Structure and function of proteins

MYOGLOBIN, HEMOGLOBIN

(heme proteins)

Reversible binding of a Protein to a Ligand: Oxygen-Binding Proteins

- MYOGLOBIN, HEMOGLOBIN
- Oxygen- poorly soluble in aqueous, diffusion ineffective-few millimeters
- No AA bind O2
- Evolution- proteins –transport and store oxygen
- Hemoproteins (heme, reversible binding of oxygen)
- Heme- Fe

Myoglobin- single binding site for O2

- Transport O2 in muscle tissue
- Globins
- Protein ligand interaction can be described **quantitatively**

In general, the reversible binding of a protein (P) to a ligand (L) can be described by a simple **equilibrium expression:**

$$P + L \rightleftharpoons PL \qquad (5\text{–}1)$$

The reaction is characterized by an equilibrium constant, K_a, such that

$$K_a = \frac{[PL]}{[P][L]} \qquad (5\text{–}2)$$

The term K_a is an **association constant** (not to be confused with the K_a that denotes an acid dissociation constant; p. 63). The association constant provides a measure of the affinity of the ligand L for the protein. K_a has units of M^{-1}; a higher value of K_a corresponds to

It is more common (and intuitively simpler), however, to consider the **dissociation constant, K_d,** which is the reciprocal of K_a ($K_d = 1/K_a$) and is given in units of molar concentration (M). K_d is the equilibrium constant for the release of ligand. The relevant expressions change to

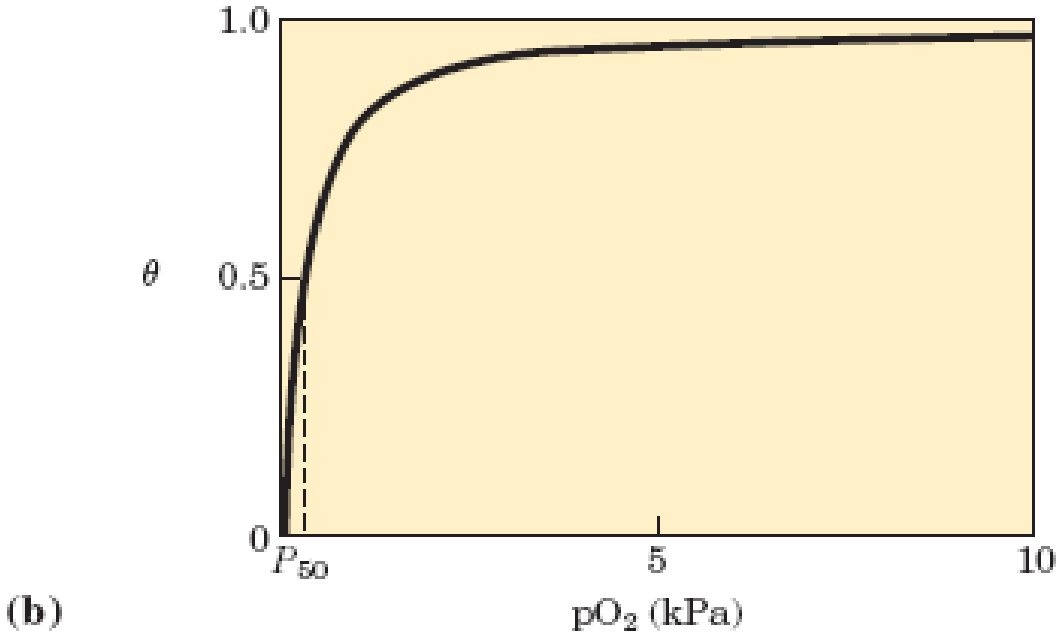
$$K_d = \frac{[P][L]}{[PL]} \qquad (5\text{–}6)$$

$$[PL] = \frac{[P][L]}{K_d} \qquad (5\text{–}7)$$

$$\theta = \frac{[L]}{[L] + K_d} \qquad (5\text{–}8)$$

When [L] is equal to K_d, half of the ligand-binding sites are occupied. As [L] falls below K_d, progressively less of the protein has ligand bound to it. In order for 90% of the available ligand-binding sites to be occupied, [L] must be nine times greater than K_d.

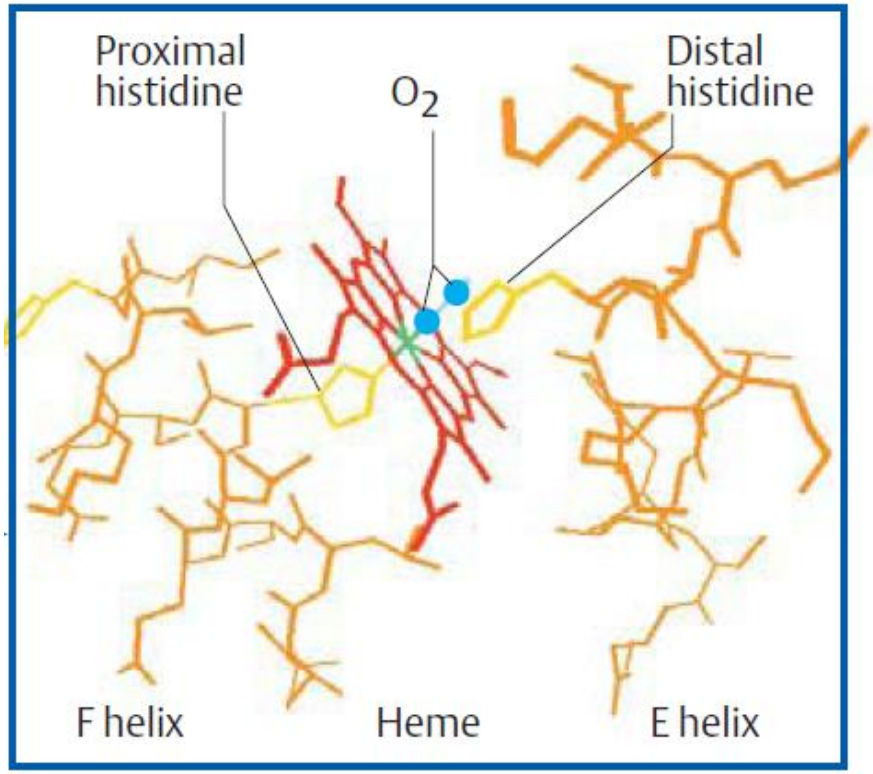
Saturation curve of myoglobin

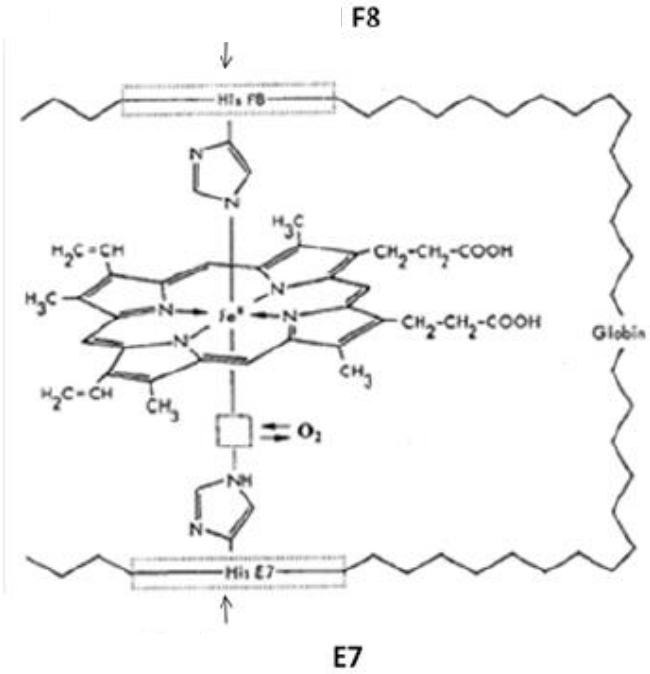


or K_d. The curve has a horizontal asymptote at $\theta = 1$ and a vertical asymptote (not shown) at $[L] = -1/K_a$. (**b**) A curve describing the binding of oxygen to myoglobin. The partial pressure of O_2 in the air above the solution is expressed in kilopascals (kPa). Oxygen binds tightly to myoglobin, with a P_{50} of only 0.26 kPa.

Coordination of Heme in Hb/Mb

- Coordination bond of Fe2+ - histidine **F8 proximal (F helix**
- Partially to **histidine E7 distal** (in E helix).





Myoglobin x hemoglobin

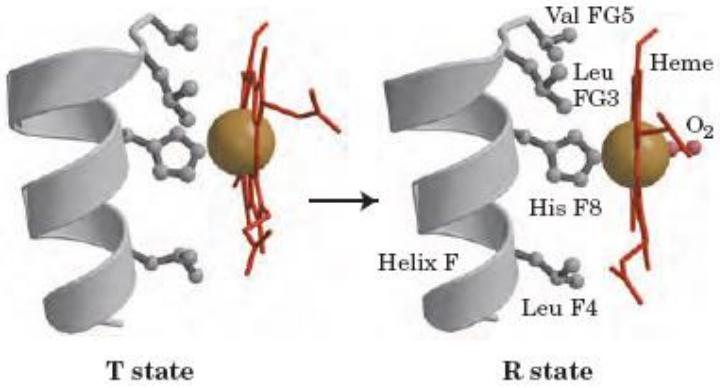
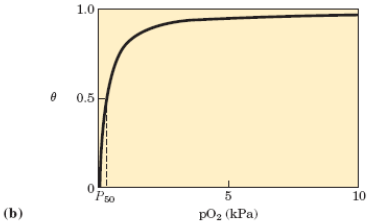


FIGURE 5–11 Changes in conformation near heme on O_2 binding to deoxyhemoglobin. (Derived from PDB ID 1HGA and 1BBB.) The shift in the position of the F helix when heme binds O_2 is thought to be one of the adjustments that triggers the T ⟶ R transition.

