Hemoglobin & Myoglobin

Myoglobin (Mb)

Intracellular heme protein found in most cells

Stores and facilitate oxygen diffusion in muscles especially in heart and skeletal muscle.

It binds the O_2 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.

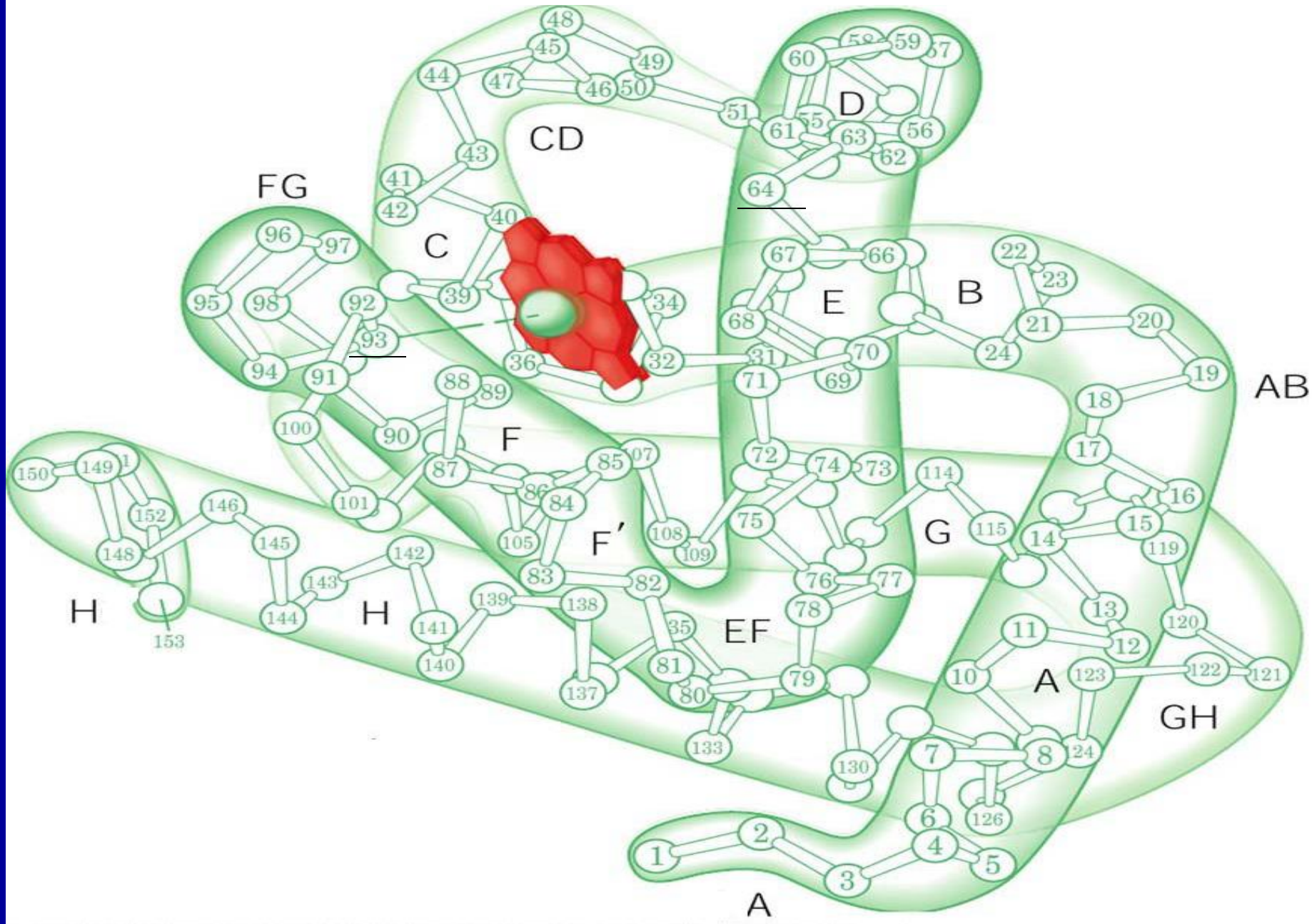
Each helical segment is terminated either by the presence of proline or by β-bends and loops.

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 O_2.

The heme of myoglobin lies between helices E and F.

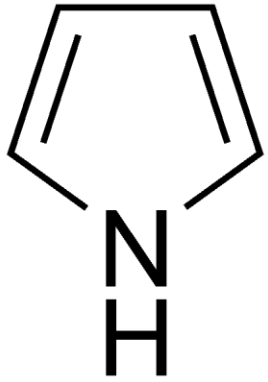
The polypeptide of myoglobin may be viewed as serving three critical functions: 1- it hold the heme group, 2- it provides a pocket into which the O2 can fit, and 3- it protects the heme iron atom from oxidation.

Myoglobin

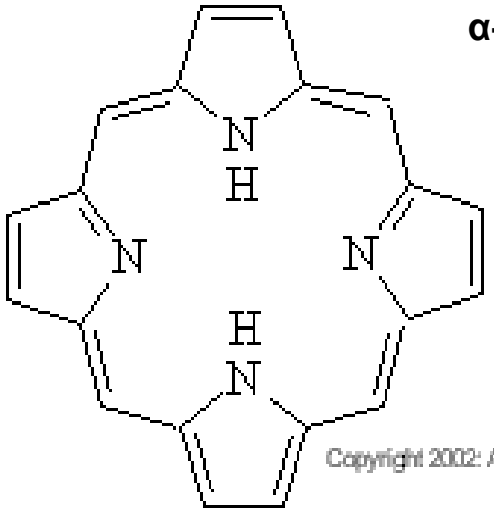


Structure of heme in myoglobin and hemoglobin

- 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 (=CH-)
- A pyrrole ring is a group of four carbon atoms and a nitrogen atom bonded together in a ring (see figure).



