Lecture 8: Heme/Non-Heme Iron Proteins and O_2 Management II

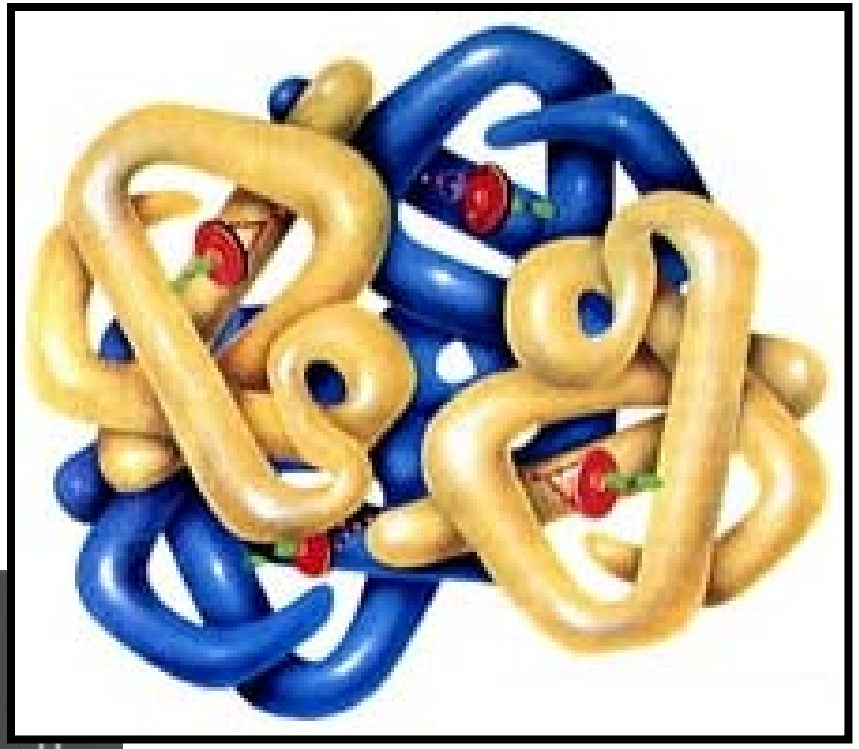
Plus a bit of catalysis in Oxygen processes

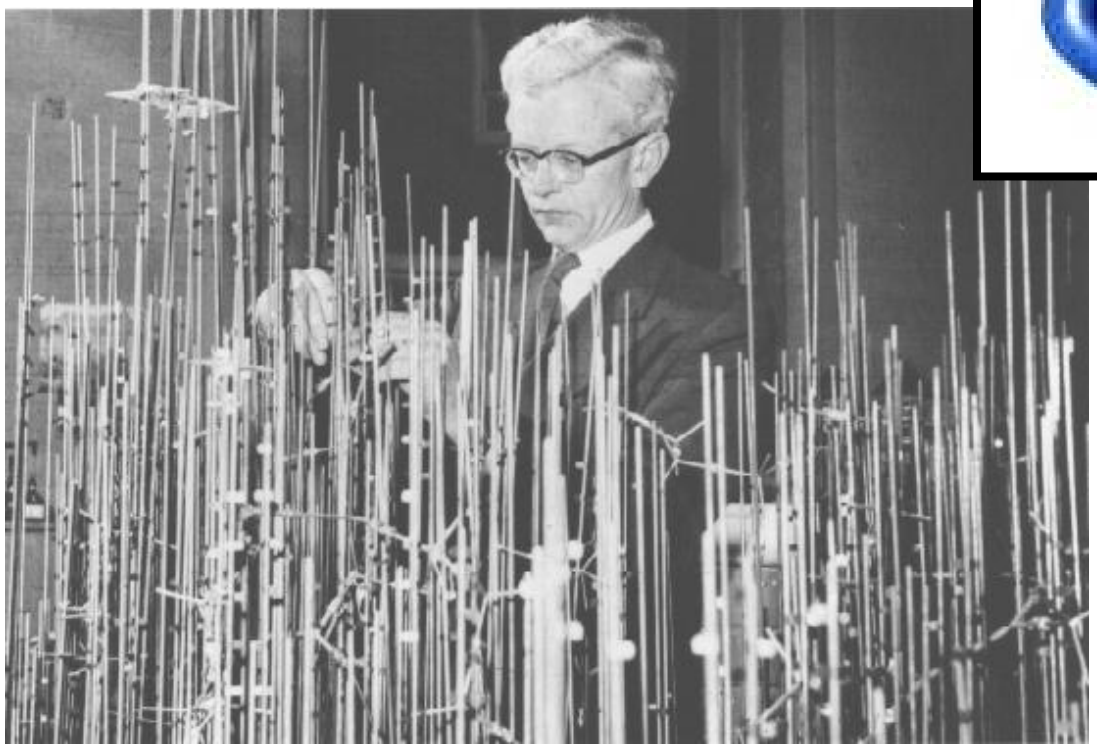
Hemoglobin- Key Properties

- **Ubiquitous O_2 transport protein**
- **A globular soluble protein, 2X2 chains (164 kDa)**
- **α and β chains 44% identical**
- **All helical secondary structure (like myoglobin)**
- **$\alpha\beta\alpha\beta$ quaternary structure**
  - **α-subunit 141 residues**
  - **β-subunit 146 residues**
- **Extensive contacts between subunits**
  - **Mix of hydrophobic, H-bond, and ionic interactions**
  - **$\alpha_1\beta_1$ ($\alpha_2\beta_2$)- 35 residues, $\alpha_1\beta_2$ ($\alpha_2\beta_1$)- 19 residues**

