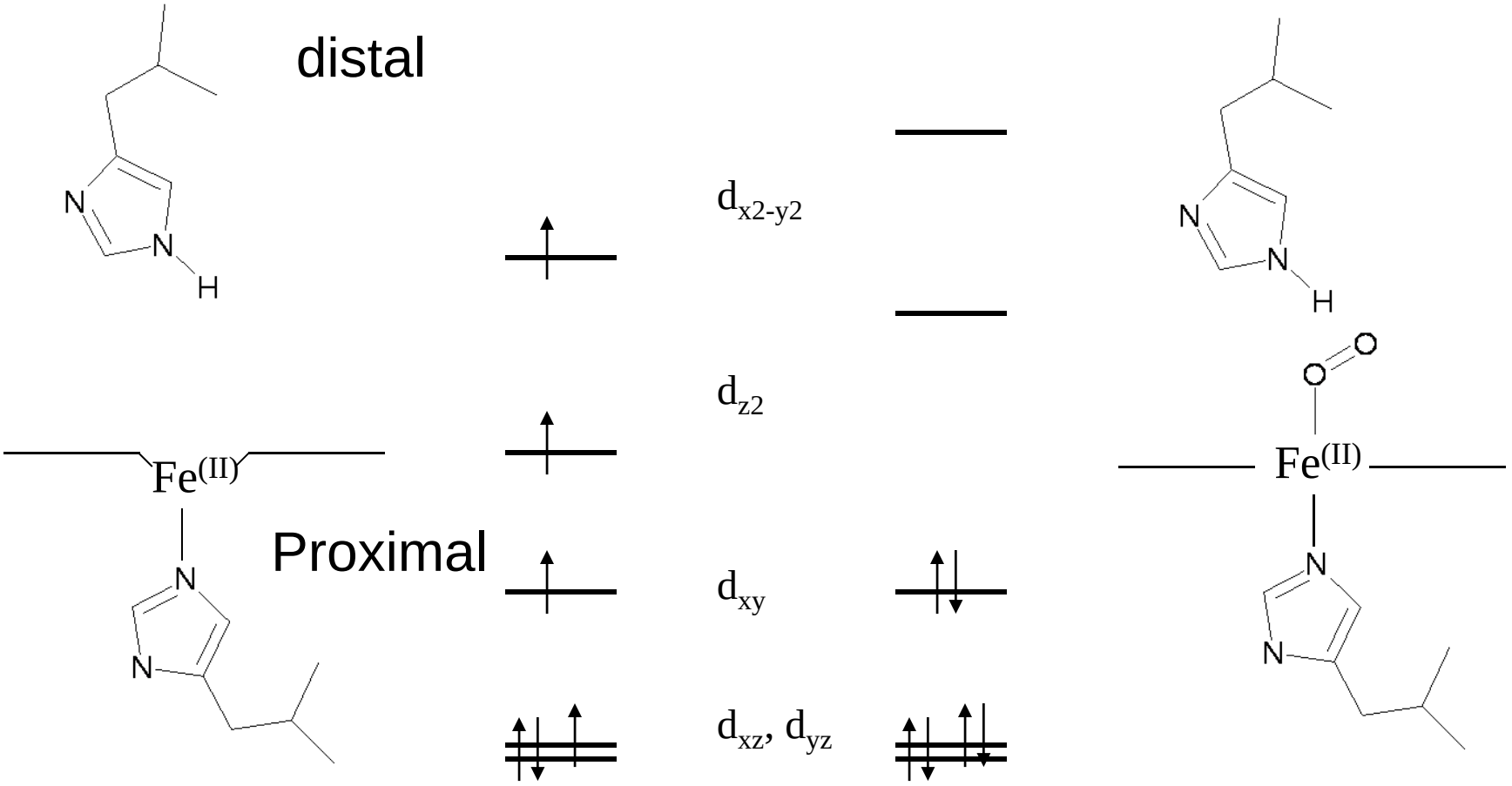
Mode of O_2 Binding in Myoglobin



$Fe^{(II)}$(HS) ionic radius = 78 pm

$Fe^{(II)}$(LS) ionic radius = 61 pm

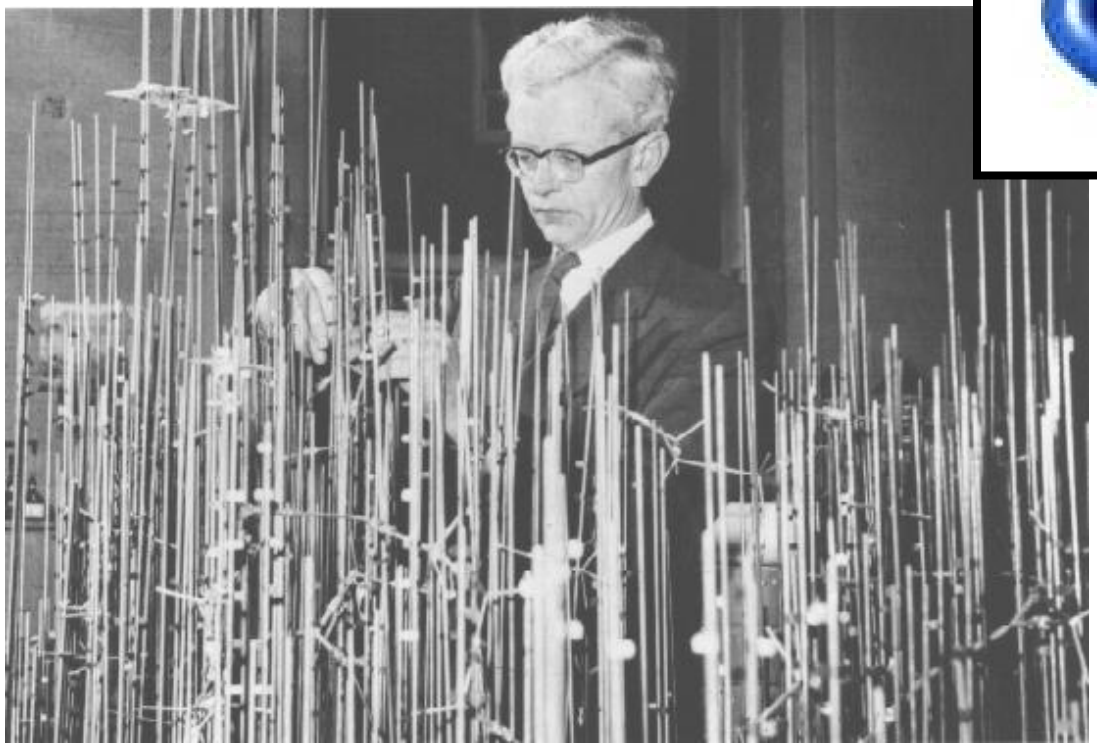
$Fe(II) + O_2 = Fe(II)\text{-}O_2 = Fe(III)\text{-}O_2^-$

Hemoglobin- Key Properties

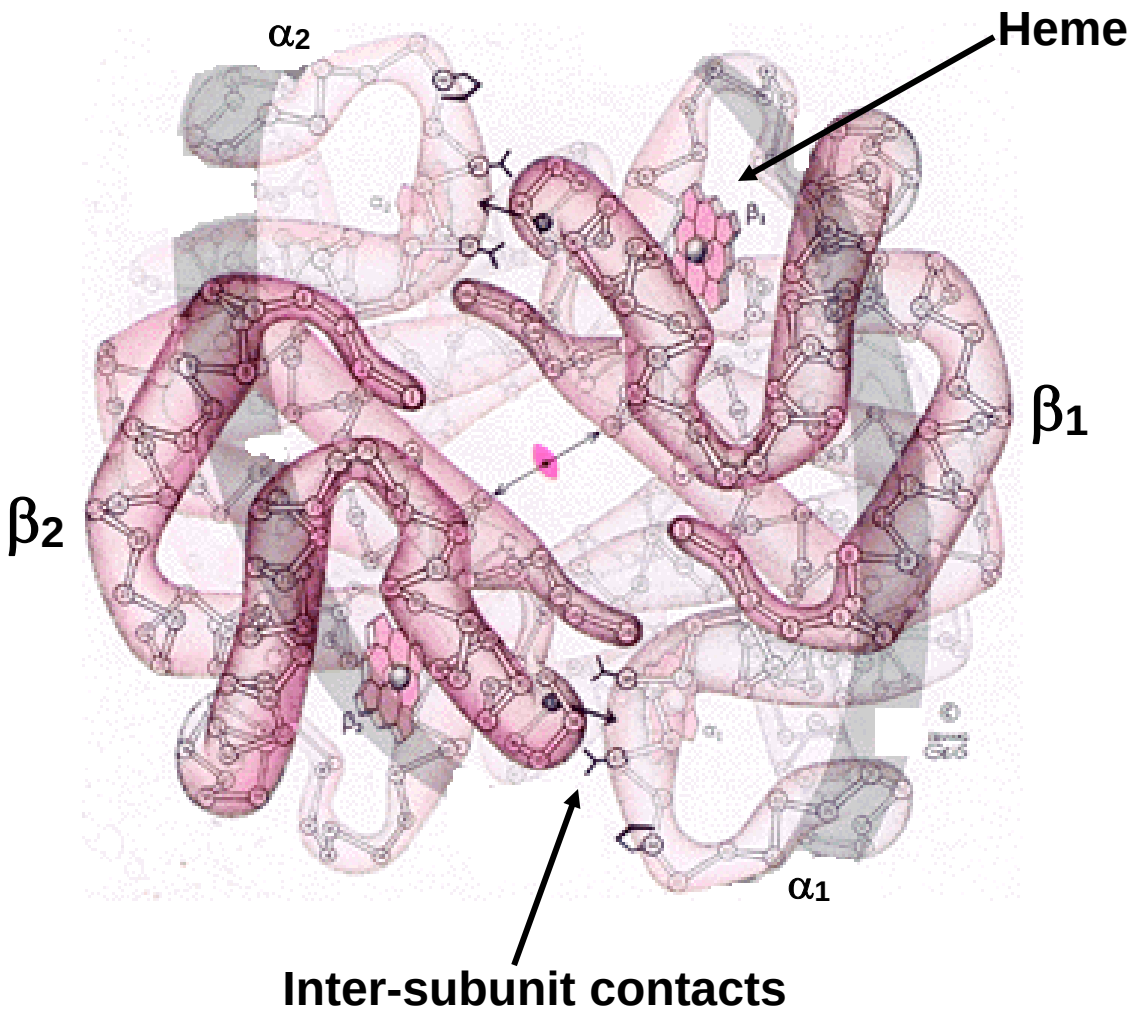
- **Ubiquitous O_2 transport protein**
- **A globular soluble protein, 2X2 chains (164 kDa)**
- **α and β chains 44% identical**
- **All helical secondary structure (like myoglobin)**
- **$\alpha\beta\alpha\beta$ quaternary structure**
  - **α-subunit 141 residues**
  - **β-subunit 146 residues**
- **Extensive 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