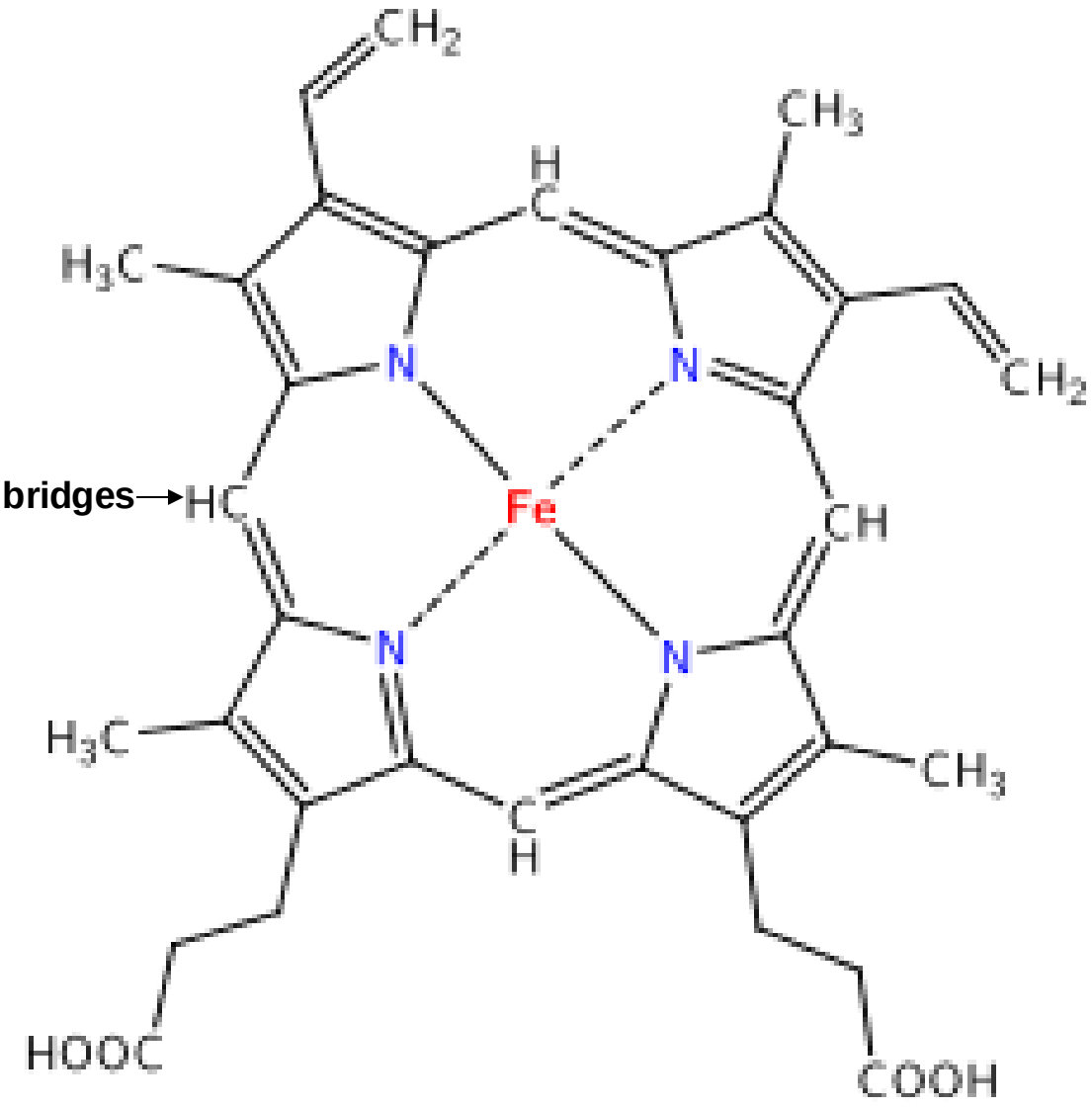
Pyrrole ring

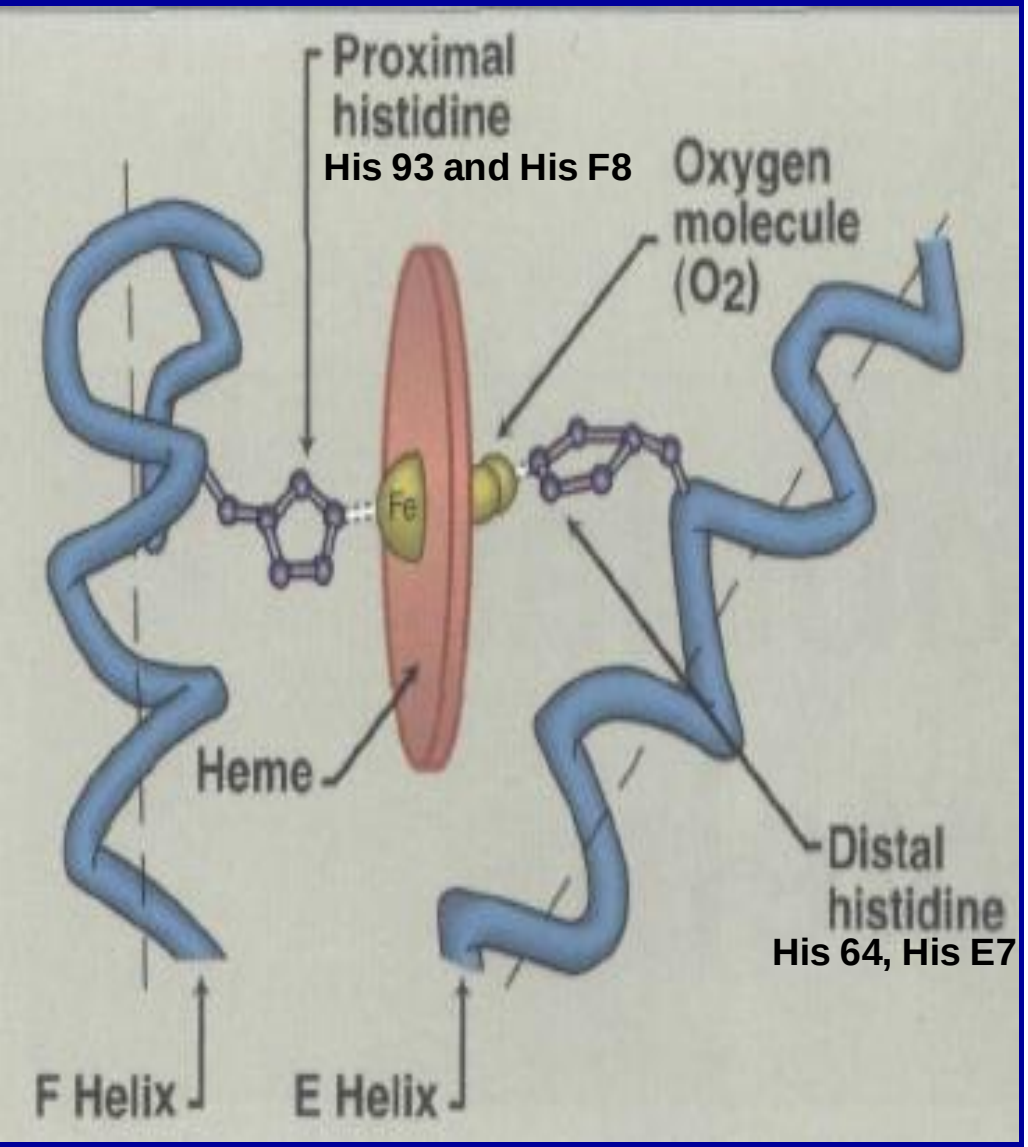


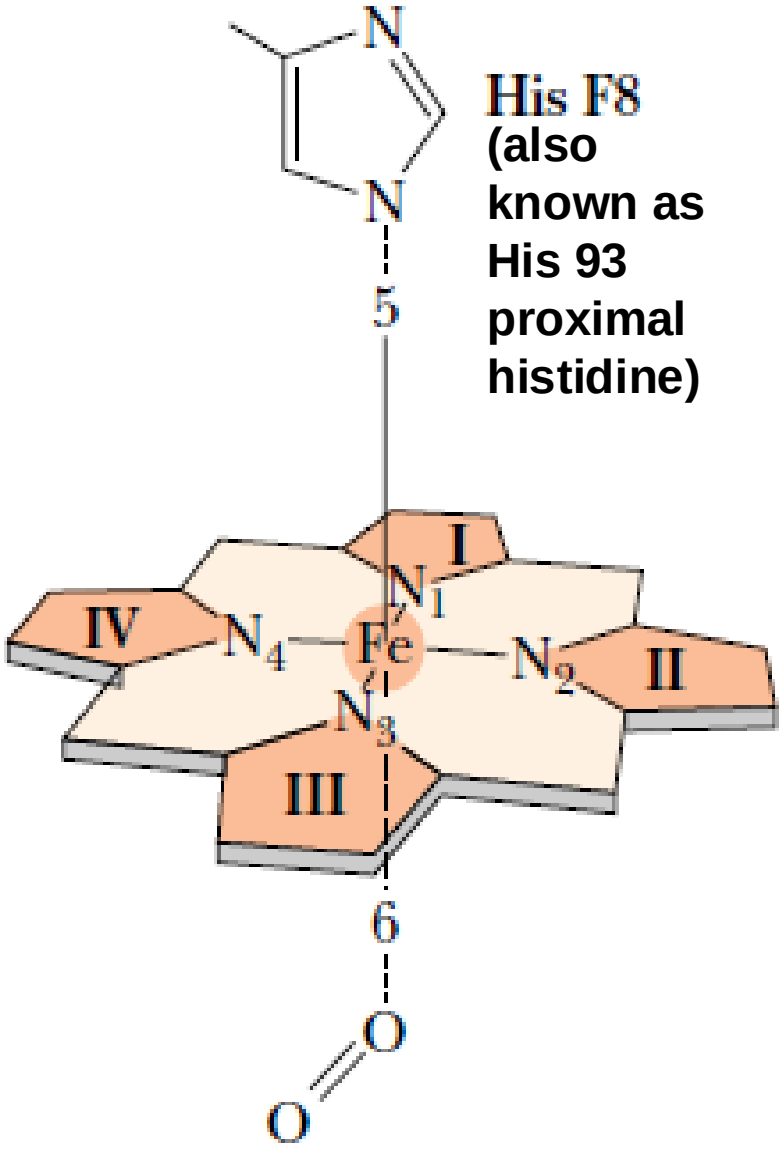
Porphyrin Ring

Heme

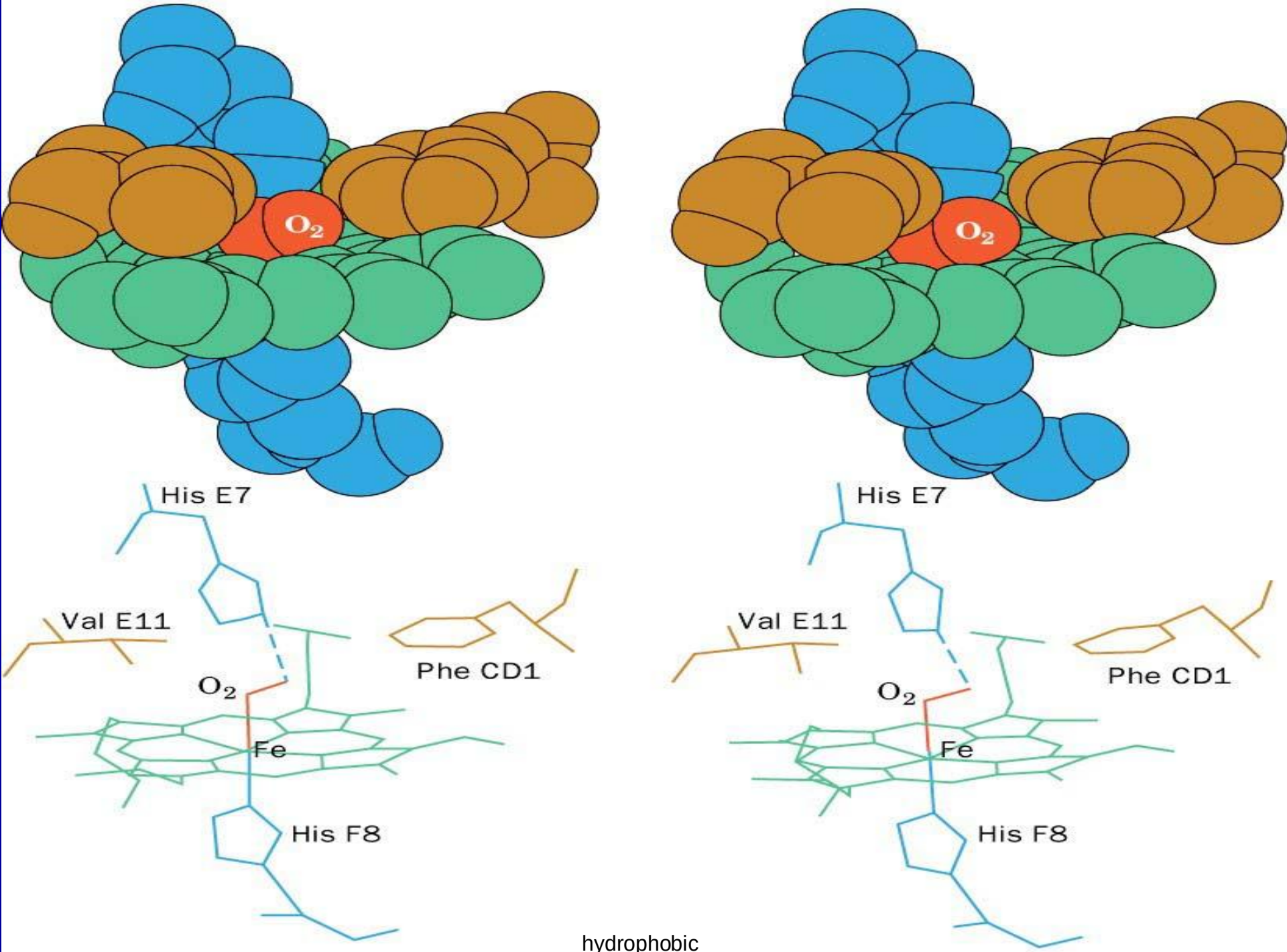
α-methylene bridges





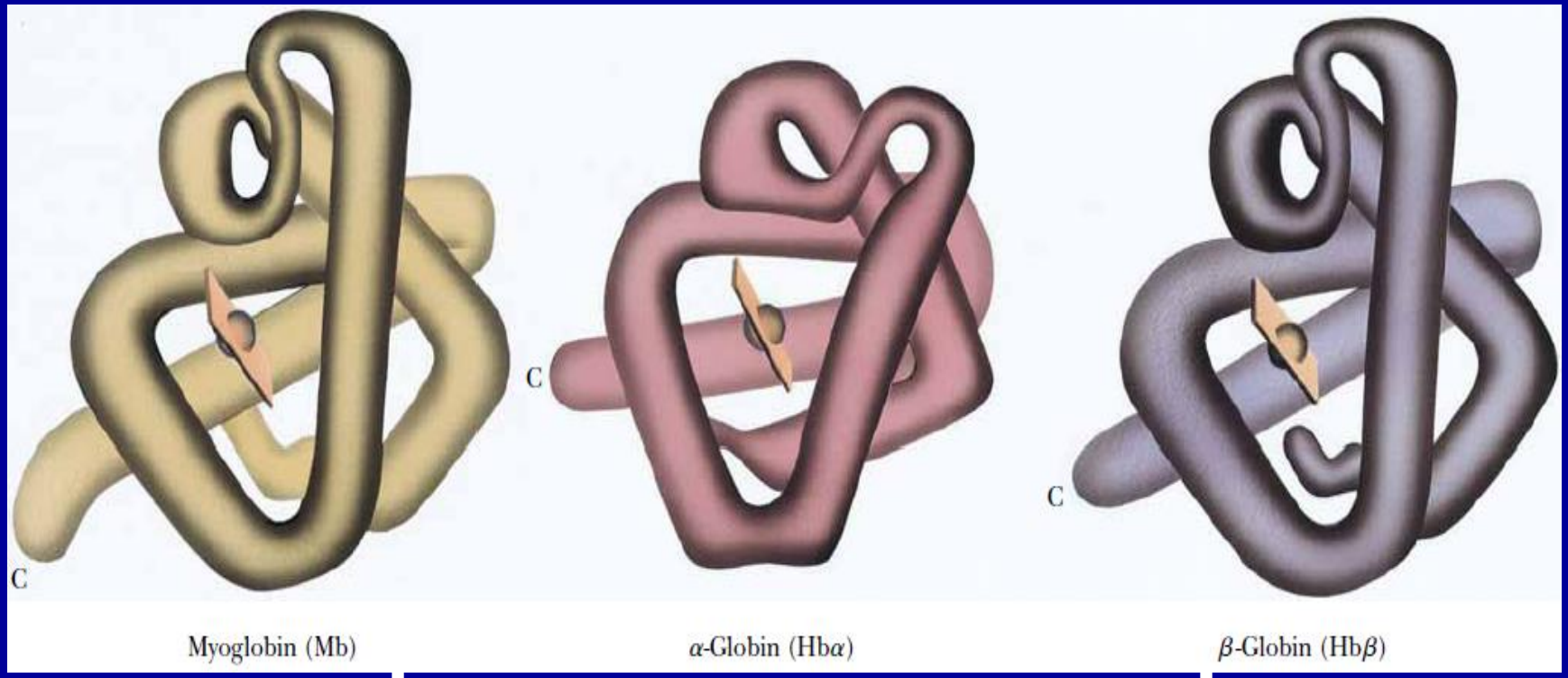


coordinate covalent bonds



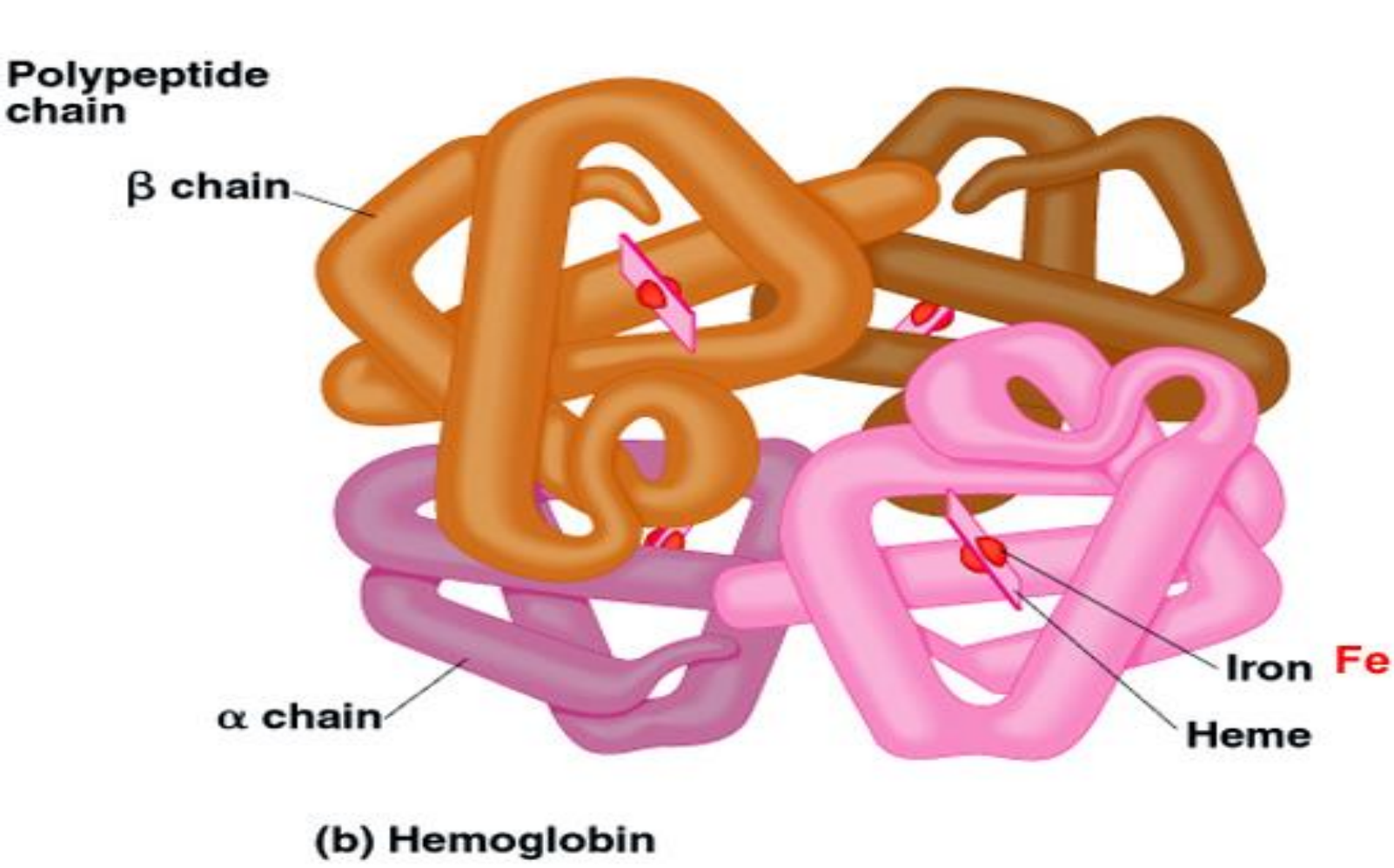
