Oxygen Transporters

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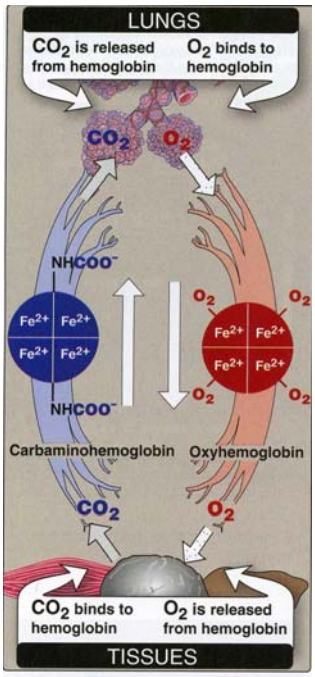
General Outline:
1) The heme prosthetic group.
2) Myoglobin versus hemoglobin.
3) Cooperativity enhances oxygen delivery in hemoglobin.
4) Allosteric effectors (2,3-BPG, Bohr effect).
5) CO_2 transport and carbonic anhydrase.
6) Hemoglobin disorders.

Reading assignment:
Meisenberg and Simmons, 3rd edition, Chapter 3



Oxygen Transporters

Daily need of oxygen: 500 g.

At an oxygen partial pressure of 90 torr only 4.1 mg of oxygen can dissolve in 1 l of blood plasma.

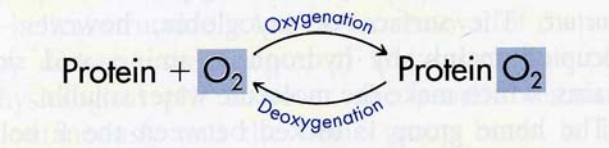
In the presence of hemoglobin (150 g/l of blood) 70-fold more oxygen (280 mg) can be dissolved and transported to satisfy the demand.

Hemoglobin:

In red blood cells carries oxygen from the lungs to pulmonary tissues.

Myoglobin:

Stores oxygen for strenuous exercise.



Oxygenation is reversible:
oxygen is bound when plentiful and released when scarce.

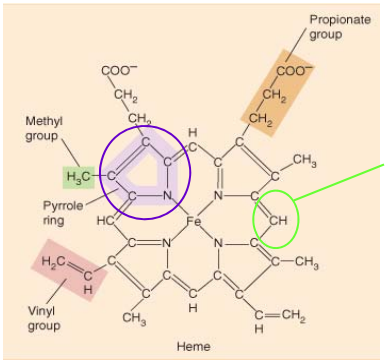
Are Myoglobin and Hemoglobin enzymes?

Enzymes:

- are highly selective catalysts, usually specific for one or a few substrates.
- bring substrates together in optimal orientation in the active site.
- catalyze reactions by stabilizing the transition state,
  but do not change the thermodynamics of chemical reactions.

Hemeproteins are specialized proteins that contain **heme** as a tightly bound prosthetic group;

the heme group is the oxygen binding site.



4x pyrrole rings

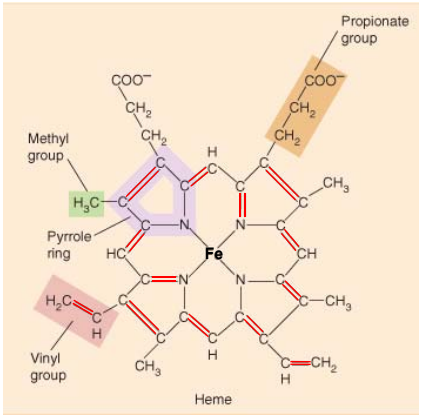
held together by methine bridges

decorated with 2 propionate (top),

4 methyl, and 2 vinyl groups.

Heme consists of porphyrin chelating a ferrous iron (Fe^{2+}) in the center.
Prosthetic group: tightly bound, nonpolypeptide “helper” group.

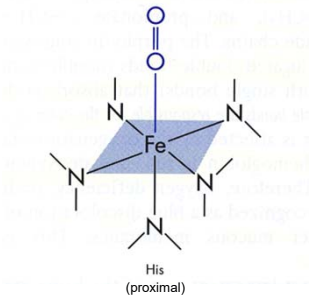
System of conjugated double bonds is responsible for the color of blood.

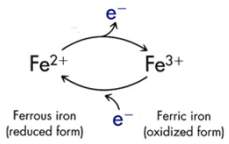


Fe^{2+} is bonded to N
in the center of the plane

Oxygenated hemoglobin is red,
Deoxygenated hemoglobin is blue.
Hypoxia (oxygen deficiency) can be recognized by "blue" lips or cyanosis.

Iron is central to oxygen binding

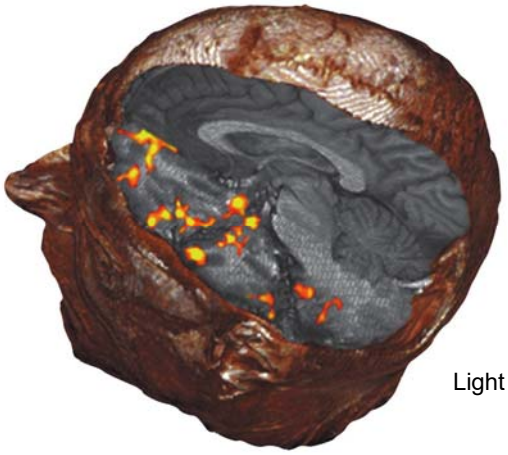




Fe^{2+} in heme is oxygenated
not oxidized.

Fe^{2+} is held in place (coordinated) by the N of the 4 pyrrole rings,
and by the proximal His (F8).
Oxygen (O_2) can bind to the 6th coordinate.

