بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

BioChemistry

وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

Final | Lecture 5

Globular Proteins pt.1

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Color coding used in the modified:

- *Black: the original slides*
- *Blue: the doctor’s explanation/words*
- *Gray: additional information and explanation*
- *Red: important information*

Globular proteins

Myoglobin and hemoglobin

Professor Mamoun Ahram

Protein Classification:

Protein can be classified in 3 ways:

1) Based on structure
Two types: Fibrous and Globular proteins

Fibrous proteins (usually structural): elongated, rigid, and hydrophobic (to be compact with no flexibility)
Examples (check the fibrous protein lecture to study them in details): Collagen, elastin, and keratin.

Globular (Globe & Rounded) proteins: has a 3D structure

2) Based on function
3) Based on localization (nuclear proteins, membrane proteins, cytosol, etc.)

Functions of myoglobin and hemoglobin

It's a monomeric protein .

- Myoglobin functions in storing O_2 in muscles. During periods of oxygen deprivation (hypoxia), oxymyoglobin releases its bound oxygen.

- Function of hemoglobin:
  - transport of O_2 and CO_2
  - blood buffering

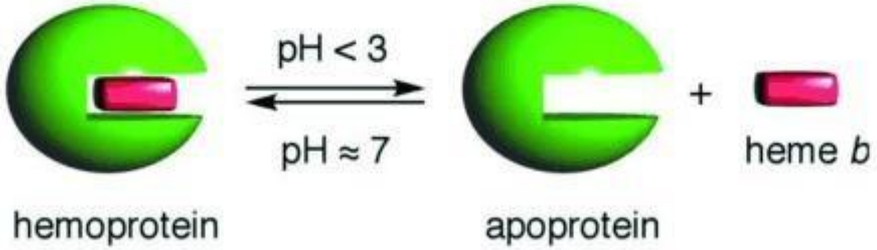
Myoglobin can be broken down into:
Myo = Muscle
Globin = Globular protein

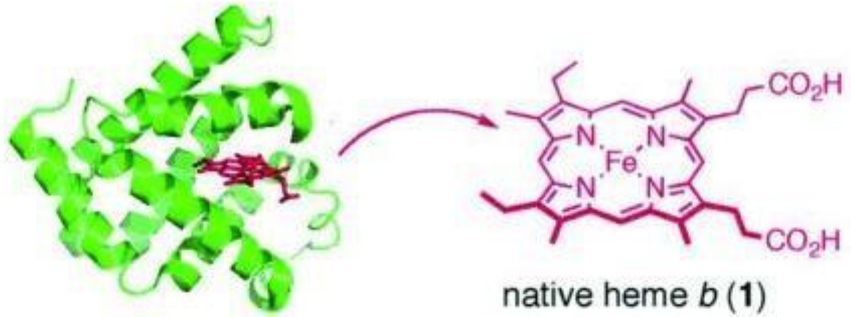
Hemoglobin transports gases (mainly oxygen) from the lungs to the tissue and it carries CO2 from the tissue to the lungs which we exhale out of our body.

Hemoproteins

Myoglobin and Hemoglobin are classified as Hemoproteins

- Many proteins have heme as a prosthetic group called hemoproteins.



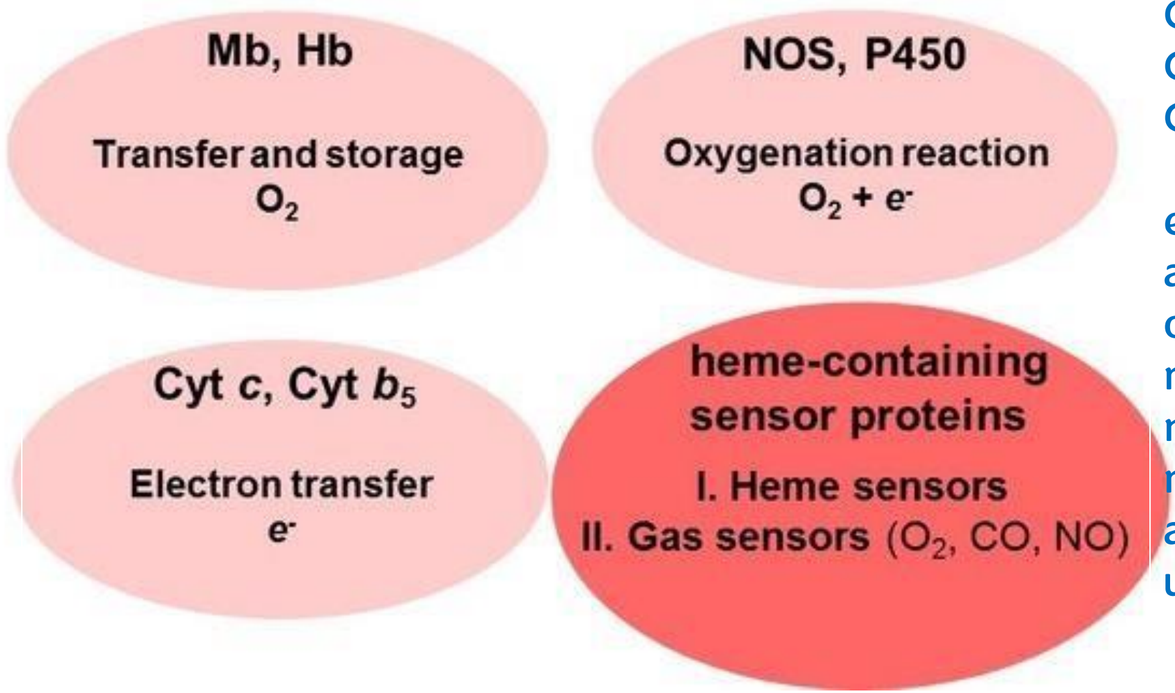


The protein environment dictates the function of the heme.

Heme is an organic molecule that is made up of 4 rings + iron in the middle
It's precursor is protoporphyrin
The heme (prosthetic group & non protein) is tightly bound covalently to the protein

***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.*

Mb: Myoglobin
Hb: Hemoglobin

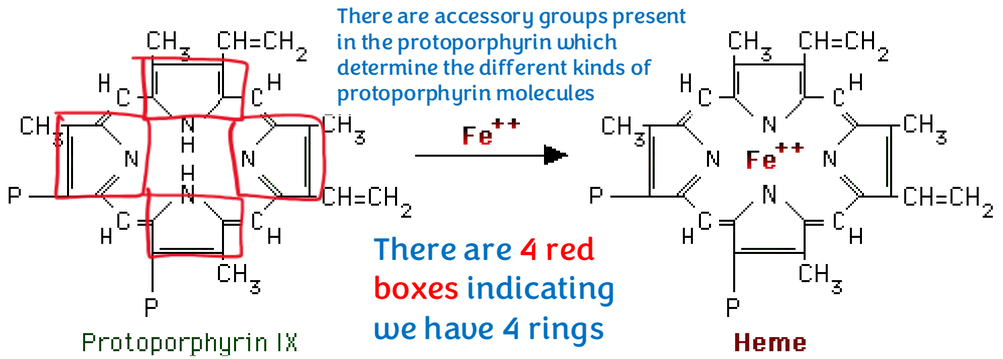


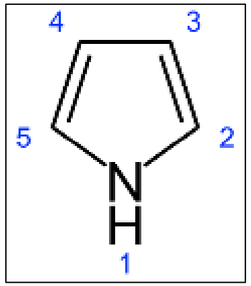
Cytochrome
Cyto: Cell
Chrome: Color
P450 are enzymes that are used in oxygenation reaction, number 450 represents its absorption wavelength

- Heme 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.

Note: The Dr accidentally said in the lecture "adding oxygen" to the protoporphyrin. This is not true as we add iron (Fe++) to form the heme which is also shown in the image ------>

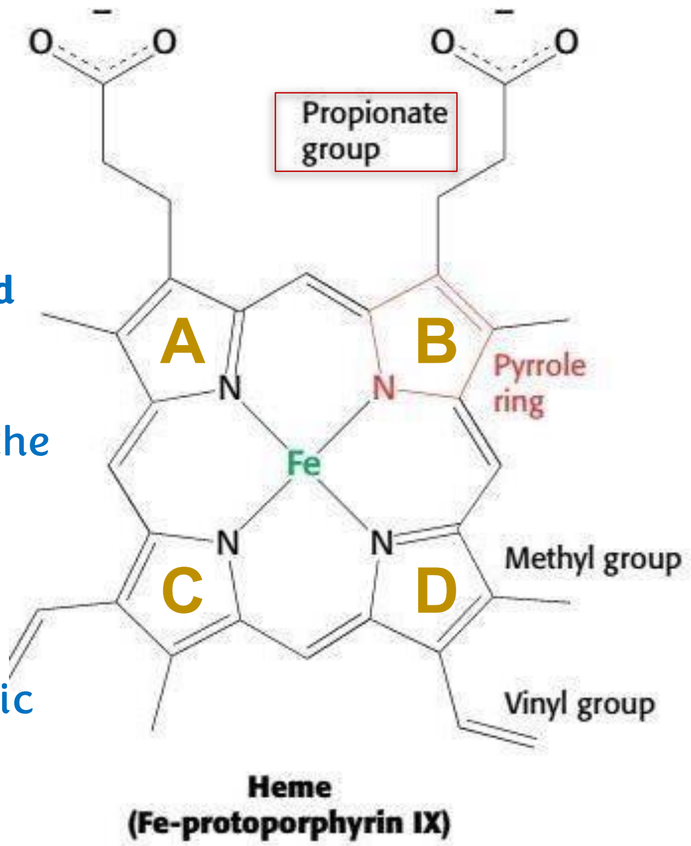
DO NOT MEMORIZE STRUCTURES





Pyrrole rings **designated as A,B,C,D**
(2 have propionate groups with methyl groups while the other 2 have vinyl groups with methyl groups)

