.

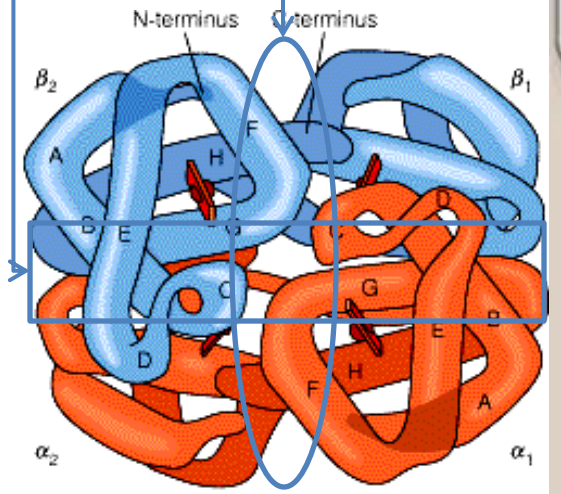
Therefore, hydrophobic amino acids can also be present on the surface.

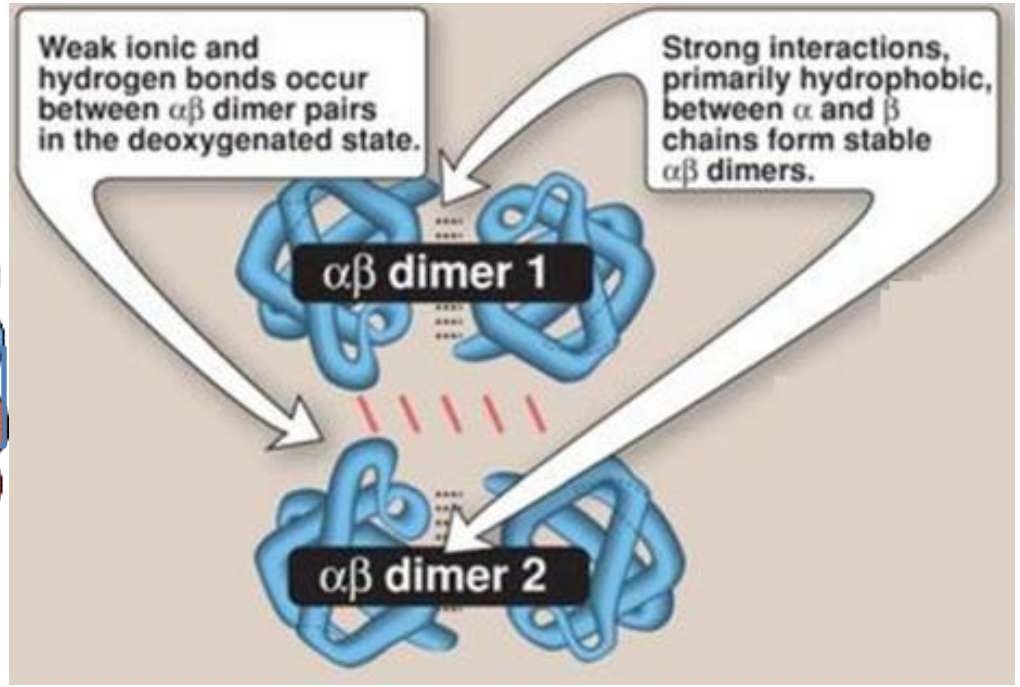
For the purpose of interaction between the four chains

Between each alpha and beta chain: hydrophobic interactions

Between alpha-beta and alpha-beta electrostatic interaction.

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

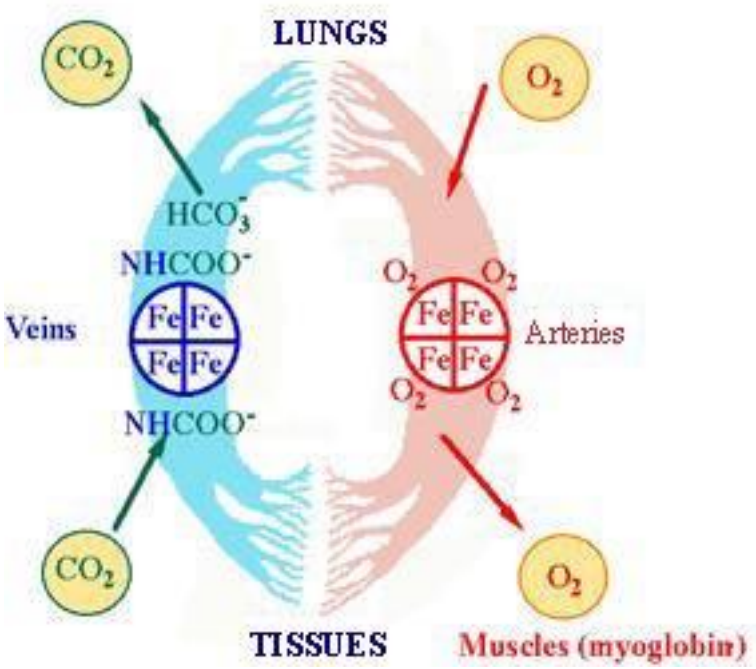




Oxygen binding to hemoglobin

-Hemoglobin must bind oxygen efficiently and become saturated at the high oxygen pressure found in the lungs (approximately 100 mm Hg). → **Saturated**

-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?