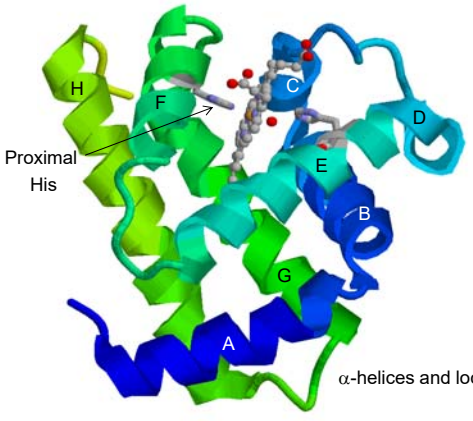
Functional magnetic resonance imaging (MRI) of the brain



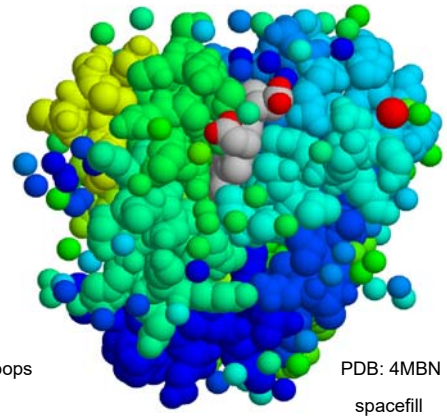
Light spots: regions activated by odorants

Detects oxygenated form of hemoglobin and thus more “active” regions of the brain

Three dimensional structure of myoglobin



α-helices and loops

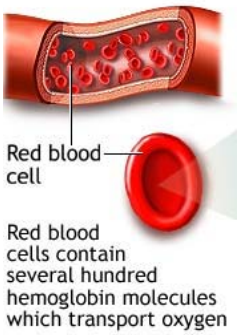


PDB: 4MBN

spacefill

Myoglobin contains 153 amino acids (aa) which form 8 α-helices (A-H). The heme group is tucked between helix E and F. The proximal His is residue #8 in helix F (F8).
75% of the structure is α-helical. Hydrophobic interactions of non-polar side chains are the major stabilizing forces; hydrophilic aa are on the surface and make it a water soluble molecule.

Hemoglobin



Hemoglobin is enclosed in red blood cells (erythrocytes).

Erythrocytes are released from bone marrow and circulate in the blood for ~ 120 days.

They have no nucleus,
no mitochondria,
are specialized to O_2 transport.

They do not consume any of the oxygen transported.

Erythrocytes are “bags” filled with hemoglobin dissolved in the cytoplasm.

Characteristics of red blood cells

Diameter of RBCs	7.3 μm
Life span of RBCs	120 days
Number of RBCs	4.2–5.4 million/mm³ (female)
	4.6–6.2 million/mm³ (male)
Intracorpuscular hemoglobin concentration	33%
Hematocrit*	38%–46% (female)
	42%–53% (male)
Hemoglobin in whole blood	12%–15% (female)
	14%–17% (male)

diagnostically important

* Hematocrit: percentage of blood volume occupied by red blood cells.

Patients with abnormally low hemoglobin concentrations are anemic.

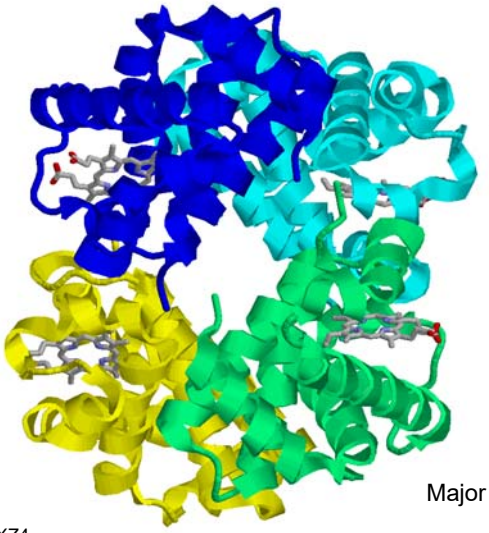
Hemoglobin is a tetramer of two α and two "non-α" chains

Human hemoglobins:

Type	Subunit structure	Importance
Major adult (HbA)	$\alpha_2\beta_2$	97% of adult hemoglobin
Minor adult (HbA_2)	$\alpha_2\delta_2$	2%-3% of adult hemoglobin
Fetal (HbF)	$\alpha_2\gamma_2$	Major hemoglobin in second and third trimesters of pregnancy

The different subunits differ in their amino acid composition but are similar in structure, and similar to myoglobin.

Three dimensional structure of the hemoglobin tetramer



Alpha 1, **Beta 1**

$\alpha_2\beta_2$

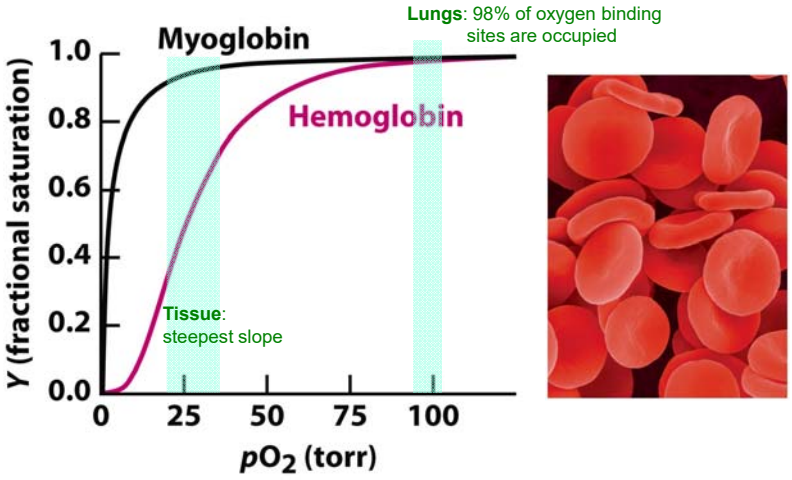
Beta 2, **Alpha 2**

Major human adult HbA

PDB: 1XZ4

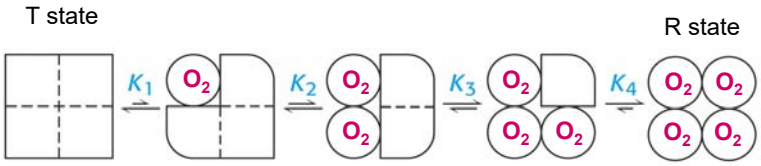
http://www.umass.edu/molvis/tutorials/hemoglobin/index.htm

