

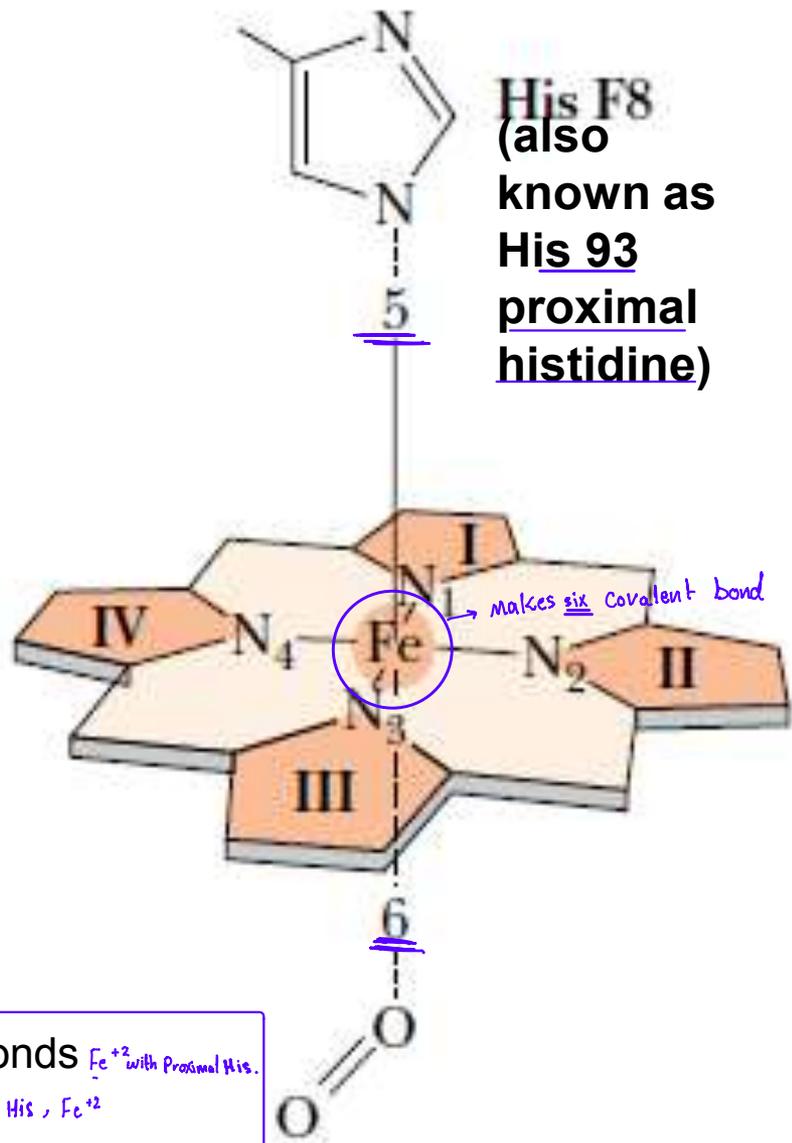
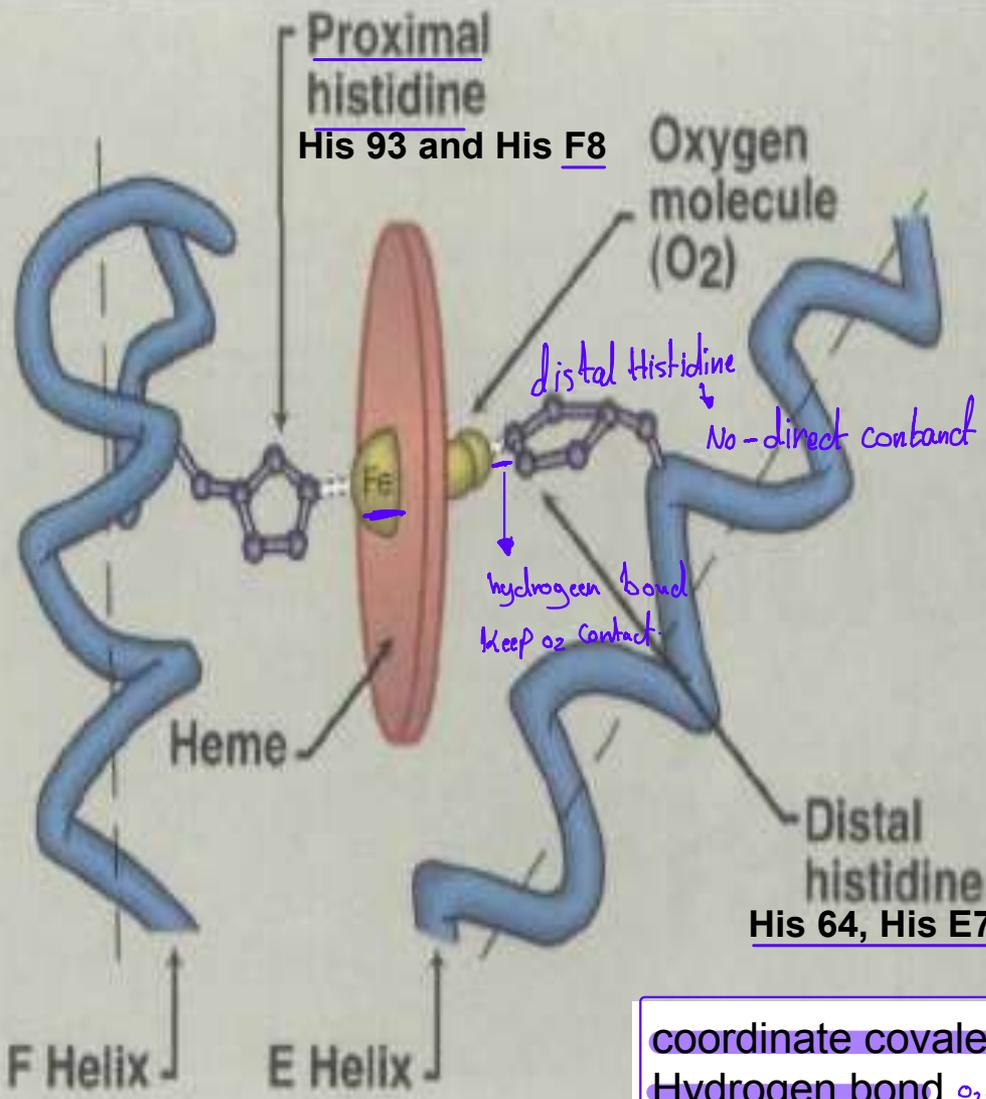
Hemoglobin & Myoglobin