Strong interaction with oxygen in the lungs
Weak interactions with oxygen in the tissues

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.

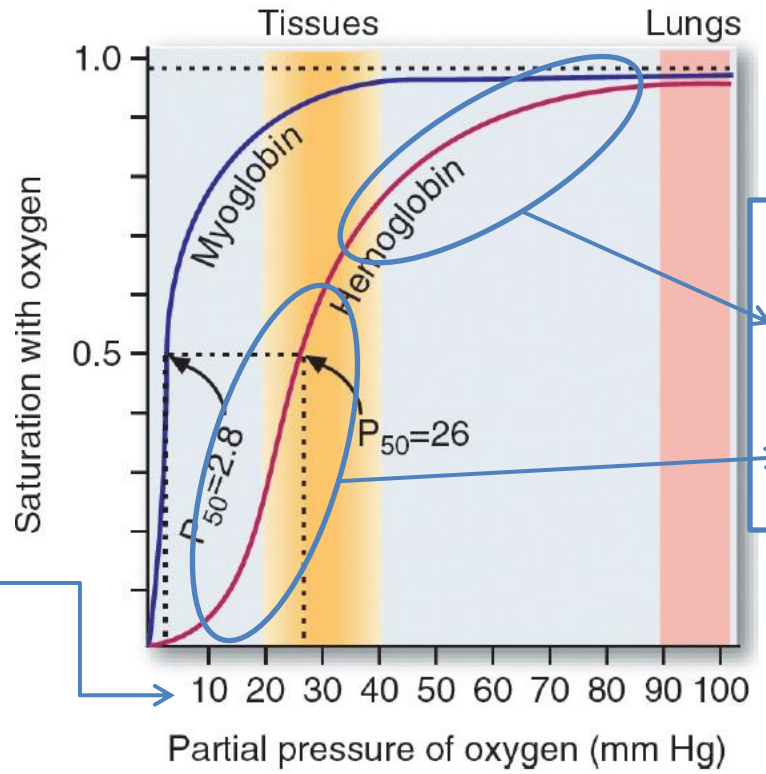
In contrast to a low p50 for myoglobin, the p50 of hemoglobin is approximately 26 mm.

Relate the value of p50 to affinity

-When you are sleeping, the hemoglobin will be mainly saturated in the tissues because you wouldn't be consuming oxygen so some oxygen will be released based on the need of the tissues.
-if you play sports (physical exercise) the oxygen level will go down to almost 20 and affinity will be very weak

Oxygen saturation curve for hemoglobin → sigmoidal shape (looks like an S)

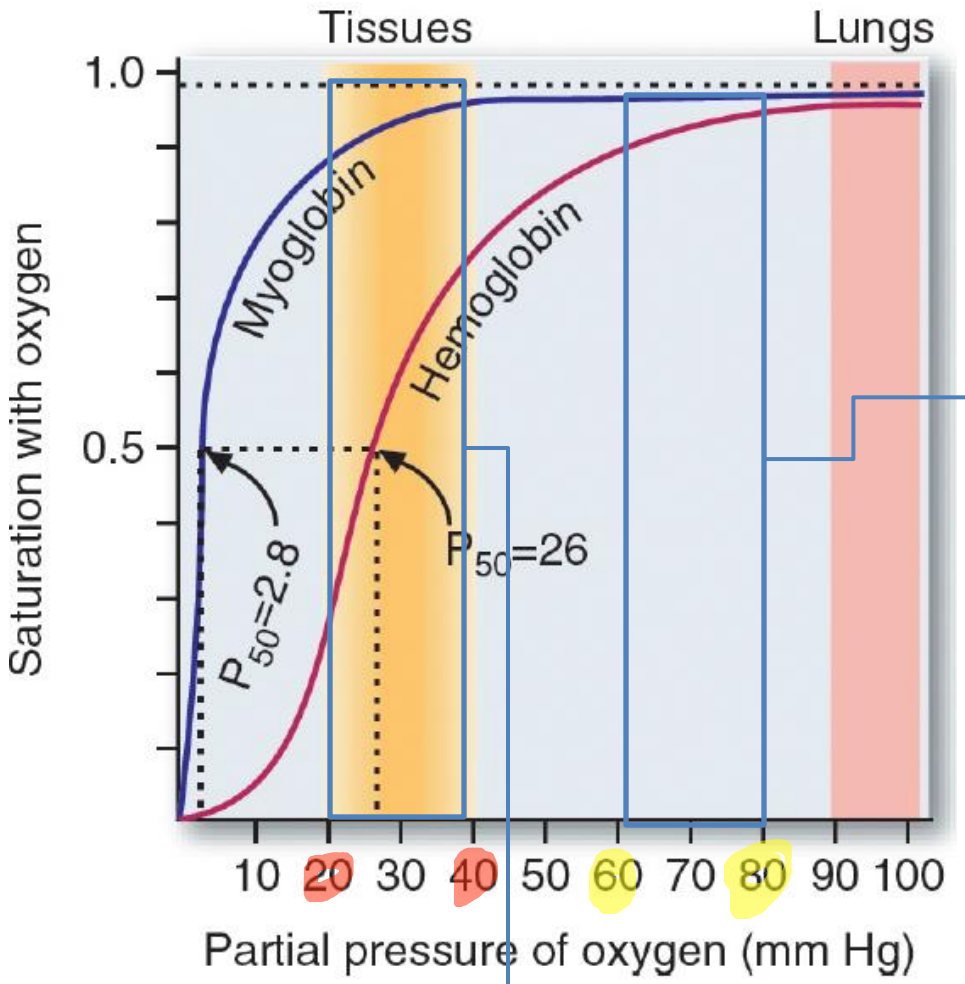
Myoglobin has a stronger affinity for oxygen than hemoglobin (serves it's purpose)