Myoglobin/Hemoglobin Comparison:

Myoglobin:
Only 1 heme and 1 polypeptide chain of 153 amino acids. MW = 17,800

Hemoglobin:
4 heme units and 4 polypeptide chains;
2 α chains with 141amino acids, and
2 β chains with 146 amino acids.
MW = 64,500



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Myoglobin – hyperbolic binding curve for oxygen

-oxygen-storage protein

-Hemoglobin- multiple subunits- O2 binding sites

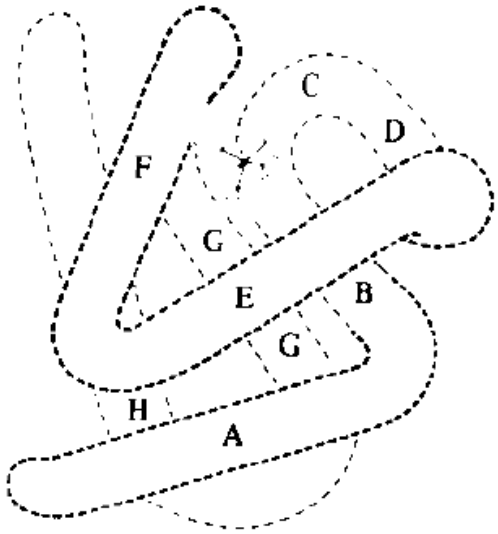
-oxygen transport

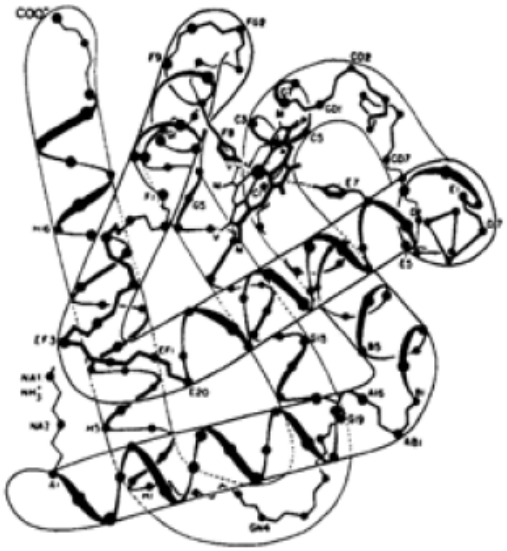
Functions of hemoglobin and myoglobin

TABLE 3-1. Characteristics of Hemoglobin and Myoglobin

	HEMOGLOBIN	MYOGLOBIN
Function	O_2 transport	O_2 storage
Location	Only in the erythrocyte	Only in skeletal muscle
O_2 affinity in tissues	Low	High
O_2 affinity in lungs	High	High
O_2 affinity change with Po_2	Yes	No
Allosteric regulation	Yes	No
Quaternary structure	Yes—tetramer	No—monomer

Hemoglobin

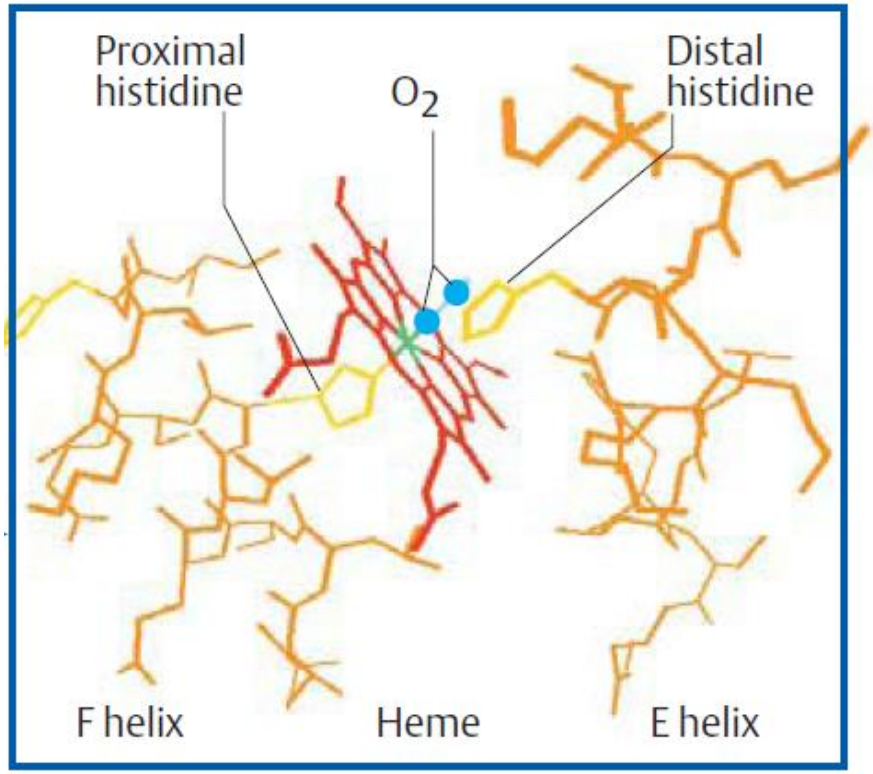


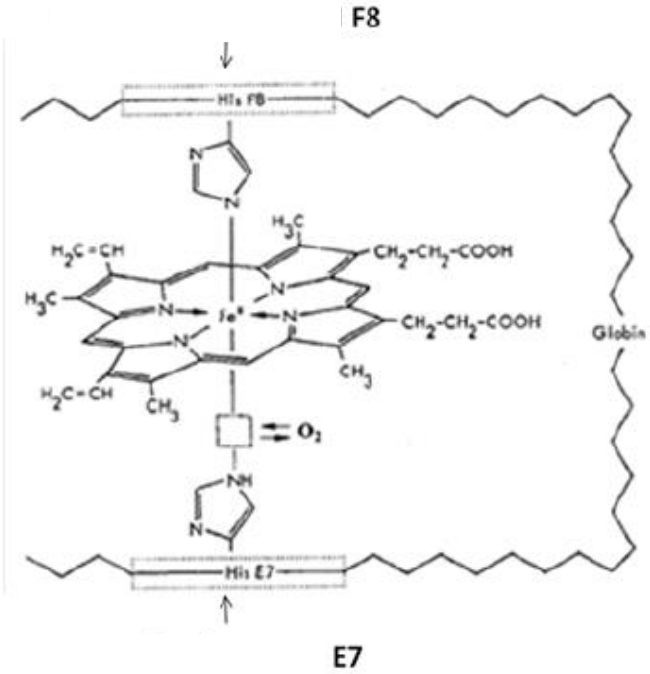


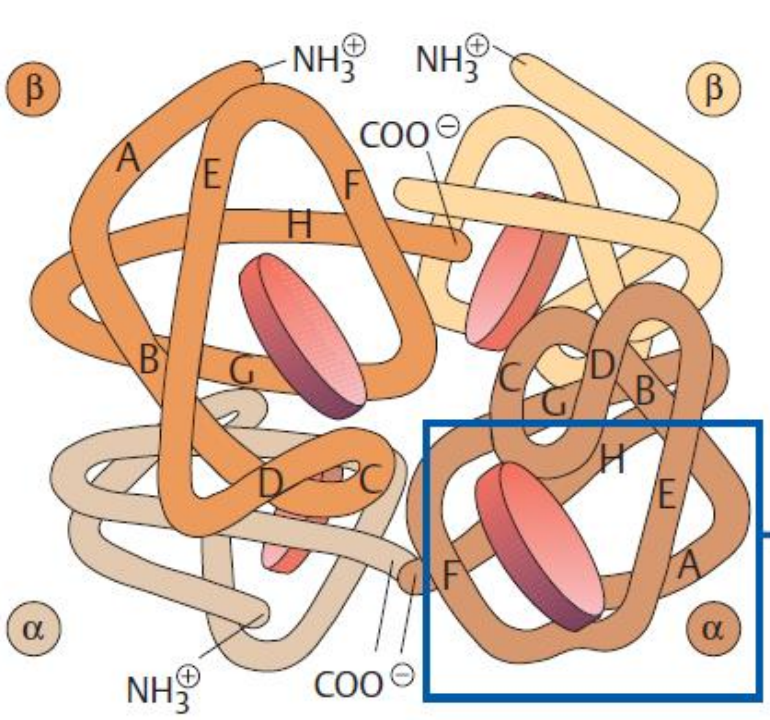
- **Structure of Hemoglobin**
- **primary – AA**
- **secondary (α helixes A-H)**
- **Tertiary (space composition …)**
- **α helixes, hydrophobic inside, hydrophilic outside, hydrophobic pocket - heme**
- **Quartery (subunits)**

Coordination of Heme in Hb/Mb

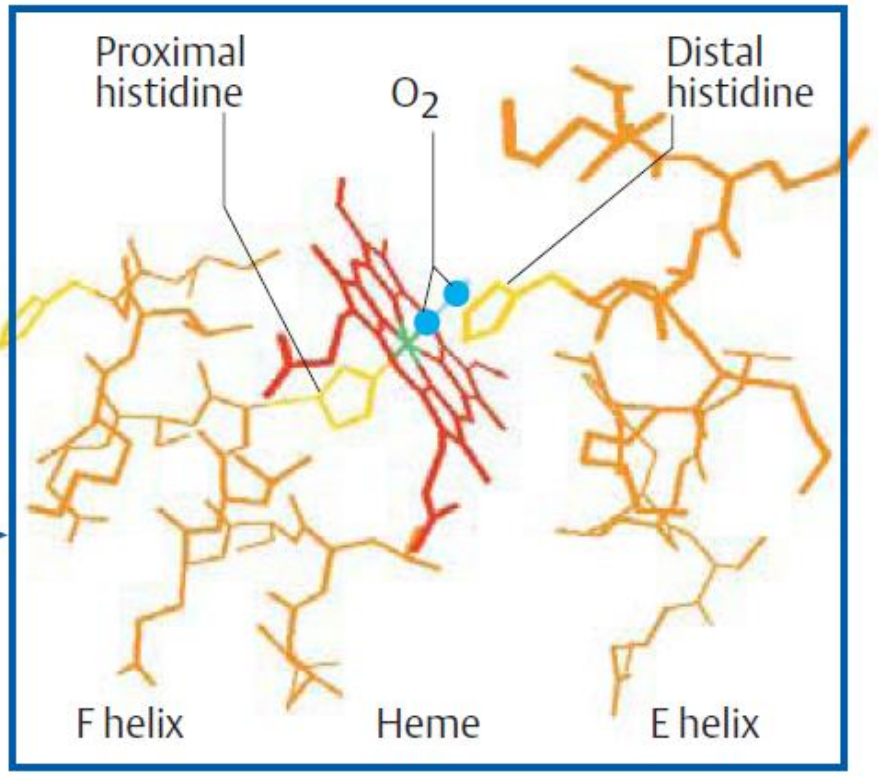
- Coordination bond of Fe2+ - histidine **F8 proximal (F helix**
- Partially to **histidine E7 distal** (in E helix).