Myoglobin (Mb) store O₂

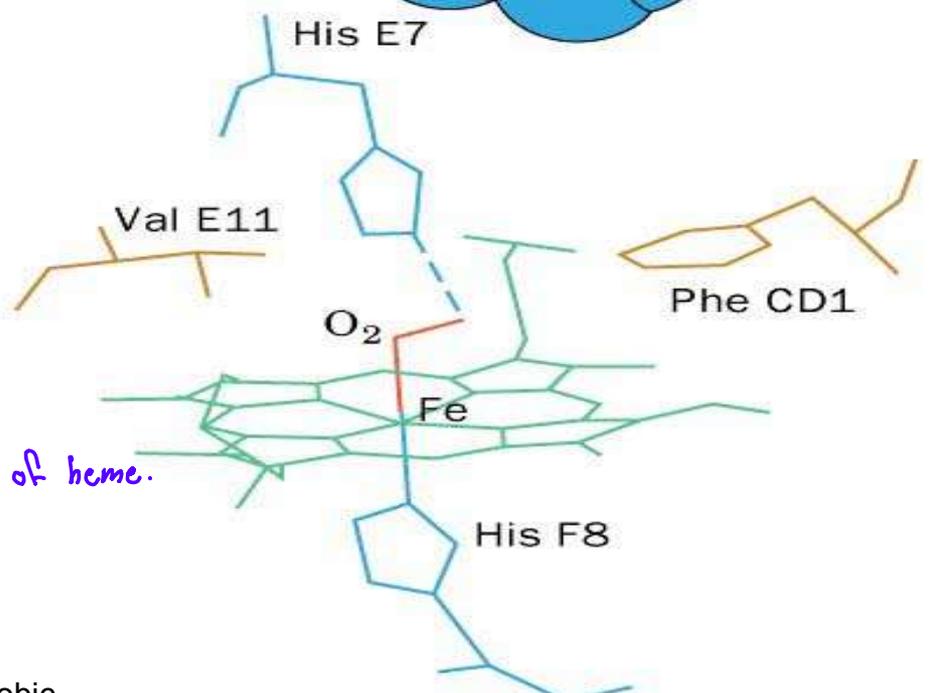
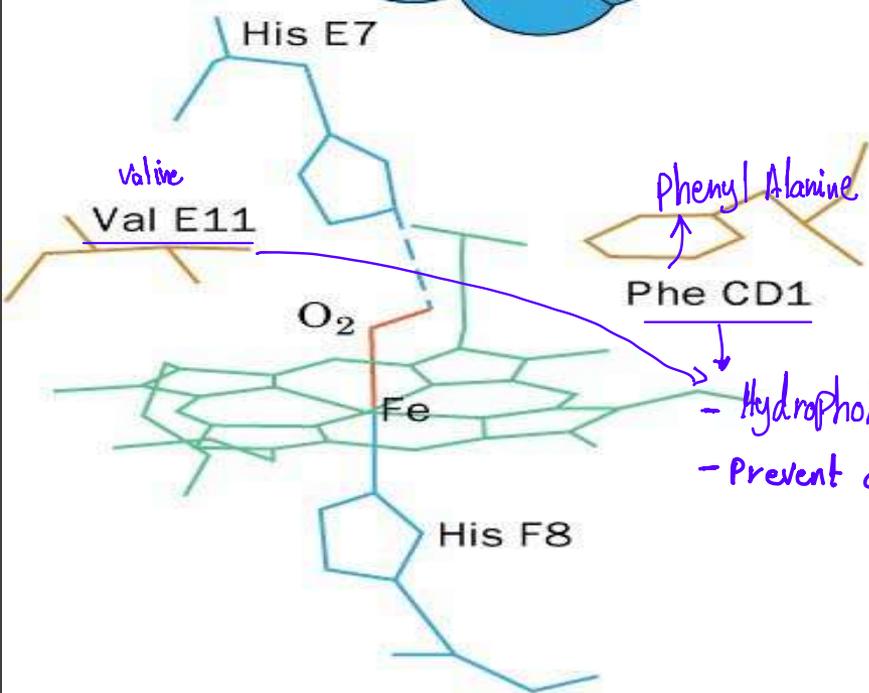
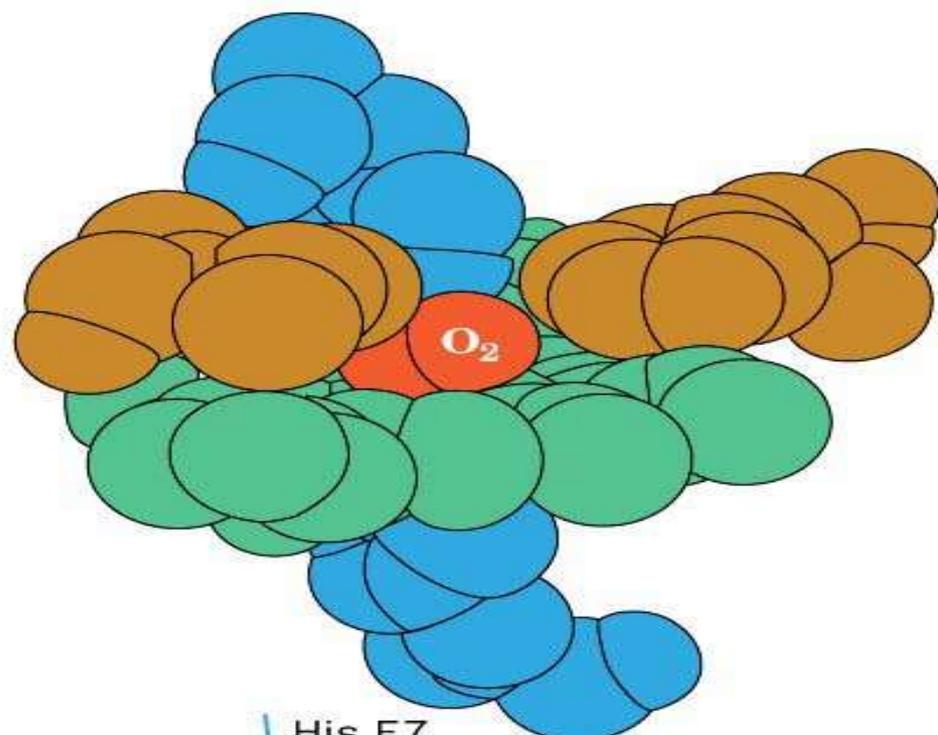
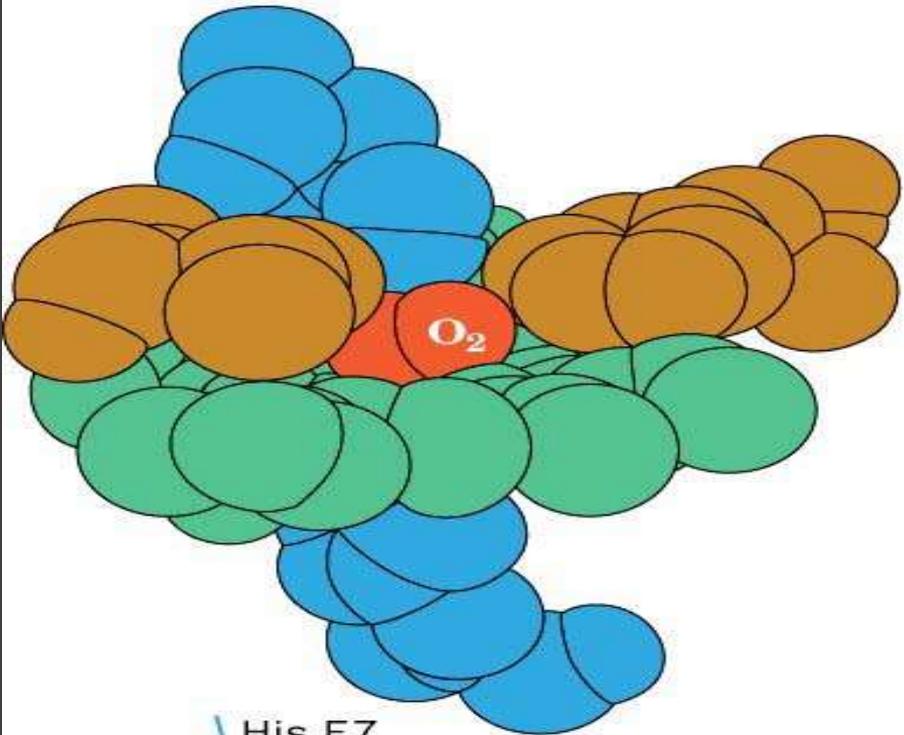
- # Intracellular heme protein found in most cells
- # Stores and facilitate oxygen diffusion in muscles especially in heart and skeletal muscle. → “Hb releases O₂ to the tissues, where Mb binds and stores it, then delivers it to mitochondria when needed.”
- # It binds the O₂ released by hemoglobin
- # Myoglobin consists of a single polypeptide chain of 153 amino acids attached to a single heme group
- # About 80% of myoglobin proteins are α helix.
- # It consists of eight α helical segments, these are termed helices A–H.
- # Each helical segment is terminated either by the presence of proline or by β-bends and loops.
- # The eight α helical segments are folded into a globular structure, creating a cradle (box) and within this cradle lies a single heme group and the binding site of O₂.
- # The heme of myoglobin lies between helices E and F.
- # The polypeptide of myoglobin may be viewed as serving three critical functions: 1- it hold the heme group, 2- it provides a pocket into which the O₂ can fit, and 3- it protects the heme iron atom from oxidation.