hydrophobic

- **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.



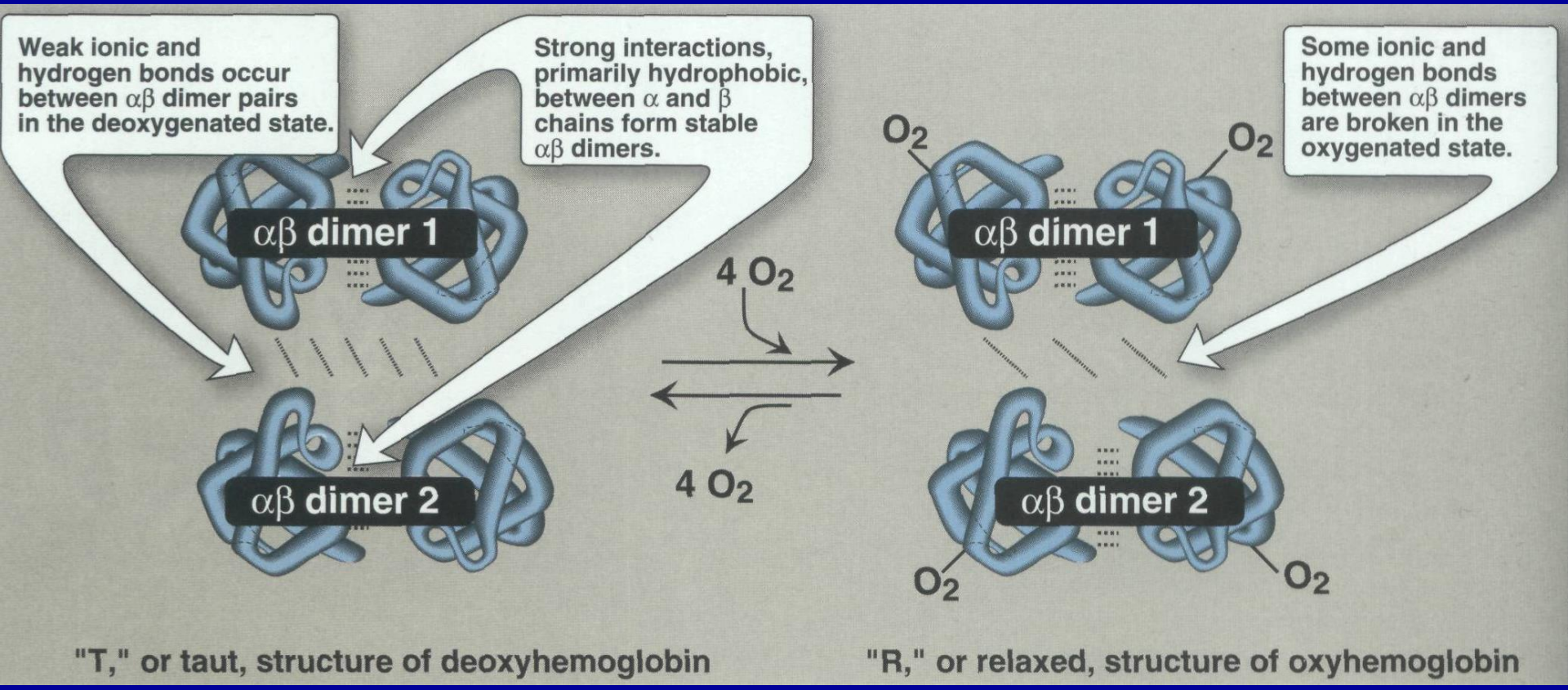
Myoglobin	α- globin in Hb	β-globin in Hb
153 aa	**141 aa** shortened H helix and lacks the D helix	**146** H helix is shorter

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, dimmer one α1β1 and dimmer two α2β2.
- The two polypeptide chains within each dimmer are held tightly together, primarily by hydrophobic interactions although Ionic and hydrogen bonds play a role.
- The type of interaction between dimmer 1 and dimmer 2 is a weak ionic and hydrogen bonds.
- The ionic bond is a relatively weak ionic bond and is called salt bridge (salt bond).