Hemoglobin:
Cooperativity enhances oxygen delivery



Marked sigmoidal = steep slope indicative of cooperativity

Quaternary structure and
allosteric regulation of hemoglobin



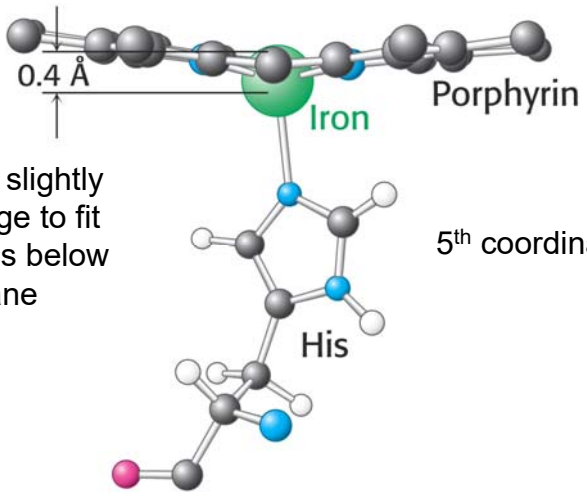
T conformation
(tense)
in absence of O_2

R conformation
(relaxed)
fully oxygenated form
R binds O_2 ~300-times more tightly than T

<u>Transition from T to R:</u>
Binding of O_2 to the first heme increases the affinity of the neighboring heme, which increases the affinity of the next neighbor, etc.

This is called: Positive cooperativity.

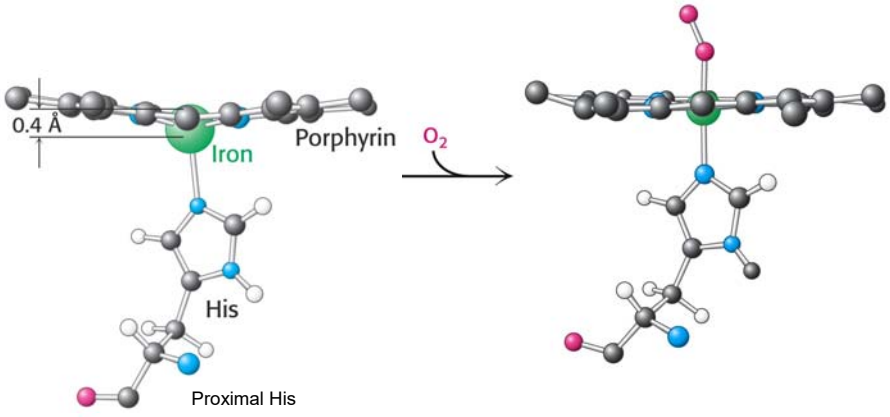
Deoxyhemoglobin

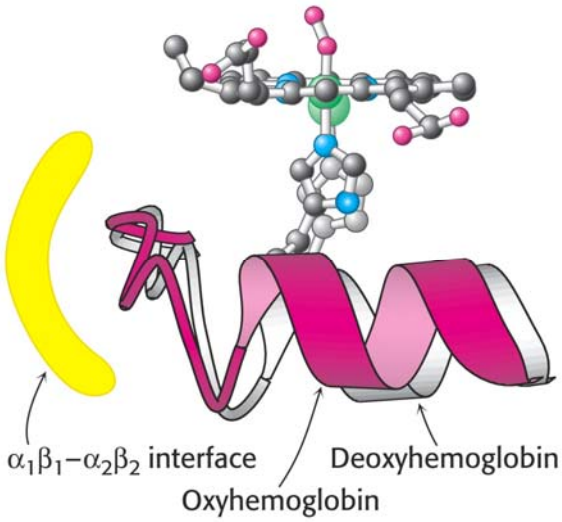


Fe^{2+} is slightly too large to fit and lies below the plane

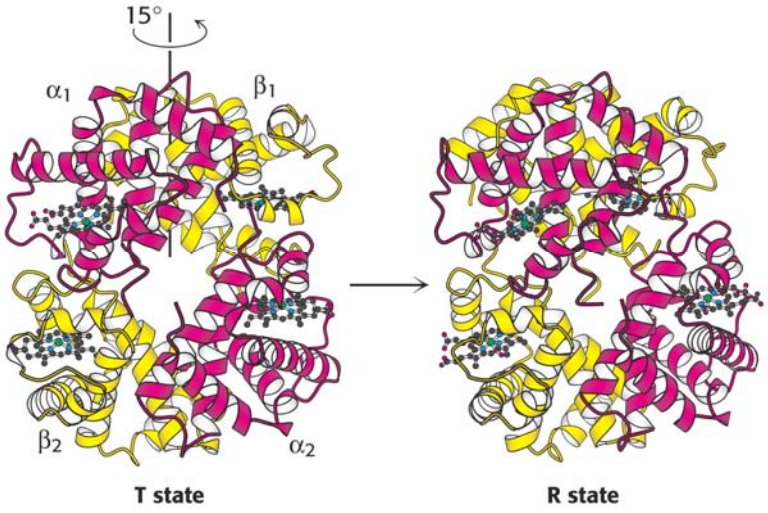
5^{th} coordination

Oxygen (O_2) binds to 6^{th} coordination, "pulls" electron density away from Fe^{2+}, and iron can now fit into the tetrapyrrole ring



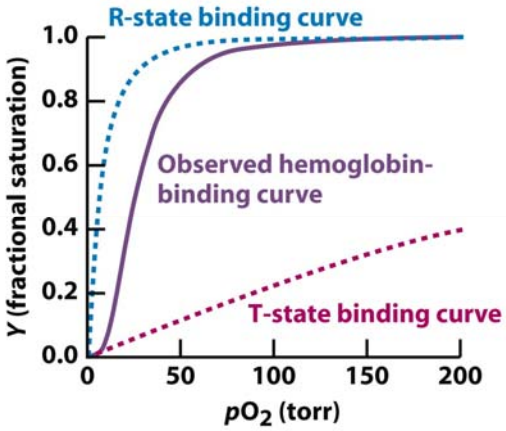


O_2 binding pulls the Fe^{2+} into the plane and moves the His up, His is part of an α–helix (F-helix) which moves with it. The COOH terminus of this α-helix lies in the interface between the two dimers leading to quaternary rearrangements.