The heme molecule is predominantly hydrophobic (it is also hydrophilic because of the propionate groups)



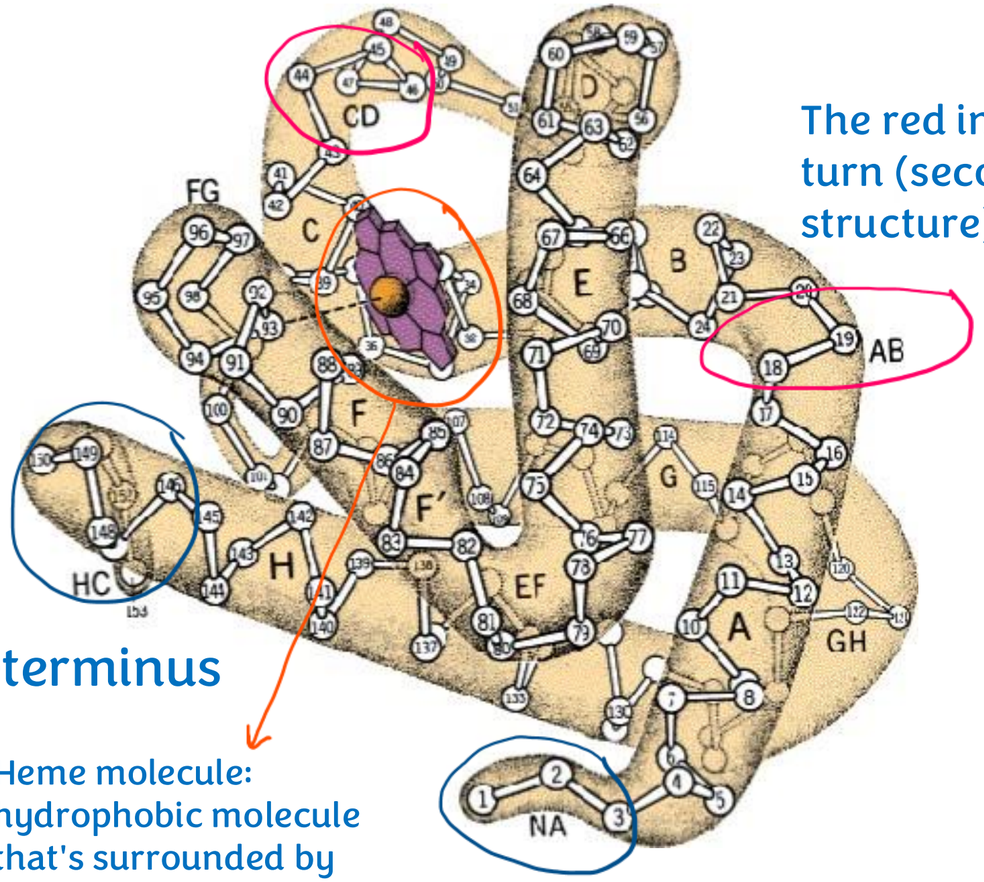
Heme
(Fe-protoporphyrin IX)

Iron (Fe++) is found in the middle and it can form 6 covalent bonds (coordinates) 4 of them are used for the pyrrole rings
1 for the proximal histidine
1 for O2 binding site (will be explained in details later)

The factor that determines the function of the heme group is the amino acids (protein) that surround it

- Myoglobin is a monomeric protein that is mainly found in muscle tissue.
- The tertiary structure of myoglobin contains 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 pocket.
- It contains an internal heme as a prosthetic group.
- Myoglobin can be present in two forms:
  - oxymyoglobin (oxygen-bound)
  - deoxymyoglobin (oxygen-free)

Structure of myoglobin



The red indicates a turn (secondary structure)

C-terminus

Heme molecule: hydrophobic molecule that's surrounded by hydrophobic amino acids for stabilization & positioning

N-terminus

It is a globular protein (3D structure)
On the surface (mainly): hydrophilic polar amino acids
On the inside (mainly): hydrophobic non-polar amino acids
However we do have hydrophobic amino acids on the outside & hydrophilic amino acids on the inside but in low amounts.

The heme has iron in the middle which binds with oxygen, this causes myoglobin to have two forms (either oxymyoglobin or deoxymyoglobin)

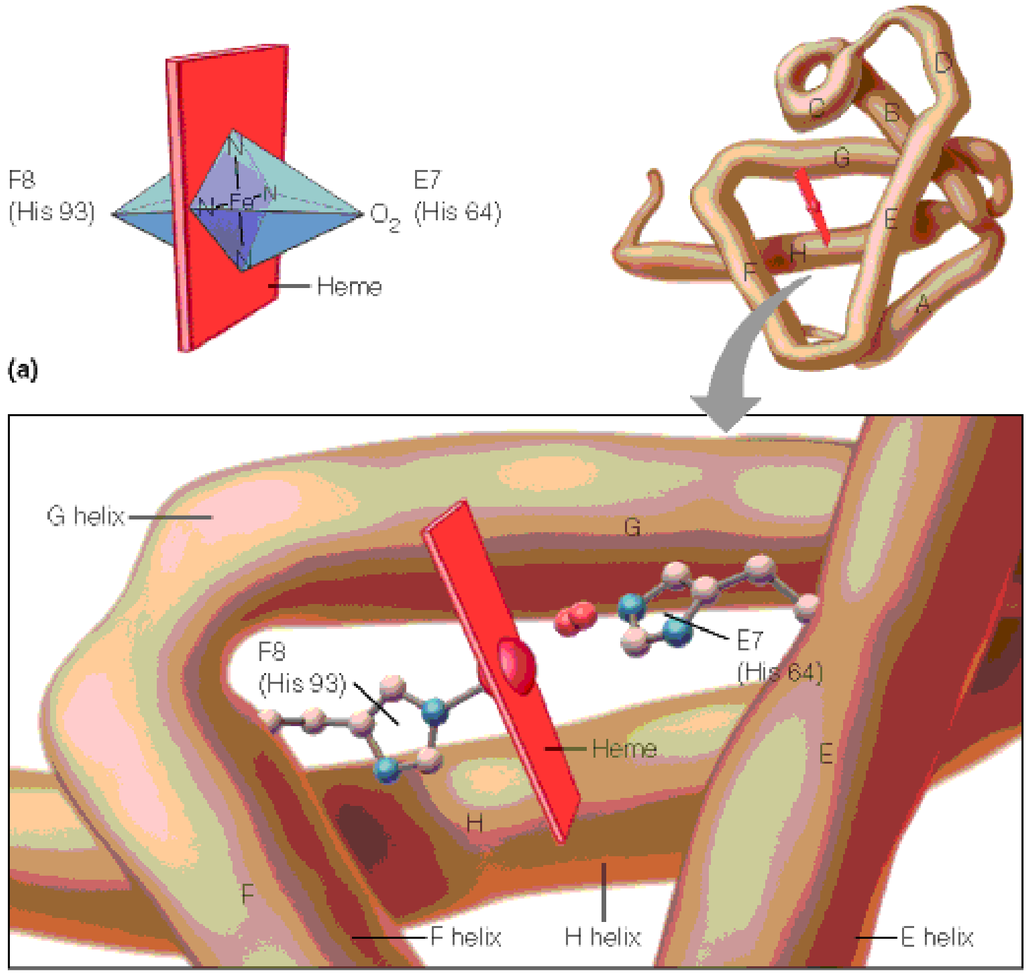
Arrangement of amino acids

Myoglobin doesn't directly bond with the oxygen , instead the iron in the heme group in myoglobin does bond with O2.

- 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.