Hemoglobin has two states:
-High affinity state (in lungs)
-Low affinity state (in tissue)

Oxygen level: lungs almost 100mmHg → hemoglobin almost saturated Tissues between 30 to 40→ low affinity but still most hemoglobin is saturated

Any sudden drop of oxygen level in the tissues the affinity will immediately change into the low affinity state even if the change is minor.



Between 60-80: it was 95% saturated with oxygen and it didn't change much from the drop between 80 to 60 (almost became 90%)

But in contrast between (20-40) the saturation of oxygen dropped much more due to the change of oxygen level from 40 to 20

Sigmoidal shape= like an S which means it changes from a state to a state
In hemoglobin it is from high affinity state to low affinity state when the oxygen level is low but not hypoxic like myoglobin

This is the feature of proteins with two states, the change from high affinity state to low affinity state releases alot of oxygen (advantage

Hemoglobin is allosteric

All allosteric proteins are multimeric; but not all multimeric proteins are allosteric

Remember:
Stereoisomers→ stereo=shape, iso=similar, mer=unit

-Hemoglobin is an allosteric protein (from Greek "allos" = "other", and "stereos" = "shape").

Different

Allosteric proteins: proteins with different structures (slight change in structure) which is the characteristic of multi-subunit protein.

→ Myoglobin is not an allosteric structure since it isn't multi-subunit

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

low affinity

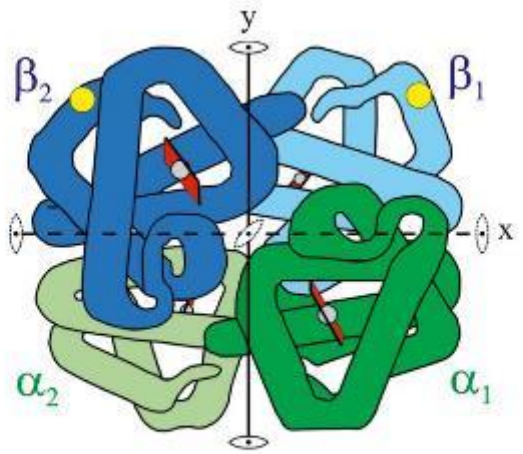
The T-state is also known as the "taut" or "tense" state and it has a low binding affinity to oxygen.

-The R-state is known as the "relaxed" state, and it has 500 times higher affinity to oxygen than the T conformation.