Weak ionic and hydrogen bonds occur between αβ dimer pairs in the deoxygenated state.
Strong interactions, primarily hydrophobic, between α and β chains form stable αβ dimers.
αβ dimer 1
αβ dimer 2
4 O2
4 O2
Some ionic and hydrogen bonds between αβ dimers are broken in the oxygenated state.
O2
O2
αβ dimer 1
αβ dimer 2
O2
O2
"T," or taut, structure of deoxyhemoglobin
"R," or relaxed, structure of oxyhemoglobin

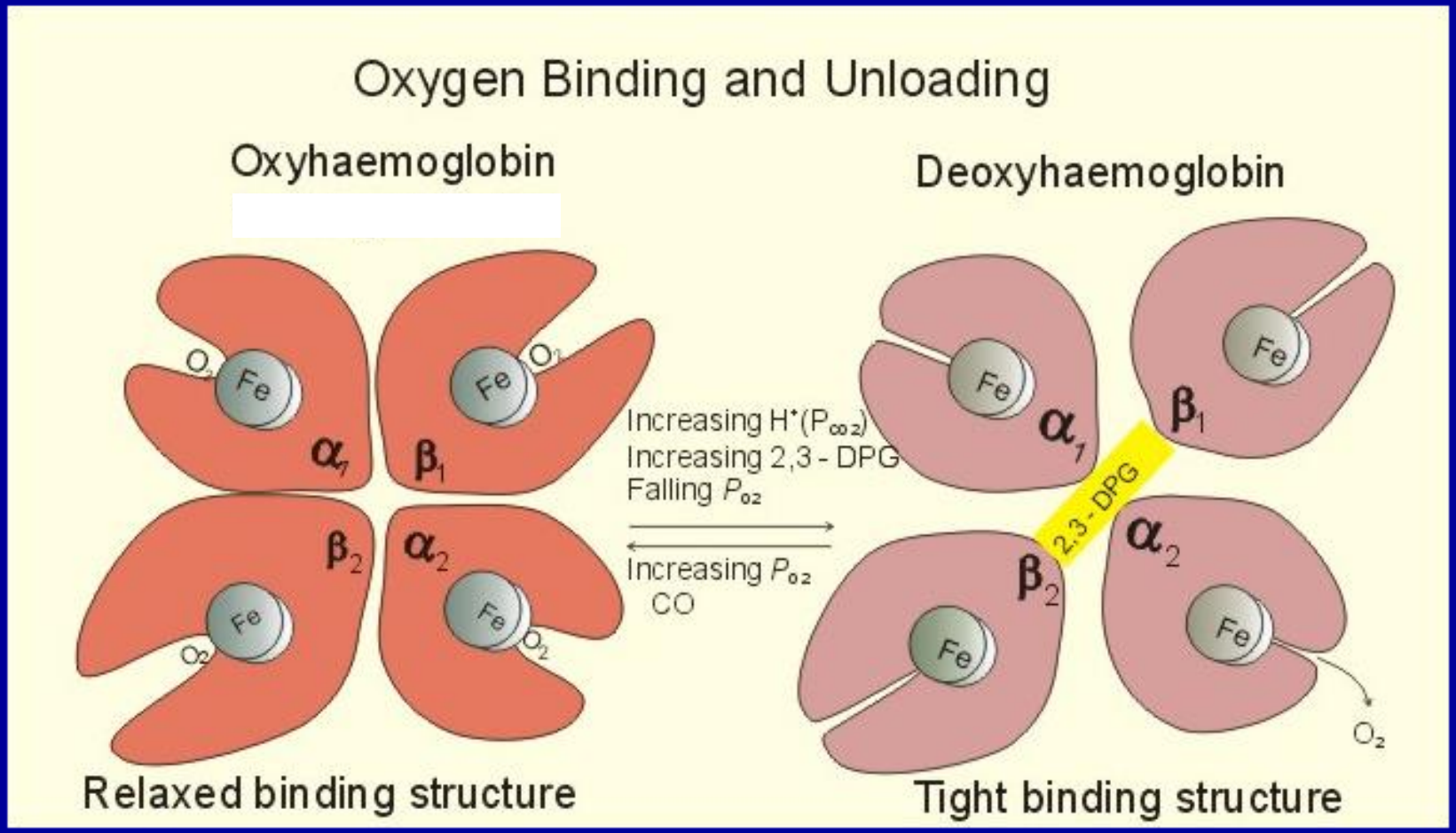
- **T & R forms of Hemoglobin**

a. T form: The deoxy form of hemoglobin is called the "T" **(tense)** form.

- In the T form, the two αβ 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.

b. R form: The binding of oxygen to hemoglobin causes the rupture of some of the ionic bonds and hydrogen bonds between the αβ 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.

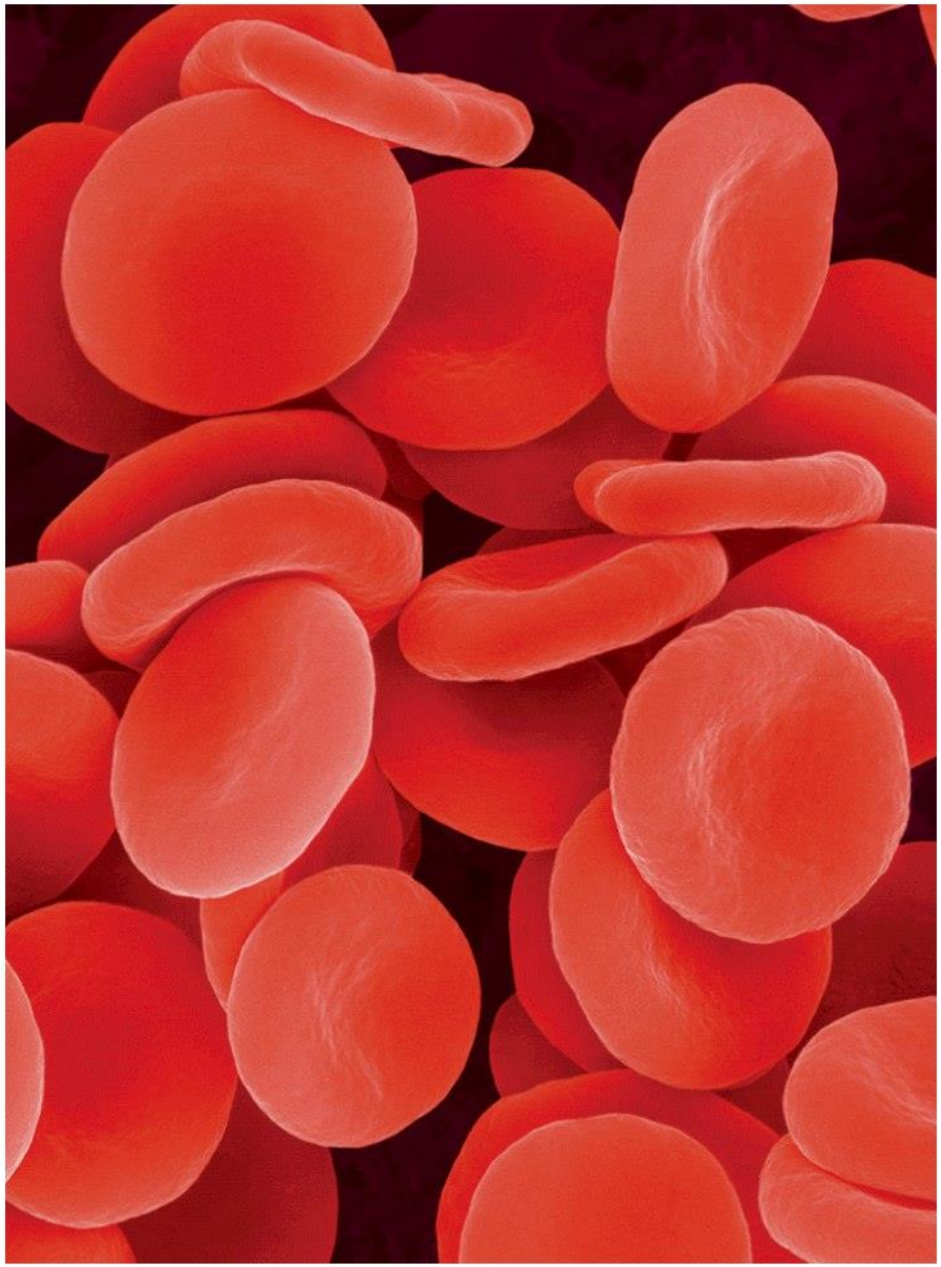
Hemoglobin Structure Changes



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

- **Carbon monoxide** binds to heme on the same place as that of O_2.
- Carbon monoxide (CO) has a greater affinity for hemoglobin than oxygen.
- 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