E7 is the distal histidine because it is much more further away from the heme group compared to F8

There is a covalent bond between the proximal histidine and iron

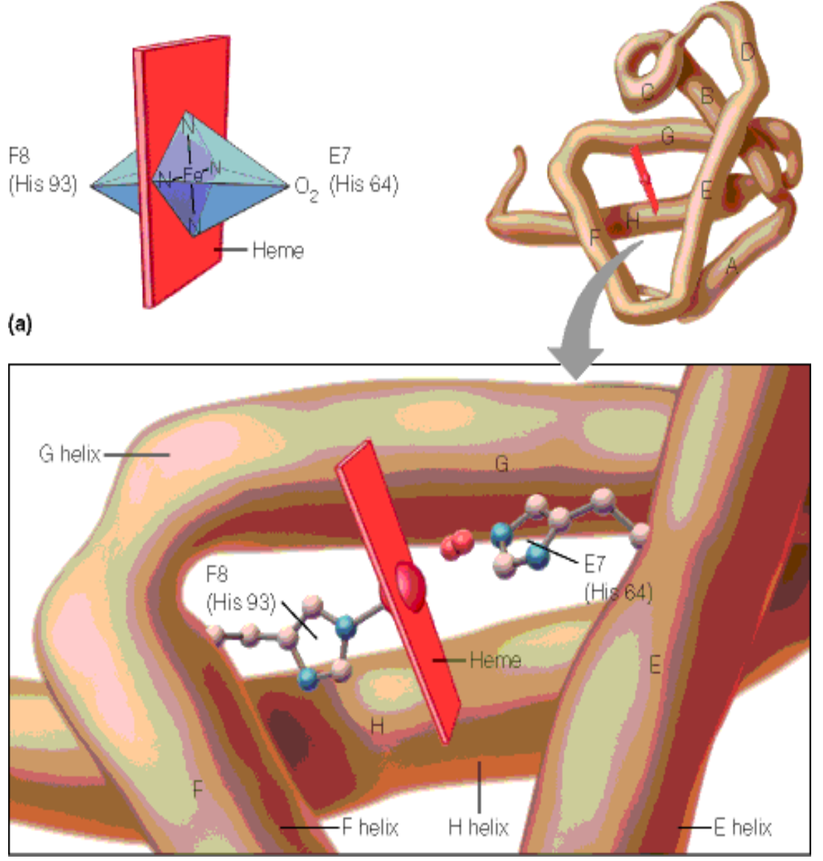


F8 = the amino acid in on helix "F" and it's the 8th amino acid in the F helix. (it is also called His 93 which is its order in the whole protein.)

DO NOT MEMORIZE HISTIDINE NUMBERS

Iron

For iron to bind with oxygen It should be reduced Fe+2 (ferrous state). If the iron is oxidized or the ferric state (Fe+3) Or the ferric state it can not bind to O2.

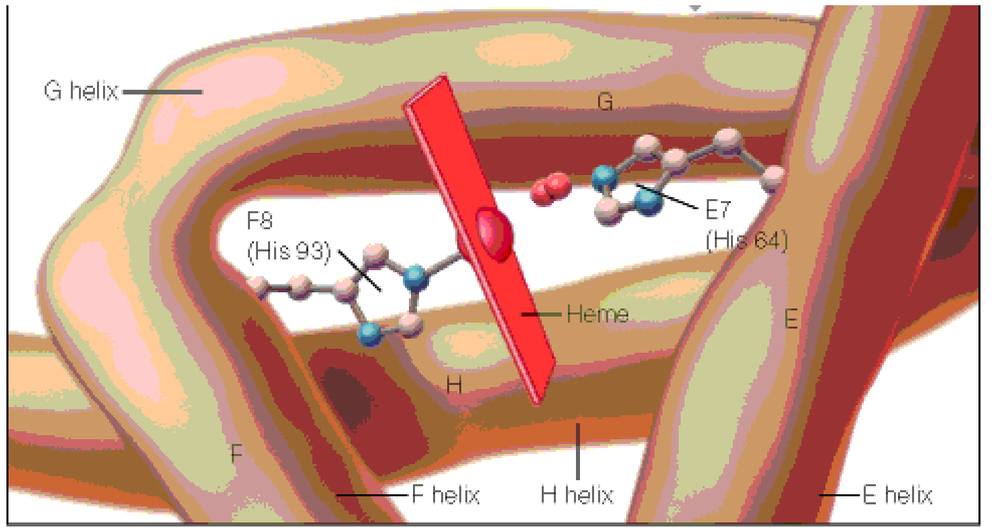


- Fe is in the ferrous state (Fe^{2+} and can form 6 bonds:
  - 4 bonds with the nitrogen of the rings,
  - One bond (known as the fifth coordinate) with the nitrogen of the proximal His.
  - Another one with O_2 (the sixth coordinate) when O_2 is there
- Upon absorption of light, heme gives a deep red color. (The red color in muscles or the meat of animals and humans isn't blood, instead it is the color of the heme group when it absorbs light).
- Oxidation of iron to the **Fe^{3+}**, the ferric state cannot bind to **O_2**.

Structure-function relationship (myoglobin)

Heme's position in myoglobin is stabilized by the presence of hydrophobic amino acids around it.

- 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^{2+} state and can bind to another O_2.
- The distal histidine acts as a gatekeeper that opens and closes as O_2 enters the hydrophobic pocket to bind to the heme.
- It also stabilizes the interaction with oxygen.



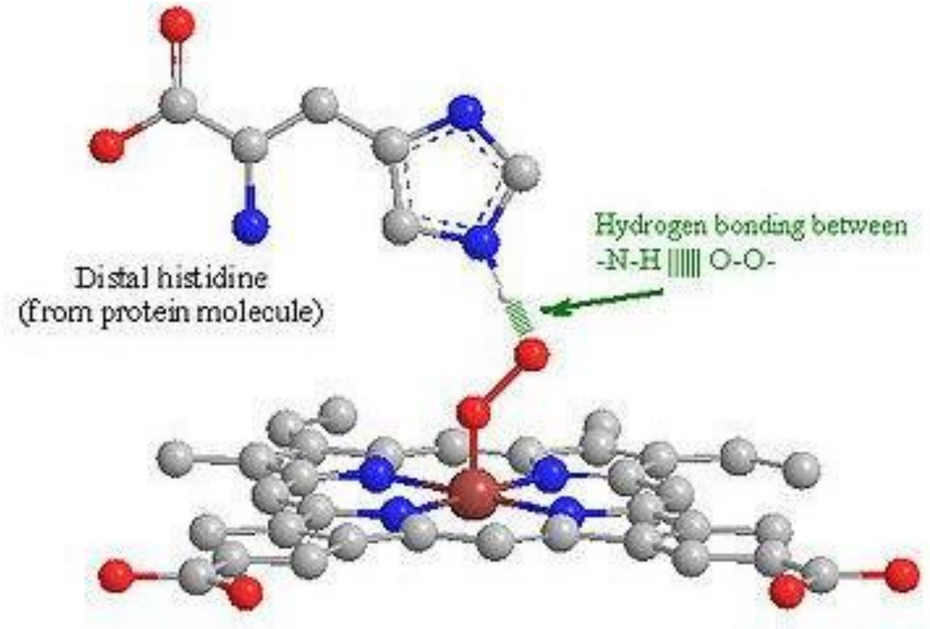
While The role of the proximal histidine is to "anchor" the heme molecule

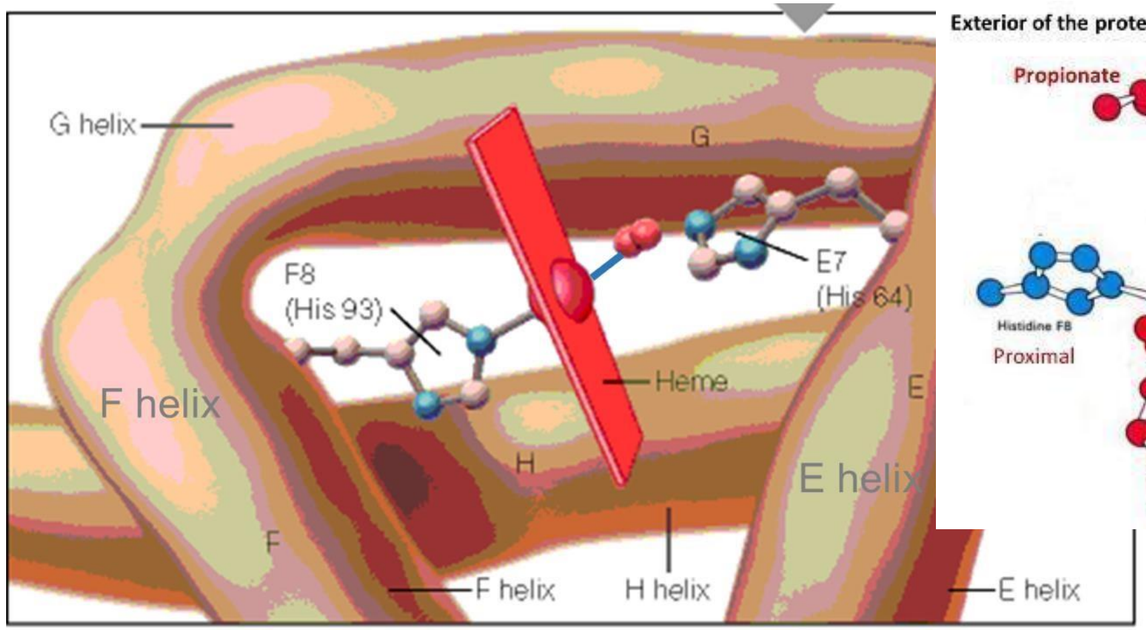
Usually when iron is oxidized and O2 is released from it, it becomes (Fe+3), but this doesn't happen here because the hydrophobic amino acids around iron prevent the oxidation of iron and iron stays in the ferrous state which is important to bind to O2.