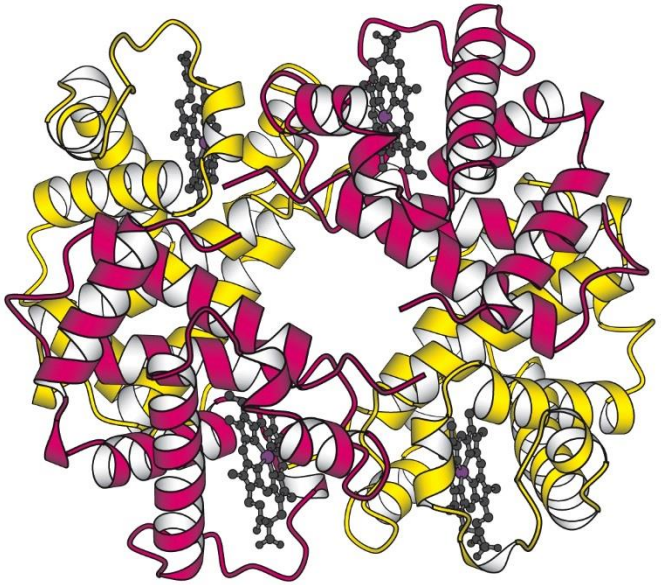




Hemoglobin A $(\alpha_2 \beta_2)$ M: 65 kDa



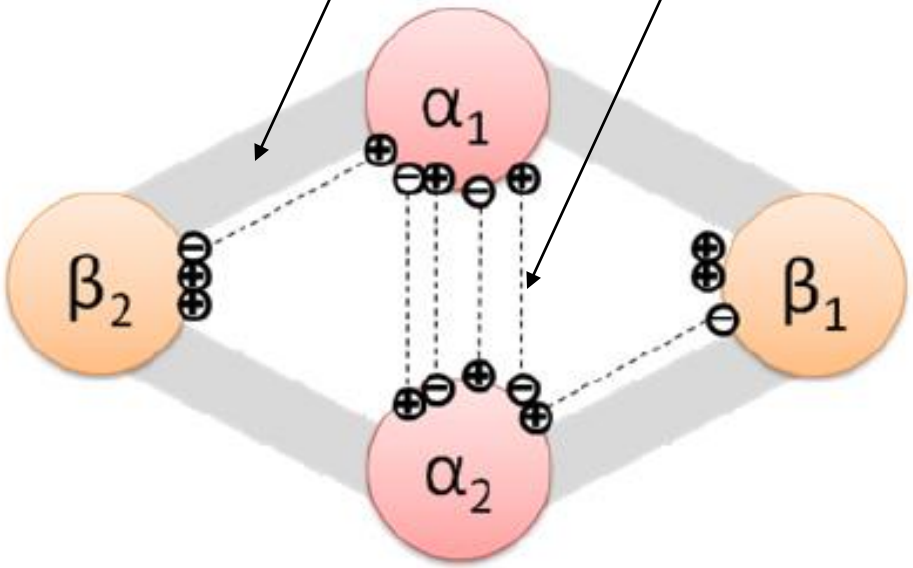
Quaternary structure, tetramer



Human
hemoglobin,
two alpha(red)
two beta(yellow)
subunits,

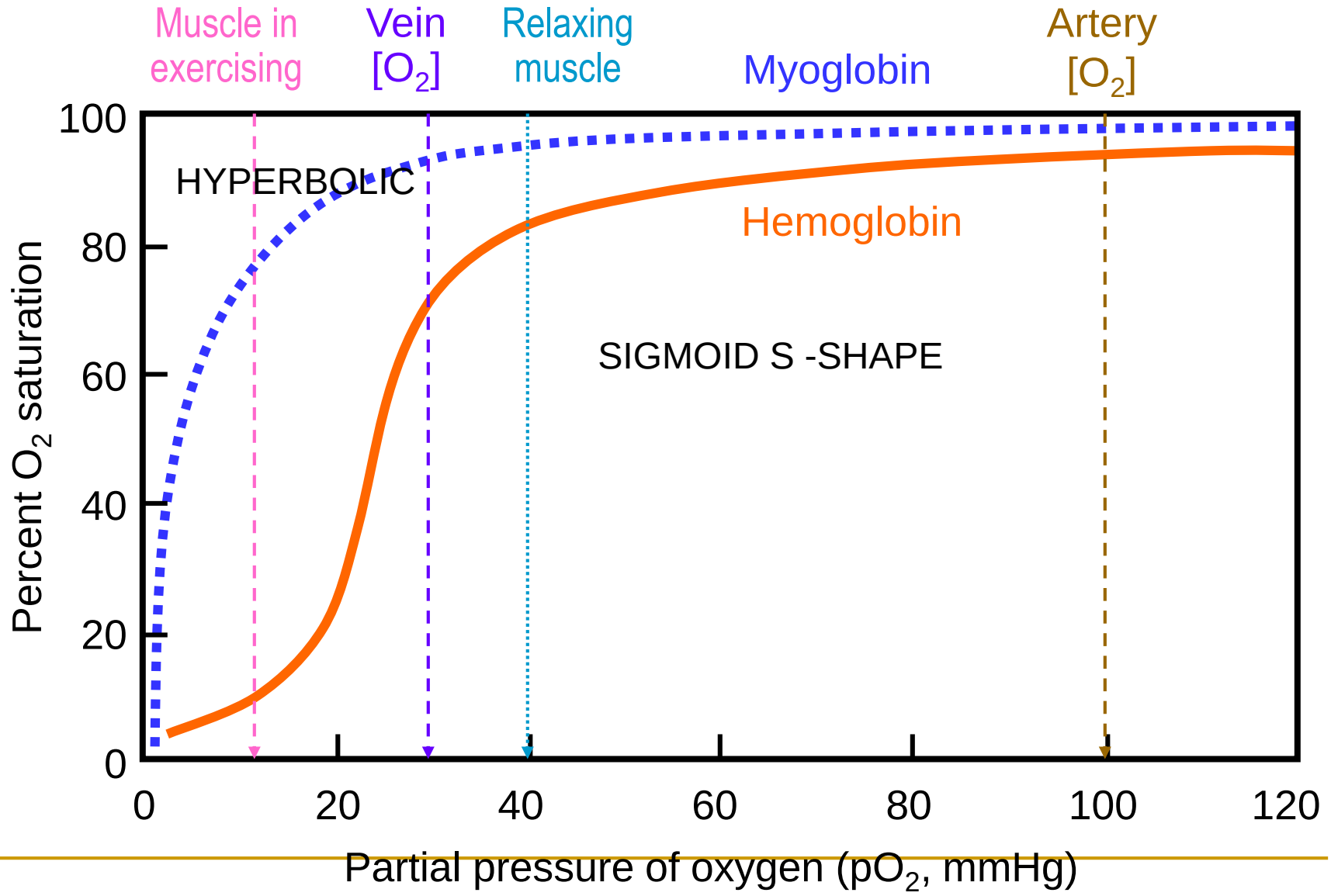
4 subunits

- hydrophobic
- electrostatic interactions



4 heme groups

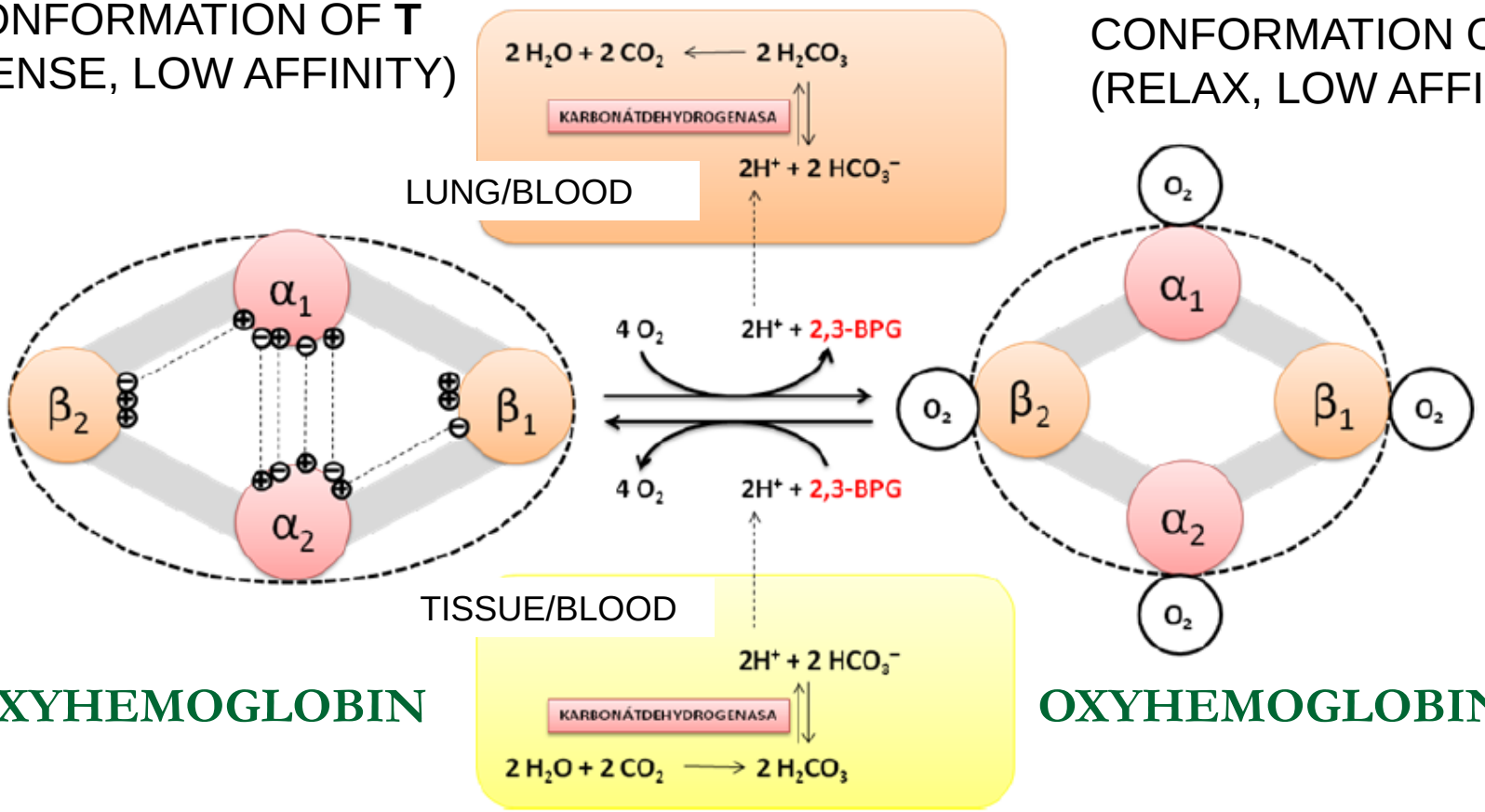
Environmental Oxygen Effects Binding Affinity



Adapted from Garrett & Grisham (1999) Biochemistry (2e) p.480

CONFORMATION OF **T**
(TENSE, LOW AFFINITY)

CONFORMATION OF **R**
(RELAX, LOW AFFINITY)



DEOXYHEMOGLOBIN

OXYHEMOGLOBIN

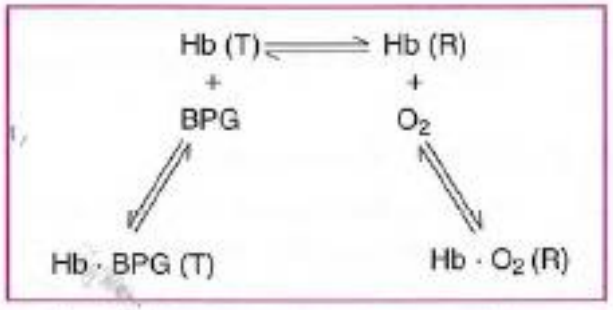
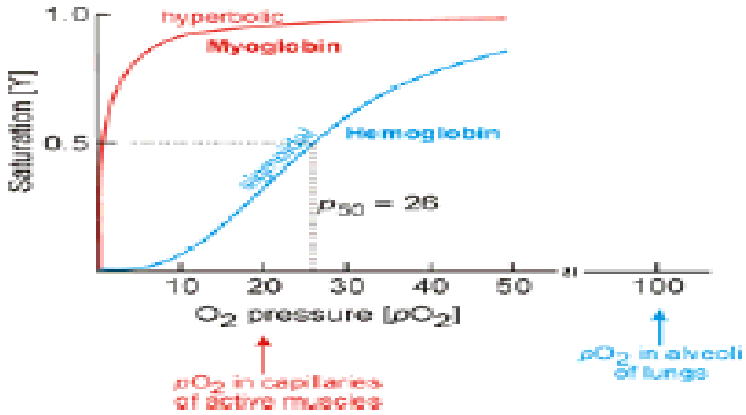


Figure 3-10. Equilibrium between the tense (*T*) and relaxed (*R*) forms of hemoglobin.

Dioxygen: Uptake, Transport & Storage

Hb binds dioxygen and transports O_2 to the tissue.
How does Mb manages to get O_2 transferred from Hb?



Hemoglobin is a much more intricate and sentient molecule than Myoglobin is!

Hb transports H^+ and CO_2 in addition to O_2

O_2 binding properties in Hb are regulated by interactions between separate, non-adjacent sites.

Hemoglobin is an allosteric protein; whereas Myoglobin is not!

Dioxygen binds cooperatively to hemoglobin!

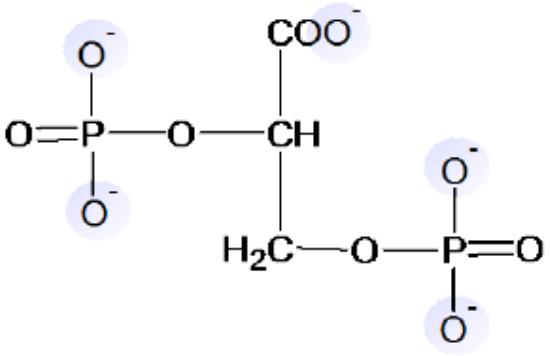
The binding of O2 to hemoglobin enhances the binding of additional O2 to the same hemoglobin (take advantage of high concentrations of O2 in the lungs; **sigmoid curve**). Binding of O2 to Myoglobin is not cooperative; hyperbolic cure).