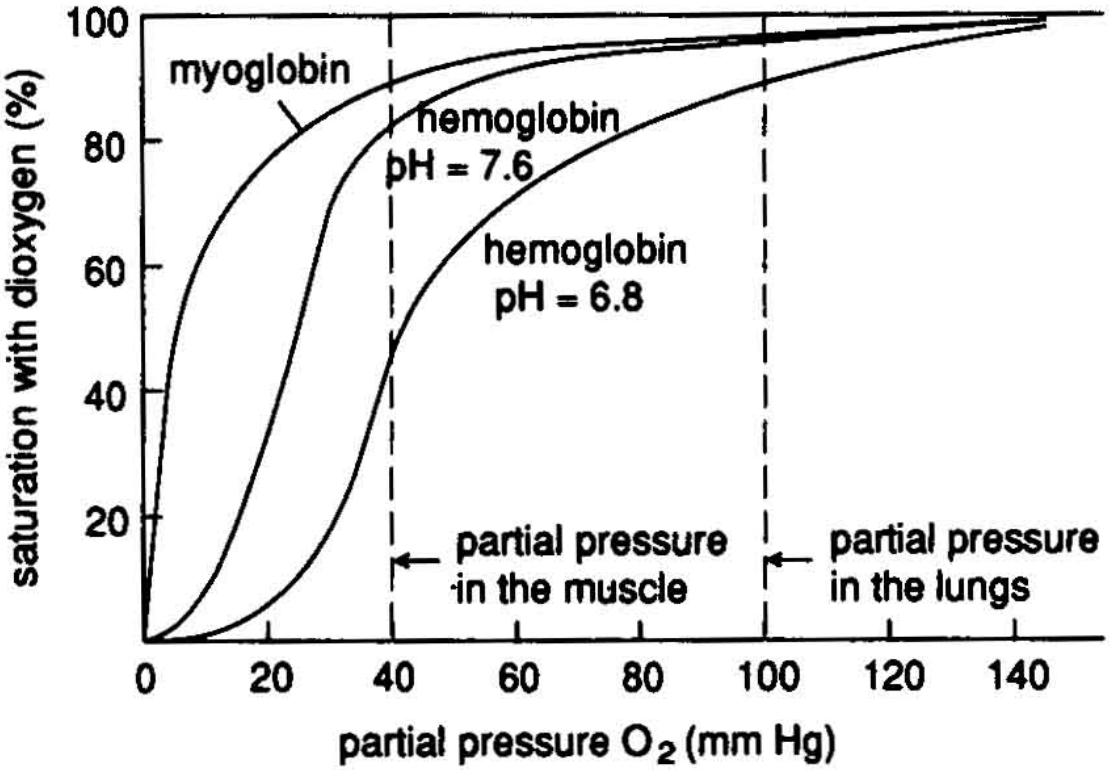


Figure 5.3
Oxygen saturation curves of myoglobin and hemoglobin at different pH values

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

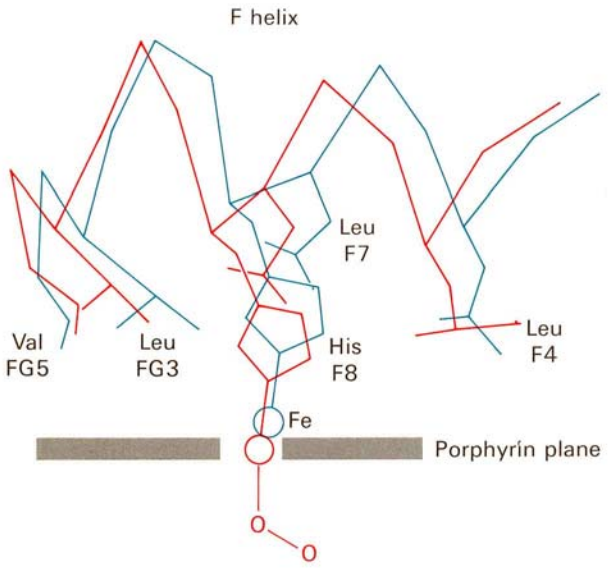


Figure 7-34
Conformational changes induced by the movement of the iron atom on oxygenation. The oxygenated structure is shown in red and the deoxygenated structure in blue. [After J. Baldwin and C. Chothia. *J. Mol. Biol.* 129(1979):192.]

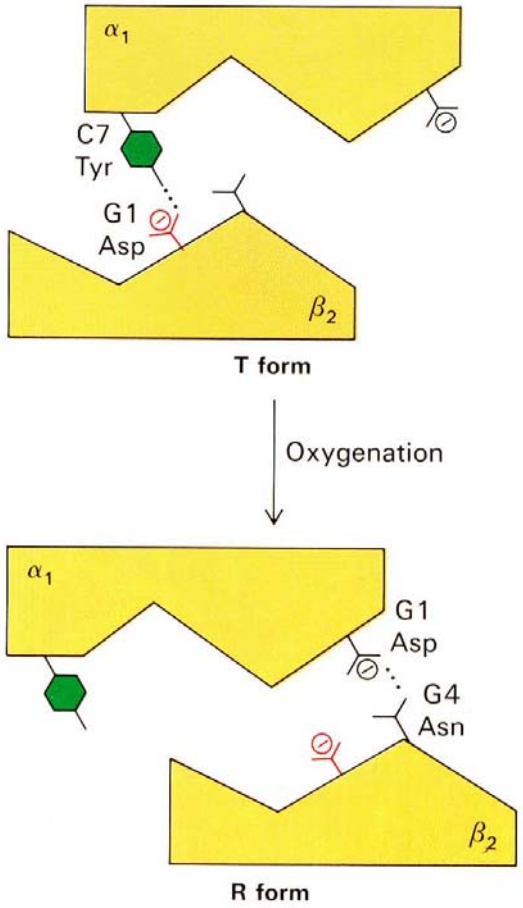
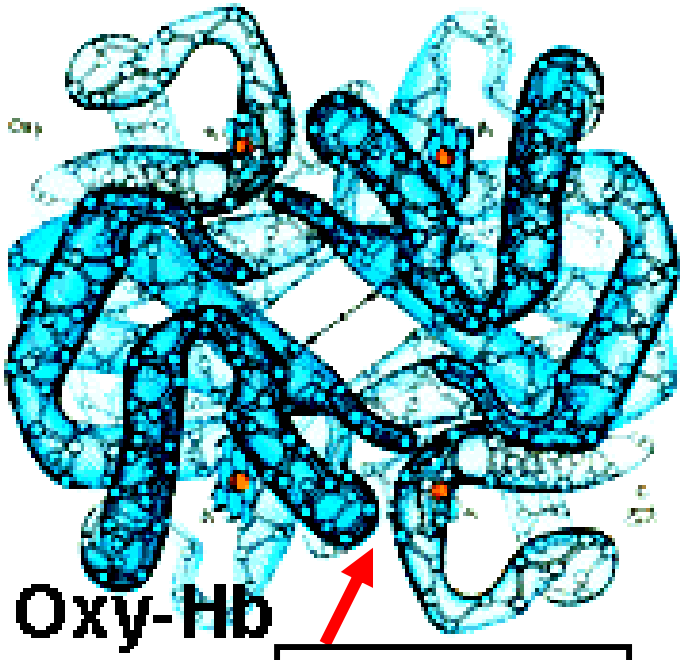


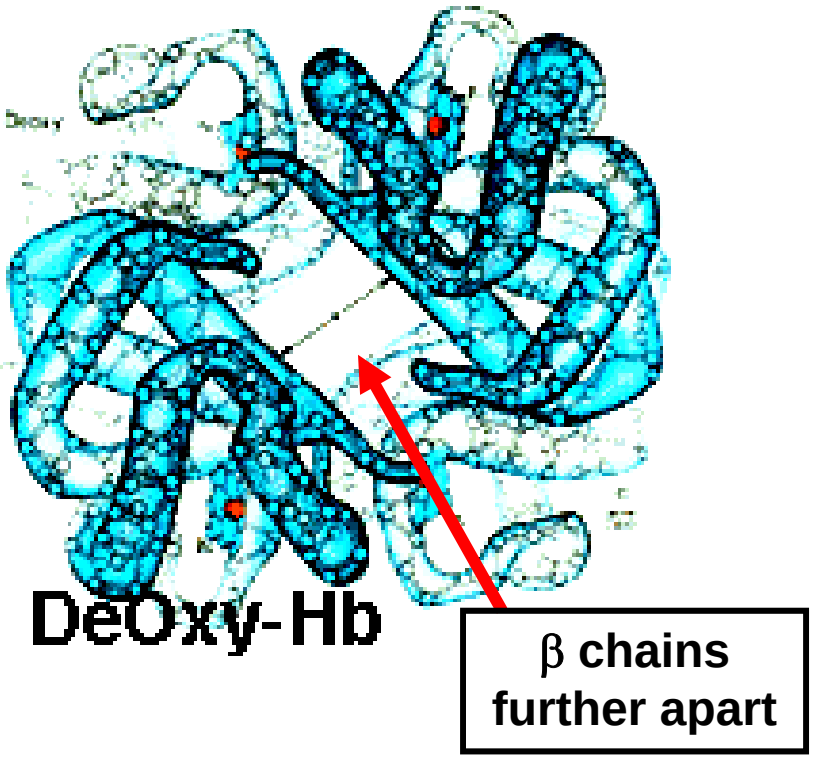
Figure 7-31
The $\alpha_1\beta_2$ interface switches from the T to the R form on oxygenation. The dove-tailed construction of this interface allows the subunits to readily adopt either of the two forms.