Structure of heme in myoglobin and hemoglobin

- Both myoglobin and hemoglobin have heme.
- Heme has similar structure in myoglobin and hemoglobin
- Heme is a complex of porphyrin and ferrous iron (Fe^{2+}).
- Porphyrins are a group of organic compound that have four pyrrole subunits interconnected via α -methylene bridges ($=\text{CH}-$)
- A pyrrole ring is a group of four carbon atoms and a nitrogen atom bonded together in a ring (see figure).



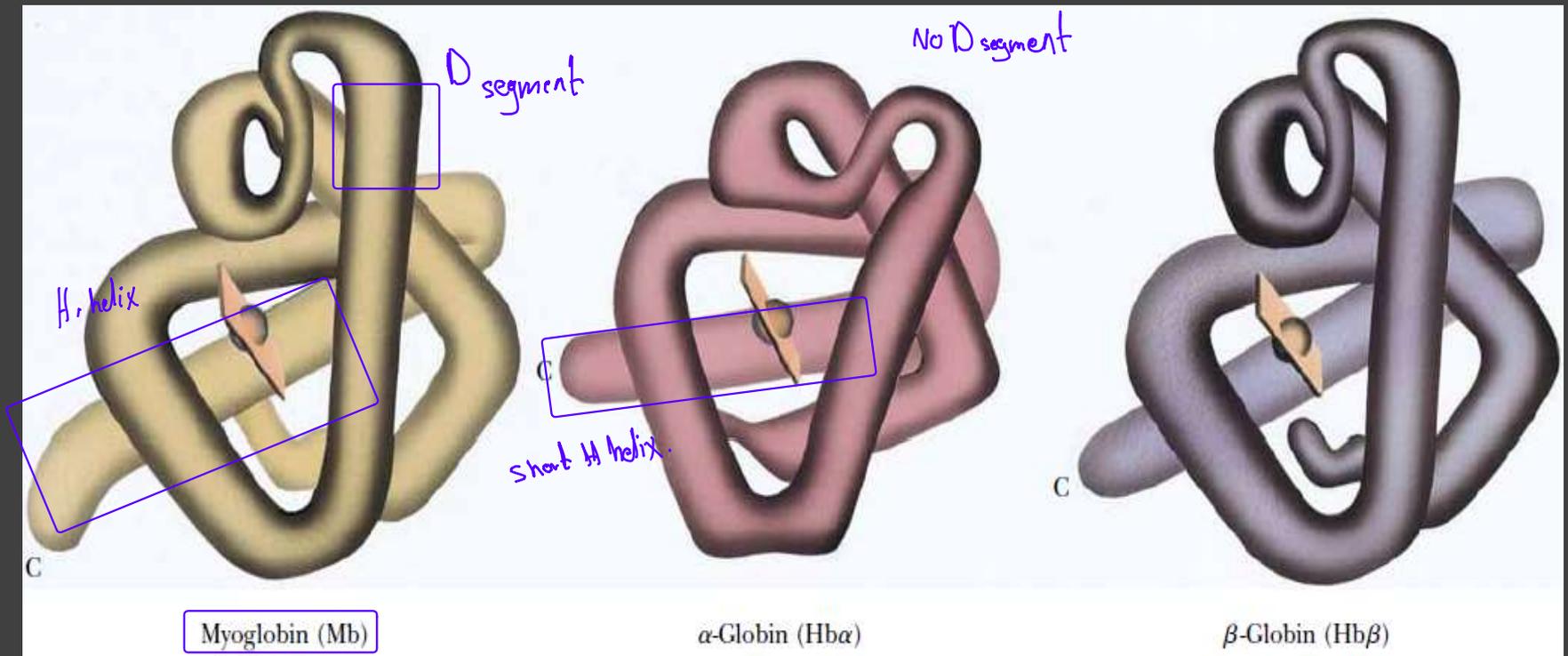
coordinate covalent bonds Fe⁺² with Proximal His.
Hydrogen bond O₂ with distal His, Fe⁺²



hydrophobic

- **Hemoglobin (Hb)**

- Hemoglobin (Hb) is a globular protein.
- Each human red blood cell contains approximately 270 million hemoglobin biomolecules
- A single hemoglobin consists of four polypeptide chains, 2 α chains and 2 of β chains, each of which is very similar structurally to the myoglobin polypeptide chain, and each bears a heme group.
- The α and β subunits differ in primary structure (i.e., they have different sequences of amino acids and are encoded by different genes).
- The β chain at 146 amino acid residues is shorter than the myoglobin chain (153 residues), mainly because the H helix is shorter.
- The α -chain at 141 residues also has a shortened H helix and lacks the D helix.



Myoglobin
153 aa

α- globin in Hb
 ← 141 aa
shortened H helix and lacks the D helix

12 A.A. shorted

153 → 141 ↑

β-globin in Hb
146
H helix is shorter

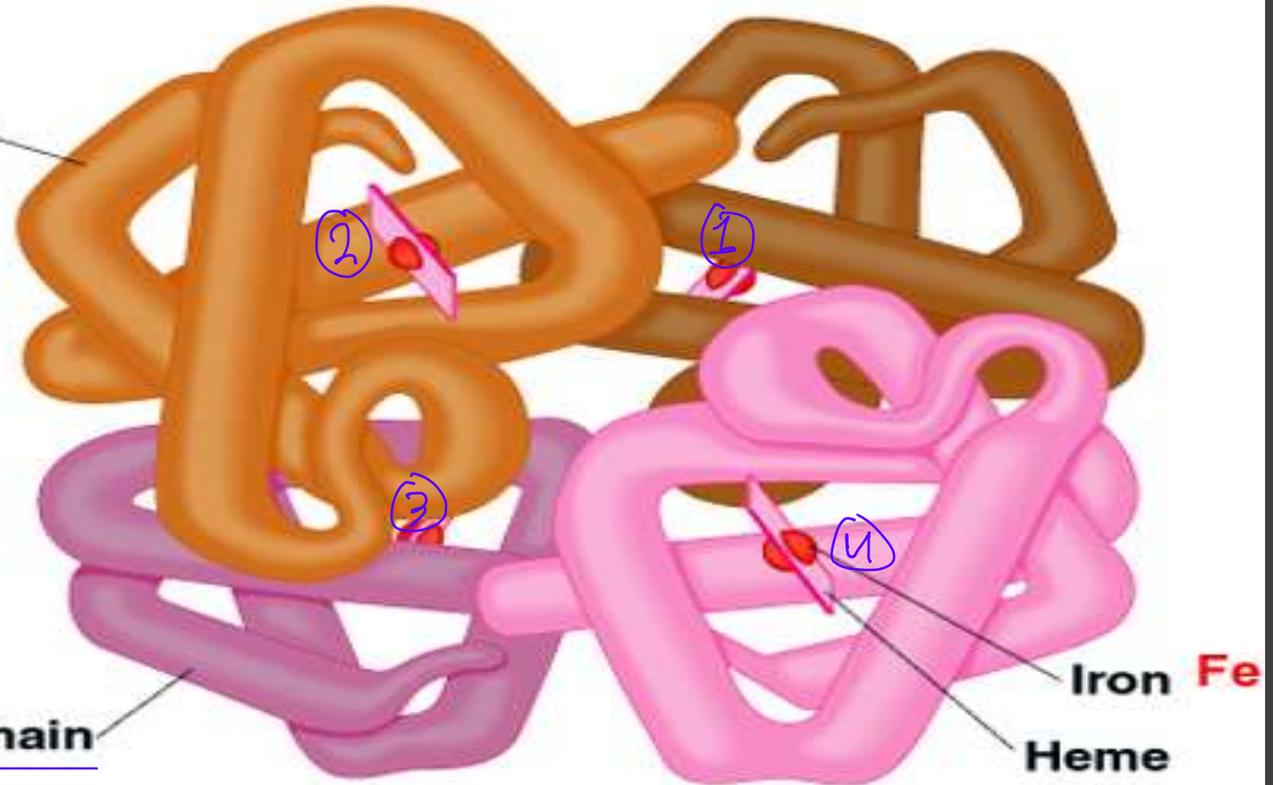
Hemoglobin

one HB can bind 4 O₂.

