

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)



HLS BioChemistry | MID 2

Hemoglobin

An overview and more



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Resources

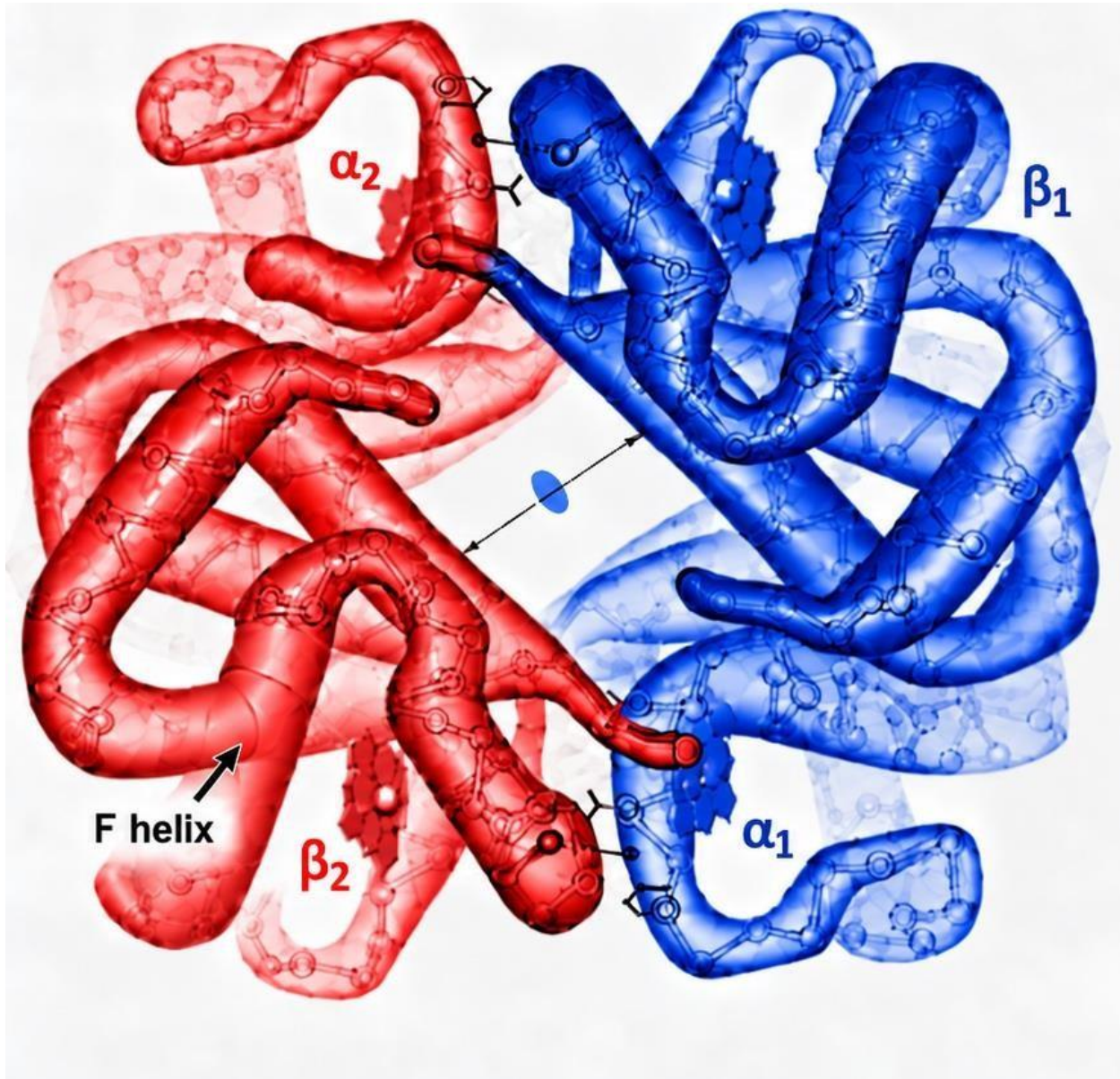
- Voet and Voet Biochemistry
- Hemoglobin O₂ Binding and Allosteric Properties of Hemoglobin
(http://home.sandiego.edu/~josephprovost/Chem331%20Lect%207_8%20Myo%20Hemoglobin.pdf)
- Lecture 3: Cooperative behaviour of hemoglobin
(https://www.chem.uwec.edu/chem452_f12/pages/lecture_materials/unit_III/lecture-3/overheads/Chem452-lecture_3-part_1-overheads.pdf)

What we are covering

- In this lecture we will be covering:
 - 1 Hemoglobin (Globin and Heme structures)
 - A- The 2 forms of hemoglobin
 - B- Mutations of globin residues
 - C- Heme structure
 - D- Mutations of heme
 - 2 Hemoglobin effectors



1-A-Hemoglobin



General Structure and Function of Hemoglobin:

- **Subunits:** Hemoglobin consists of **4 subunits**, which are: alpha1, alpha2, beta1, and beta2.
- **Function:** Hemoglobin functions to deliver oxygen to various parts of the body.
- **The Heme Group:**
Each subunit contains a heme group (one in each subunit, located as shown in the structure).
Oxygen binds specifically to the **heme**, not to other parts.

✓ Sequences and Amino Acids:

Sequence Identity and Similarity:

alpha1 and alpha 2 are identical in sequence, and beta1 and beta2 are identical in sequence.

Although Alpha and Beta subunits look very similar in shape, they are only **18% identical** in sequence, meaning there is a large variation in their sequence.

Length (Number of Amino Acids):

Alpha chain: Approximately **141 amino acids** (exact numbers are noted but not strictly required to memorize).

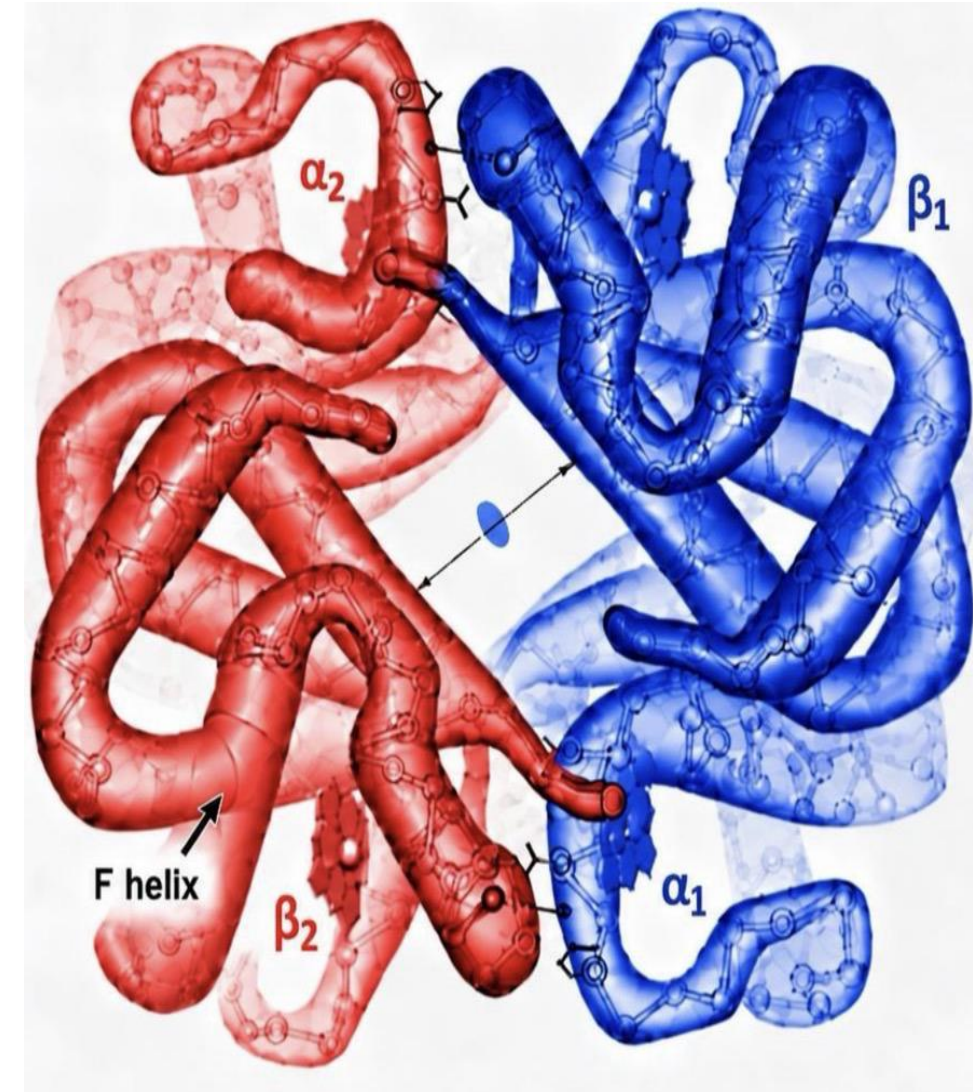
Beta chain: **146 amino acids**.

Last Amino Acids and Charges:

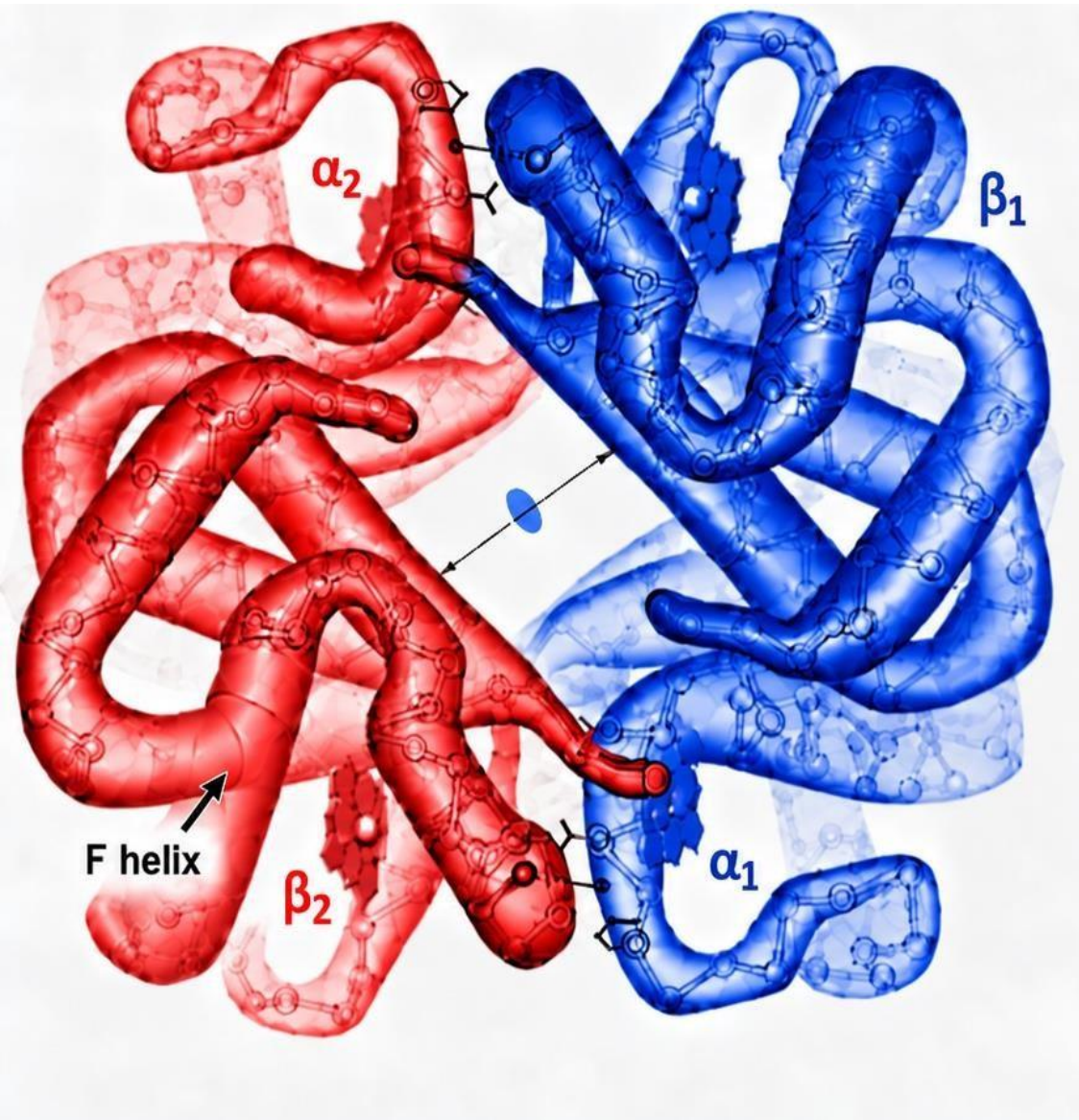
Alpha's Last Amino Acid: **Arginine** (position 141).

Beta's Last Amino Acid: **Histidine** (position 146).

Charge: Both carry a **positive charge** (positive-charged) and will recur later in the course material.



1-A-Hemoglobin



- Hb is a globular protein that is used to transport O_2 to the body. It is composed of 2 dimers: $\alpha_1\beta_1$ and $\alpha_2\beta_2$
 - α polypeptide = 141 amino acids (Last aa Arg141)
 - β polypeptide = 146 amino acids (Last aa His146 → It participates in Bohr's effect)
 - The first amino acid in both is valine.
- Each monomer binds to a heme group

✓ Subunit Arrangement and Bonds

- **Spatial Arrangement:**

beta1 and beta2 face each other (they are not right next to each other).

Similarly, alpha1 and alpha2 face each other rather than being directly adjacent.