Affinity of hemoglobin for O2 is pH dependent!

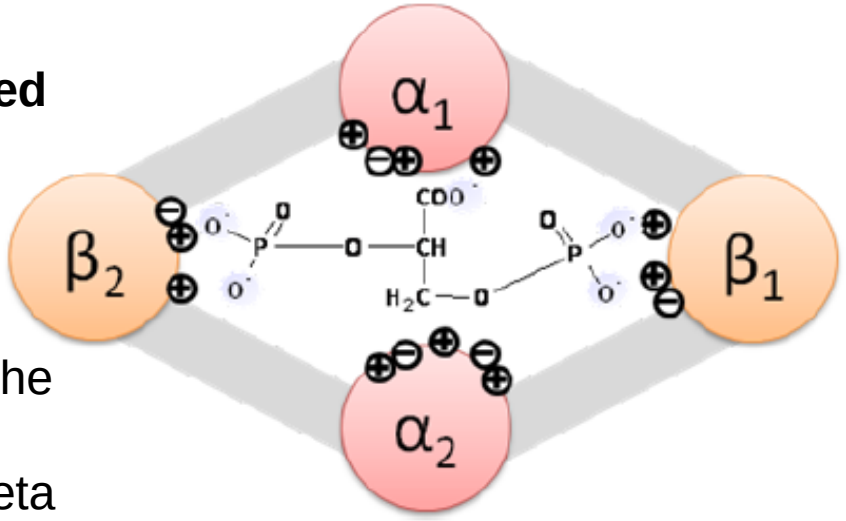
H+ and CO2 promote the release of bound dioxygen (for instance in active tissues such as in muscles). Reciprocally, higher concentrations of O2 promote the release of CO2 (e.g. in the lungs).

Dioxygen affinity of the tetrameric hemoglobin is regulated by 2,3-**B**i**P**hospho**G**lycerate (**lowered by the presence of BPG**)!

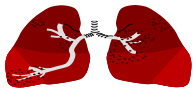
Importance of 2,3-Bisphosphoglyceric acid (2,3-Bisphosphoglycerate or 2,3-BPG, also known a diphosphoglycerate or 2,3-DPG)



-glycolytic intermediate 1,3-bisphosphoglyceric acid (1,3-BPG).
-2,3-BPG is present in human red blood cells at approximately 5 mmol/L.
-It binds with **greater affinity to deoxygenated hemoglobin** (e.g. when the red cell is near respiring tissue) than it does to oxygenated hemoglobin (e.g., in the lungs) due to spatial changes: 2,3-BPG fits in the deoxygenated hemoglobin configuration), but not as well in the oxygenated
-It interacts with deoxygenated hemoglobin beta subunits by decreasing their affinity for oxygen, so it allosterically promotes the release of the remaining oxygen molecules bound to the hemoglobin, thus enhancing the ability of RBCs to release oxygen near tissues that need it most.
2,3-BPG is thus an **allosteric effector.**



The Transportation of Blood Oxygen



Any one subunit receives an oxygen molecule will increase the oxygen-binding affinity of the others

