



Hemoglobin

An overview and more

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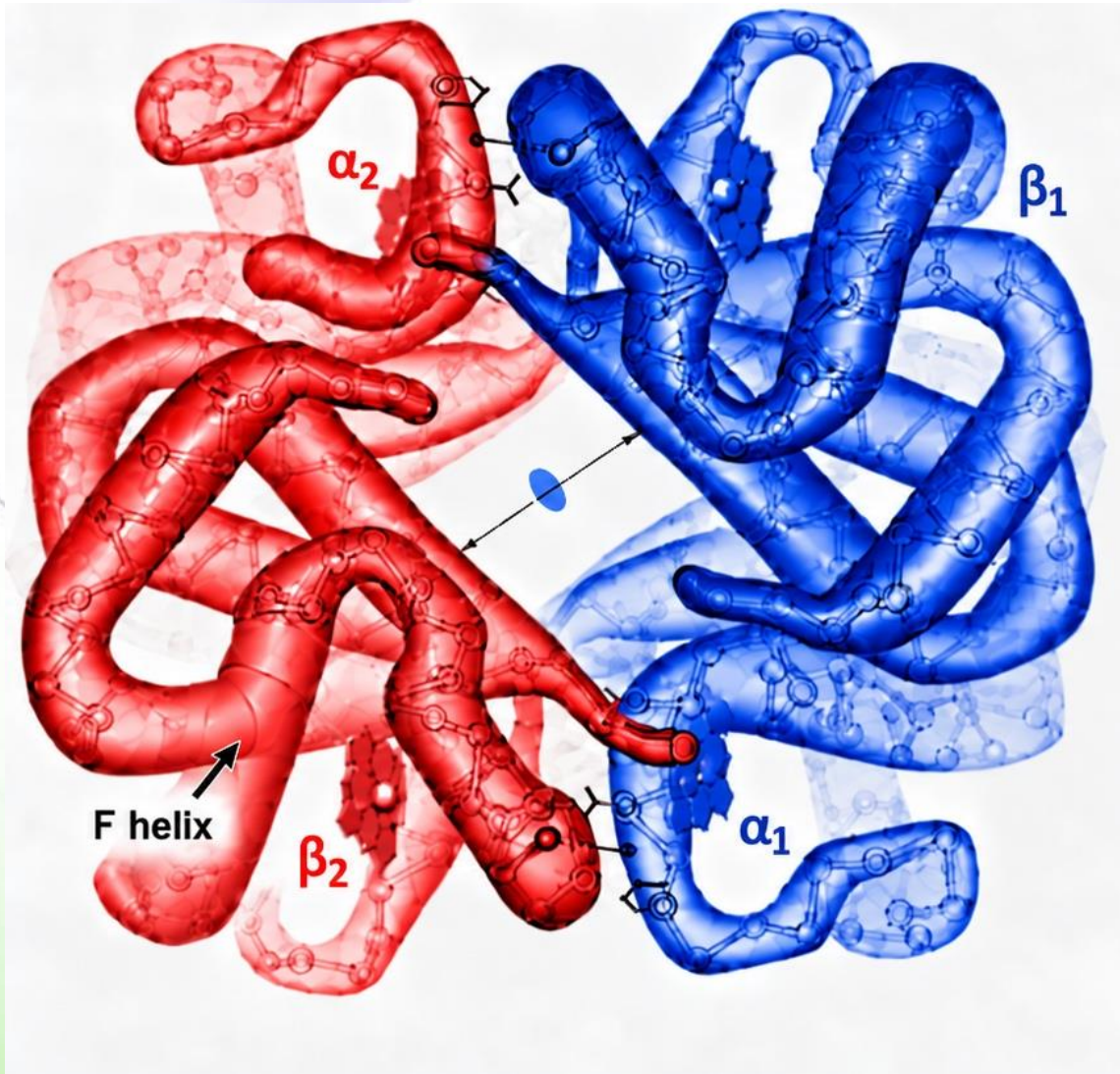
- Voet and Voet Biochemistry
- Hemoglobin O₂ Binding and Allosteric Properties of Hemoglobin
(http://home.sandiego.edu/~josephprovost/Chem331%20Lect%207_8%20Myo%20Hemoglobin.pdf)
- Lecture 3: Cooperative behaviour of hemoglobin
(https://www.chem.uwec.edu/chem452_f12/pages/lecture_materials/unit_III/lecture-3/overheads/Chem452-lecture_3-part_1-overheads.pdf)

What we are covering

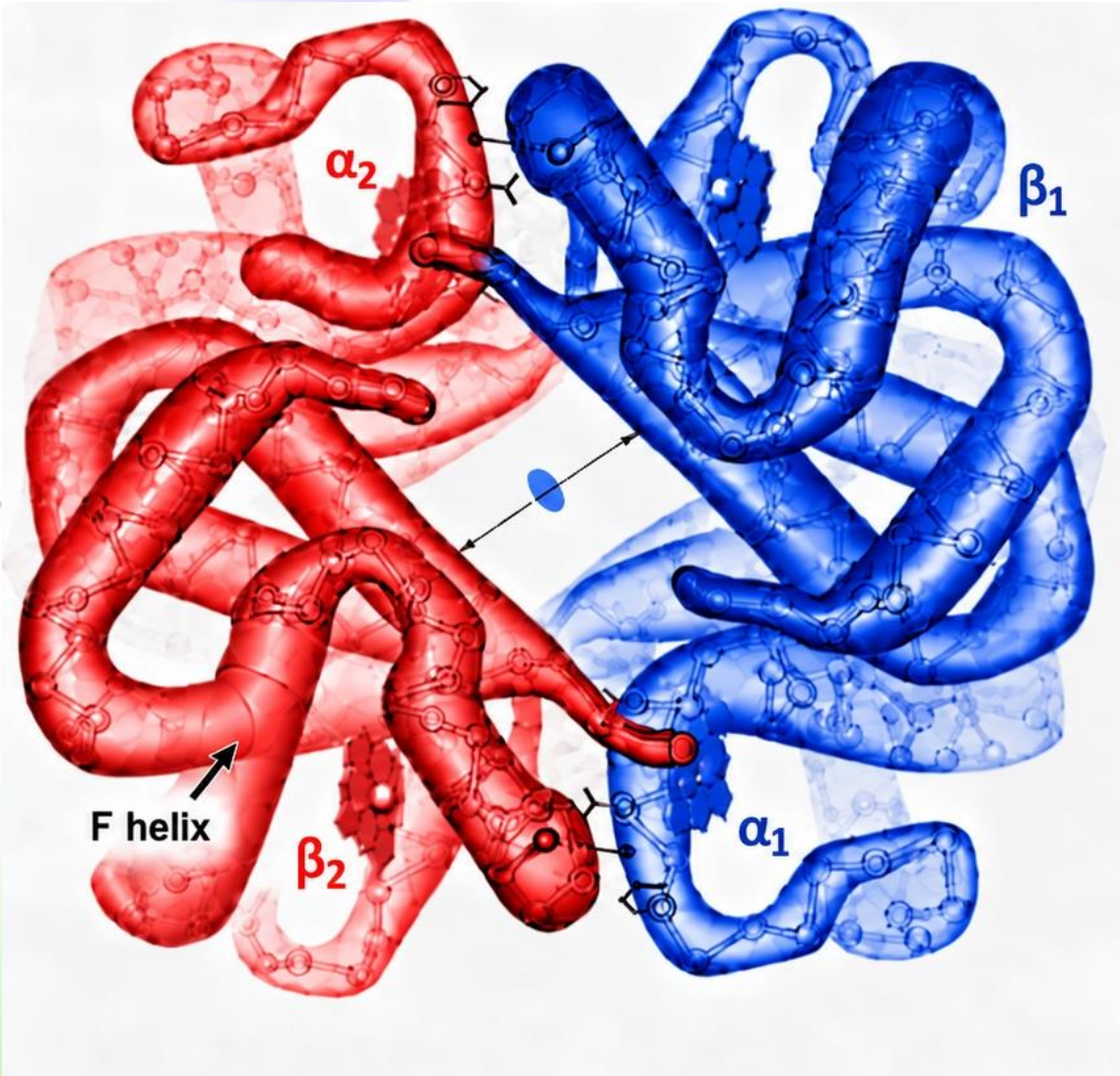


- In this lecture we will be covering:
 - 1- Hemoglobin (Globin and Heme structures)
 - A- The 2 forms of hemoglobin
 - B- Mutations of globin residues
 - C- Heme structure
 - D- Mutations of heme
 - 2- Hemoglobin effectors

1-A-Hemoglobin

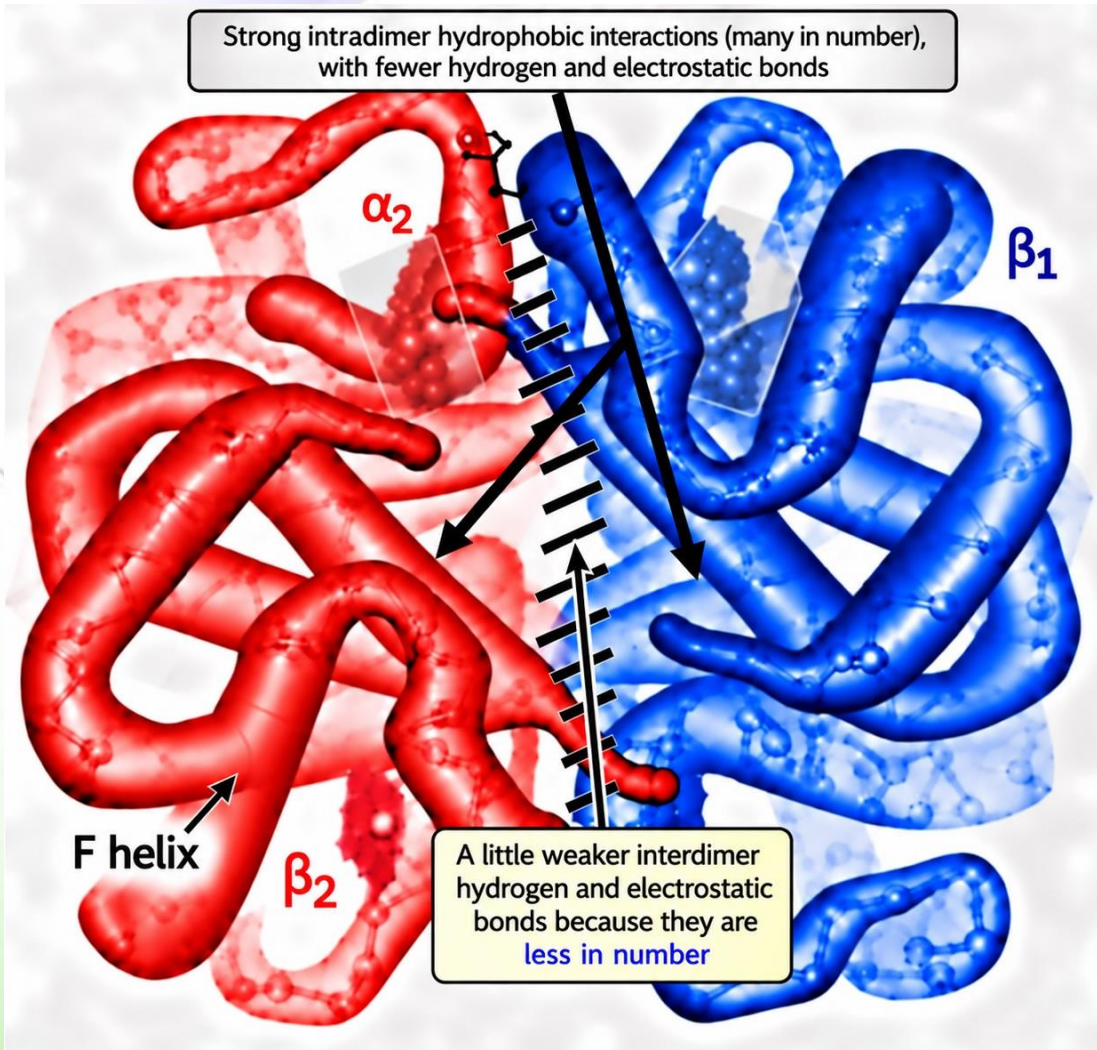


1-A-Hemoglobin



- Hb is a globular protein that is used to transport O₂ 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