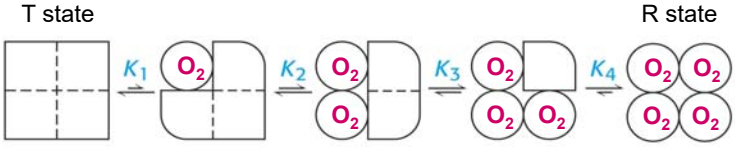


Transition from T to R state; one pair of $\alpha\beta$ subunits rotates by 15°

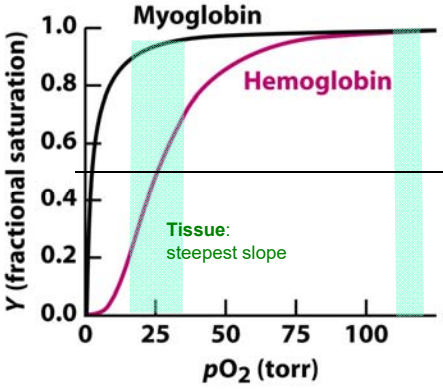
Cooperativity enhances oxygen delivery



Sigmoidal (greek: S-shaped) curve is best described as transition from the T-state to R-state curve.



Oxygen binding curves



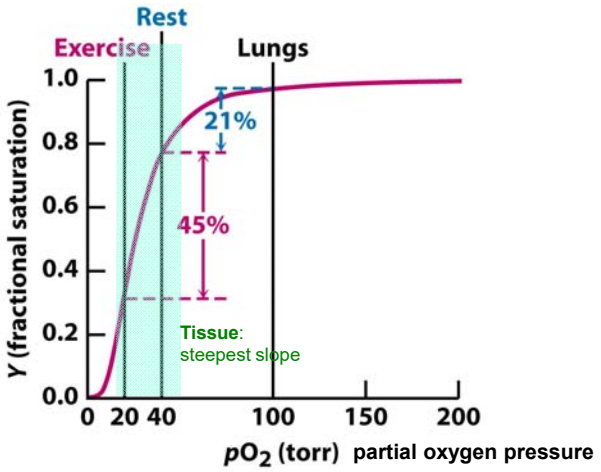
Lungs: 98% of oxygen binding sites are occupied

Tissue: steepest slope

P_{50}: oxygen partial pressure at which half of the heme groups are oxygenated.

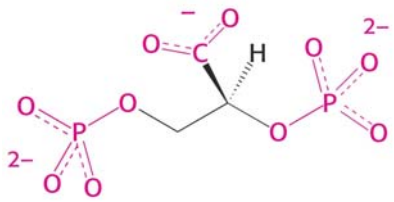
- Myoglobin binds O_2 much tighter (p50 ~ 1 torr) than hemoglobin. Shape of the curve is typical for a simple equilibrium reaction (Michaelis-Menten kinetics).
- In contrast, hemoglobin binds and releases O_2 with positive cooperativity. This allows for more efficient release of O_2 into tissue, and facilitates transfer of O_2 from hemoglobin to myoglobin (p50 ~ 26 torr).

Cooperativity enhances oxygen delivery

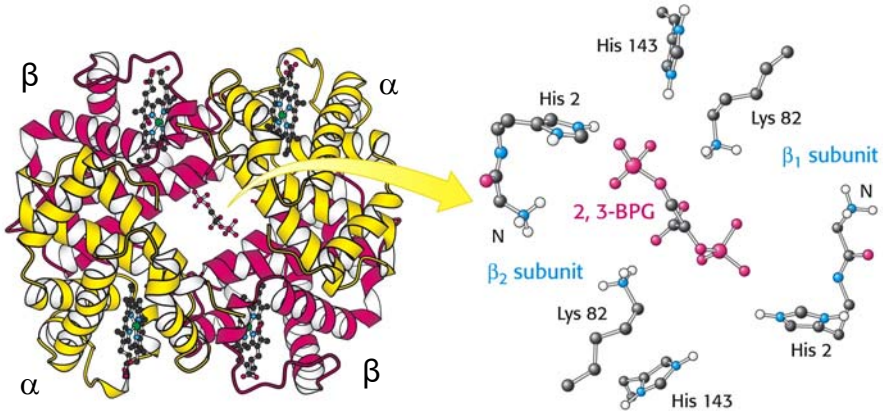


The oxygen binding/dissociation curve is steepest at the oxygen concentrations that occur in the tissue.
This permits oxygen delivery to respond to small changes in pO_2.

How is O_2 released in the blood?



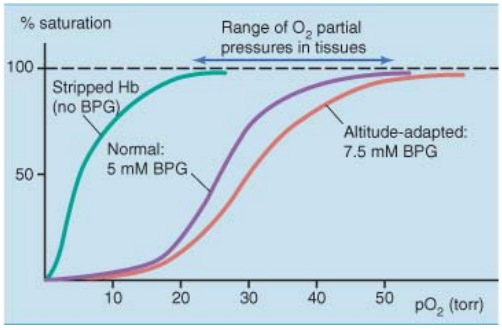
- Concentration of BPG in red blood cells is 5 mM.
- Is an important regulator
- Is a negative allosteric effector.
- Allosteric: binds to a site different than O_2, and acts through conformational changes.
- 2,3-BPG dramatically decreases affinity of hemoglobin for O_2



2,3-BPG binds to the center cavity of deoxyhemoglobin only and shifts the equilibrium to the T state effectively reducing the O_2 affinity.

Three positively charged residues in each β-chain are involved in binding.

Effects of 2,3-BPG on the oxygen binding affinity of hemoglobin

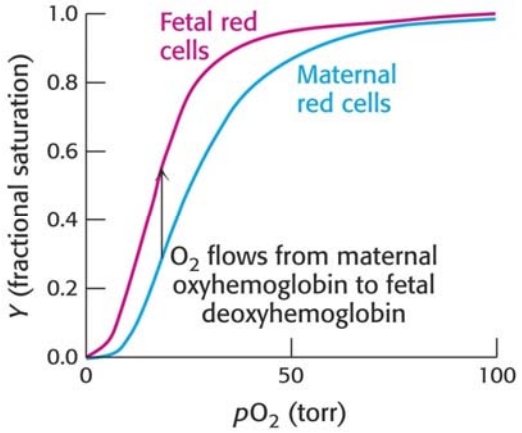


BPG enhances unloading of oxygen in the tissues whose oxygen partial pressure are in the steep part of the oxygen binding curve.