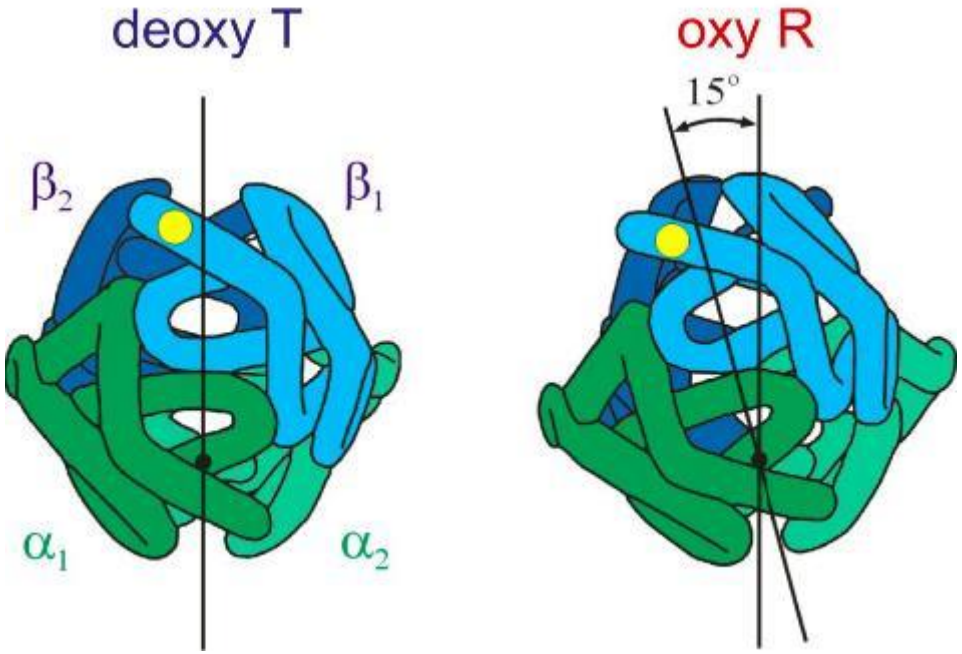
Multimeric proteins can homomultimeric (all same peptide)

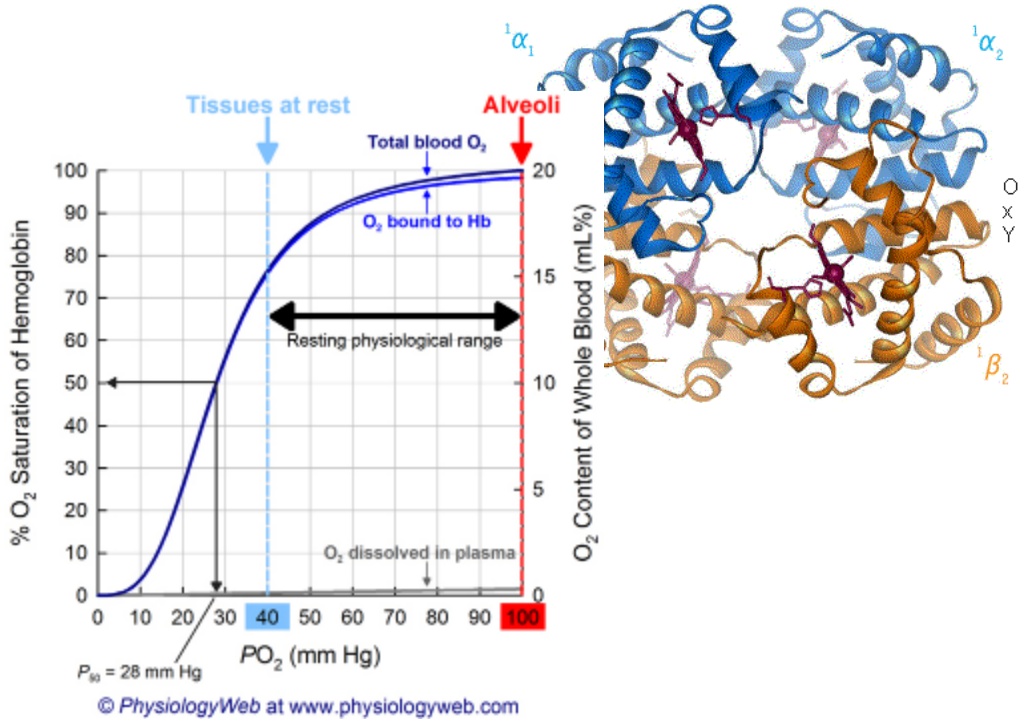
-Binding of O_2 causes conformational changes in hemoglobin, converting it from the low-affinity T-state to the high-affinity R-state .

Structural change of hemoglobin



The oxygen changes the hemoglobin structure. When oxygen binds to hemoglobin molecule the structure changes from T→R (low affinity to high affinity)





How does the structure change? (1)

Heme molecule isn't flat, it is domed structure and the iron is right in the middle