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.

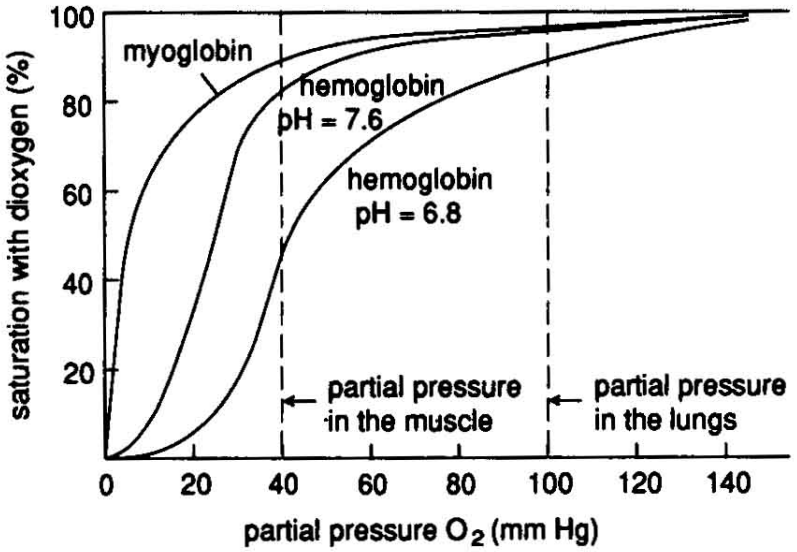


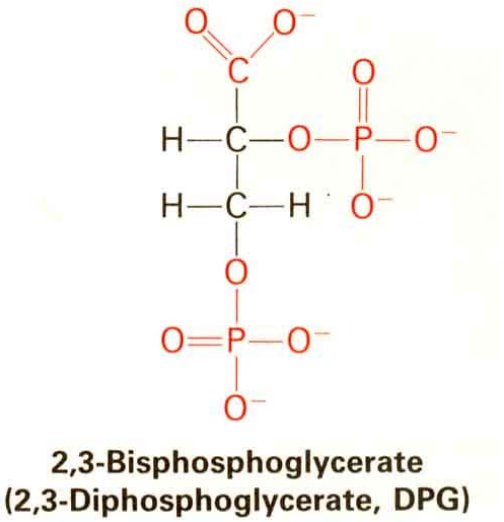
Figure 5.3
Oxygen saturation curves of myoglobin and hemoglobin at different pH values

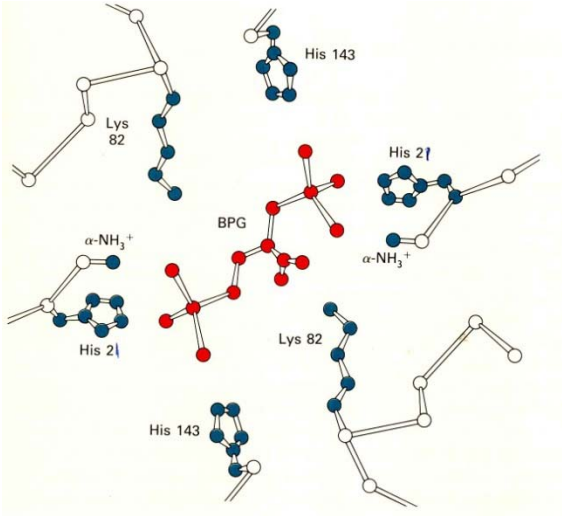
2. CO_2. Release of CO_2 lowers pH via conversion to HCO_3^-:

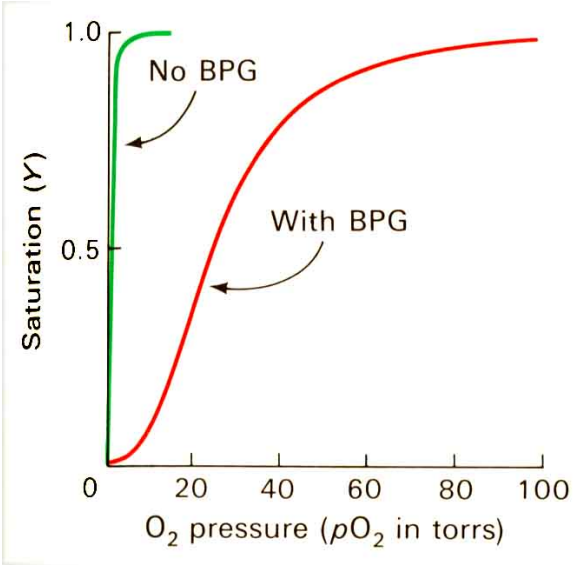
$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$. Reinforces Bohr Effect

Allosteric Effects

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



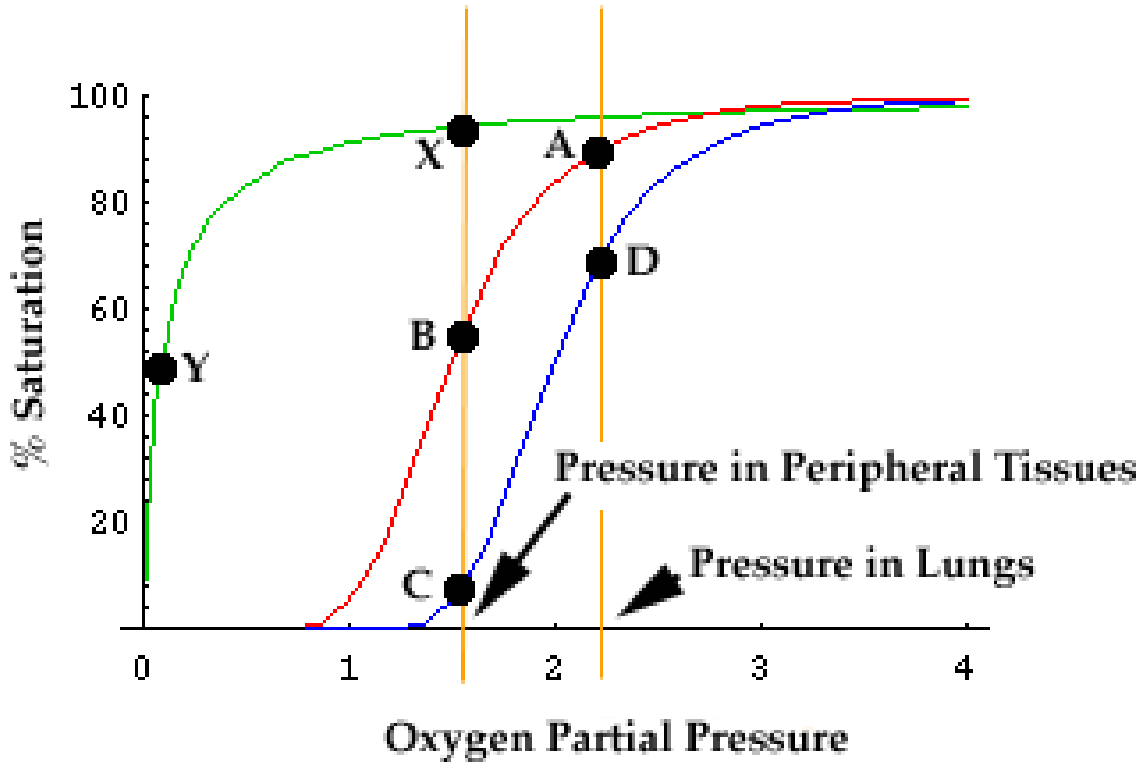




Increase in levels of BPG helps adaptation to high altitude- faster than making more hemoglobin.

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

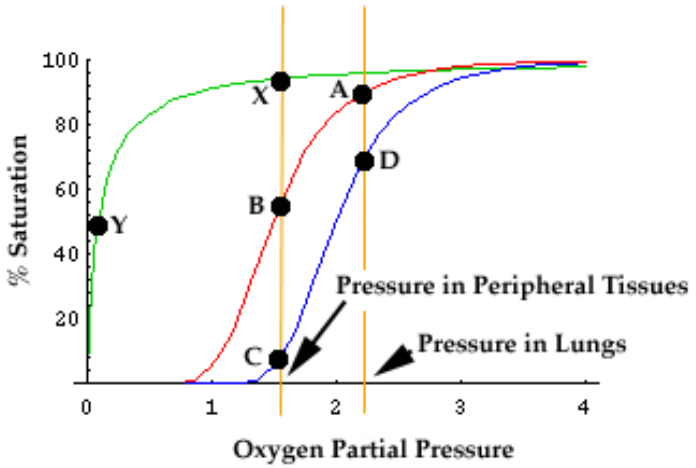
1. O_2 diffuses from the alveoli of the lungs into the capillaries of the bloodstream then into the red blood cells

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.

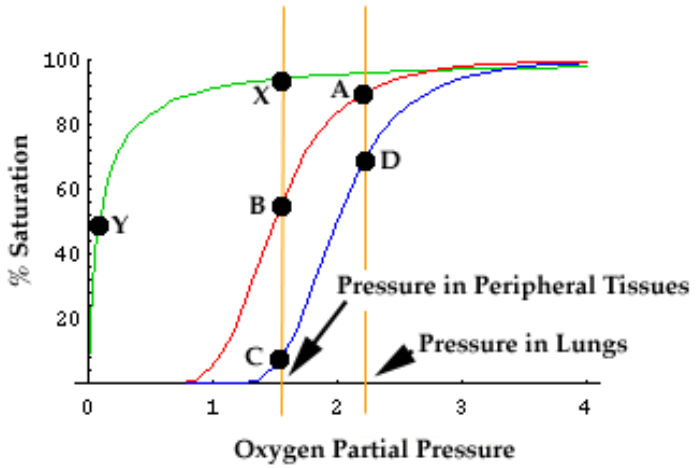
B. pO_2 decreases because O_2 USED by the tissues.

