



● Sheet

○ Slides

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Globular Proteins

We will talk about two proteins in this lecture, fascinating proteins which are great examples to see how the structure is related to function.

1) Myoglobin.

2) Hemoglobin.

- Both of them are Holoproteins.

Recall that Holoproteins are proteins with their non-protein components.

- The non-protein component in both of them is HEME, in the center of it we have iron.
- The protein component is known as globin;
The first is in the muscles and it's called **myoglobin**.
The other is in red blood cells and it's called **hemoglobin**.

Functions of Myoglobin

Myoglobin's function is storage of O_2 in muscles. During periods of oxygen deprivation, oxymyoglobin releases its bound oxygen: **It stores oxygen which is released in case of emergencies like hypoxia; low oxygen in tissues. [Hypo = less/low, Oxia = Oxygen].**

Functions of Hemoglobin

1) Transport of O_2 and CO_2

It's its main function, transport of O_2 from lungs to all peripheral tissue by binding to iron which is associated with heme which is bound to globin. Transfer of CO_2 from peripheral tissues to lungs. BUT, CO_2 doesn't bind to iron in heme, it binds to the protein part.

2) Blood buffering

Recall that, in general, major buffering system in our body is the bicarbonate system buffering. However, we have millions of red blood cells and since every red blood cell contains hemoglobin molecules, we have millions of Hemoglobins. Also, recall that you have learned that amino acids function as buffers since they can donate and accept protons whether by carboxyl group, amino group or ionizable R group. So, Hemoglobin can function as a buffering system as well.

- 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).
 - Prosthetic group: Any non-protein group that is bound **covalently** to a protein.
- The protein environment dictates the function of the heme.
- Hemoproteins participate in oxidation-reduction reactions; they accept and donate electrons; thus they are important in enzymatic reactions.

Myoglobin

Structure of Myoglobin

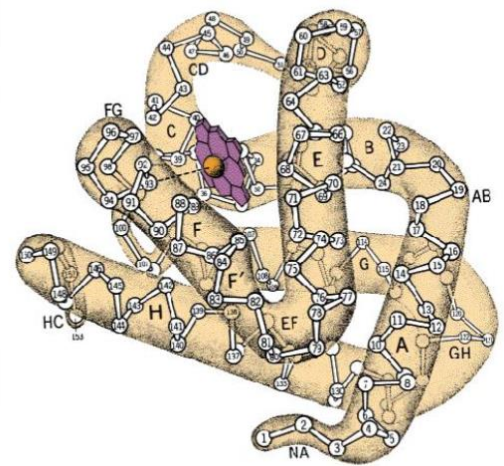
Myoglobin is a monomeric protein (made of one polypeptide) that is mainly found in muscle tissues.

It includes a prosthetic group; the heme group that is embedded inside in the core of the protein. And it can be present in **two forms**:

- 1) Oxymyoglobin (oxygen-bound)
- 2) Deoxymyoglobin (oxygen-free)

The tertiary structure of myoglobin is **8** α -helices, designated from A to H, that are connected by short non-helical regions.

Look at the image...

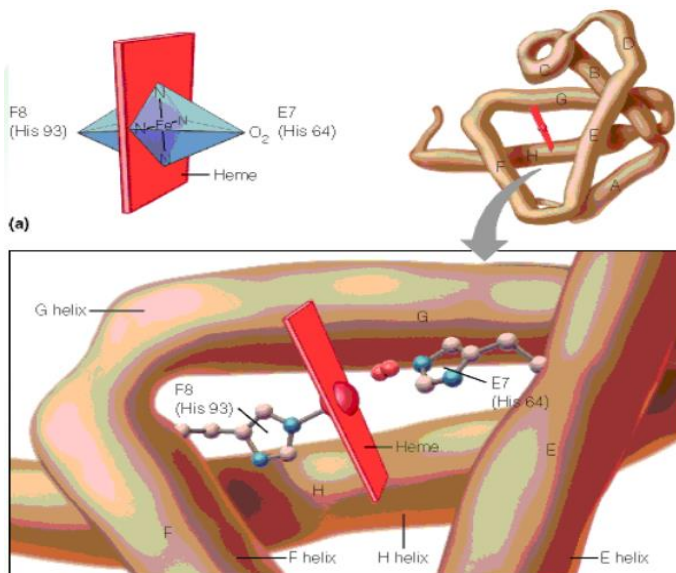


We start with amino terminus, we find first Alpha helix (A) then a turn then another Alpha helix (B) then a turn again etc. until we reach the C terminus of the last alpha helix.

The heme group is present between Alpha (B) and Alpha (C).

Arrangement of Amino acids.

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



Whenever you see a hydrophilic, charged, and polar amino acids in the interior, or hydrophobic on the surface:

Always remember that they have a specific and a particular function.