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

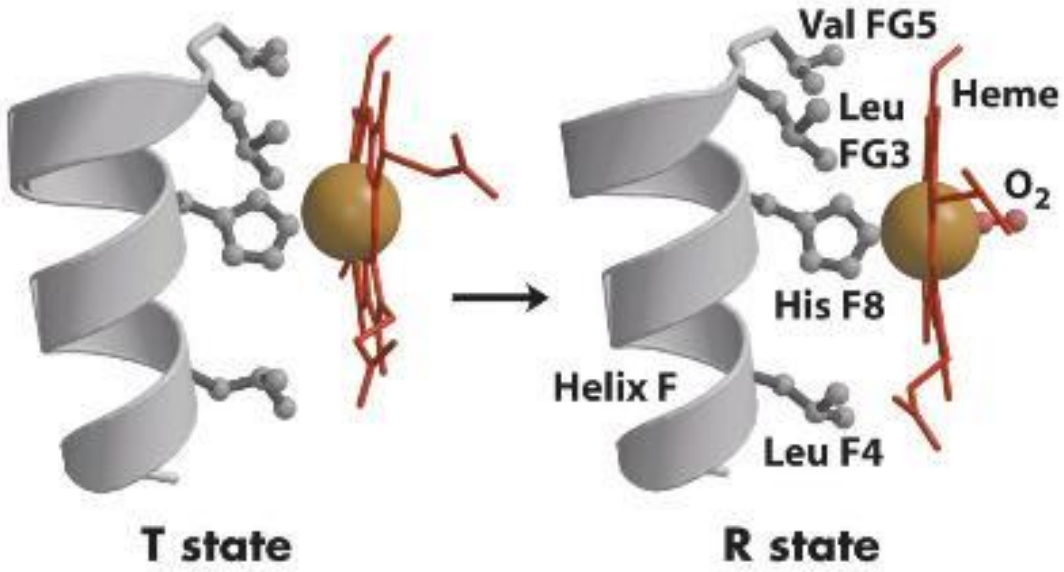
Because the hydrophobic heme is repelled by the proximal His.

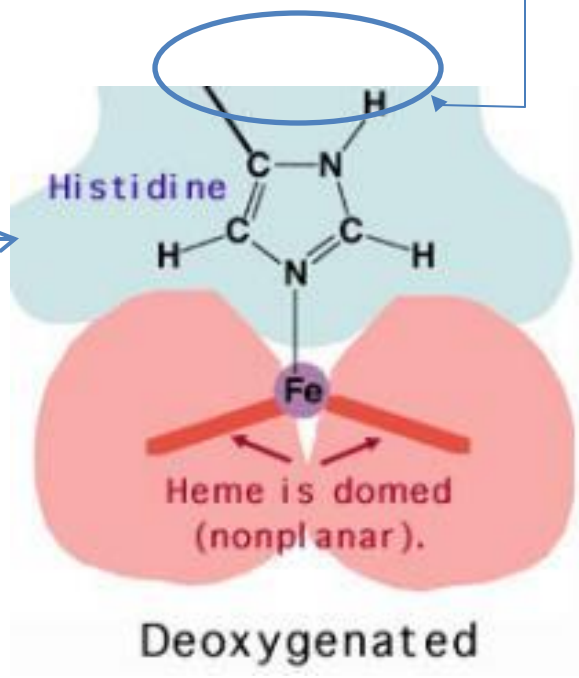
When oxygen binds to an iron atom, heme adopts a planar structure and the iron moves into the plane of the heme pulling proximal histidine (F8) along with it.

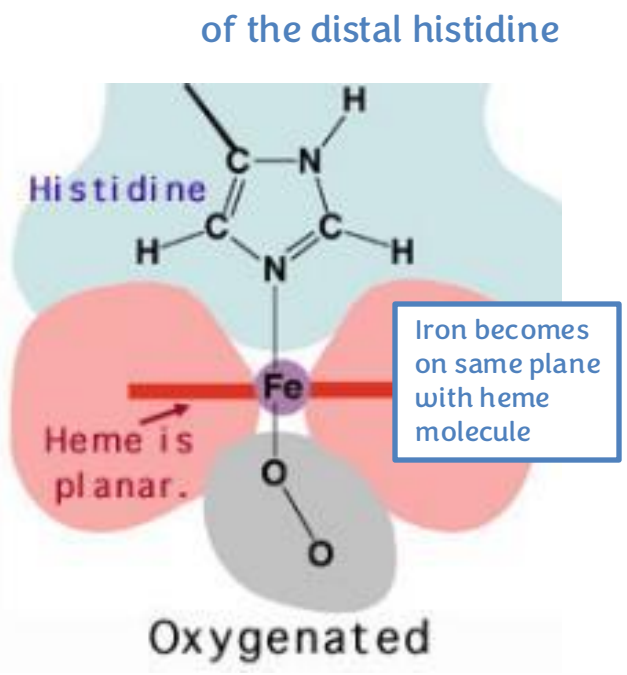
The oxygen will form a hydrogen bond with distal histidine so it will come closer

The dome shaped is because of the repulsion between amino acids and heme molecule

When oxygen binds to the iron, it becomes flat because of the distal histidine







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.