Polypeptide
chain

2 β chain

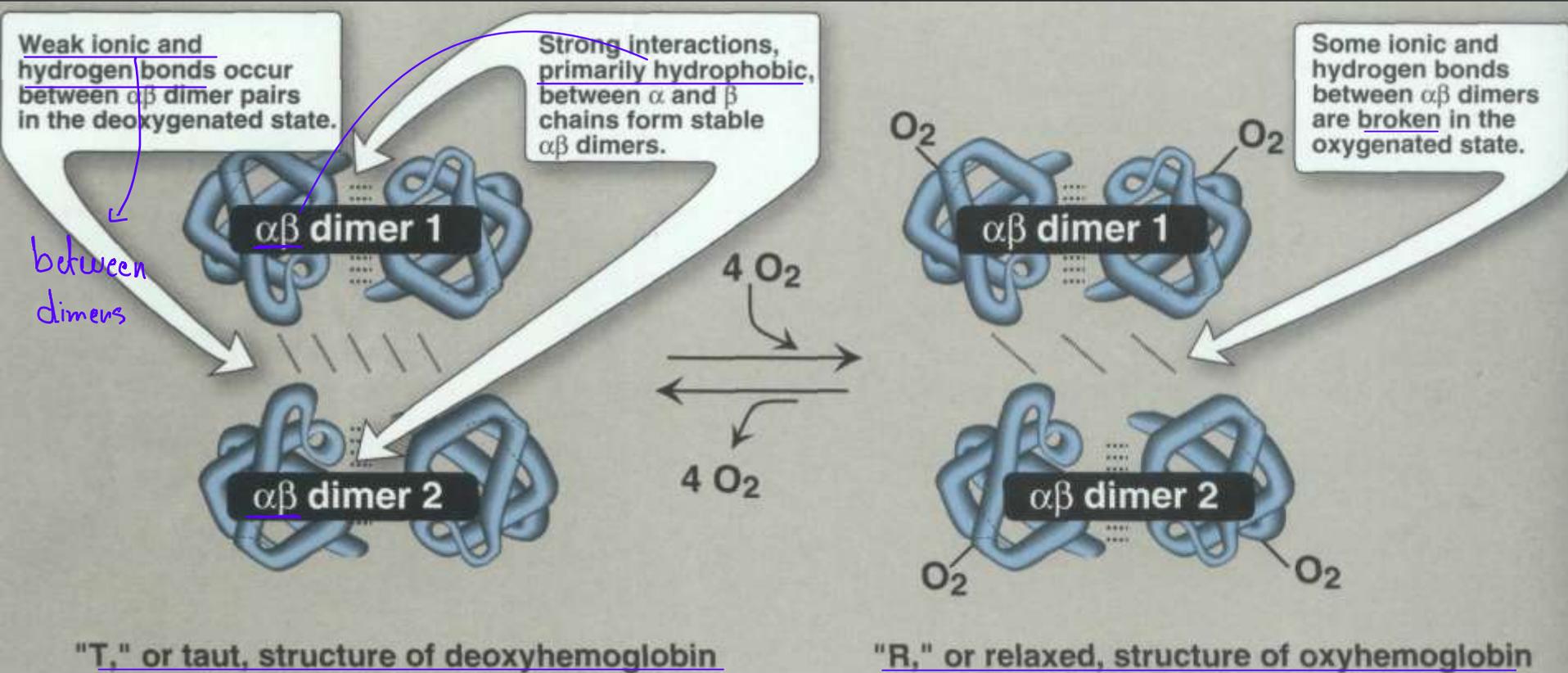
2 α chain



(b) Hemoglobin

Quaternary structure of hemoglobin

- The subunit interactions are mostly between dissimilar chains: each of the α -chains is in contact with both β -chains.
- Therefore there are two identical dimmers, dimer one $\alpha_1\beta_1$ and dimer two $\alpha_2\beta_2$.
- The two polypeptide chains within each dimer are held tightly together, primarily by hydrophobic interactions although ionic and hydrogen bonds play a role.
- The type of interaction between dimer 1 and dimer 2 is a weak ionic and hydrogen bonds.
- The ionic bond is a relatively weak ionic bond and is called salt bridge (salt bond).



- Globin chains can assume two formations
- **Taut form (T)**
 - Tends to release O_2
 - Favored form in tissues
 - Allows more release of O_2
- **Relaxed form (R)**
 - Holds on to O_2
 - Favored form in lungs
 - Allows more binding of O_2

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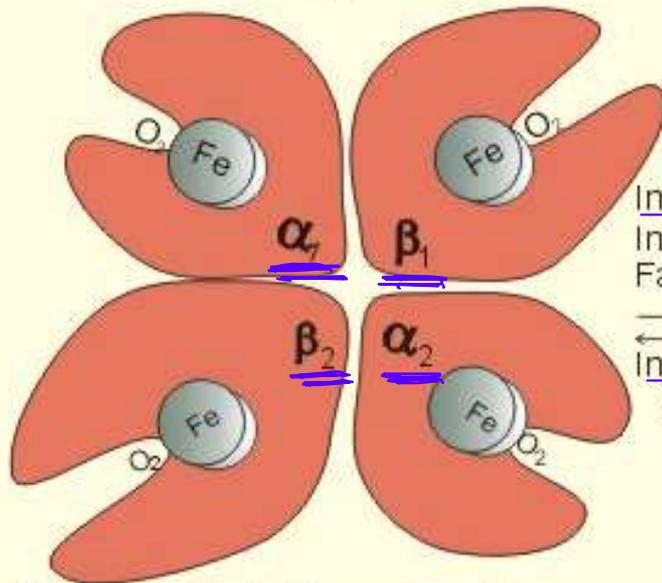
- **T & R forms of Hemoglobin**

- T form:** The deoxy form of hemoglobin is called the "T" (tense) form.
 - In the T form, the two $\alpha\beta$ dimmers interact through a network of ionic bonds and hydrogen bonds that constrain the movement of the polypeptide chains. The T form is the low oxygen-affinity form of hemoglobin.
- R form:** The binding of oxygen to hemoglobin causes the rupture of some of the ionic bonds and hydrogen bonds between the $\alpha\beta$ dimmers. This leads to a structure called the "R," or relaxed form, in which the polypeptide chains have more freedom of movement. The R form is the high oxygen-affinity form of hemoglobin.