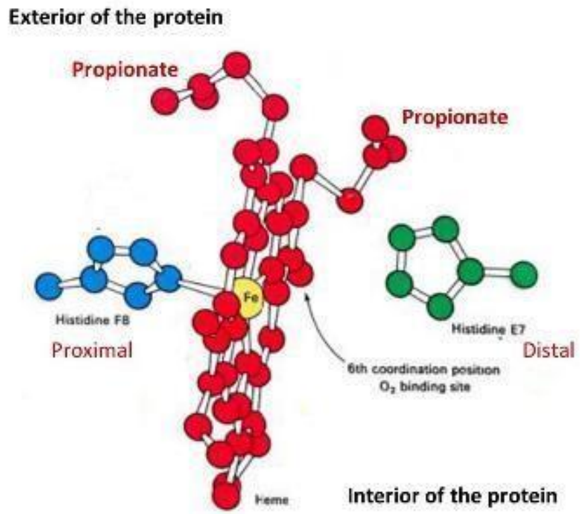
By forming a hydrogen bond with O2 that is binded to the iron in heme group, this also stabilizes the heme group. This interaction is reffered to as dipole-charge interaction as the fe in heme donates the electron density and the whole 'O2' molecule become polarised (partial negative) making it possible to form hydrogen bonds

Structure-function relationship (heme)

- The heme group stabilizes the tertiary structure of myoglobin.
- The planar heme group fits into a hydrophobic pocket of the protein and the myoglobin-heme interaction is stabilized by hydrophobic attractions.
- The hydrophilic propionate groups (**charged**) extend outward, interacting with the hydrophilic amino acids on the surface. **Which also stabilizes the heme group in the protein**







- Myoglobin function: storage of O2 in msucle tissues, and oxygen is released in cases of hypoxia (low oxygen amounts in muscles).

- PO2 in muscle tissues is approximately **20 Torr= 20mmHg.** The heme affinity of binding to O2 should be high so that it's able to store O2 (when PO2 is high). Only In cases of emergency (hypoxia) where the PO2 is low, as muscle activity increases and PO2 decreases, myoglobin releases O_2 to meet the muscle's oxygen demand.

- Physiologists, biochemistry and pharmacology professors often use terms that have to do with (50%) such as:

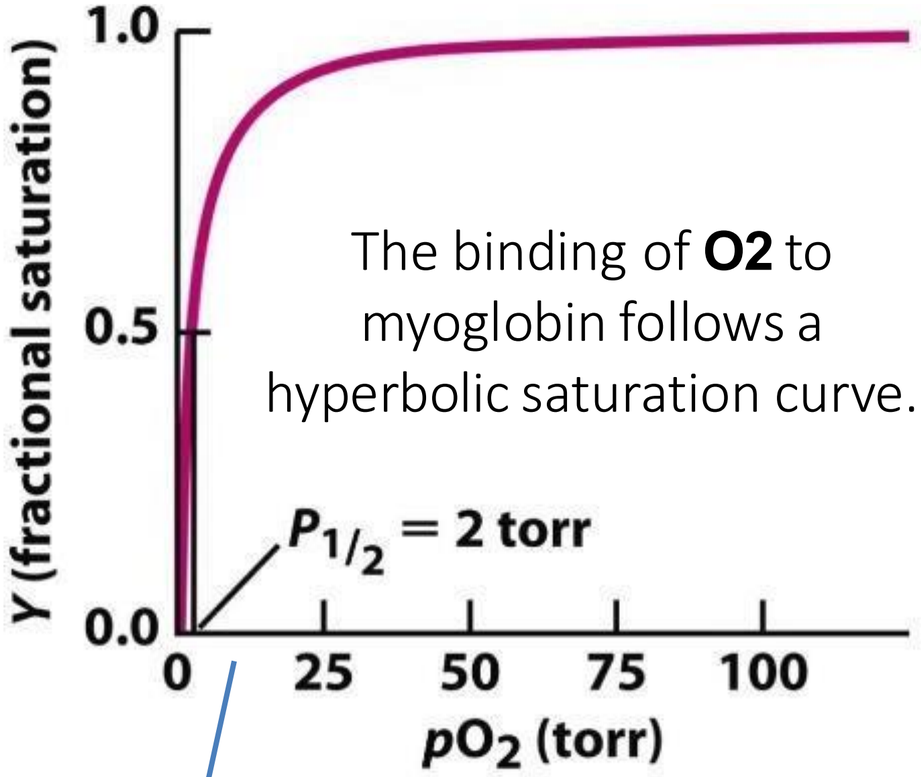
1. IC50: the concentration of a drug that makes it able to kill 50% of cells.

2. P50: oxygen pressure that is required to fill 50% of myoglobin/hemoglobin molecules.

This curve is an indicator of the strength of the affinity of the interaction .

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 20-40 mm Hg, it is almost fully saturated with oxygen at normal conditions.



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

O2 saturation curve.

The P50 of myoglobin is approximately 2 Torr. Also at 20 Torr, the saturation is almost 100% which means that the affinity is really high. Rule: the affinity of a molecule increases when it's P50 decreases.

Extra explanation : you might think that there is kind of contradiction between this rule and the info in the previous slide which states that" the heme affinity Of binding to O2 should be high so that it's able to store O2 (when PO2 is high). But notice that the P50 is a fixed value indicates how much pressure is needed to reach 50% saturation (in other words we can compare different affinities to different molecules or proteins. On the other hand, in the previous slide , we explain why the affinity should be high and when oxygen is bonded to the protein

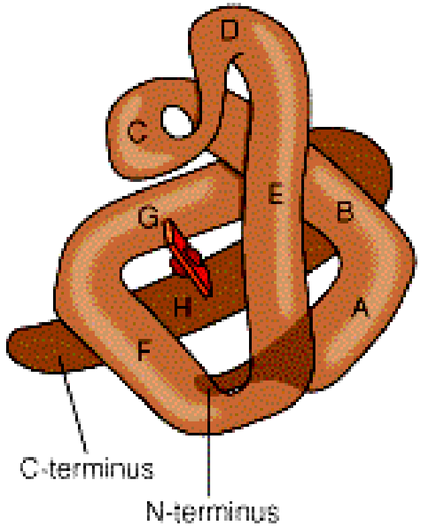
This curve is called a hyperbolic curve (it goes up really quick then becomes flat). The hyperbolic curve usually means the affinity is high for a molecule.

Hemoglobin

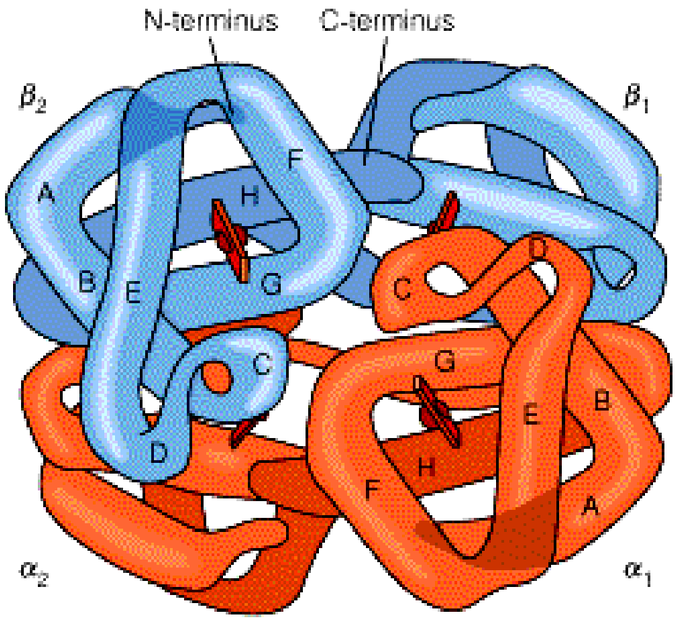
Hemoglobin structure It can be considered as a helical protein.