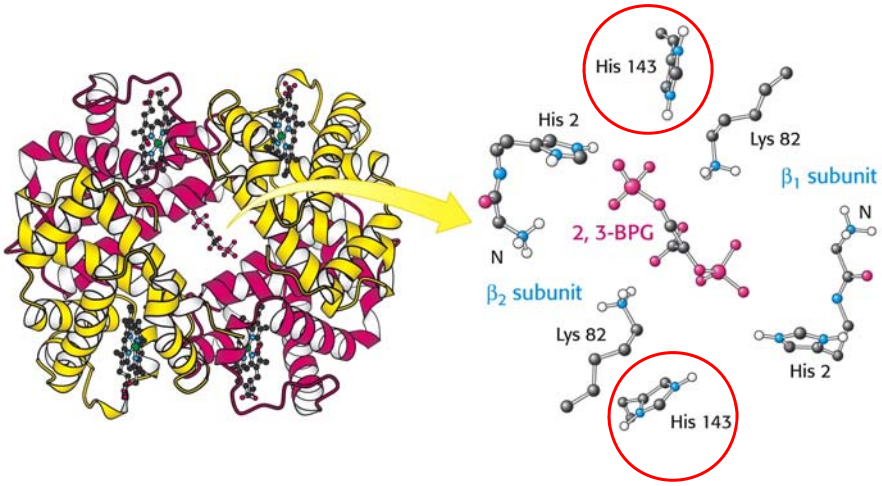
Fetal hemoglobin binds BPG less tightly, thus facilitating transfer of O_2 from the maternal to the fetal blood.

His143 in β-chains is Ser in fetal γ-chains, this removes 2 charges from the BPG binding site.

Oxygen affinity of fetal red blood cells



Fetal hemoglobin has 2 α and 2 γ chains
γ has much lower affinity for 2,3-BPG



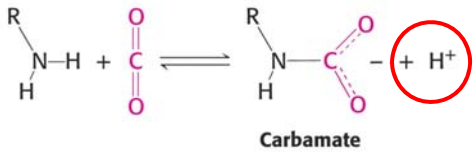
In fetal hemoglobin His143 (on γ-hemoglobin) is substituted by a Ser
reducing the affinity for 2,3-BPG

Other negative allosteric effectors

Bohr effect:

Protons (low pH) lower the O_2 binding affinity of hemoglobin and favor release of O_2. Protons are involved in intersubunit salt bonds in the T form.

Carbon dioxide (CO_2), the product of oxidative metabolism, also decreases O_2 affinity of hemoglobin. It binds to N-terminal groups of the α and β chains resulting in carbamino-hemoglobin:

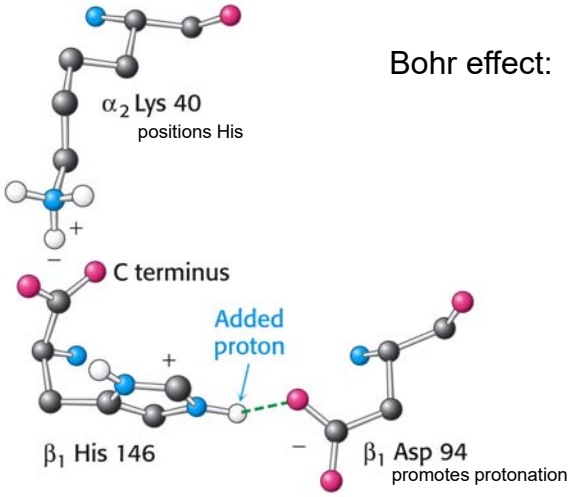


Carbamate

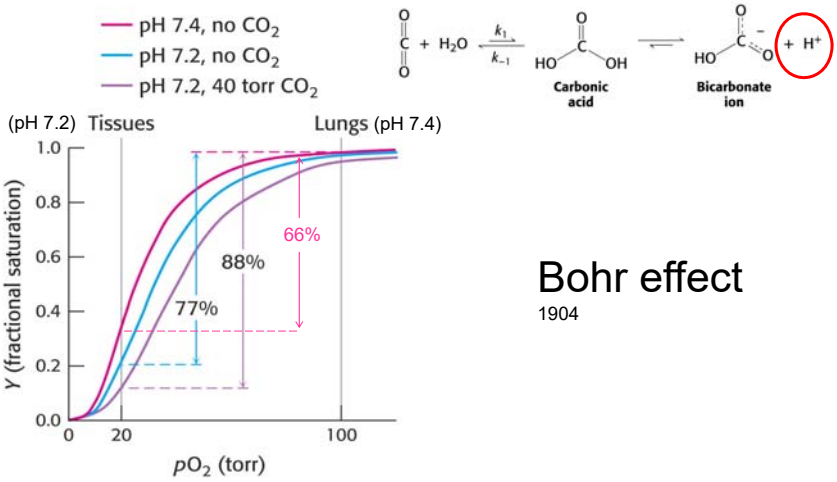
pH and CO_2 ensure that oxygen is released preferentially in actively metabolizing tissue where it is most needed.