- **Bond Strengths (Ranked from weakest to strongest):**

1. **Weakest Bonds:**

- The weakest interactions—or lack of direct bonding—occur between identical/homogeneous subunits (such as between alpha1 and alpha2, or between beta1 and beta2) due to the large distance separating them across the central cavity; whereas the inter-dimer interactions take place between non-identical subunits (between alpha1 and beta2, and between alpha2 and beta1)

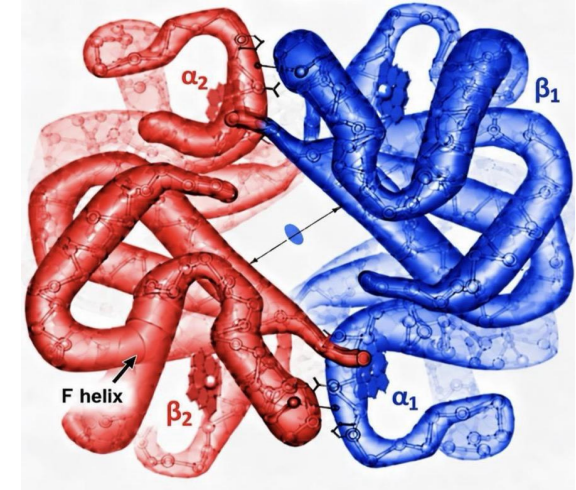
2. **Moderate Bonds:**

Bonds that are slightly stronger than the weakest ones (between alpha2 and beta1, and between beta2 and alpha1)

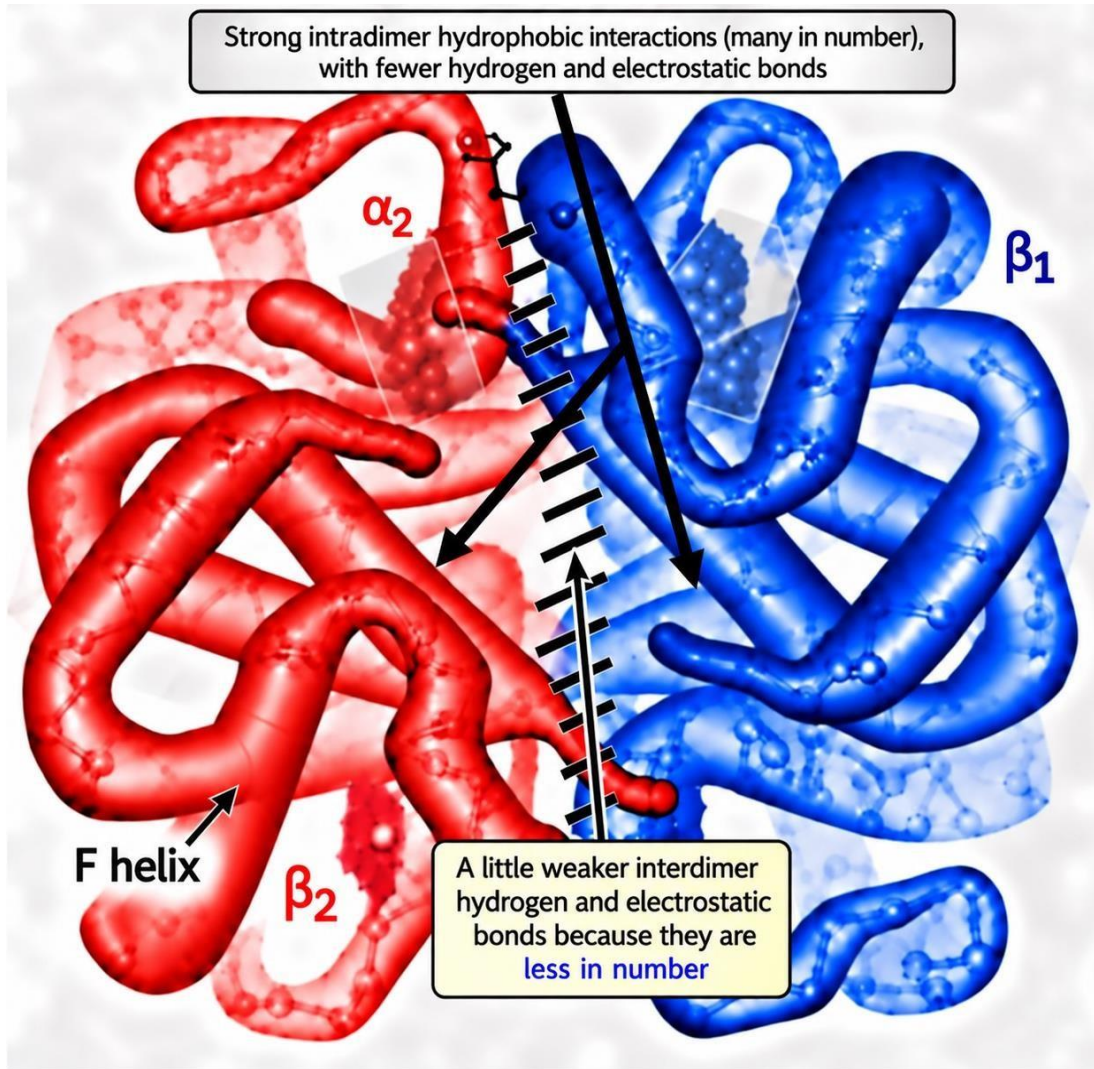
3. **Strongest Bonds:**

The strongest bonds are the intradimer bonds, which are present between the α_1 and β_1 monomers within one dimer, and between the α_2 and β_2 monomers within the other dimer

General Rule: Bonds between **different** (heterogeneous) elements are stronger than bonds between similar elements.



Hemoglobin

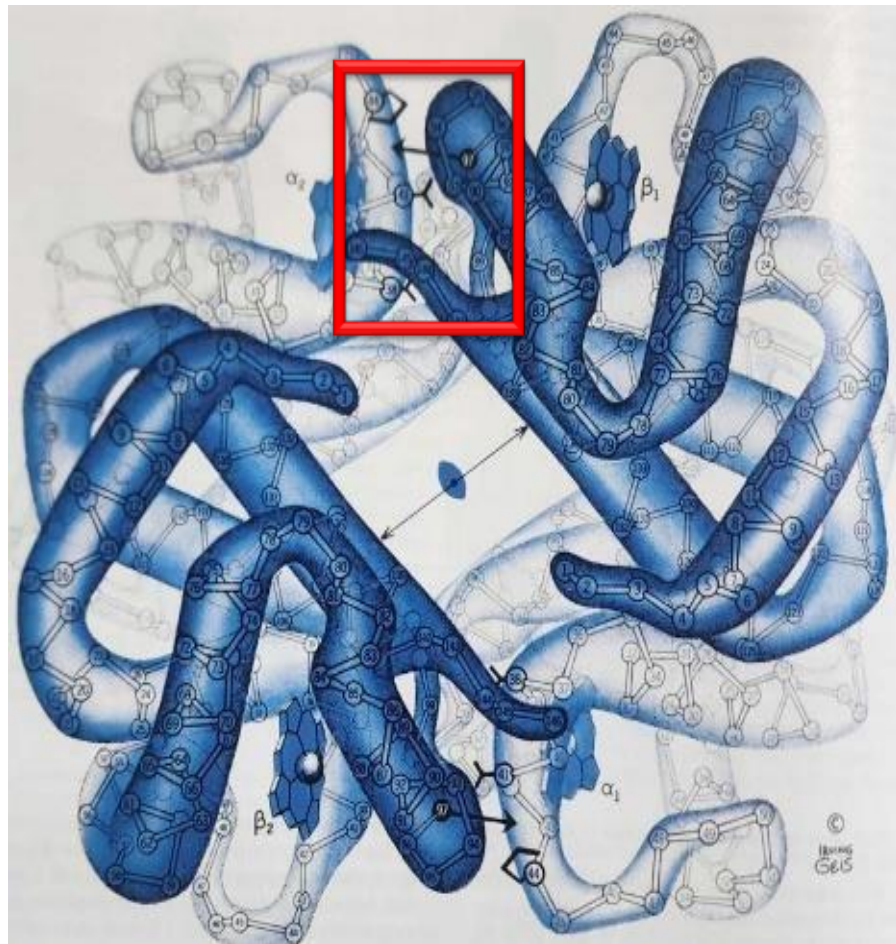


- **There are 3 types of bonds:**
- 1 Strong intradimer hydrophobic and electrostatic bonds (more in number about 35 at each interface) **$\alpha_1\beta_1$, $\alpha_2\beta_2$**
- 2 A little weaker interdimer hydrogen bonds and electrostatic bonds (less in number about 19 at each interface) **$\alpha_1\beta_2$, $\alpha_2\beta_1$**
- 3 The weakest are the long bonds between similar **$\alpha_1\alpha_2$** subunits and **$\beta_1\beta_2$** subunits

The two forms of hemoglobin

- **Deoxygenated Tense “T” state: stabilized by the interdimer bonds**
- **Oxygenated Relaxed “R” state: stabilized by oxygen binding.**
- **Oxygen binding breaks some interdimer bonds and induces the T state to reform to the R state.**
- ***Positive cooperativity towards oxygen. The last Oxygen has 100-250 fold stronger propensity for binding to hemoglobin than the first hemoglobin.***

Oxygen binding induces Reformation of the interdimer bonds upon



The Tense T state (deoxygenated) is stabilized by the interdimer bonds.

✓ Mechanism of Oxygen Binding to Hemoglobin:

When oxygen binds to hemoglobin, bonds begin to break, causing a transition from the T state to the R state.

The first oxygen molecule binds to hemoglobin, followed by the binding of the second molecule, then the third. As the number of bound oxygen molecules increases, the rate and ease of binding increase.

The final oxygen molecule binds with an affinity that is about 150 to 250 times greater compared to the first molecule that binds.

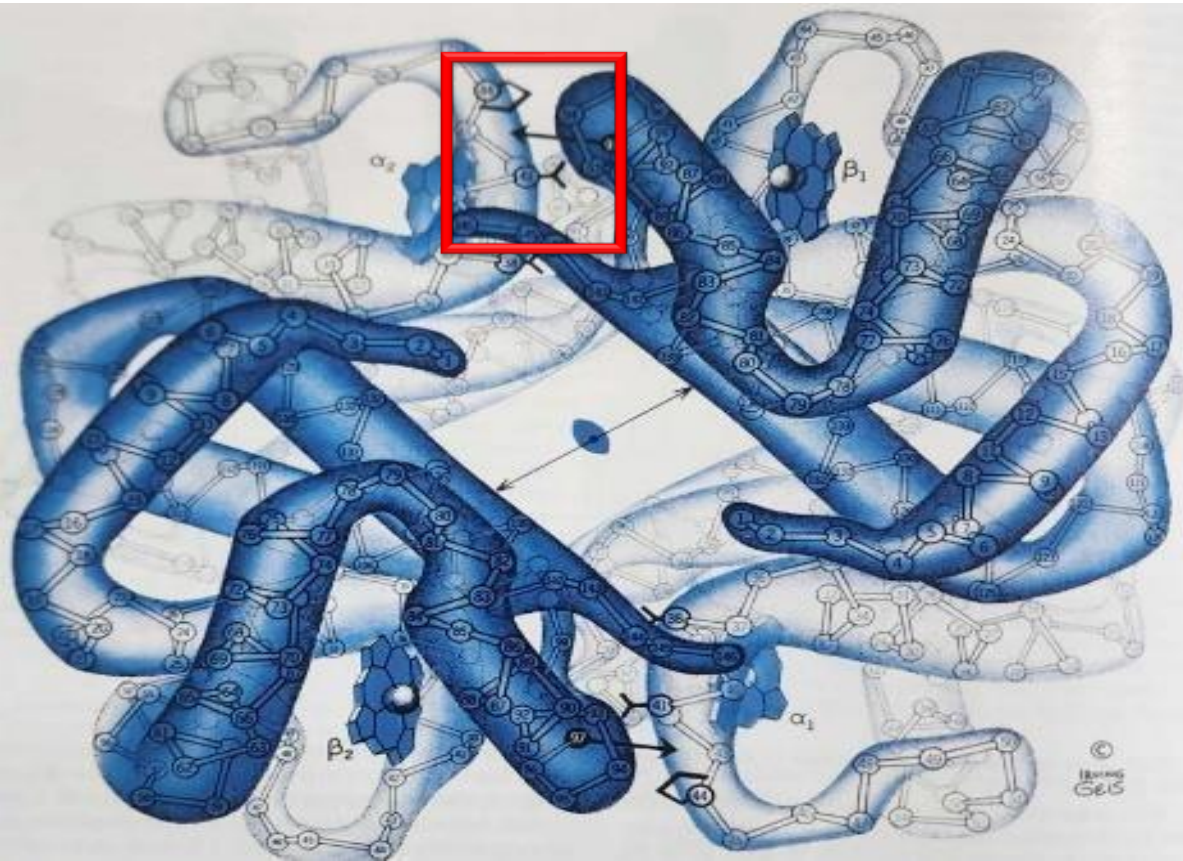
The Reason: Hemoglobin is composed of subunits (alpha and beta), and between these subunits, there are strong bonds specifically those highlighted with red squares (the bonds responsible for stabilizing the dimer).

Once oxygen binds to hemoglobin, these bonds break slightly, the dimer moves with the oxygen binding, and the movement of the entire molecule starts from the heme.

This breakage in the upper bonds leads to a slight difference in the distance between the molecules on the left and right (we notice some molecules are closer while others move slightly downward due to the breakage of some bonds).

This process starts with oxygen and heme and ends with the breakage of bonds, leading to the promotion of further oxygen binding to hemoglobin.

Oxygen binding induces Reformation of the interdimer bonds upon

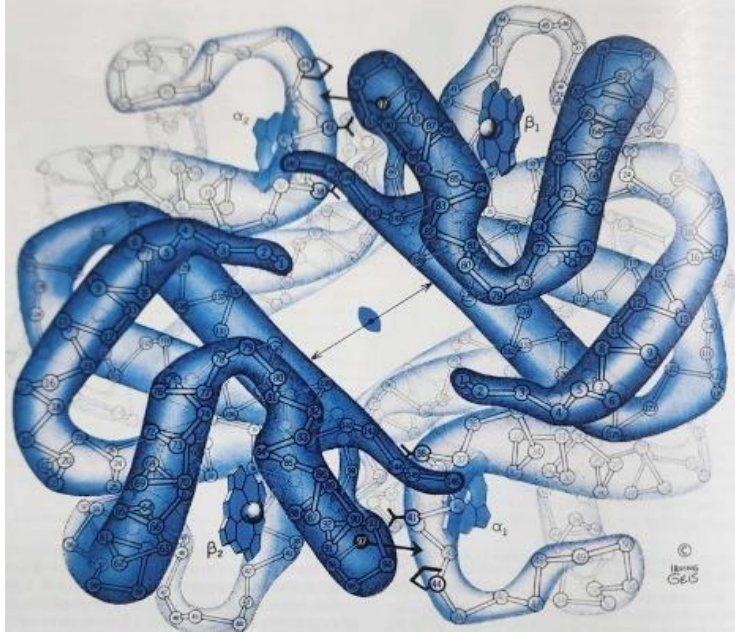


The Tense T state (deoxygenated) is stabilized by the interdimer bonds.