→When the heme becomes flat, the proximal histidine will also be pulled

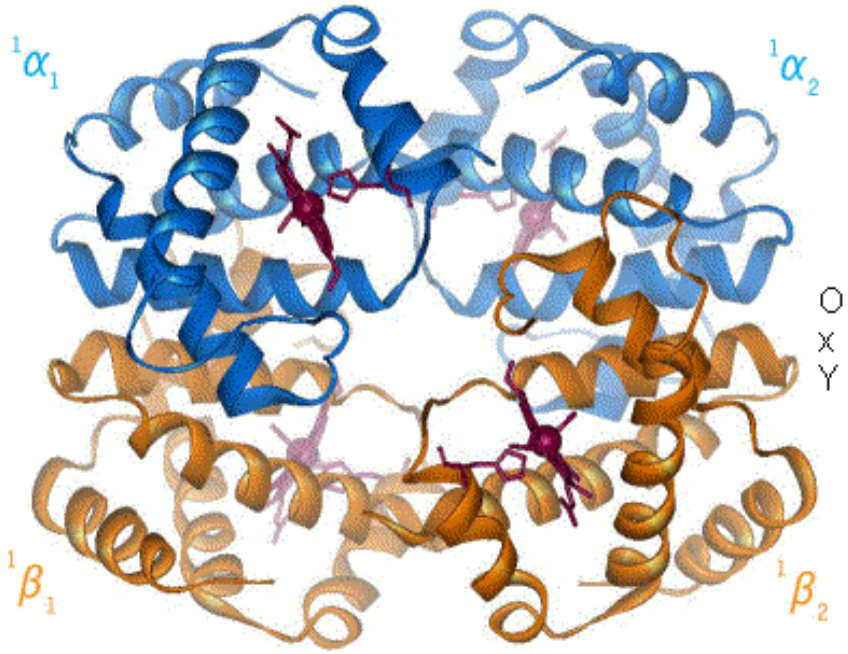
→the proximal histidine is in the helix, the helix will also be pulled

→this will change the tertiary structure of the polypeptide that the oxygen binded to

→when the tertiary structure changes it will break the electrostatic interactions between alpha-beta and alpha-beta

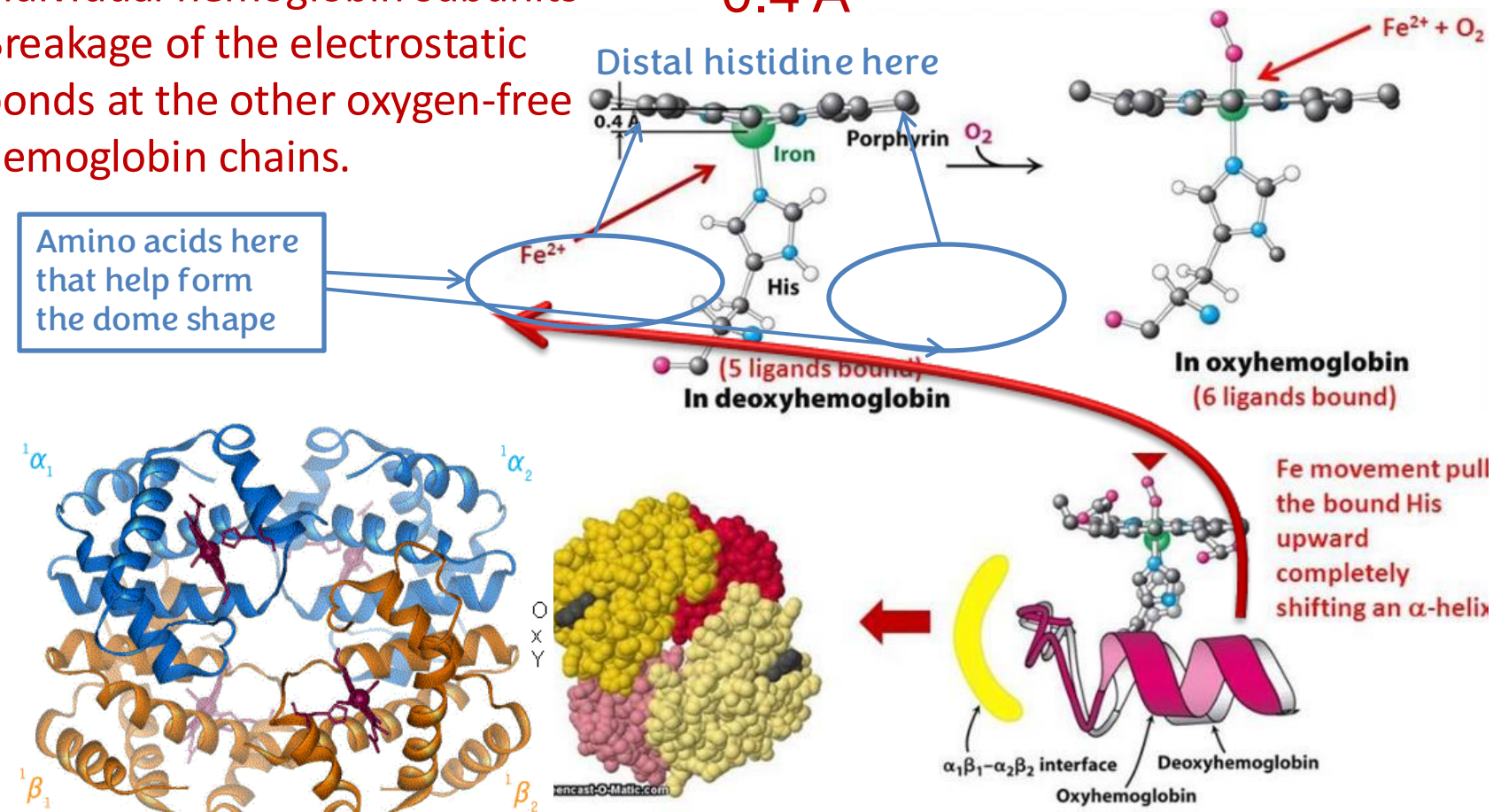
→this will change the Quaternary structure and move 15 degrees