Note: only 14% of CO_2 is transported by hemoglobin
CO_2 is also transported in the blood as bicarbonate (HCO_3^-)
hydrated by carbonic anhydrase

Bohr effect:



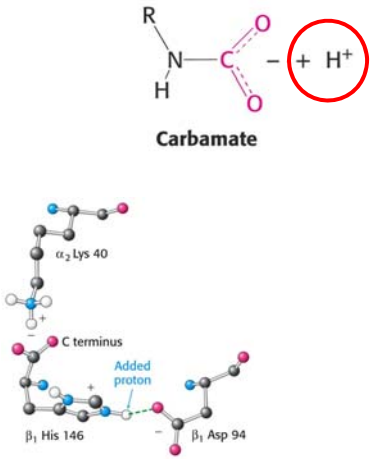
His146: pK_a ~ 7.0
At low pH salt bridge stabilizes quaternary structure of deoxyhemoglobin

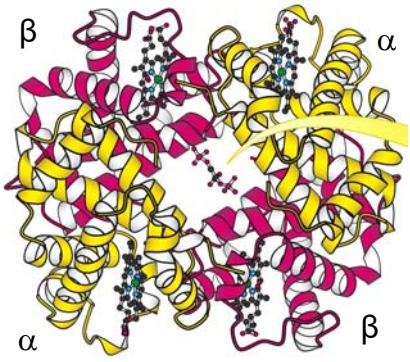


Bohr effect

1904

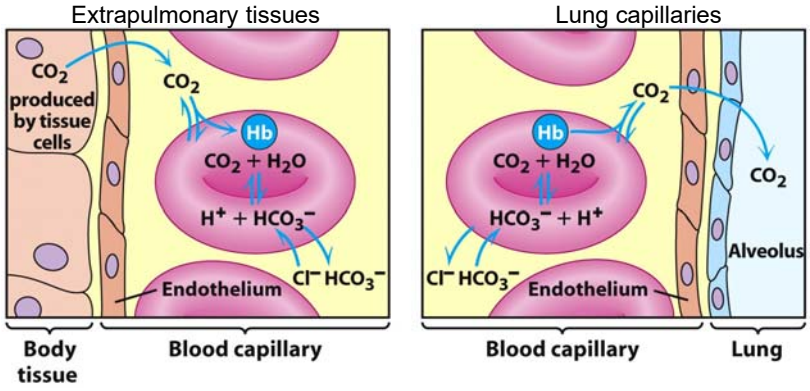
Heterotropic effectors:
Hydrogen ions and CO_2 promote release of oxygen (negative allosteric effectors).





Bohr effect: lower pH and high CO_2 concentrations increase subunit interactions and shift the enzyme to the T-state, which in turn facilitates 2,3-BPG binding to the center cavity and further shifts the equilibrium to the T state effectively reducing the O_2 affinity.

Carbon dioxide (CO_2) transport

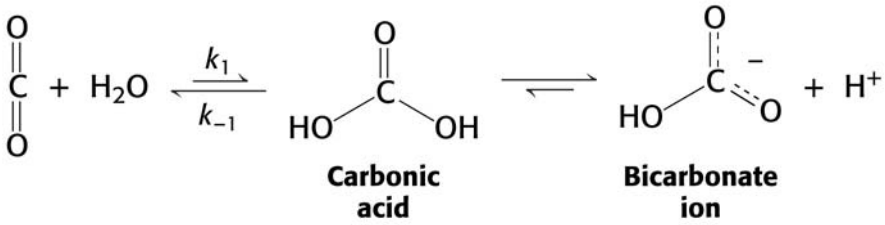


CO_2 is hydrated to carbonic acid by carbonic anhydrase, leaves the cell in exchange for chloride ion, and is transported back to the lungs dissolved in plasma.

In the lungs CO2 is released by the reverse process.

~80% of CO_2 is transported as inorganic bicarbonate, other as carbamino-hemoglobin.

Carbonic anhydrase: making a fast reaction faster



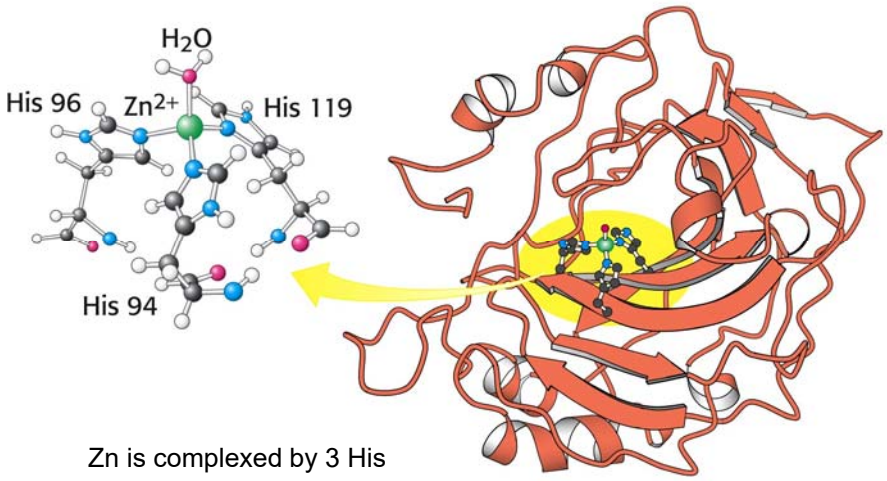
Non-enzymatic rate: 0.15 s^{-1} (~1 every 7 seconds)

k_1 = 0.15 s^{-1}
k_{-1} = 50 s^{-1}

$$K_{equ.} = \frac{k_1}{k_{-1}} = \frac{[H_2CO_3]}{[CO_2]} = \frac{1}{340}$$

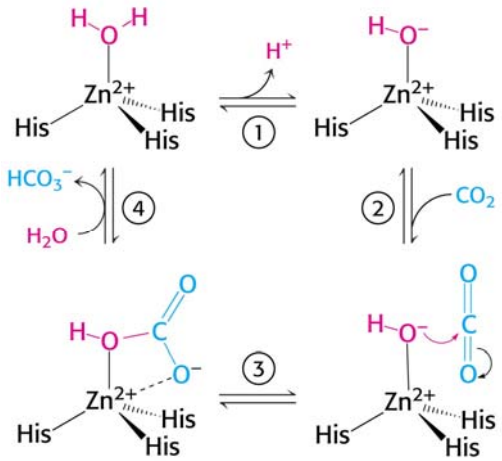
Turnover number (k_{cat}) of Carbonic anhydrase: 600,000 s^{-1}
Rate enhancement with Carbonic anhydrase: ~4,000,000
K_M: 8 mM
K_{cat}/K_M: 7.5 x 10^7 $s^{-1}M^{-1}$
Close to kinetic perfection!