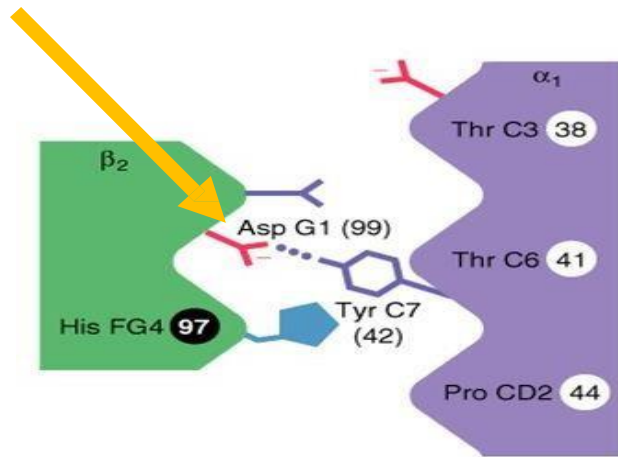
The Relaxed R state (oxygenated) is stabilized by the oxygen. Note the reformation of the bonds upon oxygen binding

Oxygen binding induces Reformation of the interdimer bonds upon



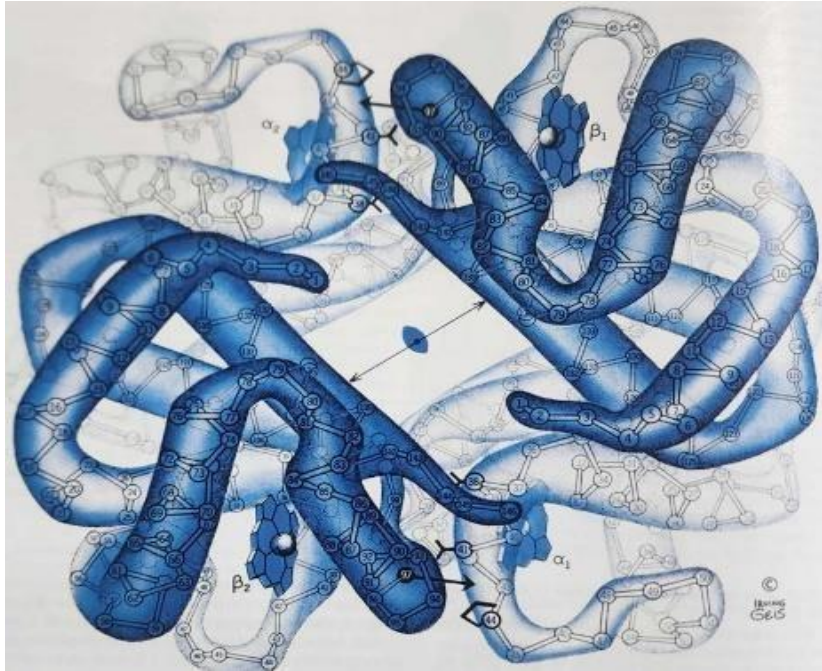
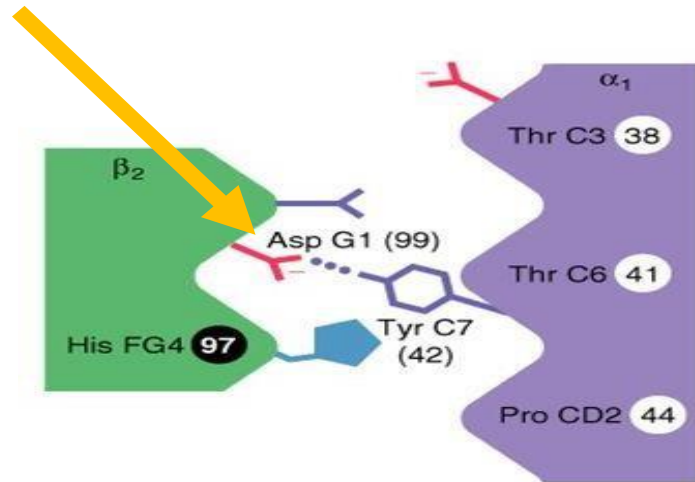
- **Nature of Bonds in Hemoglobin:**

* Hemoglobin consists of four subunits: two α and two β chains, arranged as two $\alpha\beta$ dimers.* The subunits are held together by numerous non-covalent interactions, including hydrophobic interactions, hydrogen bonds, and electrostatic interactions (ionic interactions).* The $\alpha_1\beta_1$ and $\alpha_2\beta_2$ interfaces are stabilized by strong interactions, while the $\alpha_1\beta_2$ and $\alpha_2\beta_1$ interfaces undergo important changes during oxygen binding.* In the deoxygenated T state (tense), multiple ionic interactions and hydrogen bonds help stabilize the structure.* When oxygen binds, hemoglobin undergoes a conformational change toward the R state (relaxed), altering interactions between its subunits and increasing its affinity for oxygen. Example: Aspartate 99* β Asp99 (Aspartate at position 99 of the β chain) participates in intersubunit hydrogen-bonding interactions, particularly with α Tyr42.* These interactions contribute to stabilizing the T state and help regulate hemoglobin's affinity for oxygen.

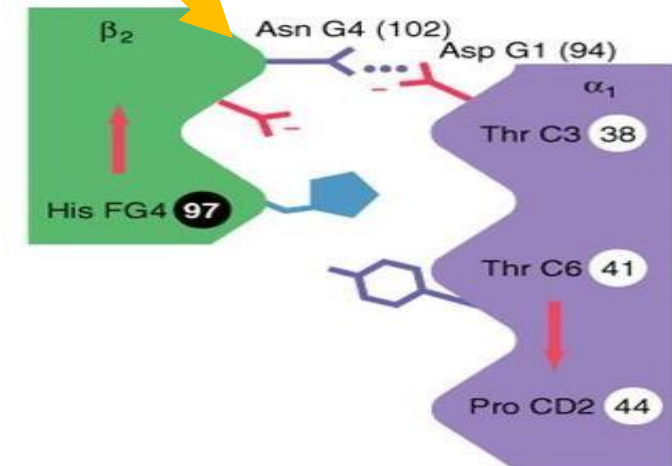


(a) T Form (deoxy)

Oxygen binding induces Reformation of the interdimer bonds upon



(a) T Form (deoxy)



(b) R Form (oxy)

- **Effect of Mutations on Hemoglobin:**

When a mutation occurs in hemoglobin, it directly affects this state.

If hemoglobin maintains the T state and undergoes a mutation, it will transform into the R state, and vice versa.

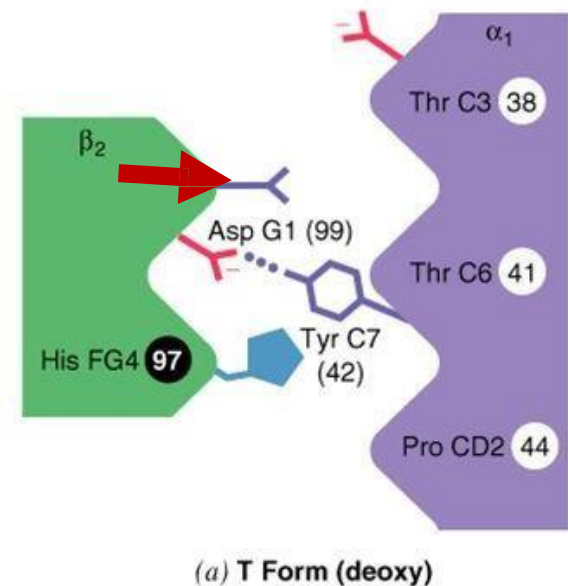
Therefore, if a mutation occurs that keeps hemoglobin stable in the T state, it will neither bind oxygen nor release it as required, because the T state does not bind oxygen well, whereas the R state is the one that binds it and releases it in various tissues.

If hemoglobin remains in the R state, it will bind oxygen and not release it to the tissues, and vice versa if it remains in the T state.

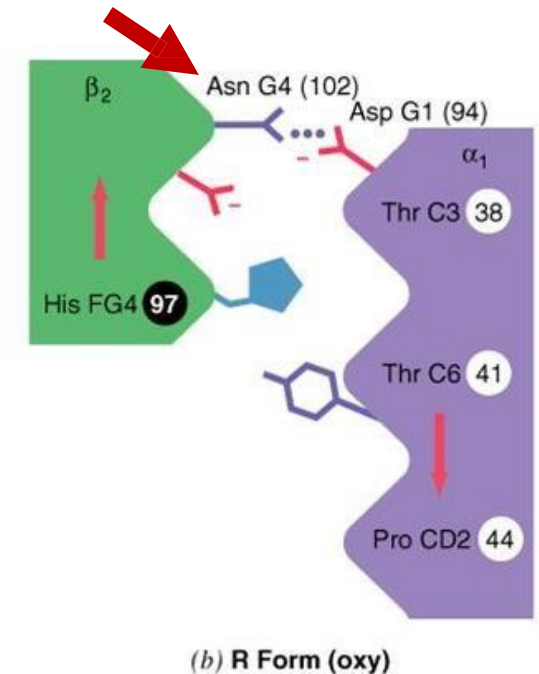
Examples of these mutations that affect these bonds and alter the normal behavior of hemoglobin include a mutation named **Kansas** and another called **Hikari**.

Mutations at $\alpha 1$ - $\beta 2$ contacts

- Eliminating hydrogen bonds between the chains can also alter the quaternary structure and affects cooperativity.
- Hb Yakima: stabilization of the R state (Asp G1 (99) to His).



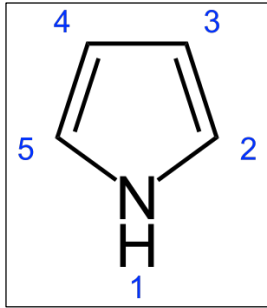
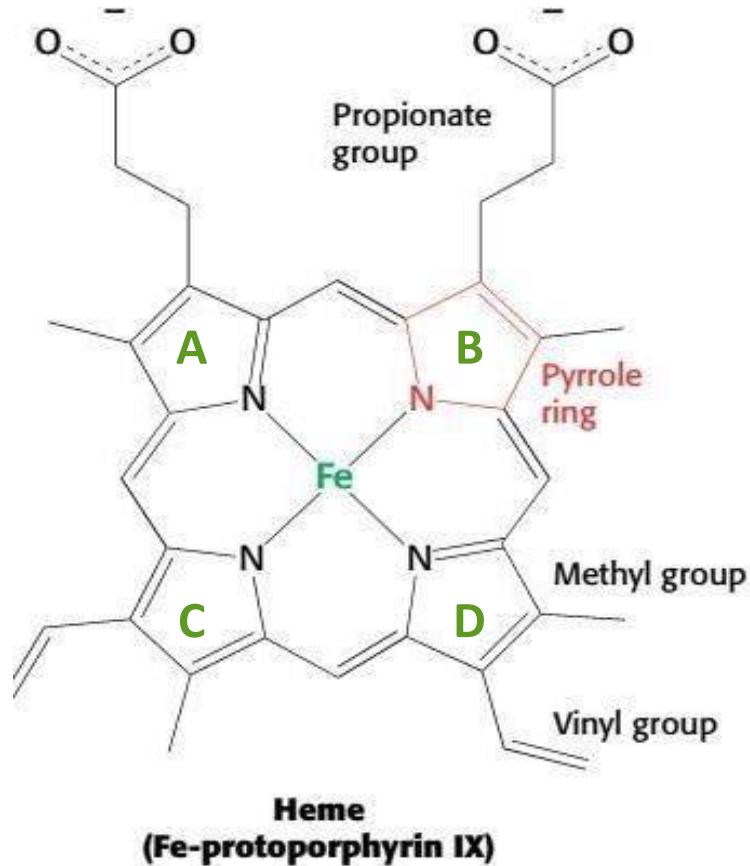
-Hb Kansas: stabilization of the T state (Asn G4 (102) to Thr).



- Heme

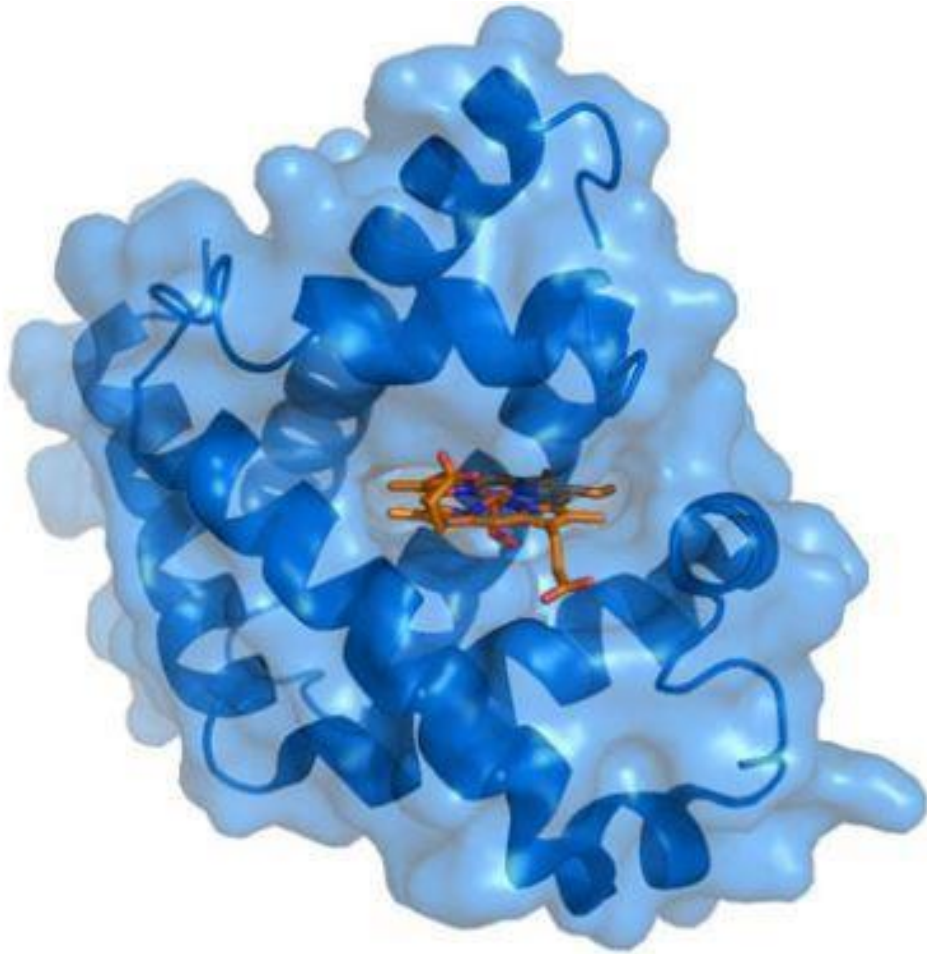
1-C-Heme structure

Pyrrole ring



- The heme is composed of:
- 1 Fe²⁺
- 2 Protoporphyrin (which is composed of):
 - a. Pyrrole rings
 - b. Two of the pyrrole rings have a propionate group each.
 - c. Vinyl groups
 - d. Methyl groups
- *Note: the molecule is hydrophobic.*
- Fe has six coordinates of binding.