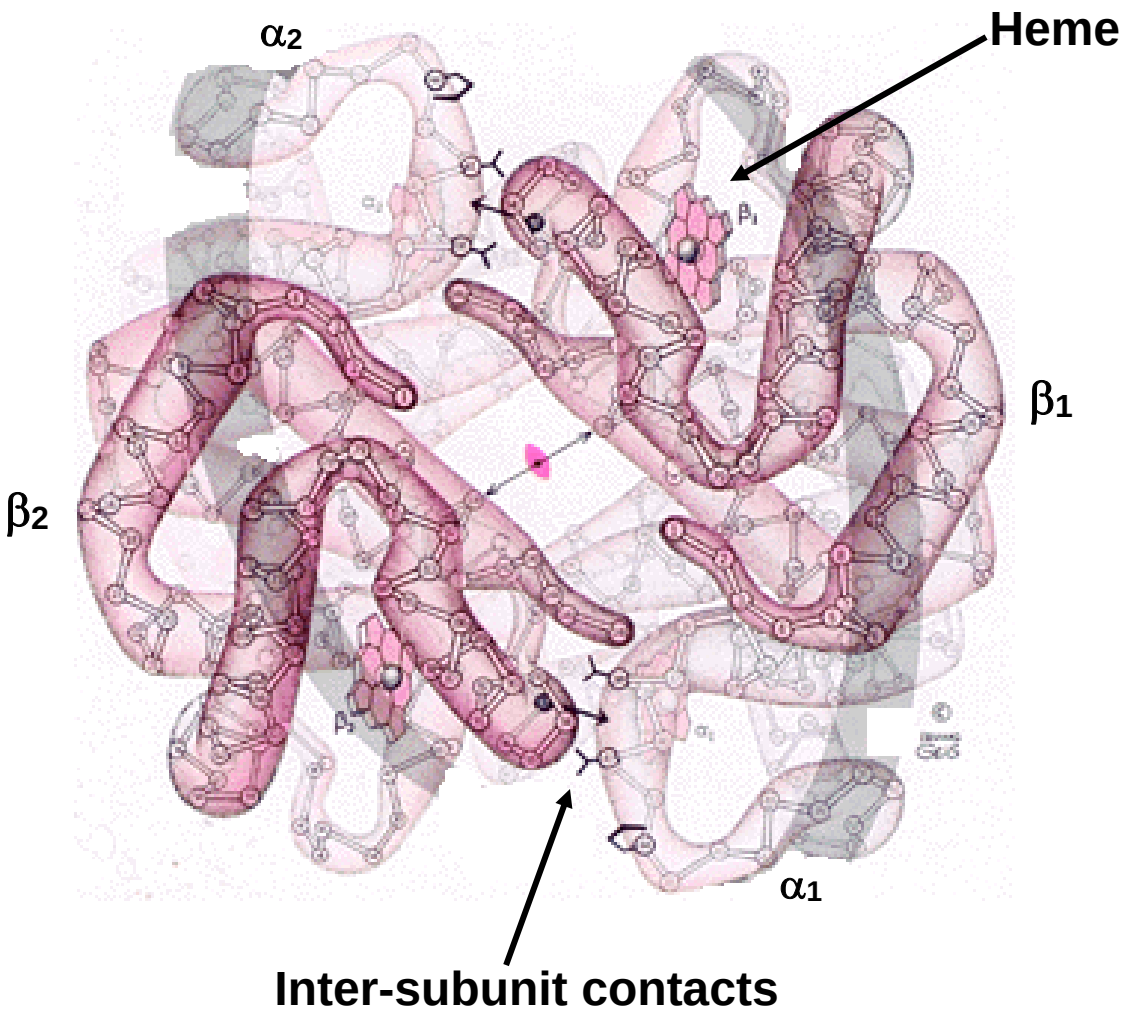
First Protein Complex

- Hemoglobin.
- Two copies each of α & β chains of myoglobin in a complex.
- Solved by John Kendrew.





Structure of Hemoglobin



Cooperative binding

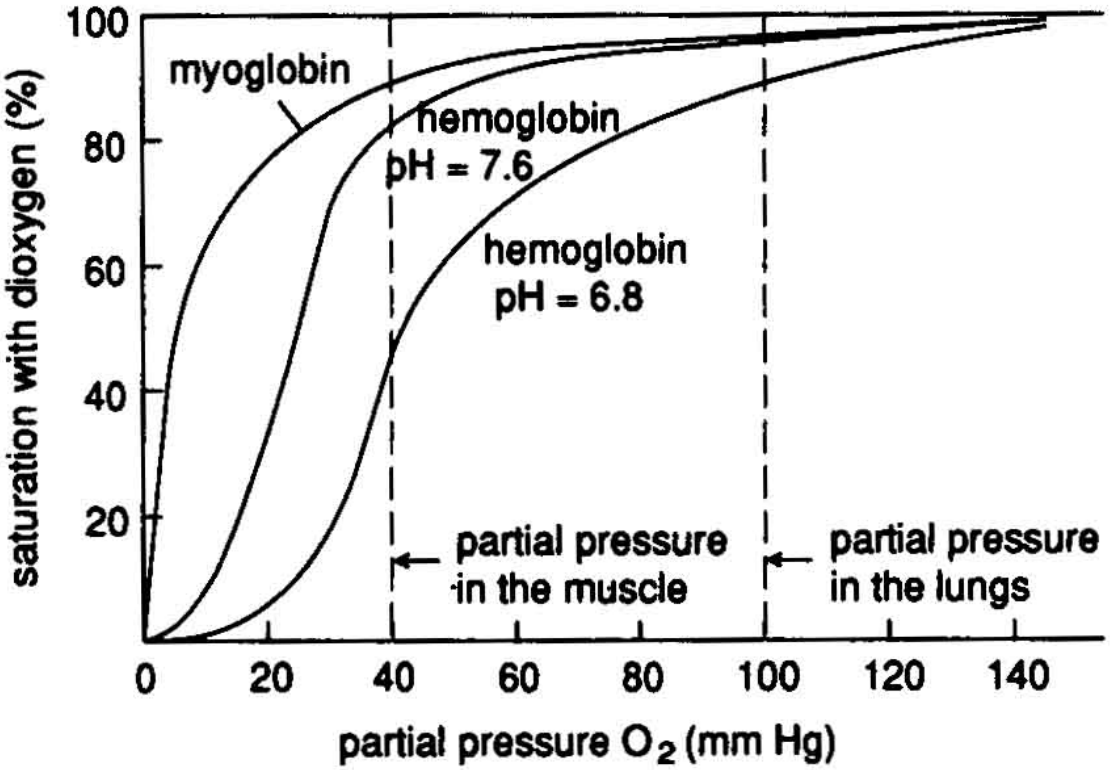


Figure 5.3
Oxygen saturation curves of myoglobin and hemoglobin at different pH values

Cooperativity in Binding O_2

The sigmoidal shape is a consequence of the 4 subunits of hemoglobin "cooperating" in the binding of O_2.