Carbonic anhydrase and its zinc site



Zn is complexed by 3 His

Mechanism of carbonic anhydrase

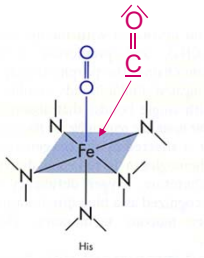


Binding of water to Zn reduces the pK_a of water from 15.7 to 7

Carbon monoxide (CO)

CO: byproduct of incomplete combustion,
is present in gas heaters and furnaces, automobile exhaust, burning buildings and other materials, cigarette smoke, etc.

CO is a competitive antagonist: competes with oxygen for binding to the heme group



-binds to Fe^{2+} in hemoglobin just like O_2 but with 200 fold higher affinity

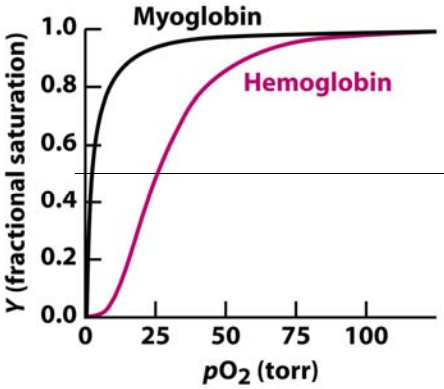
-Throbbing, headache, confusion, fainting occur when 30-50% of the hemes are occupied by CO, 80% is fatal.

-binding is very slowly reversible

-poisoning can be treated with hyperbaric oxygen, O_2 will slowly replace CO

CO-hemoglobin has a cherry-red color (not cyanosed).

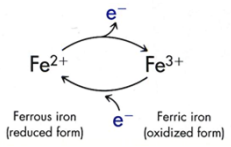
The binding of carbon monoxide at one of the four sites increases the oxygen affinity of the remaining three sites, which causes the hemoglobin molecule to retain oxygen that would otherwise be delivered to the tissue. This situation is described as carbon monoxide shifting the oxygen dissociation curve to the left. Because of the increased affinity between hemoglobin and oxygen during carbon monoxide poisoning, the blood oxygen content is increased. But because all the oxygen stays in the hemoglobin, none is delivered to the tissues. This causes hypoxic tissue injury. Hemoglobin acquires a bright red color when converted into carboxyhemoglobin, so poisoned patients have been described as looking pink-cheeked and healthy. However, this cherry-red appearance is rarely seen in living patients so is not considered a reliable diagnostic sign.



Lungs: 98% of oxygen binding sites are occupied

Draw an oxygen binding curve of hemoglobin with one CO molecule bound. How does the curve differ?

Methemoglobin contains the oxidized form of ferric iron (Fe^{3+}) which is useless as an oxygen transporter.



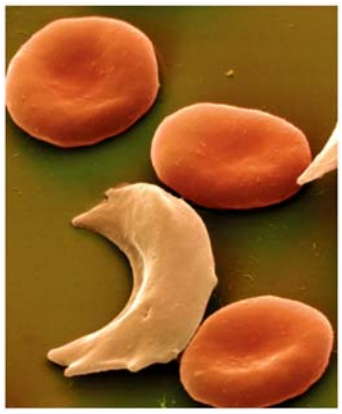
Natural protection:
-erythrocytes contain ascorbic acid and glutathion as reducing agents.
-binding of heme to the apo-protein creates a protective environment.
-methemoglobin reductase reduces methemoglobin back to normal hemoglobin.

Exposure to strong oxidants that overwhelm the reductase system cause methemoglobin formation. These include nitrates (typically from fertilizer-contaminated water, wells), chemicals like aniline dyes, aromatic nitro compounds (naphthalene, in mothballs), local anesthetics (lidocaine), antimalarials (chloroquine), and sulfonamides. Blood is dark and "chocolate-colored", and does not turn red on exposure to O_2.

Methemoglobinemia is treated with methylene blue which reduces ferric iron back to ferrous iron.

Hemoglobin disorders

Mutations in the α or β chains:



Sickle hemoglobin:
mutation in β-chain (Glu6Val)

Thalassemias:
Hemoglobin chain imbalance

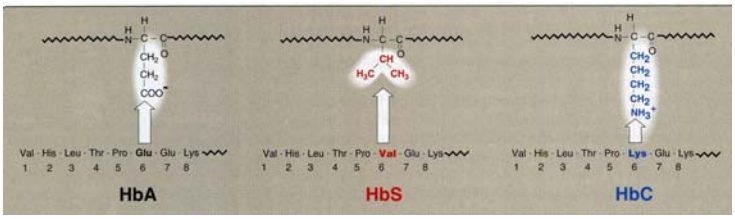
Abnormal Hemoglobin Isotypes

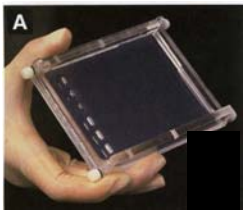
	Disease	Genotype	Hemoglobin Expressed
α-Thalassemia	Hydrops fetalis	All four α genes deleted.	HbH (δ_4) and Hb Barts (γ_4). Death in utero.
	HbH disease	Three α genes deleted.	HbH and Hb Barts, some HbA_2. Death by age 8.
	Thalassemia trait	Two α genes deleted.	HbA_2 and Hb Barts early in life. Mild phenotype.
	Carrier	One α gene deleted.	Normal HbA_1 content. Silent phenotype.
β-Thalassemia	Thalassemia major	Both β genes affected by a severe mutation (so that no or little β produced).	HbF and HbA_2 are the main isotypes available. HbA_1 reduced or absent.
	Thalassemia intermedia	Both β genes affected by a mild mutation.	As in thalassemia major, but more HbA_1.
	Thalassemia trait	Only one β gene affected by a mutation (mild or severe).	Normal HbA_1, but increased HbA_2.
Sickle cell anemia		Both β genes have a mutation at position 6 (glutamate → valine).	HbSS present, no HbA_1. HbF increased.
Sickle cell anemia carrier		One β gene with the above mutation.	HBSS present along with HbA_1.
Diabetes		Normal hemoglobin genes.	Normal hemoglobin pattern in addition to HbA_{1c}, a glycated form of HbA_1.