Except for two histidine residues in helices E and F (known as E7 and F8). This indicates that they play an important function for them to be found there.

F8 His is designated as proximal His, whereas E7 His is known as distal His.

Proximal Histidine: called proximal because it is near/linked to heme group via iron (indirectly linked). It is bound to F helix and covalently linked to the iron (heme).

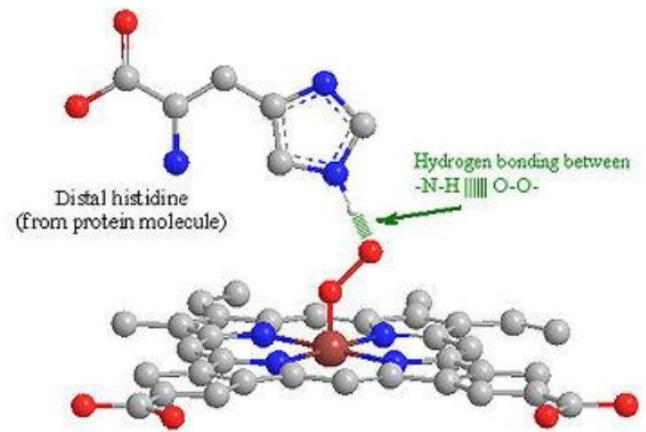
Distal Histidine: called distal because it is far from the heme group. It is bound to E helix. It is **NOT** covalently linked to the iron (heme). Instead, oxygen is bound to distal histidine via hydrogen bonds.

The following picture shows Distal His whereas Proximal His is located below and thus it is not shown.

Notice the interaction between distal His and the oxygen which is a hydrogen bond.

Oxygen molecule is bent not straight.

Note: Recall from sheet 12 that histidine is a positively-charged amino acid.



Heme group

Both proteins contain a heme group, it is a prosthetic group (a non-protein group **covalently** attached to a protein).

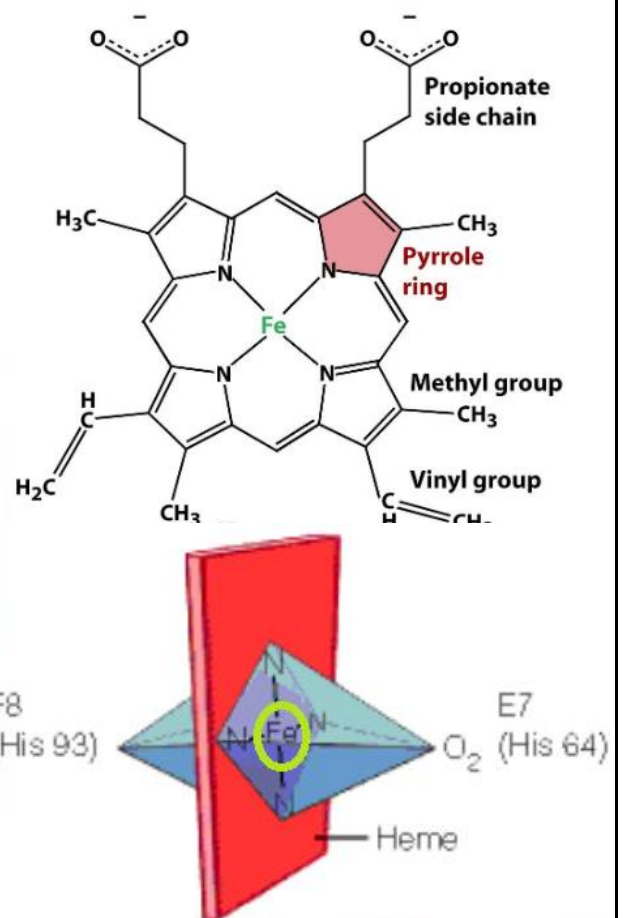
Heme is a flat molecule that has four cyclic groups known as pyrrole rings.

Iron

Iron can bind in the center of the four rings.

Fe in the ferrous state (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 O_2 (the sixth coordinate)



Upon absorption of light, heme gives a deep red color (iron color when it is reacted with oxygen).

Oxidation of iron to the Fe^{3+} , ferric state, makes the molecule incapable of normal O_2 binding. So, Iron can be present in two ionic forms, in order for it to bind to oxygen it **MUST** be in ferrous state.