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

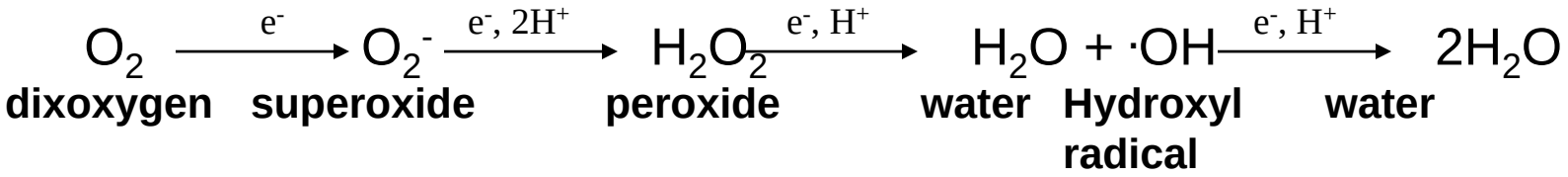
Assigned readings:

Bertini Book, Chapter XI: XI1, XI3

Dioxygen Reactions

1. Importance of O_2 reaction
   Energy (respiration)
   Activation of C-H bond (functional group)

2. O_2 redox chemistry: O_2 is a powerful oxidant!



Reaction	E^o, V vs. NHE, pH 7, 25°C
$O_2 + e^- \rightarrow O_2^-$	-0.33
$O_2^- + e^- + 2H^+ \rightarrow H_2O_2$	+0.89
$H_2O_2 + e^- + H^+ \rightarrow H_2O + OH$	+0.38
$OH + e^- + H^+ \rightarrow H_2O$	+2.31
$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$	+0.281
$H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O$	+1.349
$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$	+0.815

So why does O_2 not react with everything?

Kinetics of Dioxygen Reactions

1. Small theromodynamic barrier

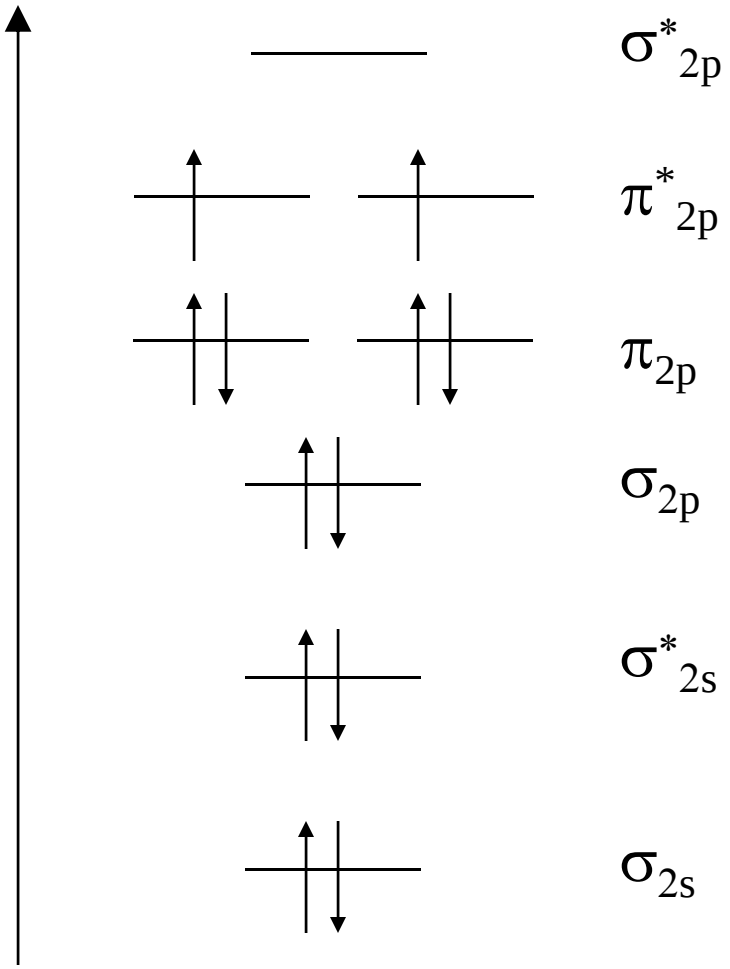
$O_2 + e^- \rightarrow O_2^-$ $\quad E° = - 0.33$ V

2. Large kinetic barrier:

slow

$\frac{1}{2}\ {}^3O_2 + {}^1X \rightarrow {}^1XO$

Triplet dioxygen	Singlet substrate	Singlet product



Solutions to increase O_2 reactivity

1. through excited triplet state

1) $\frac{1}{2}\ {}^3O_2 + {}^1X \rightarrow {}^3XO$

2) ${}^3XO \xrightarrow{\text{slow}} {}^1XO$

$E_{activation}$ > 40-70 kcal/mol

2. Through excited singlet O_2

1) ${}^3O_2 \rightarrow {}^1O_2$

2) $\frac{1}{2}\ {}^1O_2 + {}^1X \rightarrow {}^1XO$

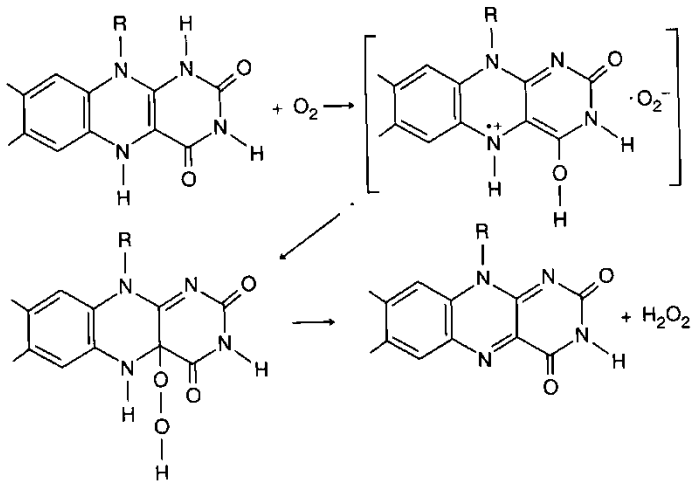
$E_{activation}$ > 22.5 kcal/mol

Solutions to increase O_2 reactivity

3. Through electron transfer (reduction)

1) $^3O_2 + {}^1X \rightarrow {}^2O_2^- + {}^2X^+$

2) $^2O_2^- + {}^2X^+ \rightarrow {}^2O_2^- + {}^2X^+ \rightarrow {}^1XO_2$



Need an unusually strong reductant

Solutions to increase O_2 reactivity

4. Organic radical reactions

Initiation: $X_2 \rightarrow 2X\cdot$

$X\cdot + RH \rightarrow XH + R\cdot$

Propagation: $R\cdot + O_2 \rightarrow ROO\cdot$

$ROO\cdot + RH \rightarrow ROOH + R\cdot$

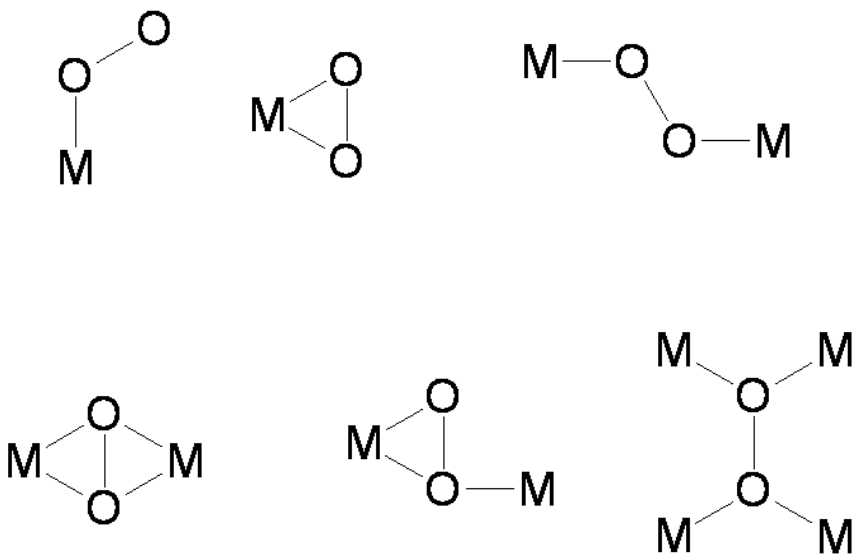
Termination: $R\cdot + ROO\cdot \rightarrow ROOR$