Hemoglobin



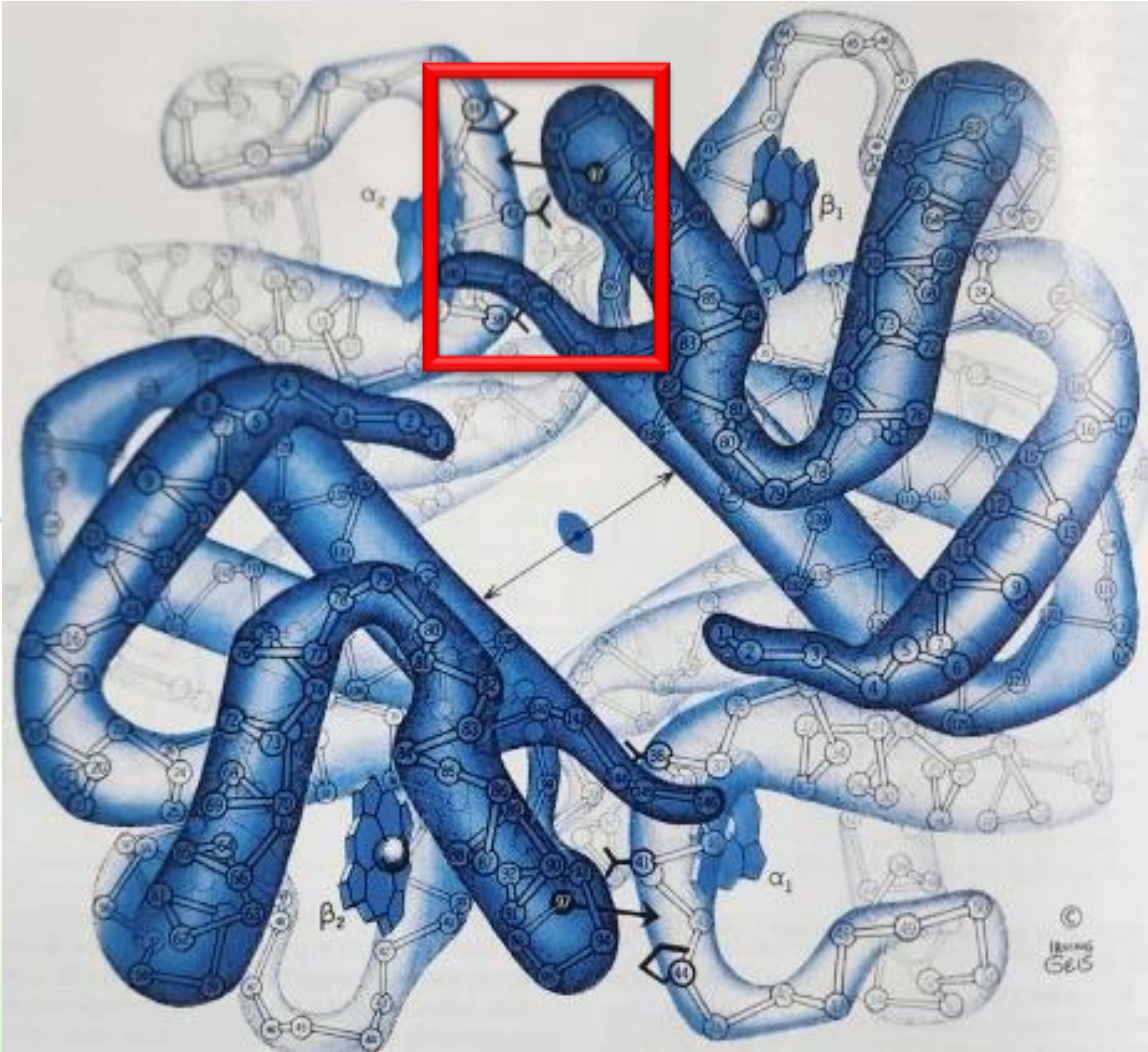
- **There are 3 types of bonds:**
- 1- Strong intradimer hydrophobic and electrostatic bonds (more in number about 35 at each interface) $\alpha_1\beta_1$, $\alpha_2\beta_2$
- 2- A little weaker interdimer hydrogen bonds and electrostatic bonds (less in number about 19 at each interface) $\alpha_1\beta_2$,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



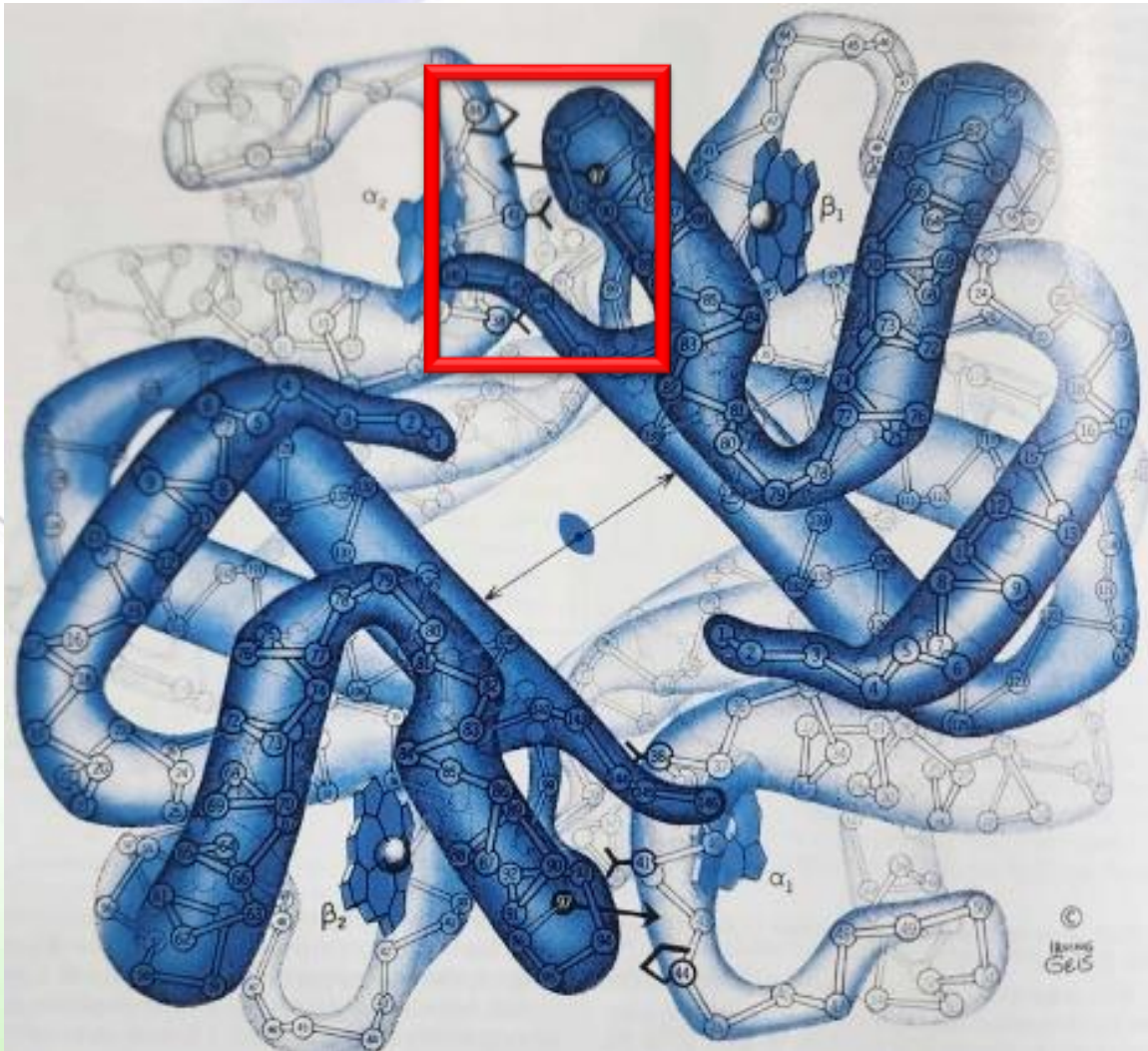
- **Deoxygenated Tense “T” state: stabilized by the interdimer bonds**
- **Oxygenated Relaxed “R” state: stabilized by oxygen binding.**
- **Oxygen binding breaks some interdimer bonds and induces the T state to reform to the R state.**
- ***Positive cooperativity towards oxygen. The last Oxygen has 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.

Oxygen binding induces Reformation of the interdimer bonds upon

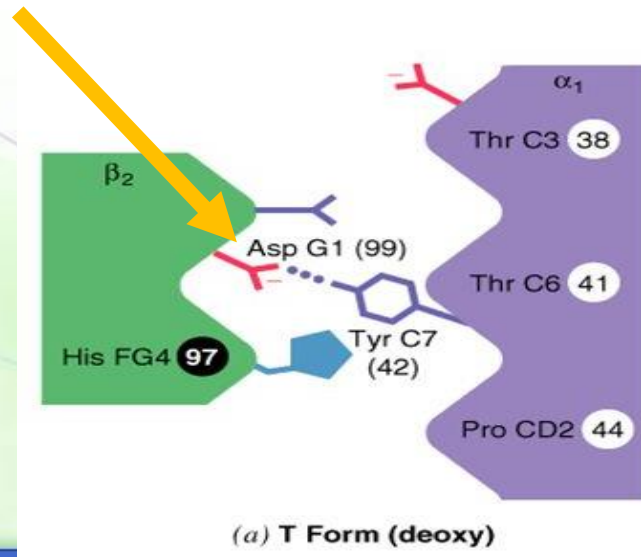
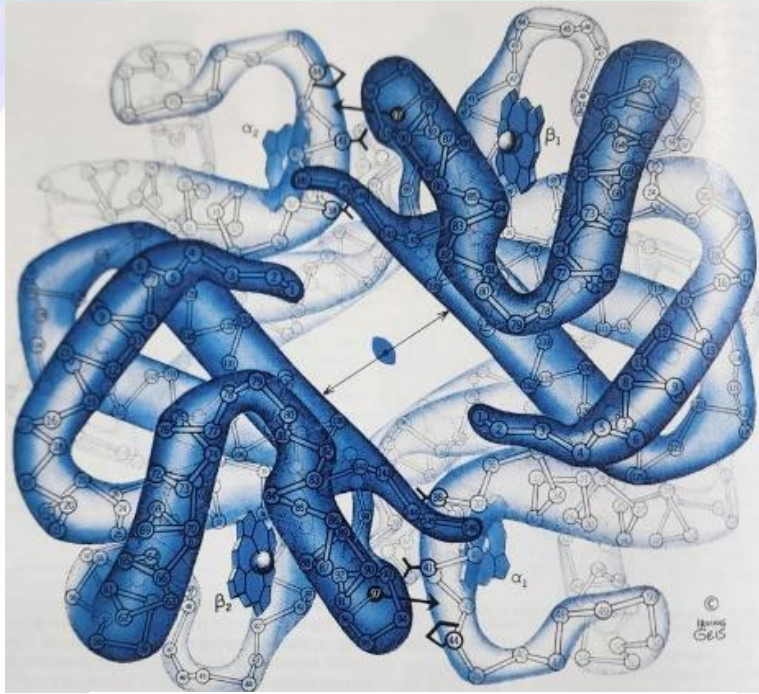


The Tense T state (deoxygenated) is stabilized by the interdimer bonds.