Does this mean that it can't bind to another O_2 !!!!?? ↓↓

Structure-Function Relationship

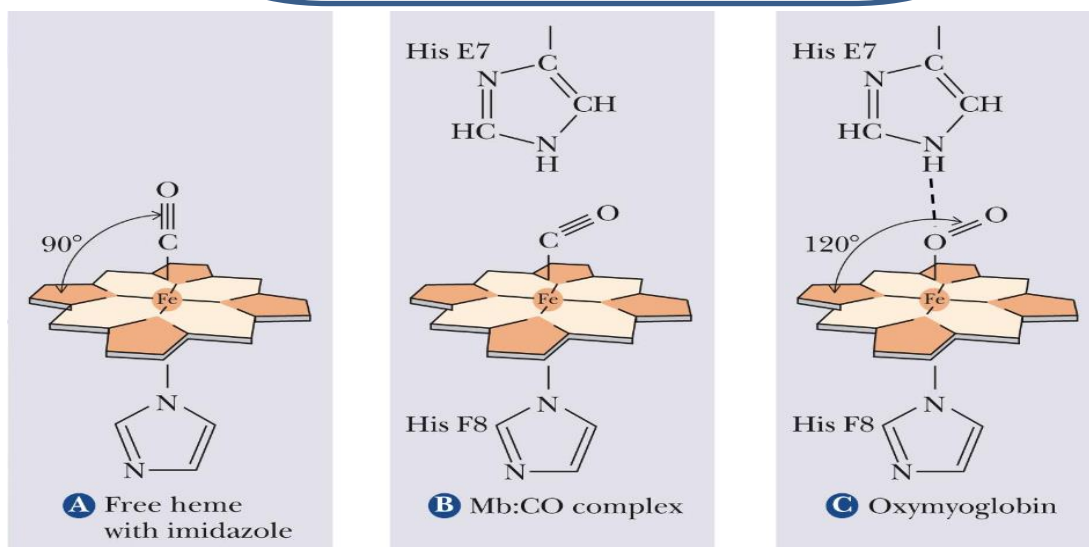
- When the oxygen that is bound to iron is released, iron itself gets oxidized; it loses an electron and becomes in the ferric state, so Iron can't bind to another oxygen molecule until it is reduced, **how?**

This is the importance of the environment/structure in maintaining the function of binding to oxygen again, iron is reduced by the hydrophobic amino acids that surround it, electrons transfer from one atom to another until it reaches the iron and reduce it. (After iron released the oxygen it immediately accepts another one from the surrounding amino acids).

- 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.

10:45

Another significance to distal histidine



(A) Free heme with imidazole:

If we take this free heme and add oxygen and carbon monoxide, there will be a competition between CO binding to iron and oxygen binding to iron. However, CO has much higher affinity towards heme than oxygen. (in case of free heme), which leads to

carbon monoxide being bound rather than oxygen. (if this happens in reality it is disastrous since any small amount of CO in surrounding air might cause death.

(B) Myoglobin:CO Complex

Because of the presence of the heme near the distal histidine of the globin there is some repulsion, steric hindrance between CO and distal histidine, therefore CO is bent. This bending weakens the interaction between carbon monoxide and iron. (it destabilizes and reduces the binding affinity of CO to heme).

(C) Oxy-myoglobin

Conclusion: if we have heme within a protein, the interaction/binding affinity of CO is less than binding affinity of oxygen due to the presence of the distal histidine.

(Another important significance for the surrounding environment around heme)

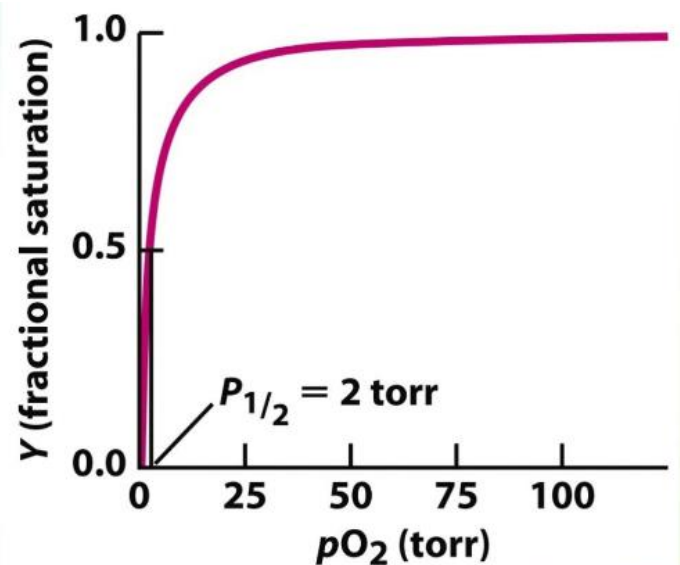
Exception: This might not be the case if carbon monoxide level is extremely high (there is much more CO than oxygen), which will lead to unwanted binding of CO causing serious life-threatening problems. For example, choking of a family due to gas leakage with doors being closed or switching on the car in a closed garage.

Oxygen binding to myoglobin

This is called saturation curve for myoglobin which reflects oxygen binding to protein. The binding of O₂ to myoglobin follows a hyperbolic saturation curve.