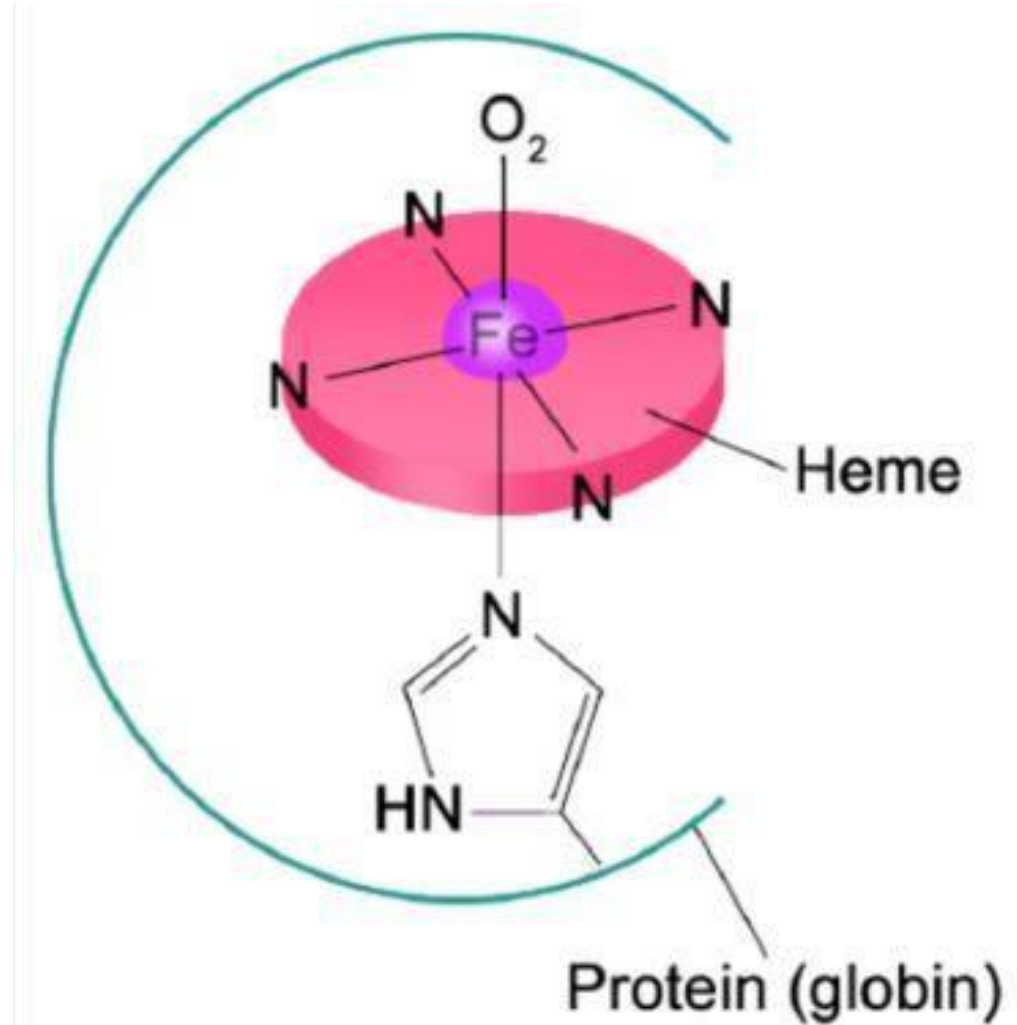
Heme inside a hydrophobic pocket



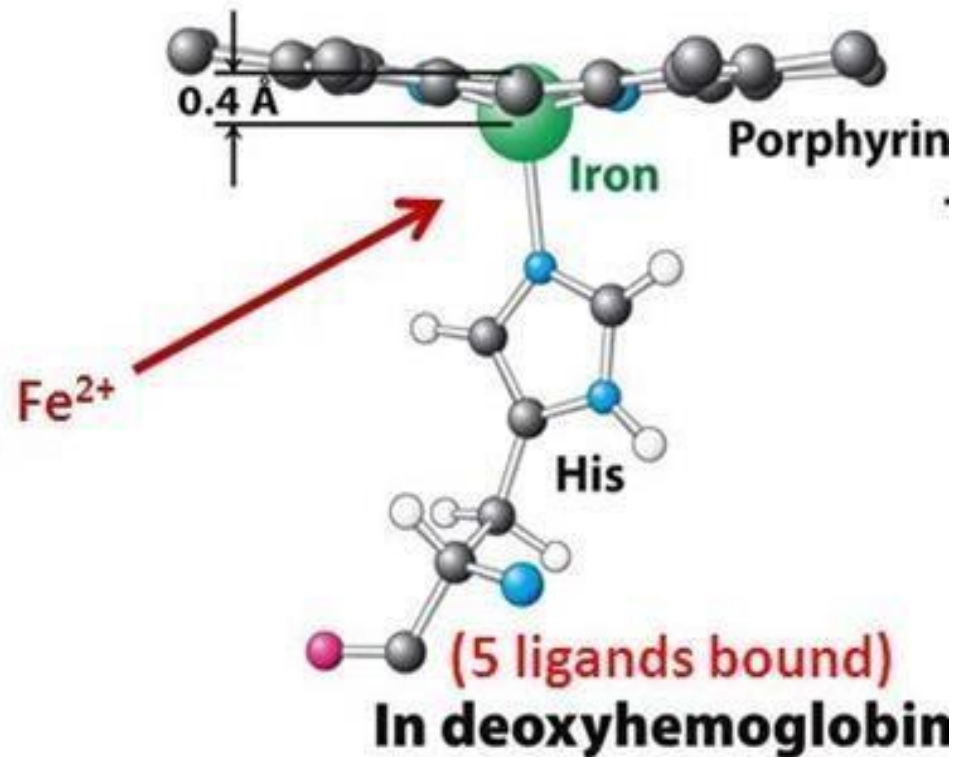
Heme is held inside a hydrophobic pocket (hydrophobic interactions), but the propionate groups (ionic bonds) also participate in stabilizing the heme inside the Hemoglobin.

Fe+2 attachments

- Fe+2 makes 5 bonds. 4 with the protoporphyrin and the 5th with the globin protein through the proximal Histidine, it is a covalent bond (This is a very important bond)
- In the presence of Oxygen, Fe+2 makes a 6th bond with Oxygen.



Structural amplification change



F-Helix Involvement:

There is a component called the **F-helix** which is linked to heme and the proximal histidine.

When oxygen binds, it pulls the proximal histidine along with it, which moves the F-helix and triggers a chain reaction breaking and shifting bonds across the structure.

The Movement and Conformational Change (the 0.4Å Shift):

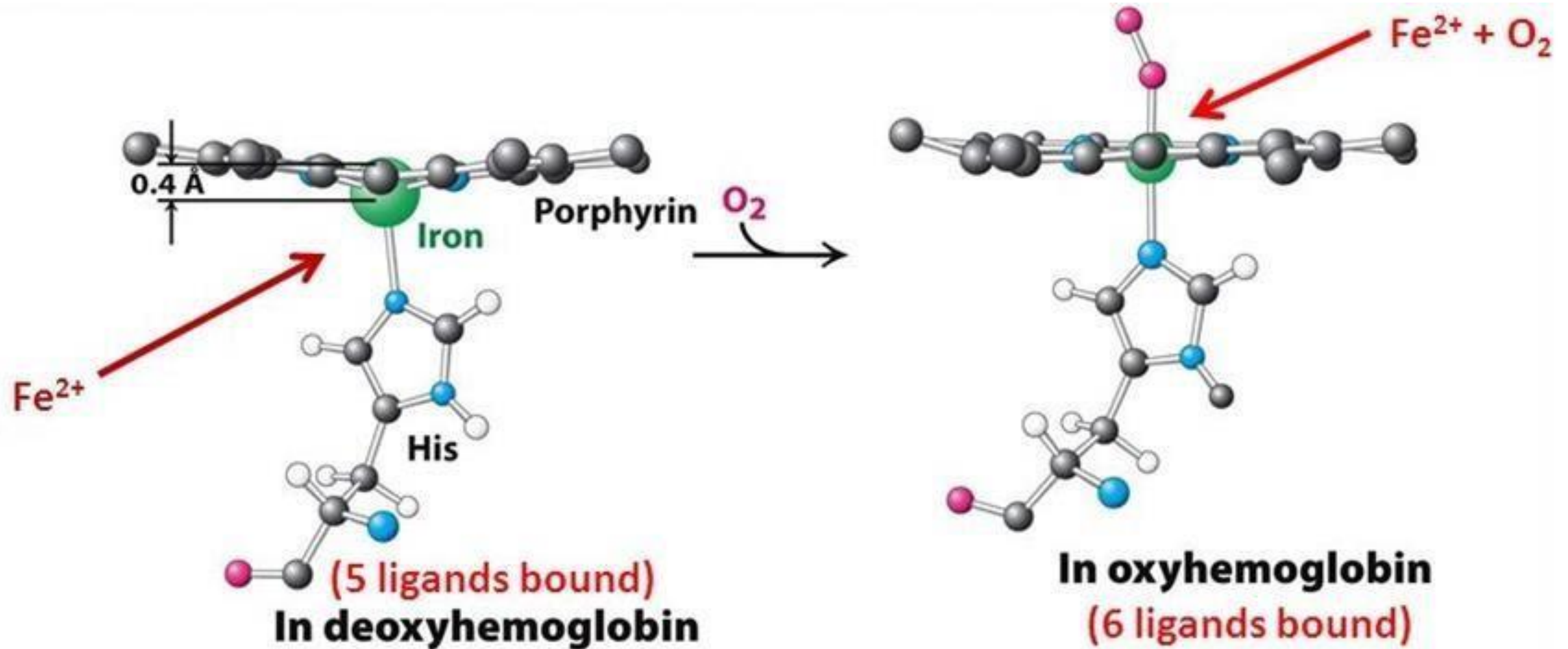
The 0.4Å Point: A tiny shift of 0.4Å (angstroms) causes all the structural changes.

Umbrella-like Shape: Heme has iron and porphyrin, shaped somewhat like an umbrella not completely flat on a single level, but pushed down slightly (the 0.4Å shift is minor but impactful).

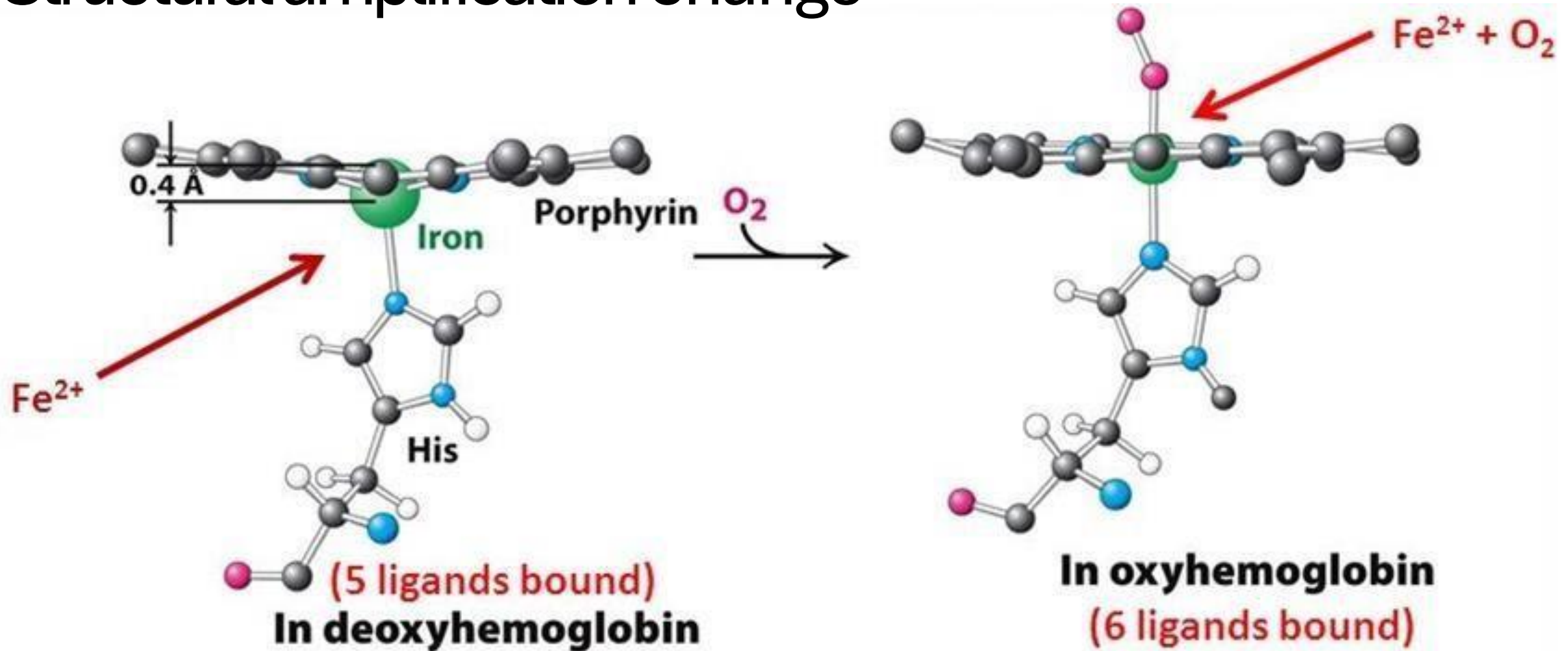
Oxygen Binding & Movement: When oxygen binds to the first subunit, it lifts that 0.4Å point, pulling the iron up to approximately the level of the porphyrin ring.