Hemoglobin Structure Changes

Oxygen Binding and Unloading

Oxyhaemoglobin

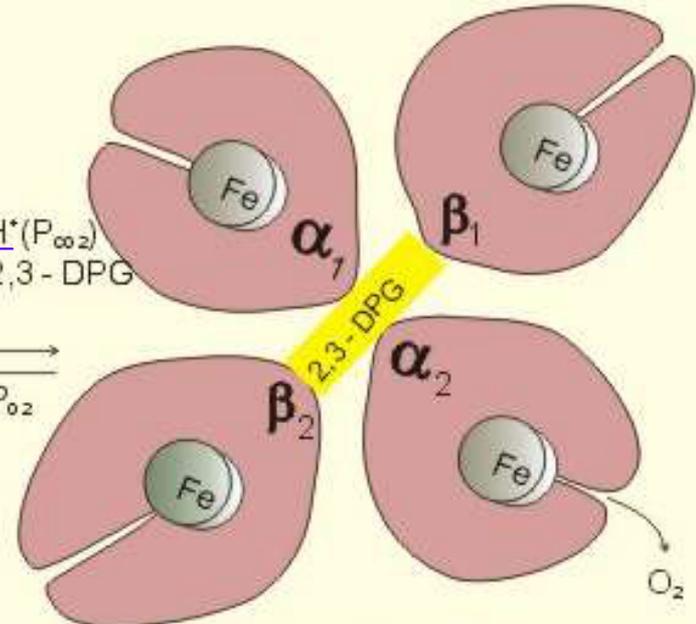


Relaxed binding structure

Increasing H^+ (P_{CO_2})
Increasing 2,3 - DPG
Falling P_{O_2}

Increasing P_{O_2}
CO

Deoxyhaemoglobin



Tight binding structure

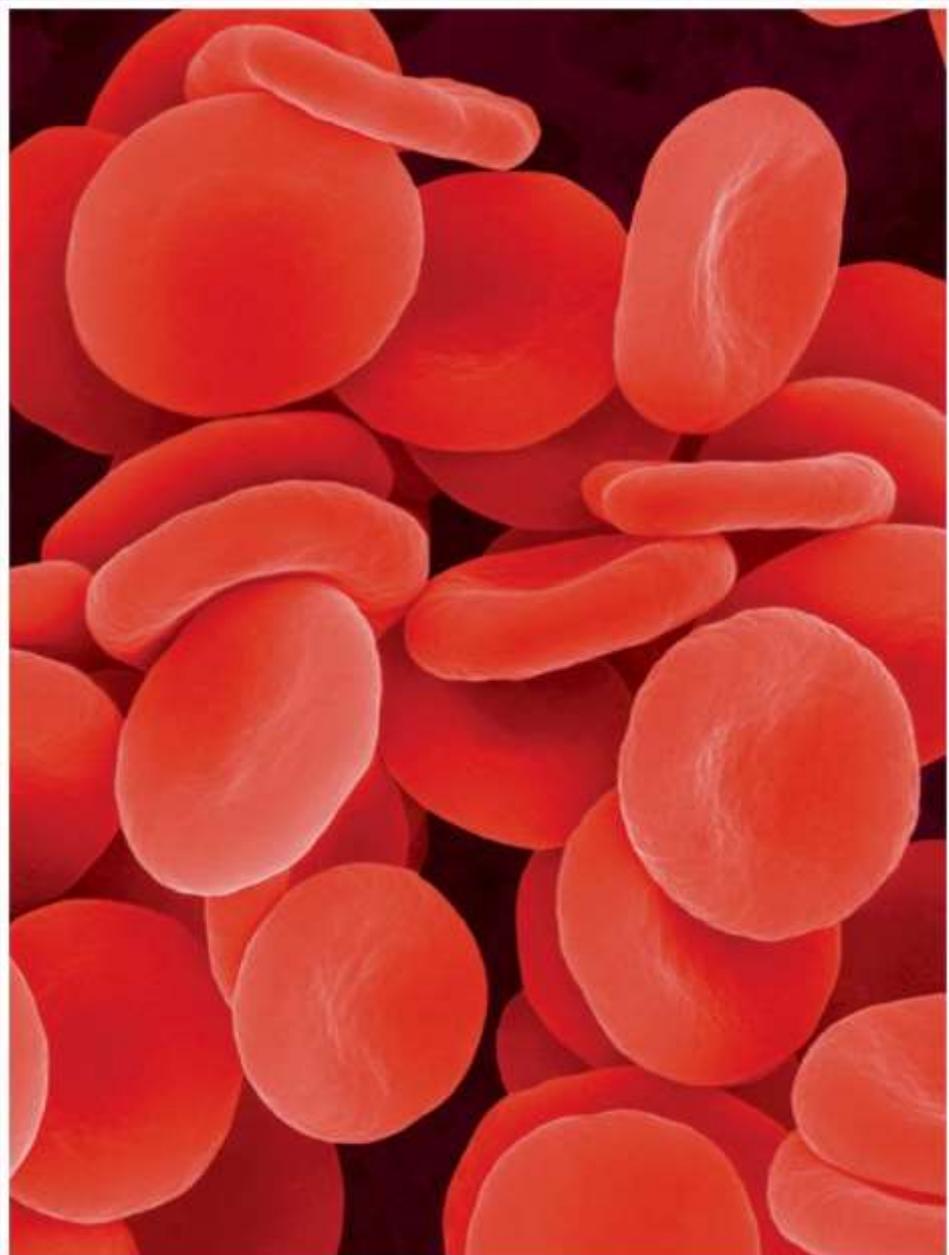
(Tense)

- RBCs

- are typically shaped as biconcave disks
- Biconcave shape gives them a much greater surface area & flexibility to squeeze through tiny capillaries. ① ②

- Carbon monoxide^(CO) binds to heme on the same place as that of O₂.
- Carbon monoxide (CO) has a greater affinity for hemoglobin than oxygen. (200 - 300) times greater Affinity
- Therefore the haemoglobin is no longer available for oxygen transportation causing hypoxia tissue death.
- To reverse the effects of carbon monoxide, pure oxygen is needed to be introduced

Erythrocytes (Red cells)



Chapter 7 Opener part 1

Biochemistry, Sixth Edition

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Methemoglobin

To bind oxygen, the iron of hemoglobin must be in the ferrous (Fe^{2+}) state.

Reactive oxygen species can oxidize the iron to the ferric (Fe^{3+}) state, producing methemoglobin

Methemoglobin is useless in transporting oxygen.

Red blood cell possesses an effective system for reducing heme Fe^{3+} back to the Fe^{2+} state.

This system consists of

- 1- NADH (generated by glycolysis),
- 2- Cytochrome b5 reductase (also known as methemoglobin reductase)
- 3- Cytochrome b5.

Cytochrome b5 reduces (transfer an electron) the Fe^{3+} of methemoglobin. The oxidized cytochrome b5 is then reduced by cytochrome b5 reductase, using NADH as the reducing agent. ($\text{Fe}^{3+} + e^- \rightarrow \text{Fe}^{2+}$)



→ source of electron.

Binding of oxygen to myoglobin and hemoglobin:

[Allosteric effects] → binding of molecule on site affect other molecule on the other site positively or negatively

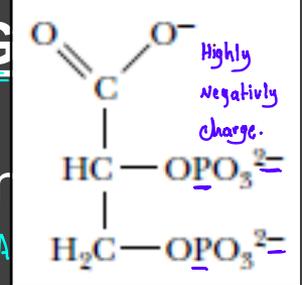
The oxygen-binding properties of hemoglobin are regulated by interaction with allosteric effectors.

The ability of hemoglobin to reversibly bind oxygen is affected by the pO₂, the pH of the environment, the pCO₂ and the availability of 2,3-bisphosphoglycerate (2,3-BPG)

These are collectively called allosteric ("other site") effectors, because their interaction at one site on the hemoglobin molecule affects the binding of oxygen to heme groups at other locations on the molecule.

The binding of oxygen to myoglobin is not influenced by the allosteric effectors of hemoglobin.

2,3-Bisphosphoglycerate (2,3-BPG also 2,3-DPG)



- BPG a three carbon atom (glycolysis intermediate) is an important regulator of the binding of oxygen to hemoglobin.
 - low O_2 in tissue \rightarrow synthesize BPG_n \rightarrow  \rightarrow bind to positive AA
- A low pO_2 in peripheral tissues promotes the synthesis in RBC of 2,3-bisphosphoglycerate (BPG).
- The binding of BPG to partially deoxygenated Hb lowers its affinity for oxygen and promotes (allosterically upregulates) the release of remaining O_2 by stabilizing the quaternary structure of deoxyhemoglobin.
- The Hb molecule has one binding site for BPG. This site is situated within the central cavity formed by the association of four amino acids, the strongly negative BPG molecule binds to these positively charged amino acid. Once bounded, BPG cross-links the two β -subunits. The ionic bonds between BPG and the two β -chains aid in stabilizing the conformation of Hb in its deoxy form, thereby favouring the dissociation of oxygen. Thus, BPG and O_2 are mutually exclusive allosteric effectors for Hb, even though their binding sites are physically distinct.
- The phosphate groups of 2, 3-BPG form ionic bonds with N-terminus on carbons 1 and 2 in amino acid 143 Histidine in addition to binding of the carboxyl group of 2, 3-BPG to 82 Lysine.