Recall that function of myoglobin is storage of oxygen and oxygen is released in case of hypoxia so do you expect oxygen binding to myoglobin to be with high or low affinity?

Affinity is indication for strength of binding, so Myoglobin binds O₂ with high affinity.



In lungs it is about 100 torr (filled with oxygen) in peripheral tissues it is obviously less than that, approximately 20-30 torr. Y axis reflects the /percentage of myoglobin that is bound to oxygen. At 100 torr (lungs) almost 100% of myoglobin is bound to oxygen. At 25 torr (tissues) almost 100% of myoglobin is bound to oxygen too!!!

When do we have release of oxygen? When the pressure decreases much lower.

P50: this is a commonly used measure that is used in pharmacology, biochemistry and many other industries, it is a measure of affinity to take the 50% of the protein that is bound, that is free, that does this or that.... etc. (this is an important term for enzymes)

P50 that reflects oxygen bound to myoglobin is 2.8 torr, at 2.8 torr, 50% of myoglobin is bound to oxygen, which indicates this is a really strong affinity. At 2 torr, myoglobin's affinity decreases in order to get O₂ for an emergency.

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

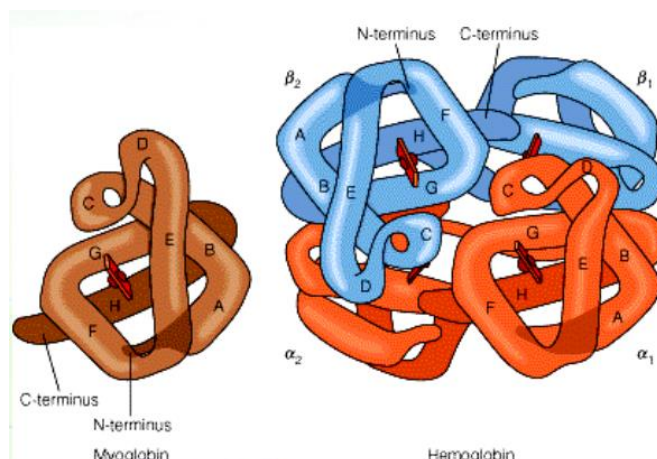
Given that O₂ pressure in tissues is normally 20 mm Hg, it is almost fully saturated with oxygen at normal conditions.

Hemoglobin

Hemoglobin Structure

Hemoglobin is a hetero-tetrameric hemeprotein (four protein chains known as globins with each of them bound to heme).

In adults, the four globin proteins are of two different types known as α and β , alphas are identical, and betas are identical. So, a hemoglobin protein is an $\alpha_2\beta_2$ globin protein.



Note: Beta/alpha is not respected to hemoglobin only, in insulin we have 2 peptide chains; alpha and beta which are different from alpha and beta of hemoglobin so there are used as designation only.

The α and β chains contain multiple α -helices where α contains 7 α -helices and β contains 8 α -helices (similar to myoglobin, almost identical, just a few differences in the amino acid sequence; primary structure).

- How many hemes does hemoglobin have?

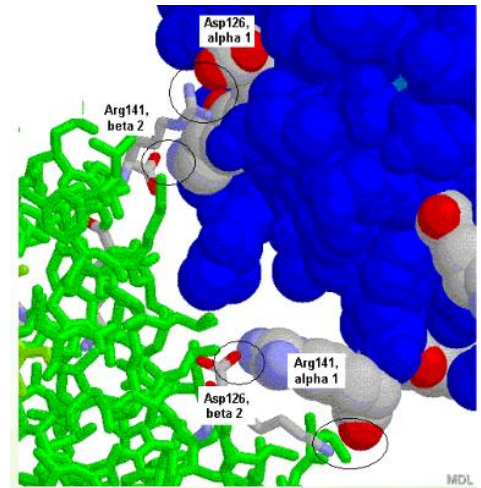
Each polypeptide has a heme which means we have 4 hemes. So, hemoglobin is capable of binding to 4 oxygen atoms.

And that is the structure of hemoglobin in adults. (fetal hemoglobin differs from adult 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 to link between each other.



→→Remember the note from page 3

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

How can proteins with quaternary structure have their polypeptides connected to each other? It depends on the protein.

- **Immunoglobulin** chains are attached covalently by disulfide bonds.
- Hemoglobin chains are attached via non-covalent interactions, mainly hydrophobic interactions, and also few hydrogen bonds and electrostatic interactions (salt bridges).