Cooperativity: This simple 0.4Å movement causes a conformational change that increases the affinity of the remaining subunits for oxygen. Thus, oxygen binding makes the second unit bind easier than the first, the third easier than the second, and the fourth has the highest affinity

Structural amplification change



Structural amplification change



- The small move of Iron upon binding to Oxygen and the pull on the proximal His a.a. propagates through the protein and causes breakage of the electrostatic bonds at the other oxygen-free hemoglobin chains.
- It causes a change in the tertiary structure of individual hemoglobin subunits, allowing them to bind to Oxygen more readily.

Reformation of hydrogen bonds

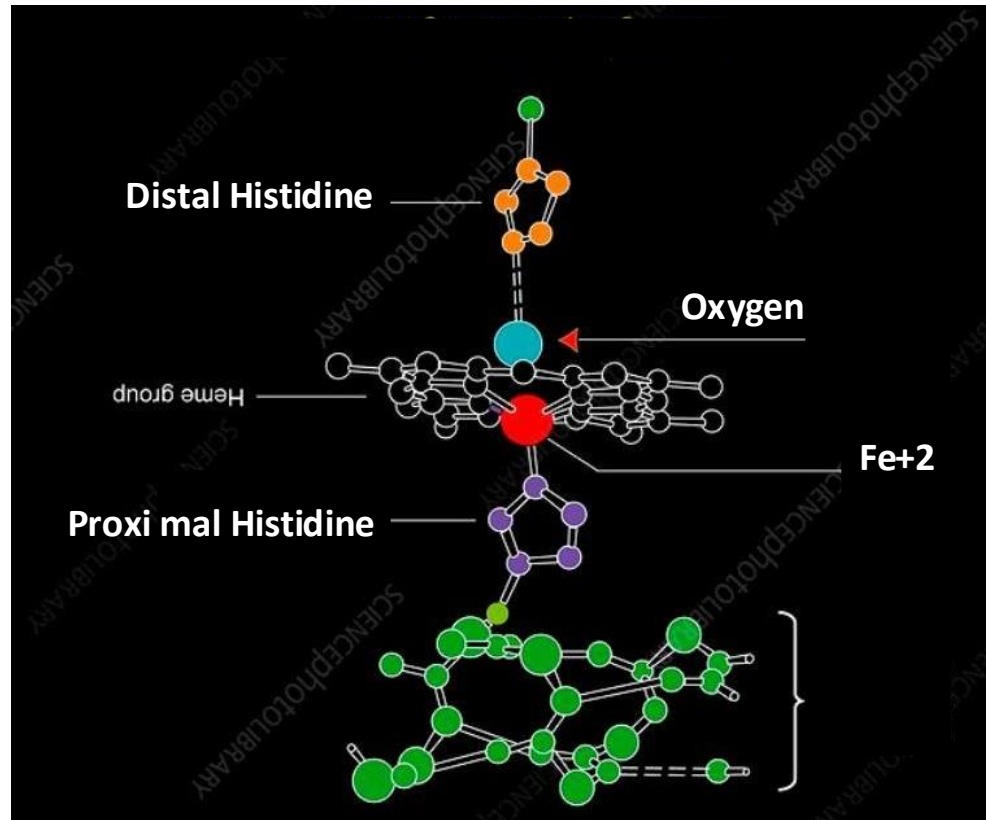
 Note: It is not the sliding that causes the Oxygen to bind more strongly to the other subunits. It is the total 3-D conformational change that does so. It could be a change to the binding site on each subunit where the Fe+2 becomes situated closer to the plane of the heme, or it could be a better opening of the Oxygen gate to allow better passage of Oxygen, or both.

Last piece of information about the structure

There is another Histidine called the distal Histidine that has several functions:

- It works as a gate keeper that allows the passage of Oxygen only.
- It binds to Oxygen and prevents it (the Oxygen) from oxidizing the Fe^{+2} (Ferrous) iron to Fe^{+3} (Ferric) by stabilizing the Oxygen (sharing an electron).

Proximal Histidine:
Serves as the primary structural link that drives the mechanical movement and keeps iron in the (ferrous) state. It must *not* become (ferric), because if it becomes Fe^{3+} it becomes non-functional.



Distal Histidine: Has two main functions:

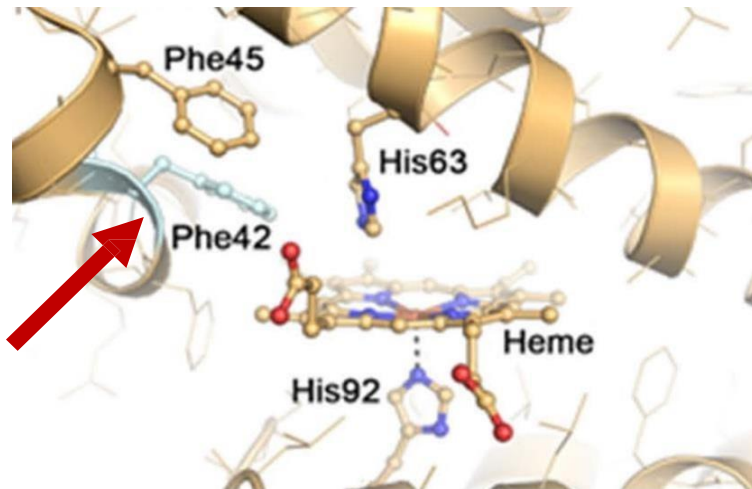
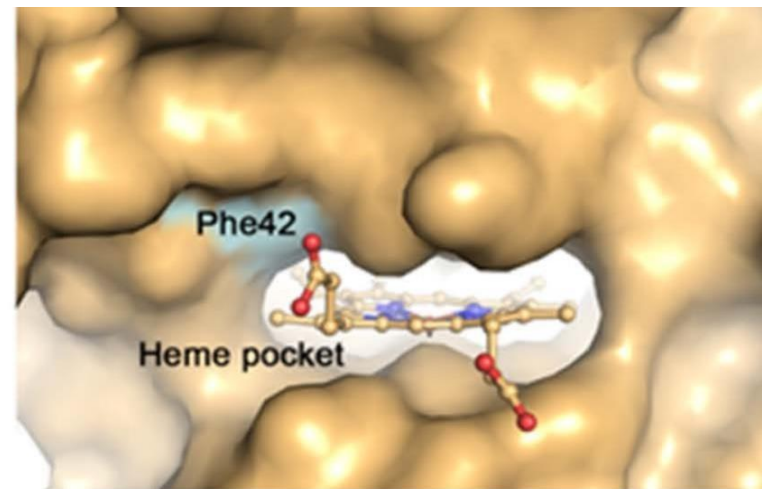
- Acts as a gatekeeper that restricts how oxygen passes through.
- Keeps oxygen inactive/stabilized. If too much electron transfer occurs, iron could convert from Fe^{2+} to Fe^{3+} and escape. The distal histidine protects the complex so that iron remains Fe^{2+} and prevents the formation of harmful reactive oxygen species (or Fe^{3+}) conversion, which leads to major clinical problems

- 1-D-Heme mutations:
 - 1 - Hammersmith
 - 2 - Methemoglobin Reversible → Reagents
 - 3 - Methemoglobin non-reversible Boston and Iwate

These mutations reduce the efficiency
of oxygen delivery to cells

1-D-1 - Hb Hammersmith

- Hb Hammersmith results from a point mutation that leads to formation of unstable hemoglobin and denaturation of the globin protein.
- The most common point mutation of Hb Hammersmith substitutes an internal phenylalanine with a serine within the β globin, reducing the hydrophobicity of the heme-binding pocket, heme positioning, and lower oxygen binding affinity causing cyanosis **and poor oxygen delivery to the body.**



1-D-2- Methemoglobin (HbM)-Reversible

- Both proximal histidine and distal histidine work to maintain the iron atom in its reduced divalent oxidation state (Fe^{2+}), preventing oxygen from converting into free radicals or iron from oxidizing into its trivalent state (Fe^{3+}).
- Hemoglobin can be reversibly oxygenated because iron remains in the reduced (ferrous, Fe^{+2}) state.
- Oxygen binding to Fe^{+2} may cause the oxidation of Fe^{+2} to Fe^{+3} , forming methemoglobin (HbM), except that the enzyme *methemoglobin reductase* reduces iron back using NADH.



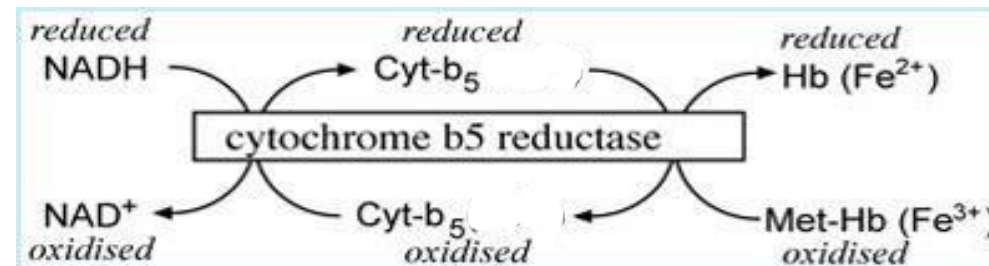
Normal Blood



Chocolate Brown coloured Blood



*Methemoglobin reductase AKA
NADH-Cytochrome b5 reductase*



1-D-2- Methemoglobin (HbM)-Reversible

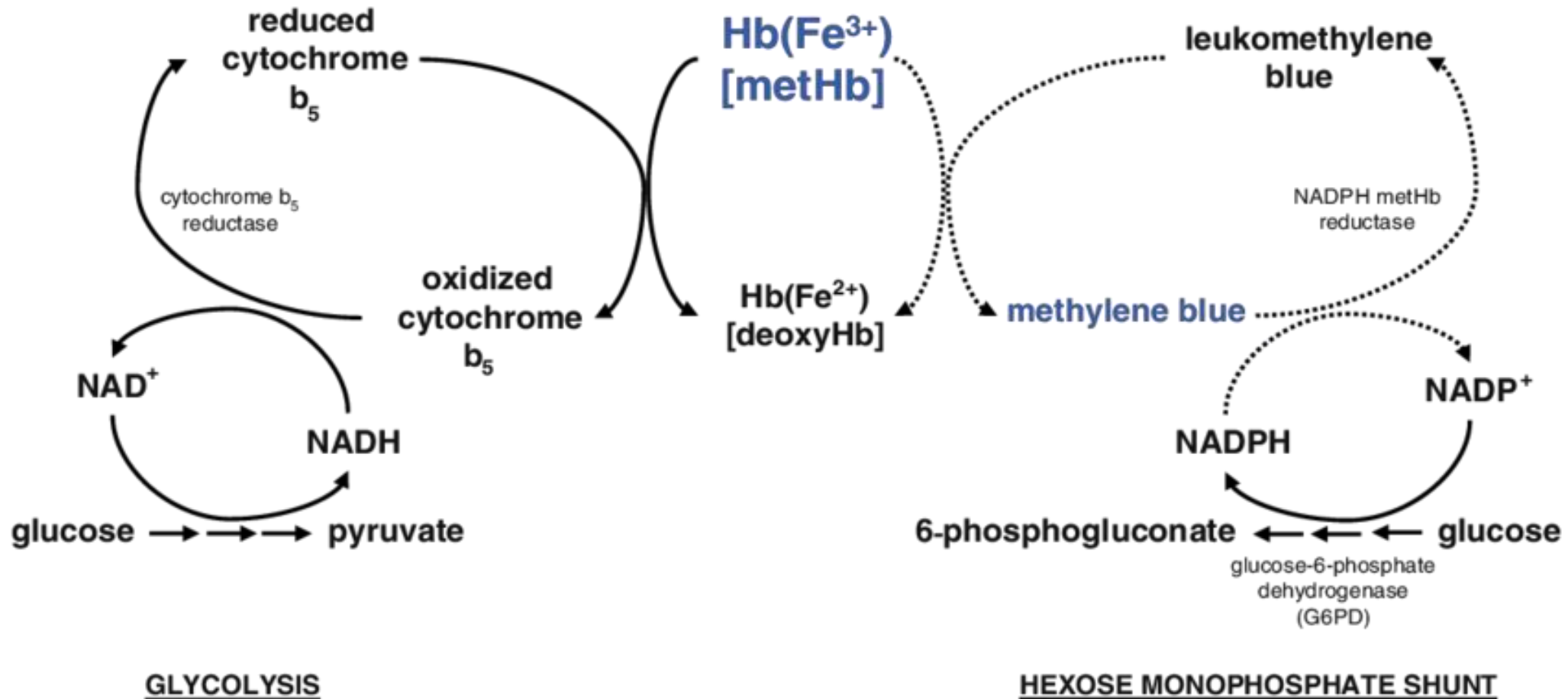
Symptoms are related to the degree of tissue hypoxia, and include anxiety, headache, and dyspnea. In rare cases, coma and death can occur.

Treatment is with methylene blue, which is oxidized as Fe^{+3} is reduced

The erythrocytes of newborns have approximately half the capacity of those of adults to reduce methemoglobin.