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. ($Fe^{3+} + e \longrightarrow Fe^{2+}$)

Binding of oxygen to myoglobin and hemoglobin: Allosteric effects

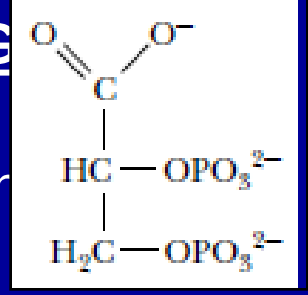
The oxygen-binding properties of hemoglobin are regulated by interaction with **allosteric effectors.**

The ability of hemoglobin to reversibly bind oxygen is affected by the pO_2, the pH of the environment, the pCO_2 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.

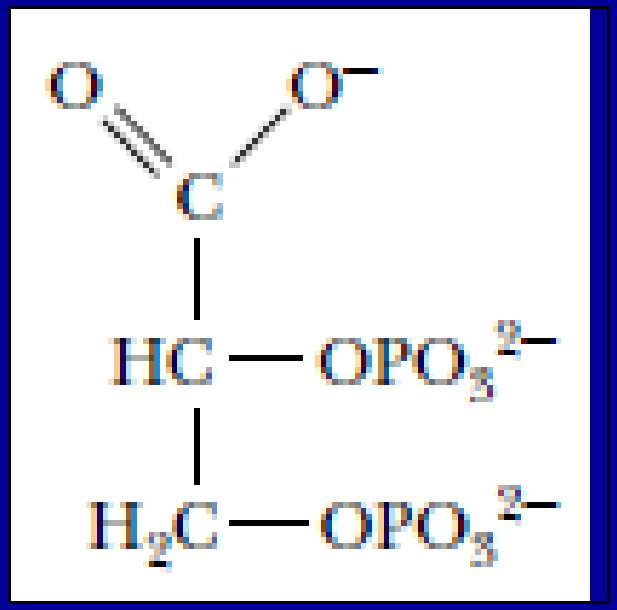
The binding of oxygen to myoglobin is not influenced by the allosteric effectors of hemoglobin.

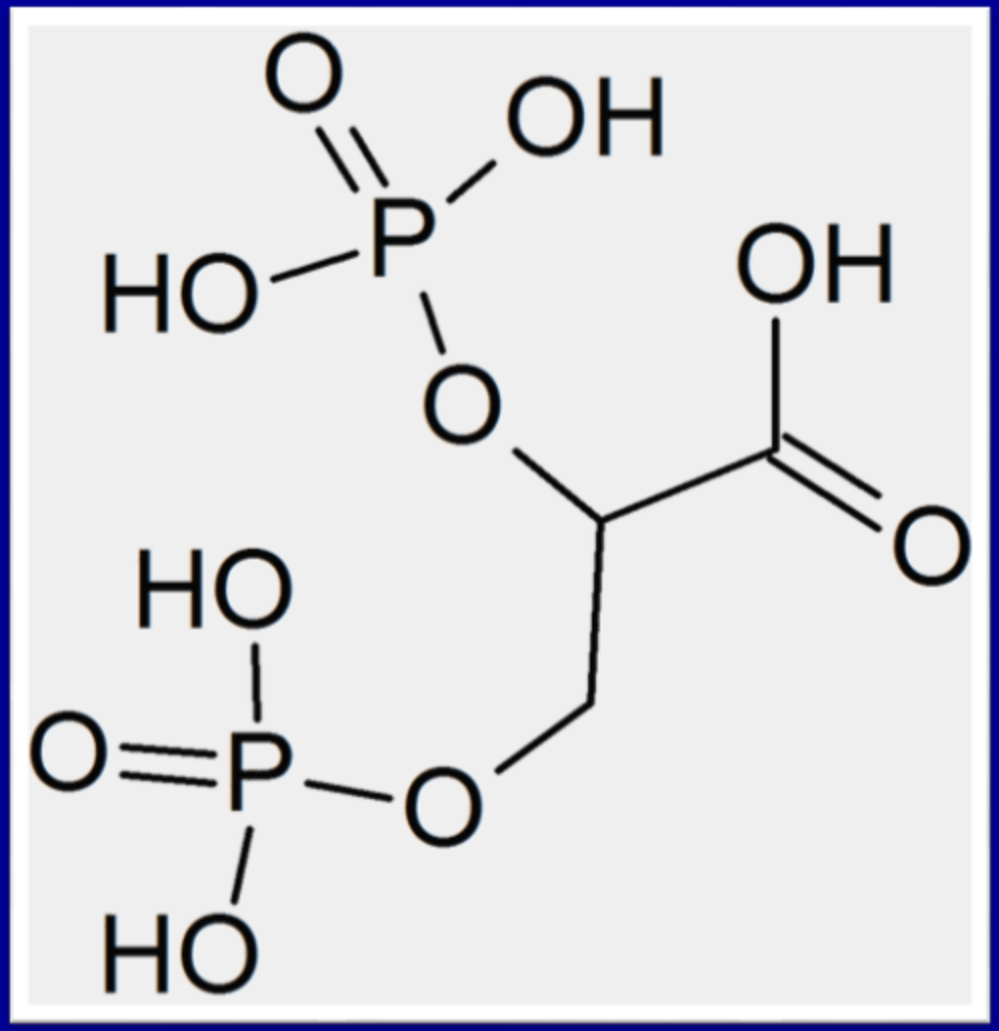
2,3-Bisphosphoglycerate (2,3-BPG also 2,3-DPG