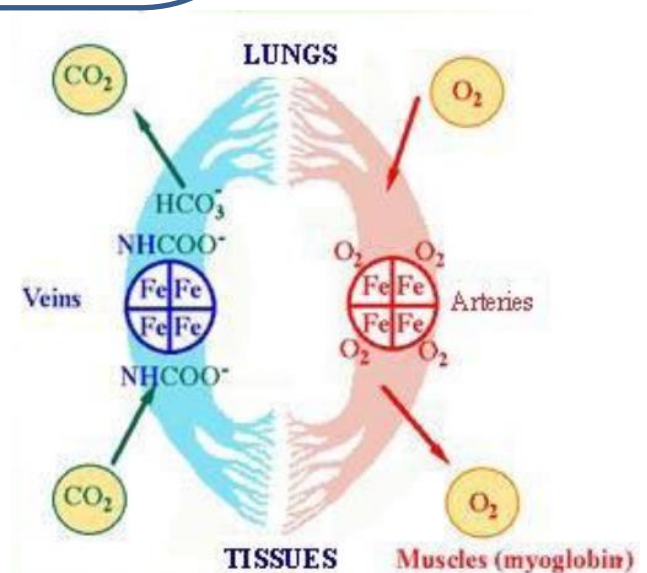
Oxygen binding to hemoglobin

Would u expect the binding of hemoglobin to oxygen to be with high affinity or low affinity?

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).

So, it depends on the place in which hemoglobin is present in.



If it is on low affinity in lungs, it will not be saturated and if it goes to peripheral tissues with high affinity it won't be able to release oxygen that is why its affinity is not fixed

but **dependable** on the place; we want it to have a high affinity in lungs and low affinity in peripheral tissues in order for lungs to be filled with oxygen and for oxygen to be released when hemoglobin reaches peripheral tissues.

When heme is bound to oxygen its color is red (that's why our blood is red in color).

Binding of hemoglobin to oxygen can be extremely weak due to a disease, thus hemoglobin is not saturated and therefore, it won't give a deep red color, some people call this abnormality: Blue blood.

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Important Notes: Keep in mind the following:

- Oxygen is released, and hemoglobin becomes unsaturated in tissues where the oxygen pressure is low (about 20-30 mm Hg, an average of 25 torr).
- Oxygen is bound efficiently, and hemoglobin becomes saturated at the high oxygen pressure found in lungs (approximately 100 mm Hg).
- If you have noticed, P50 value for myoglobin was given as 2 torr and 2.8 torr, why is that? Our slides are taken from different sources, so the doctor said to not really focus about the exact number, but u have to keep in mind it is between that range.

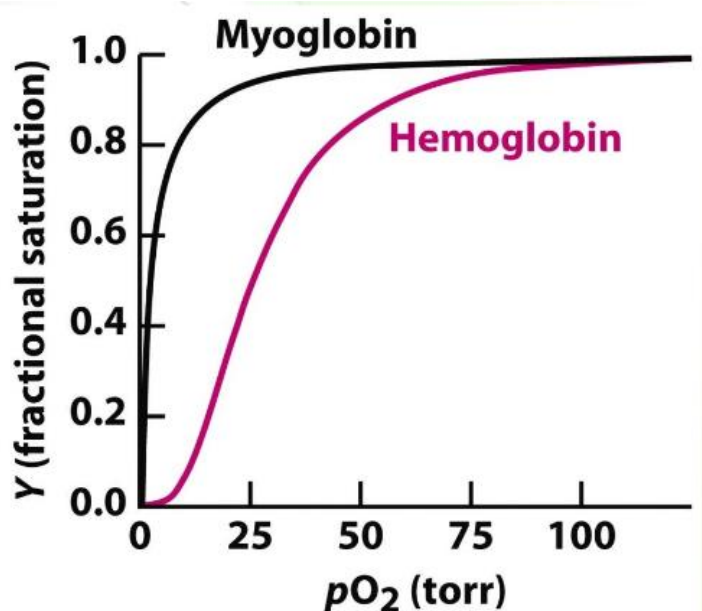
The Saturation Curve

For Hemoglobin:

- The saturation curve of hemoglobin binding to O₂ has a sigmoidal shape. (looks like an S).
- At 100 mm Hg, hemoglobin is 95-98% saturated (oxyhemoglobin).
- As the oxygen pressure falls, oxygen is released to the cells.

For Myoglobin:

- When the pressure of oxygen is high, it has a high affinity
- When the pressure of oxygen is too low (2 torr), it has a low affinity.



BUT, in the case of hemoglobin:

When pressure of oxygen is high, hemoglobin has a high affinity. However, when pressure of oxygen is decreased a bit low, affinity becomes very low which can be noticed unlike the curve of myoglobin. When affinity becomes very low, oxygen is released at about 20-30 torr in peripheral tissues (p50 is about 26 torr).