$2ROO\cdot \rightarrow ROOOOR \rightarrow O_2 + ROOR$

Needs initiators
Difficult to control selectivitiy
Can damage biomolecules

Solutions to increase O_2 reactivity

5. Through metal centers



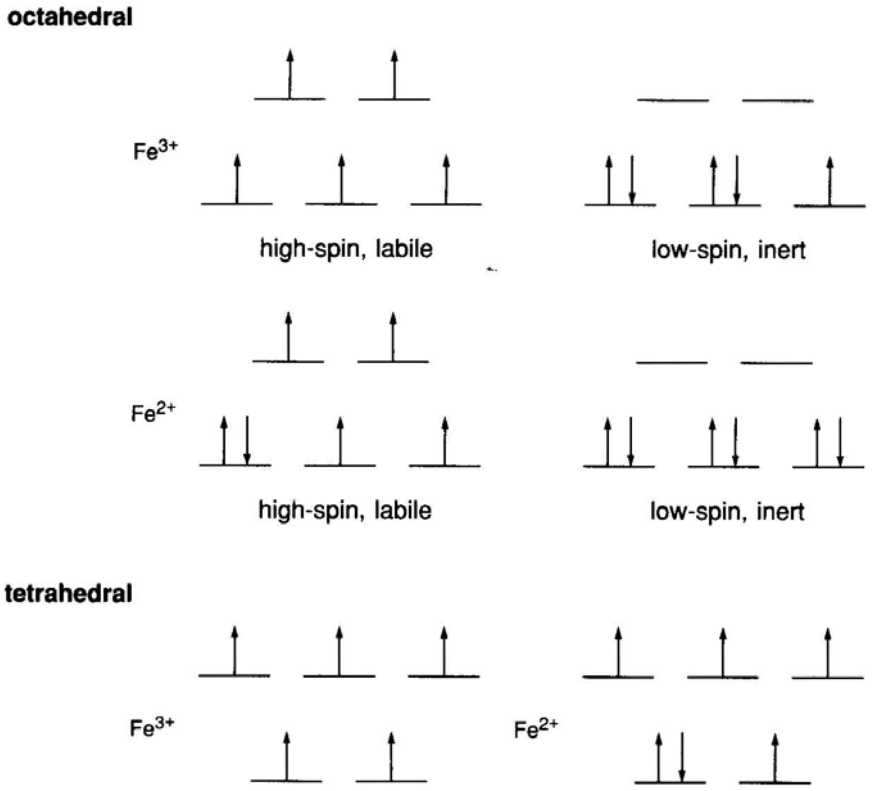
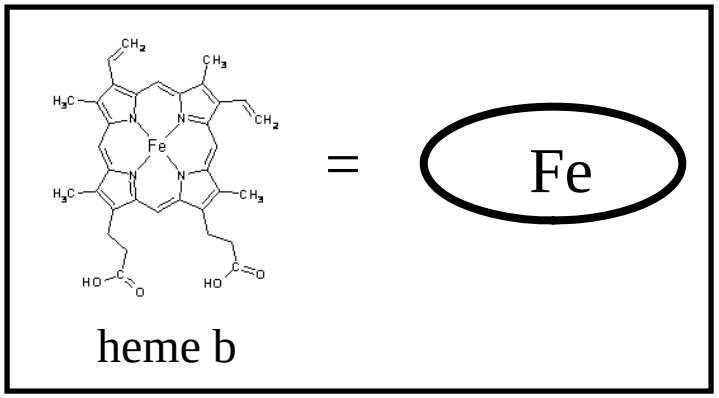
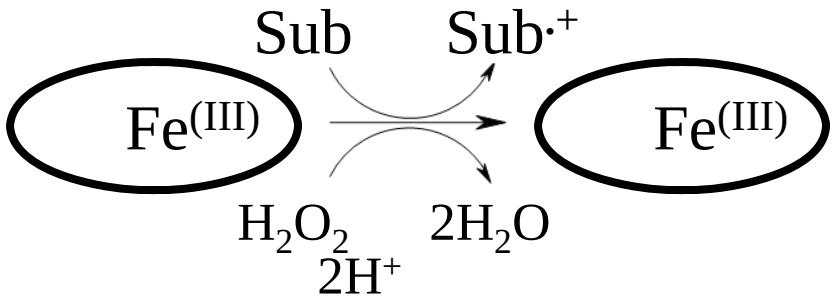


Figure 1.3
Versatility of Fe coordination complexes.

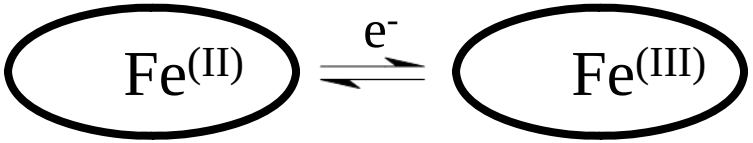
Paramagnetic metal ions can overcome spin restriction