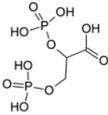
(COO⁻)

2,3-BPG Structure

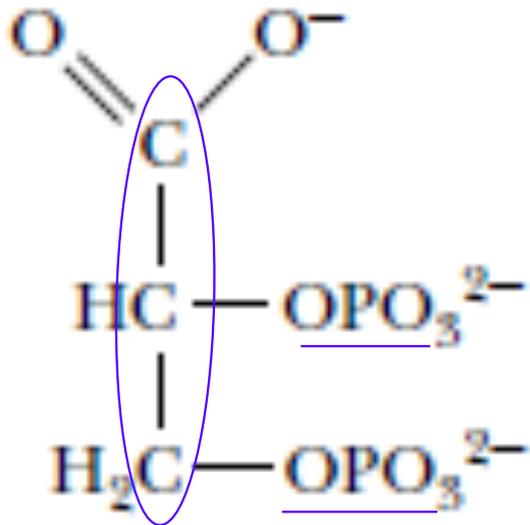
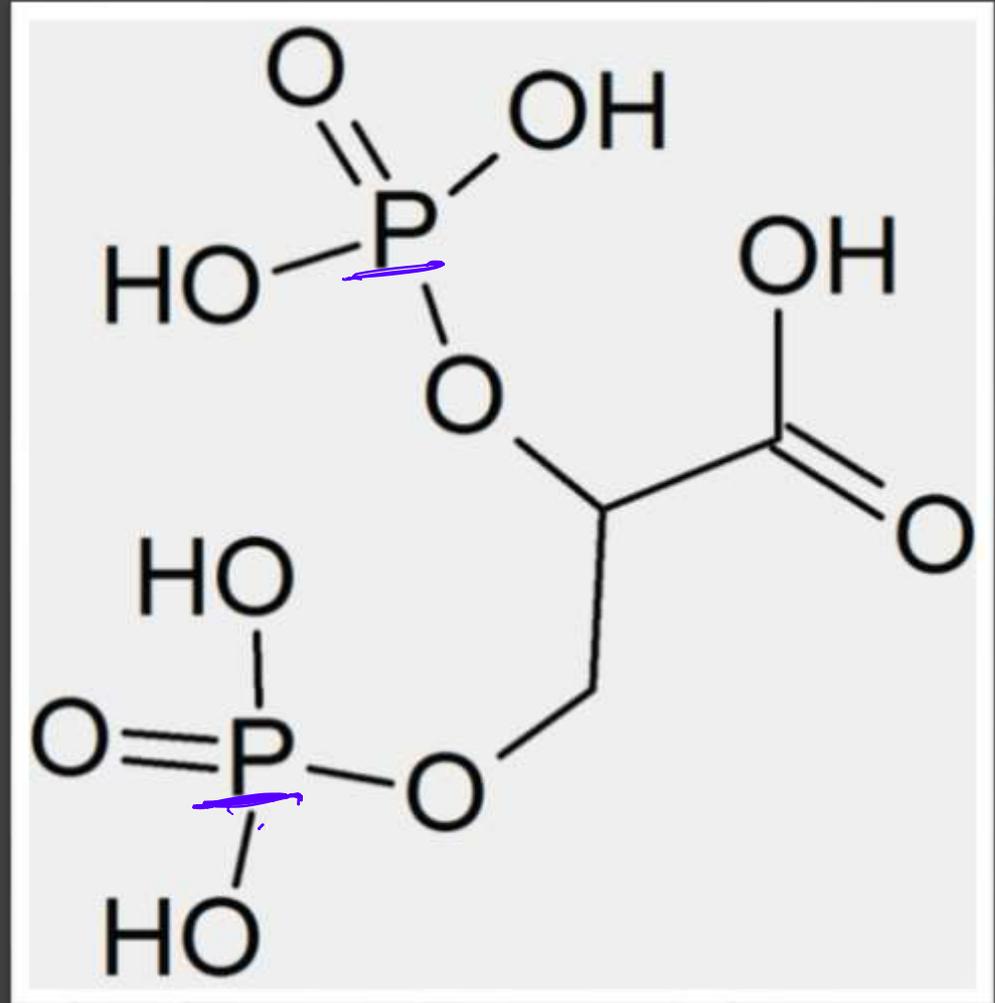
2,3-Bisphosphoglycerate

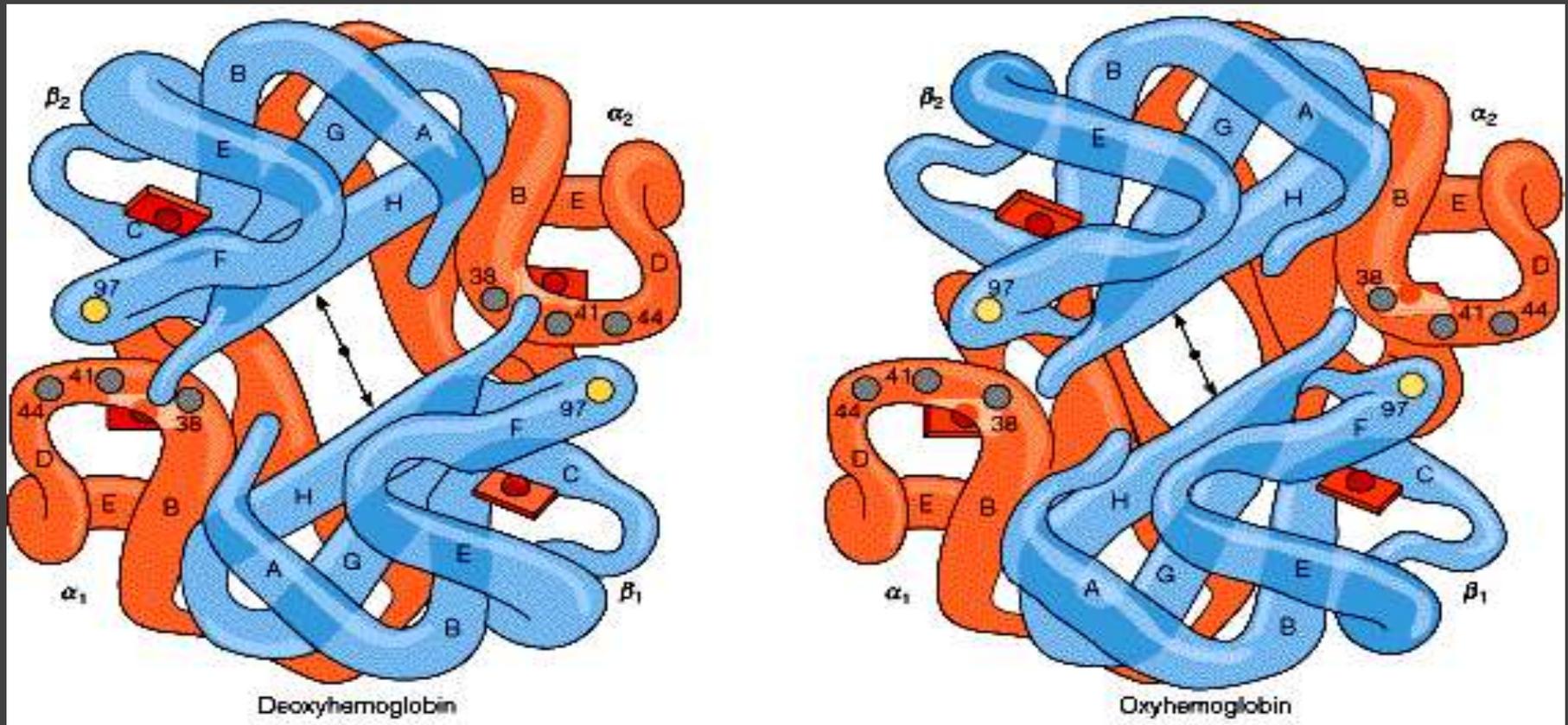
2,3 BPG

- Found in RBCs
- Promotes O₂ release from hemoglobin
- *Negative* allosteric effector
- Increasing levels:
 - Decrease oxygen affinity of hemoglobin
 - Increase delivery oxygen to tissues