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

	Myoglobin	Hemoglobin
Structure	Monomeric hemoprotein 8 α -helices.	heterotetrameric hemoprotein. $\alpha_2\beta_2$ globin protein. α contains 7 α -helices and β contains 8 α -helices.
Saturation curve	Hyperbolic	Sigmoidal
Allosteric	Not Allosteric	Allosteric
Place	Muscles	Red Blood Cells
Function	Storage and Release of Oxygen	1) Transport of Oxygen and Carbon Dioxide. 2) Blood Buffering
P50	2 torr	26 torr
Number of oxygen molecules bound	One	Four

When we look at the structure of hemoglobin we have two structures

A structure at the high affinity state and a structure at the low affinity state and we also have the transition state

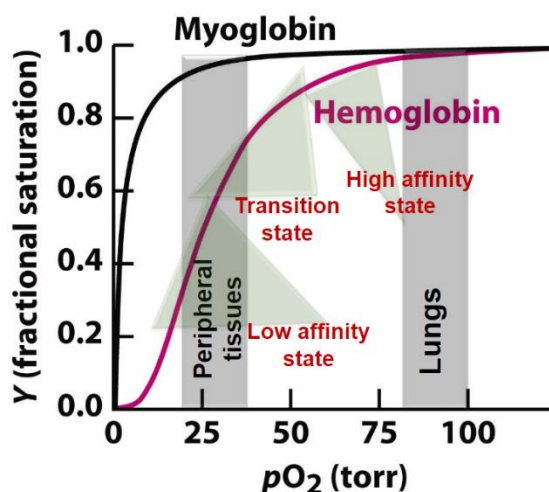
How can it have two structures?

Hemoglobin is allosteric; have different structures.

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.

Proteins that have quaternary structure are Allosteric proteins.



So, hemoglobin is considered allosteric since it has 2-3 structures(have a sigmoidal curve).

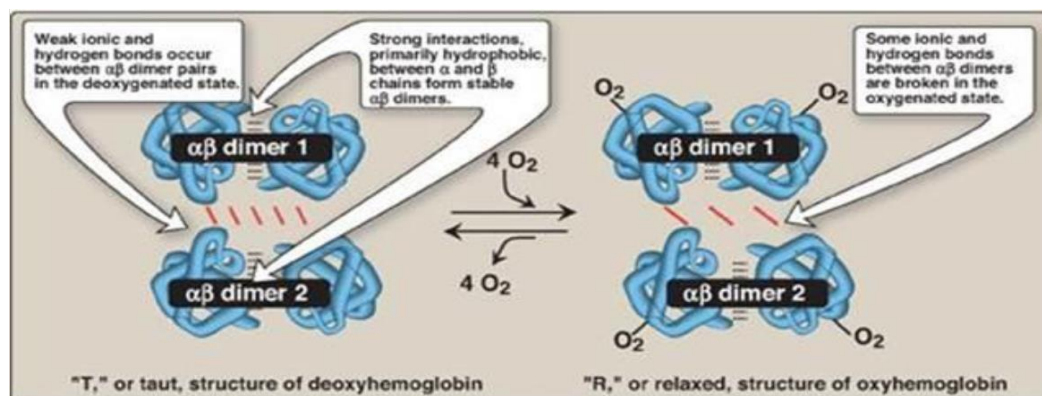
That is why, unlike hemoglobin, Myoglobin is a monomeric protein, so it is **NOT** an allosteric protein which is why it doesn't have two structures.

Hemoglobin exists in two forms, T-state and R-state:

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

The high affinity structure is called **R-state** which is known as the "relaxed" state and it has 500 times higher affinity to oxygen than as the T conformation.

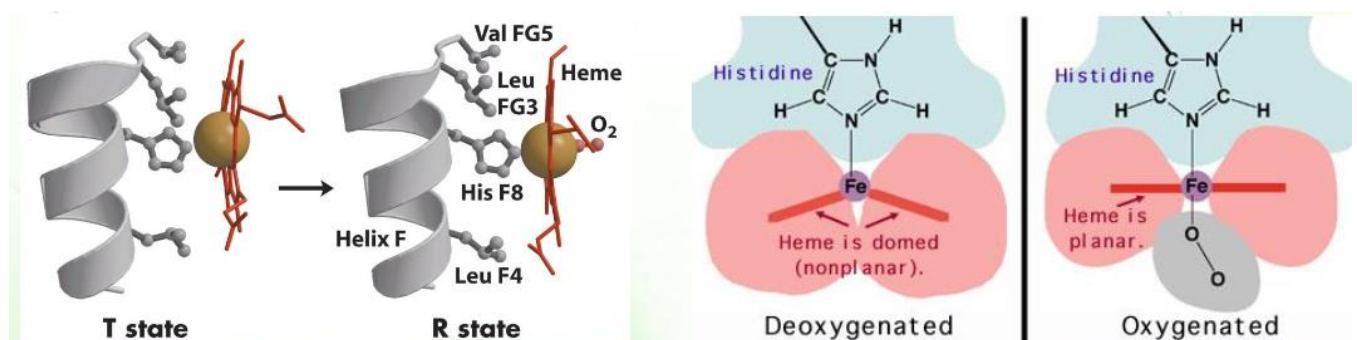
Binding of O₂ causes conformational changes in hemoglobin, converting it from the low affinity T-state to the high affinity R-state.