Reactions of Heme Proteins

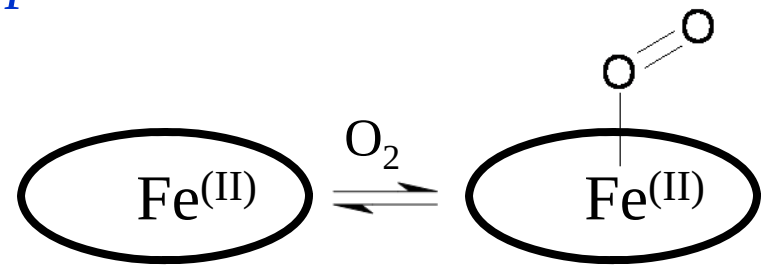




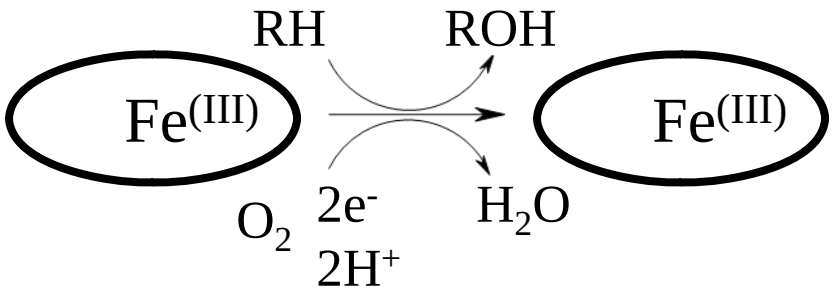
peroxidases



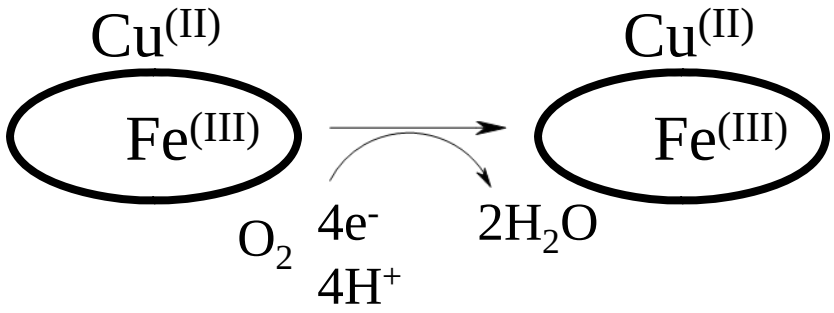
cytochromes



hemoglobin/myoglobin

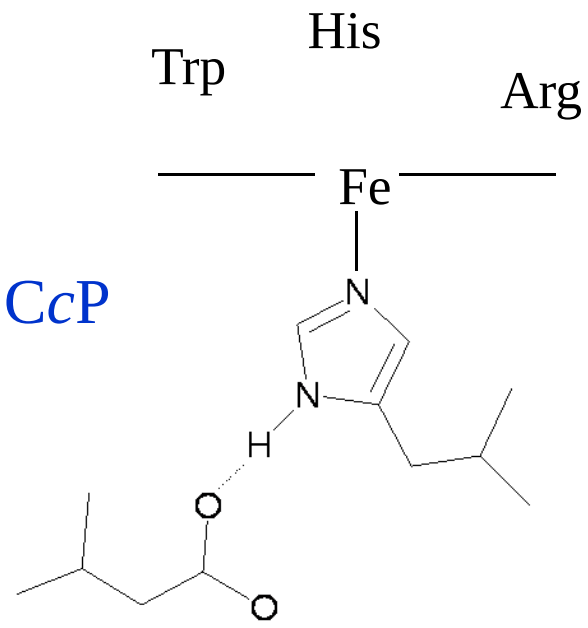


cytochromes P450

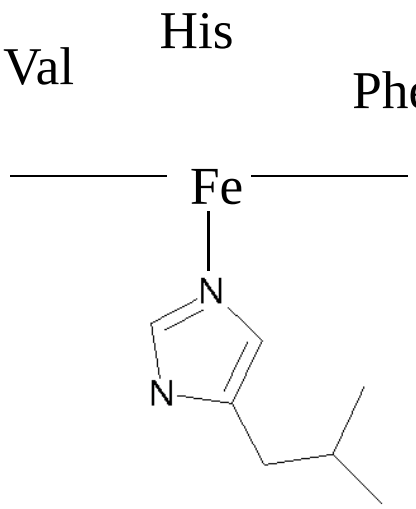


cytochrome c oxidase

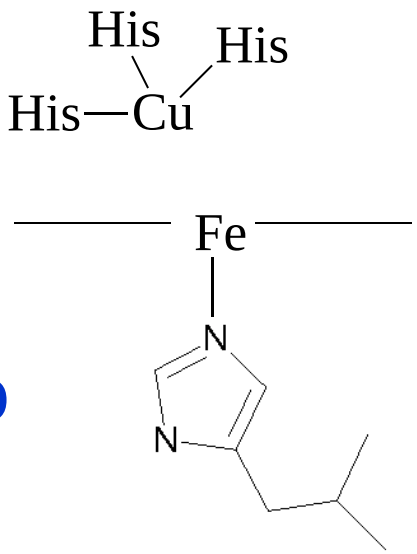
The Protein's Influence



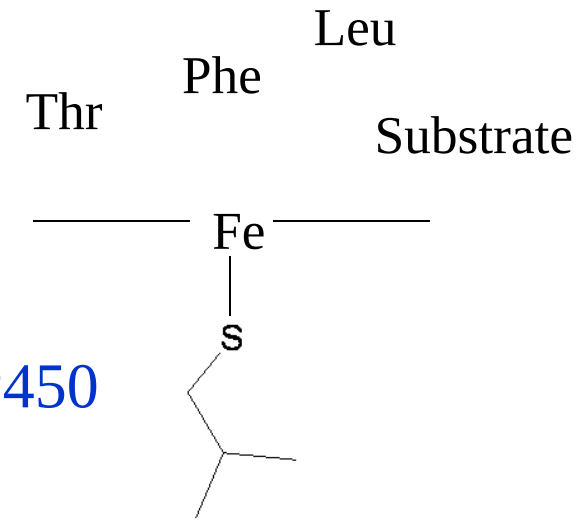
C*c*P



Mb



C*c*O



Cyt P450

Hydroperoxidase functions

1. Catalases

$$H_2O_2 + H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

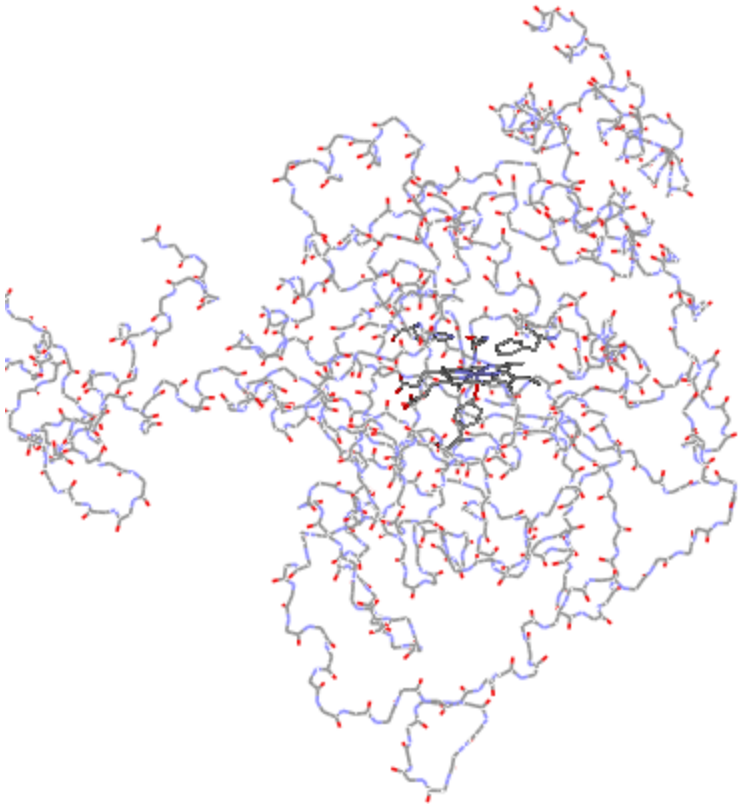
2. Peroxidases

$$H_2O_2 + AH_2 \xrightarrow{\text{peroxidase}} 2H_2O + A$$

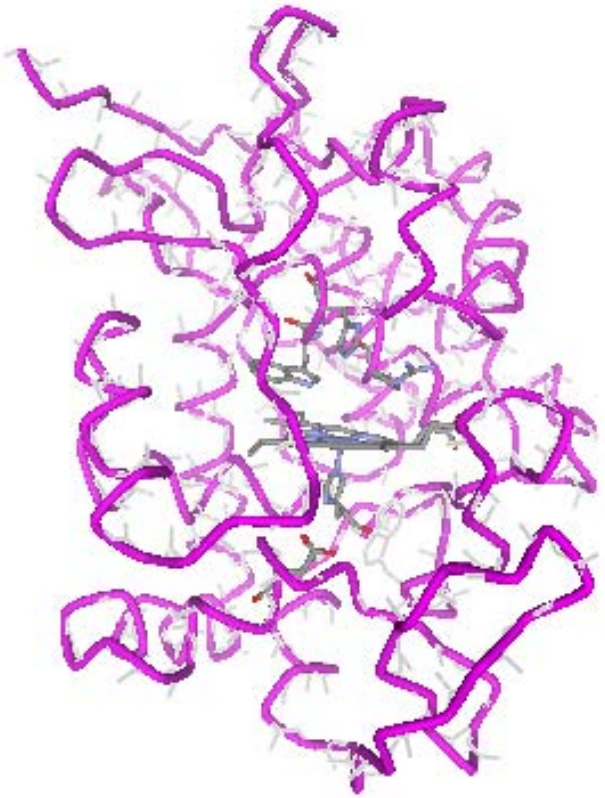
3. Haloperoxidases

$$H_2O_2 + H + X + HR \xrightarrow{\text{haloperoxidase}} 2H_2O + X\text{-}R$$

Hydroperoxidase structures

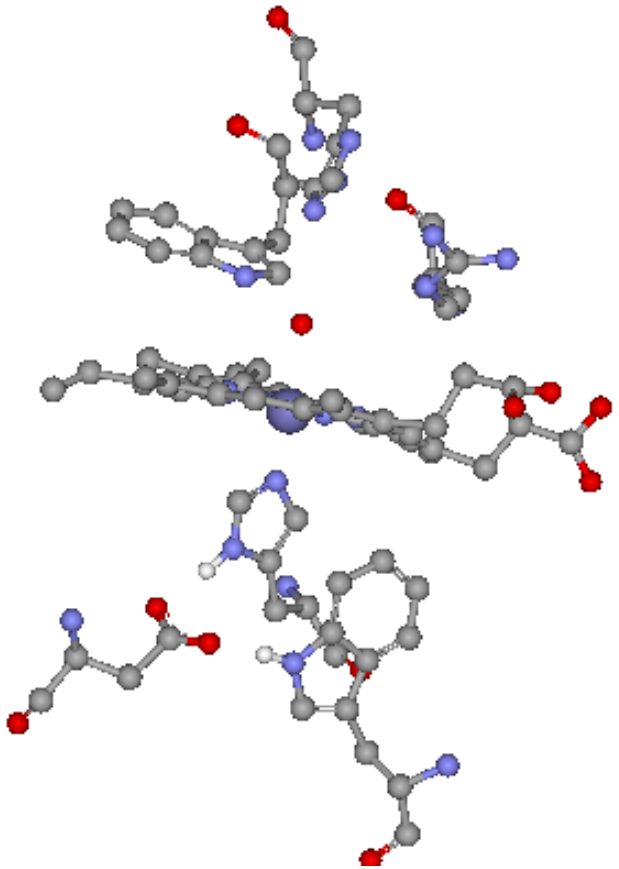


Catalase

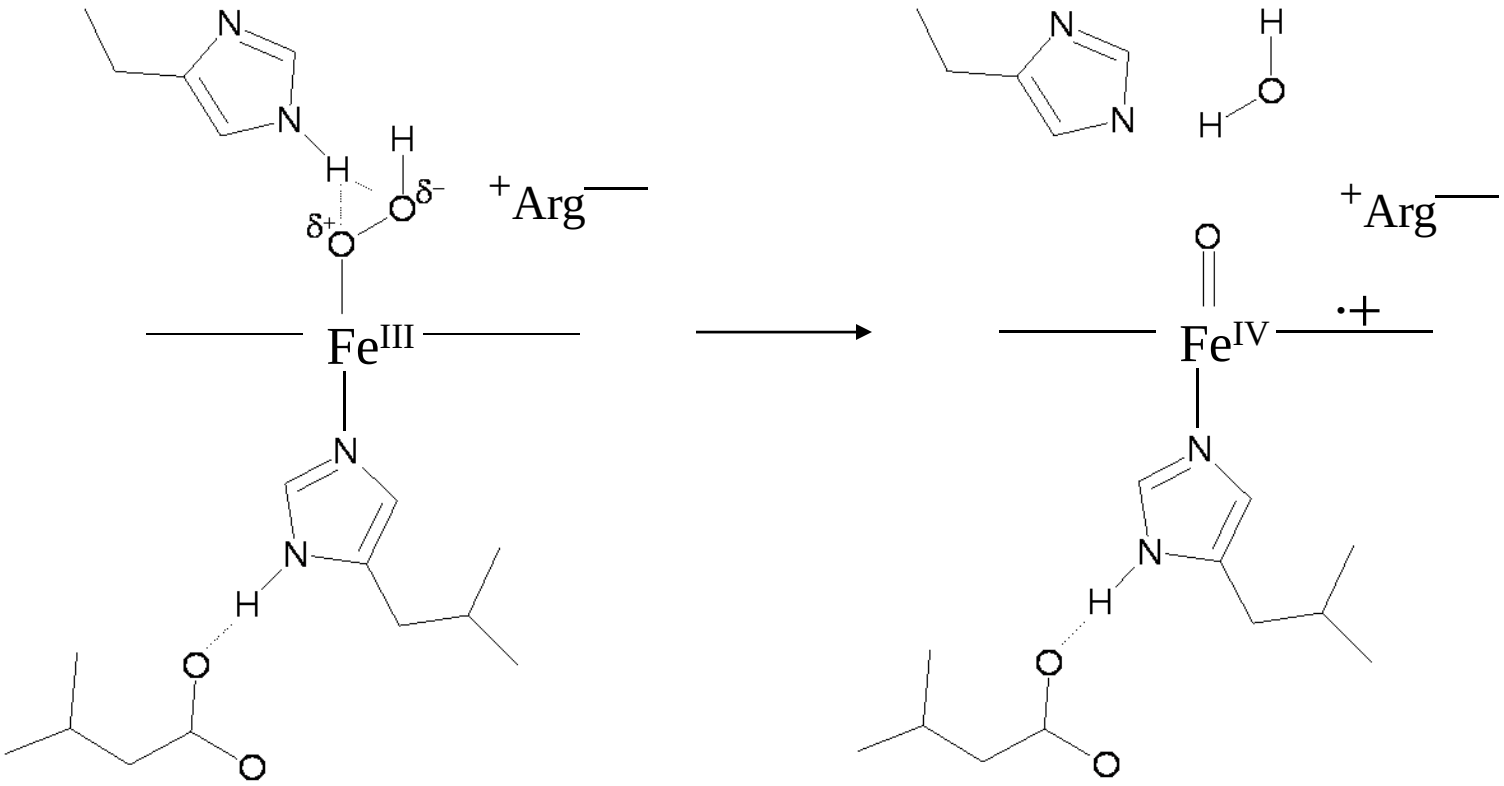


Cytochrome c peroxidase

CcP active site



Distal Effect



Mechanism

$$Fe^{III}(P)^{+} + H_2O_2 \rightarrow Fe^{III}(P)(H_2O_2)^{+} \rightarrow Fe^{IV}(P^{\cdot -})(O)^{+} + H_2O$$

compound I

Catalase: $Fe^{IV}(P^{\cdot -})(O)^{+} + H_2O_2 \rightarrow Fe^{III}(P)^{+} + H_2O + O_2$

Peroxidase: $Fe^{IV}(P^{\cdot -})(O)^{+} + AH_2 \rightarrow Fe^{IV}(P)(O) + HA\cdot + H^{+}$