O_2

Lung

(R)
relaxed state

Artery

When environmental $[O_2]$ decreases, Hb releases oxygen to Mb

When environmental $[O_2]$ increases, Hb binds oxygen efficiently

Muscle

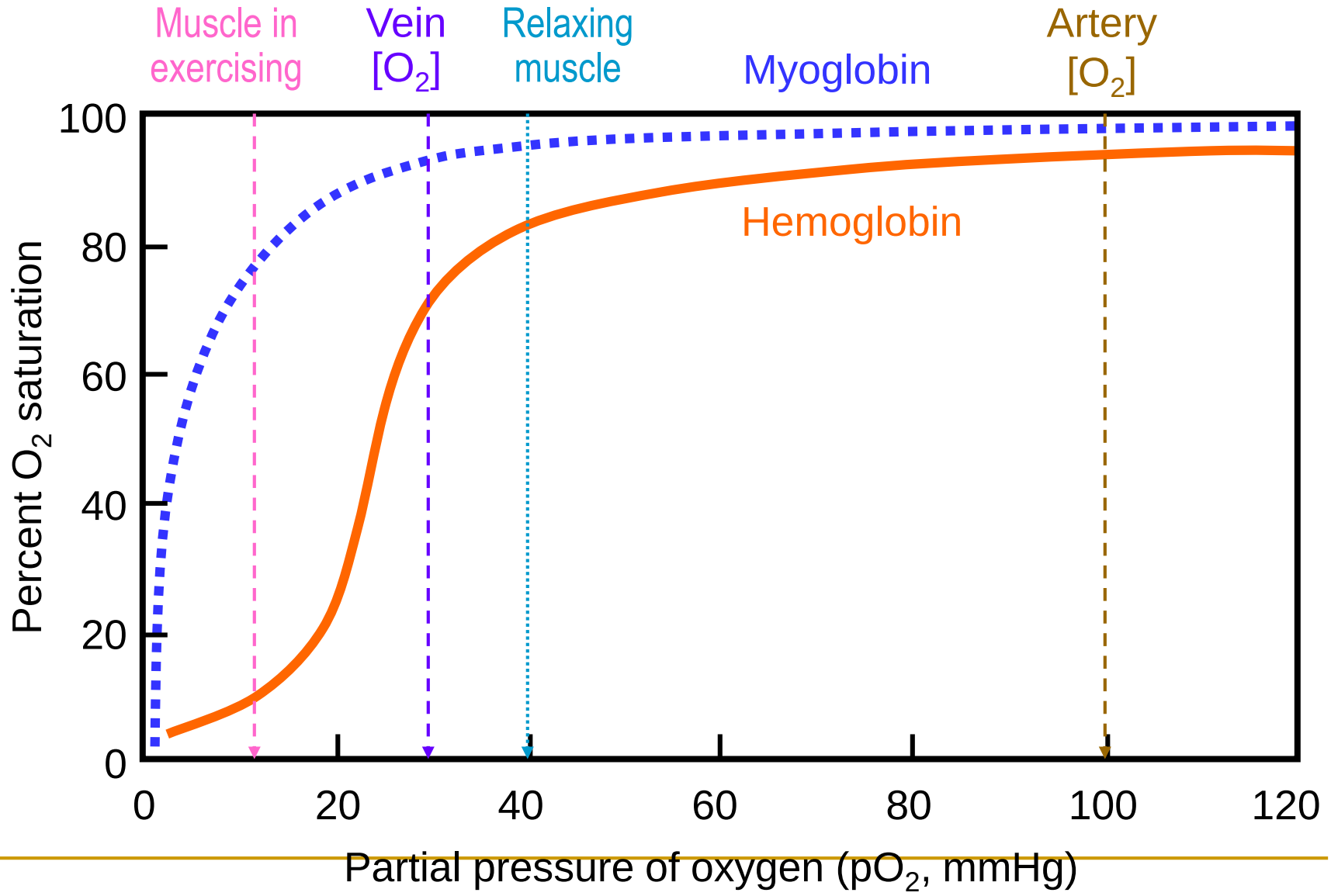
Vein

Hemoglobin

(T)
tense state

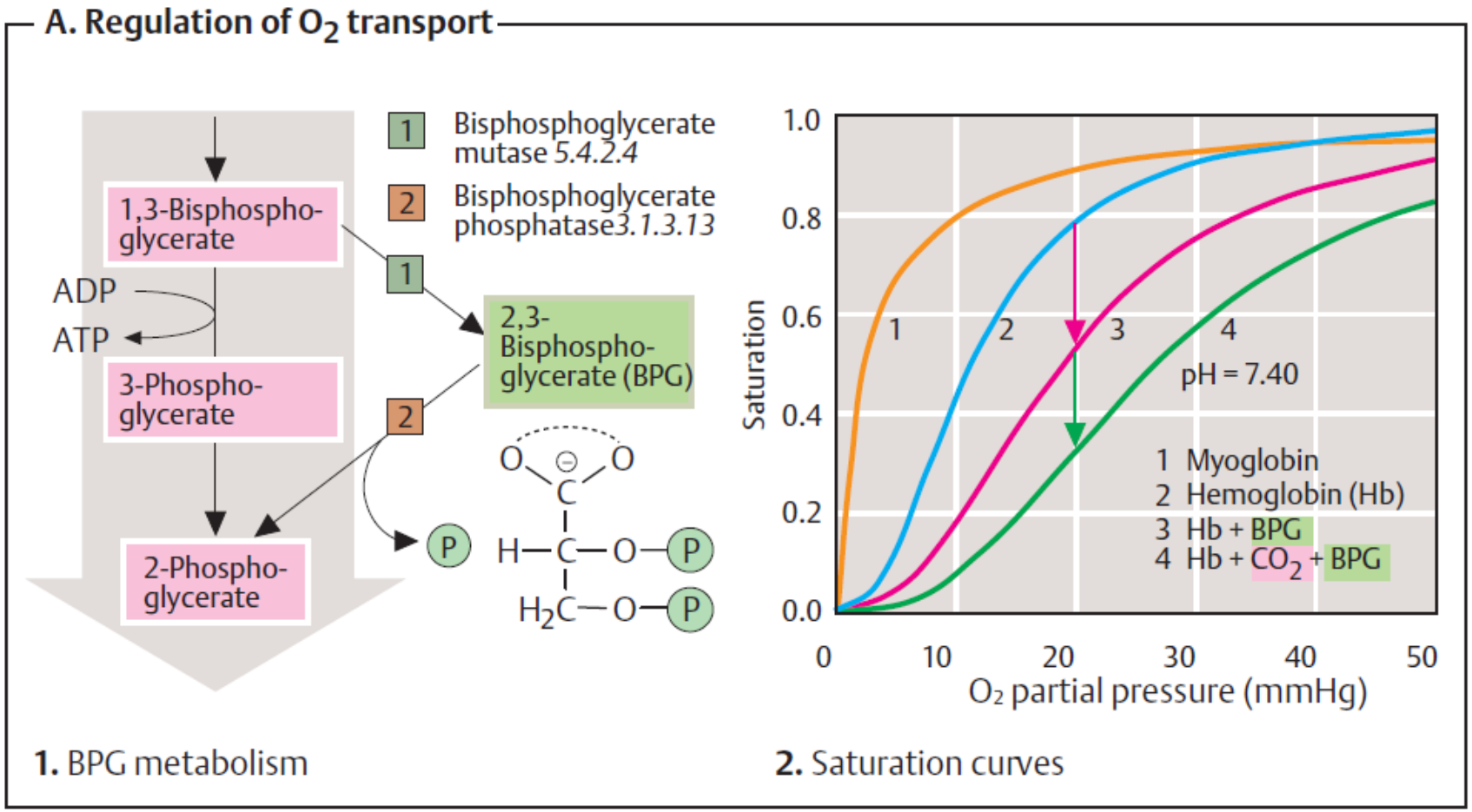
Myoglobin

Environmental Oxygen Effects Binding Affinity



Adapted from Garrett & Grisham (1999) Biochemistry (2e) p.480

ALLOSTERISM

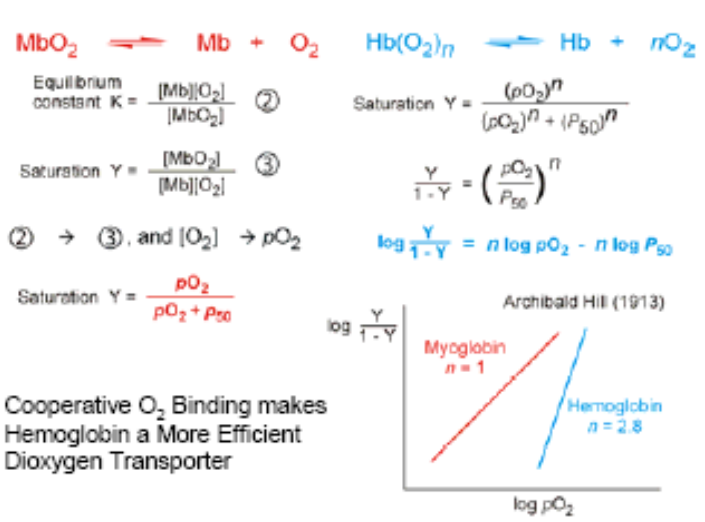


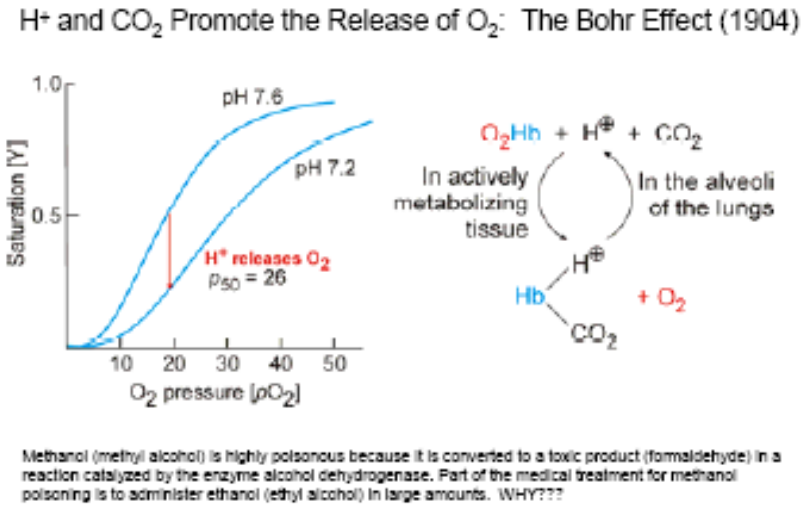
Hemoglobin transport H+ and CO2

- Products of cellular oxidation H+, CO2
- From the tissues to lung and kidneys

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

This reaction is catalyzed by **carbonic anhydrase**, an enzyme particularly abundant in erythrocytes. Carbon

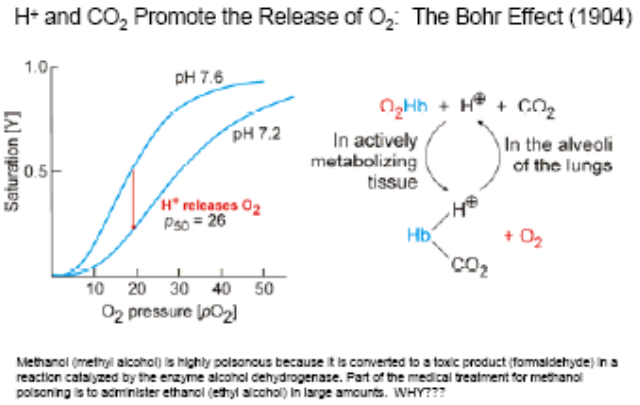




Methanol (methyl alcohol) is highly poisonous because it is converted to a toxic product (formaldehyde) in a reaction catalyzed by the enzyme alcohol dehydrogenase. Part of the medical treatment for methanol poisoning is to administer ethanol (ethyl alcohol) in large amounts. WHY???

The Bohr Effect

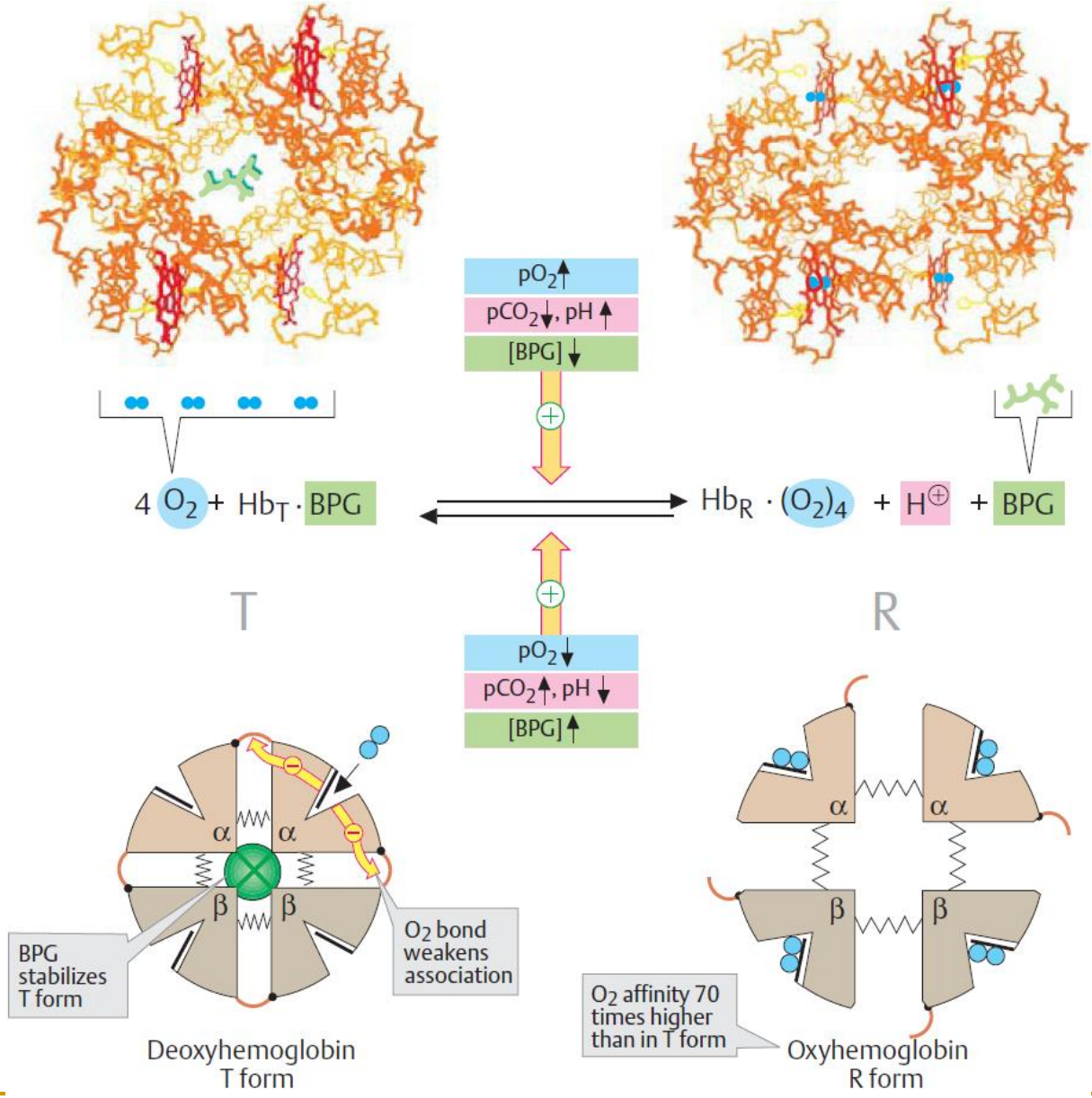
a decrease in blood pH or an increase in blood CO2 concentration will result in hemoglobin proteins releasing their loads of oxygen and a decrease in carbon dioxide or increase in pH will result in hemoglobin picking up more oxygen. Since carbon dioxide reacts with water to form carbonic acid, an increase in CO2 results in a decrease in blood pH.

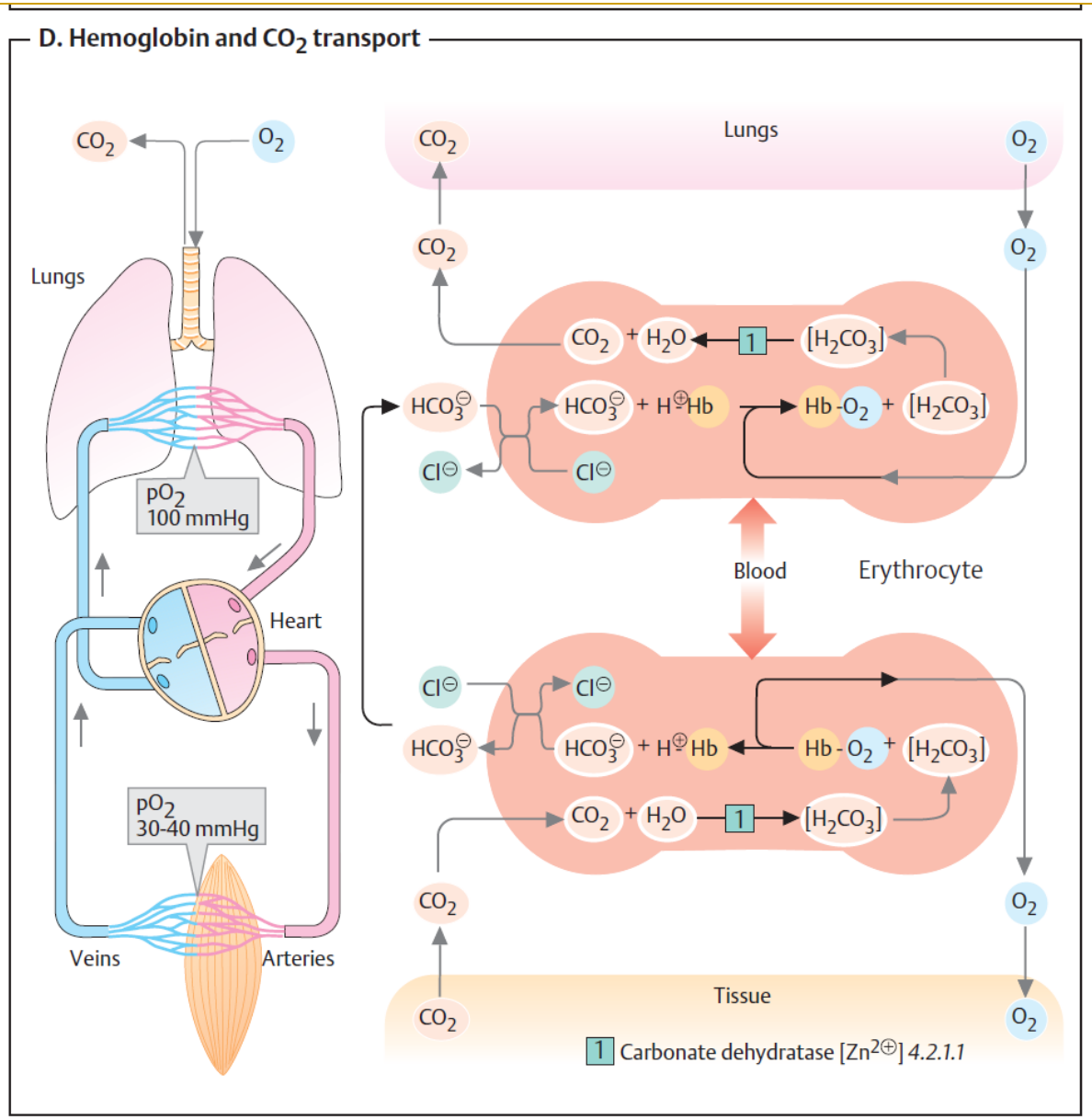


- Most of the CO2 is transported as bicarbonate, which is formed within
- red blood cells by the action of **carbonic anhydrase**:
- CO2 + H2O ⟶ HCO3− + H+

- The major portion of the Bohr Effect is due to the fact that
- increasing *p*(CO2) causes a decreased red cell pH (acidosis).
- A secondary part of the Bohr Effect is due to the fact that CO2
- reacts covalently with hemoglobin to form *carbamino*-hemoglobin
- which has a reduced O2 affinity.