How does the structure change?

If you keep twisting a finger of a muscular guy, his hand will be forced to rotate/twist too, therefore his arm is twisted and lastly the whole body.

Same thing happens with hemoglobin,

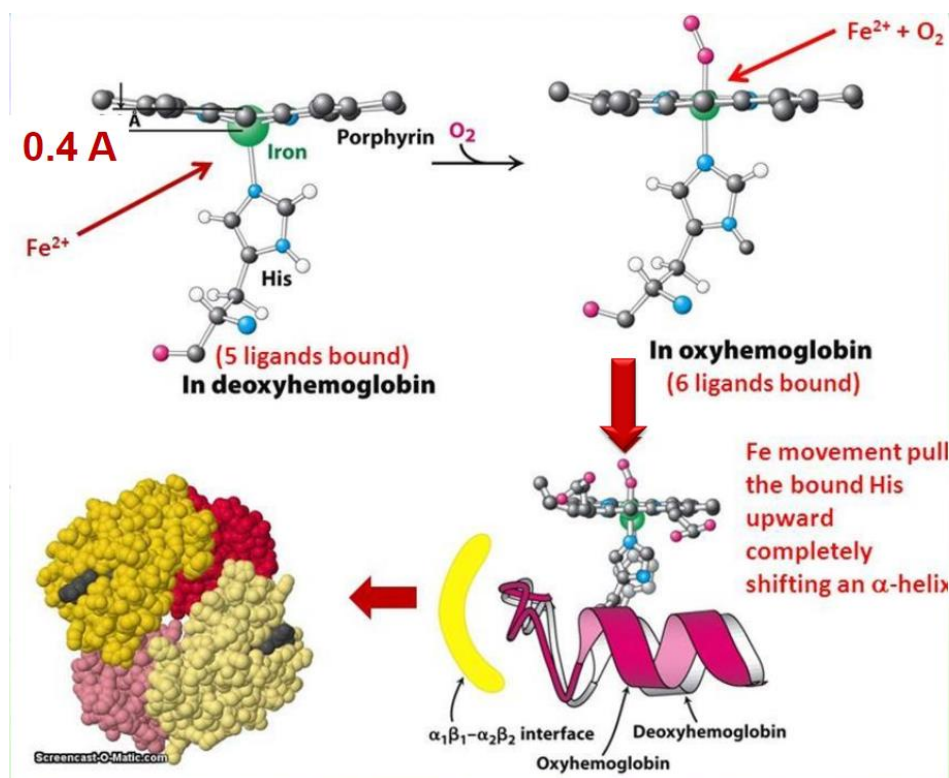


When heme is free of oxygen (in deoxygenated state), it has a **domed structure** and iron is outside the plane of the heme group.

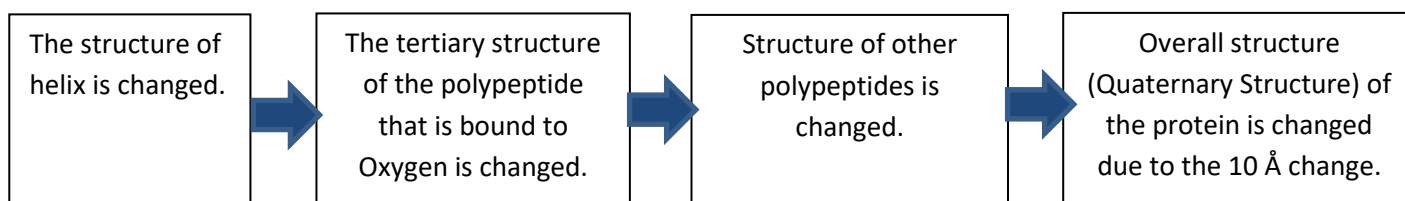
When oxygen binds to an iron atom, heme adopts a **planar structure (flat)** and the iron moves into the plane of the heme pulling proximal histidine (F8) along with it.