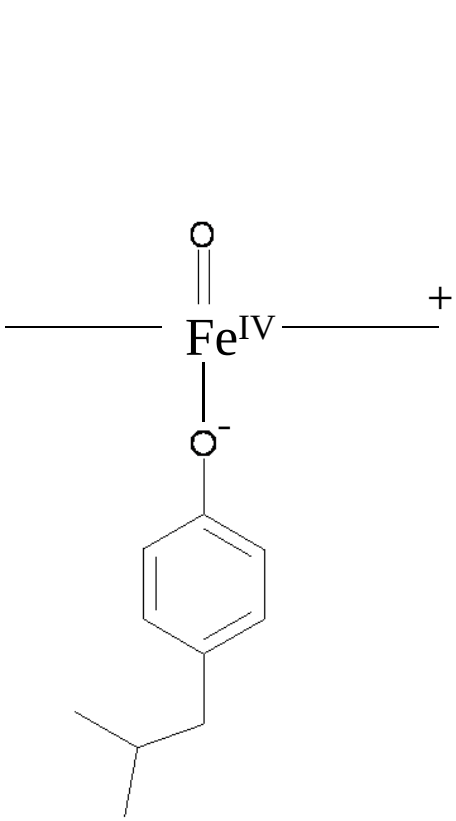
compound I compound II

$$Fe^{IV}(P)(O) + AH_2 \rightarrow Fe^{III}(P)^{+} + HA\cdot + OH^{-}$$

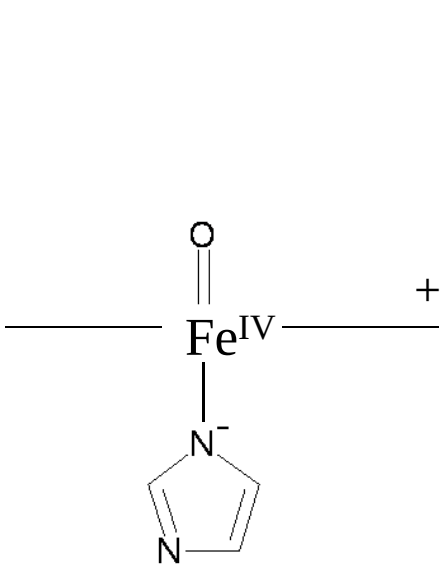
$$2HA\cdot \rightarrow A + AH_2$$

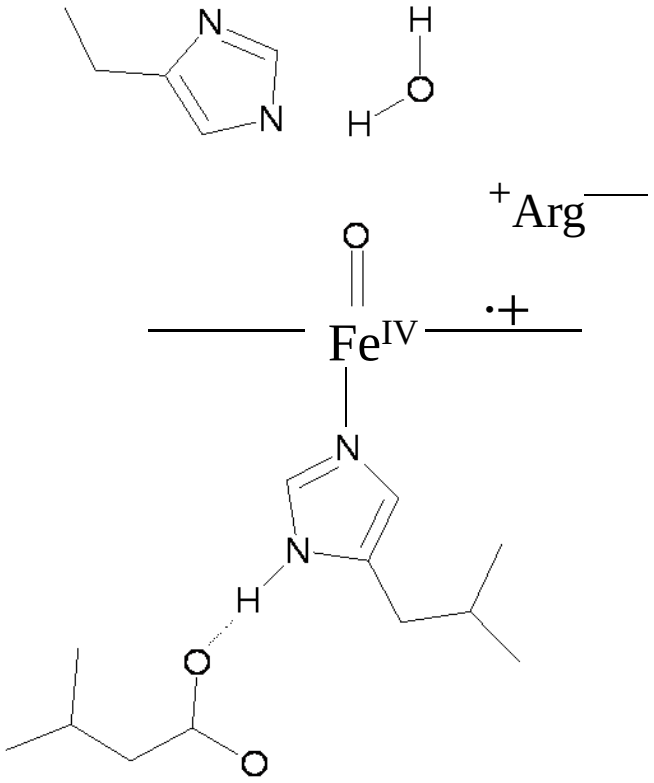
$$2HA\cdot \rightarrow HA\text{-}HA$$

Nature of Compound I



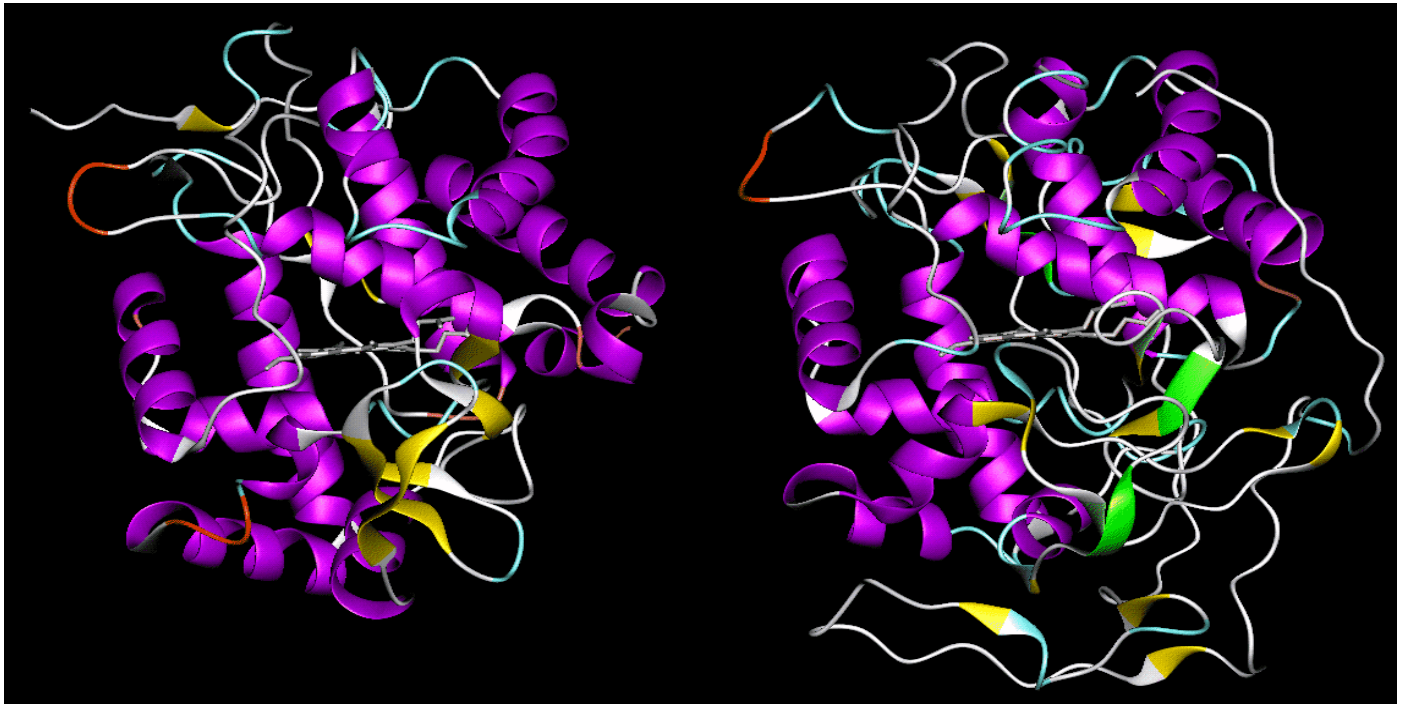
catalase





peroxidase

Two peroxidases: cytochrome c peroxidase (CcP) and Manganese peroxidase (MnP)



CcP

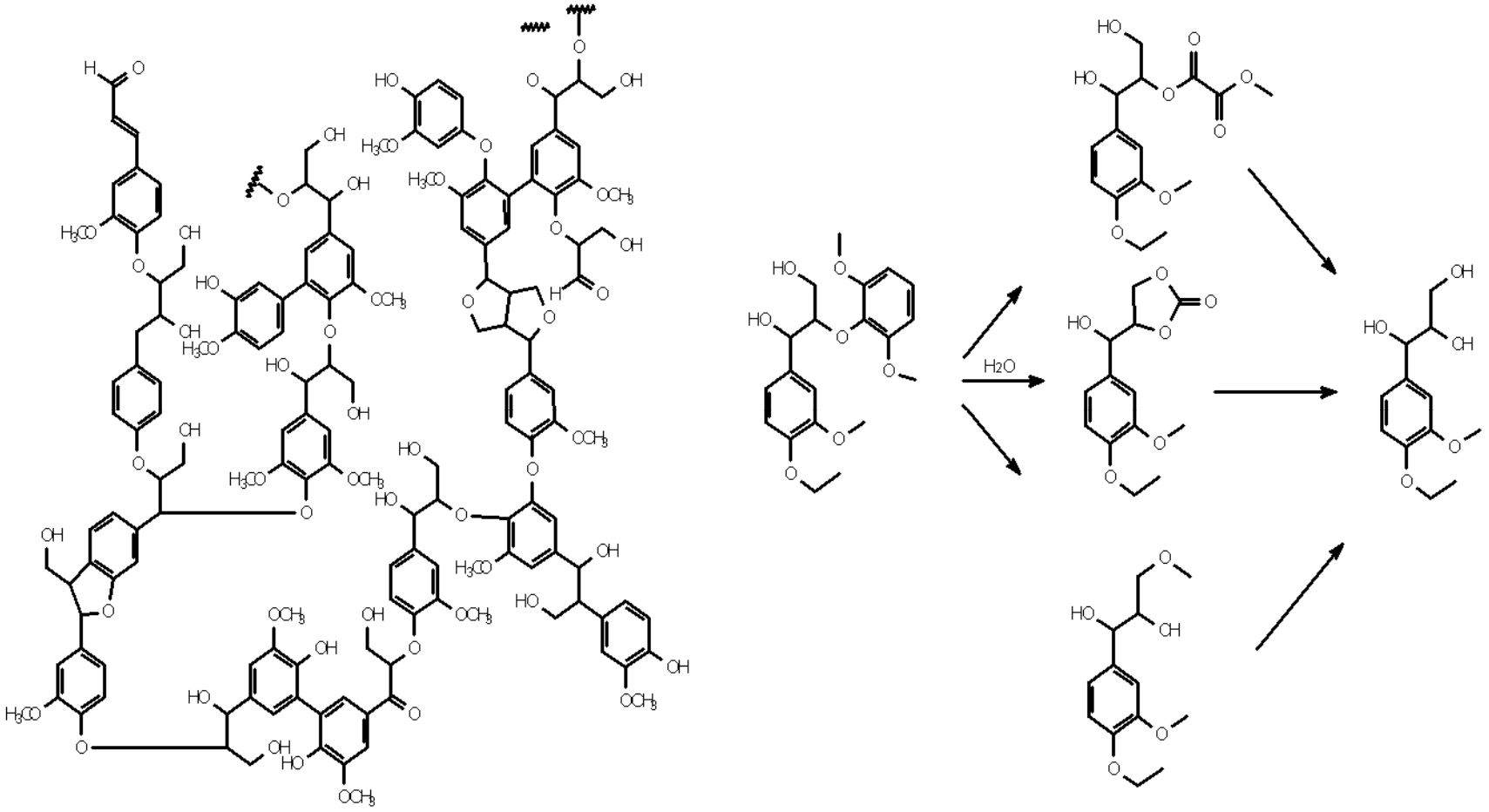
Oxidation of cyt *c*

MnP

Degradation of lignin and many aromatic pollutants

1. B. C. Finzel, T. L. Poulos, and J. Kraut, *J. Biol. Chem. 259,* 13027 (1984).
2. M. Sundaramoorthy, K. Kishi, M. H. Gold, and T. L. Poulos, *J. Biol. Chem.269*, 32759 (1994).