- Hemoglobin is a **heterotetrameric 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 α polypeptide has 7α– helices, while the β polypeptide has 8 helices. And there structure overall is quite similar to the myoglobin polypeptide.**



Myoglobin

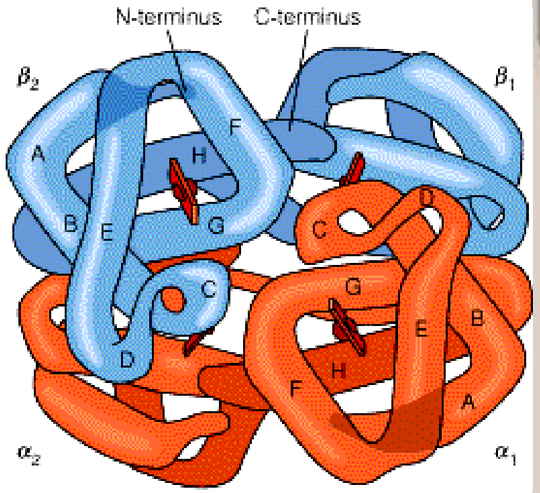


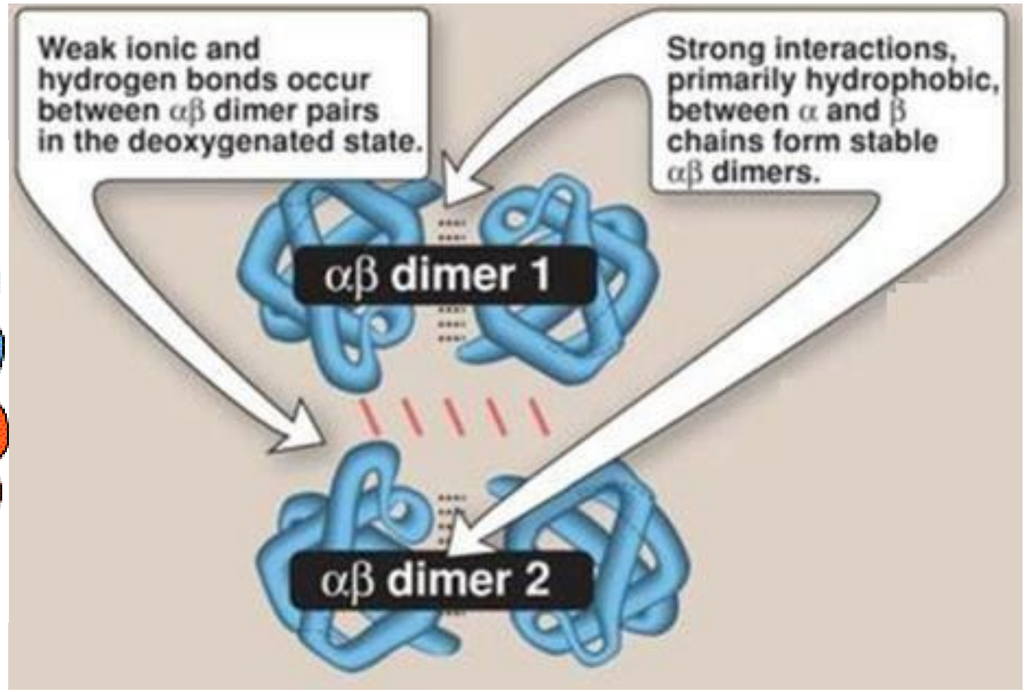
Hemoglobin

How are the subunits bound?

- A dimer of dimers (I made up this term) OR two αβ-protomers
  - $(\alpha\text{-}\beta)_2$
- The chains interact with each other via hydrophobic interactions. Mainly
  - Therefore, hydrophobic amino acids can also be present on the surface.

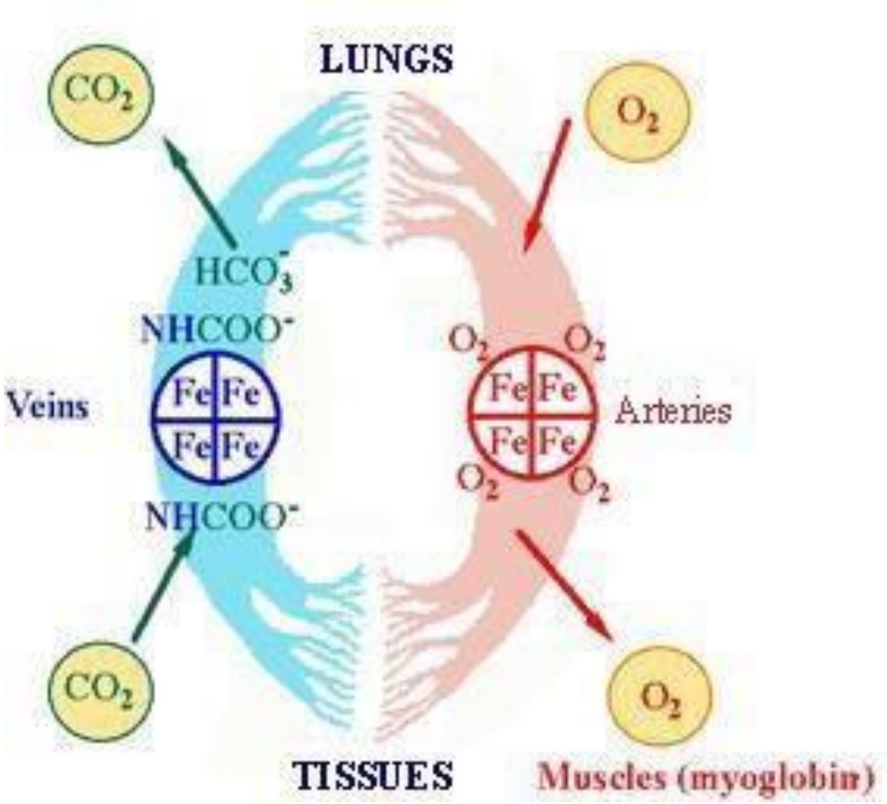
- Electrostatic interactions (salt bridges) and hydrogen bonds also exist between the two different chains.





Oxygen binding to hemoglobin

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

Both (depending on the location)

If a hemoglobin molecule had a low affinity it won't bind with a sufficient amount of oxygen and when it goes to the tissues it will be mostly empty

If it had a high affinity it will bind with oxygen efficiently at the lungs but it won't release oxygen where it is needed in the tissues ,so how can we overcome such obstacle??!!

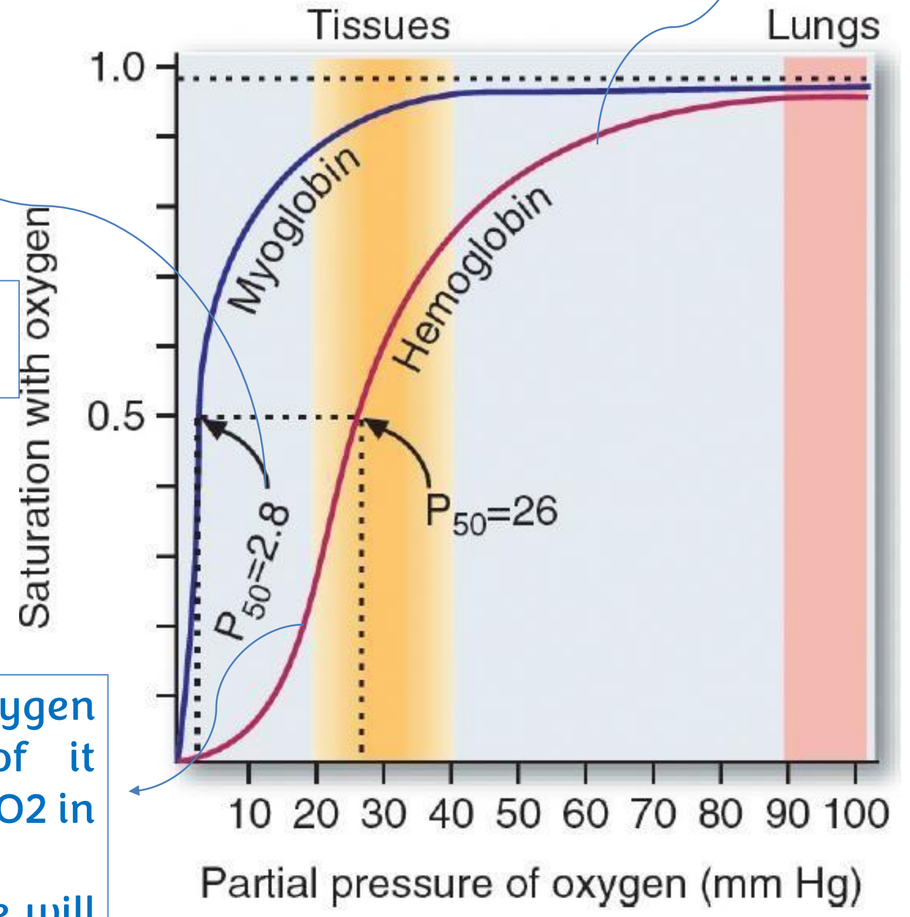
Actually, hemoglobin has 2 states high affinity state and a low affinity state meaning it has 2 structure a high affinity structure that is present in the lungs where it become saturated with oxygen and a low affinity structure that releases oxygen in the tissues

- The saturation curve of hemoglobin binding to O_2 has a sigmoidal shape like the letter S.
  - 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

Notice the difference in the P50 between hemoglobin and myoglobin u can indicate that myoglobing has a higher affinity since u need a small amount of O2 for 50% of the myoglobin to be saturated while U need a much much higher amount of O2 to saturate 50% of hemoglobin

U can notice that a great decrease in P O2 is countered by a smal, decrease in the degree of saturation this is due to high affinity