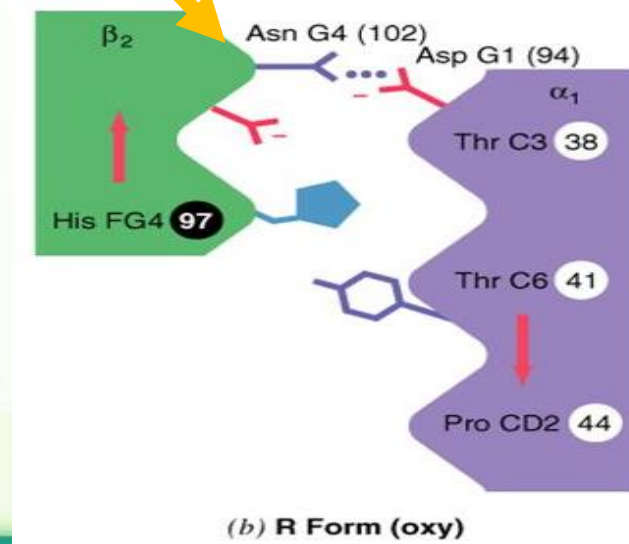
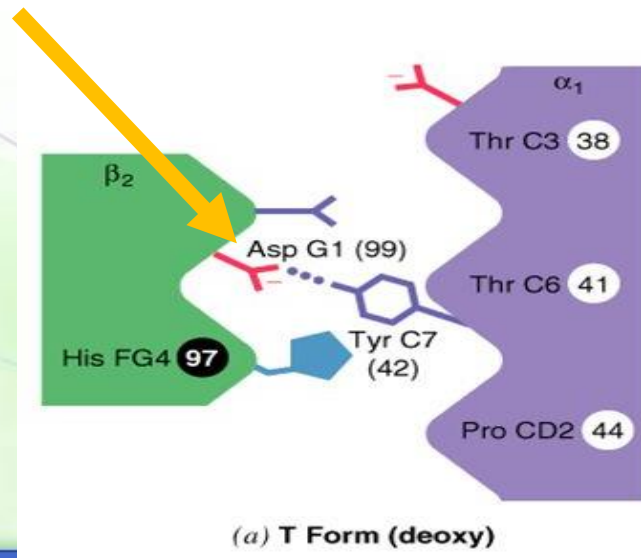
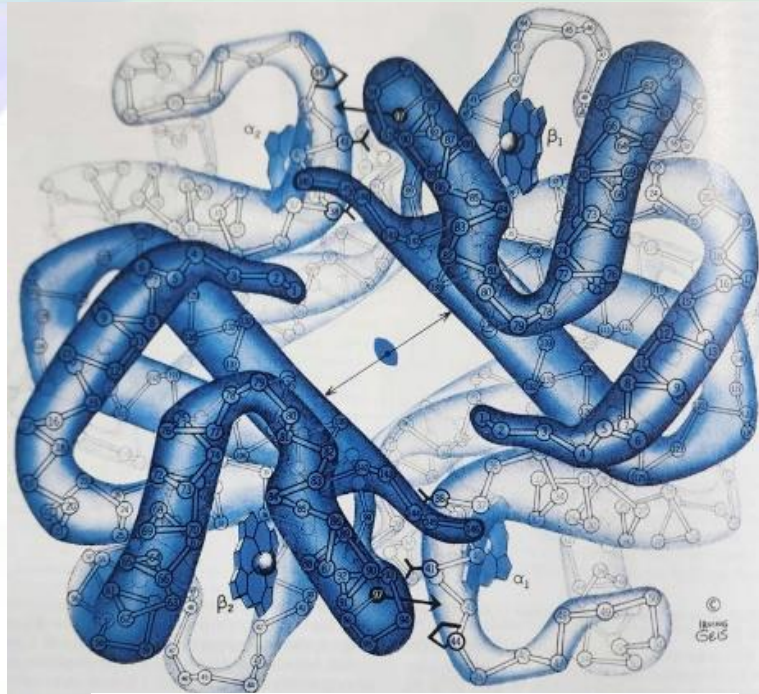


The Relaxed R state (oxygenated) is stabilized by the oxygen. Note the reformation of the bonds upon oxygen binding

Oxygen binding induces Reformation of the interdimer bonds upon



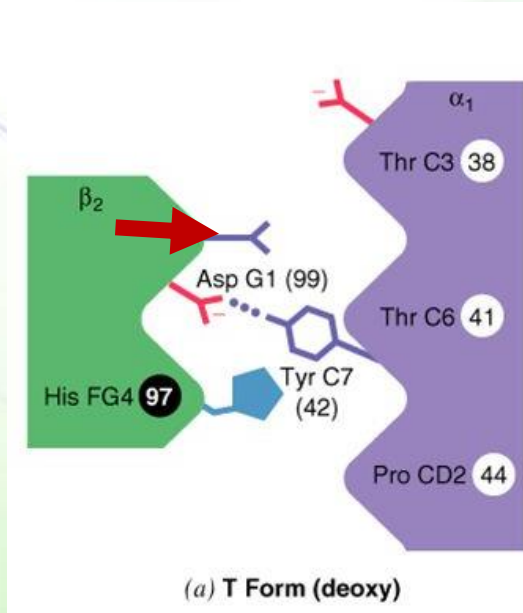
Oxygen binding induces Reformation of the interdimer bonds upon



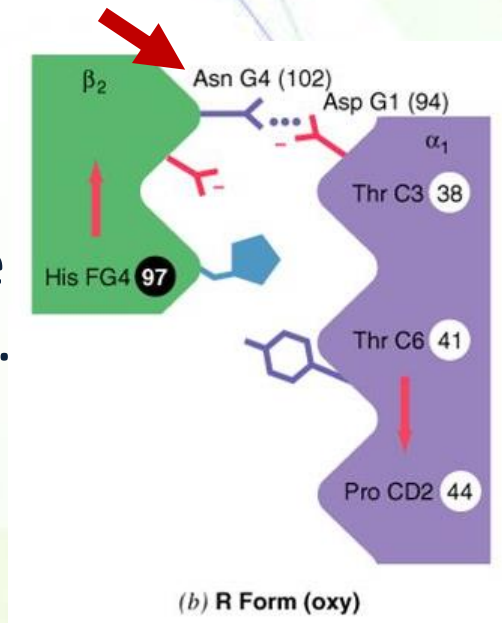
Mutations at $\alpha 1$ - $\beta 2$ contacts



- 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).



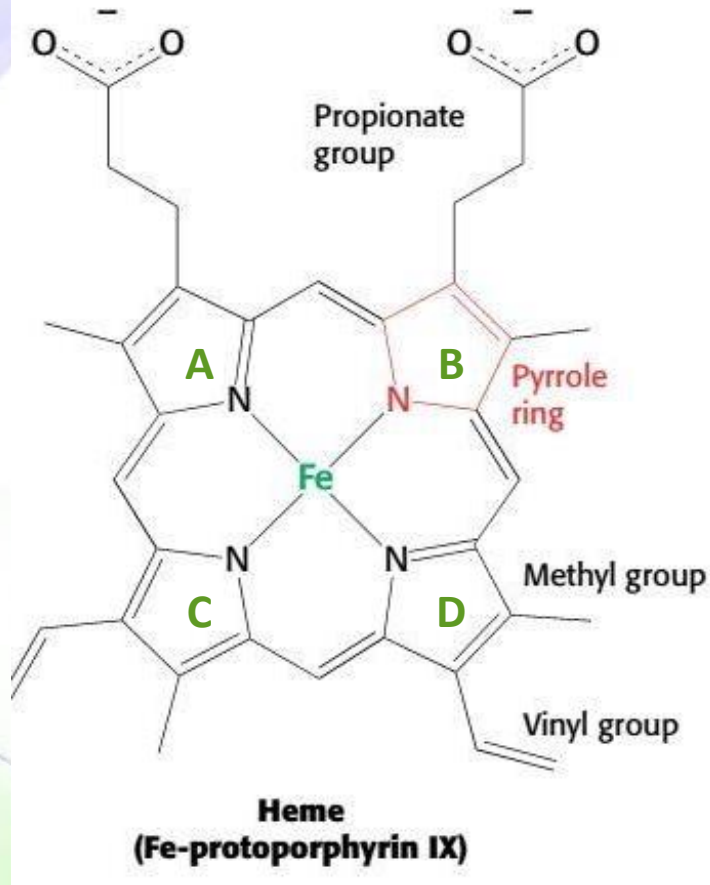
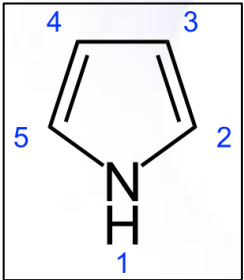


- Heme

1-C-Heme structure

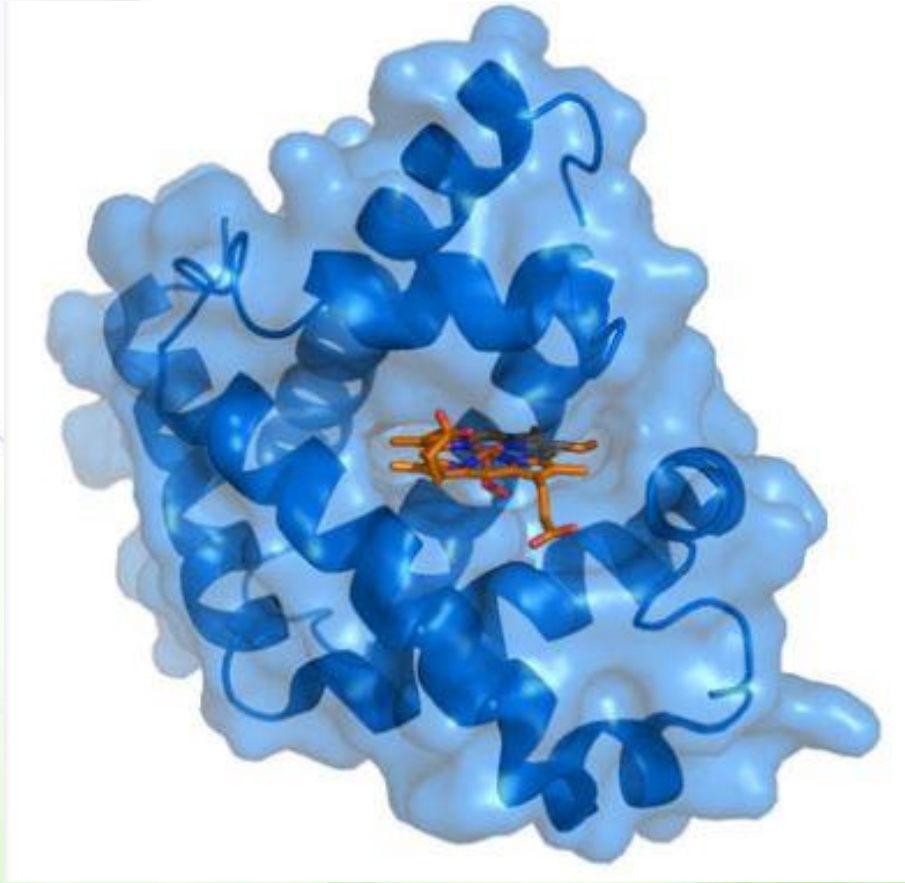


Pyrrole ring



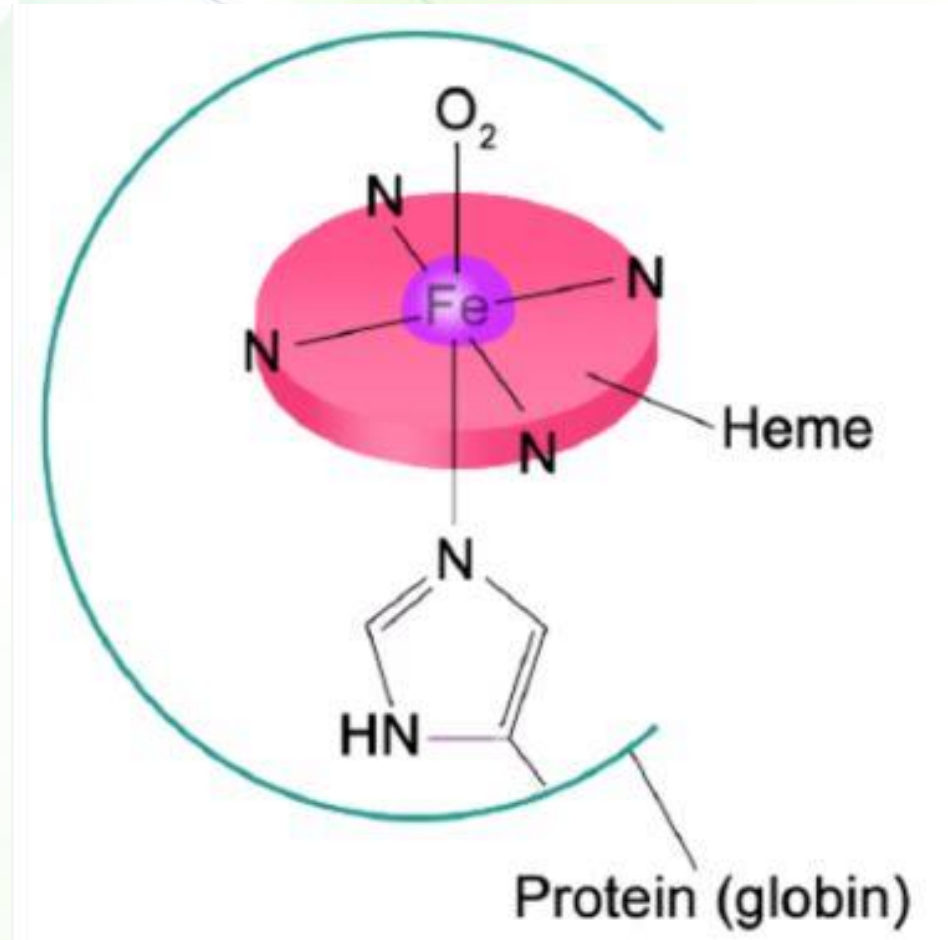
- The heme is composed of:
- 1-Fe+2
- 2-Protoporphyrin (which is composed of):
- a- Pyrrole rings
- b- Two of the pyrrole rings have a propionate group each.
- c-Vinyl groups
- d-Methyl groups
- *Note: the molecule is hydrophobic.*
- Fe has six coordinates of binding.

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.

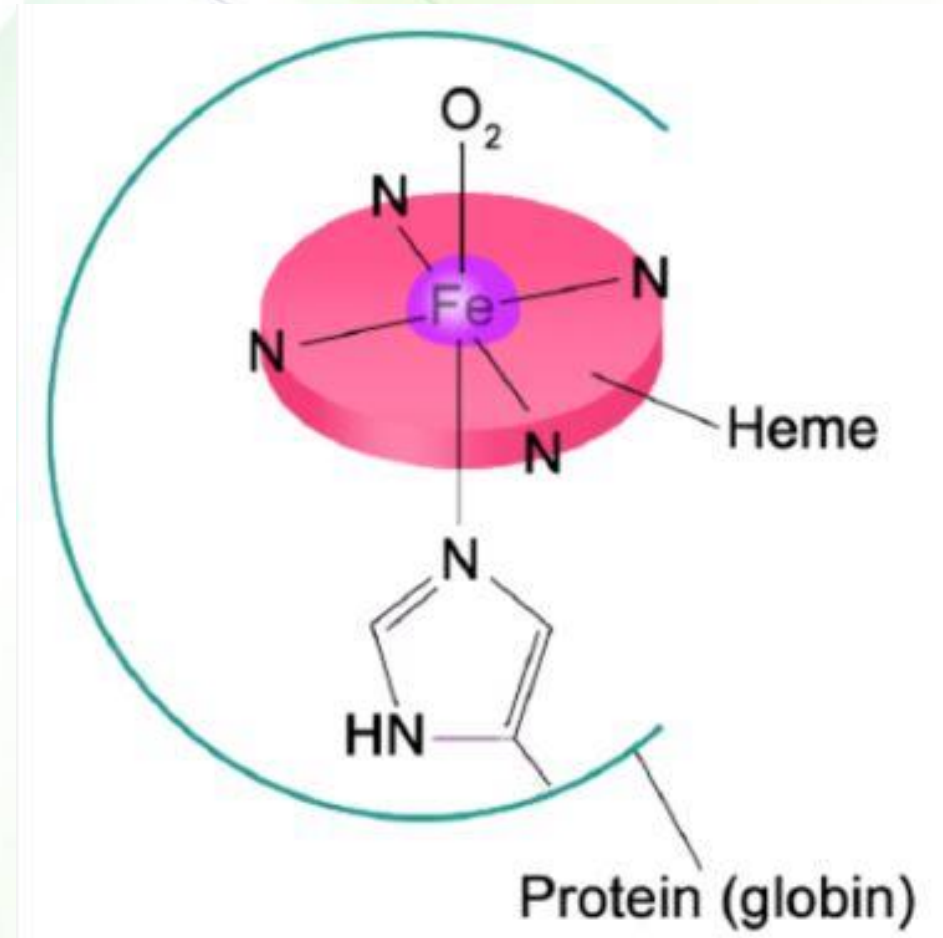
Fe+2 attachments



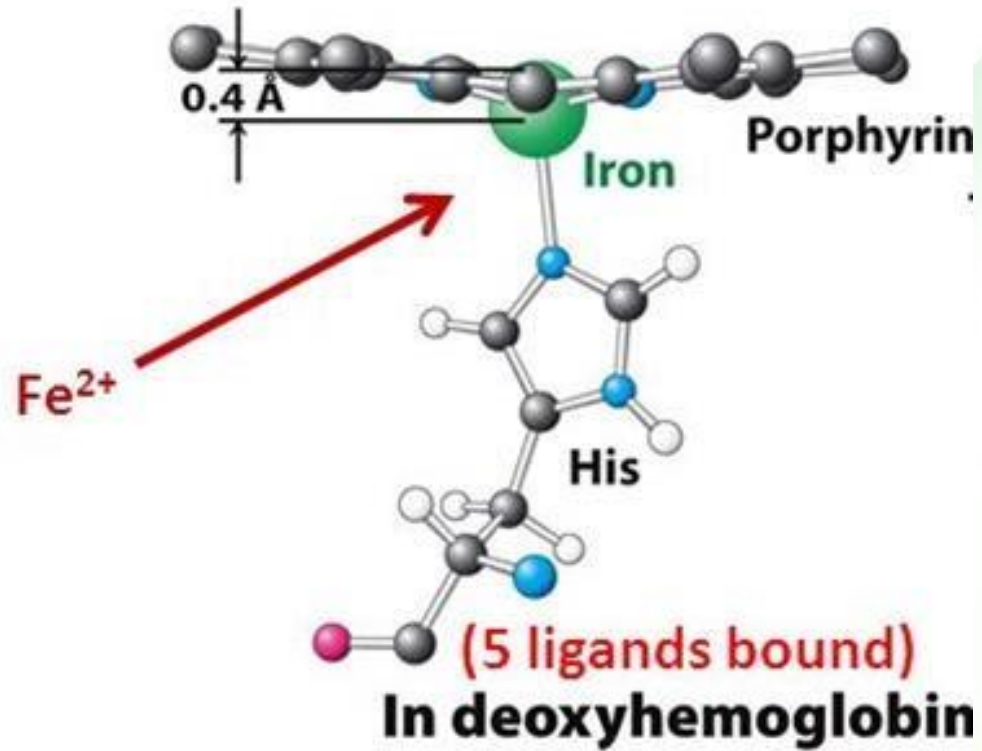
Fe+2 attachments



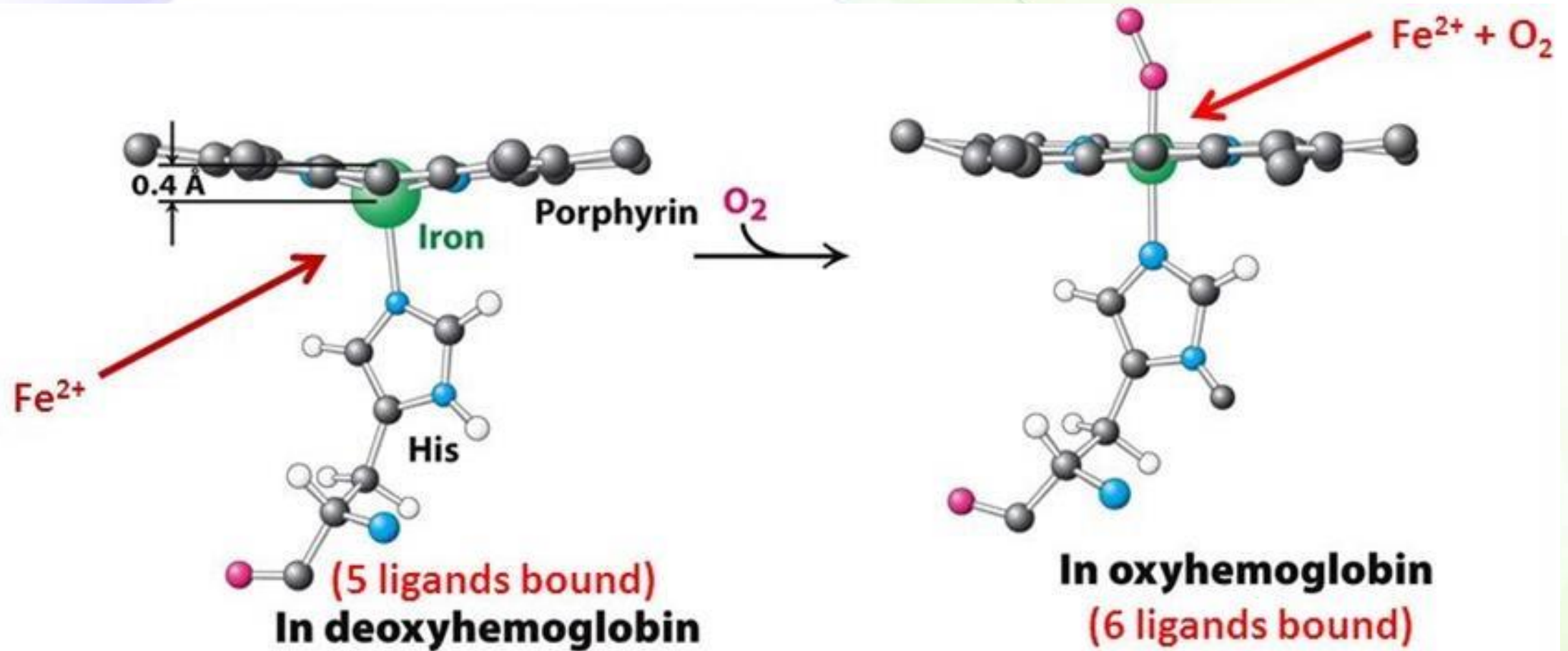
- Fe+2 makes 5 bonds. 4 with the protoporphoryn and the 5th with the globin protein through the proximal Histidine, it is a covalent bond (This is a very important bond)
- In the presence of Oxygen, Fe+2 makes a 6th bond with Oxygen.



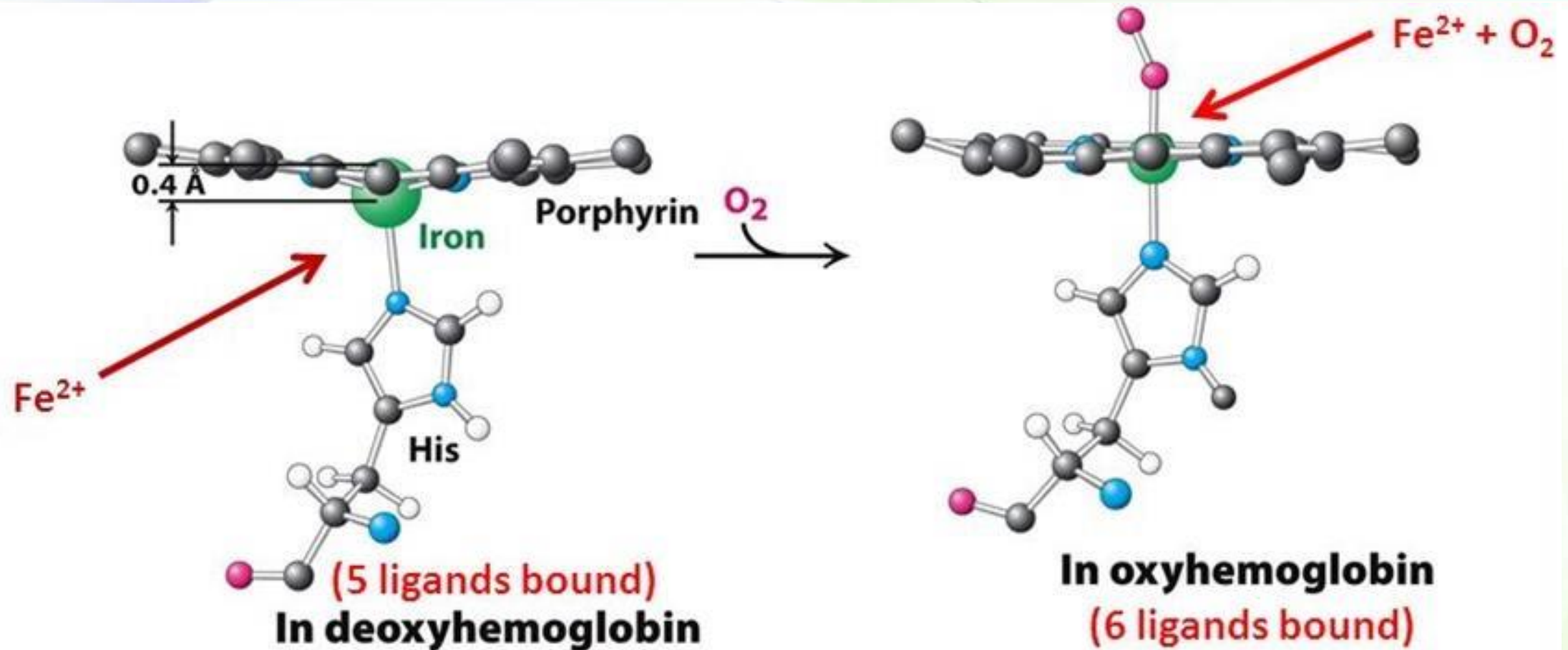
Structural amplification change



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

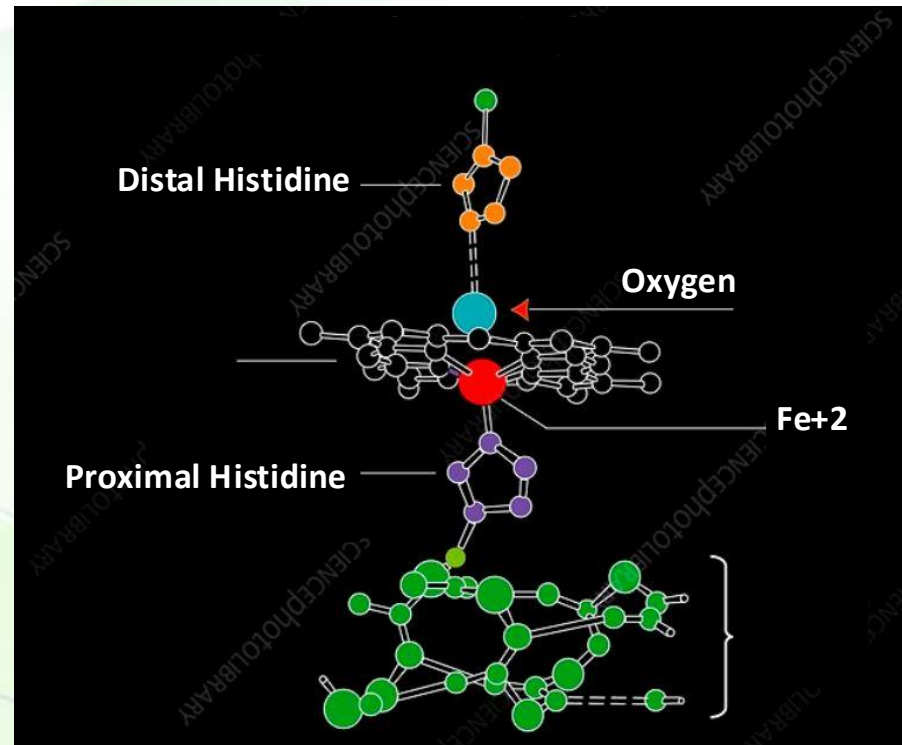


- Note: It is not the sliding that causes the Oxygen to bind more strongly to the other subunits. It is the total 3-D conformational change that does so. It could be a change to the binding site on each subunit where the Fe^{+2} becomes situated closer to the plane of the heme, or it could be a better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



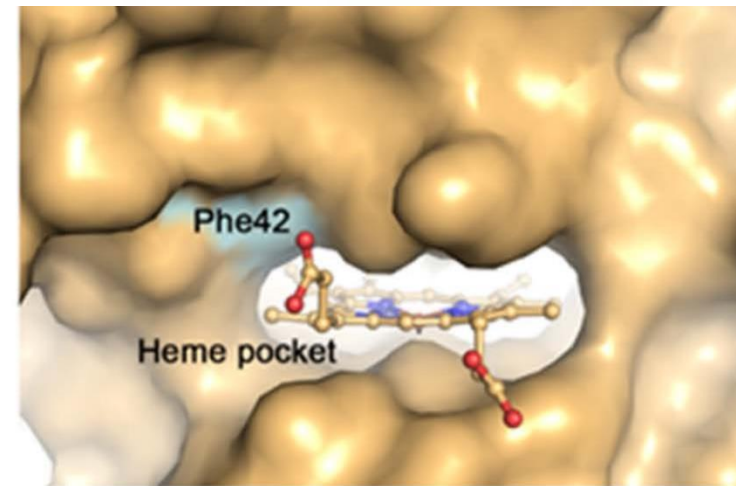
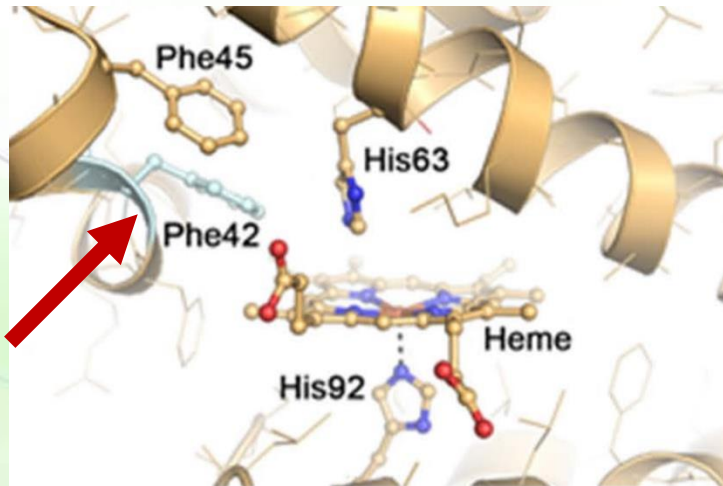


- 1-D-Heme mutations:
 - 1- Hammersmith
 - 2- Methemoglobin Reversible → Reagents
 - 3- Methemoglobin non-reversible Boston and Iwate

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.



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



- 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.
- 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.



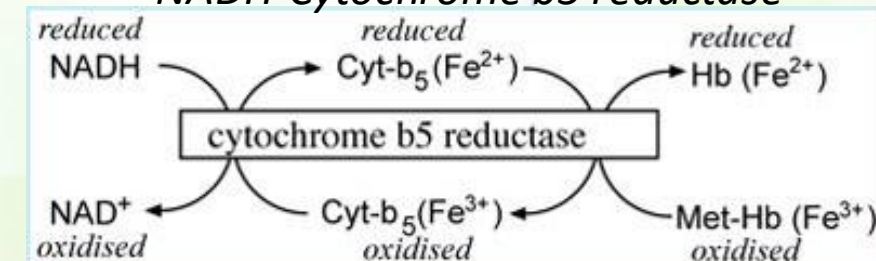
Normal Blood



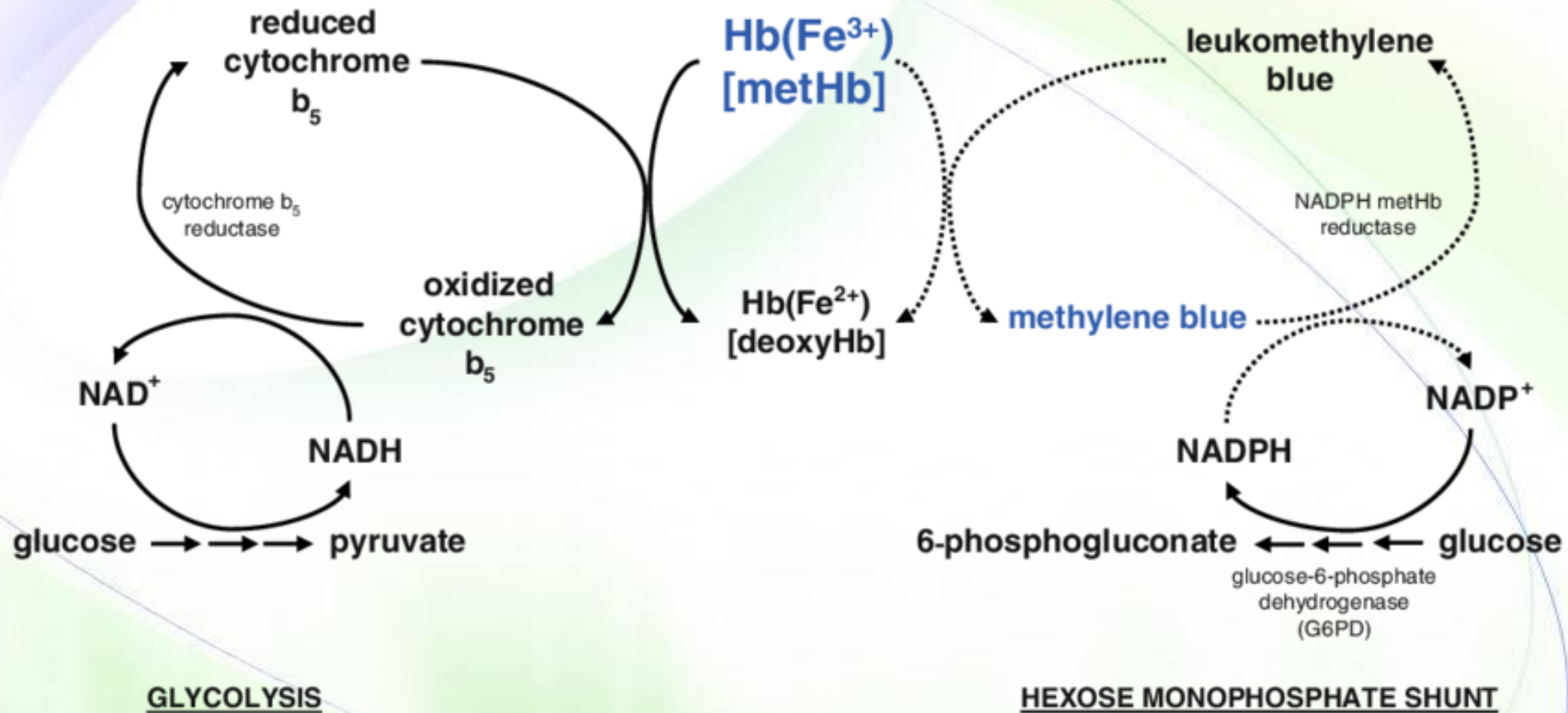
Chocolate Brown coloured Blood



*Methemoglobin reductase AKA
NADH-Cytochrome b5 reductase*



Treatment (methylene blue)



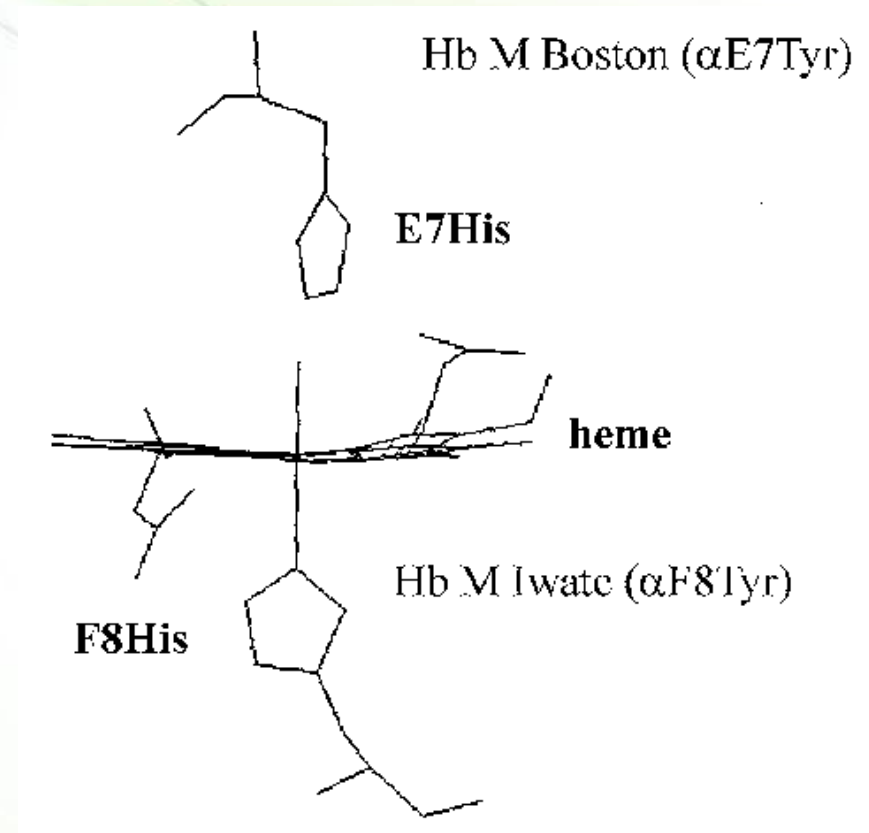
Solid arrows (→) represent normal physiology. Dotted arrows (....→) indicate pathway only active in presence of methylene blue.

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



- 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.





2- Hemoglobin effectors

Effectors on Hemoglobin

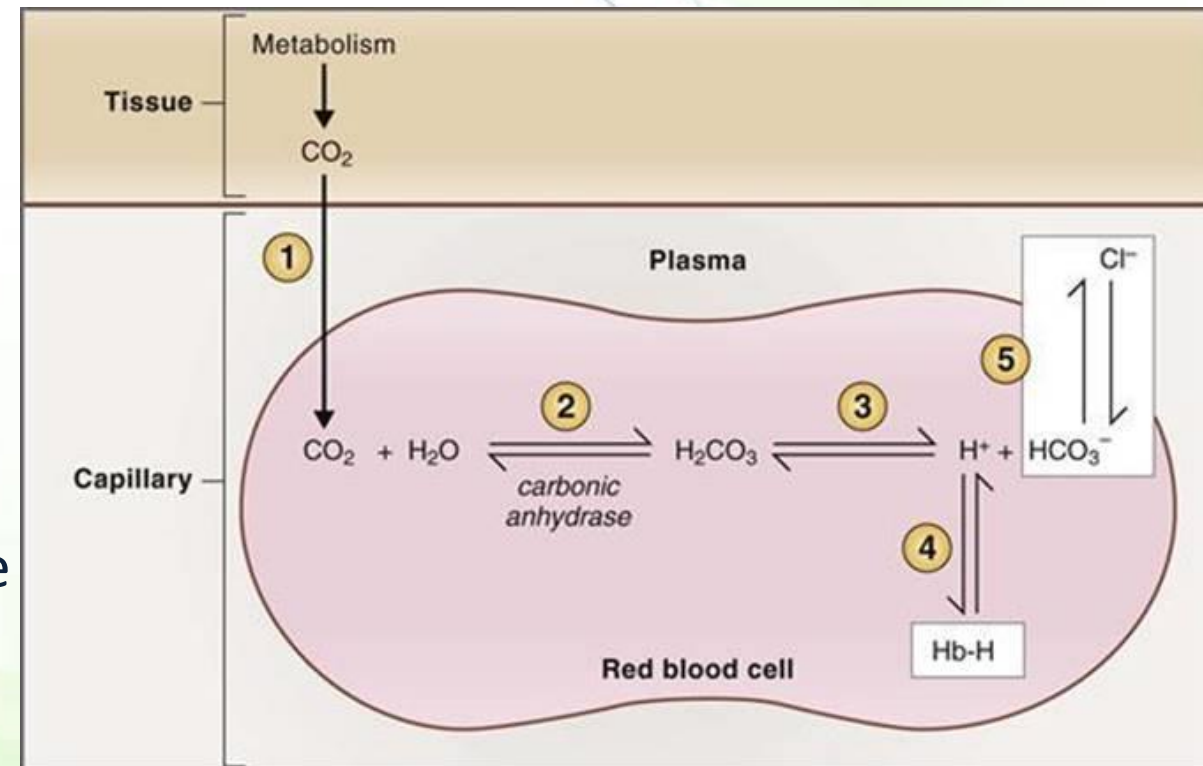


- The major heterotropic effectors of hemoglobin which happen to be T-state stabilizers:
 - Hydrogen ion, Bohr effect
 - Carbon dioxide
 - Chloride ions
 - 2,3-Bisphosphoglycerate
- A competitive inhibitor
 - Carbon monoxide
- An inhibitor
 - Heat

Where do protons come from?



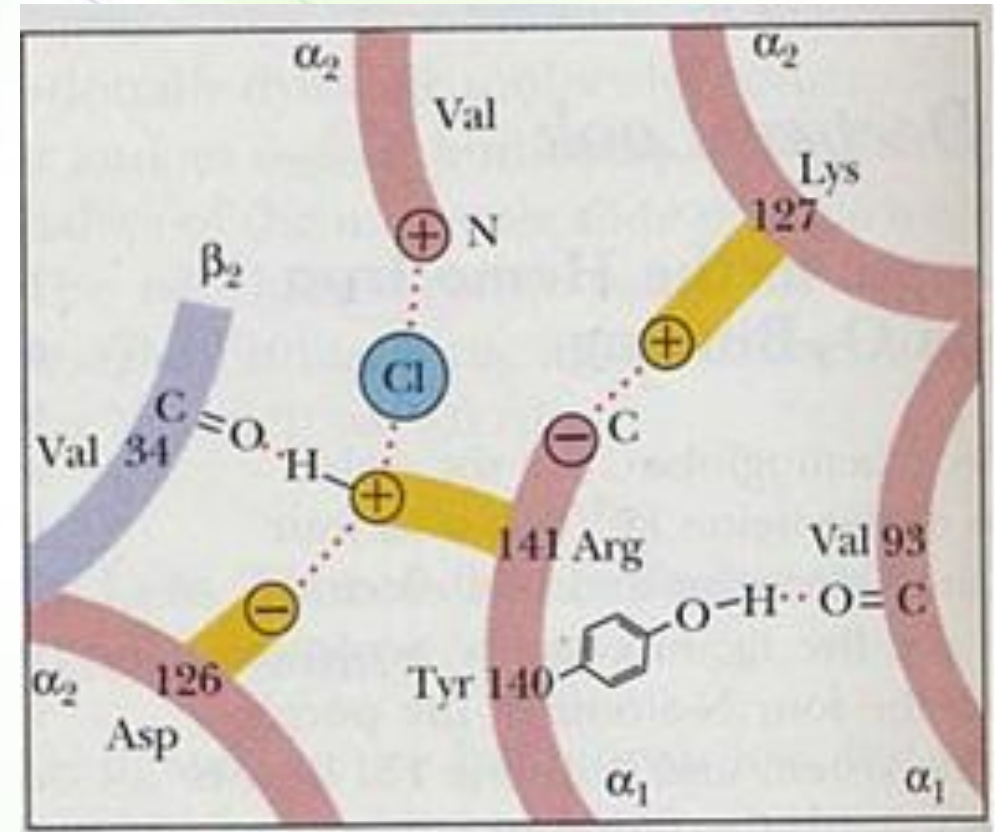
- CO₂ is produced at high levels in metabolically active tissues. Then it enters the RBCs and reacts with H₂O through carbonic anhydrase as shown in the figure and the equation to the right to produce H₂CO₃ (carbonic acid) which breaks to H⁺ and HCO₃⁻ (bicarbonate).
- 1- The H⁺ binds to Arg-141 on the α monomers inducing the hemoglobin to release the O₂. This is called Bohr effect and it stabilizes the T-state. Major effector in releasing O₂.**
- 2- The H₂CO₃ that is produced is converted to H⁺ + HCO₃⁻, the HCO₃⁻ leaves the cell and is replaced by Cl⁻ which binds to the hemoglobin inducing the T-state.**
- 3- Some CO₂ binds directly to the Hemoglobin as carbamate and is transported to the lungs as CO₂. This CO₂ promotes the T-state.**
- Note: At the lungs, the high partial pressure of O₂ induces the lungs to bind the O₂ and release the H⁺ (part of Bohr effect).



Effect of chloride ions



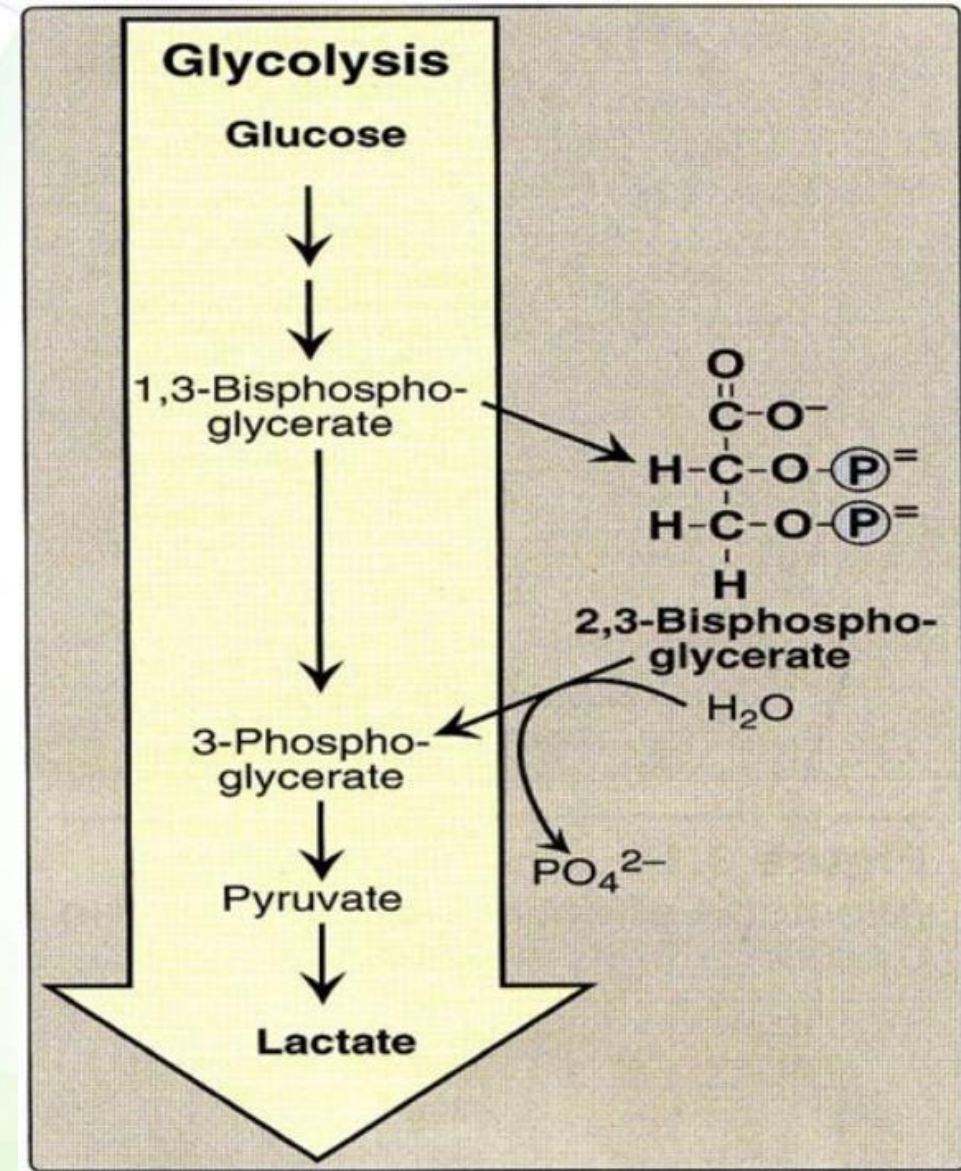
- Chloride ions interact with both the N-terminus of α_2 chain and Arg141 of α_1 chain stabilizing the T-state of hemoglobin.
- Increasing the concentration of chloride ions (Cl^-) shifts the oxygen dissociation curve to the right (lower affinity).



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



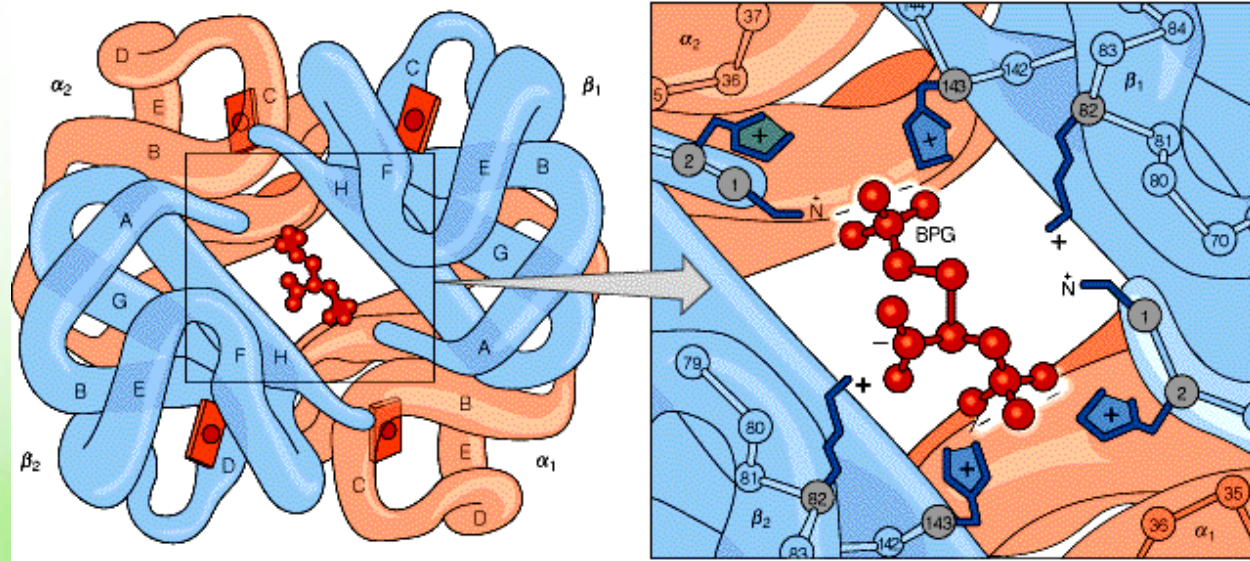
- 2,3-Bisphosphoglycerate (2,3-BPG) is produced as a by-product of glucose metabolism in the red blood cells.
- It binds to hemoglobin and reduces its affinity towards oxygen.



2,3-BPG –hemoglobin interaction

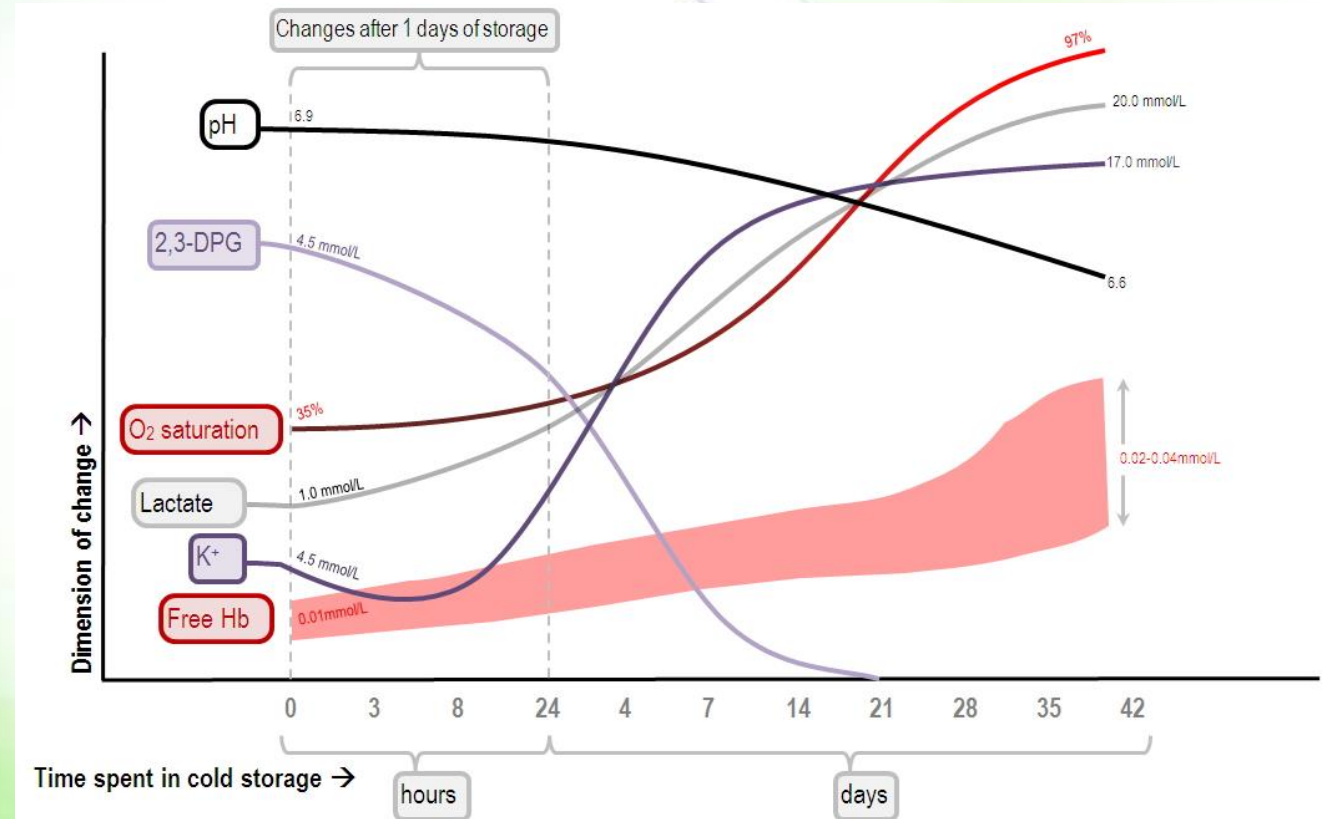
- 2,3-BPG binds in the central cavity of deoxyhemoglobin only in a ratio of 1 2,3-BPG/hemoglobin tetramer.
- This binding stabilizes the T-state hemoglobin reducing the binding of oxygen to hemoglobin and facilitating oxygen release.

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.



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.*

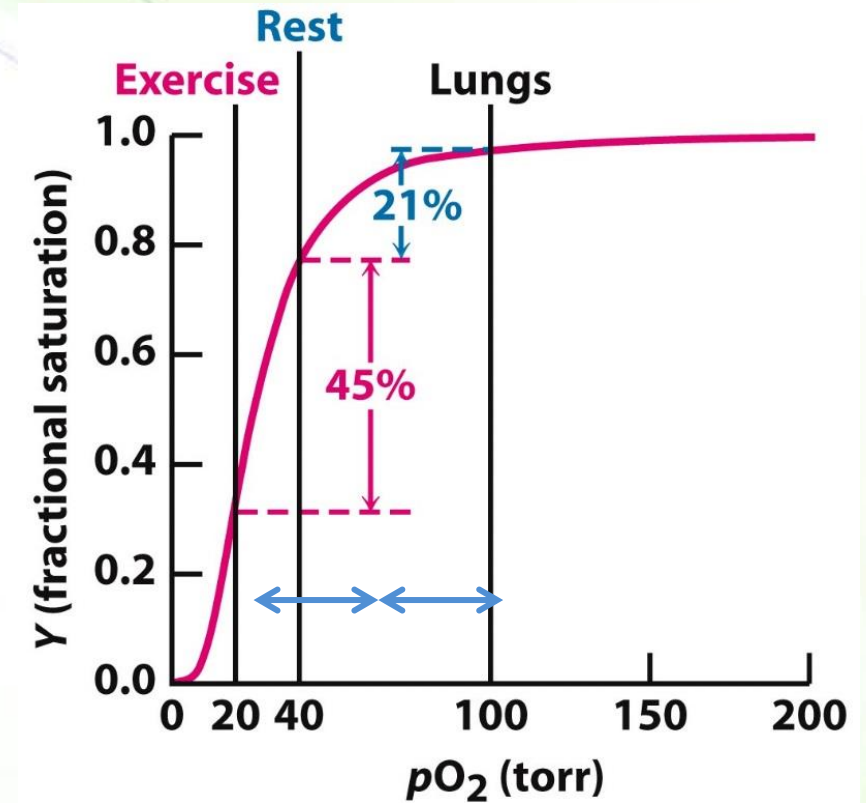
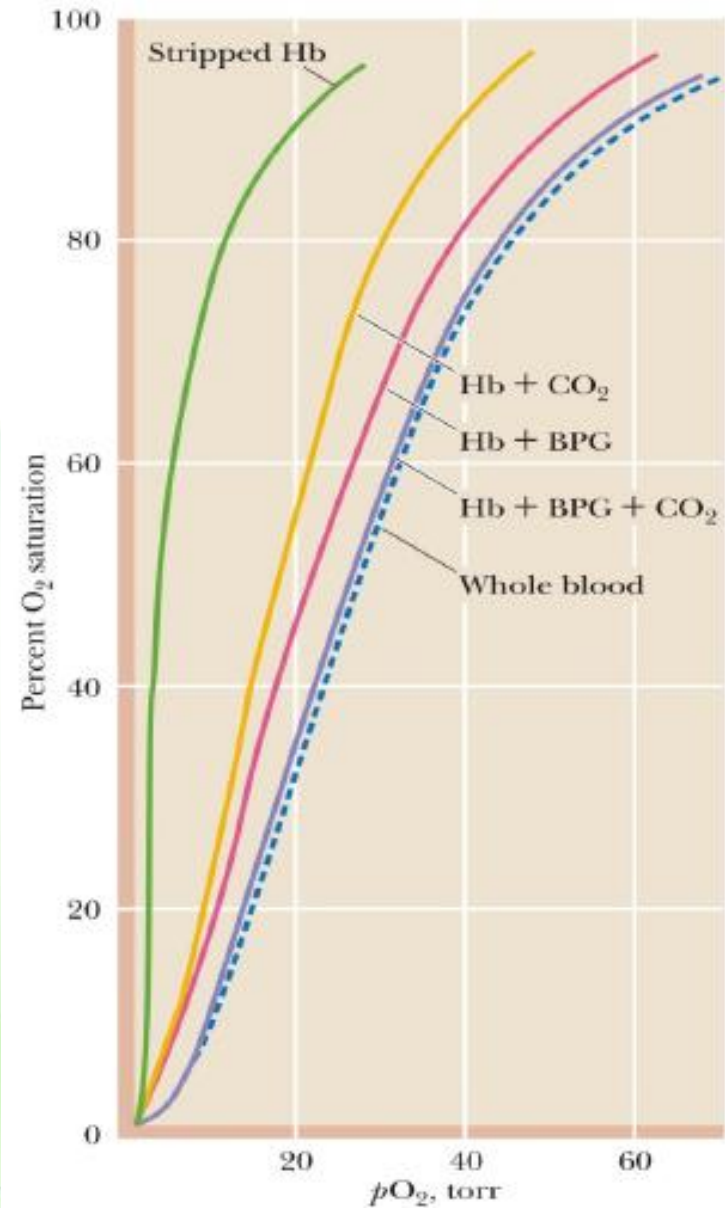


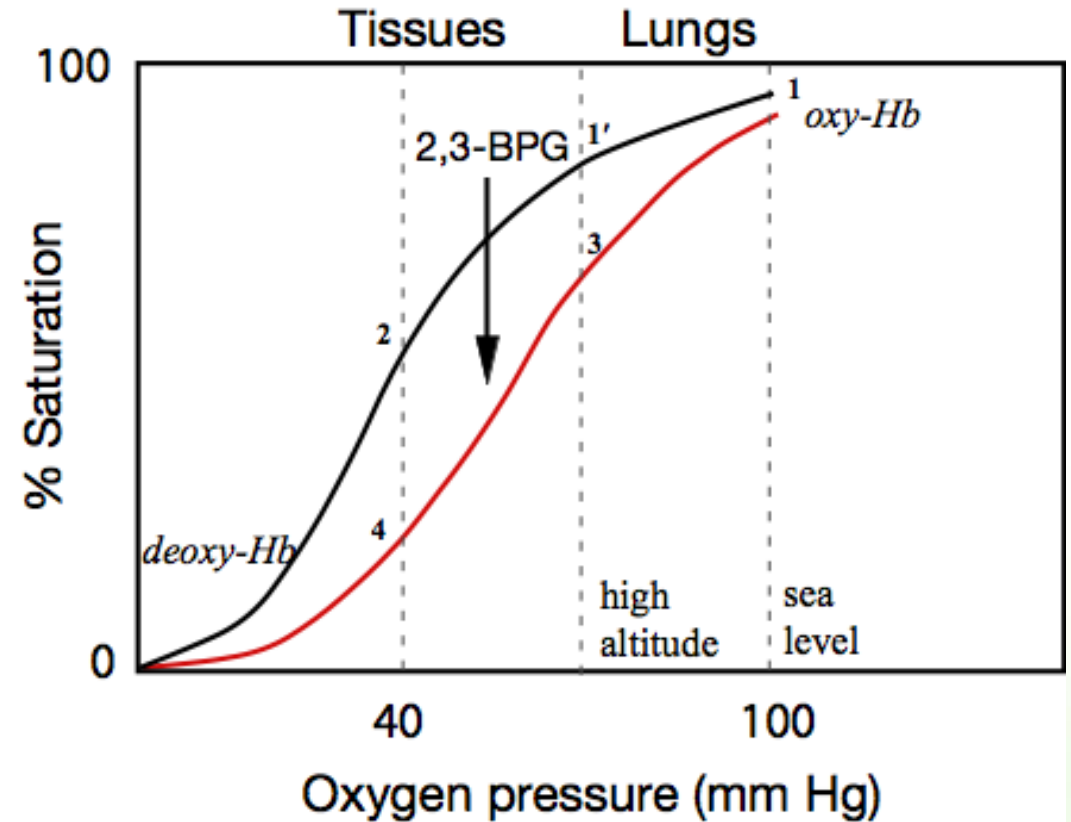
Figure 7.10
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2,3-BPG and CO₂ are important players



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.





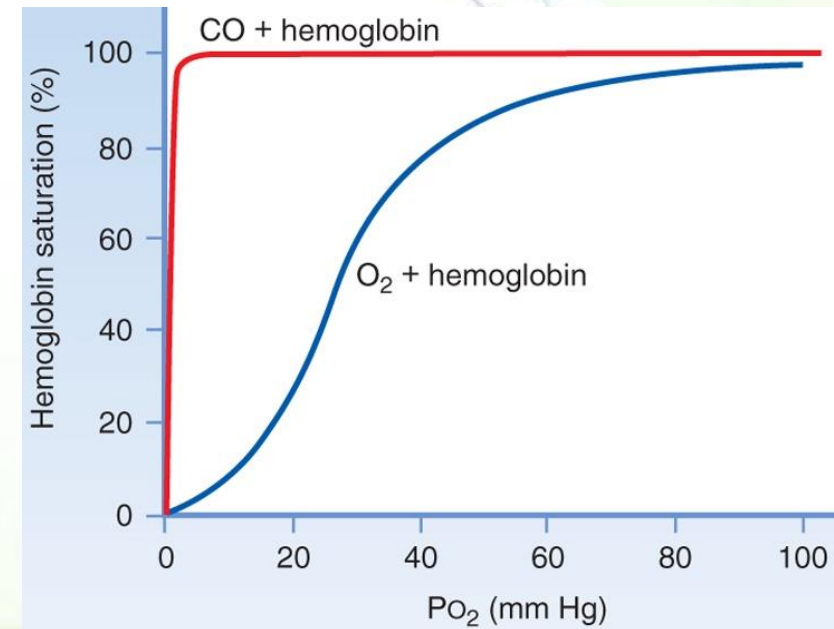
Other factors: B- Effect of CO

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.

(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 HbO_2 .