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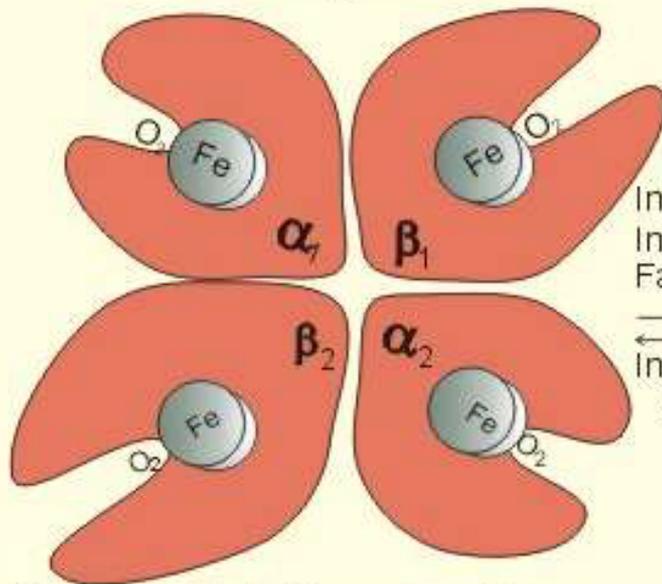


- The change in hemoglobin quaternary structure during oxygenation
- the cavity opening is much narrower in oxyhemoglobin than in deoxyhemoglobin, in fact, 2,3-BPG cannot be accommodated in the oxy form.

Hemoglobin Structure Changes

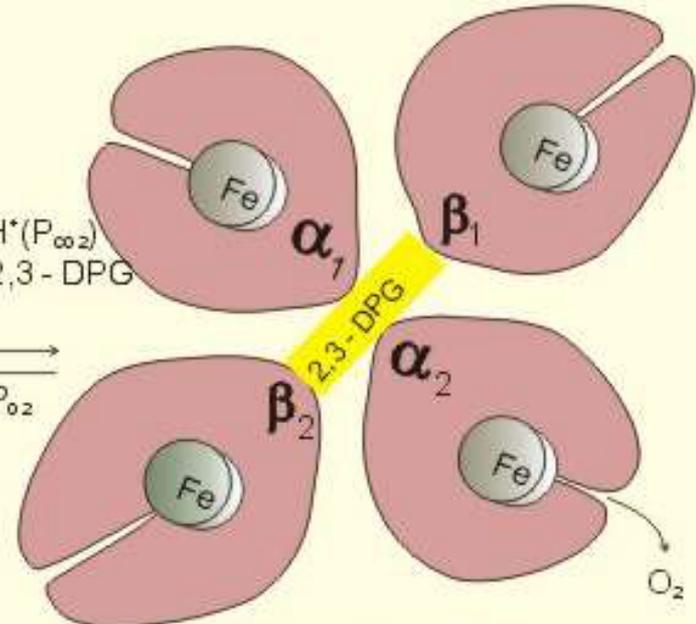
Oxygen Binding and Unloading

Oxyhaemoglobin



Relaxed binding structure

Deoxyhaemoglobin

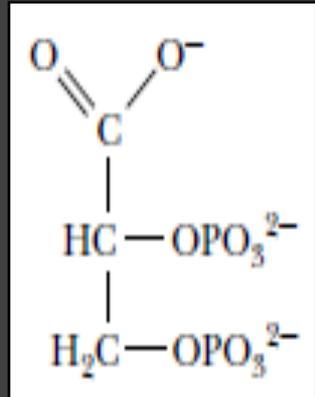
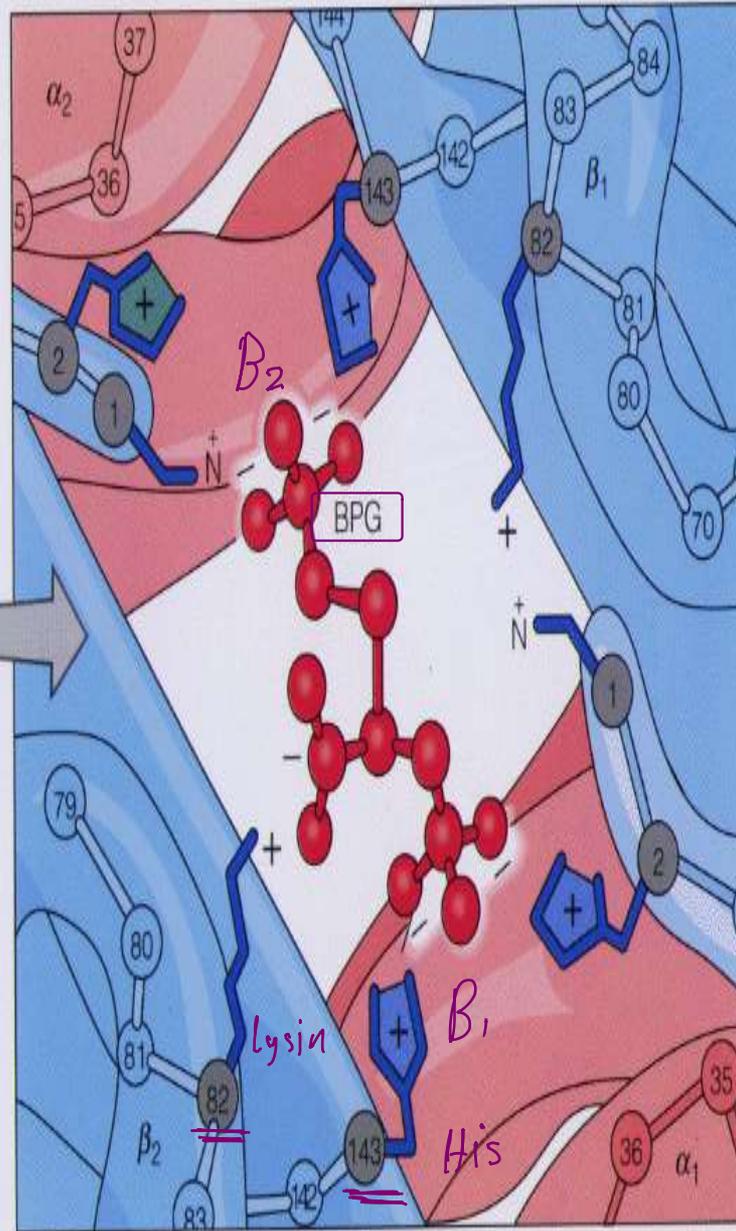
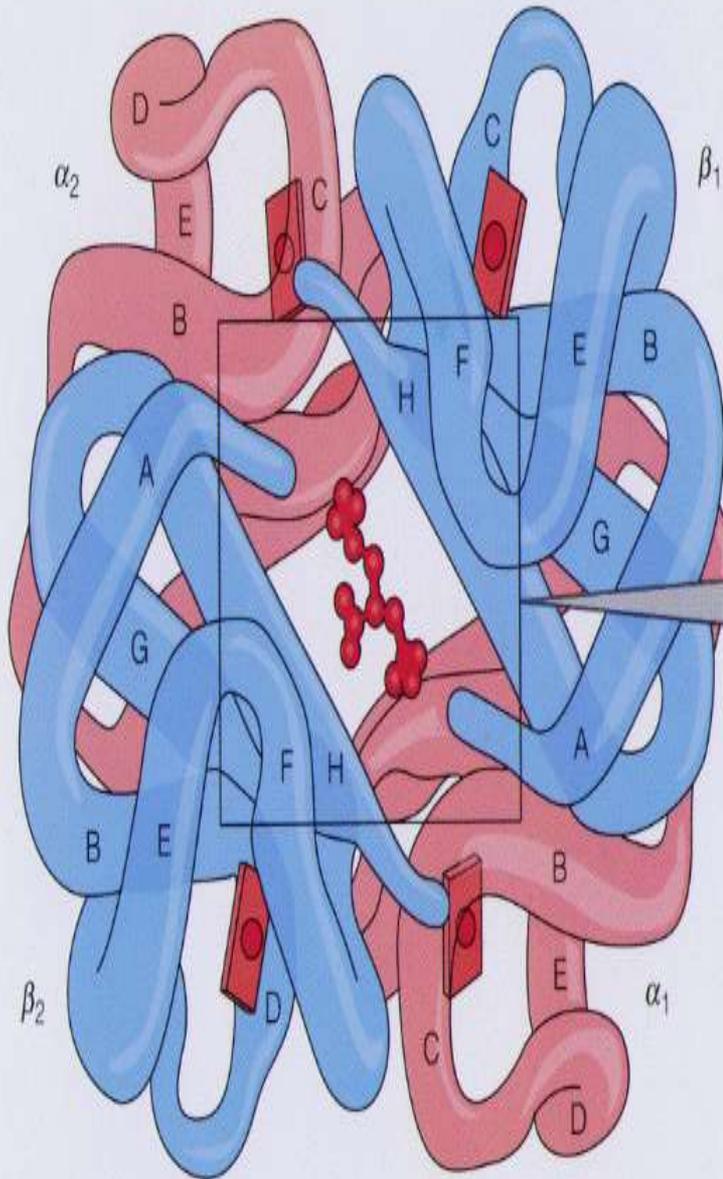