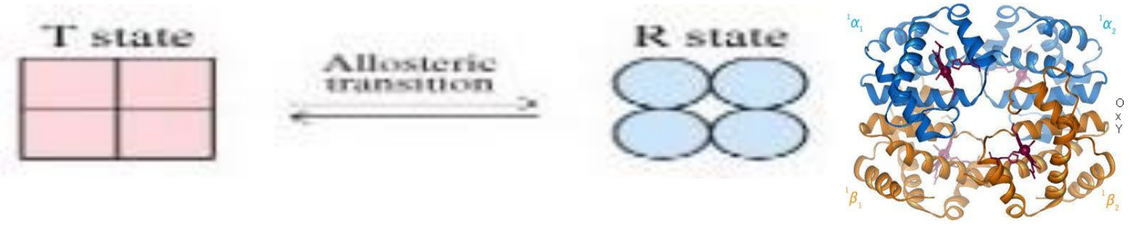
The oxygen saturation curve



Remember myoglobin structure is hyperpolic

The hemoglobin curve indicates it has 2 structures ons with a very high affinity for oxygen ,u can notice it in high pressures on O2
And the second structure is low affinity of O2 where which is present in low pressures of O2

And In a low pressure of oxygen there is a low affinity of it resulting in a swift release of O2 in the tissue
So when u reduce PO2 there will be huge decline in the saturation of Hemoglobin due to low affinity



We will study this concept thoroughly in enzyme lectures

Hemoglobin is allosteric

- Hemoglobin is an allosteric protein (from Greek "allos" = "other", and "stereos" = "shape").
  - 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 slightly changing its structure.
- Hemoglobin exists in two allosteric forms, T-state and R-state
  - The T-state, known as the "taut" or "tense" state, has a low binding affinity to oxygen.
  - The R-state, known as the "relaxed" state, has 500 times higher affinity to oxygen than the T conformation.
- The binding of O_2 causes conformational changes in hemoglobin, converting it from the low-affinity T-state to the high-affinity R-state.

The change between High affinity state and Low affinity state require a change in the structure and usually this change is subtle ;

-When the protein is in the high affinity state we say it is in R (relaxed) state

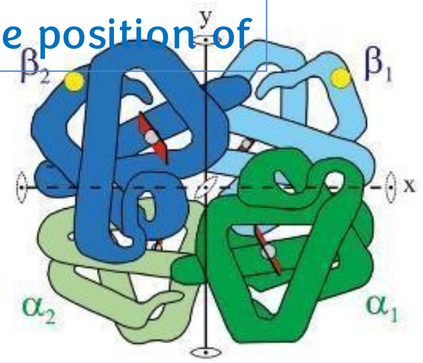
-When it is in low affinity state we say it is in T (taut/tense) State

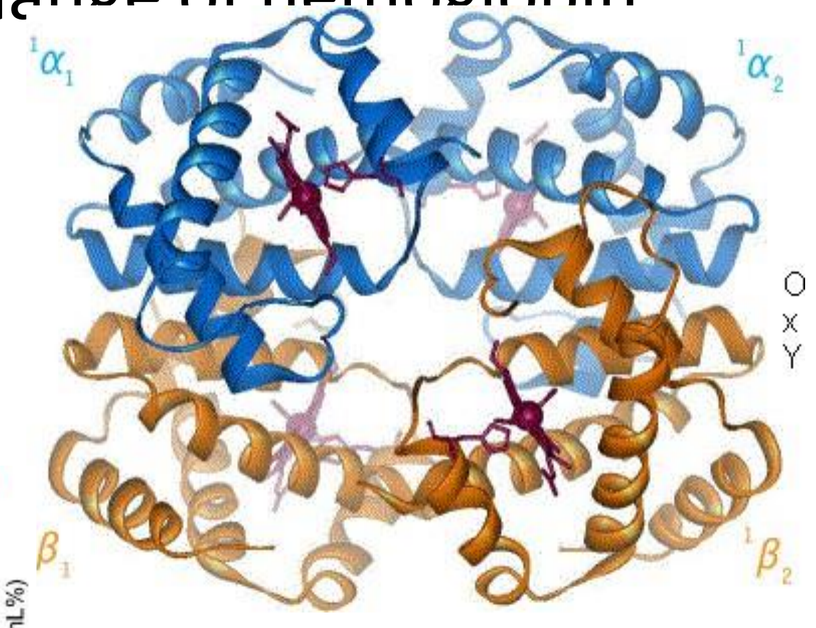
When a protein has 2 states high affinity and low affinity like hemoglobin or high activity and low activity like enzymes we call it an allosteric protein (protein with different structures). Allosteric proteins are multimeric (quaternary structure).

When there is much O2 singing Hemoglobin is in R state and when there isn't that much O2 as in tissues the Hemoglobin is in T state

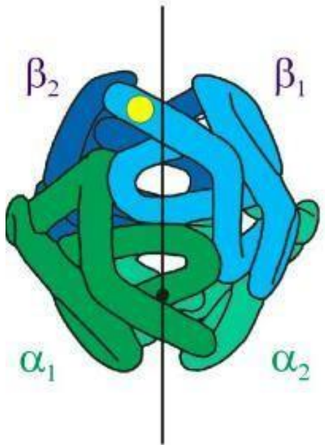
Notice a small movement results in the conversion between T and R states when we talk in distance it is about (0.4Å)m which is0.4 × 10^(−10) meters
The change is in the position of the atoms