- **As pO_2 increases and $[O_2]$ increases, increasing probability that at least 1 subunit has bound O_2.**

Binding of O_2 to a subunit INCREASES the probability that empty subunits will be able to bind an O_2!!

- **As pO_2 increases even further, the probability that remaining binding sites will have O_2 bound increases.**
- **Eventually, a plateau is reached: when most hemoglobins are filled there are few sites left to bind to, so not much increase, even if the pO_2 is very high.**

Structural basis for the allosteric effect

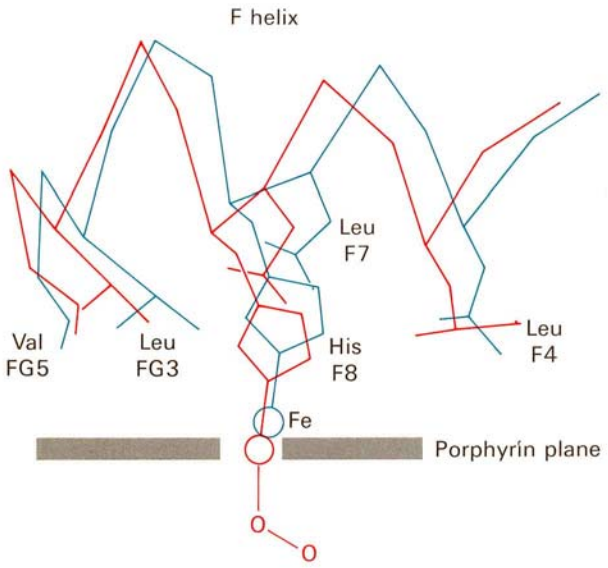


Figure 7-34
Conformational changes induced by the movement of the iron atom on oxygenation. The oxygenated structure is shown in red and the deoxygenated structure in blue. [After J. Baldwin and C. Chothia. *J. Mol. Biol.* 129(1979):192.]

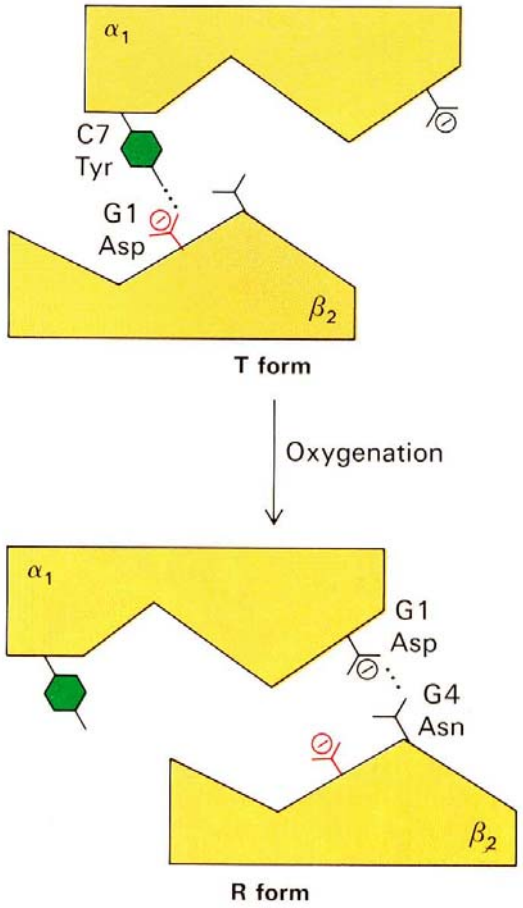
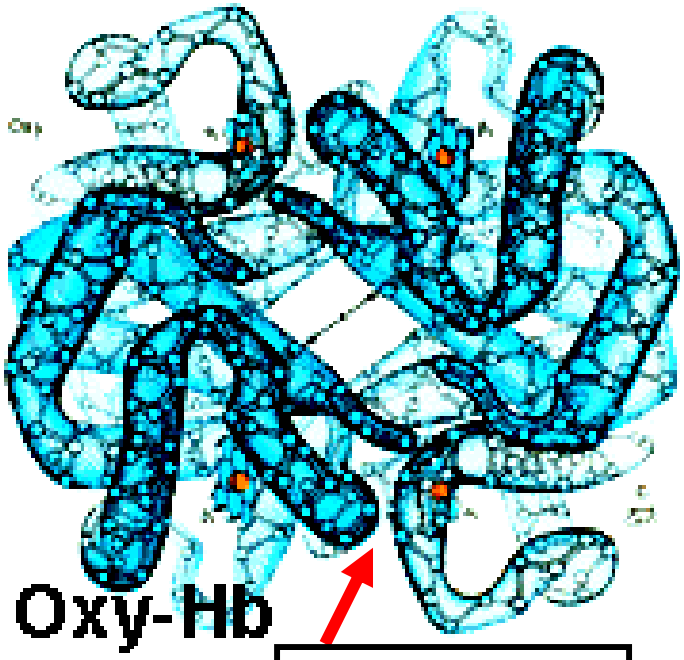