Treatment (methylene blue)

Reversible

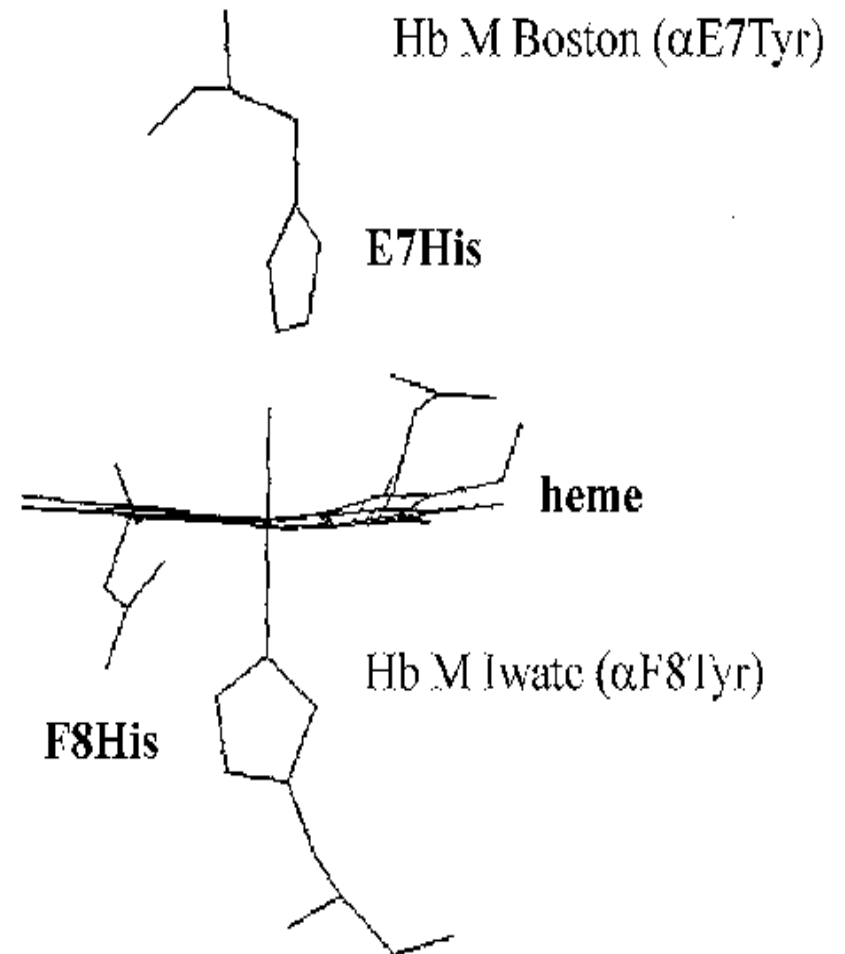


Solid arrows (→) represent normal physiology. Dotted arrows (....→) indicate pathway only active in presence of methylene blue.

NADPH reduces methylene blues to leukomethylene blue which reduces Fe³⁺ to Fe²⁺,
NADPH comes from the Hexose monophosphate shunt

1-D-3-Methemoglobin mutations-irreversible Not sever symptoms

- Some mutant globins (α and β) bond with heme in such a way as to resist the reductase, (non-reversible):
- A- Hb Boston: distal histidine is mutated into a tyrosine resulting in oxidation of ferrous iron by tyrosine's oxygen.
- B- HbM Iwate: proximal histidine is replaced by a tyrosine.



*Names of diseases are not required

2- Hemoglobin effectors

Effectors on Hemoglobin

- The major heterotropic effectors of hemoglobin which happen to be T-state stabilizers:
 - Hydrogen ion, Bohr effect
 - Carbon dioxide
 - Chloride ions
 - 2,3-Bisphosphoglycerate
 - A competitive inhibitor
 - Carbon monoxide
 - An inhibitor
 - Heat
- T state is Induced and stabilized by a group of effectors that help hemoglobin release oxygen to the tissues.**

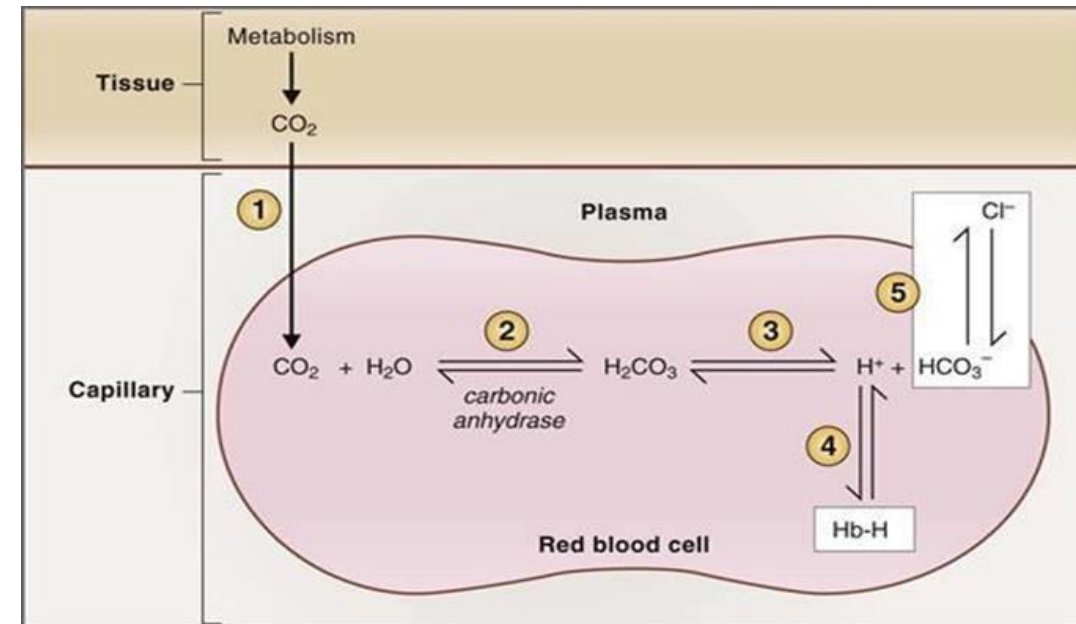
Where do protons come from?

CO₂ is produced at high levels in metabolically active tissues. Then it enters the RBCs and reacts with H₂O through carbonic anhydrase as shown in the figure and the equation to the right to produce H₂CO₃ (carbonic acid) which breaks to H⁺ and HCO₃⁻ (bicarbonate).

1 - The H⁺ binds to Arg-141 on the α monomers inducing the hemoglobin to release the Oxygen. This is called Bohr effect and it stabilizes the T-state. Major effector in releasing O₂.

Bohr effect is the binding of hydrogen ions to the C-terminus of hemoglobin—specifically to "Arginine 141" on the alpha chain and "Histidine 146" on the beta chain. This shifts hemoglobin from the "R" state to the "T" state, facilitating oxygen release.

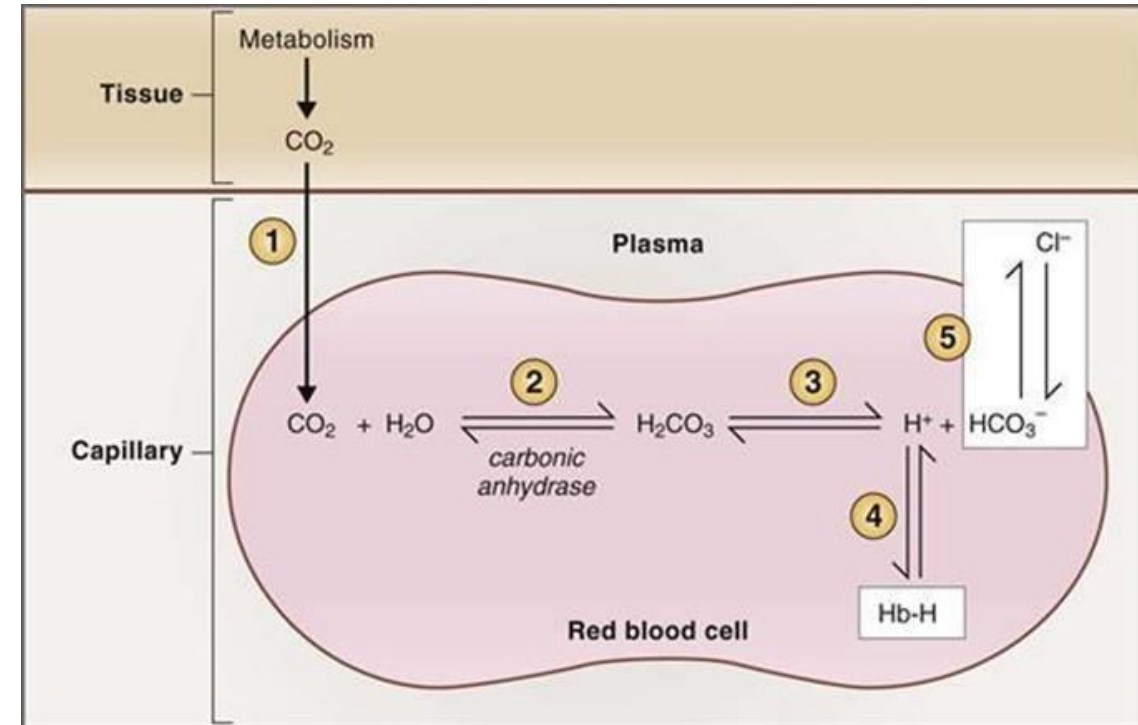
A mutation at these sites causes "Hemoglobin Cowtown," where hemoglobin remains stuck in the "R" state insufficient hydrogen binding.



2 – The H_2CO_3 that is produced is converted to H^+ + HCO_3^- , the HCO_3^- leaves the cell and is replaced by Cl^- (to maintain the electrical charge and, osmolarity of the cell) which binds to the hemoglobin inducing the T-state.

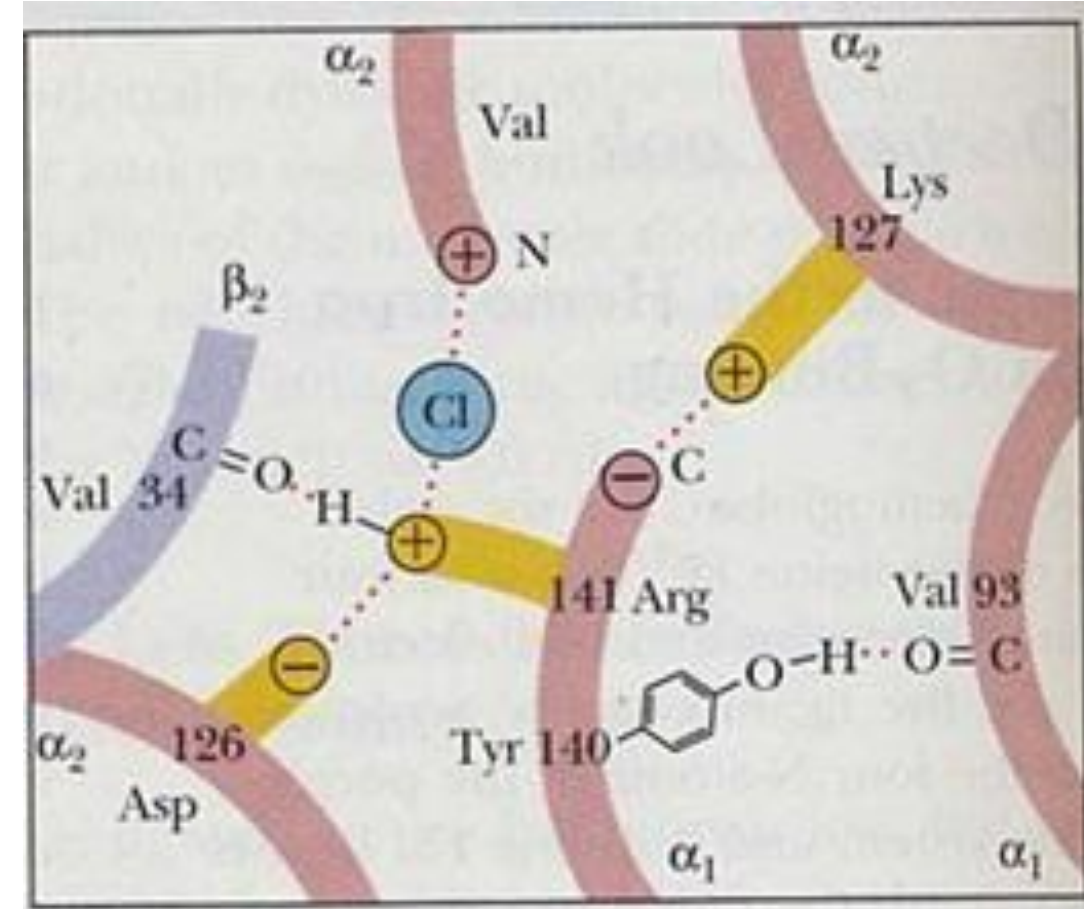
3 - Most CO_2 is transported as HCO_3^- in the plasma. Some CO_2 binds directly to the Hemoglobin as carbamate and is transported to the lungs as CO_2 . This CO_2 promotes the T-state.

Note: At the lungs, the high partial pressure of O_2 induces the lungs to bind the O_2 and release the H^+ (part of Bohr effect).