HbH (δ_4) Hb Barts (γ_4) are embryonic forms of hemoglobin

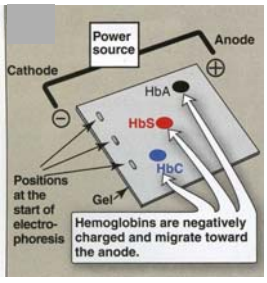
Taken from First Aid, Basic Sciences

Amino acid substitutions in HbS and HbC

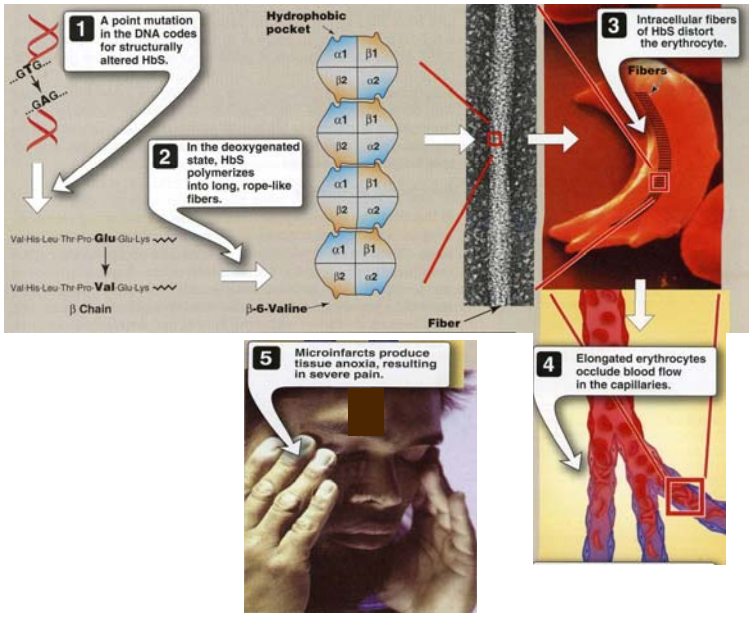




Photograph of a gel
prior to electrophoresis



Molecular and cellular events leading to sickle cell crisis

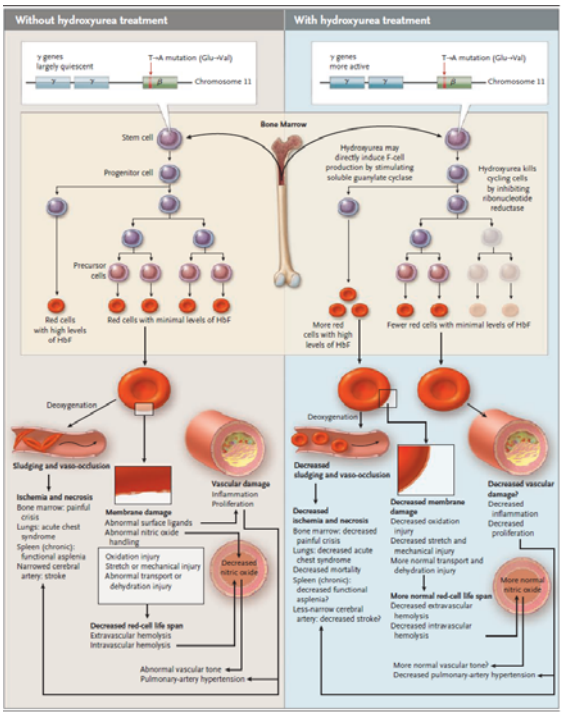


-An 18-year-old woman with sickle cell anemia presents with recurrent painful crises and episodes of the acute chest syndrome. She was hospitalized three times in the past year. A hematologist recommends that treatment with hydroxyurea be started.

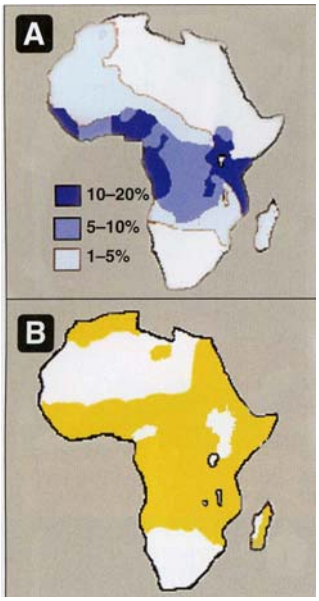
-Hydroxyurea causes a shift toward the production of red cells containing fetal hemoglobin.

-Hemoglobin F (HbF), or fetal hemoglobin, is a tetramer composed of $\alpha 2\gamma 2$ globin chains. γ-Globin chains prevent the formation of sickle linear aggregates because they do not have the valine substitution that attaches to the sticky spot on adjacent tetramers.

Hydroxyurea usage led to a small, statistically significant reduction in daily pain, analgesic use, and utilization in adults in MSH, corroborating previously shown larger reductions in crises and mortality. The degree of daily symptomatic reduction was related to the size of the HbF treatment response, further confirming HbF response as a useful laboratory correlate. **Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia,** Pain Med. 2011 .



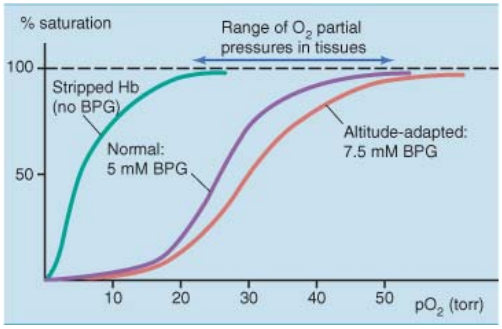
Taken from:
Platt, N Engl J Med. 2008



Distribution of sickle cell in Africa expressed as percentage of the population with disease

Distribution of malaria in Africa

-The malaria parasite has a complex life cycle and spends part of it in red blood cells. In a carrier, the presence of the malaria parasite causes the red blood cells with defective haemoglobin to rupture prematurely, making the plasmodium unable to reproduce. Further, the polymerization of Hb affects the ability of the parasite to digest Hb in the first place. Therefore, in areas where malaria is a problem, people's chances of survival actually increase if they carry sickle-cell trait (selection for the heterozygote).



Altitude adaptation and sickle cell treat?

2,3-BPG is already right-shifted in people with sickle cell trait, no more room for further adaptation