Structural change of hemoglobin

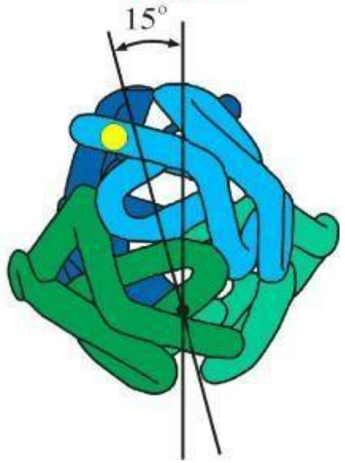


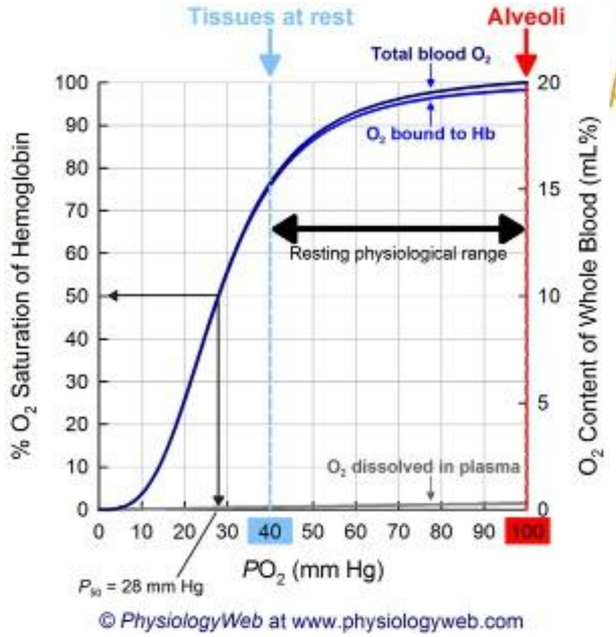


deoxy T



oxy R

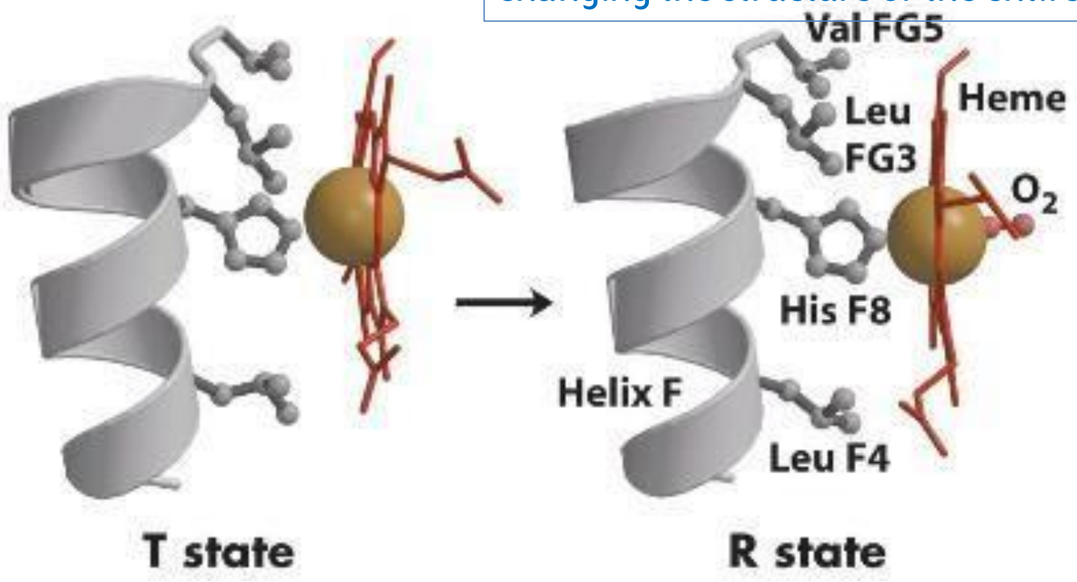


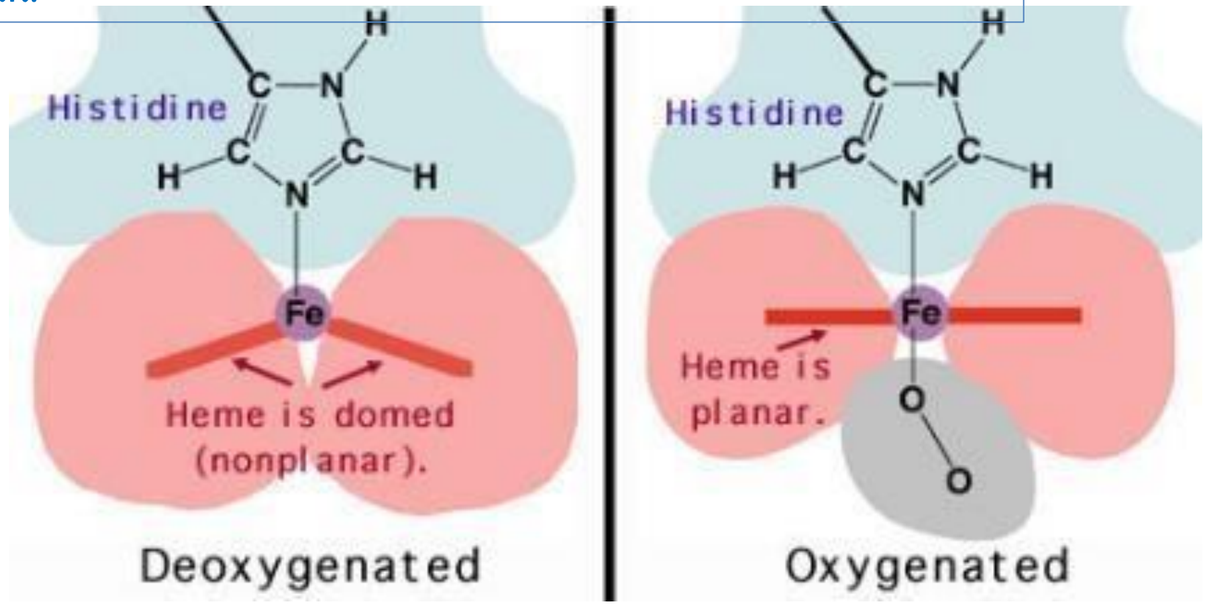


How does the structure change? (1)

- When heme is free of oxygen, it has a domed structure and iron is outside the plane of the heme group.
  - Because the hydrophobic heme is repelled by the proximal His.
- When oxygen binds to an iron atom, it is attracted to the distal His heme adopts a planar structure and the iron moves into the plane of the heme pulling proximal histidine (F8) along with it.

In (derby structure) notice the proximal histidine is attached directly to the iron atom. You can see that the heme group is bent around iron atom because it is hydrophobic and doesn't like the hydrophilic nature of histidine , but when the O2 molecule binds to Fe(in the oxygenated structure) it binds to the distal histidine through hydrogen bonding which stretches the heme group making it planar and stretching the helix of the proximal histidine changing the tertiary structure in about 0.4Å collectively changing the structure of the entire protein.

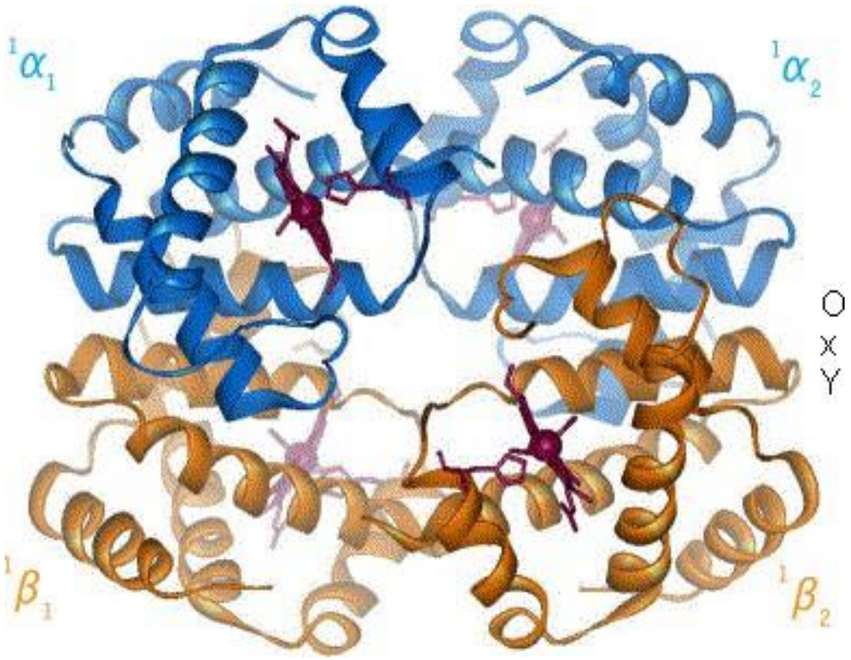




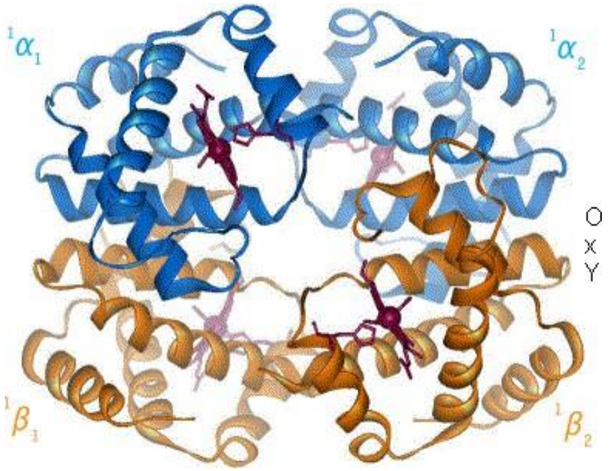
How does the structure change? (2)

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

In myoglobin, the movement of the helix does not affect the function of the protein because it is not allosteric.