Effect of chloride ions

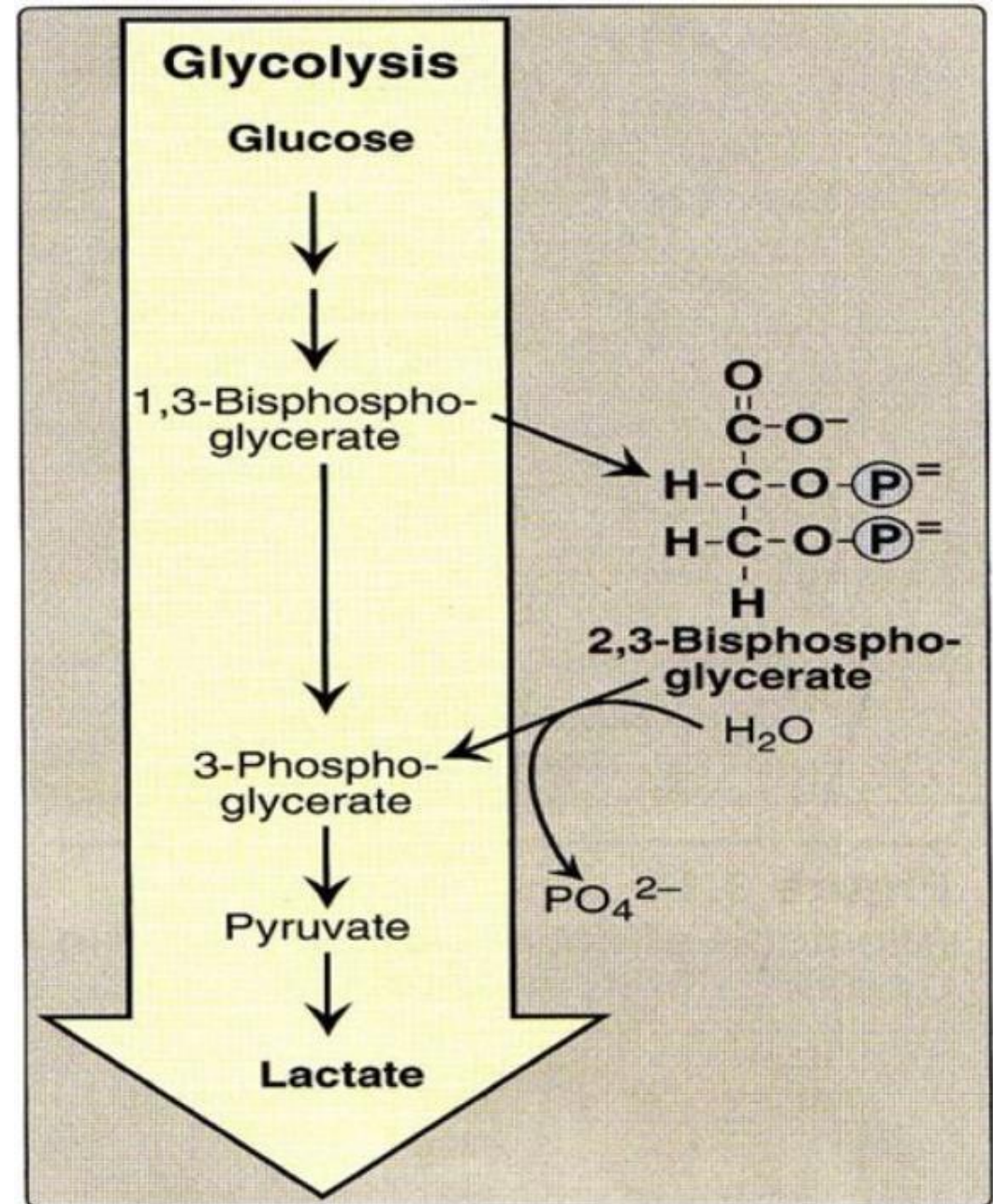
- Chloride ions interact with both the N-terminus of α_2 chain and Arg141 of α_1 chain stabilizing the T-state of hemoglobin.
- Increasing the concentration of chloride ions (Cl^-) shifts the oxygen dissociation curve to the right (lower affinity).



No.4 2,3-bisphosphoglycerate (2,3-BPG)

Scientists observed that isolated, pure hemoglobin binds oxygen extremely tightly and refuses to release it, whereas hemoglobin within the blood releases it easily even without chloride and CO₂. This observation led to the discovery of 2,3-BPG's role.

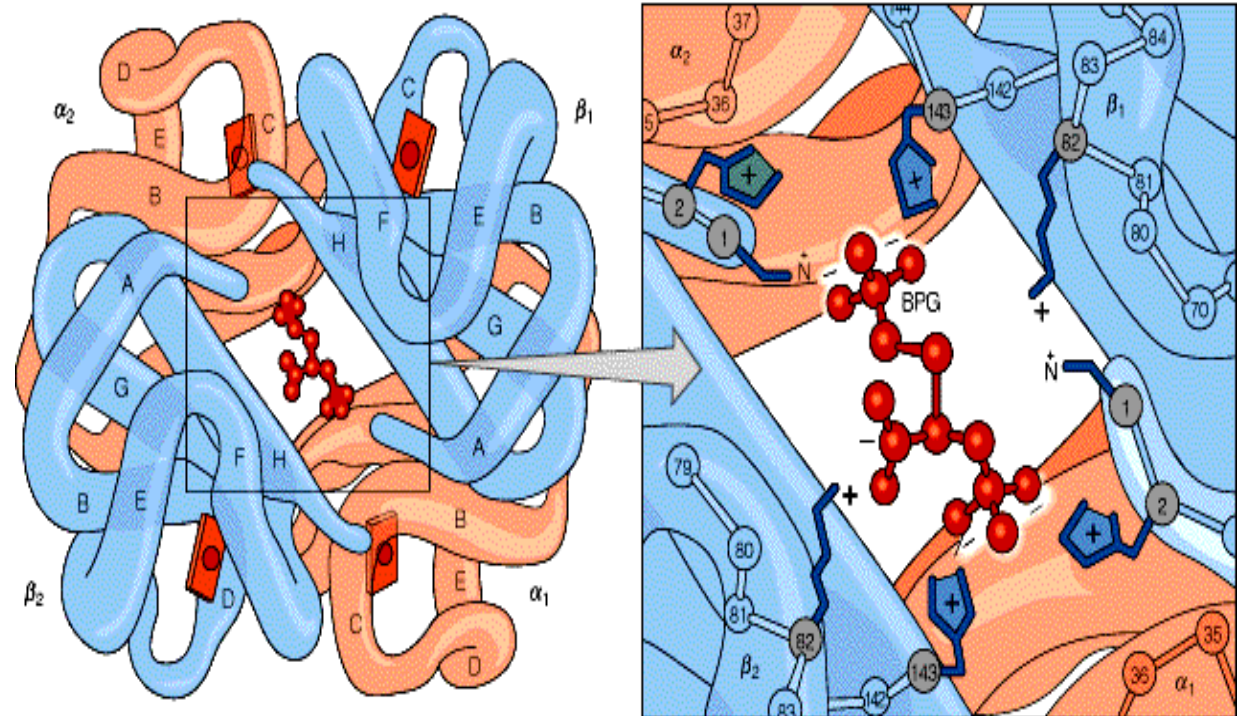
- 2,3-Bisphosphoglycerate (2,3-BPG) is produced as a by-product of glucose metabolism in the red blood cells.
- It binds to hemoglobin and reduces its affinity towards oxygen.



2,3-BPG–hemoglobin interaction

- 2,3-BPG binds in the central cavity of deoxyhemoglobin only in a ratio of 1 (2,3-BPG/hemoglobin) tetramer.
- This binding stabilizes the T-state hemoglobin reducing the binding of oxygen to hemoglobin and facilitating oxygen release.

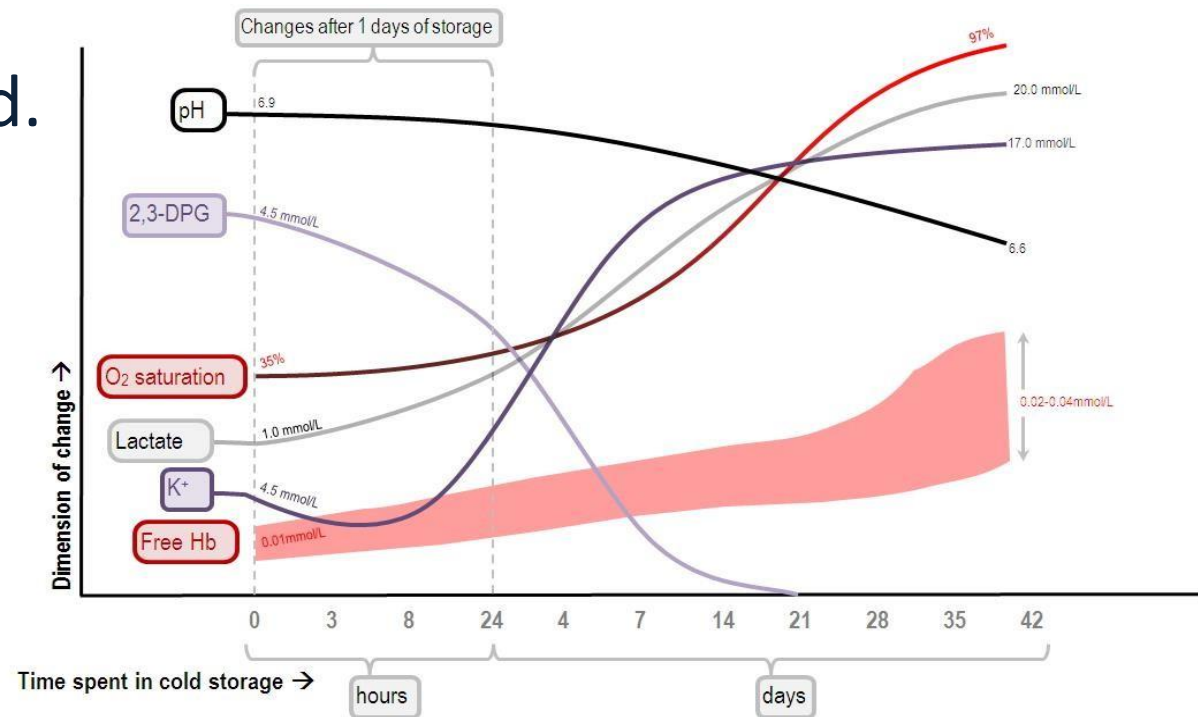
2,3-BPG forms salt bridges with the terminal amino groups of both β chains and with a lysine and His143.



2,3-BPG in transfused blood

- Storing blood results in a decrease in 2,3-BPG (and ATP), hence hemoglobin acts as an oxygen “trap”, not an oxygen transporter.
- Transfused RBCs are able to restore the depleted supplies of 2,3-BPG in 6–24 hours.
- Severely ill patients may be compromised.
- Both 2,3-BPG and ATP are rejuvenated.

Direct addition of 2,3-BPG is ineffective because it cannot cross the red blood cell membrane. Instead, precursors and rejuvenating additives—such as inosine, dextrose, and phosphate—must be added to stimulate the intracellular synthesis of 2,3-BPG and ATP.



Oxygen saturation curve

- The saturation curve of hemoglobin binding to O_2 has a sigmoidal shape.
- It is cooperative.
- At 100 mm Hg (torr), hemoglobin is 97% saturated (oxyhemoglobin).
- As the oxygen pressure falls to 40 torr at rest in the tissues, hemoglobin is saturated at 77%.
- With exercise where the oxygen drops to 20 torr, hemoglobin becomes at 37% saturation which is a big drop to satisfy tissues' needs at exercise.
- Note: at high altitude (~5000 m), alveolar pO_2 = 75 mmHg.*

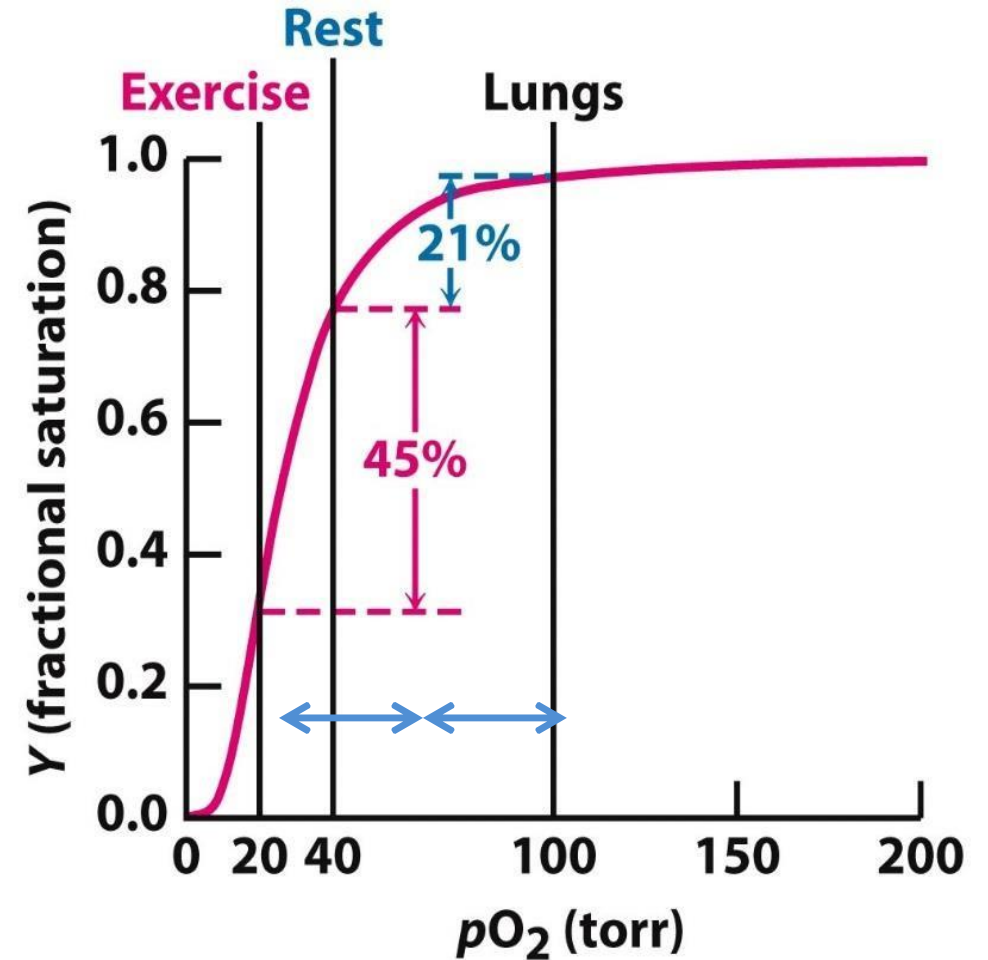
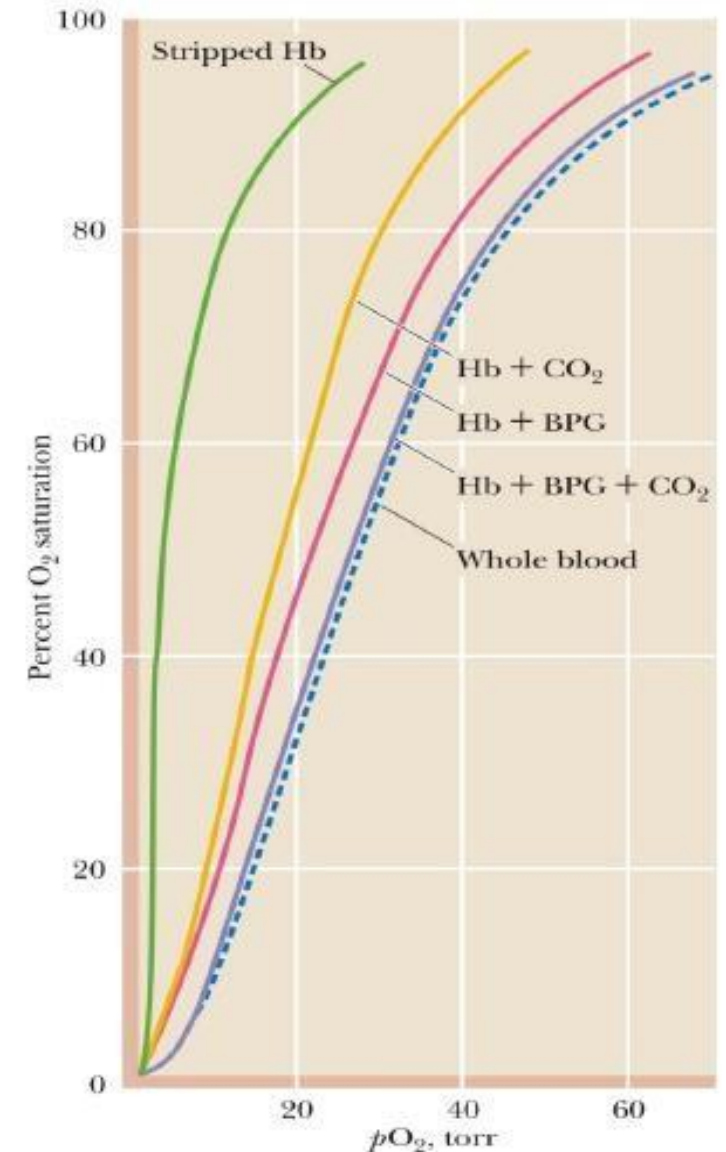