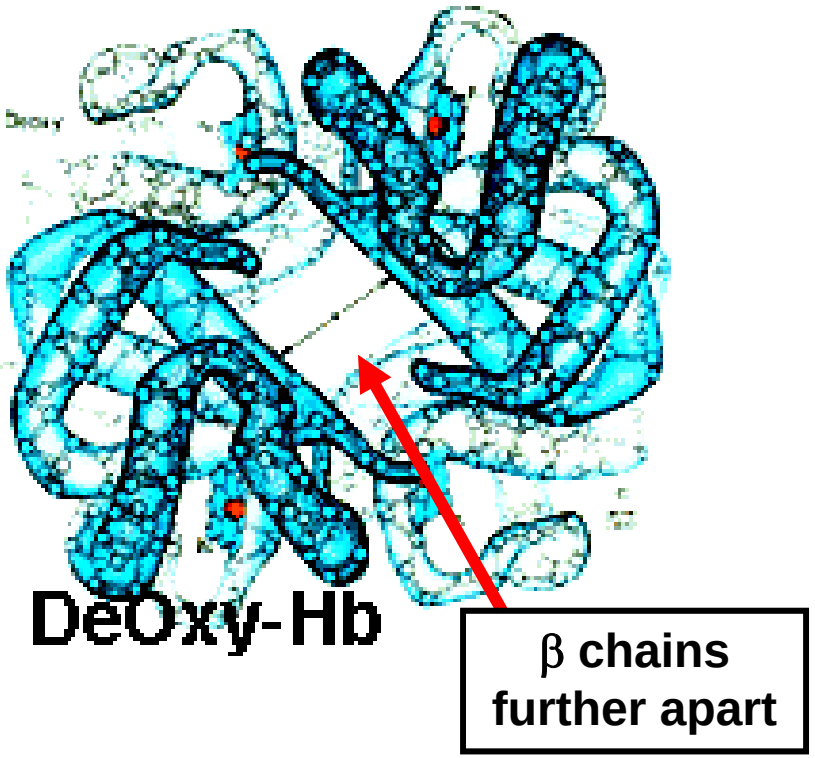
Figure 7-31
The $\alpha_1\beta_2$ interface switches from the T to the R form on oxygenation. The dove-tailed construction of this interface allows the subunits to readily adopt either of the two forms.

Binding of O_2 to the Heme Changes the Whole Structure of Hemoglobin

R state



T state



The T to R State Transition

- **Binding of O_2 causes a series of shifts in all subunits**
- **Change in heme structure upon binding O_2**
- **Since His F8 is covalently attached, all of F helix shifts**
- **Reorganization of helix alters tertiary structure, which in turn alters the quaternary structure- 4 chains behave as a single cooperative structural unit**
  - **Changes in packing of hydrophobic side chain**
  - **Changes in pairing of charged side chains**

The change in conformation of Hemoglobin from the T to the R state increases O_2 affinity at ALL sites

Allosteric Effects

- **The R or T state can be stabilized by the binding of ligands other than O_2.**

1. H^+. Lower pH favors the T state which causes Hb to release bound O_2. This is known as the Bohr Effect.

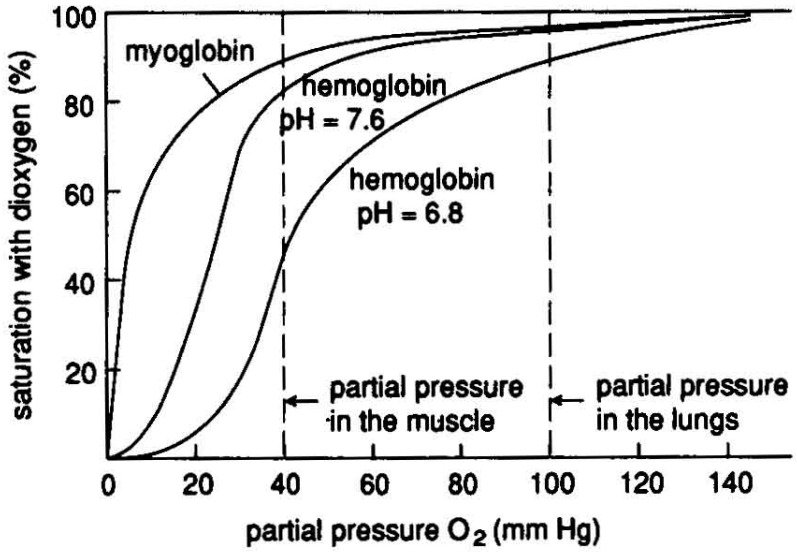


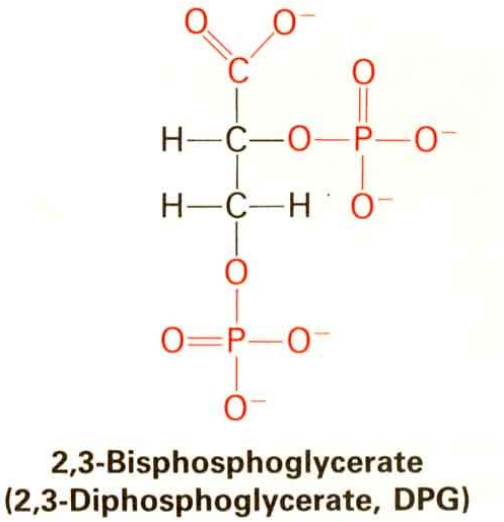
Figure 5.3
Oxygen saturation curves of myoglobin and hemoglobin at different pH values

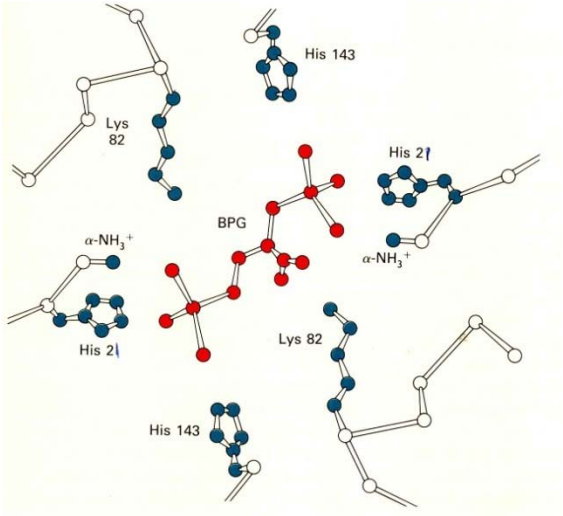
2. CO_2. Release of CO_2 lowers pH via conversion to HCO_3^-:

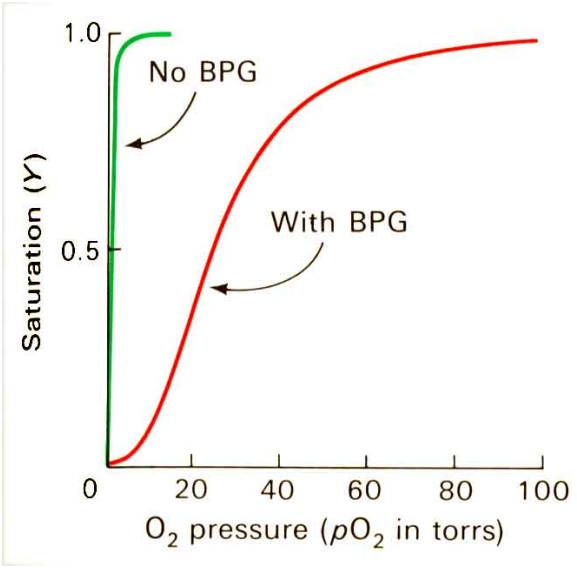
$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$. Reinforces Bohr Effect