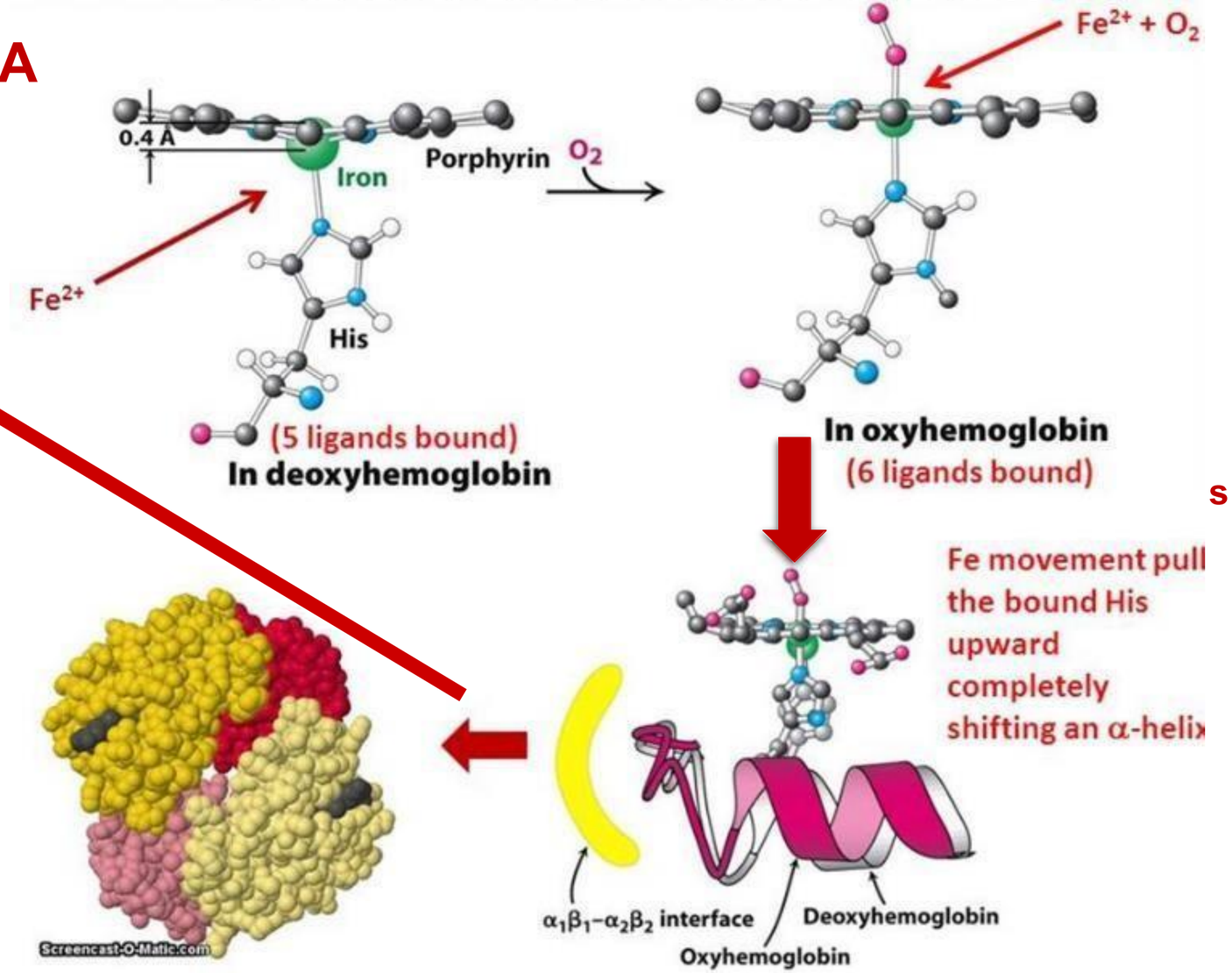


- **Changes in tertiary structure of individual hemoglobin subunits**
- **Breakage of the electrostatic bonds at the other oxygen-free hemoglobin chains.**

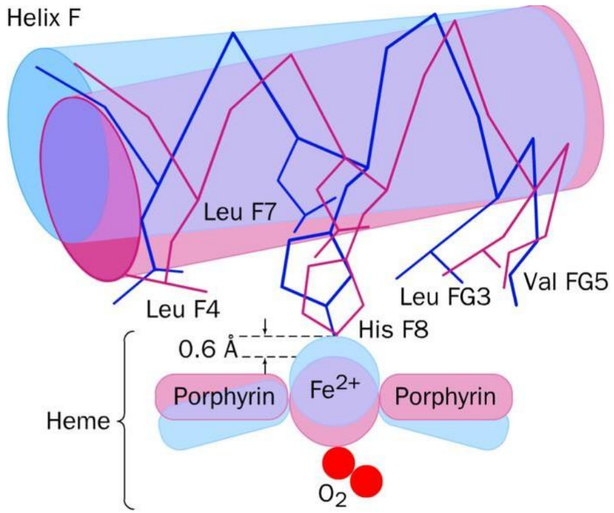


Structural amplification change

0.4 °A



Flat with oxygen

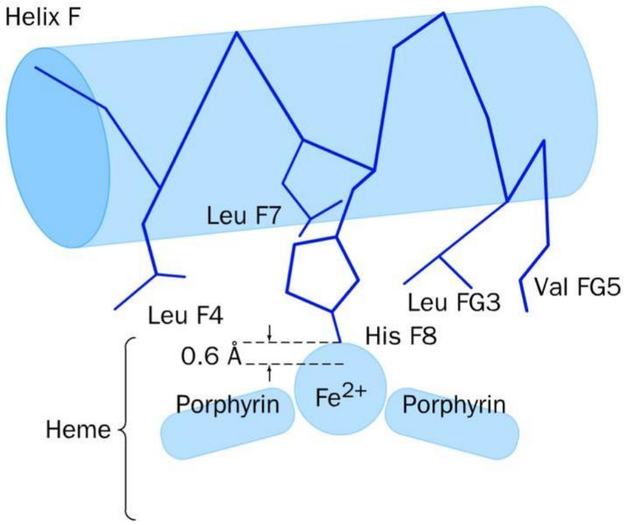


Another look at it

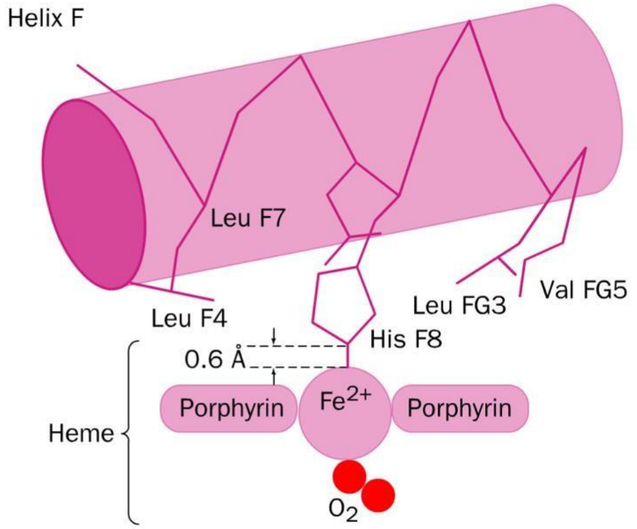
Movements of the hemoglobin's heme and F helix during the T → R transition.

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.

Bent without oxygen



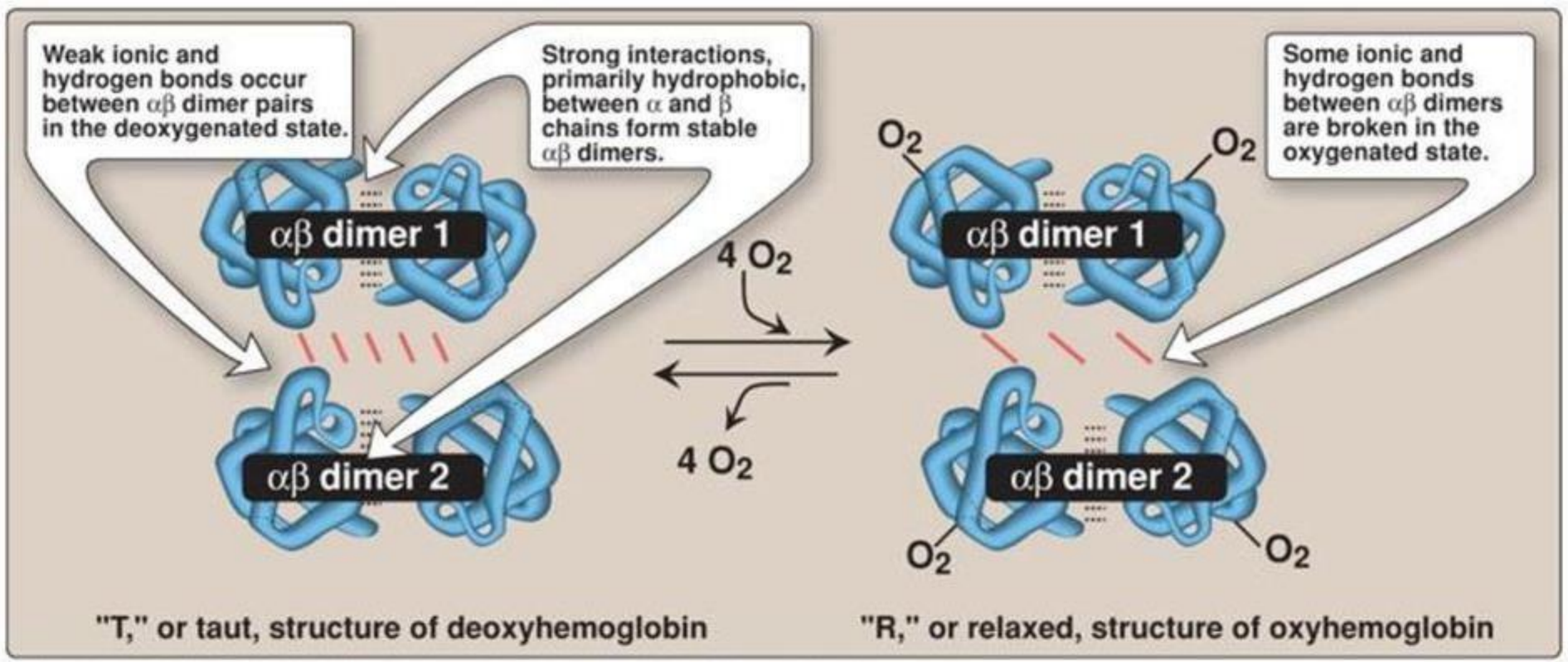
In the absence of bound O_2 ,the Fe(II) lacks a sixth ligand, and resides about 0.6 Angstrom out of the plane of the heme toward its His ligand (the proximal His).



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.

Between each a-B dimers there are electrostatic interactions when we move the heme slightly which moves the helix this results in breaking some electrostatic interactions between the dimers so the protein will change from T state to R state

Electrostatic interactions are broken



Quiz time

https://forms.gle/4TxD6YHTS9oD7yMJA

Good luck!!

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	13 28	Oxygen affinity of binding to O2 moves the helix this results in breaking some hydrophobic interactions between the dimers	Heme affinity of binding to O2 moves the helix this results in breaking some electrostatic interactions between the dimers
V1 → V2			

Additional Resources:

Reference Used:

Prof. Mamoun Ahram recorded lecture

Extra References for the Reader to Use:

Marks’ Basic Medical Biochemistry (6th edition)

رسالة من الفريق العلمي: