GENERAL BIOCHEMISTRY MODULE 1

- **Biological macromolecules:**

✓ **Carbohydrates**

✓ **Lipids**

✓ **Amino acids and Peptides**

✓ **Proteins**

  - **myoglobin and hemoglobin**
  - **enzymes and enzymology**

NUCLEIC ACIDS – GENERAL BIOCHEMISTRY
MODULE 2

- **Structure and Function of Nucleic Acids**
- **Genes and Chromosomes (brief summary)**
- **DNA metabolism (replication)**
- **RNA metabolism (synthesis and transcription)**
- **Protein Synthesis (translation)**

BIOCHEMISTRY LABORATORY

- December 19th 2025, 9-11 AULA MAGNA DERMATOLOGIA -Sant'Orsola
  Prof. Vadim Viviani «Bioluminescence color modulating luciferases for cellular biosensing and environmental analysis»

- January 9th 2025, 11-13 AULA MAGNA DERMATOLOGIA -Sant'Orsola
  Clinical case presentations (4/5 people per group- G1 and G2)

- January 13th 2025, 9-11 AULA MAGNA DERMATOLOGIA - Sant'Orsola
  Clinical case presentations (4/5 people per group- G3 and G4)

- January 15th 2025, 11-13 AULA MAGNA DERMATOLOGIA - Sant'Orsola
  Prof. Antonio Pannuti «RNA in diagnostics and therapy»

- January 17th 2025, 9-11 AULA MAGNA DERMATOLOGIA - Sant'Orsola
  «Mock exam – Chemistry and Biochemistry (with results discussion)»

- January 27th 2025, 9-11 AULA MAGNA DERMATOLOGIA - Sant'Orsola
  Dr. Akram Ghantous «Epigenomics and Big Data: Linking the Environment with Health and Disease"

Examples of globular proteins

Myoglobin; Hemoglobin

The reversible binding of a ligand to a protein

- ✓ The functions of many proteins involve the reversible binding of other molecules. A molecule bound reversibly by a protein is called a **ligand** (any kind of molecule, including another protein). The transient nature of protein-ligand interactions is critical to life, allowing an organism to respond rapidly and reversibly to changing environmental and metabolic circumstances.

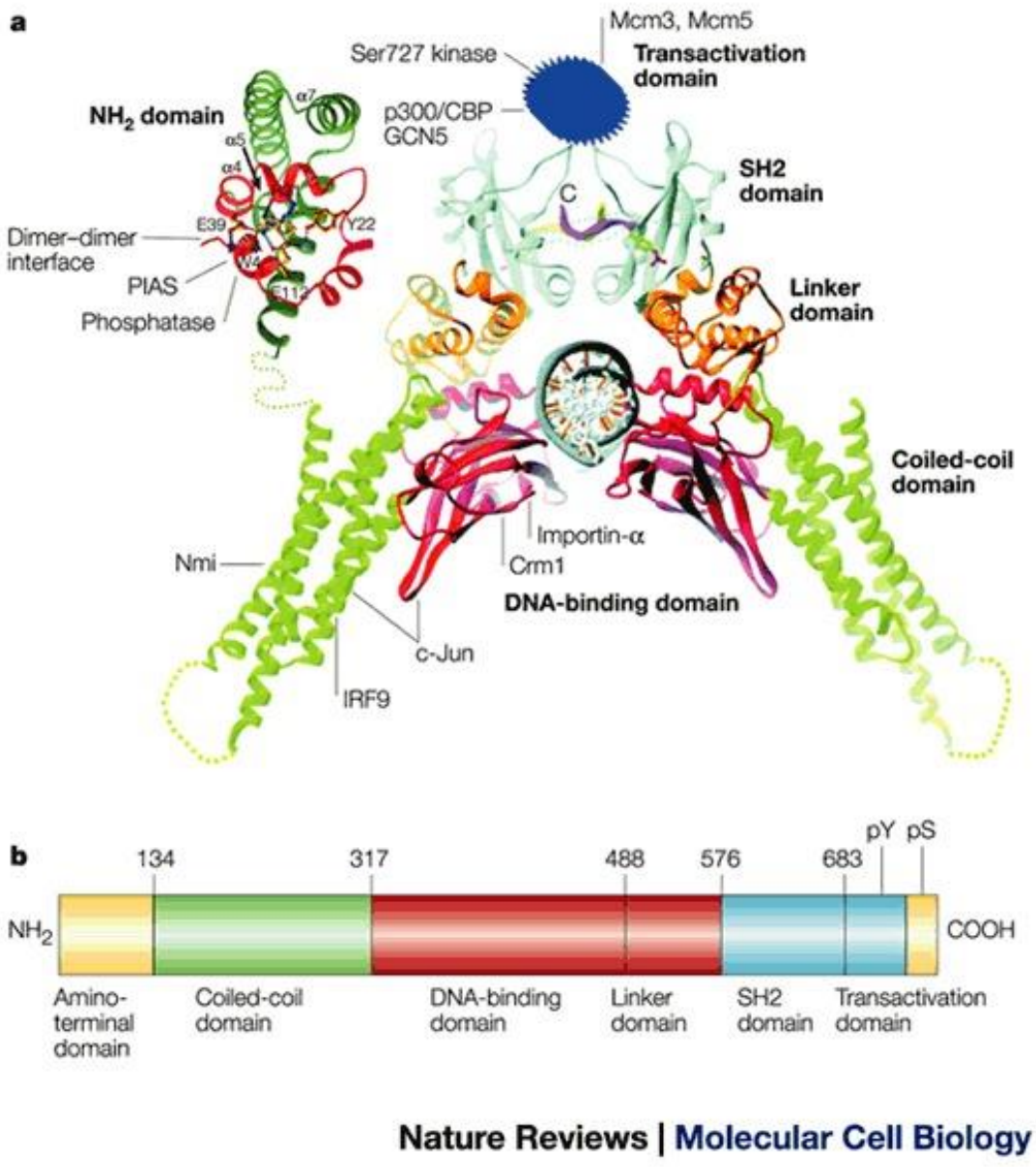
- ✓ A ligand binds at a site on the protein called the **binding site**, which is complementary to the ligand in size, shape, charge, and hydrophobic or hydrophilic character; the interaction is specific. A given protein may have separate binding sites for several different ligands.

- ✓ The binding of a protein and ligand is often coupled to a **conformational change** that makes the binding site more complementary to the ligand, permitting tighter binding. The structural adaptation that occurs between protein and ligand is called **induced fit**. In a multi-subunit protein, a conformational change in one subunit often affects the conformation of other subunits. Interactions between ligands and proteins may be regulated, usually through specific interactions with one or more additional ligands. These other ligands may cause conformational changes in the protein that affect the binding of the first ligand.

Binding site *vs* Domain

Key Differences:

Feature	Binding Site	Domain
Size	Small, often a few amino acids	Large, typically 50-300 amino acids
Structure	Part of the protein surface or interior	Independent folding unit
Function	Specific interaction with a ligand/molecule	Broader structural or functional role
Independence	Depends on the overall protein structure	Can fold and function independently
Relationship	Can be part of a domain	May contain multiple binding sites



Nature Reviews | Molecular Cell Biology

WHY OXYGEN IS IMPORTANT FOR OUR LIFE?

- The transition from anaerobic to aerobic life was one of the most important stages in the evolution, as it made possible to product more energy thanks to its metabolism.
- Oxidation of **glucose** under **anaerobic** conditions provides **2 molecules of ATP**
- The same oxidation under **aerobic** conditions provides **38 (2+36) molecules of ATP**
- Small living organisms absorb O_2 directly from the air or the surrounding aqueous environment, while larger organisms require the presence of a **circulatory system** that **distributes O_2 to the cells of different tissues and organs**.

OXYGEN CAN BIND TO A HEME PROSTHETIC GROUP

- Oxygen is poorly soluble in aqueous solutions and cannot be carried to tissues in sufficient quantity if it is simply dissolved in blood serum. Also, diffusion of oxygen through tissues is ineffective over distances greater than a few millimeters.

- The evolution of larger, multicellular animals depended on the evolution of proteins that could transport and store oxygen. However, none of the amino acid side chains in proteins are suited for the reversible binding of oxygen molecules. This role is filled by certain transition metals, among them iron and copper, that have a strong tendency to bind oxygen.

- Multicellular organisms exploit the properties of metals, most commonly iron, for oxygen transport. However, free iron promotes the formation of highly reactive oxygen species such as hydroxyl radicals that can damage DNA and other macromolecules.

- Iron used in cells is therefore bound in forms that sequester it and/or make it less reactive. In multicellular organisms, iron is often incorporated into a protein-bound prosthetic group called **heme.**

The structure of Myoglobin and Hemoglobin

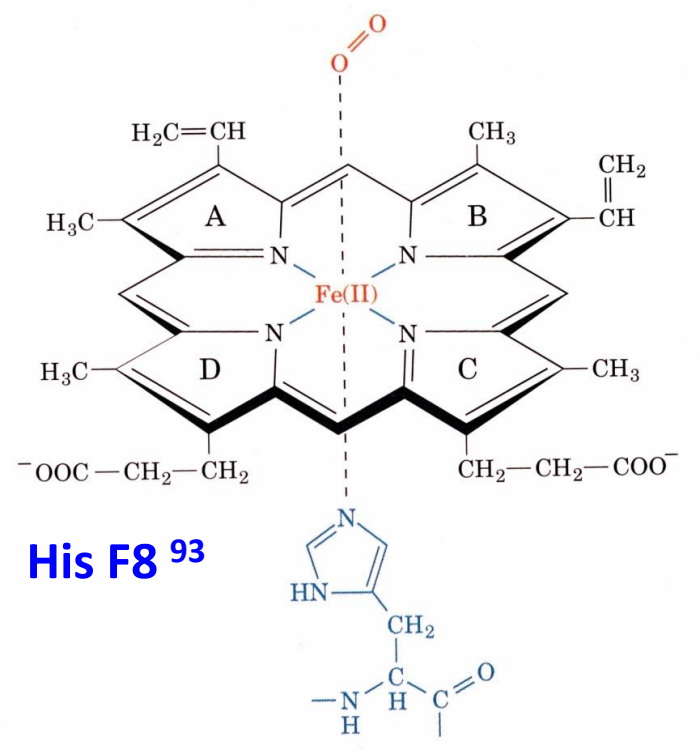
Andrew Kendrew and Max Perutz solved the structure of these molecules in 1959 to 1968

The questions are simple: "What chemistry is responsible for oxygen binding, cooperativity and allosterism and what alterations in activity do single mutations have on structure and function?"

The Heme group

Each subunit of hemoglobin or myoglobin contains **a heme group**

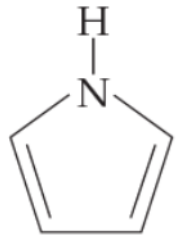
- Binds one molecule of oxygen.
- Heterocyclic porphyrin ring, to which is bound a single iron atom in its ferrous (Fe^{2+}) state.



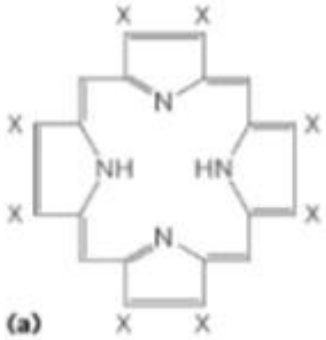
The iron atom has six coordination bonds, four to nitrogen atoms that are part of the flat porphyrin ring system and two perpendicular to the porphyrin.

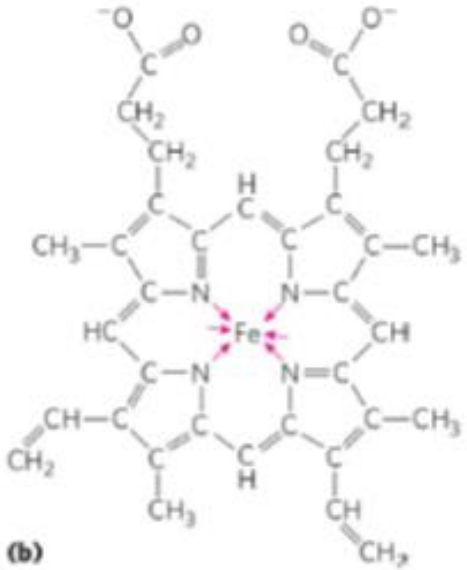
The N imidazole of the histidine residue located in helix F (Proximal histidine) in myoglobin binds the Fe^{2+}.

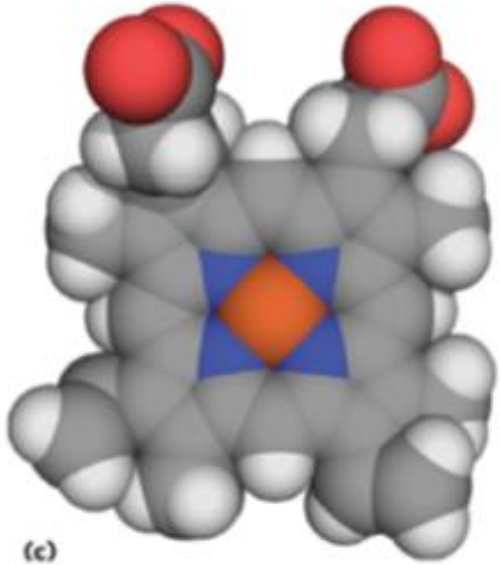
The Heme group

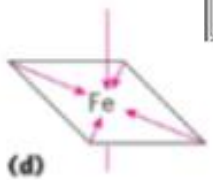


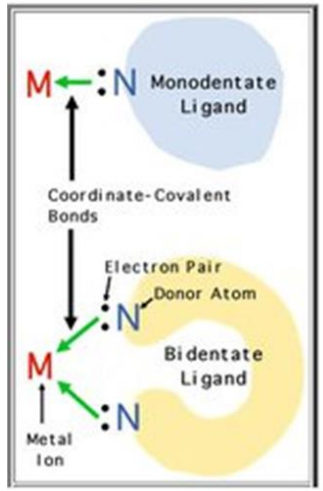
Pyrrole











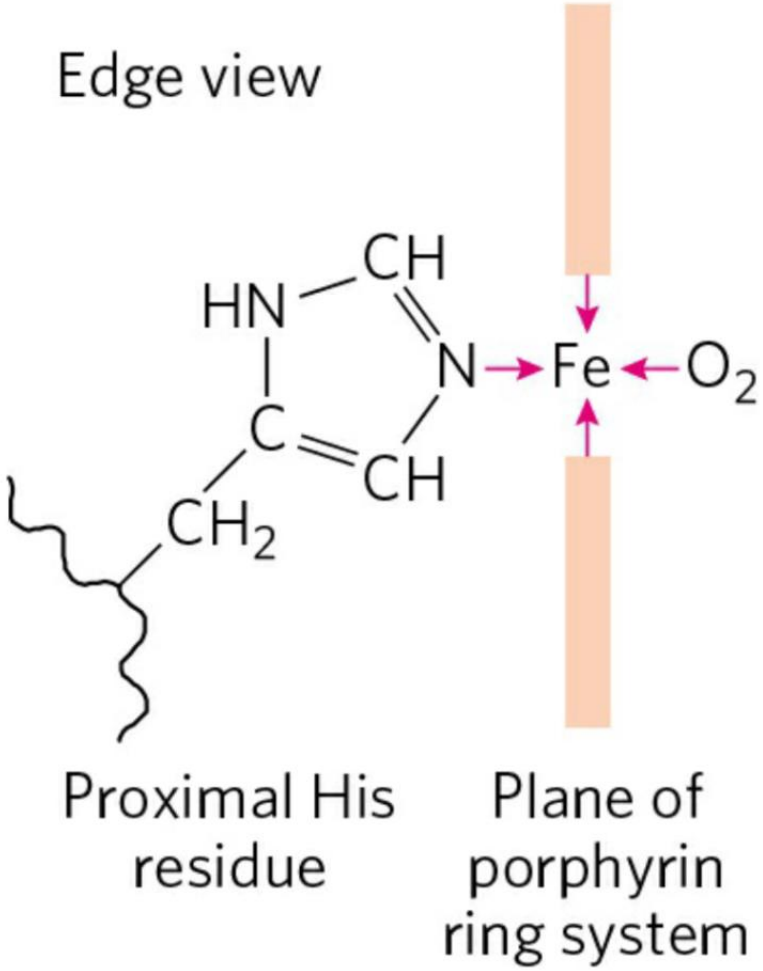
Porphyrin ring

The iron atom of heme has six coordination bonds: four in the plane of, and bonded to, the flat porphyrin ring system, and two perpendicular to it.

Binding of O_2 to Heme

- Binding of O_2 to a FREE heme group is irreversible
- Enclosure in a protein allows reversible binding
  - O_2 has only limited solubility (1×10^{-4} M) in water
  - Solubility problem overcome by binding to proteins
  - Also increases diffusion

The Heme group

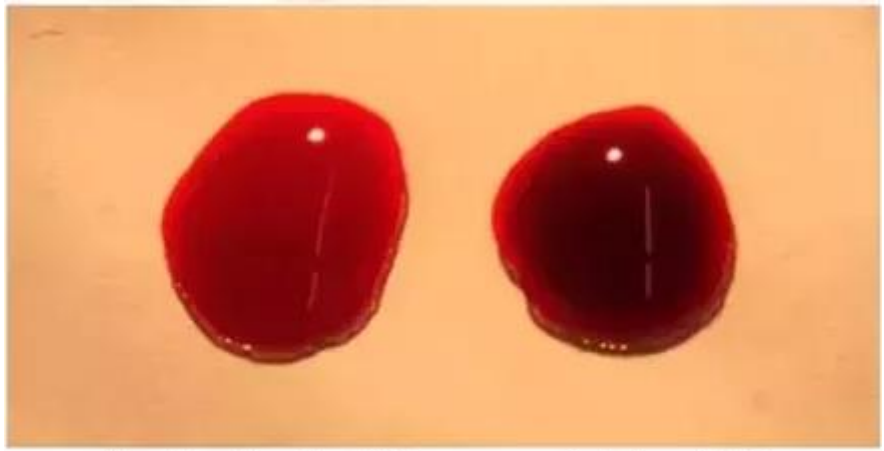


Two coordination bonds to Fe^{2+} that are perpendicular to the porphyrin ring system.

- **One is occupied by a His residue called the proximal His, the 93rd residue from the amino-terminal end of the myoglobin polypeptide sequence) also called His F8 (the 8th residue in α helix F).**
- **The other is the binding site for oxygen.**
- **The remaining four coordination bonds are in the plane of, and bonded to, the flat porphyrin ring system.**

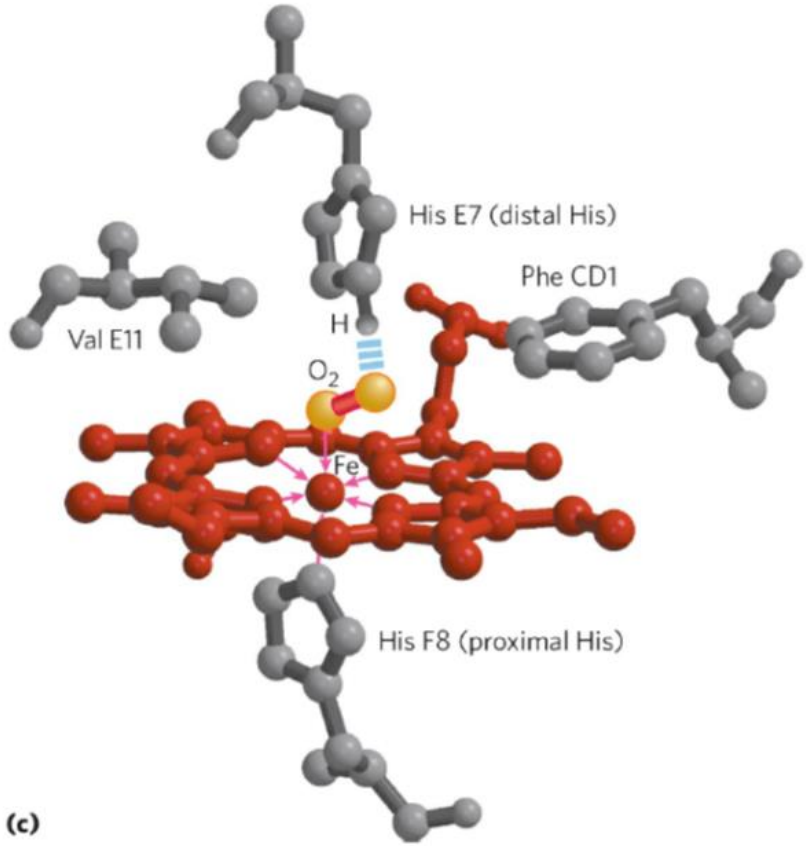
The Heme group

When oxygen binds a heme group, the color of blood changes (from the dark purple of oxygen-depleted venous blood to the bright red of oxygen-rich arterial blood).
Some small molecules, such as carbon monoxide (CO) and nitric oxide (NO), coordinate to heme iron with greater affinity than O_2 does. When a molecule of CO is bound to heme, O_2 is excluded, which is why CO is highly toxic to aerobic organisms.



The blood on the left is oxygenated, the right is deoxygenated blood (from a vein)

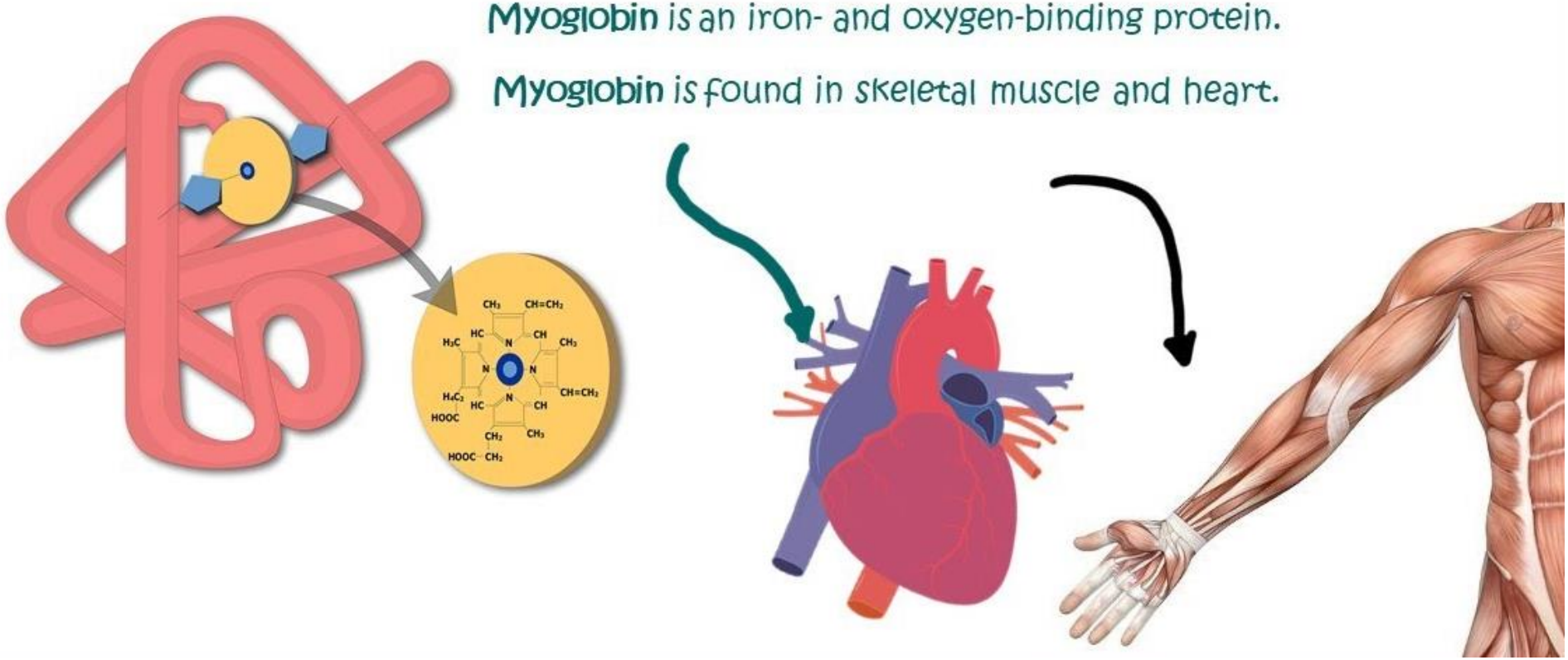
The presence of the distal His (His64, or His E7 in myoglobin) selectively increased the affinity for O_2 of heme group



MYOGLOBIN

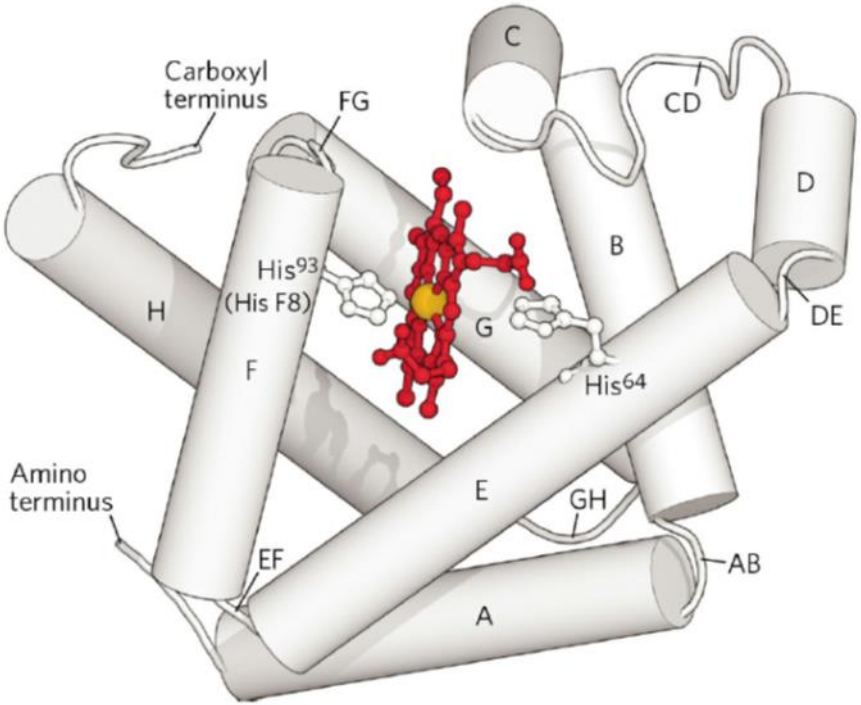
Myoglobin is an iron- and oxygen-binding protein.

Myoglobin is found in skeletal muscle and heart.



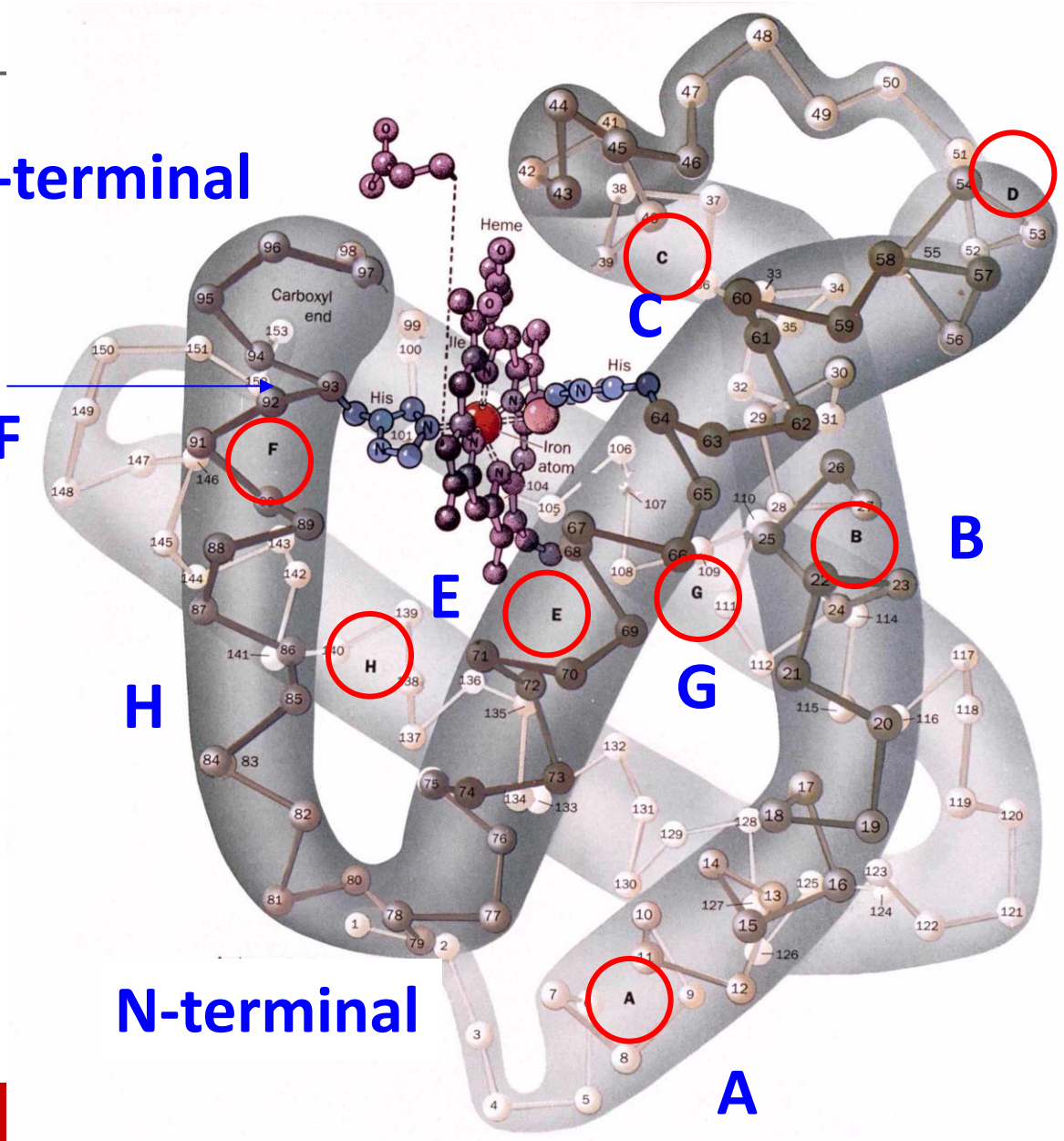
MYOGLOBIN

Globular protein linked to a PROSTHETIC GROUP (HEME) that binds oxygen; it is present in all tissues particularly in MUSCLES

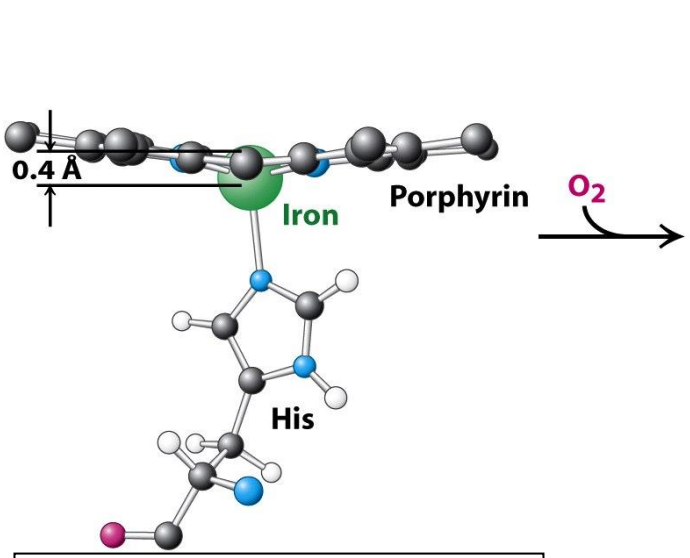


- Myoglobin is a single polypeptide of 153 amino acid residues with one molecule of heme. As is typical for a globin, it is made up of eight α-helical segments connected by loops.
- 121 residues (78%) are in an α-helix. Helices are named A, B, C, …G (7). The heme pocket is surrounded by E and F but not B, C, G, also H is near the heme.
- The bends in the structure are designated AB, CD, EF, FG, and so forth, reflecting the α- helical segments they connect.
- The His residue coordinated to the heme in myoglobin—the proximal His—is His^{93} (the 93^{rd} residue from the amino-terminal end of the myoglobin polypeptide sequence) and is also called His F8 (the 8th residue in α helix F).

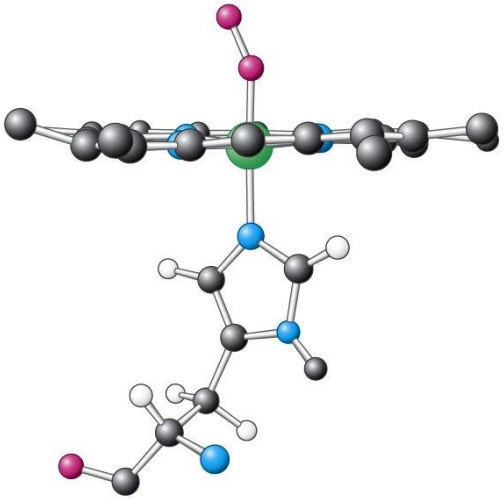
The Backbone structure of Myoglobin



The Heme complex in myoglobin



O_2



Deoxymyoglobin

Oxymyoglobin

Fe^{2+} forms six coordination bonds, the sixth with O_2

https://youtu.be/ByiR_0fpIN4

Protein-Ligand Dissociation Constants

In general, the reversible binding of a protein (P) to a ligand (L) can be described by a simple **equilibrium expression.** From the dissociation reaction **PL → P + L**, we can identify **the dissociation constant (Kd)**

$$K_d = \frac{[P]\,[L]}{[PL]}$$

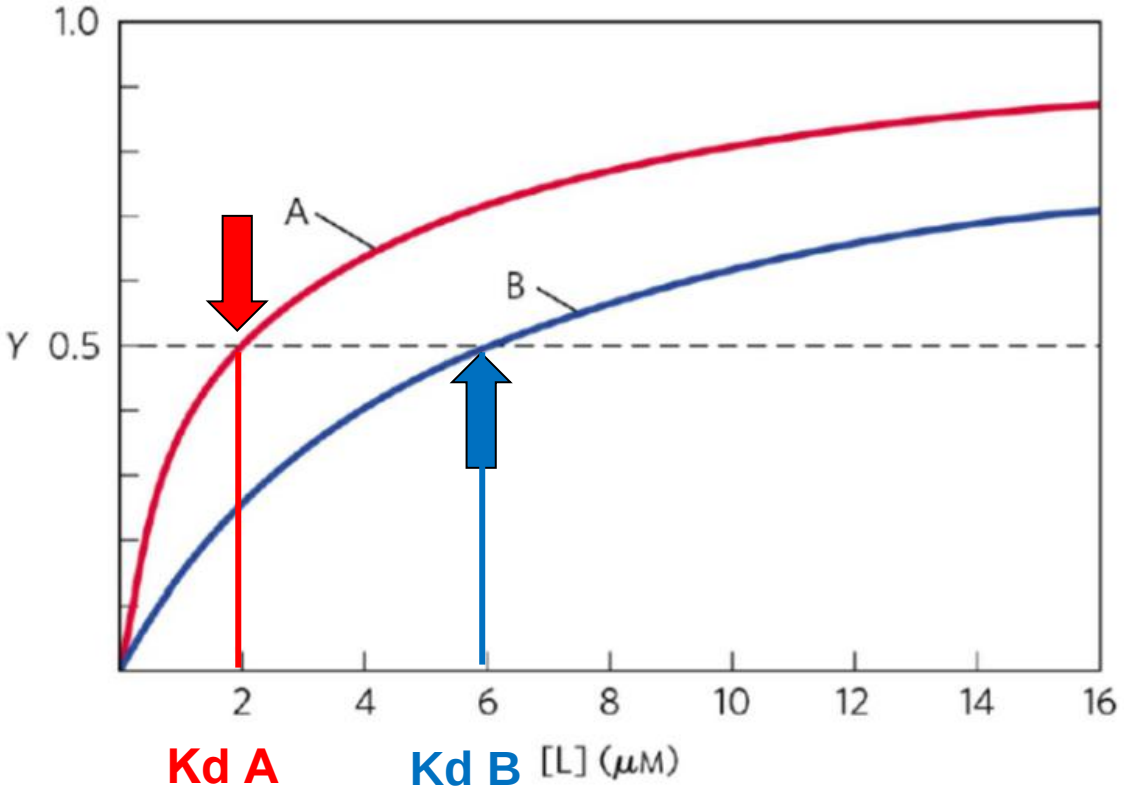
Y represents the **fraction of binding sites occupied by the ligand at equilibrium**.

$$Y = \frac{\text{binding sites occupied}}{\text{total binding sites}} \qquad Y = \frac{[L]}{[L]+K_d}$$

- **When [L] = Kd,** half of the ligand-binding sites are occupied**.**
- The mathematics can be reduced to simple statements: **Kd is equivalent to the molar concentration of ligand at which half of the available binding sites are occupied. At this point, the protein reaches half-saturation to its ligand. The more tightly a protein binds a ligand, the lower the concentration of ligand required for half the binding sites to be occupied, and thus the lower the value of Kd.**
- **Kd is used to express the affinity of a protein for a ligand**. Note that **a lower value of Kd corresponds to a higher ligand affinity for the protein**. As **[L] < Kd,** progressively less of the protein has ligand bound to it.

Protein-Ligand Dissociation Constants EXAMPLE

Two proteins, A and B, bind to the same ligand, L, with these binding curves. Which protein (A or B) has a greater affinity for ligand L?



The concentration of the ligand at which half the binding sites are occupied, for A = 2 *μ*M; for B = 6 *μ*M. Because A is half saturated at a lower [L], it has a higher affinity for the ligand.

NOTE
The concentration of the ligand at which half the binding sites are occupied—that is, the point where the binding curve crosses the line where Y = 0.5— is the dissociation constant. For A, Kd = 2 μM; for B, Kd = 6 μM.

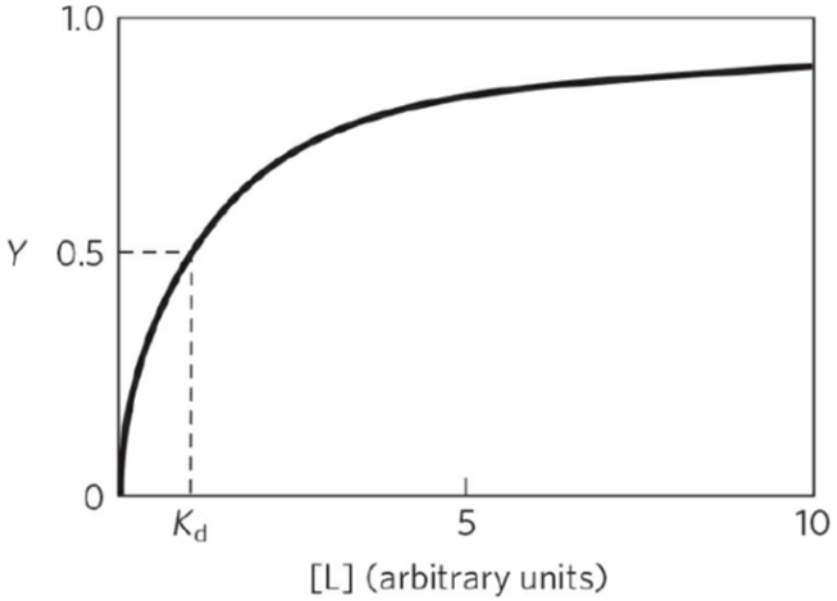
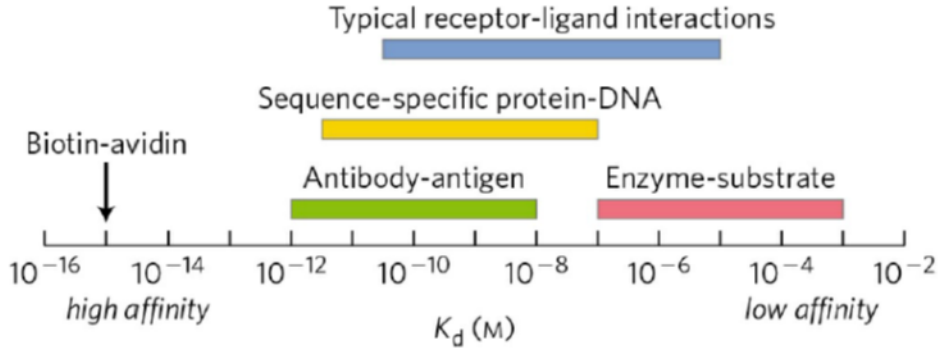


TABLE 5-1 Protein Dissociation Constants: Some Examples and Range

Protein	Ligand	K_d (M)[a]
Avidin (egg white)	Biotin	1×10^{-15}
Insulin receptor (human)	Insulin	1×10^{-10}
Anti-HIV immunoglobulin (human)[b]	gp41 (HIV-1 surface protein)	4×10^{-10}
Nickel-binding protein (*E. coli*)	Ni^{2+}	1×10^{-7}
Calmodulin (rat)[c]	Ca^{2+}	3×10^{-6}
		2×10^{-5}

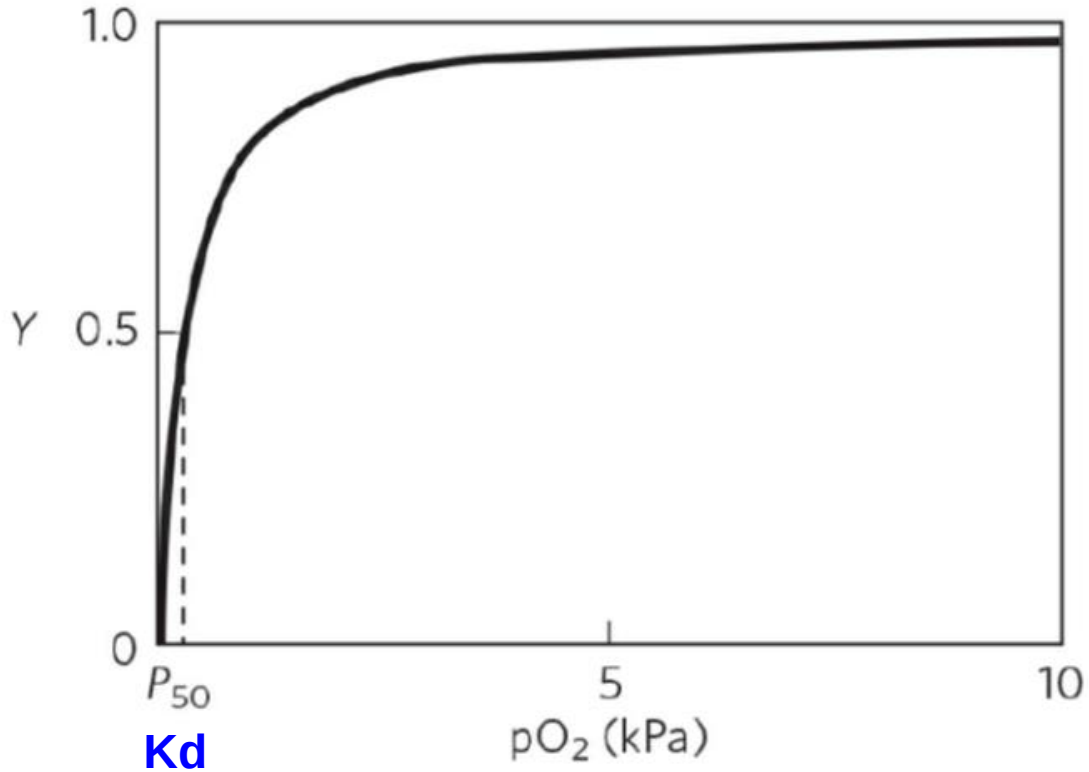


The binding of oxygen to myoglobin

- As pO_2 increases, binding increases
- Binding curve is hyperbolic, not linear

Low $pO_2 \rightarrow$ little binding to protein; High $pO_2 \rightarrow$ saturation of protein

- At half-saturation pO_2 = P_{50} (myoglobin) = 2.8 mmHg = 130 Pa



Kd

$$Y = \frac{[O_2]}{[O_2] + K_d}$$

Since the concentration of a gas in solution is always proportional to the local partial pressure of the gas, we can define the partial pressure of oxygen at Y=0.5 as P_{50}.

$$Y = \frac{pO_2}{pO_2 + P_{50}}$$

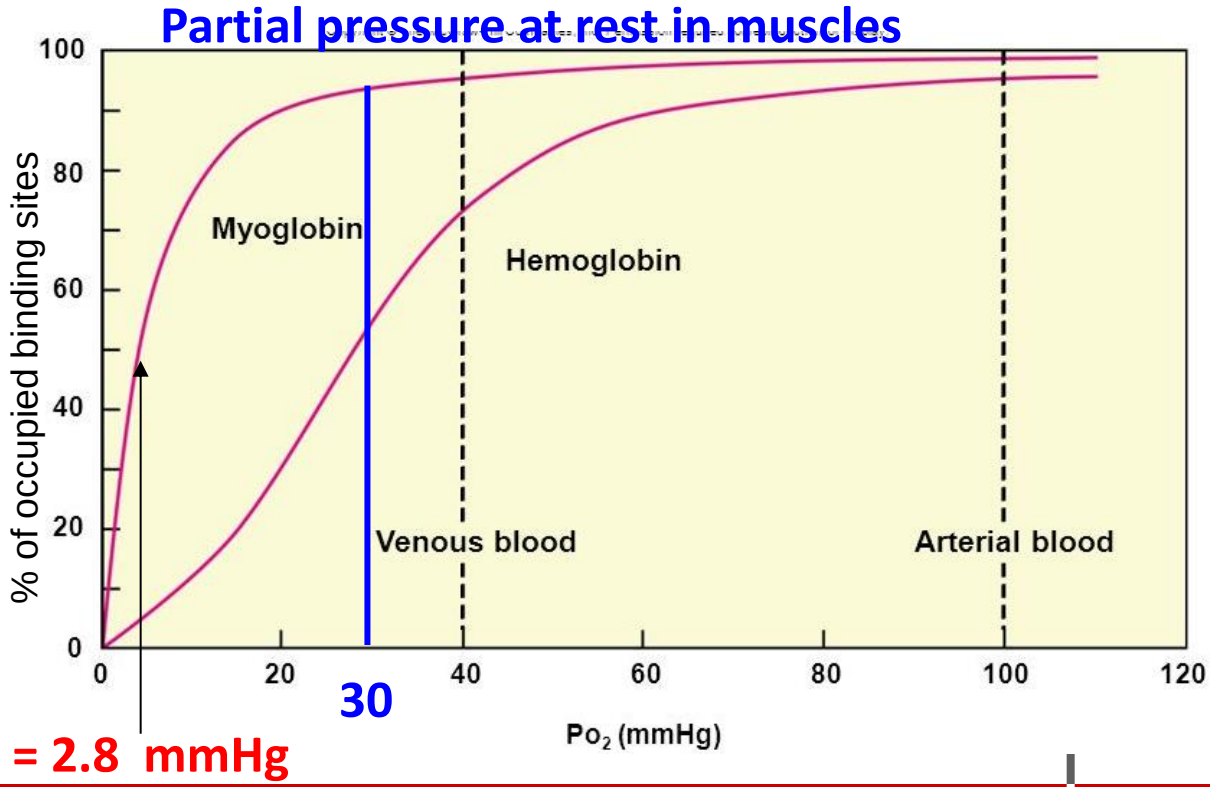
Myoglobin O_2 Binding Properties

$Mb + O_2 \leftrightarrow MbO_2$

- The affinity of myoglobin for oxygen almost doesn't vary with the variation of pO_2, in the range 30-100 mmHg, which corresponds to the values of pO_2 present in arterial and venous blood and in the pulmonary alveoli.

Its high affinity for oxygen is useful in muscles, where myoglobin actively binds O_2 from hemoglobin.

In muscles, myoglobin represents an oxygen-storage protein; under intense working conditions, when pO_2 drops to such low values O_2 dissociates from myoglobin and can be used by mitochondria.

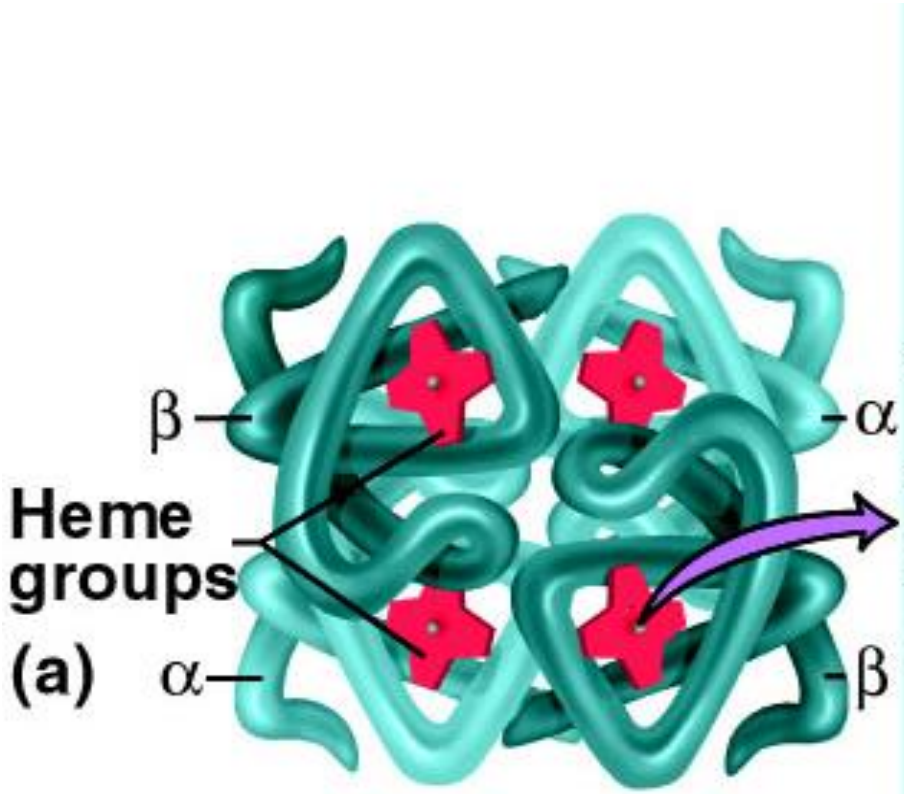


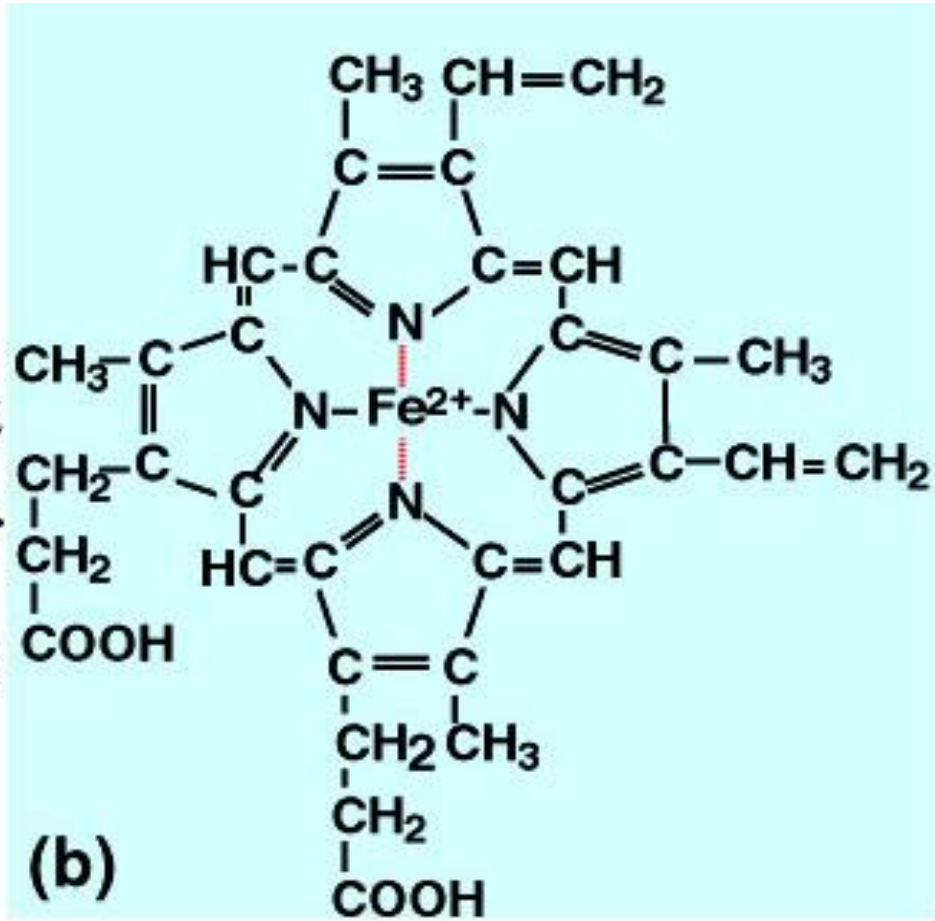
From what we have seen we can deduce that myoglobin could NEVER function as an oxygen-carrying protein because in physiological conditions it would never release it

Hemoglobin

Hemoglobin Structure (human; adult)

Hemoglobin (Mr 64,500; abbreviated Hb) is a tetrameric protein containing four heme prosthetic groups, one associated with each polypeptide chain, present in red blood cells. Adult hemoglobin contains two types of globin, two α chains (141 residues each) and two β chains (146 residues each), $\alpha_2 \beta_2$.





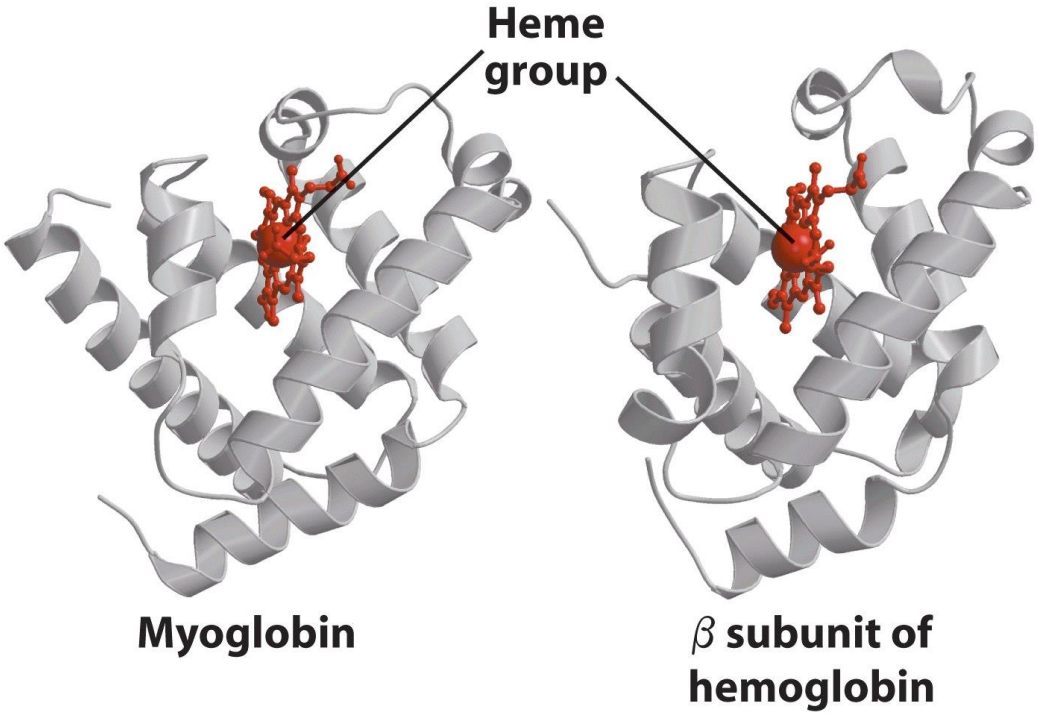
Hemoglobin (Hb) in adults $\alpha_2\beta_2$

- Found in erythrocytes
  ~34% by weight
  750 gr Hb/70 Kg body weight

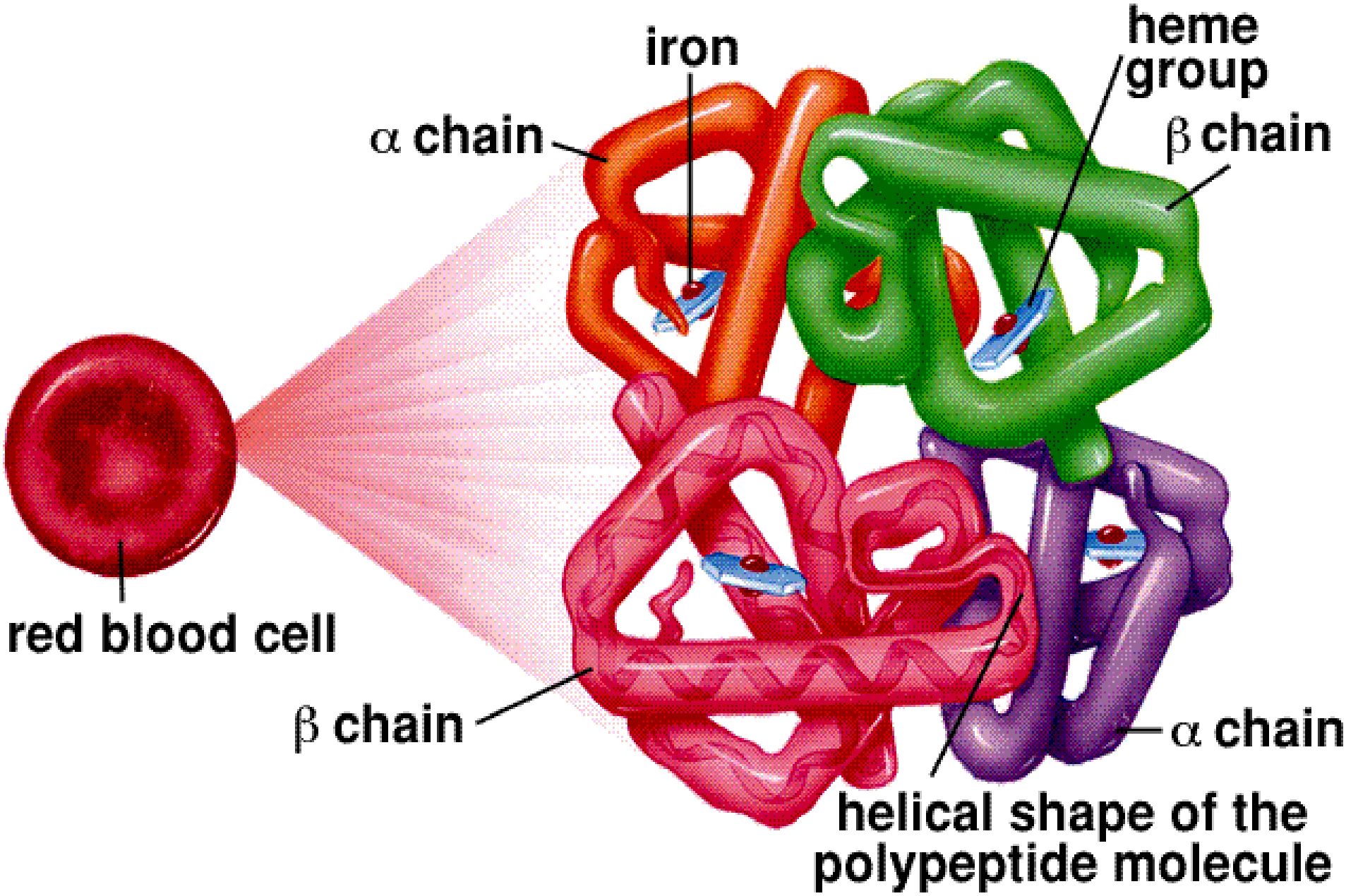
- $\alpha_2\beta_2$ tetramer (quaternary structure)

- Each subunit is structurally similar to myoglobin.

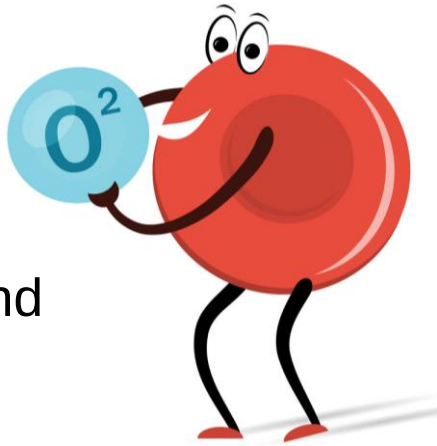
- Sensitive to small changes in $[O_2]$ thanks to the quaternary structure.



Hemoglobin Molecule



ERYTHROCYTES



- Nearly all the oxygen carried by whole blood in animals is bound and transported by hemoglobin in erythrocytes (red blood cells).

- In the maturation process of their precursors called hemocytoblasts, erythrocytes form large amounts of hemoglobin and then **lose their organelles—nuclei, mitochondria, and endoplasmic reticulum.**
- Erythrocytes are thus incomplete cells, unable to reproduce and, in humans, destined to survive for only about **120 days**.

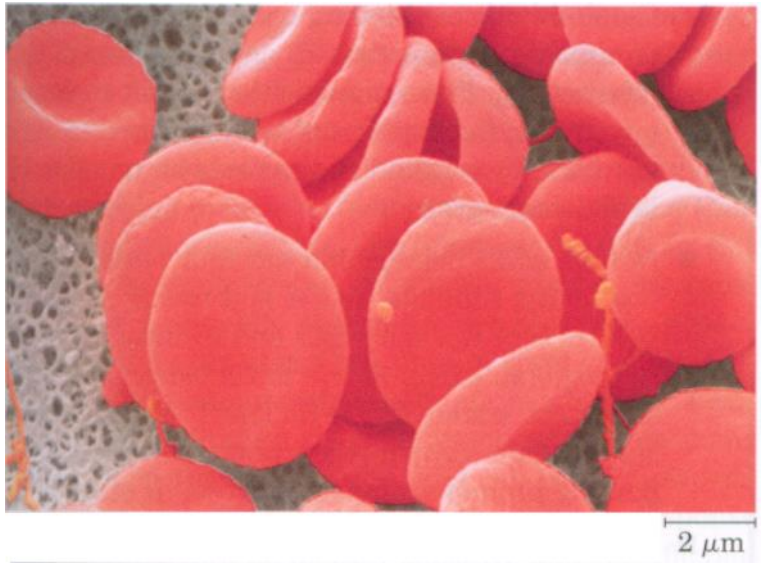
- **Their main function is to carry hemoglobin, which is dissolved in the cytosol at a very high concentration (~34% by weight).**

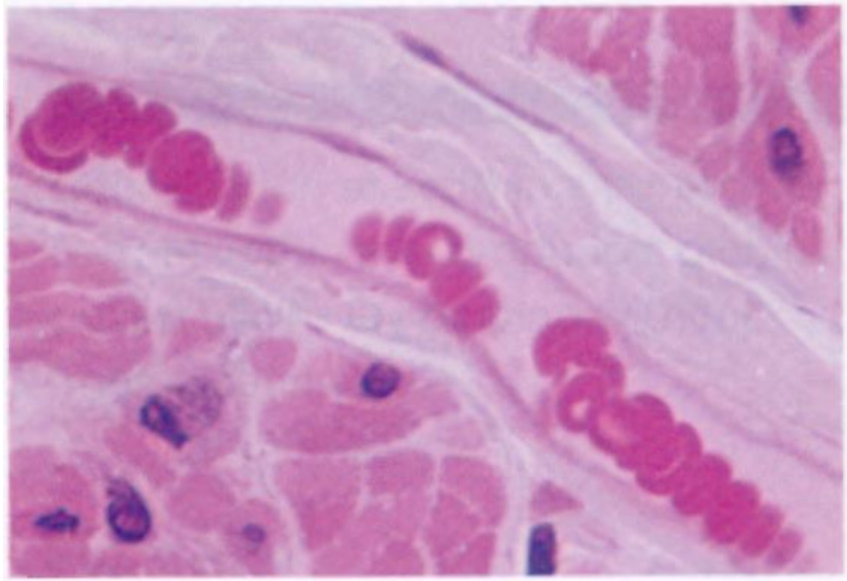
- **In arterial blood passing from the lungs through the heart to the peripheral tissues, hemoglobin is about 96% saturated with oxygen. In the venous blood returning to the heart, hemoglobin is only about 64% saturated.**

Hemoglobin is located in erythrocytes, where it greatly increases oxygen solubility, facilitating as much as 68 times higher oxygen concentrations than in water alone

O_2 has only limited solubility (1 X 10^{-4} M) in water:

Hemoglobin concentration in:
Women: 12 to 16 grams per deciliter (g/dl)
Men: 13.5 to 18 g/dl





Human erythrocytes. These red blood cells, or erythrocytes, are shown moving in a capillary. Each erythrocyte contains about 300 million hemoglobin molecules.

OXYHEMOGLOBIN FORMATION

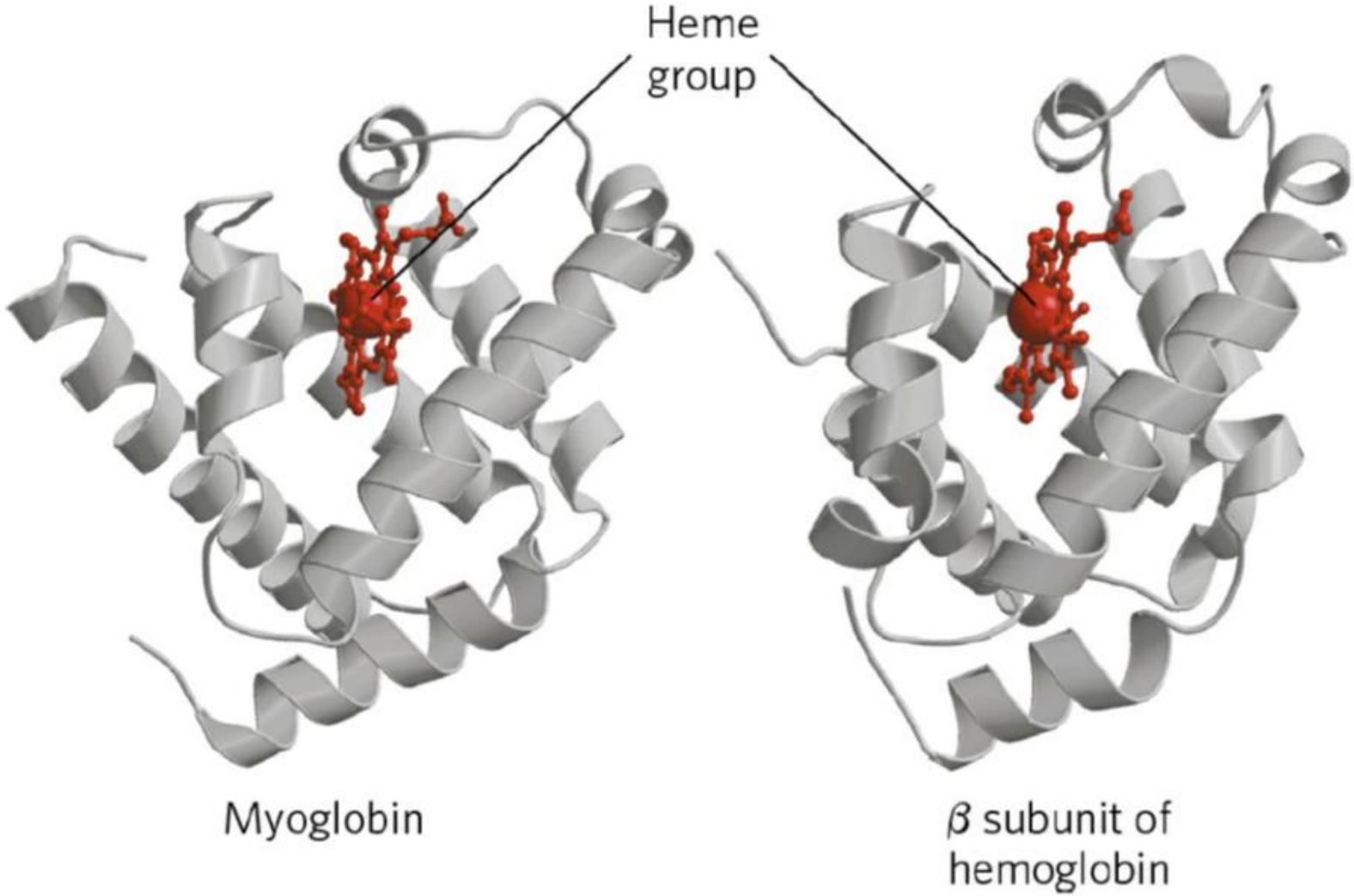
- An oxygen molecule reversibly attaches to the heme portion of hemoglobin.
- The heme unit contains Fe^{2+} which provides the attractive force

$$4\,O_2 + Hb\,(Fe^{2+}) \rightleftharpoons Hb\,(Fe^{2+} - O_2)_4$$

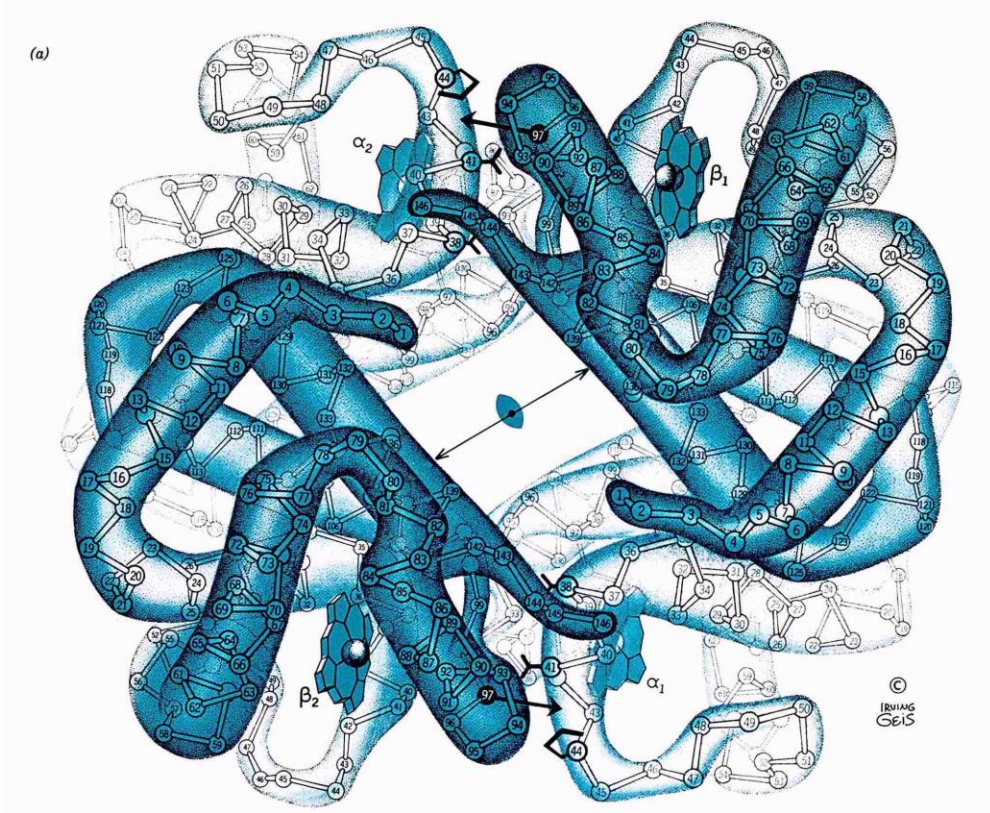
Hemoglobin- Key Properties

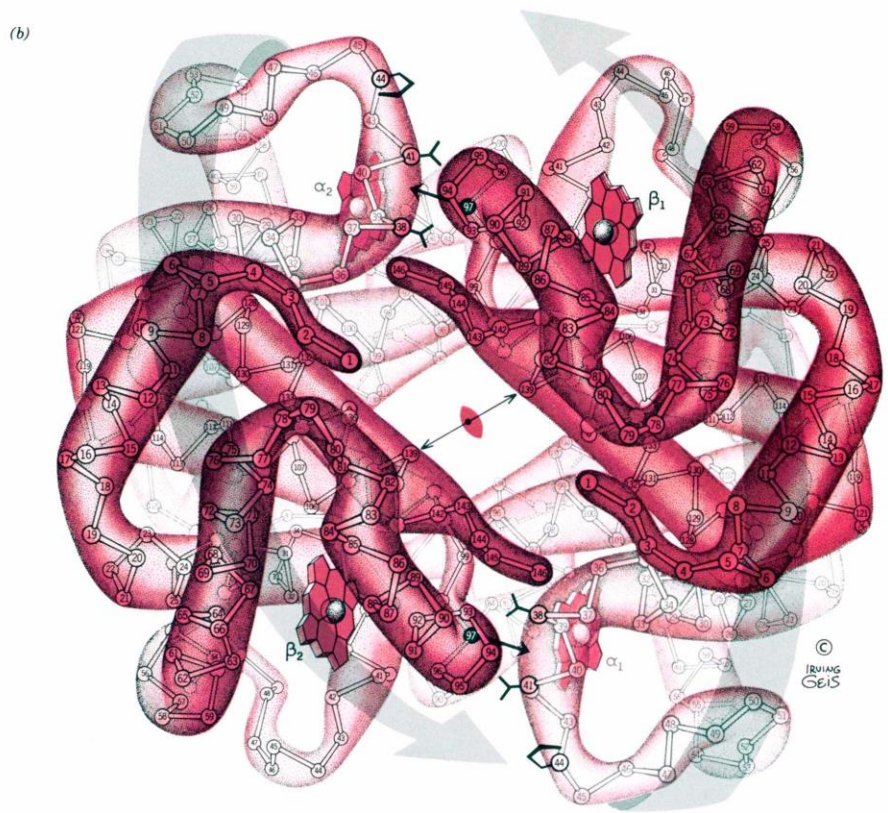
$$Hb + nO_2 \leftrightarrow Hb(O_2)_1 + Hb(O_2)_2 + Hb(O_2)_3 + Hb(O_2)_4$$

- Ubiquitous O_2 transport protein
- A globular soluble protein, 2x2 chains (64.5 kDa)
- α and β chains 44% identical
- All α-helical secondary structure (like myoglobin)
- $\alpha_1\beta_2$ $\alpha_2\beta_1$ quaternary structure from two dimers
  - α-subunit 141 residues
  - β-subunit 146 residues
- Extensive contacts between subunits
  - Mix of hydrophobic, H-bond, and ionic interactions (salt bridges)


Heme
group
Myoglobin
β subunit of
hemoglobin

Quaternary structure of deoxy- and oxyhemoglobin





T-state

R-state

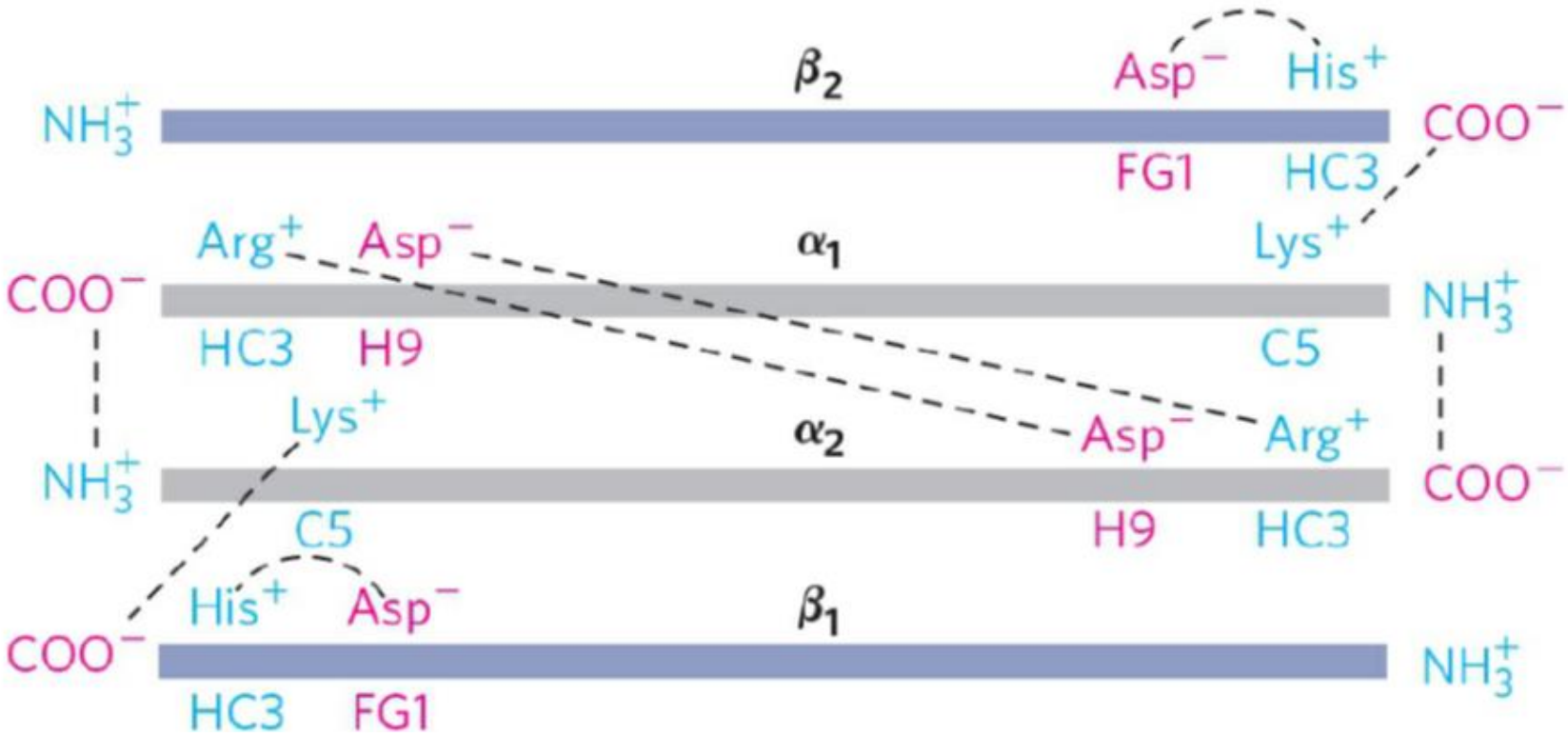
Conformations of hemoglobin: the R state and the T state

- T and R denoted “tense” and “relaxed” conformations, respectively, because the T state is stabilized by a greater number of ion pairs, many of which lie at the α1β2 (and α2β1) interface.

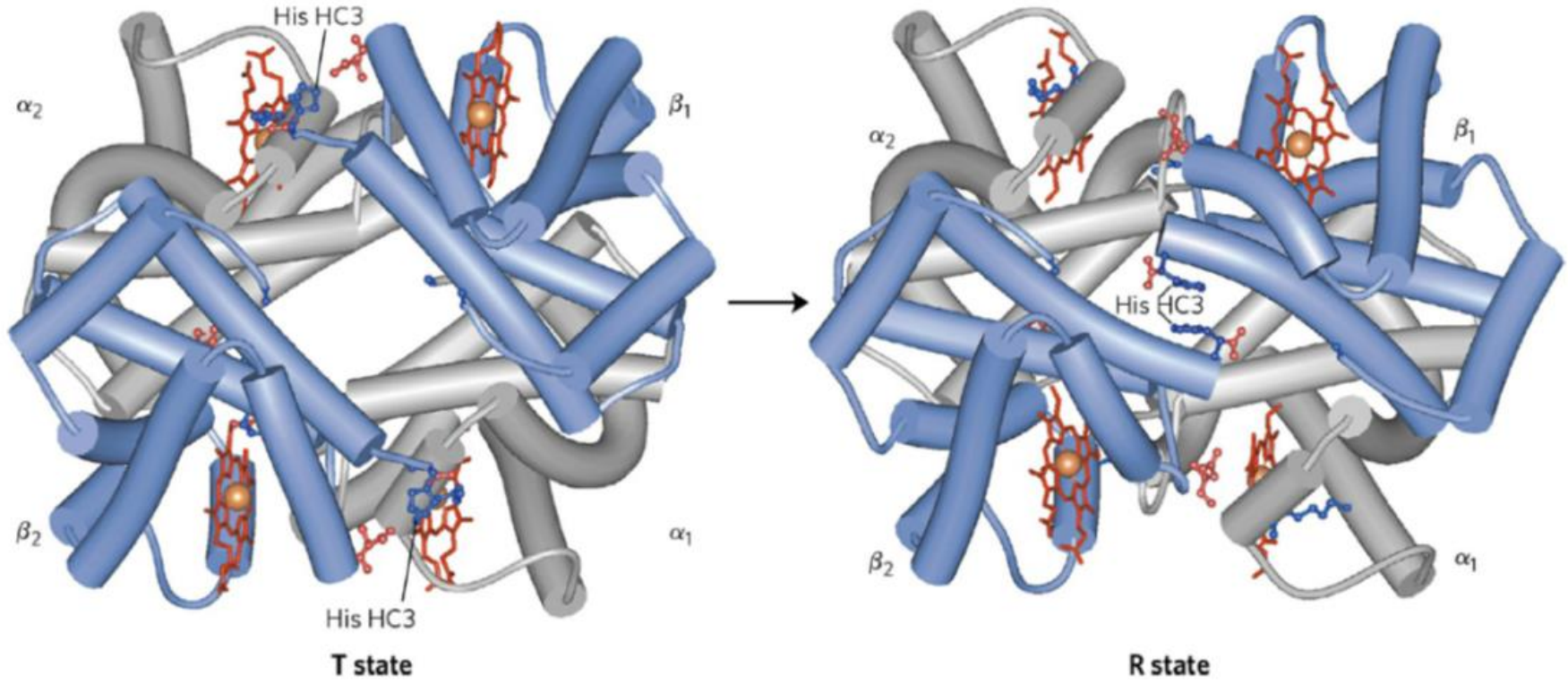
- Although oxygen binds to hemoglobin in either state, it has a significantly higher affinity for hemoglobin in the R state.

- When the entire protein undergoes **transition from T to R**, the structures of the individual subunits slightly change. In this process, some of the ion pairs that stabilize the T state are broken and some new ones are formed.

Ion pairs interactions between the four subunits of (T) hemoglobin



Max Perutz proposed that the T → R transition is triggered by changes in the positions of key amino acid side chains surrounding the heme.



The transition from the T state to the R state shifts the subunit pairs substantially, affecting certain ion pairs.

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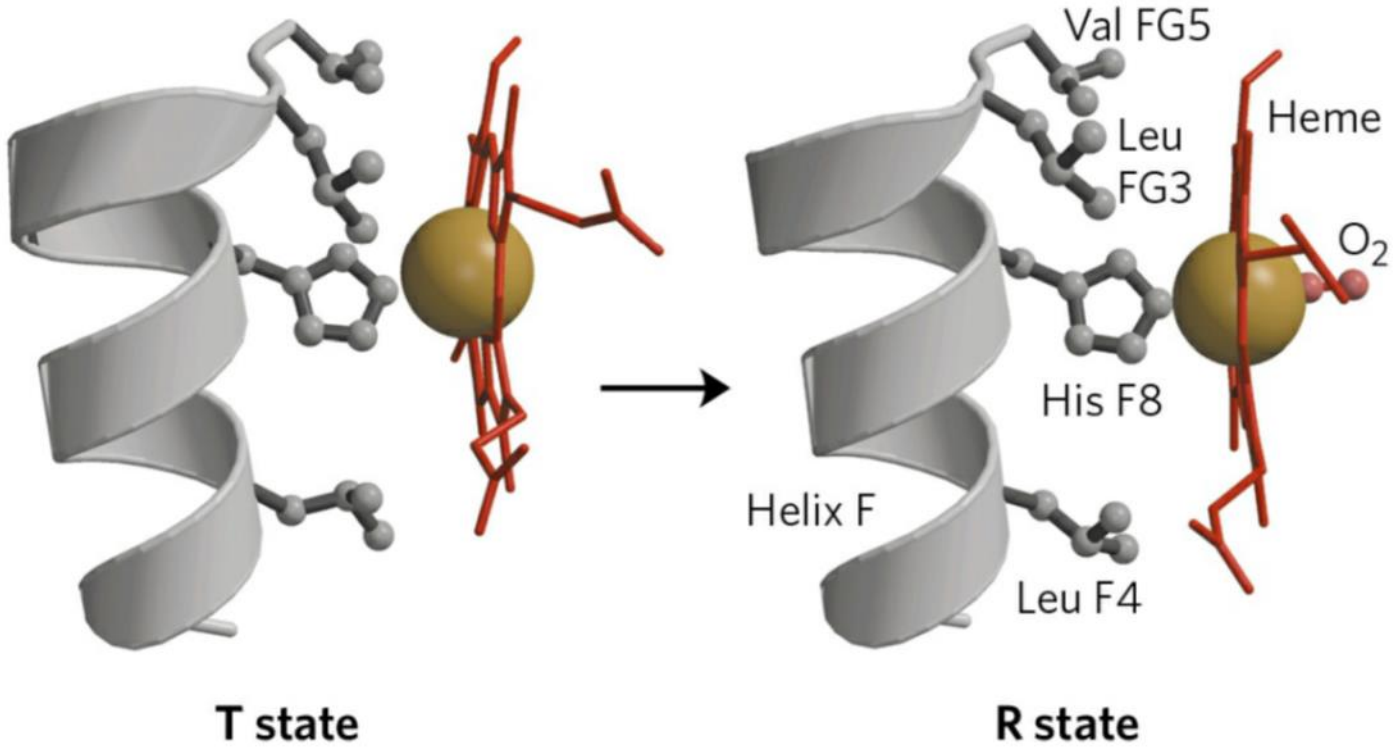


In the T state, the porphyrin is slightly puckered, causing the heme iron to protrude somewhat on the proximal His (His F8) side. The binding of O_2 causes the heme to assume a more planar conformation, shifting the position of the proximal His and the attached F helix. These changes lead to adjustments in the ion pairs at the α1β2 interface.

E-learning

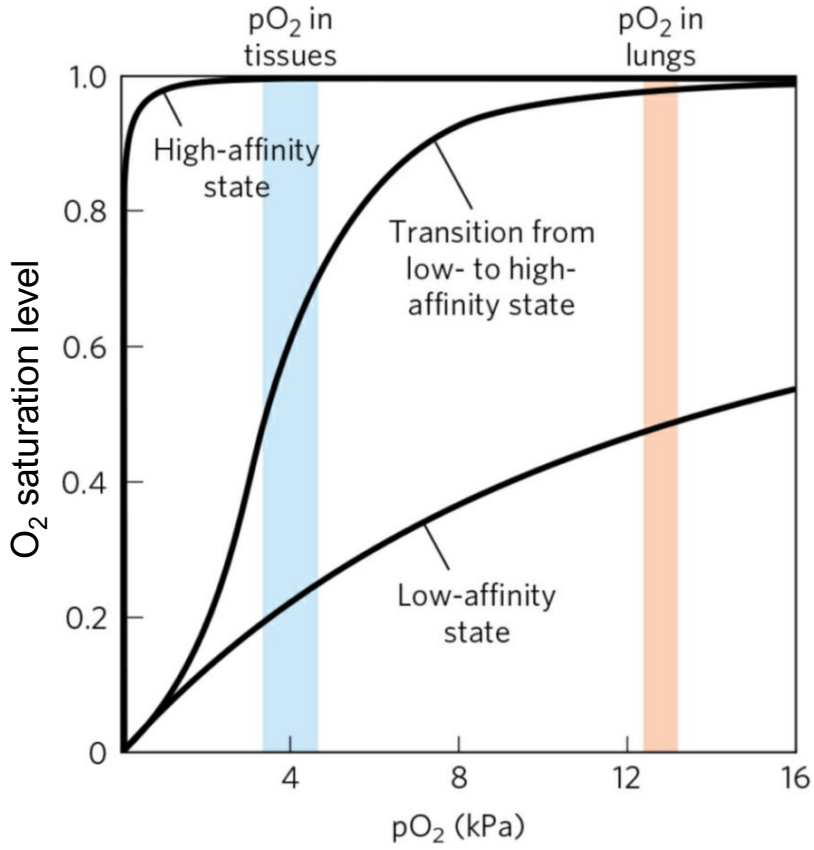
https://www.youtube.com/watch?v=H3DHvJ_MEtk

https://youtu.be/6d39yPB5yw4

https://youtu.be/Qv-KExGKAYw

Hemoglobin must bind oxygen efficiently in the lungs, where the pO_2 is about 13.3 kPa (around 100 mmHg), and release oxygen in the tissues, where the pO_2 is about 4 kPa (30 mmHg).

A protein that bound O_2 with high affinity would bind it efficiently in the lungs but would not release much of it in the tissues (such as myoglobin). If the protein bound oxygen with a sufficiently low affinity to release it in the tissues, it would not pick up much oxygen in the lungs.



Hemoglobin solves the problem by undergoing a transition from a low affinity state (T state) to a high-affinity state (R state) as more O_2 molecules are bound. As a result, hemoglobin has a hybrid sigmoid binding curve for oxygen. A sigmoid binding curve is diagnostic of cooperative binding.

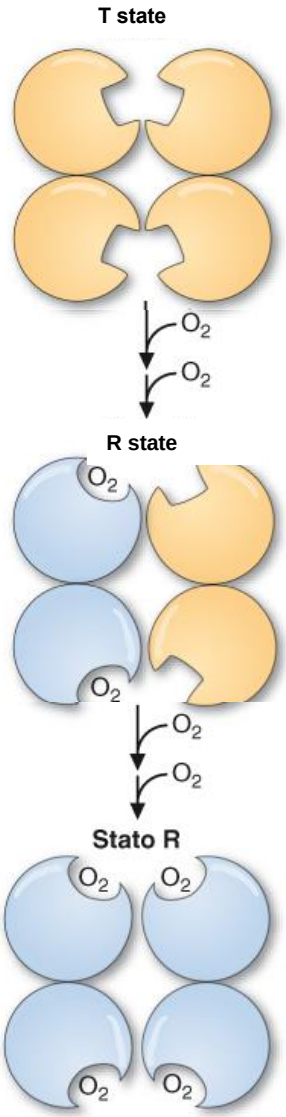
Cooperative binding of O_2 with Hb

O_2 binding to individual subunits of hemoglobin can alter the affinity for O_2 in adjacent subunits.

T state, low affinity for O_2

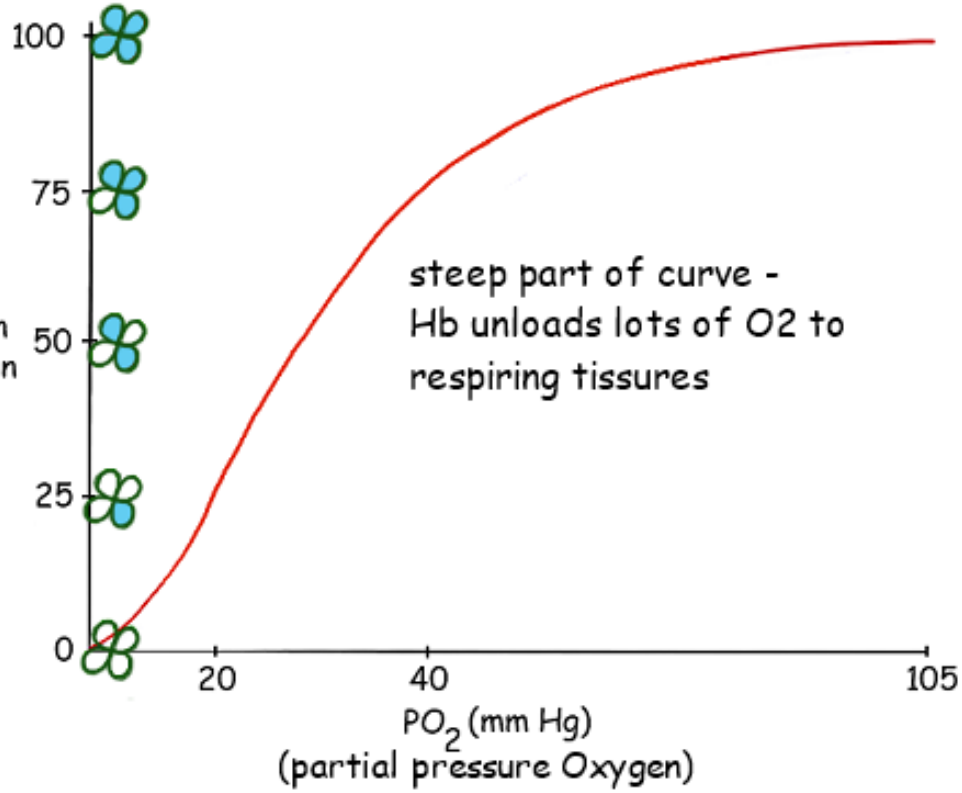
The first molecule of O_2 that interacts with deoxyhemoglobin binds weakly, because it binds to a subunit in the T state. Its binding, however, leads to conformational changes that are communicated to adjacent subunits, making it easier for additional molecules of O_2 to bind (the T → R transition).

The last (fourth) O_2 molecule binds to a heme in a subunit that is already in the R state.
R state, high affinity for O_2



Oxygen-Hemoglobin Dissociation Curve
(sigmoid-shaped curve of the relationship between Hb saturation with O_2 at different partial pressures)

High Hb saturation occurs with high PO_2 in the lungs
(Hb - High affinity for O_2)



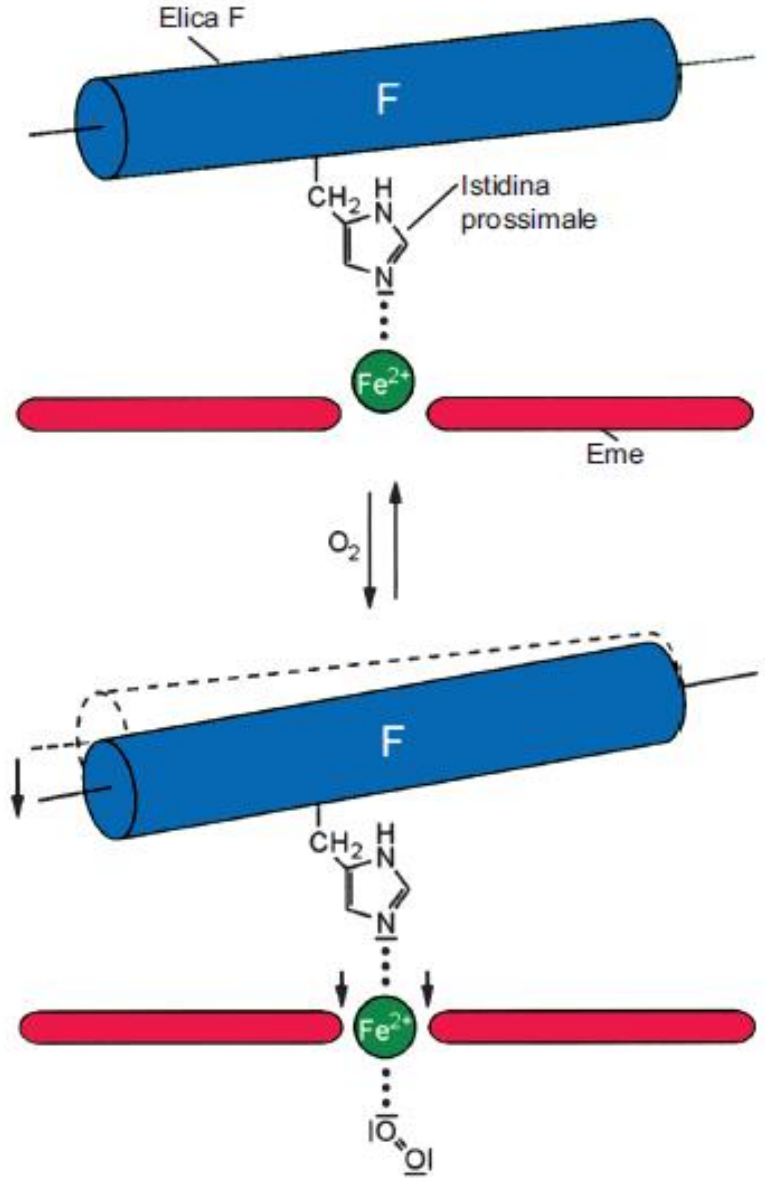
plateau part of graph exists in pulmonary capillaries where max O_2 saturation occurs

steep part of curve - Hb unloads lots of O2 to respiring tissues

hemoglobin
oxygen

Low Hb saturation occurs in respiring tissue where PO_2 is low
(Hb - Low affinity for O_2)

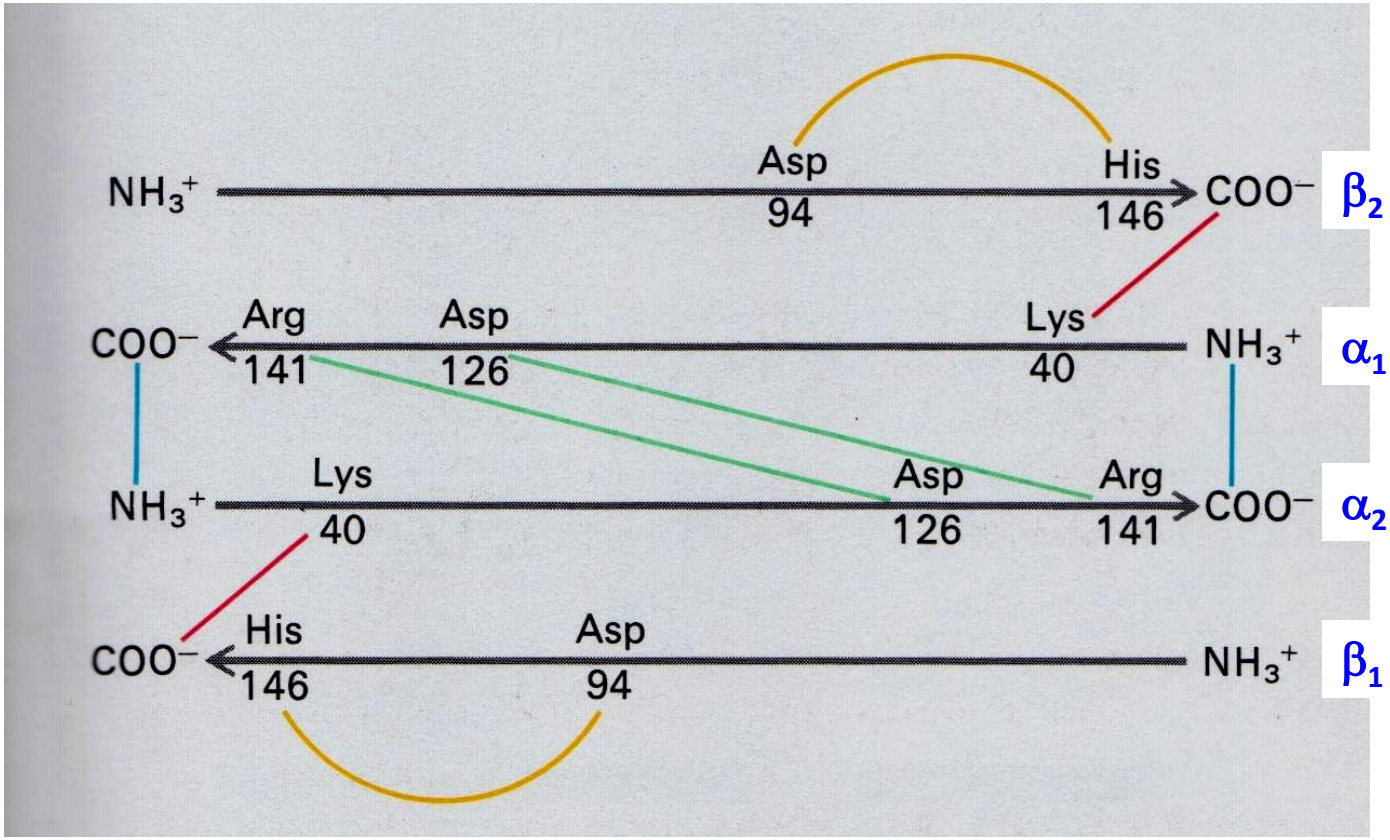
Molecular mechanism of Hb oxygenation



Oxygen binding to heme causes the Fe atom to move about 0.54 Å (0.05 nm) into the plane of the heme

This displaces the proximal histidine (His F8) and helix F to which it is attached.

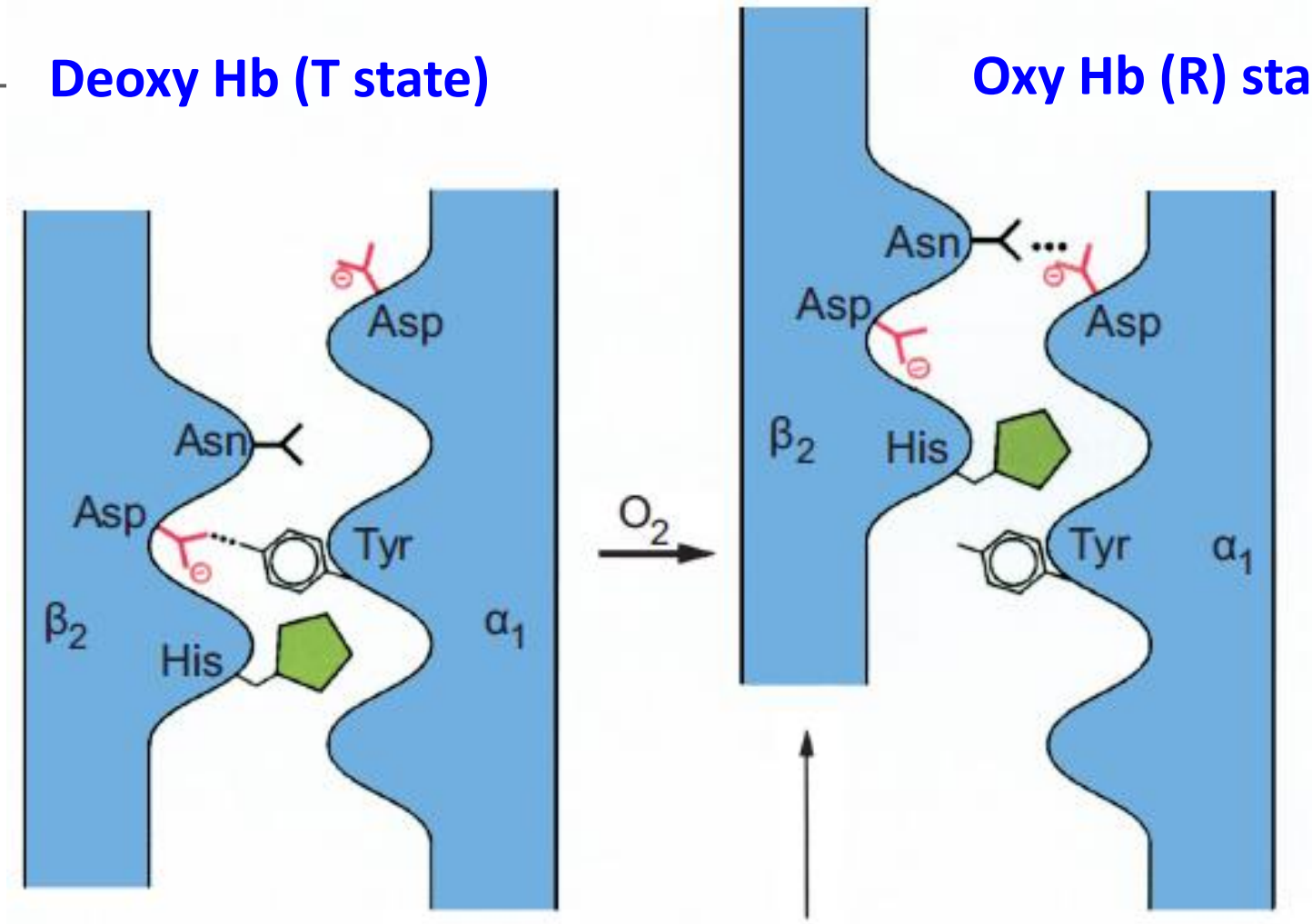
Molecular mechanism of Hb co-operativity



The conformational changes, initiated by movement of helix F, alters the network of electrostatic interactions between subunits (across α–α and α–β interfaces).

This helps to relax (R) the contacts between subunits

Conformational changes in α1β2 unit during T → R transition



Conformational change induced by the ligand (O_2) binding

Two models for the cooperative binding of ligands to proteins with multiple binding sites have greatly influenced thinking about T - R transition.

Concerted model (MWC model):

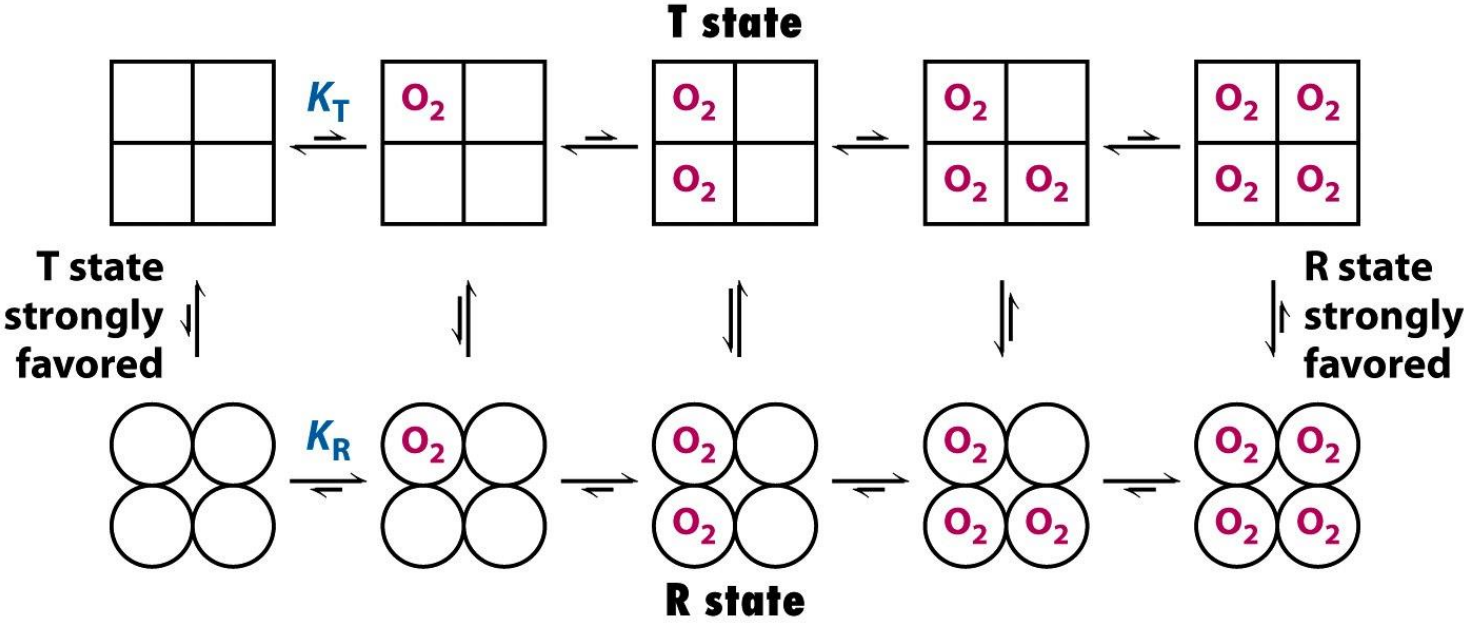
proposed by Jacques Monod, Jefferies Wyman, and Jean-Pierre Changeux in 1965

Sequential model:

proposed by Daniel Koshland and colleagues in 1966

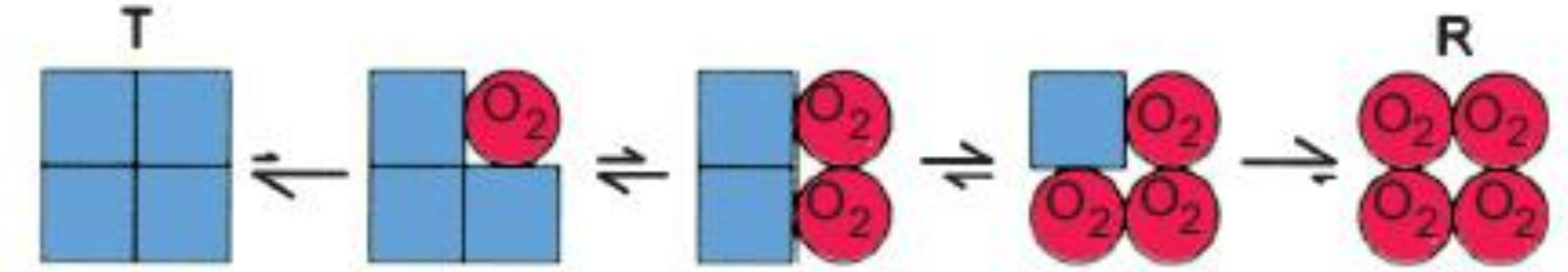
Concerted, two state model

Monod, Wyman & Changeux



- The two conformations are in equilibrium in all the subunits of Hb.
- The ligand can bind to either conformation but binds much more tightly to the R state.
- Successive binding of ligand molecules to the low-affinity conformation (which is more stable in the absence of ligand) makes a transition to the high-affinity conformation more likely.

The sequential model by Koshland



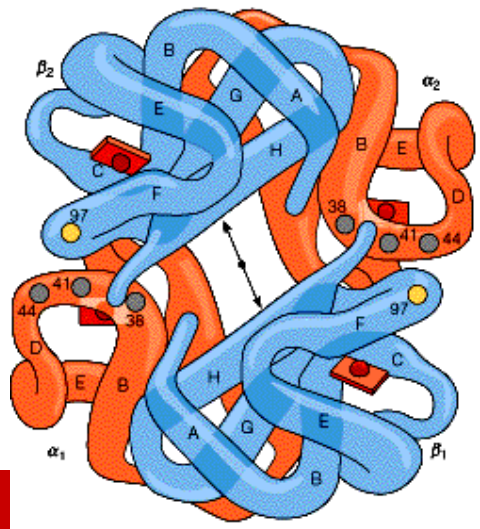
- Ligand binding can induce a change of conformation in an individual subunit.
- A conformational change in one subunit makes a similar change in an adjacent subunit, as well as the binding of a second ligand molecule, more likely.
- The two models are not mutually exclusive; the concerted model may be viewed as the “all-or-none” limiting case of the sequential model.

Molecular mechanism of Hb co-operativity

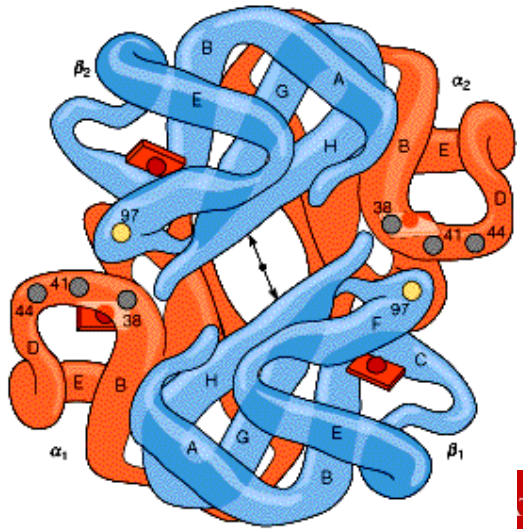
The binding energy of the first oxygen molecule is partly "consumed" in order to relax the Hb structure (loosen the intersubunit contacts); thus the initial oxygen affinity is low.

Once converted to the R-state, the barrier to oxygen binding is removed, thereby increasing oxygen affinity.

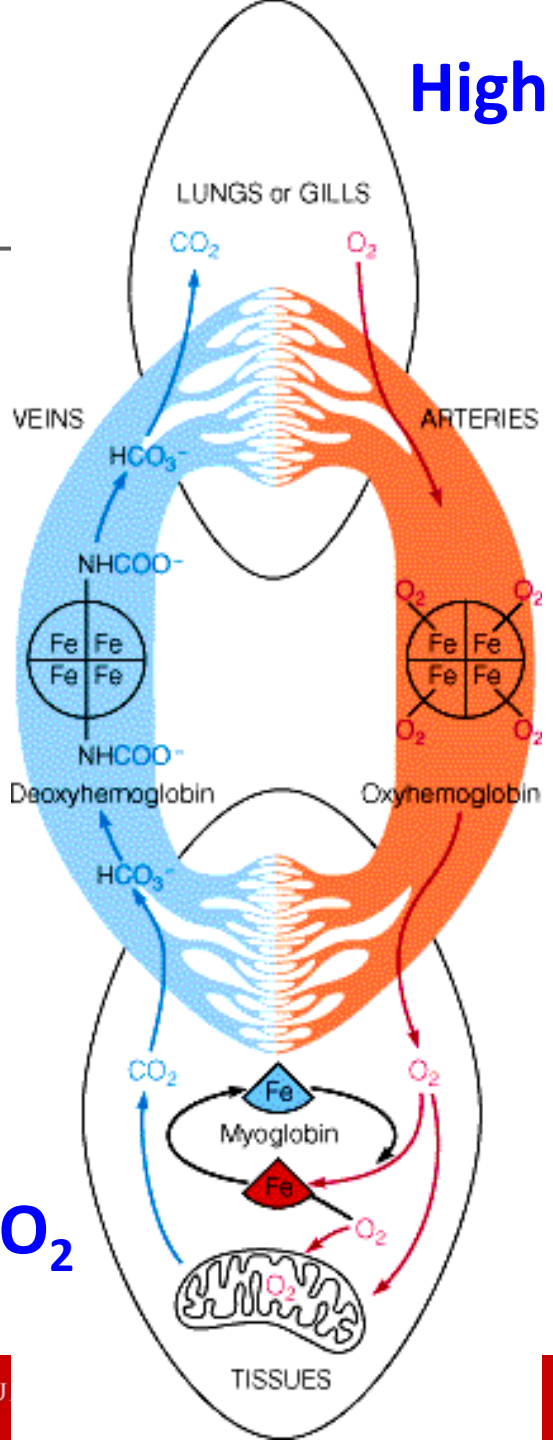
T



R



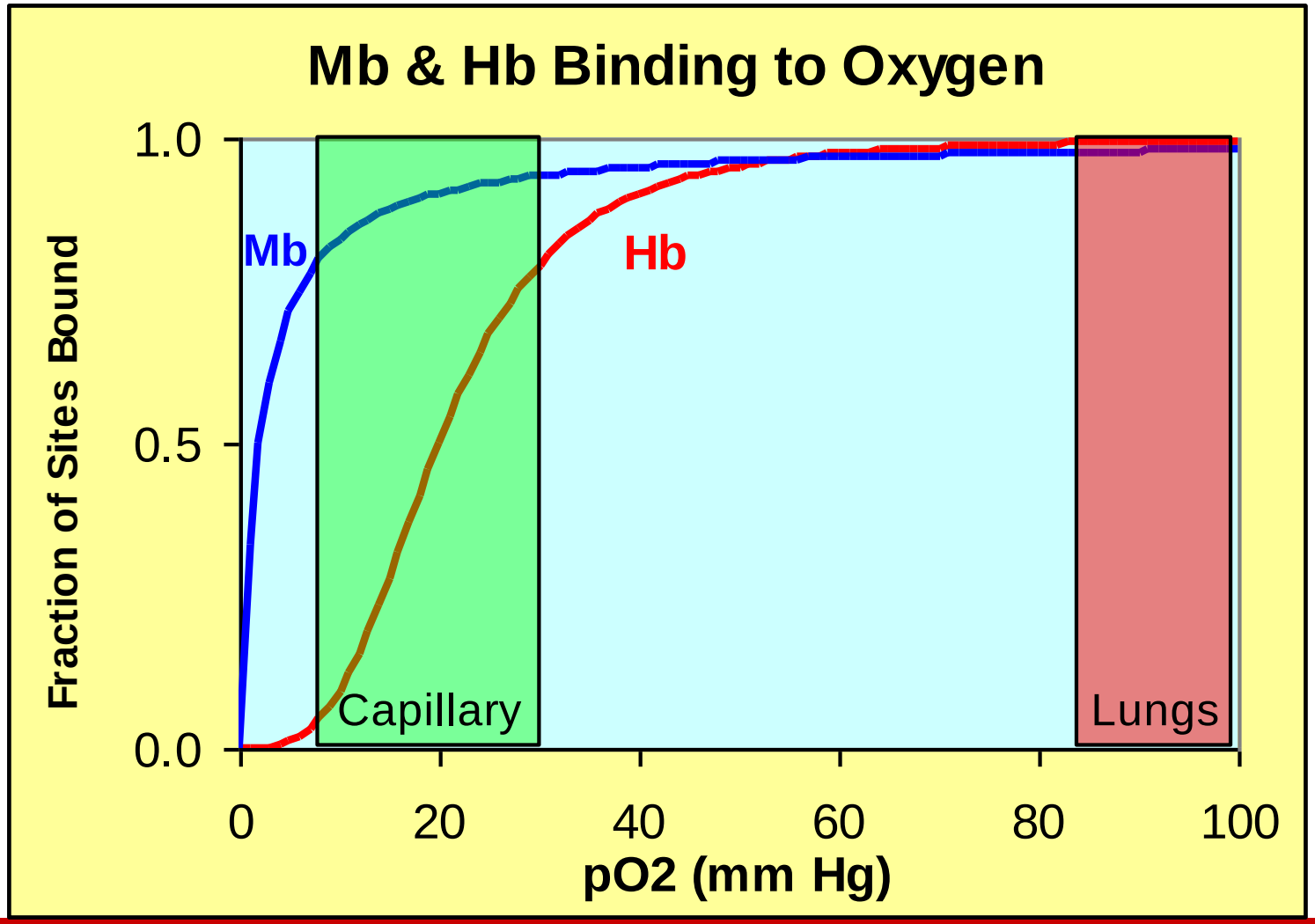
High O_2



Low O_2

At lower concentrations of oxygen (as in the capillary), myoglobin has higher affinity for oxygen than does hemoglobin

https://www.youtube.com/watch?v=91c04OoTEJs



Ligands other than oxygen can bind to hemoglobin

Carbon monoxide (CO) → Fe^{2+} **Hb** Affinity for Hb 250 times > O_2

Man in physiological condition = 1% CO-Hb
Smoker = 8-10%
Human exposure to CO can have tragic consequences;
Hb F has increase affinity for CO

Cyanide CN^- → Cyanide binds avidly to methemoglobin, forming cyanmethemoglobin. From there it is taken to the body's tissues where it inhibits to create ATP primarily through the inhibition of the mitochondrial enzyme cytochrome c oxidase

Nitric oxide NO → NO binds to the Fe (II) of the heme. NO is then yielded to the cells of the vessel during the transition from R to T-shape.
NO therefore produces its vasodilator effect.
1 molecule of Hb per 1000 participates in this mechanism.

Hemoglobin Also Transports H+ and CO_2

In Hb, O_2 binds to the iron atom of the heme groups releasing H^+

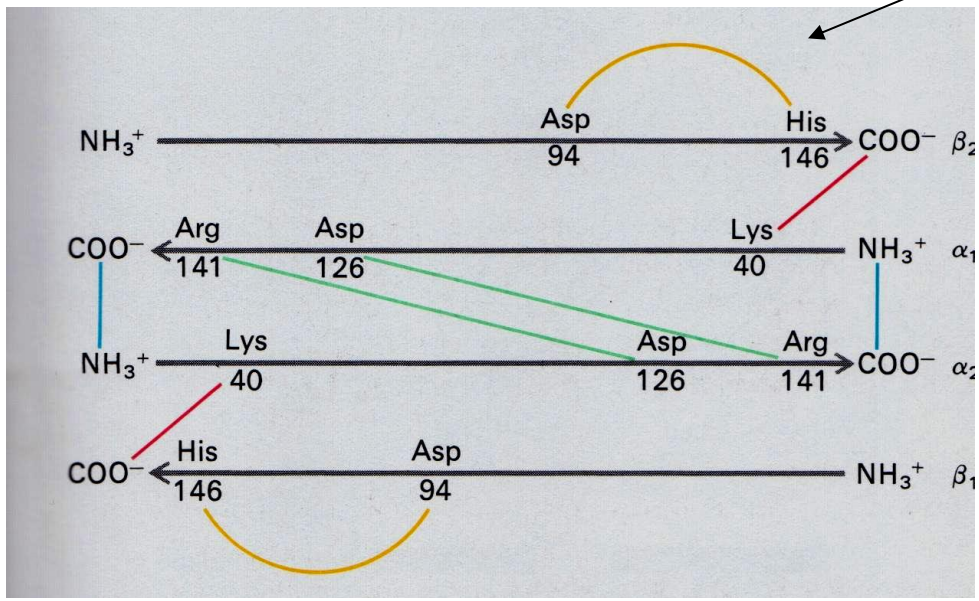
$$\mathbf{Hb(H^+)_n + 4\ O_2 \rightleftharpoons Hb\ (O_2) + \mathit{n}H^+}$$

where $Hb(H^+)_n$ denotes a protonated form of hemoglobin. The O_2-saturation curve of hemoglobin is influenced by the H+ concentration.
The effect of H^+ in the blood on binding O_2 is called the Bohr effect.

- Both O_2 and H+ are bound by hemoglobin, but with inverse affinity. When the oxygen concentration is high, as in the lungs, hemoglobin binds O_2 and releases protons. When the oxygen concentration is low, as in the peripheral tissues, H+ is bound and O_2 is released.
- Hemoglobin transports about 40% of the total H+ and 15% to 20% of the CO_2 formed in the tissues to the lungs (the remainder of the H+ is absorbed by the plasma's bicarbonate buffer; the remainder of the CO_2 is transported as dissolved in HCO_3- and CO_2)

Hemoglobin and the Bohr Effect

The Bohr Effect is the direct result of the conformational changes that occur in Hb during oxygen binding. About 50% of the effect is caused by a change in pKa of His 146 (on β subunit). In the T-state, the His is H-bound to Asp 94.



$$HbH^+ + O_2 \rightleftharpoons HbO_2 + H^+$$

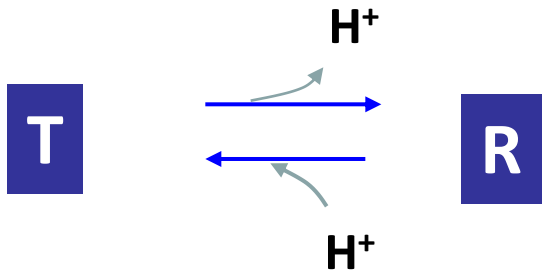
When Hb shifts from the T to R conformation, the salt-bridge to Asp 94 is disrupted, allowing a proton on the His to readily dissociate. This is the O_2 bind/H^+ release portion of the effect.

Bohr Effect

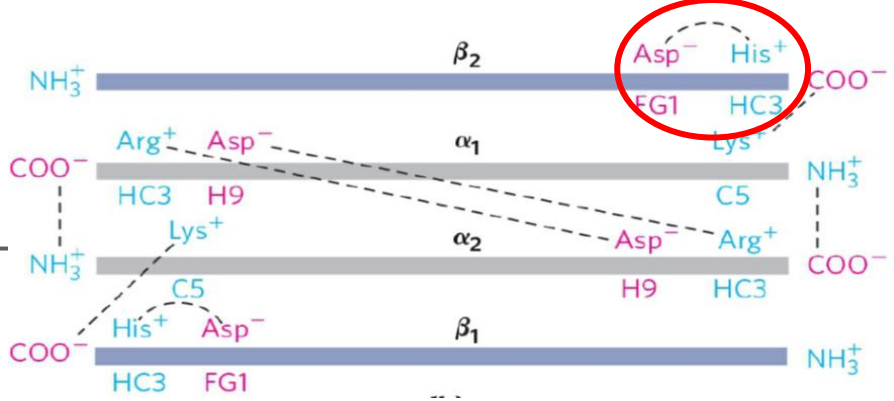
Competition between oxygen and H^+

- Hemoglobin releases H^+ when it binds O_2
- Hemoglobin binds H^+ when it releases O_2

H^+ that stabilize the T state



Bohr Effect



Oxygen and H+ are not bound at the same sites in hemoglobin.

- Oxygen binds to the iron atoms of the heme group, whereas H+ binds to any of several amino acid residues in the protein.

- A major contribution to the Bohr effect is made by **His146 (His HC3)** of the β subunits. **When protonated, this residue forms one of the ion pairs—to Asp94 (Asp FG1)—that helps stabilize deoxyhemoglobin in the T state.**

- In the R state the ion pair cannot form, and this residue is largely unprotonated in oxyhemoglobin. As the concentration of H+ rises, protonation of His HC3 promotes release of oxygen by favoring a transition to the T state.

- Protonation of the amino-terminal residues of the α subunits, certain other His residues, and perhaps other groups has a similar effect.

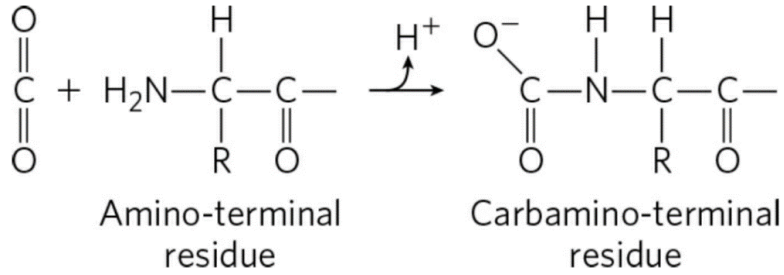
Bohr Effect

Hemoglobin also binds CO_2, again in a manner inversely related to the binding of oxygen.

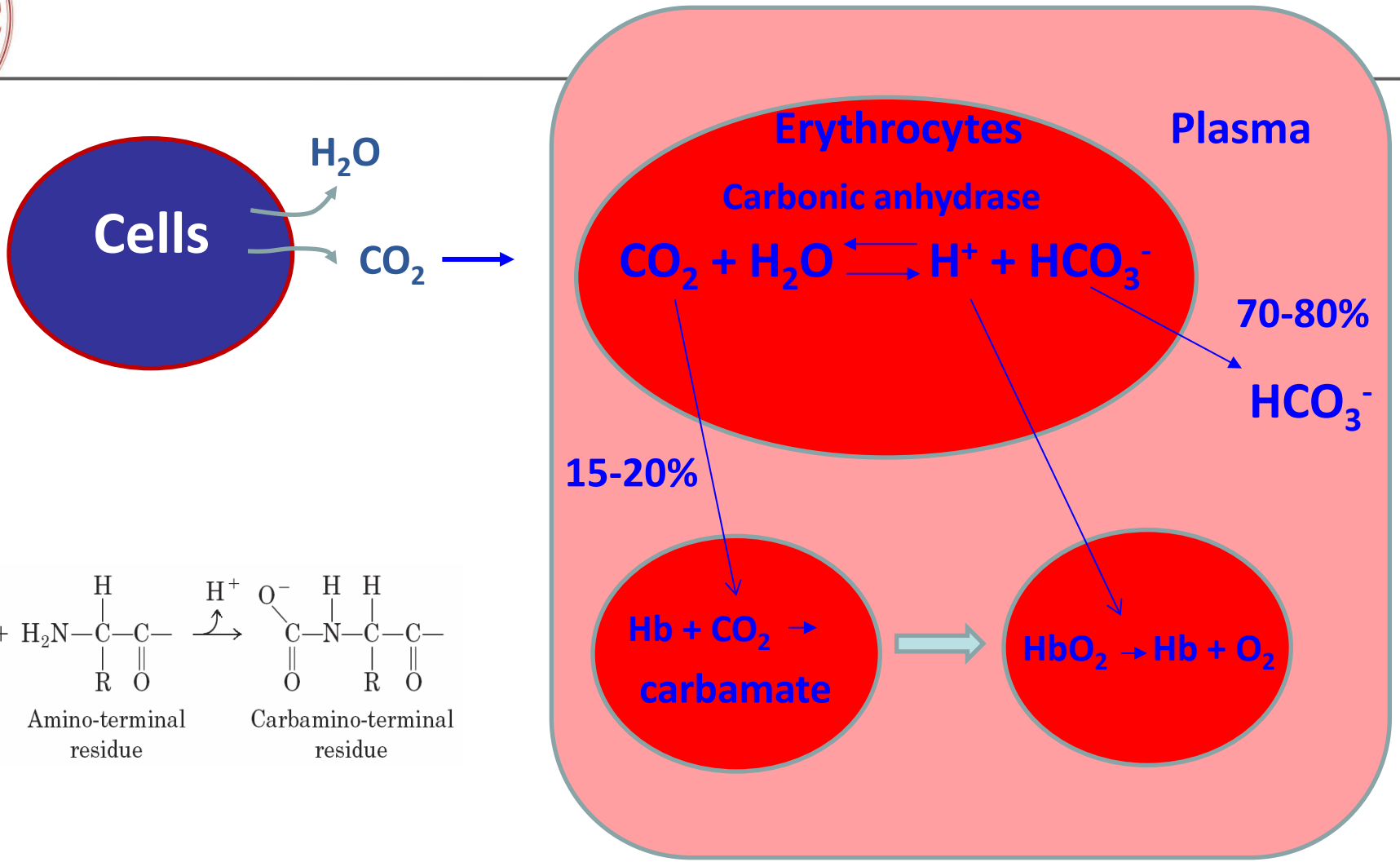
- The CO_2, produced by oxidation of organic fuels in mitochondria, is hydrated to form bicarbonate in this reaction catalyzed by carbonic anhydrase:

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$$

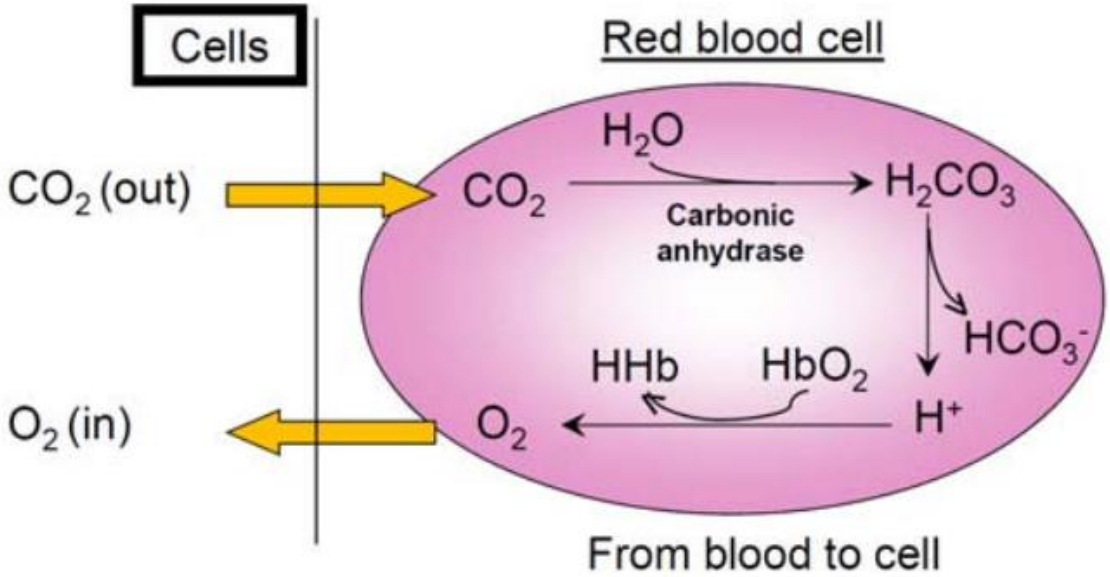
- Carbon dioxide binds as a carbamate group to **the α- amino group at the amino-terminal end of each globin chain**, forming **carbaminohemoglobin**:



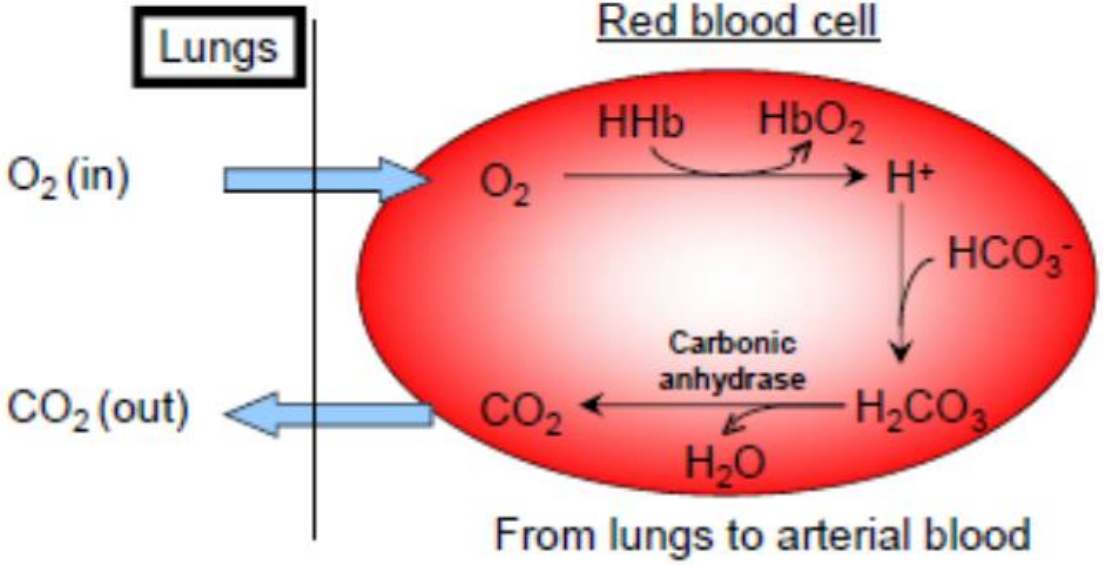
- These reactions produce H+, contributing to the Bohr effect. The bound carbamates also form additional salt bridges that help to stabilize the T state and promote the release of oxygen.



Hb transports about 40% of the total H^+ and 15-20% of the CO_2



When the concentration of carbon dioxide is high, as in peripheral tissues, CO_2 is converted to HCO_3^- and H^+ that causes the release of O_2 from Hb.



When hemoglobin reaches the lungs, oxygen binding "displaces" the H+ which is converted to H_2CO_3 and then to CO_2 and exhaled.

Hb and the Bohr Effect

- High $[H^+]$ produced from tissue metabolism is picked up by Hb as

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

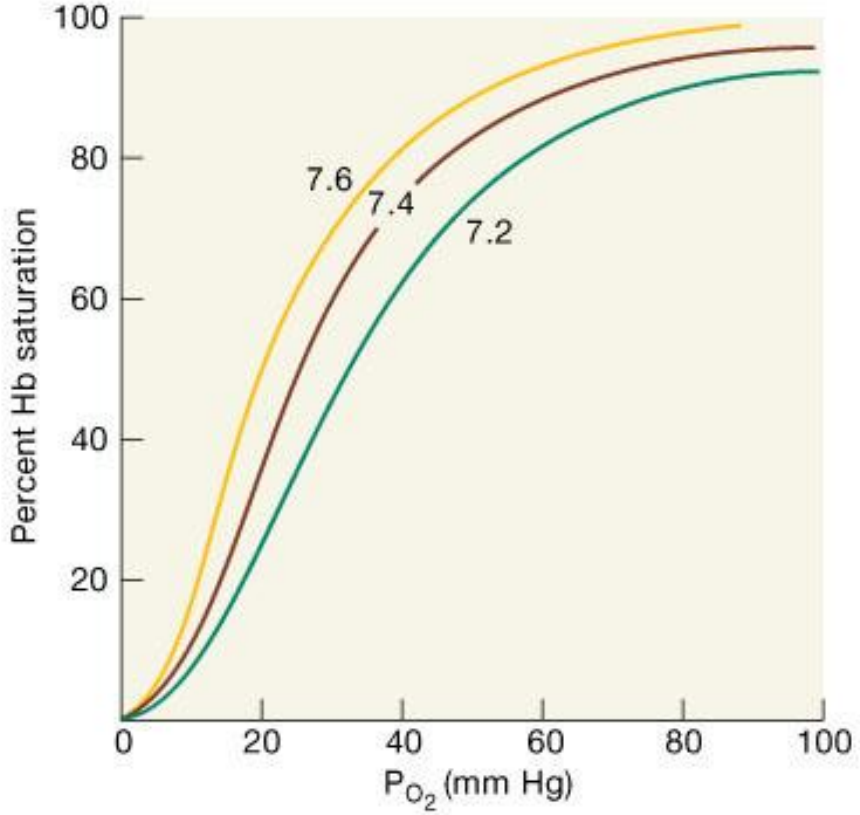
$$HbO_2 + H^+ \rightleftharpoons HbH + O_2$$

- In the lungs, oxygen binding "displaces" the H^+ which is converted to carbon dioxide and exhaled. About 30-40% of acid produced by the tissues is buffered in this way by Hb

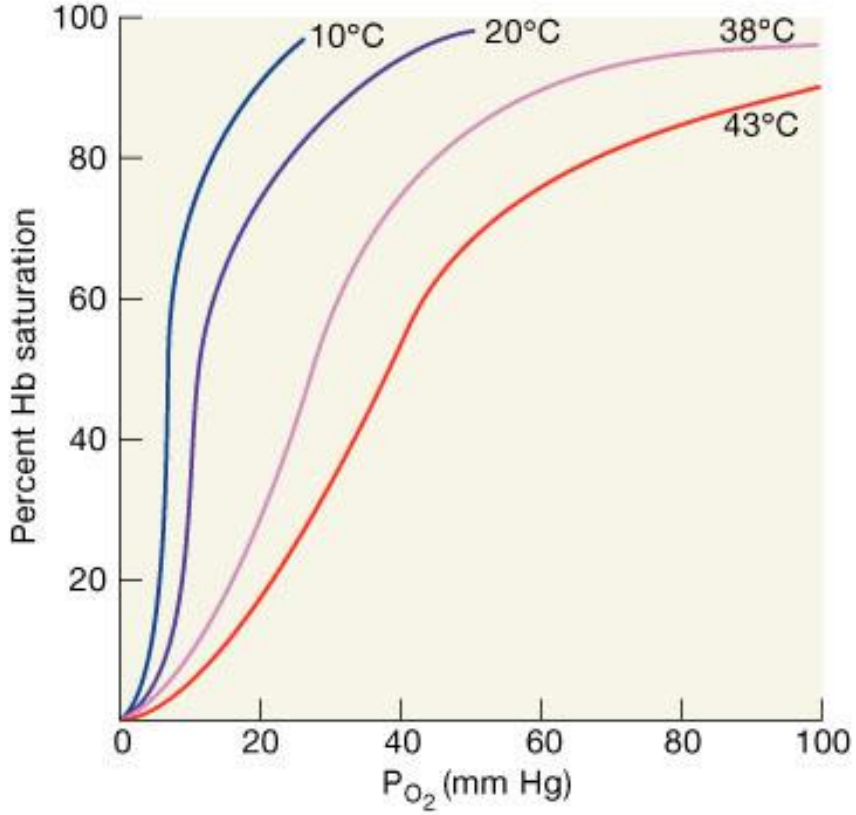
$$HbH^+ + O_2 \rightleftharpoons HbO_2 + H^+$$

$$H^+ + HCO_3^- \rightleftharpoons H_20 + CO_2$$

The Effect of pH and Temperature on Hemoglobin Saturation



(a) Effect of pH



(b) Effect of temperature

ALLOSTERIC PROTEINS

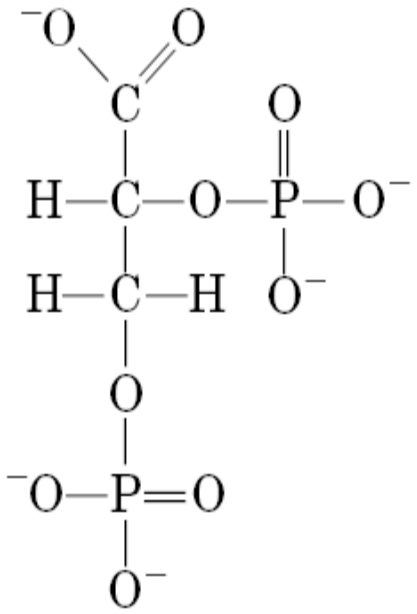
An **allosteric protein** is one in which the binding of a ligand to one site affects the binding properties of another site on the same protein. Cooperative binding of a ligand to a multimeric protein (such as Hb) is an allosteric binding.

Allosteric proteins are those having "other shapes," or conformations, induced by the binding of ligands referred to as **modulators**.

The conformational changes induced by the modulator(s) interconvert more active and less-active forms of the protein. The modulators for allosteric proteins may be either inhibitors or activators. When the normal ligand and modulator are identical, the interaction is termed **homotropic**. When the modulator is a molecule other than the normal ligand, the interaction is **heterotropic**. Some proteins have two or more modulators and therefore can have both homotropic and heterotropic interactions.

The interaction of 2,3-biphosphoglycerate (BPG) with hemoglobin molecules further refines the function of hemoglobin

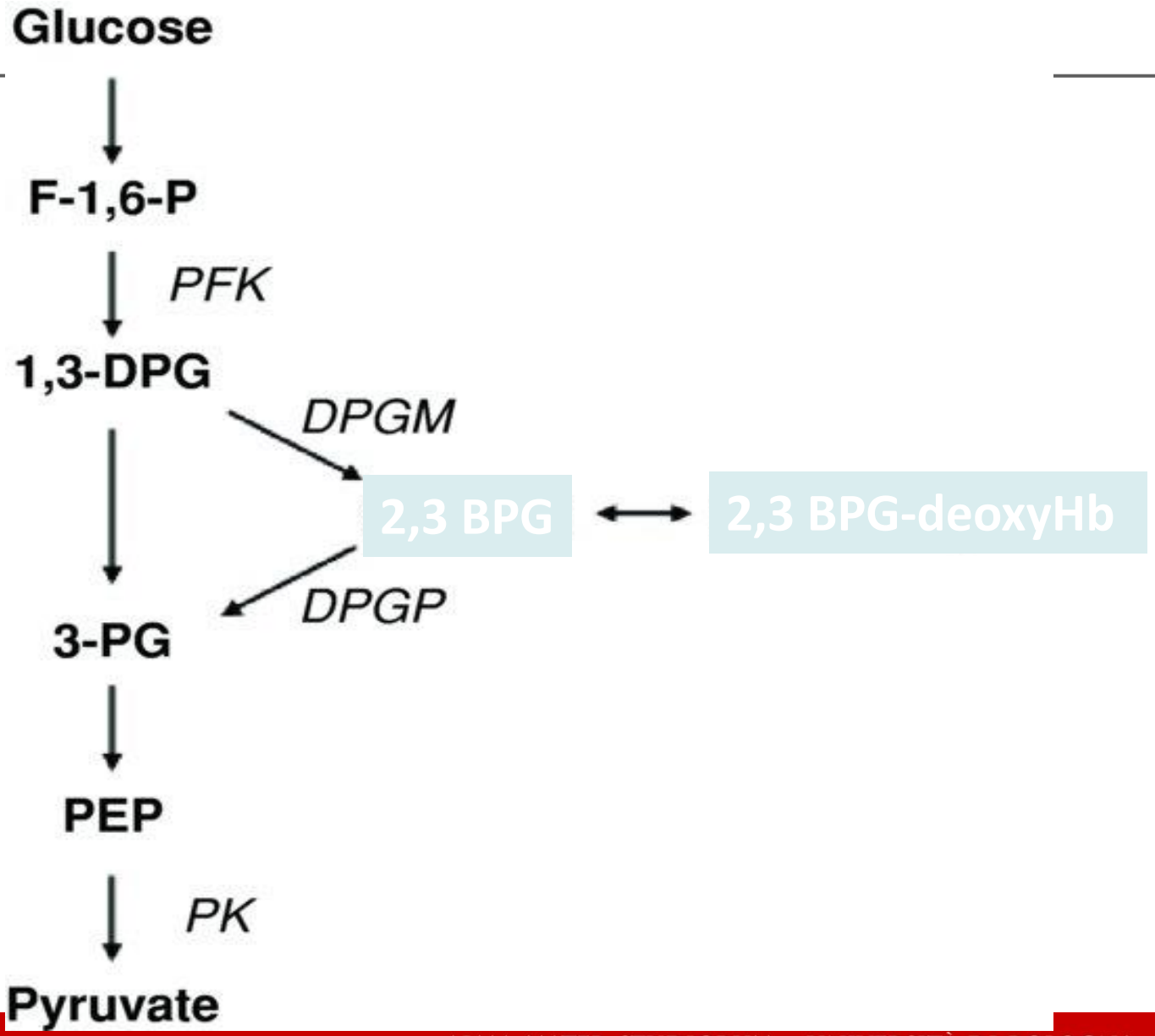
- The interaction of BPG with Hb provides an example of heterotropic allosteric modulation.
- BPG is present in relatively high concentrations in erythrocytes, linked to hemoglobin (β chains)
- BPG greatly reduces the affinity of hemoglobin for oxygen
- BPG binds at a site distant from the O_2 binding site and regulates the O_2 binding affinity of Hb in relation to the pO_2 in the lungs.



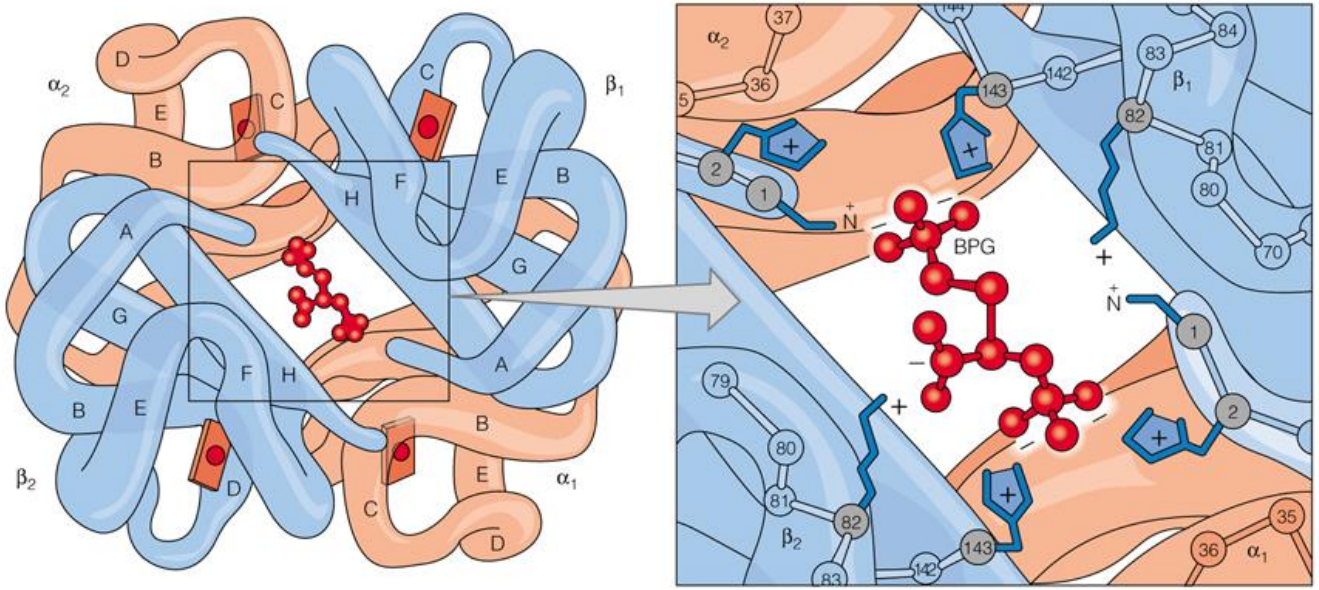
2,3-Bisphosphoglycerate

$$HbBPG + O_2 \rightleftharpoons HbO_2 + BPG$$

Formation of 2,3 BPG (2,3-biphosphoglycerate)

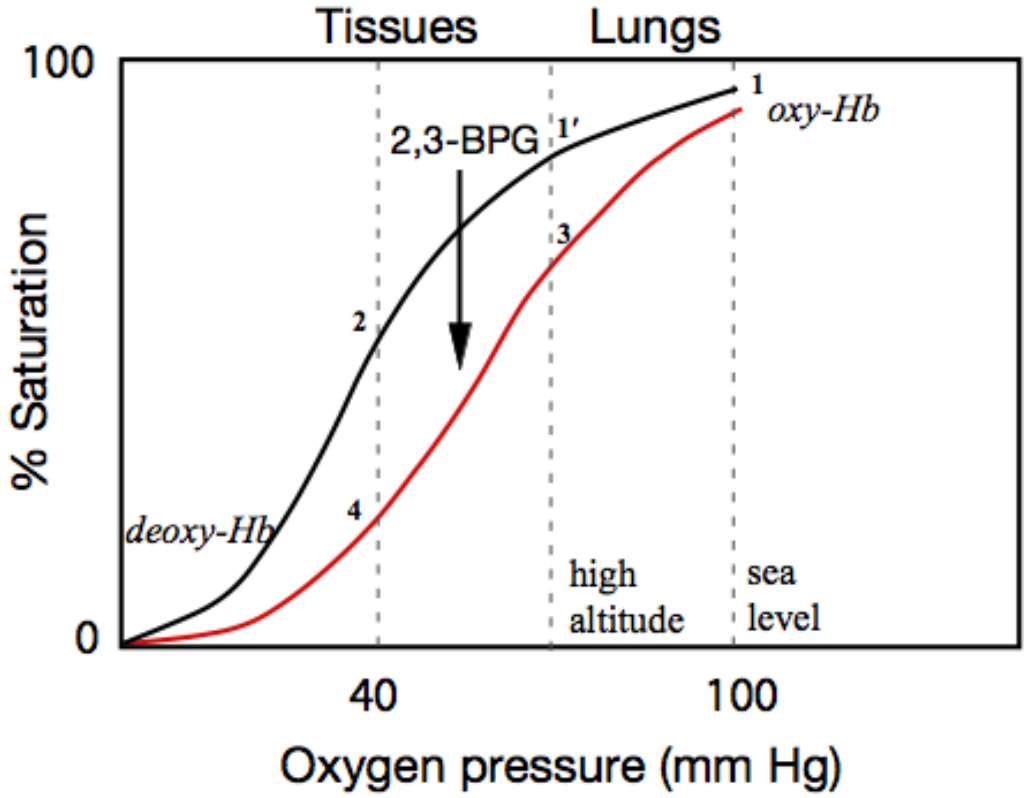


BPG lowers hemoglobin's affinity for oxygen by stabilizing the T state



- The site of BPG binding to hemoglobin is **the cavity between the *β* subunits** in the T state so only one molecule of BPG is bound to each hemoglobin tetramer.
- The transition to the R state narrows the binding pocket for BPG, precluding BPG binding. In the absence of BPG, hemoglobin is converted to the R state more easily.

BPG is important in the physiological adaptation to the lower pO_2 at high altitudes.



At sea level, hemoglobin is nearly saturated with O_2 in the lungs but is just over 60% saturated in the proximity of tissues (in arterial blood), so the amount of O_2 released in the tissues is about 38%. At high altitudes, O_2 delivery declines to 30% of maximum (due to low tissues O_2 concentration). An increase in BPG concentration, however, decreases the affinity of hemoglobin for O_2, so approximately 37% of what can be carried is again delivered to the tissues.

The BPG concentration in normal human blood is about 5 mM at sea level and about 8 mM at high altitudes.

Fetal hemoglobin

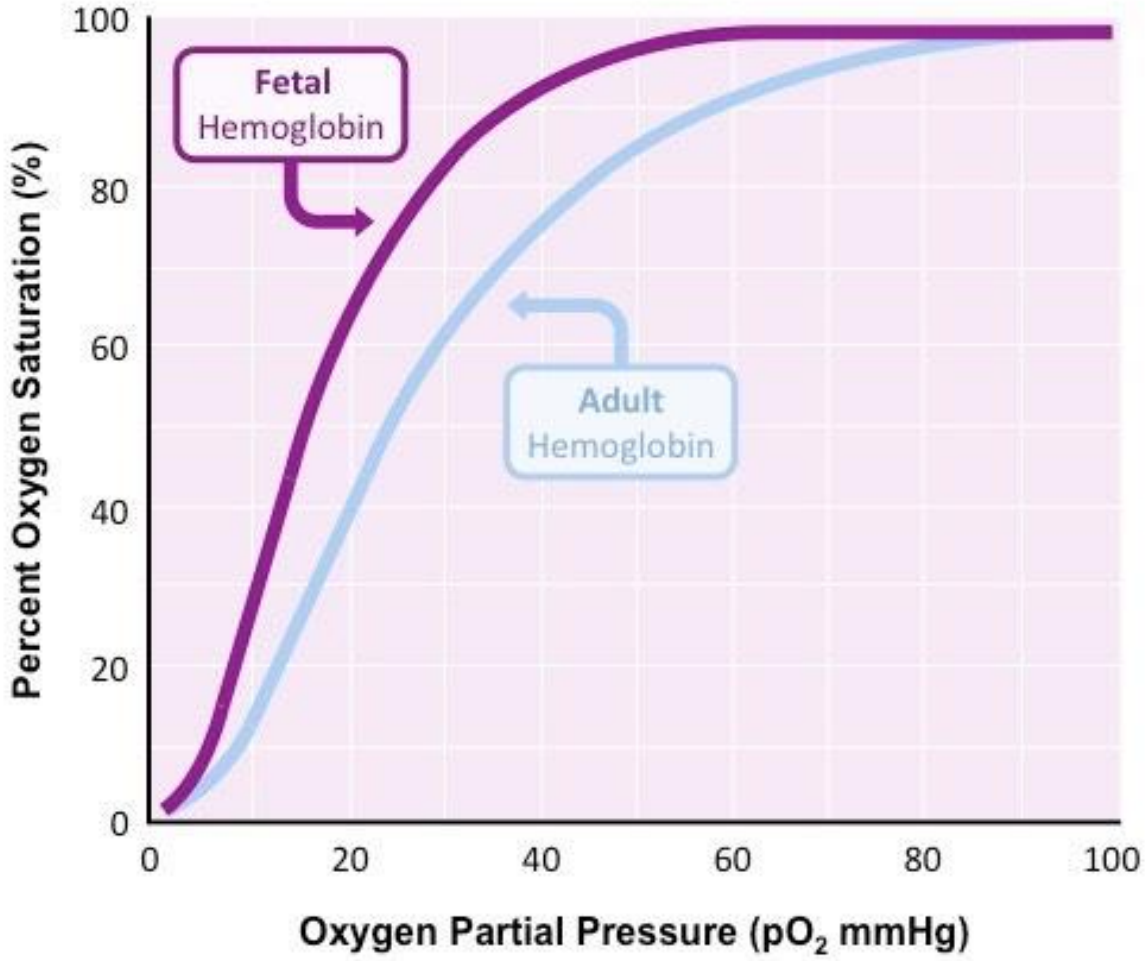
HbF: $\alpha_2\gamma_2$

Because a fetus must take oxygen from its mother's blood, fetal hemoglobin must have greater affinity than maternal hemoglobin for O_2. The fetus synthesizes **γ subunits** rather than β subunits, forming **α2γ2 hemoglobin**. Differently from normal adult hemoglobin, this tetramer cannot bind BPG, thus having a higher affinity for O_2. This way, the developing fetus is able to retrieve oxygen from the mother's bloodstream, which occurs through the placenta found in the mother's uterus.

α: 141 AA

γ: 146 AA (39 different AA in comparison to β chain)

Fetal vs. Adult hemoglobin



E-learning

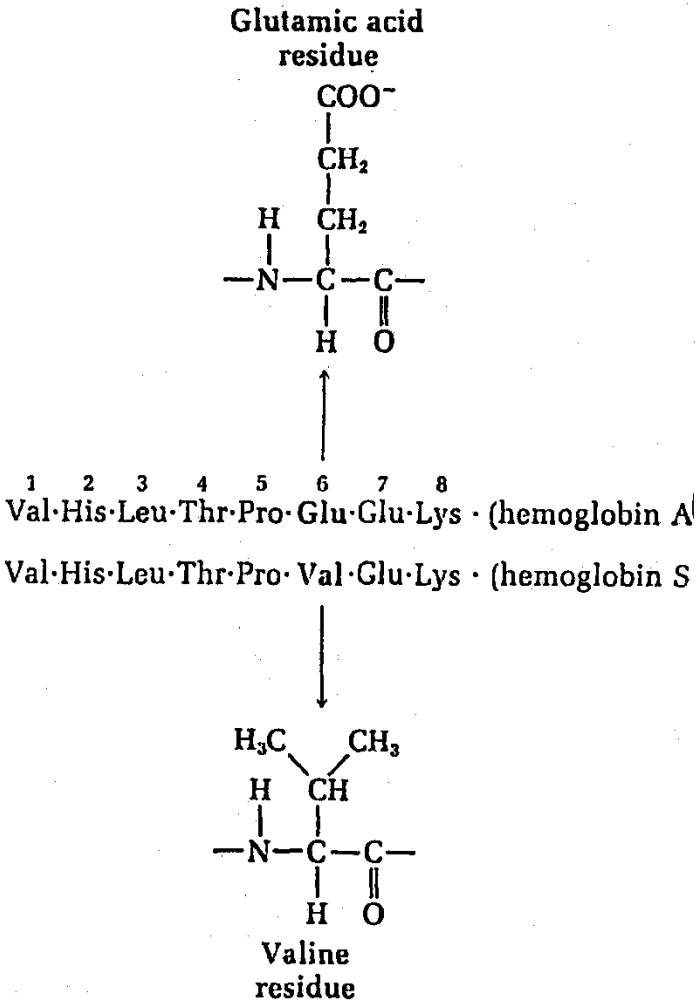
https://www.youtube.com/watch?v=Qv-KExGKAYw

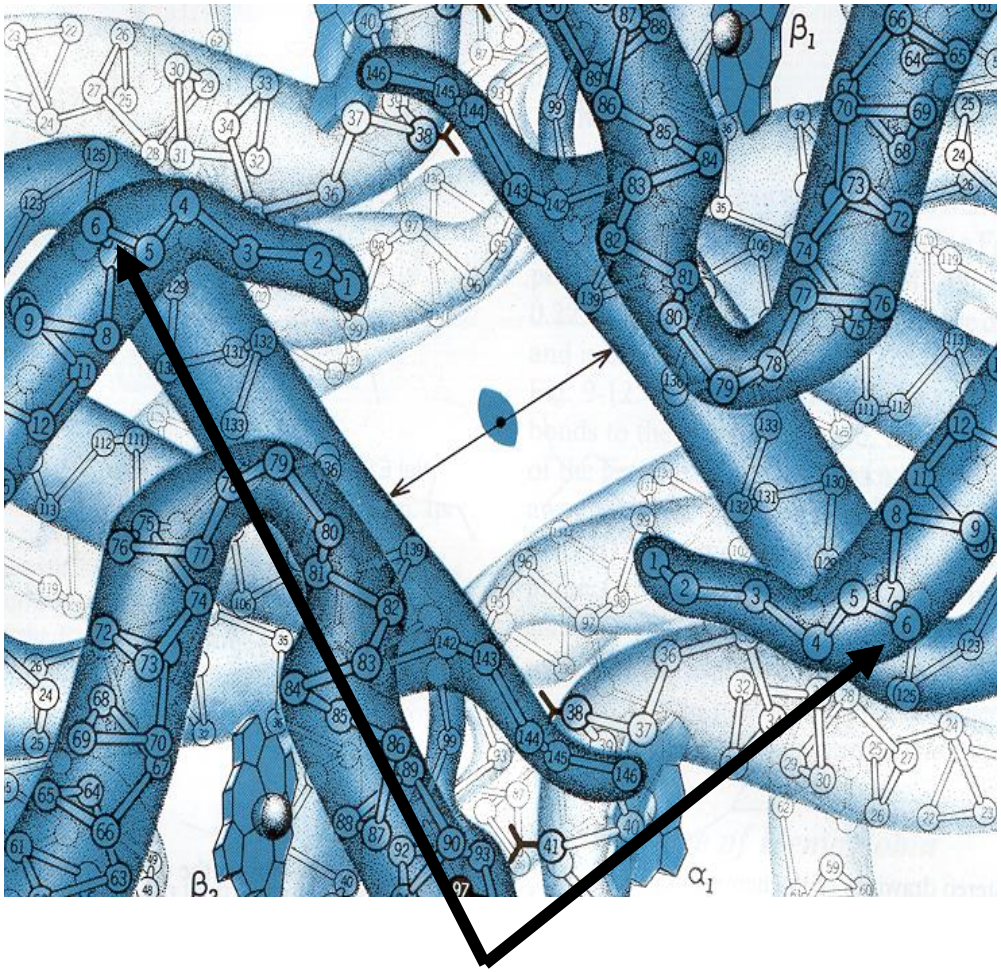
https://www.youtube.com/watch?v=b2hKDxX-KjE

Sickle Cell Anemia

- The most common form of sickle cell anemia is caused by a single amino acid substitution of **GLUTAMATE to VALINE at position 6 on the β-subunit of hemoglobin.**
- This defect is an autosomal (non-sex chromosome) recessive inherited disease, meaning both parents must be heterozygous carriers to produce a homozygous child.
- Homozygous patients die during childhood if left untreated. With a median life expectancy of 42–47 years, people with sickle cell anemia face many challenges, including severe pain episodes, stroke, and organ damage (*e.g.* brain and cardiac)
- Even heterozygous carriers can experience sickle cell symptoms after vigorous exercise or unpressurized travel at high altitudes.

Glu to Val Mutation in HbS

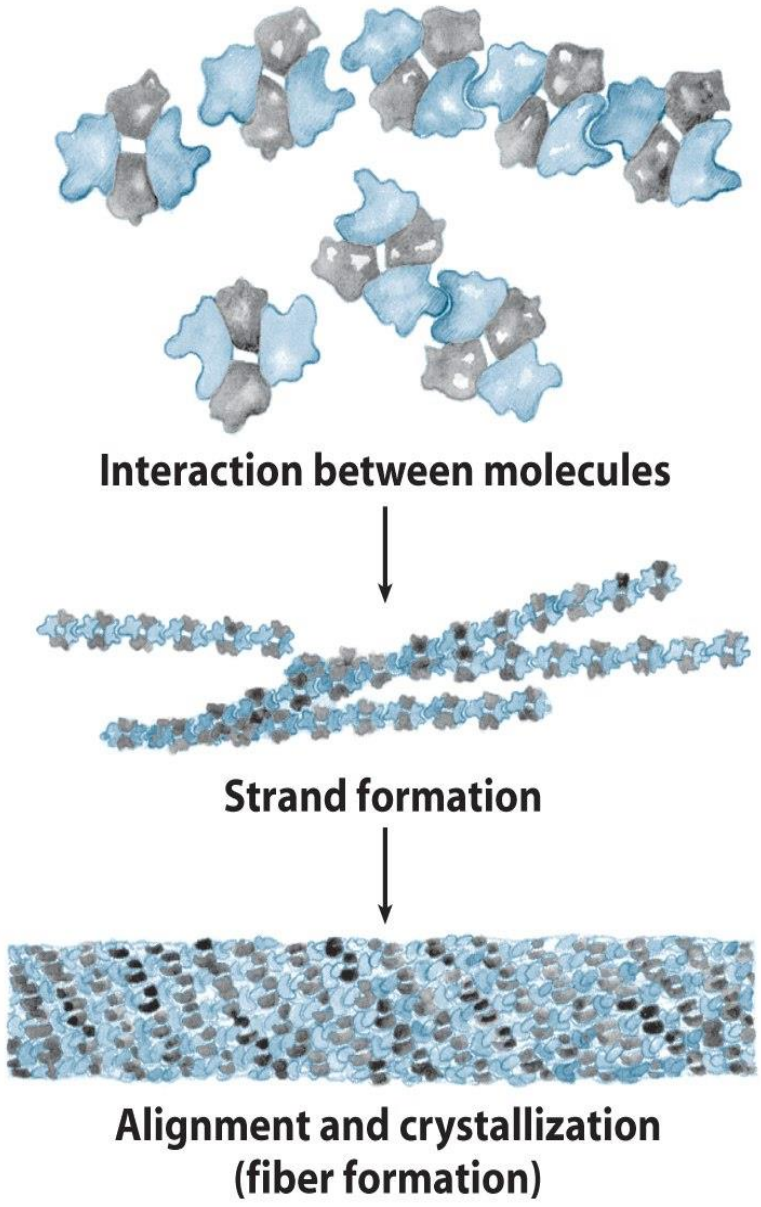




Position 6 - β-subunits

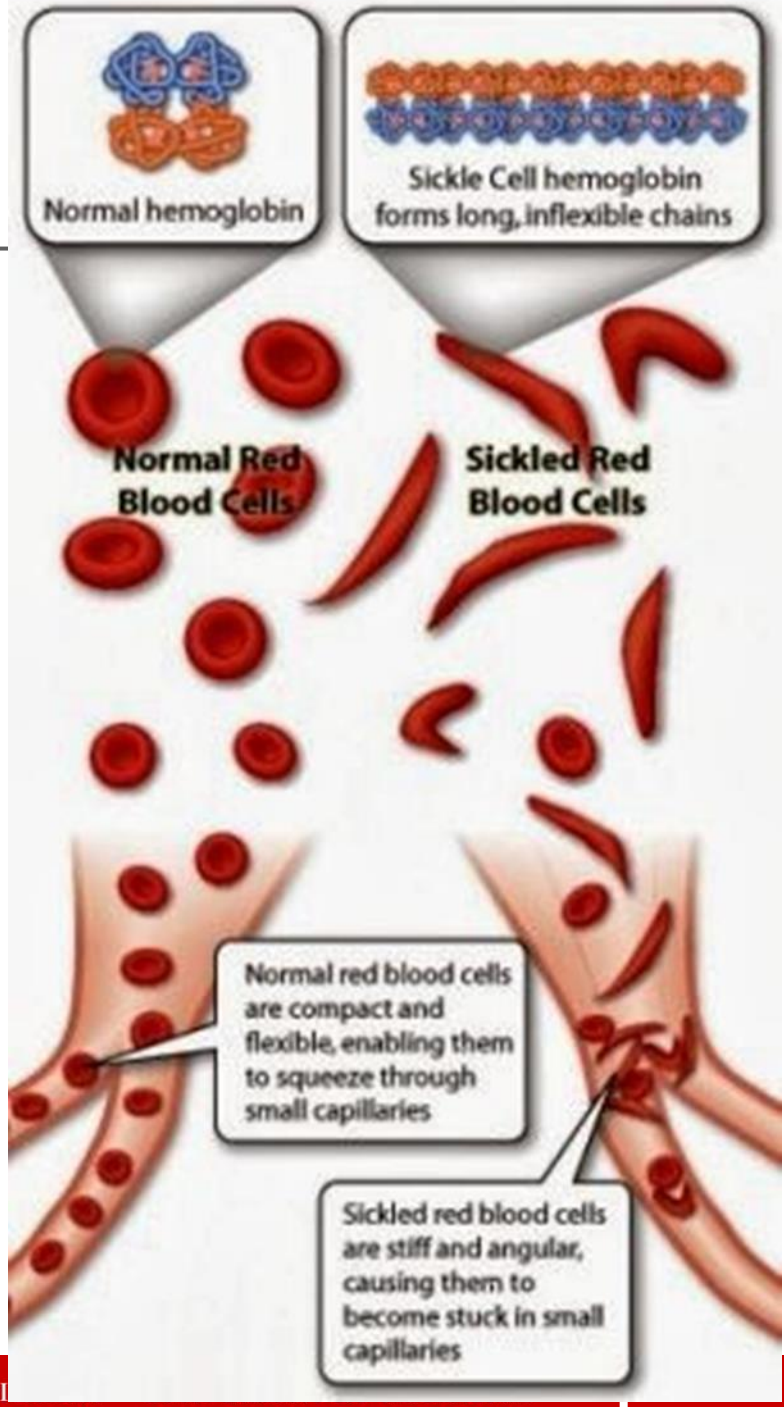
Sickle-cell hemoglobin (HbS) derives from an amino acid substitution

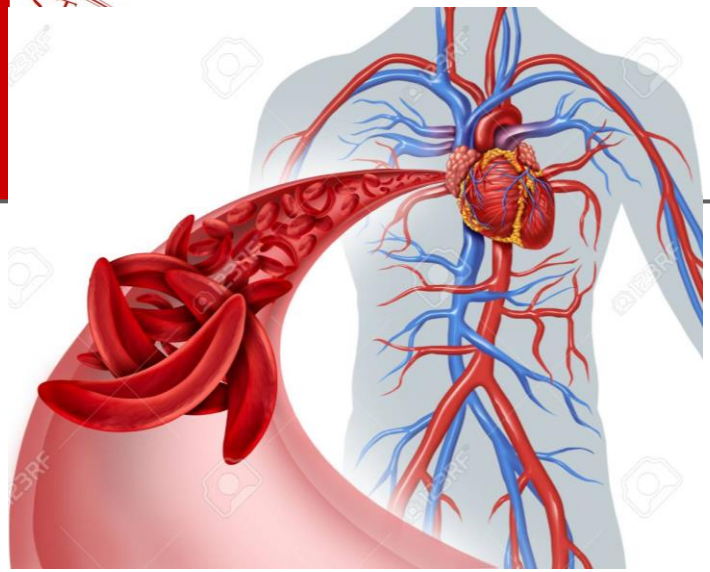
- Glu^6 to Val^6 in one β chains (heterozygous) or two β chains (homozygous).
- **The R group of Val has no electric charge, whereas Glu has a negative charge at pH 7.4.**
- **HbS has fewer negative charges than HbA. It creates a "sticky" hydrophobic contact point on the outer surface, causes DEOXY HbS to associate abnormally with each other, forming the long, fibrous aggregates that precipitate in the red blood cells.**
- This process deform the red blood cells to a "sickle" form which accumulate in the capillaries (peripheral pain and trombi)

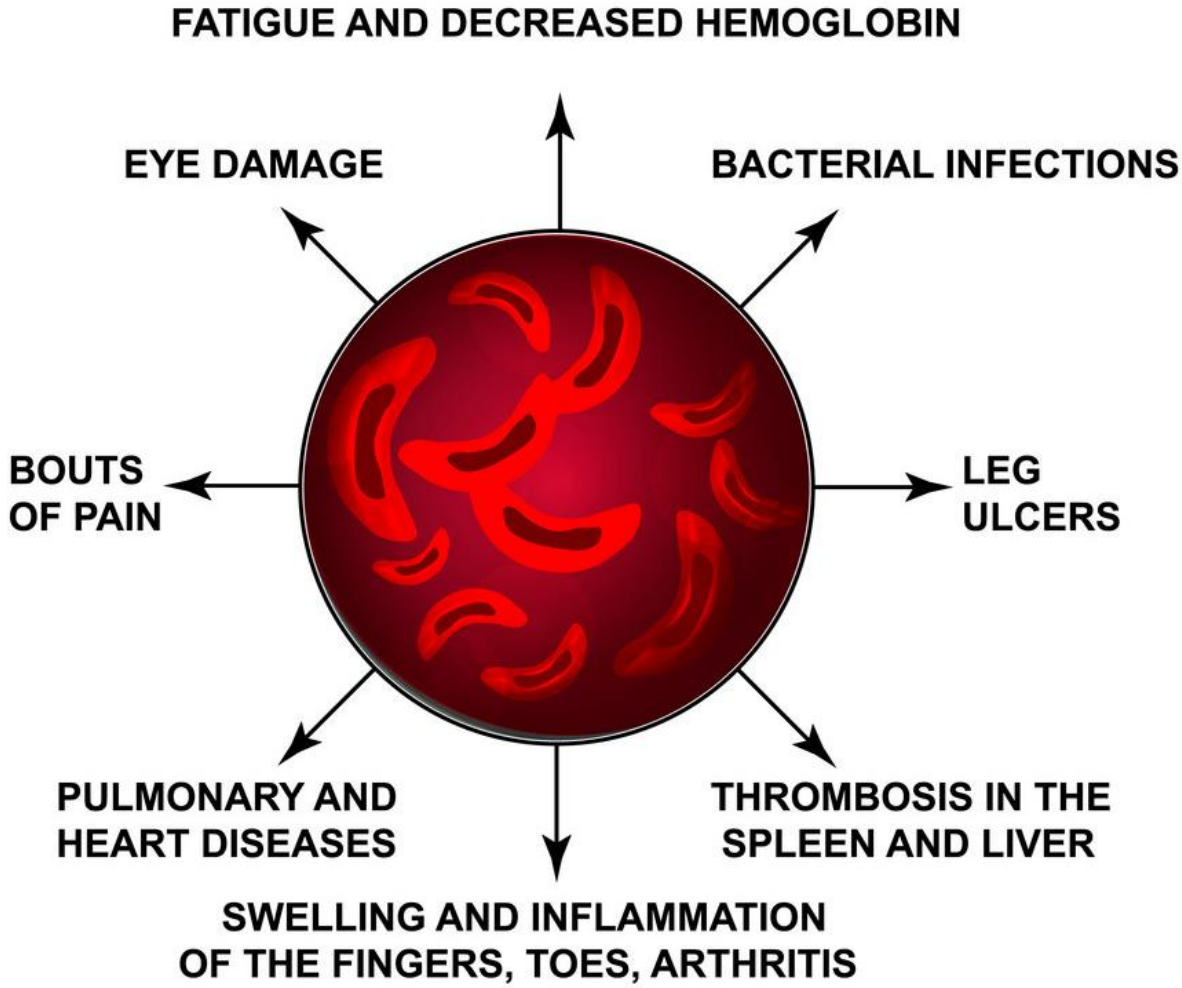


Sickle Cell Anemia

- The erythrocytes of these individuals are fewer and also abnormal. Normal hemoglobin (HbA) remains soluble on deoxygenation.
- When hemoglobin from sickle cells (HbS) is deoxygenated, it becomes insoluble and forms polymers that aggregate into tubular fibers.
- The insoluble fibers of deoxygenated HbS cause the deformed, sickle shape of the erythrocytes, and the proportion of sickled cells increases greatly as blood is deoxygenated.






FATIGUE AND DECREASED HEMOGLOBIN
EYE DAMAGE
BACTERIAL INFECTIONS
BOUTS OF PAIN
LEG ULCERS
PULMONARY AND HEART DISEASES
THROMBOSIS IN THE SPLEEN AND LIVER
SWELLING AND INFLAMMATION OF THE FINGERS, TOES, ARTHRITIS

SICKLE CELL ANEMIA TREATMENT

- **Hydroxyurea:** Increases fetal hemoglobin levels and reduces the frequency of pain crises and the need for blood transfusions.
- **Voxelotor:** Improves red blood cell flexibility and reduces anemia.
- **Crizanlizumab:** Reduces pain crises by preventing blood cell clumping.
- **Painkillers** like acetaminophen, ibuprofen, or opioids may be used for managing sickle cell crises.
- **Antibiotics:** Prevent infections, especially in children (e.g., daily penicillin until age 5).

- **blood transfusions: Reduce complications such as strokes and severe anemia.**

- **bone marrow transplant**: it's limited to patients with a suitable donor and carries significant risks. For some children and teenagers, a stem cell transplant might cure the disease.


Kahoot!