- R—NH2 + CO2 ⟶ R—NH—COO− + H+
- The bound *carbamates* form salt bridges that stabilize the T-form!
- (The **T***ense*-form of hemoglobin possesses a lower O2 affinity).


pO2↑
pCO2↓, pH↑
[BPG]↓
4 O2 + HbT · BPG
HbR · (O2)4 + H⊕ + BPG
T
R
pO2↓
pCO2↑, pH↓
[BPG]↑
α
α
β
β
BPG stabilizes T form
O2 bond weakens association
Deoxyhemoglobin T form
O2 affinity 70 times higher than in T form
Oxyhemoglobin R form


D. Hemoglobin and CO_2 transport
CO_2
O_2
Lungs
pO_2 100 mmHg
Heart
pO_2 30-40 mmHg
Veins
Arteries
Lungs
Blood
Erythrocyte
Tissue
$HCO_3^{\ominus}$
$Cl^{\ominus}$
$H^{\oplus}Hb$
Hb-O_2
$[H_2CO_3]$
$CO_2 + H_2O$
1
1 Carbonate dehydratase $[Zn^{2\oplus}]$ 4.2.1.1

Types of Hb

TABLE 3-2. Tetrameric Quaternary Structures of Hemoglobin

DEVELOPMENTAL STAGE	ABBREVIATION	QUATERNARY STRUCTURE	FRACTION OF TOTAL HEMOGLOBIN IN ADULT
Embryo	Hb Gower-2	$\alpha_2\varepsilon_2$	0
Fetus	HbF	$\alpha_2\gamma_2$	~1%
Adult	HbA	$\alpha_2\beta_2$	90%
Adult	HbA_2	$\alpha_2\delta_2$	~2%
Adult	HbA_1	$\alpha_2\beta_2$-Glucose	~5%

- Fetal Hb (HbF small increase affinity in comparison to HbA)
- HbA1c – glycosylation
- HbA1c reference value:
- □ men 140-180 g/l ; 8,1-11,2 mmol/l
- □ women: 120-160 g/l ; 7,4-9,8 mmol/l

Hemoglobin- transport oxygen in bood

- Erythrocytes (red blood cells, 6-9um in diameter)
- Precursor stem cells- hemocystoblast – maturation- large amount of hemoglobin, lose their nucleus, mitochondria, endoplasmatic reticulum
- 120 days survivor

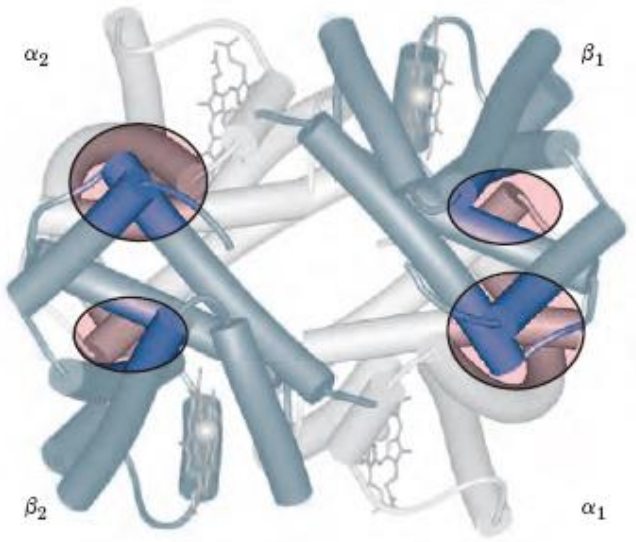


FIGURE 5–8 **Dominant interactions between hemoglobin subunits.** In this representation, α subunits are light and β subunits are dark. The strongest subunit interactions (highlighted) occur between unlike subunits. When oxygen binds, the $\alpha_1\beta_1$ contact changes little, but there is a large change at the $\alpha_1\beta_2$ contact, with several ion pairs broken (PDB ID 1HGA).

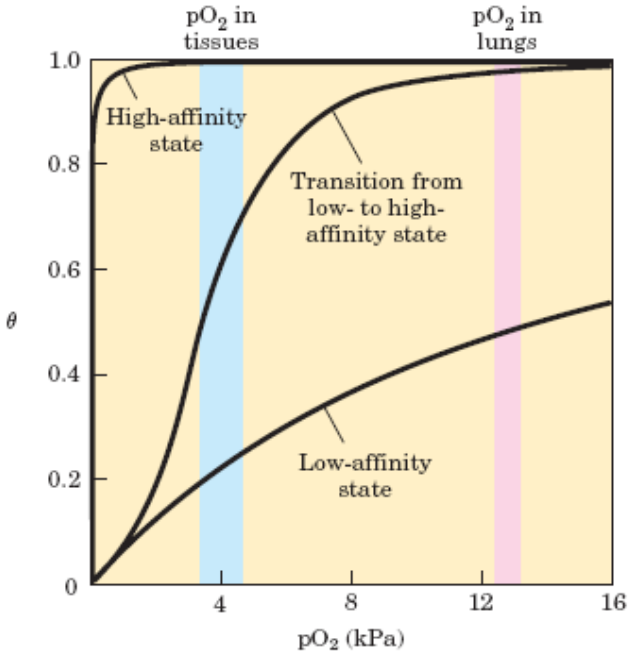
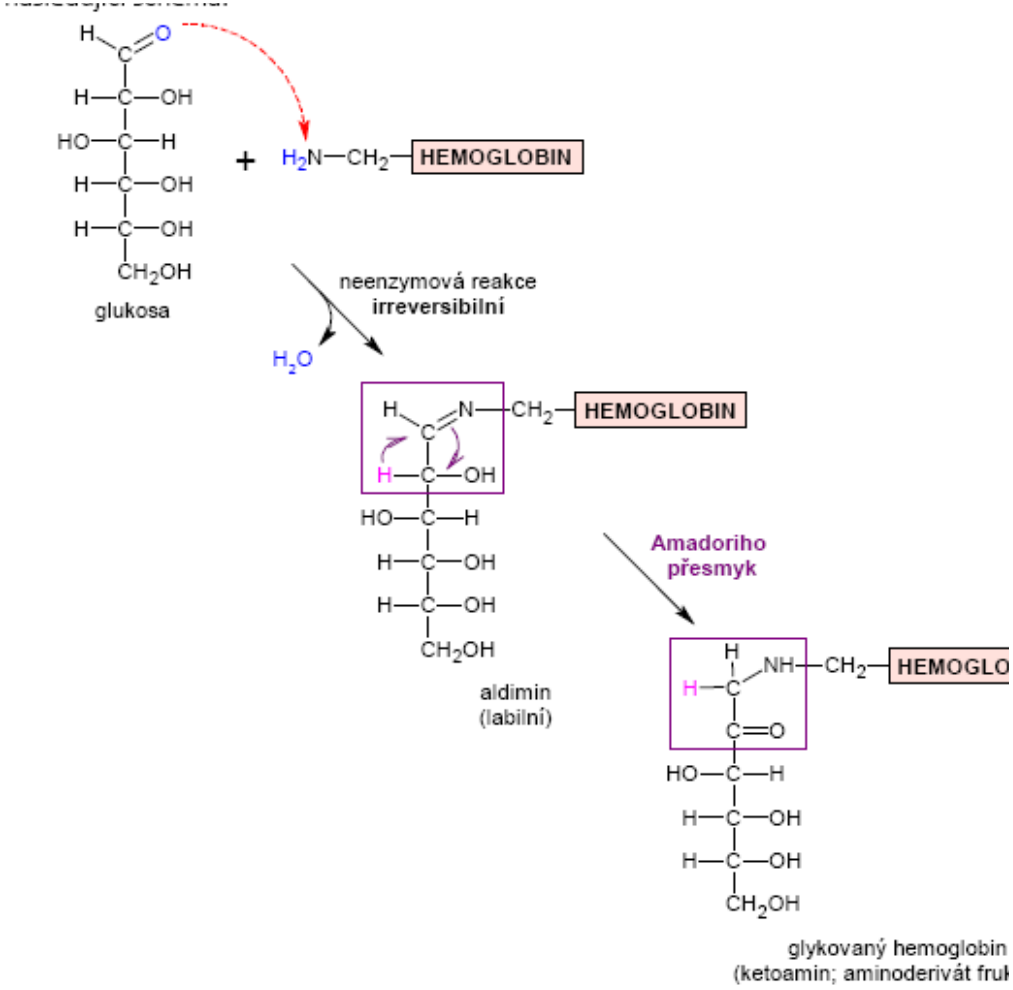


FIGURE 5–12 **A sigmoid (cooperative) binding curve.** A sigmoid binding curve can be viewed as a hybrid curve reflecting a transition from a low-affinity to a high-affinity state. Cooperative binding, as manifested by a sigmoid binding curve, renders hemoglobin more sensitive to the small differences in O_2 concentration between the tissues and the lungs, allowing hemoglobin to bind oxygen in the lungs (where pO_2 is high) and release it in the tissues (where pO_2 is low).



Glycosylated hemoglobin is the form of hemoglobin to which glucose is bound. The binding of glucose to amino acids in the hemoglobin takes place spontaneously (without the help of an enzyme) in many proteins, and is not known to serve a useful purpose. However, the binding to hemoglobin does serve as a record for average blood glucose levels over the lifetime of red cells, which is approximately 120 days.

The levels of glycosylated hemoglobin are therefore measured in order to monitor the long-term control of the chronic disease of type 2 diabetes mellitus (T2DM). Poor control of T2DM results in high levels of glycosylated hemoglobin in the red blood cells. The normal reference range is approximately 4–5.9 %. Though difficult to obtain, values less than 7% are recommended for people with T2DM. Levels greater than 9% are associated with poor control of the glycosylated hemoglobin, and levels greater than 12% are associated with very poor control.