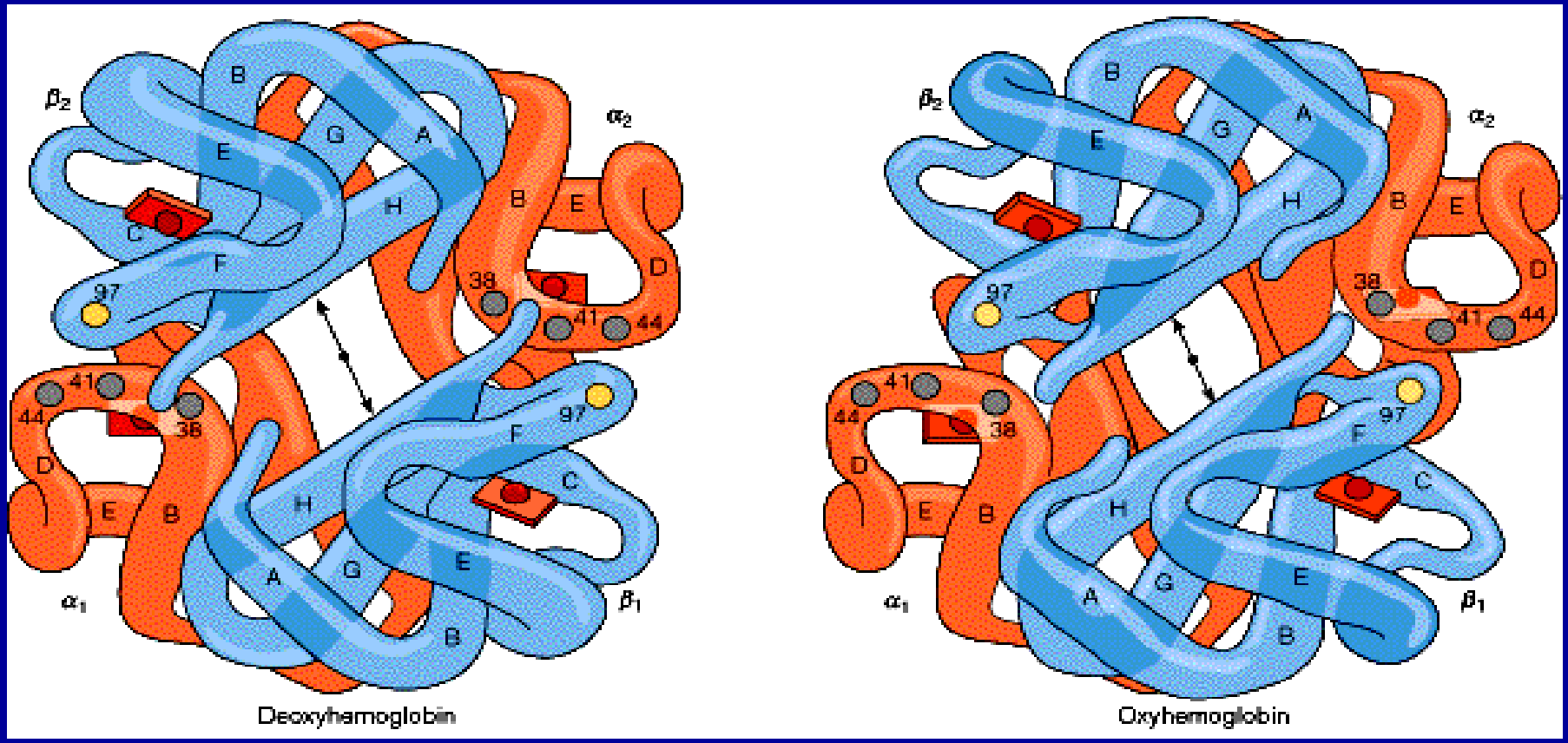


- BPG a three carbon atom (glycolysis intermediate) is an important regulator of the binding of oxygen to hemoglobin
- A low pO_2 in peripheral tissues promotes the synthesis in RBC of 2,3-bisphosphoglycerate (BPG).
- The binding of BPG to partially deoxygenated Hb lowers its affinity for oxygen and 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 O2 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 carbone 1 and 2 in amino acid 143 Histidine in addition to binding of the carboxyl group of 2, 3-BPG to 82 Lysine.