Allosteric Effects

3. Bisphosphoglycerate (BPG). Regulation of activity via binding more strongly to T state, helps to release O_2.



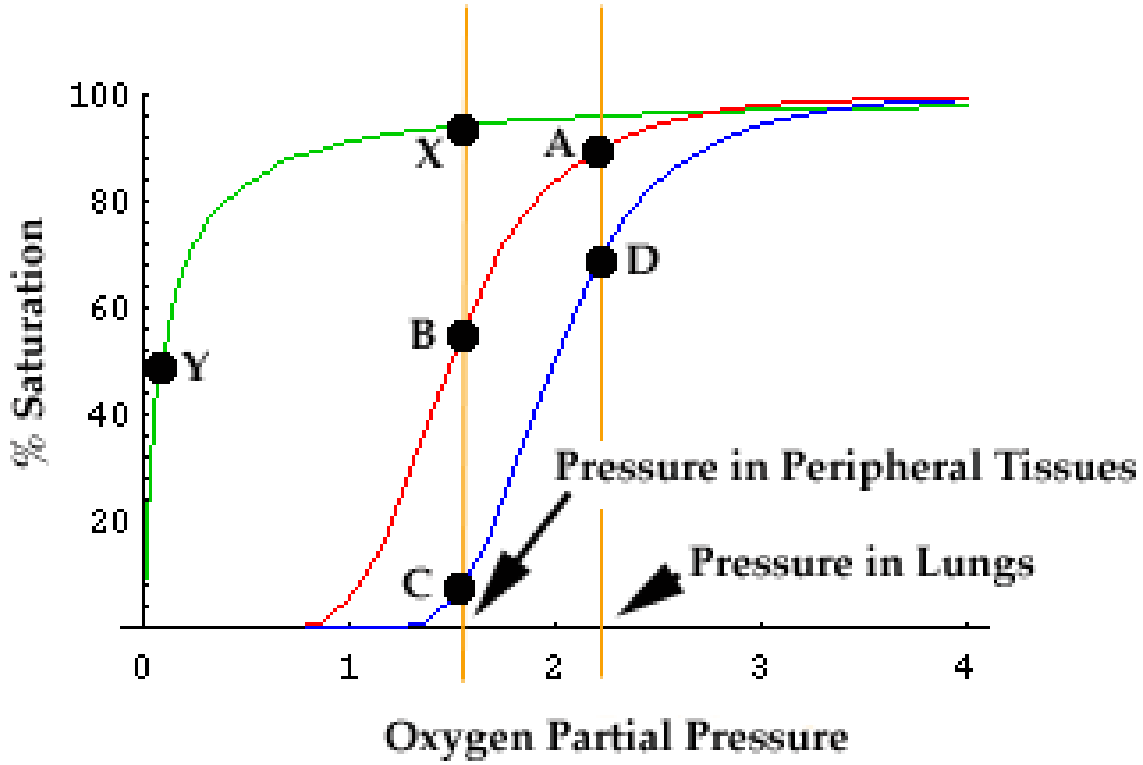




Increase in levels of BPG helps adaptation to high altitude- faster than making more hemoglobin.

Towards a More Complete Picture

Model for disucssion



- **HEMOGLOBIN at the pH (~7.6) found in the lungs.**
- **HEMOGLOBIN at the pH (~7.2) found in peripheral tissues.**
- **MYOGLOBIN in muscle (a peripheral tissue).**

Path of O_2 Flow

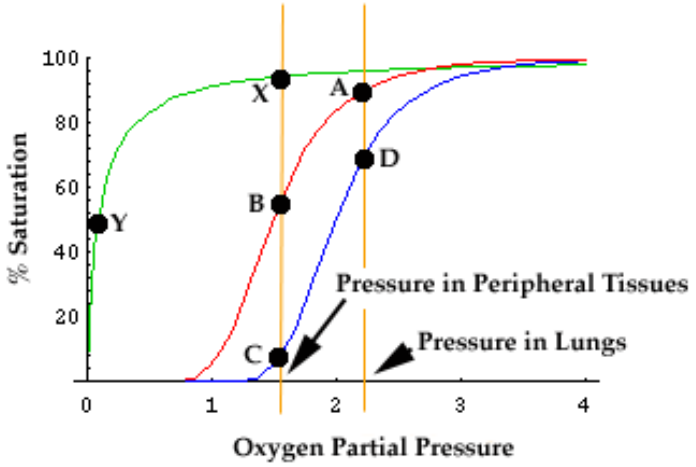
1. O_2 diffuses from the alveoli of the lungs into the capillaries of the bloodstream then into the red blood cells

2. In the red blood cells, O_2 binds to hemoglobin.

3. In parallel, CO_2 diffuses from blood into the alveoli.

4. The lower concentration of dissolved CO_2 in the blood causes higher pH (~7.6) in lungs than in the peripheral tissues (~pH 7.2) where CO_2 is being actively released.

A. High pO_2 / high pH



Why O_2 Transport Works

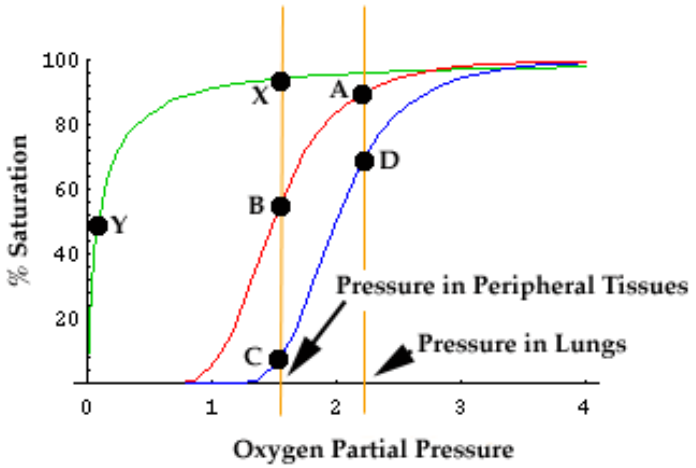
5. Red blood cells (containing O_2-hemoglobin) carried to the peripheral tissues.