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.



Again:
Oxygen gets pulled by Distal histidine because of hydrogen bonding, heme becomes flat and pulls proximal histidine, changes alpha helix position which changes tertiary structure which changes Quaternary structure by breaking some electrostatic interactions between alpha-beta and alpha-beta
The whole proteins changes it's shape (the distance is only 10 angstrom (1 nano)) and changes from low affinity state to high affinity state
1 Angstrom=10^-10

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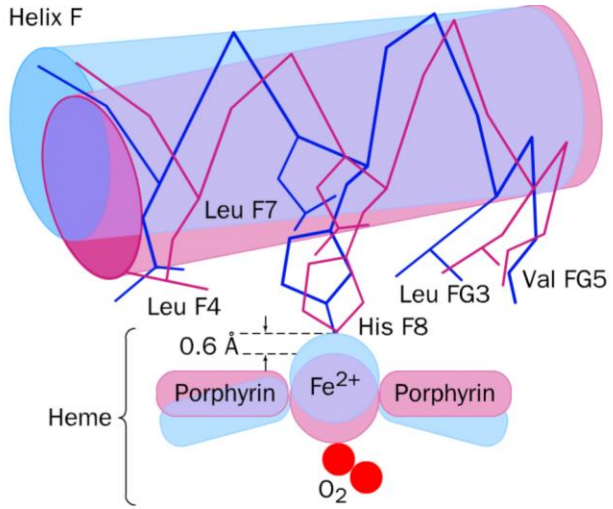
Another look at it

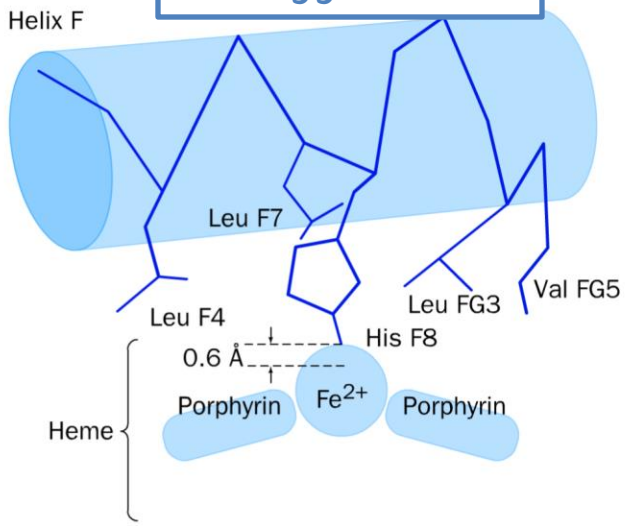
oxygen binding changes protein from low affinity state to high affinity state;
From T- state to R-state;
From tense state to relaxed state



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

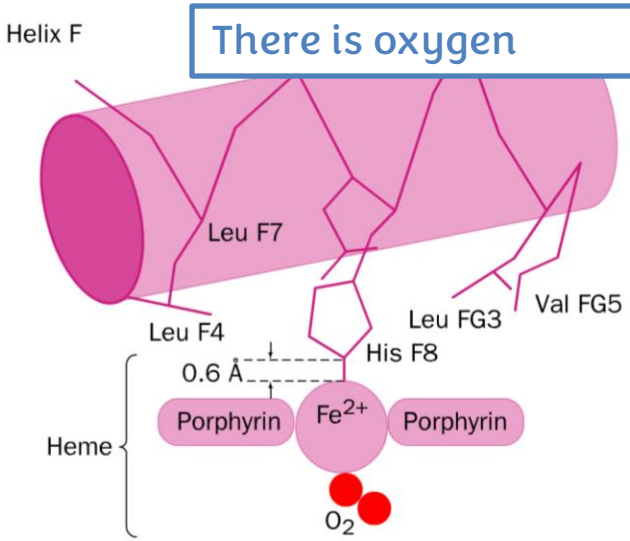
Fig. 7-9 diagrams how the binding of O_2 to one hemoglobin site induces conformation changes that influence the O_2 -binding affinity of the other sites.