2,3-BPG Structure

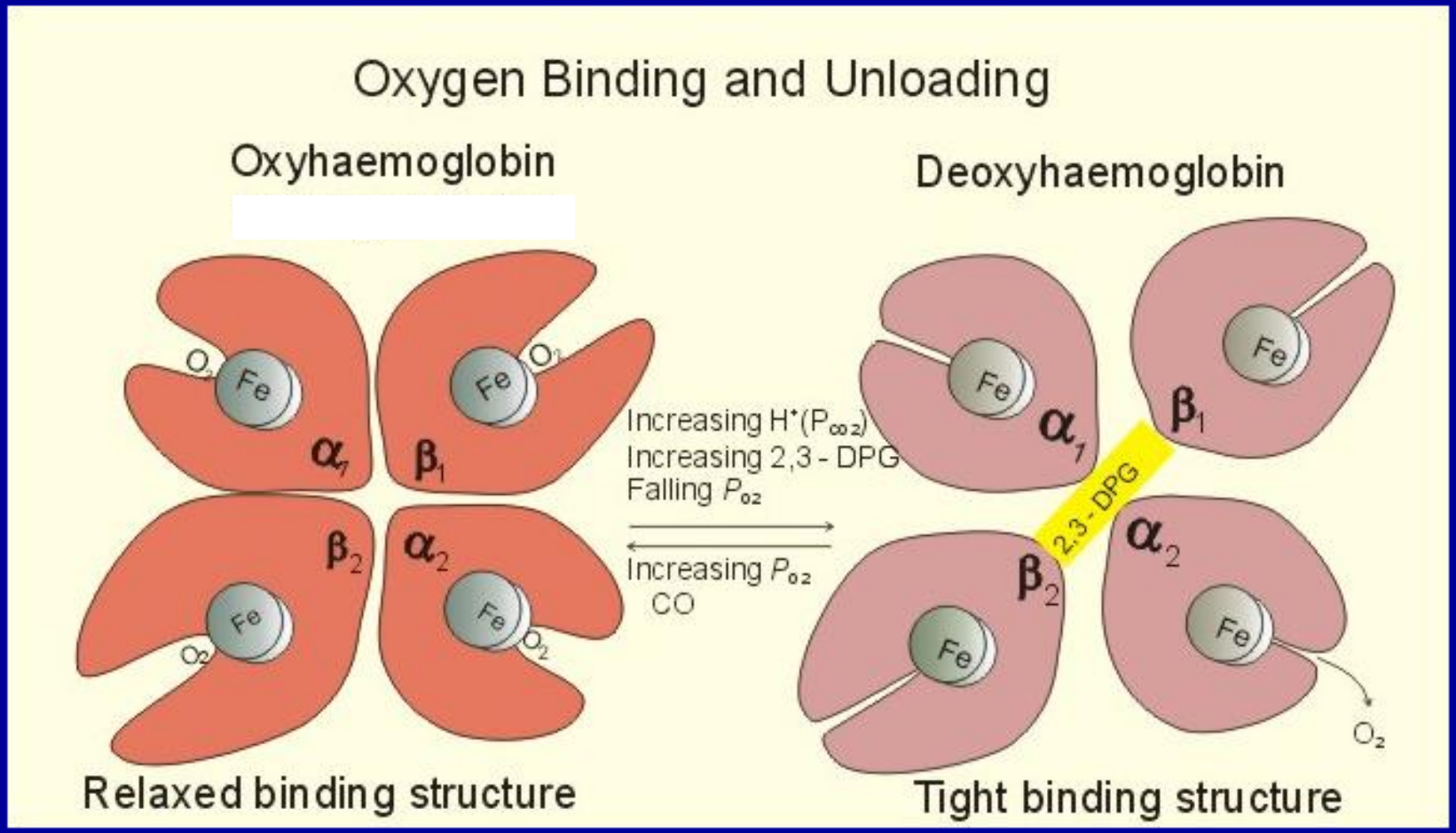




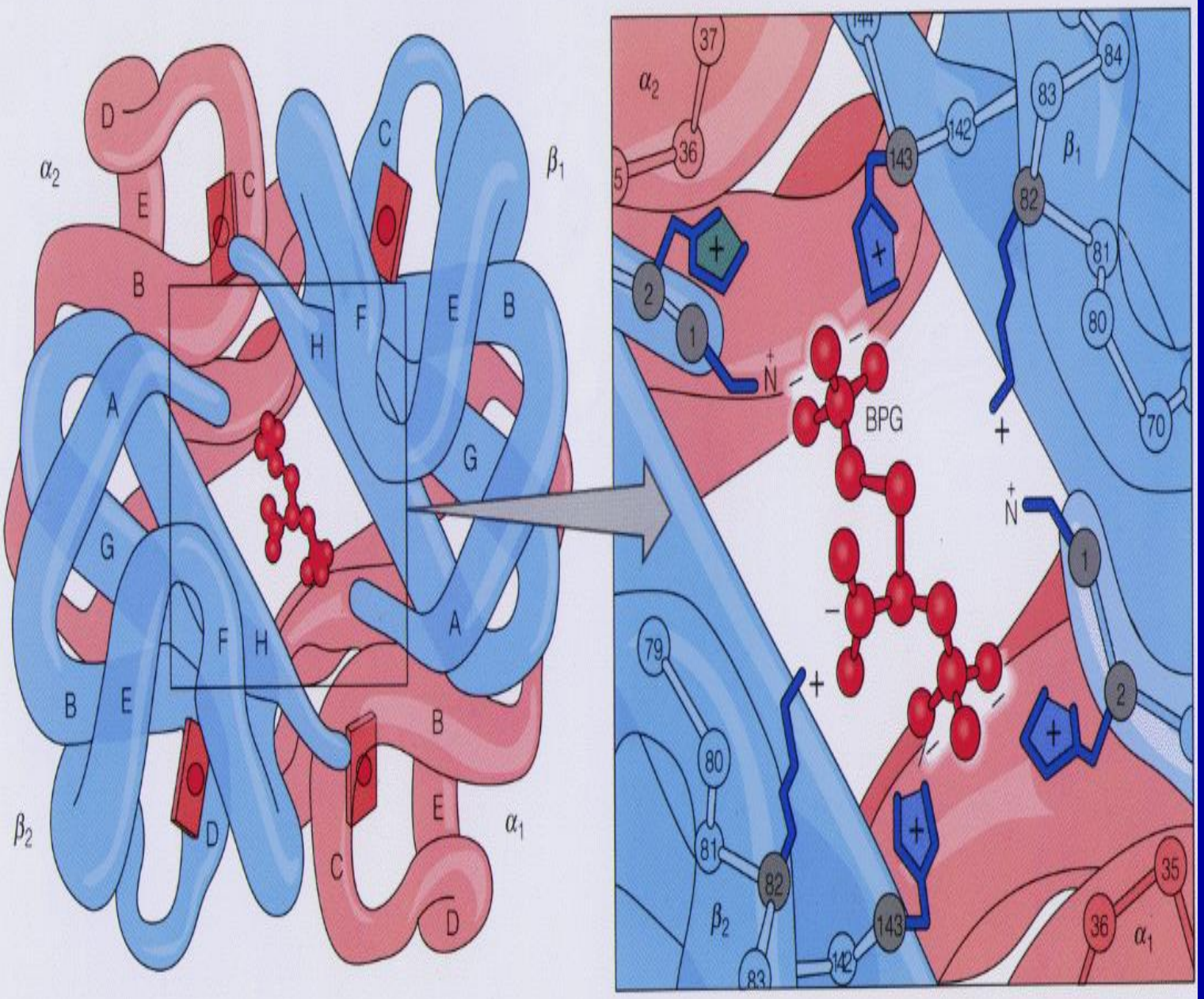


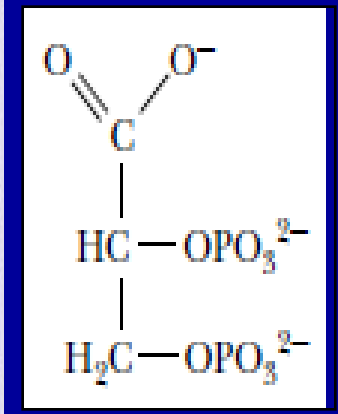
- <u>The change in hemoglobin quaternary structure during oxygenation</u>
- the cavity opening is much narrower in oxyhemoglobin than in deoxyhemoglobin, in fact, **2,3-BPG** cannot be accommodated in the oxy form.

Hemoglobin Structure Changes



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 **α2γ2**.

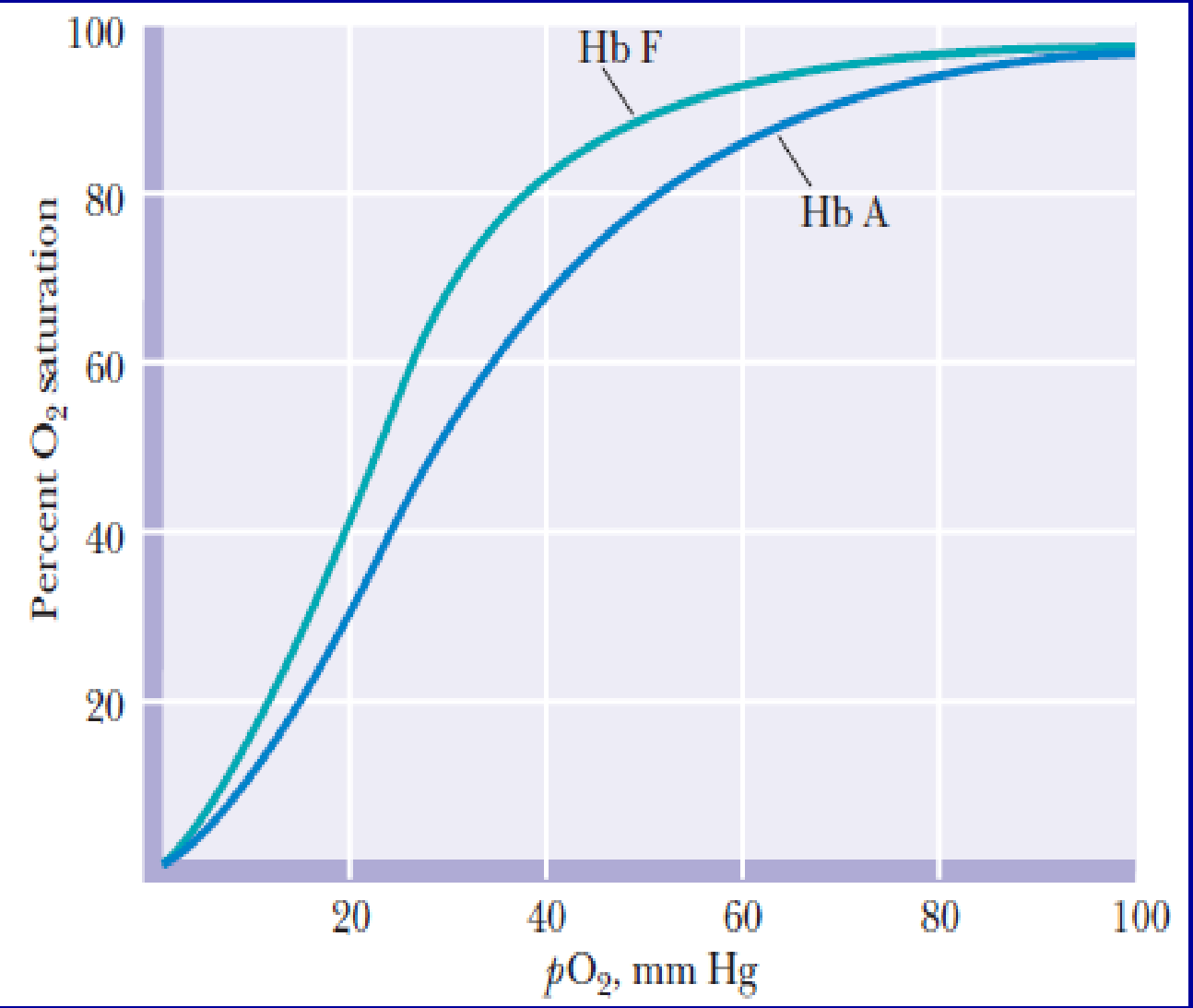
2,3-BPG binds less effectively with the γ chains of fetal Hb (also called Hb F). (Fetal γ chains have Serine (polar uncharged) instead of Histidine at position 143, and thus lack two of the positive charges in the central BPG-binding cavity).

Hemoglobin stripped of 2,3-BPG is virtually saturated with O2 at low pO2

Fetal Hb has a higher affinity for O_2 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 O_2 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

Mb	Hb
In muscle	In RBCs
Reservoir of O_2	Carrier of O_2
No quaternary structure	Has quaternary structure
Can't carry CO_2	Carries CO_2
No cooperativity of O_2 binding	Shows cooperativity
O_2 affinity is higher than Hb	O_2 affinity is lower than Mb