Figure 7.10
Biochemistry, Seventh Edition
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2,3-BPG and CO₂ are important players

- The curve has a sigmoidal (S-shaped) shape. The horizontal axis represents the partial pressure of oxygen (pO_2), and the vertical axis represents the percentage of hemoglobin saturation.
- **Stripped Hemoglobin:** Placed on the far left of the graph, it saturates at the lowest amounts of oxygen and only releases oxygen if the pressure drops close to zero.
- **Adding CO₂ or 2,3-BPG:** Gradually shifts the curve to the right. When both are present (the normal physiological state), the curve shifts further right. This means a higher oxygen pressure is required for saturation in the lungs, but it allows for rapid and easy unloading of oxygen to the cells.



Better explanation of the role of 2,3-BPG

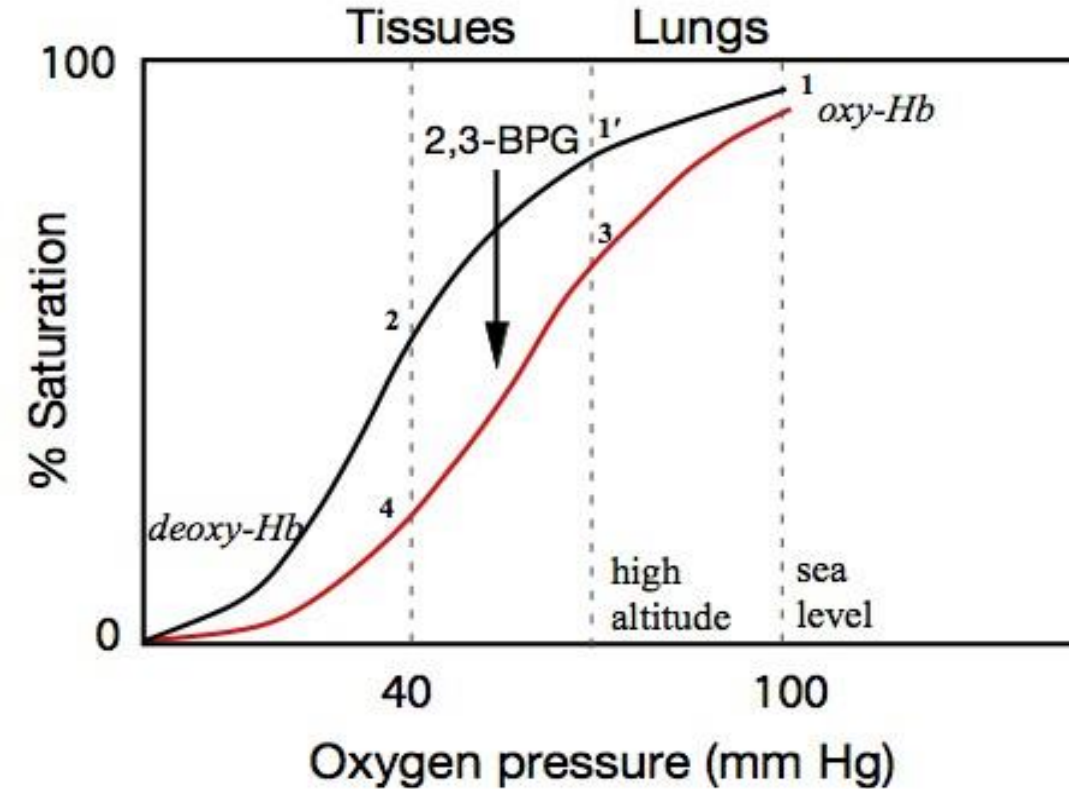
At sea level the lungs pick up oxygen with 100% saturation of Hb (1) and when the oxygen pressure drops to 40 mm Hg in the tissues (2) the Hb will be 55% saturated.

- They have released 45% of bound oxygen.

At high altitudes (in case of no adaptation), Hb is only 80% saturated (1'). Thus at 40 mm Hg in the tissues (2) when Hb is only 55% saturated, it will only have released 25% of its oxygen.

At high altitude (with increased 2,3-BPG production- in red), At the lungs (3) the Hb will be less bound with oxygen — only 70% saturation — but at 40mm Hg in the tissues (4) it will be much less saturated than on the black curve — 30%. Thus, it will have made available 40% of its oxygen.

This is not a perfect solution, but over time there is increased production of red blood cells to provide more hemoglobin to compensate for the smaller amount of oxygen it can bind.

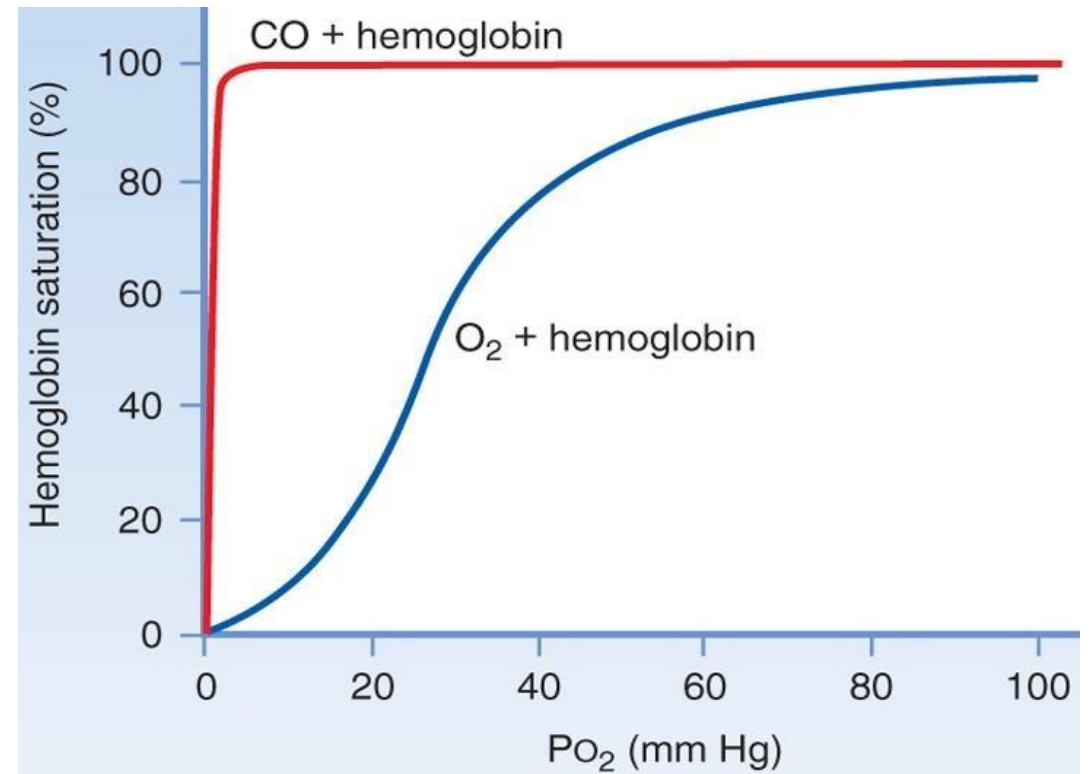


Other factors: B- Effect of CO

Effect of CO

- In addition to competing with oxygen in binding to hemoglobin, the affinity of Hb-CO towards oxygen increases resulting in less oxygen unloading in peripheral tissues **unless oxygen pressure reaches near zero.**

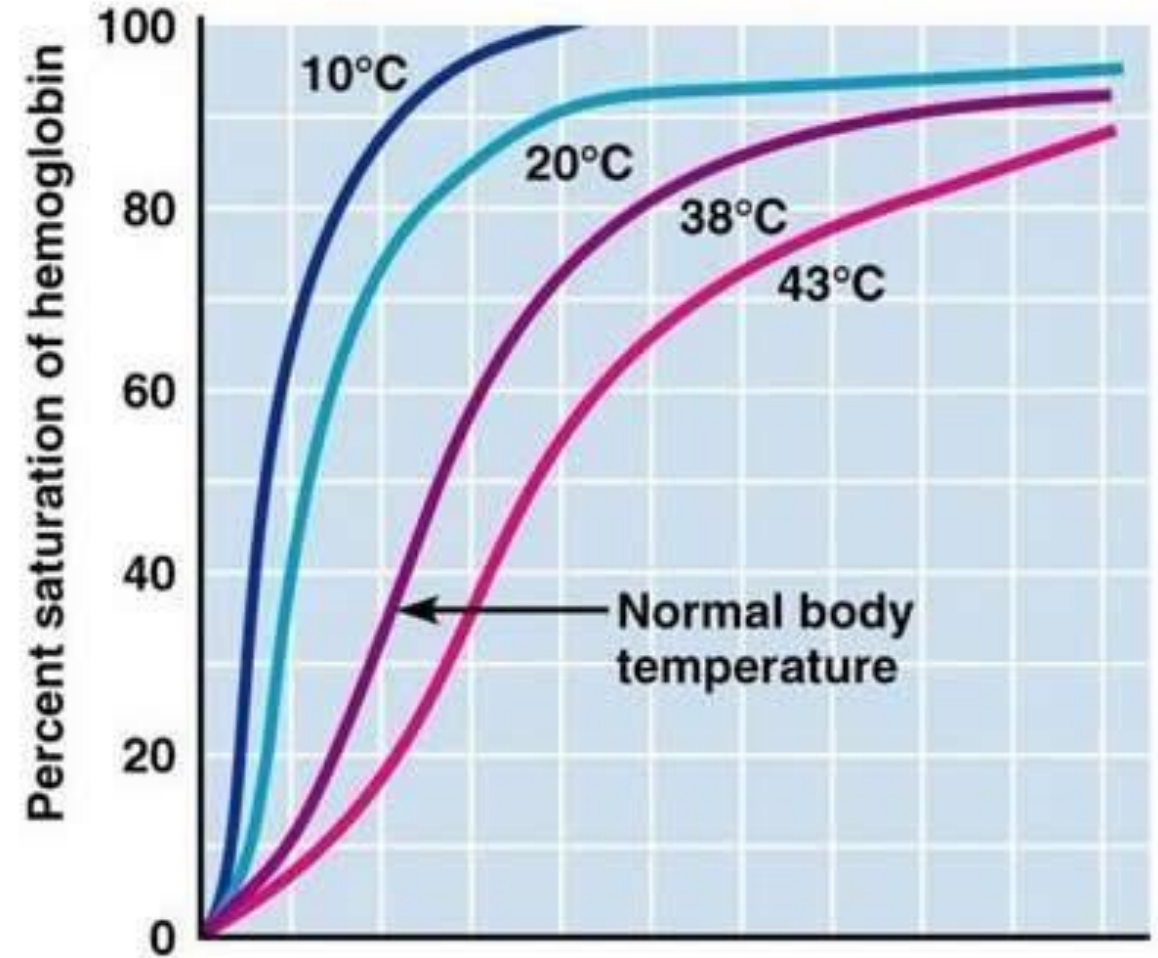
(Hb + O₂) versus (Hb + CO) → the curve illustrates the release of oxygen in the presence and in the absence of CO.



- Other factors: A-Effect of temperature

Effect of temperature

- An increase in temperature decreases oxygen affinity and therefore increases the P50.
- Increased temperature also increases the metabolic rate of RBCs, increasing the production of 2,3-BPG, which also facilitates oxygen unloading from HbO₂.



رسالة من الفريق العلمي:

"Don't stop
when your tired.
Stop when you're
done."

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	Slide 7	3. Strongest Bonds: The strongest bonds are those that bind the two dimers together: Between beta1 and alpha1 Between beta2 and alpha2	bonds are the intradimer bonds, which are present between the α_1 and β_1 monomers within one dimer, and between the α_2 and β_2 monomers within the other dimer
	Slide 12		Check the slide
V1 → V2			