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

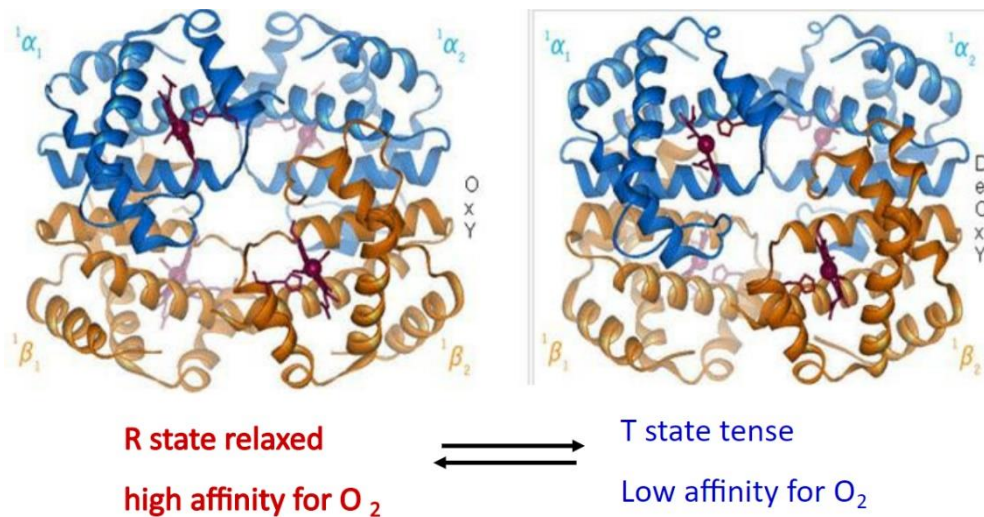
(notice the interactions that are shown in the picture on page 11).



To sum up: We are talking about a very minor change which is 10 Å ($1 \text{ Å} = 10^{-10} \text{ m}$) which causes Heme to pull down the proximal histidine and therefore:



These conformational changes, cause breakage of the electrostatic bonds, therefore hemoglobin is converted from the low affinity T-state to the high affinity R-state.



This change in structure depends on how many oxygens are bound to hemoglobin.

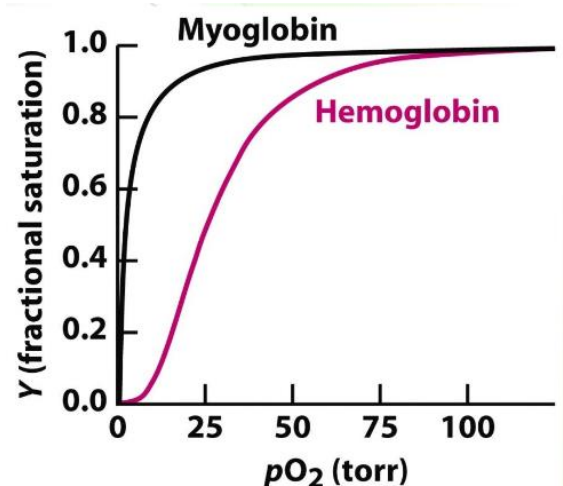
Note: In myoglobin, movement of the helix does not affect the function of the protein.

The saturation curve is sigmoidal because

Conformational changes lead to **cooperativity** among binding sites.

Binding of the first O₂ breaks some salt bridges (electrostatic) 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 a third O₂ even more, and so on.



Conclusion: Oxygen binding is cooperative.

When the 1st oxygen molecule binds to the heme in the first polypeptide of hemoglobin, it makes it easier for the 2nd oxygen molecule to bind, when the 2nd binds it makes it easier for the 3rd, when the 3rd binds it makes it easier for the 4th one to bind.

Check the sigmoidal curve and notice the following:

- When pressure of oxygen is increased, the number of oxygen molecules that bind to it increases.

1) For instance, If we have 100 hemoglobin molecules and 25 oxygens:

The percentage which represents the saturated hemoglobin molecules is very little, because we don't have many oxygens!

Saturated hemoglobin: is a hemoglobin that is bound to 4 oxygen atoms.

We will most likely have 25% of hemoglobin molecules bound to oxygen and 75% not bound. It's a proportion, most likely every hemoglobin of the 25% bound hemoglobin is bound to one oxygen.

2) BUT if we increase the pressure of oxygens a bit further to have, for example, 200 oxygen molecules to 100 hemoglobin present:

- When the percentage of oxygens that are bound to hemoglobin is increased, the affinity increase is huge, 2 oxygen atoms will be bound to hemoglobin instead of just one.
if we increase the pressure even more, 3 oxygen atoms will be bound; when 3 oxygens are bound, affinity is increased but not that much causing the 4th oxygen molecule to bind.
- Recall that when an oxygen binds to heme, we break electrostatic interactions, so it makes it easier for the 2nd one to come and bind, and so on.

100% of hemoglobin molecules are bound to oxygen atoms, but are they all relaxed? No! some may still be in the transition state.

Transition state: which is likely the state where the hemoglobin is bound to 2-3 molecules. 2 would be when hemoglobin is close to peripheral tissues, whereas 3 would be when it is close to lungs and vice versa, In lungs hemoglobin molecules are mostly likely to be found saturated, but when reaching peripheral tissues, affinity is decreased, O₂ is released. And that is the importance of having transition state, relaxed and taut state.

34:45