- **B. pO_2 decreases because O_2 USED by the tissues.**
- **C. Blood plasma becomes more acidic (lower pH) because CO_2 is released.**

The combination of lower pO_2 and pH in the peripheral tissues causes a large decrease in O_2 saturation.

O_2 is released by hemoglobin!!!!

Note: changes in pO_2 and pH are small!



Why Myoglobin in Muscle?

- **Under resting conditions, O_2 saturation is at point X on the green curve**
- **Small changes in pO_2 and pH have very little effect on saturation**
- **During extremely vigorous exercise, heart pumps blood fast and breathing is rapid to increase the intake of O_2 . Also, pH is lowered.**
- **Eventually, transport not fast enough to meet needs, i.e. pO_2 lowered because O_2 is used faster than it can be replenished. [Hemoglobin now no help!]**
- **Under extreme conditions, shift from point X to Y: saturation of the myoglobin is lowered = release of O_2.**

Defects from Hemoglobin Mutations

1. **Weakened heme binding.**
2. **Disruption of secondary structure.**
3. **Disruption of quaternary structure.**
4. **Defective oxygen transfer.**
5. **Altered affinity for oxygen.**
6. **Oxidation of Fe(II) to Fe(III).**
7. **Aggregation in the T state (Hemoglobin S). Sickle cell anemia results from aggregation of Hb into insoluble fibers causing misshapen blood cells that cannot pass through capillaries and block blood flow to tissues.**

Heme Proteins II:

Dioxygen activation: Hydroperoxidases

Assigned readings:

Bertini Book, Chapter XI: XI1, XI3

Hemerythrin

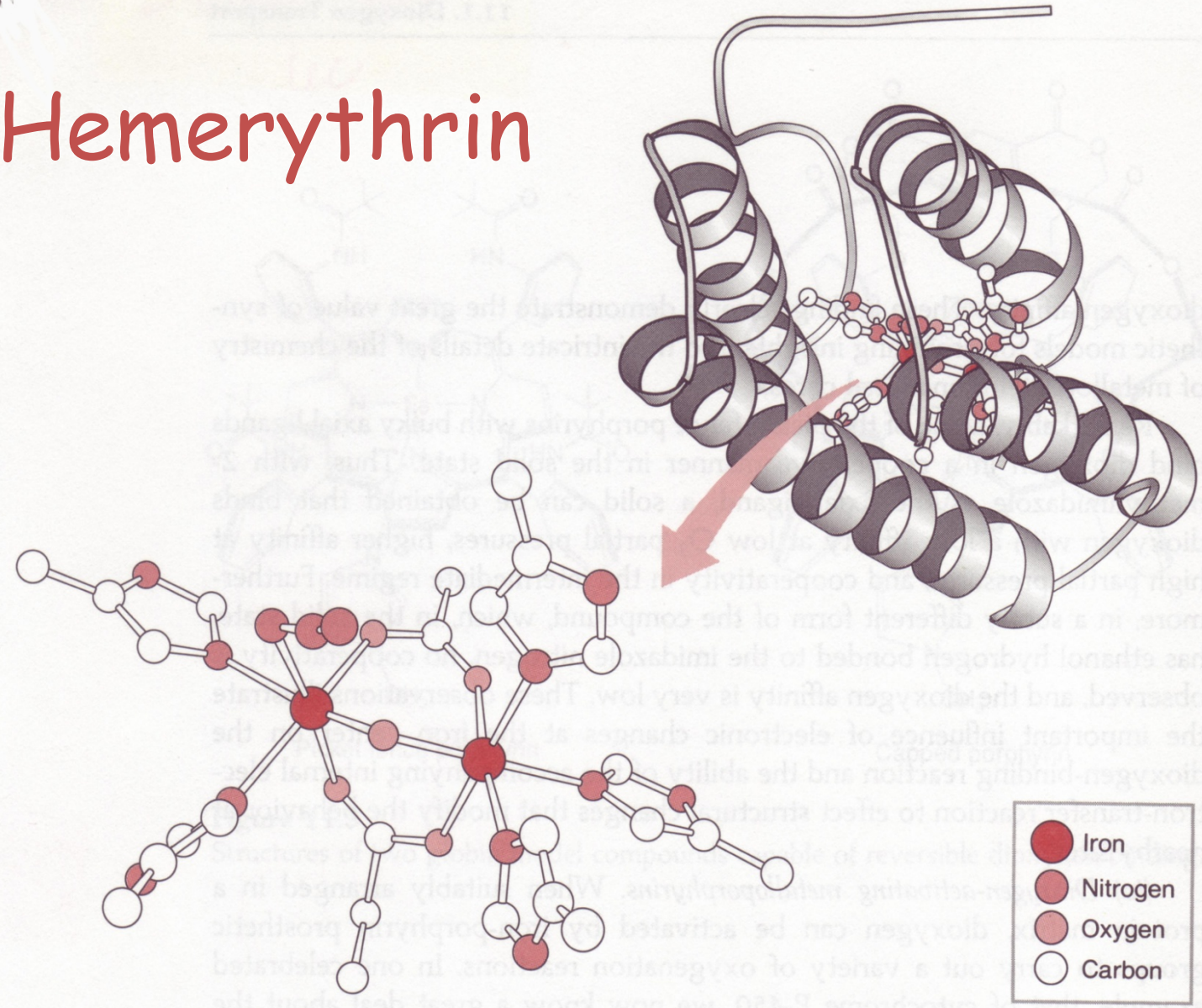


Figure 11.4
Structure of azidomethemerythrin and its dinuclear iron core. The N_3^- ion resides in the site occupied by dioxygen in oxyhemerythrin.