Tight binding structure

Increasing H^+ (P_{CO_2})
Increasing 2,3 - DPG
Falling P_{O_2}

← Increasing P_{O_2}
CO

Binding of 2, 3-bisphosphoglycerate to deoxyhemoglobin



2,3 BPG

Fetal Hemoglobin

Fetal Hb differs from adult Hb in that the β -chains are replaced by very similar, but not identical, 146-residue subunits called γ chains (gamma chains). Fetal Hb is thus $\alpha_2\gamma_2$.

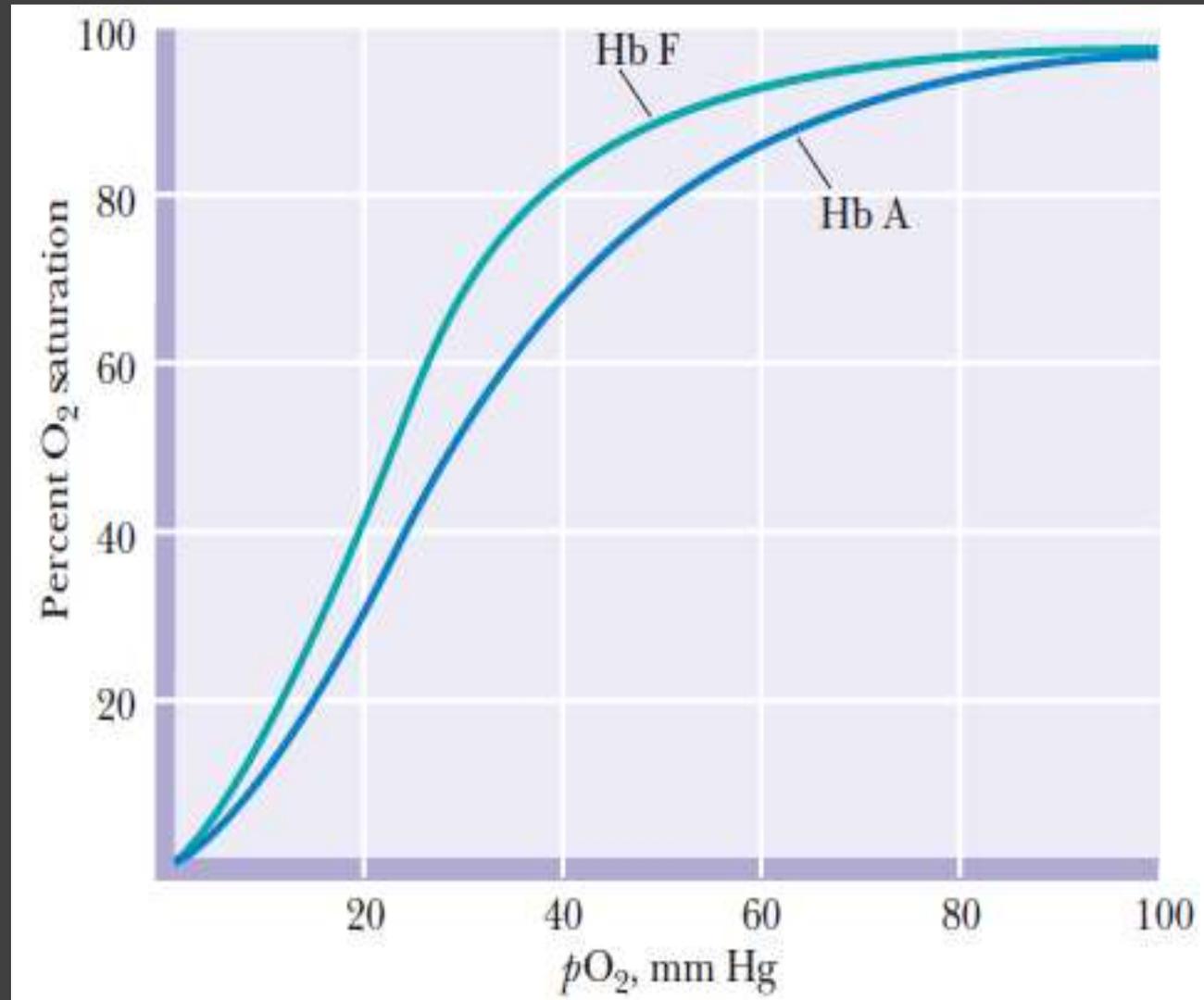
2,3-BPG binds less effectively with the γ chains of fetal Hb (also called Hb F). (Fetal γ chains have Serine (polar uncharged) instead of Histidine ^{positively charge} at position 143, and thus lack two of the positive charges in the central BPG-binding cavity).

Hemoglobin (stripped) of 2,3-BPG is virtually saturated with O₂ at low pO₂ → (لا يحمل) (منزوع)

Fetal Hb has a higher affinity for O₂ because it has a lower affinity for 2,3-BPG

The fetus depends on its mother for an adequate supply of oxygen, but its circulatory system is entirely independent. Gas exchange takes place across the placenta. Ideally fetal Hb should be able to absorb O₂ better than maternal Hb so that an effective transfer of oxygen can occur.

Figure compares the relative affinities of adult Hb (also known as Hb A) and Hb F for O₂ under similar conditions of pH and [BPG]. Note that Hb F binds O₂ at pO₂ values where most of the oxygen has dissociated from Hb A. Much of the difference can be attributed to the low capacity of Hb F to bind BPG



Comparison between Mb and Hb

<u>Mb</u>	<u>Hb</u>
In muscle	In RBCs
<u>Reservoir of O₂</u> <i>+ diffusion</i>	Carrier of O ₂
No quaternary <i>-one strand</i> structure	Has quaternary structure
Can't carry CO ₂	Carries CO ₂
No cooperativity of O ₂ binding	Shows cooperativity
<u>O₂ affinity is higher than Hb</u>	<u>O₂ affinity is lower than Mb</u>