No oxygen



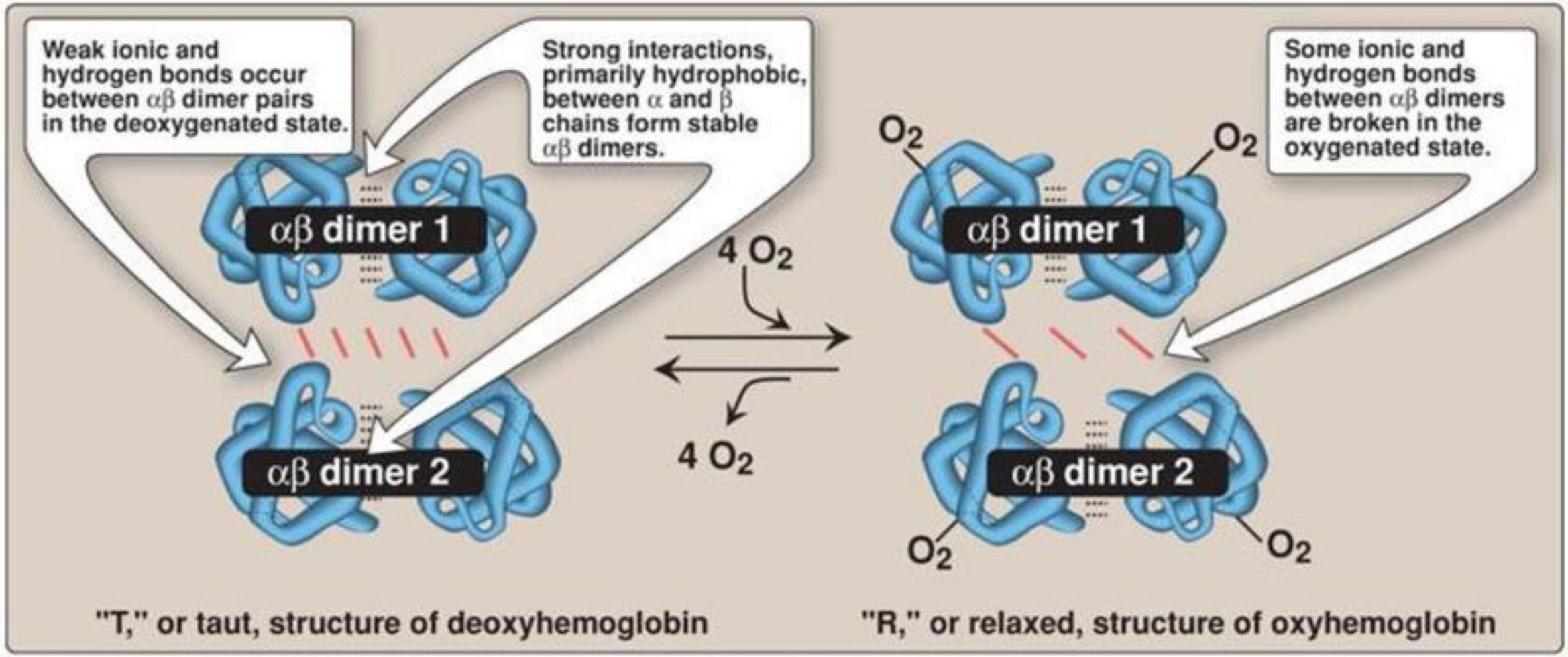
In the absence of bound O_2 ,the Fe(II) lacks a sixth ligand, and resides about 0.6 Angstrom out of the plane of the heme toward its His ligand (the proximal His).

There is oxygen



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



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

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V1 → V2	Slide 24 Slide 6	#Distal histidine gets pulled by Oxygen **#The pressure of oxygen in lungs is approximately 100 torr and myoglobin** #They extend in one direction and they are hydrophilic but the rest of the protein is hydrophobic	*Oxygen gets pulled by Distal histidine *The pressure of oxygen in lungs is approximately 100 torr and hemoglobin *They extend in one direction and they are hydrophilic but the rest of the heme is hydrophobic
V2 → V3	**Slide 12**	*The pressure of oxygen in lungs is approximately 100 torr and hemoglobin	*The pressure of oxygen in lungs is approximately 100 torr and hemoglobin or myoglobin
V3 → V4			

Additional Resources:

1. Youtube Video : summary the difference between Homoglobin and myoglobin

رسالة من الفريق العلمي:

بابُ الشَّهادةِ خير بابٍ يُقرعُ
إكليلُ غارٍ فوقَ هامِكَ يُوضَعُ
مَنْ كالشَّهيدِ وقدْ سَمَت أخلاقُهُ
هذا نِداؤهُ
للعُلا فلْتَسْمَعوا