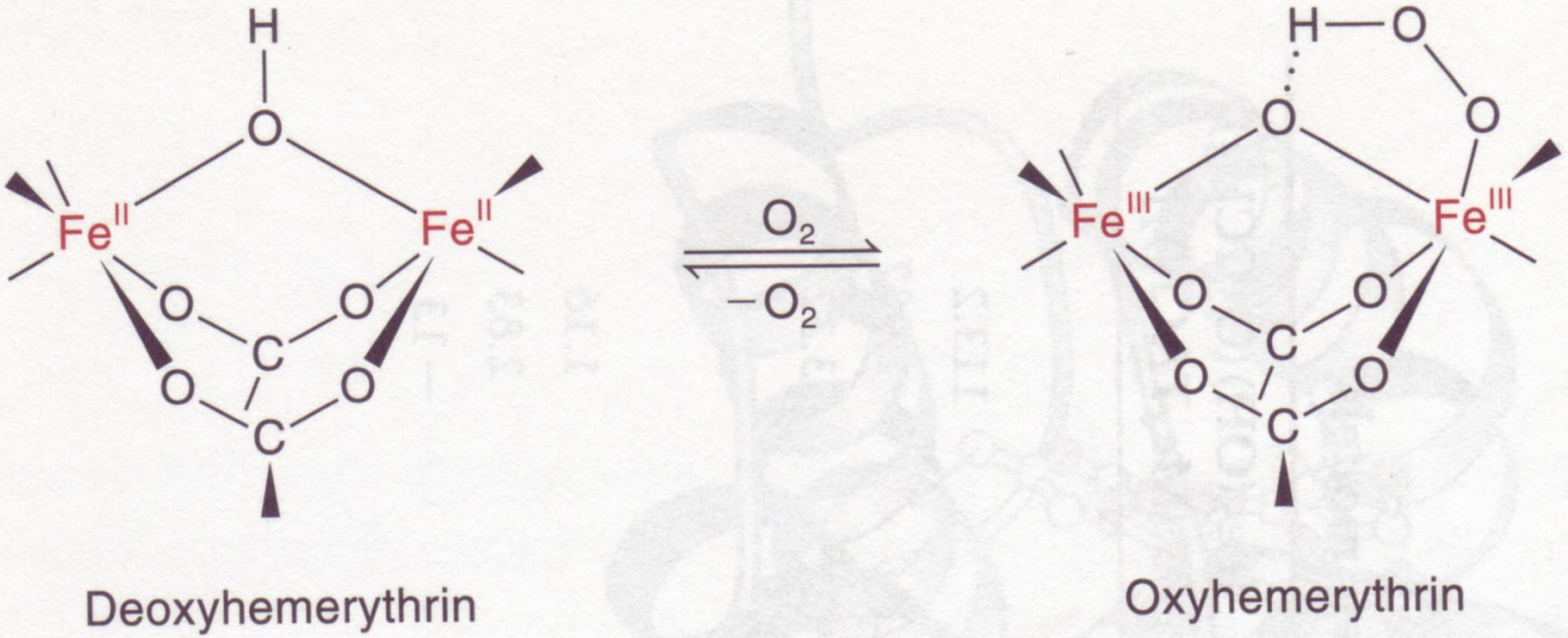
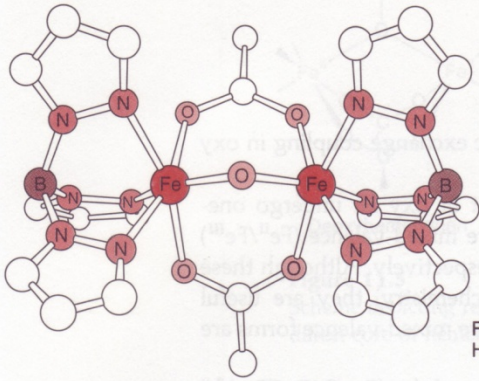
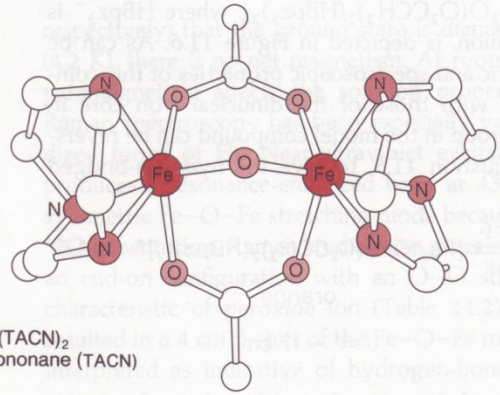


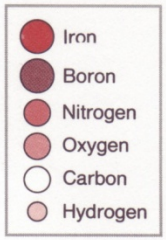
Figure 11.5
Scheme depicting redox and structural changes that occur in the diiron core of hemerythrin upon reversible dioxygen binding.

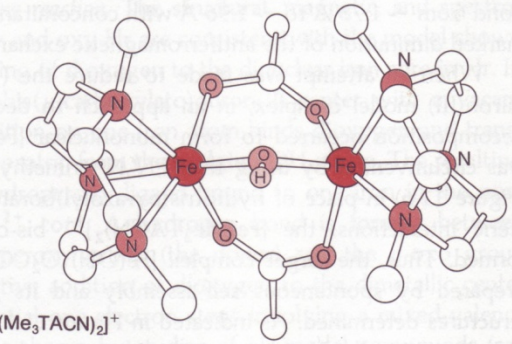


$Fe_2O(O_2CCH_3)_2(HBpz_3)_2$
Hydrotris(1-pyrazoyl)borate($HBpz_3$)



$Fe_2O(O_2CCH_3)_2(TACN)_2$
1,4,7-Triazacyclononane (TACN)





$[Fe_2(OH)(O_2CCH_3)_2(Me_3TACN)_2]^+$

Figure 11.6
Structures of two synthetic models (top, middle) for the oxidized form of hemerythrin and one (bottom) for the reduced form.