Diabetics who keep their glycosylated hemoglobin levels close to 7% have a much better chance of avoiding the complications that may accompany diabetes (than those whose levels are 8% or higher).[61] In addition, increased glycosylation of hemoglobin increases its affinity for oxygen, therefore preventing its release at the tissue and inducing a level of hypoxia in extreme cases.[62]

Role in disease

Hemoglobinopathies

- Genetic diseases (mutation in globin chain)
- Altered rate of Hb production (thalassemias)

Sickle cell hemoglobin HbS (mutation)

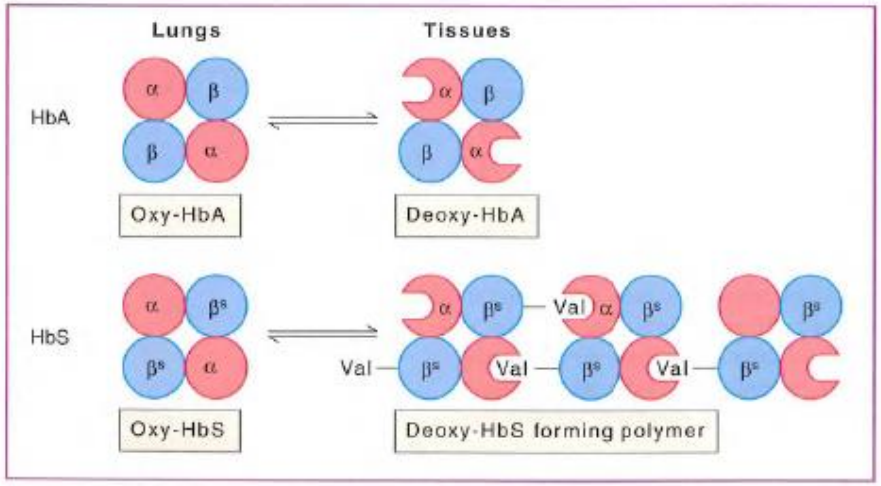
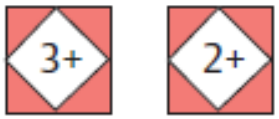


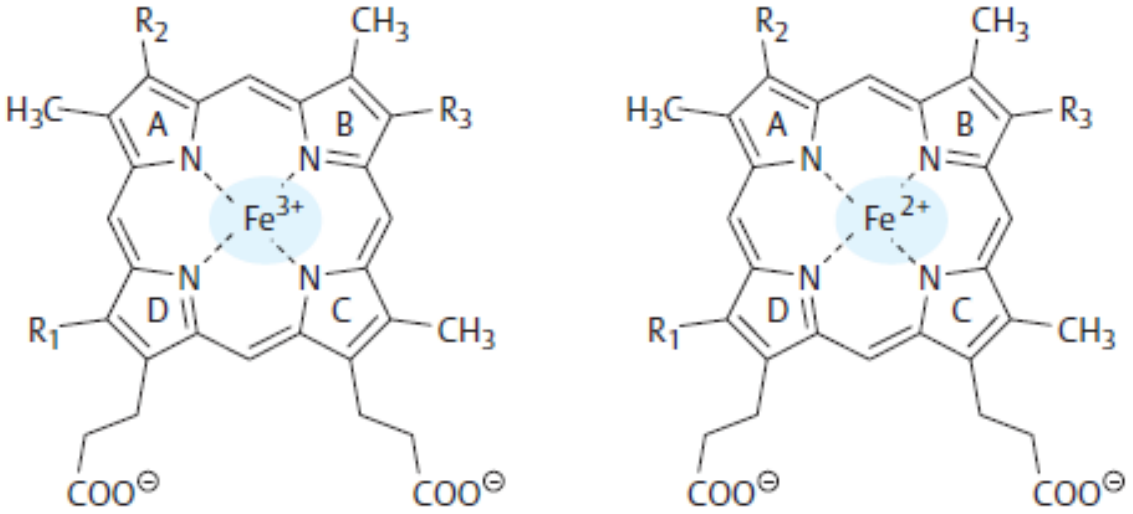
Figure 3-12. Formation of linear aggregates between molecules of sickle cell hemoglobin.

Hemoproteins

8. Heme



ox. red.



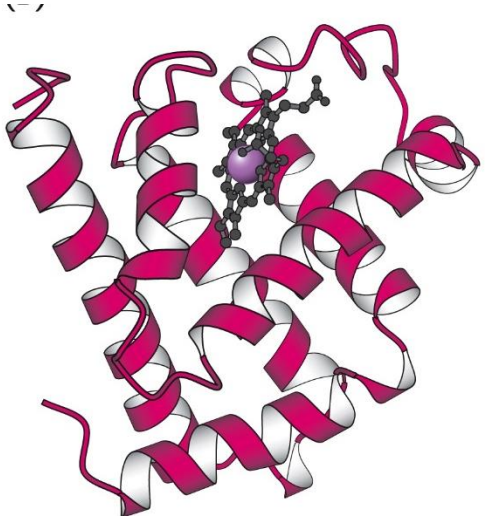
Hemoglobin je jen jedním z proteinů patřících do velké skupiny zvané **hemové proteiny** (**hemoproteiny**).

Do této skupiny dále patří, např.:

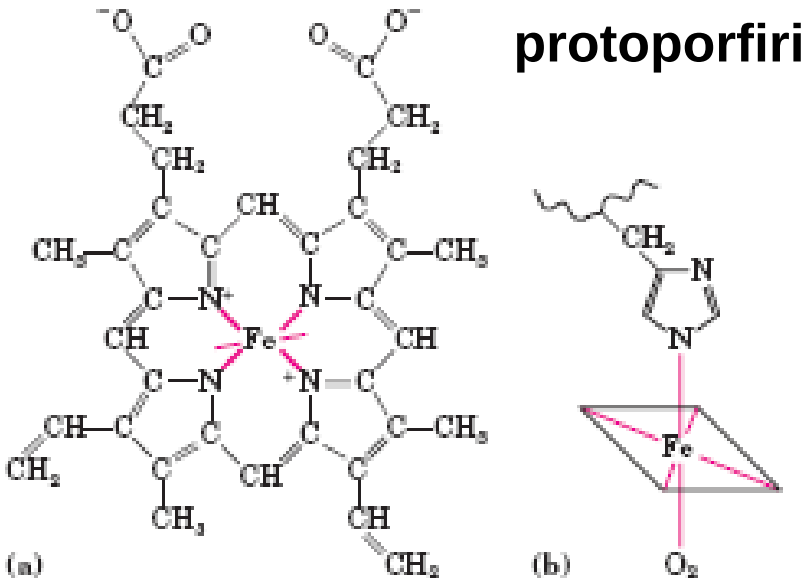
- **myoglobin** (vazba a depozice kyslíku ve svalech)
- **cytochromy** (přenašeče elektronů v elektron-transportním řetězci mitochondrií)
- **katalázy** a **peroxidázy** (rozklad a tvorba H2O2)
- **cytochrom P450** (hydroxylační systém/enzym)

Jejich společným znakem je to, že ve své molekule obsahují **hem** (cyklický tetrapyrrol).

Hemoproteins



protoporfirin



His- residue- heme coordination, oxygen on the other site

Fe2+--Fe3+, heme iron – higher affinity for CO (carbon monooxide),

- NO (nitric..) TOXIC to aerobic metabolism

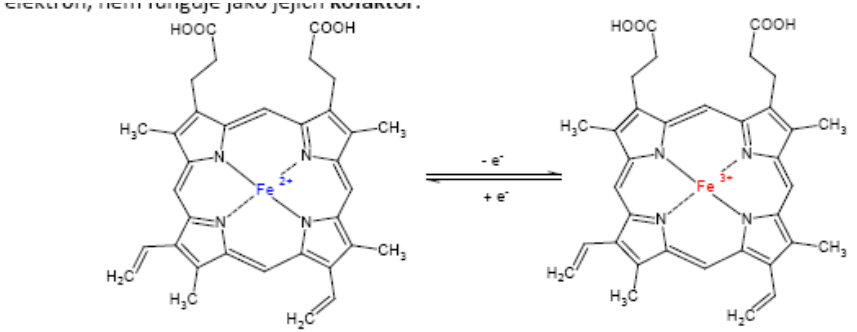
-Blood: different color- oxygen rich- bright red arterial blood

-- dark purple oxygen depleted venous blood

Other hemoproteins

Catalysis

- Cytochrome P450s
- cytochrome c oxidase
- peroxidases
- **Electron transfer/transport**
- cyctochrome a
- Cytochrome b
- cytochrome c



The **cytochrome P450** superfamily (officially abbreviated as **CYP**) is a large and diverse group of enzymes that catalyze the oxidation of organic substances. The substrates of CYP enzymes include metabolic intermediates such as lipids and steroidal hormones, as well as xenobiotic substances such as drugs and other toxic chemicals. CYPs are the major enzymes involved in drug metabolism and bioactivation, accounting for about 75% of the total number of different metabolic reactions.[1]

The most common reaction catalyzed by cytochromes P450 is a monooxygenase reaction, e.g., insertion of one atom of oxygen into an organic substrate (RH) while the other oxygen atom is reduced to water:

$$RH + O2 + NADPH + H+ \rightarrow ROH + H2O + NADP+$$

Cytochromes P450 (CYPs) belong to the superfamily of proteins containing a heme cofactor and, therefore, are hemoproteins. CYPs use a variety of small and large molecules as substrates in enzymatic reactions. Often, they form part of multi-component electron transfer chains, called P450-containing systems. The letter in *P450* represents the word pigment as these enzymes are red because of their heme group. The number 450 reflects wavelength of the absorption maximum of the enzyme when it is in the reduced state and complexed with CO.

Peroxidases (EC number 1.11.1.x) are a large family of enzymes that typically catalyze a reaction of the form:

ROOR' + electron donor (2 e-) + 2H+ → ROH + R'OH

For many of these enzymes the optimal substrate is hydrogen peroxide, but others are more active with organic hydroperoxides such as lipid peroxides. Peroxidases can contain a heme cofactor in their active sites, or alternately redox-active cysteine or selenocysteine residues.