Hemocyanin

and

Oxyhemocyanin

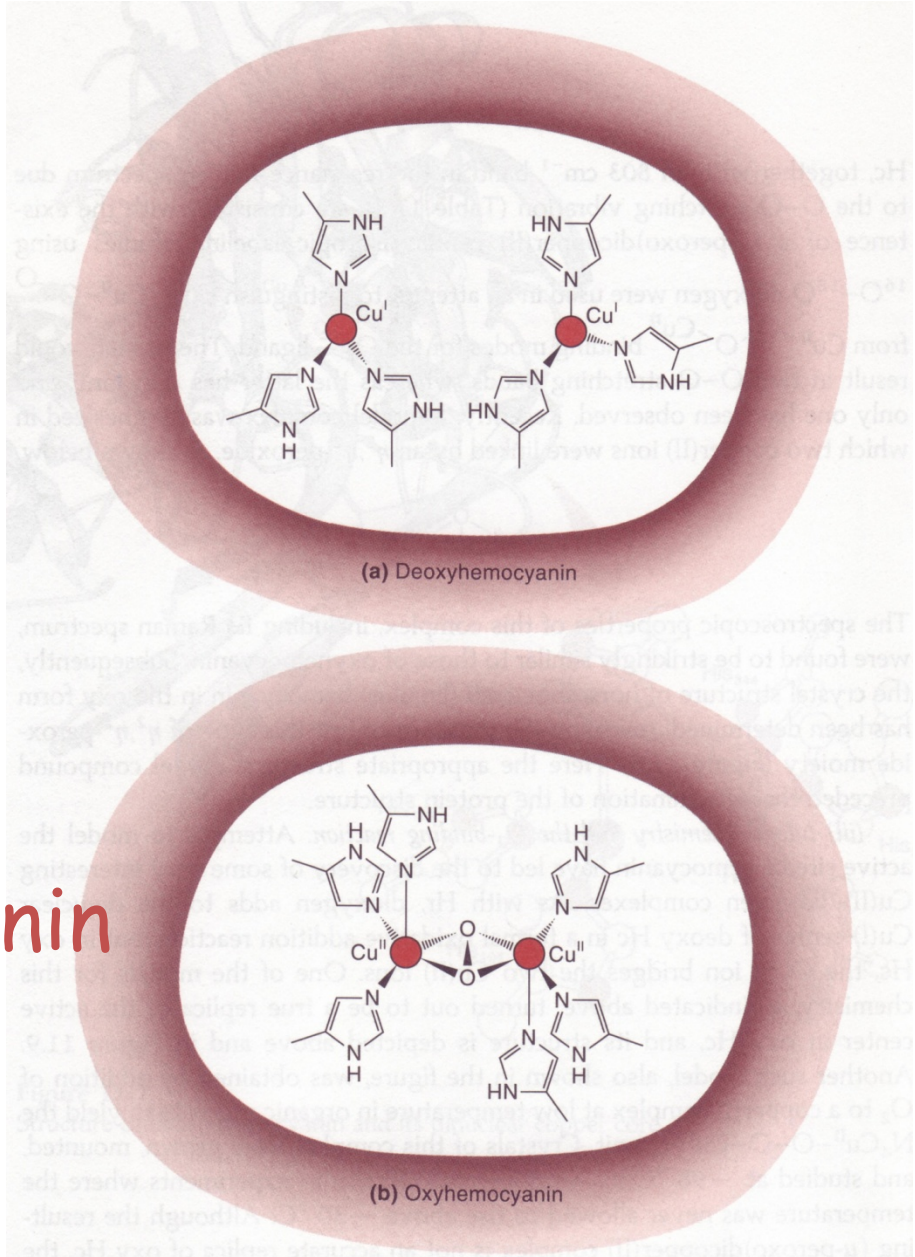
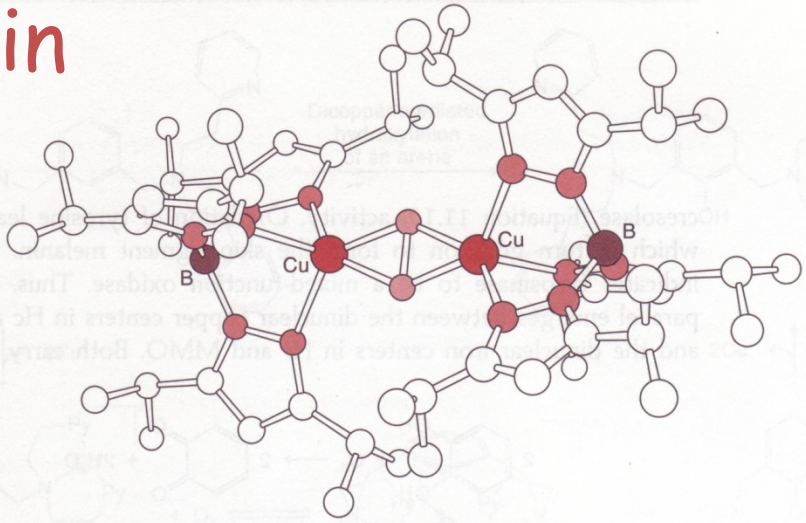
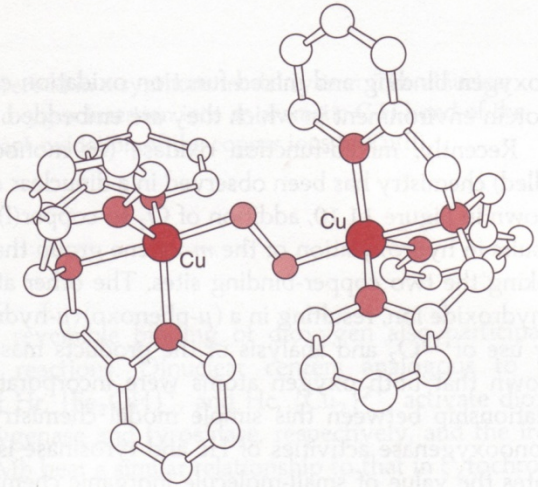


Figure 11.8
Schematic views of the structures of deoxyhemocyanin (top) and oxyhemocyanin (bottom) showing the binding of dioxygen as an η^2, η^2-peroxide.

Oxyhemocyanin Models.



$[Cu(HB(3,5\text{-}i\text{-}Pr_2pz)_3]_2\ (O_2)$



$[\{LCu\}_2O_2]^{2+}$

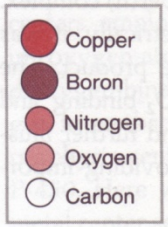


Figure 11.9
Two oxyhemocyanin models, one (top) containing the η^2, η^2-structure found in the protein and the other (bottom) having an alternative η^1, η^1-bridged attachment.