Lignin and Lignin Biodegradation



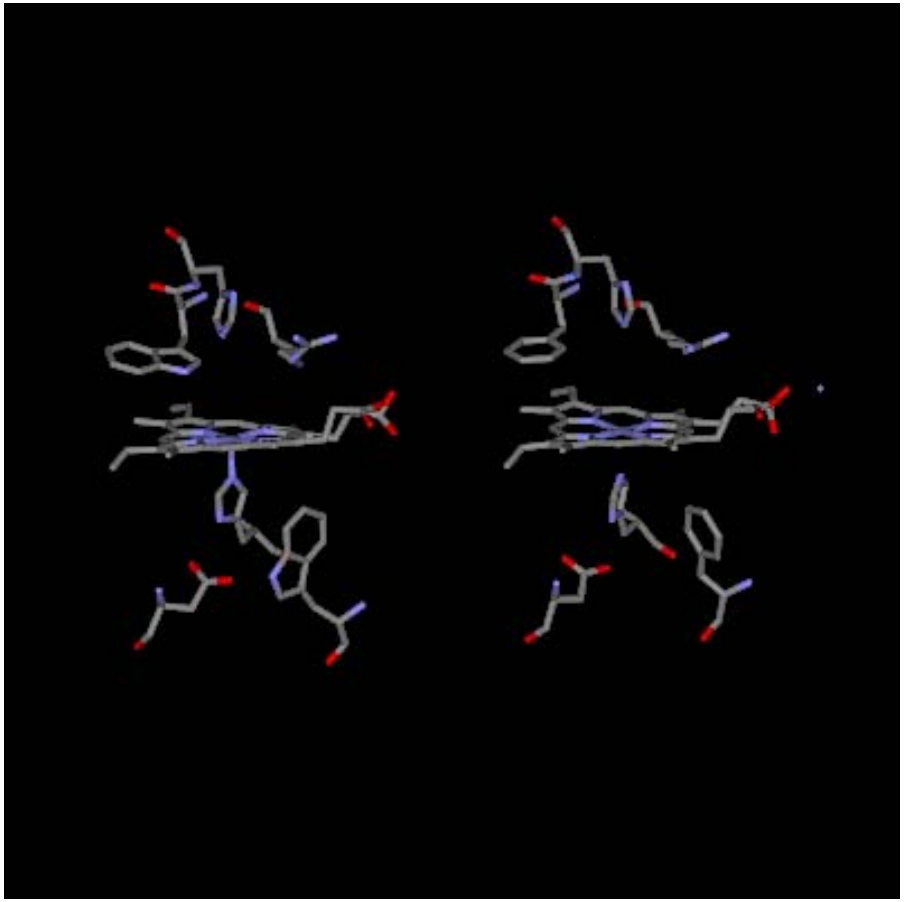
MnP catalyzes the initial one-electron oxidation of Lignin

MnP can Degrade Many Aromatic Pollutants

Class	Selected Examples
Biopolymers	Lignin Cellulose 3,4-Dichloroaniline-lignin conjugate
Lignin model compounds	Veratrylglycerol-b-(O-methoxyphenyl) ether Dehydrodiconiferyl alcohol Dehydrodivanillin
Aromatic compounds	2,6-Dihydroxybenzoic acid 2'-Hydroxy-3'-methoxyacetophenone Veratryl alcohol
Polycyclic aromatic compounds	Benzo[a]pyrene
Chlorinated aromatic compounds	4-Chlorobenzoic acid 2,4,6,-Trichlorophenol 4,5-Dichloroguaiacol 3,4-Dichloroaniline
Polyclyclic chlorinated aromatic compounds	DDT (1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin 2,4,5,2',4',5',-Hexachlororbiphenyl
Non-aromatic chlorinated compounds	1,2,3,4,5,6-Hexachlorocyclohexane (Lindane)

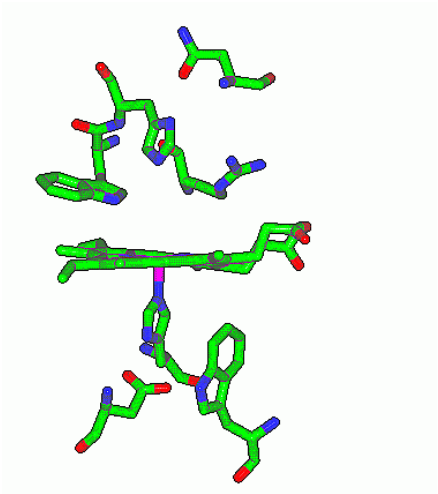
Adapted from J. A. Bumpus & S. D. Aust, *BioEssays 6,* 166 (1986).

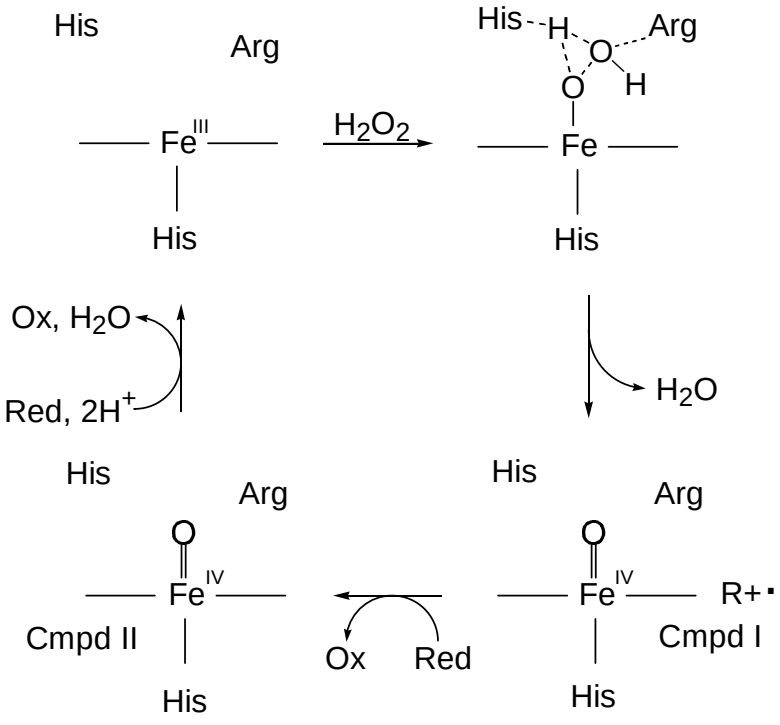
Similarities and differences



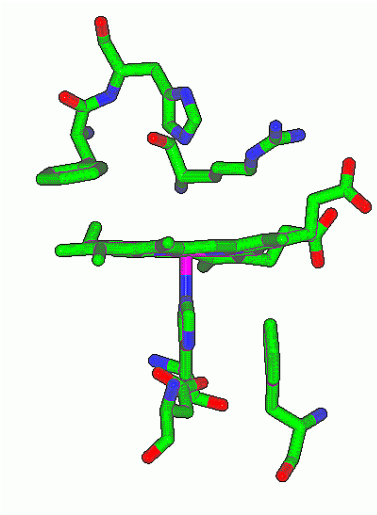
Peroxidase Mechanism

Cytochrome *c* Peroxidase





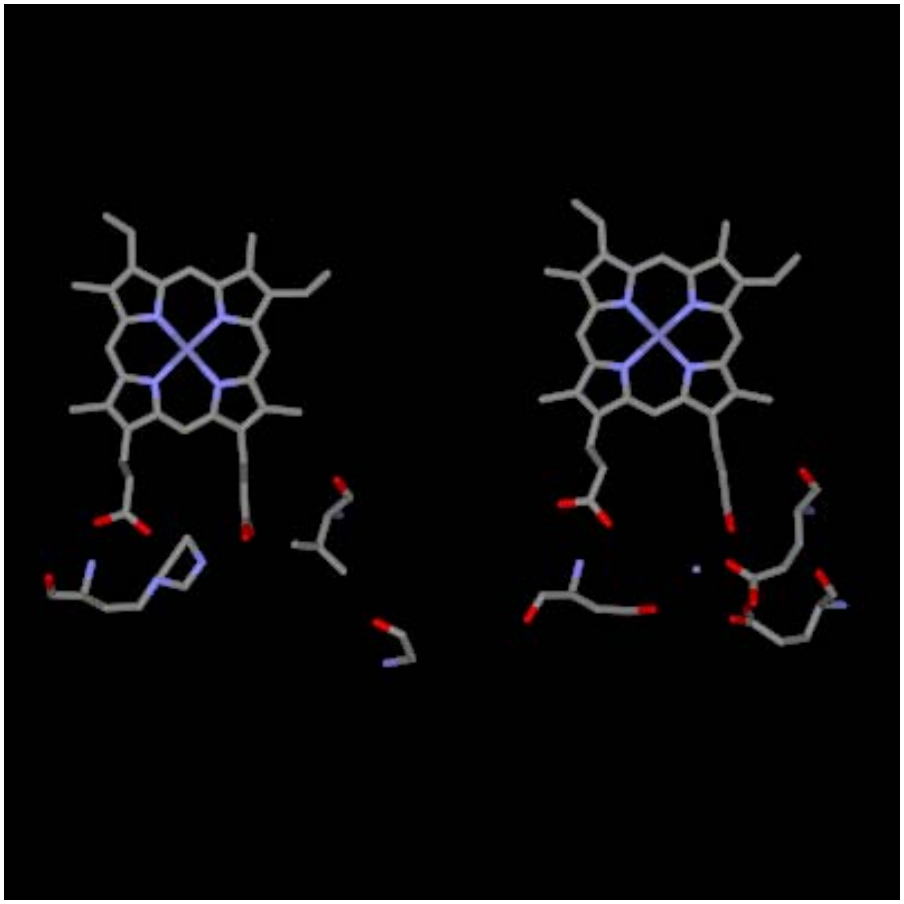
Lignin Peroxidase



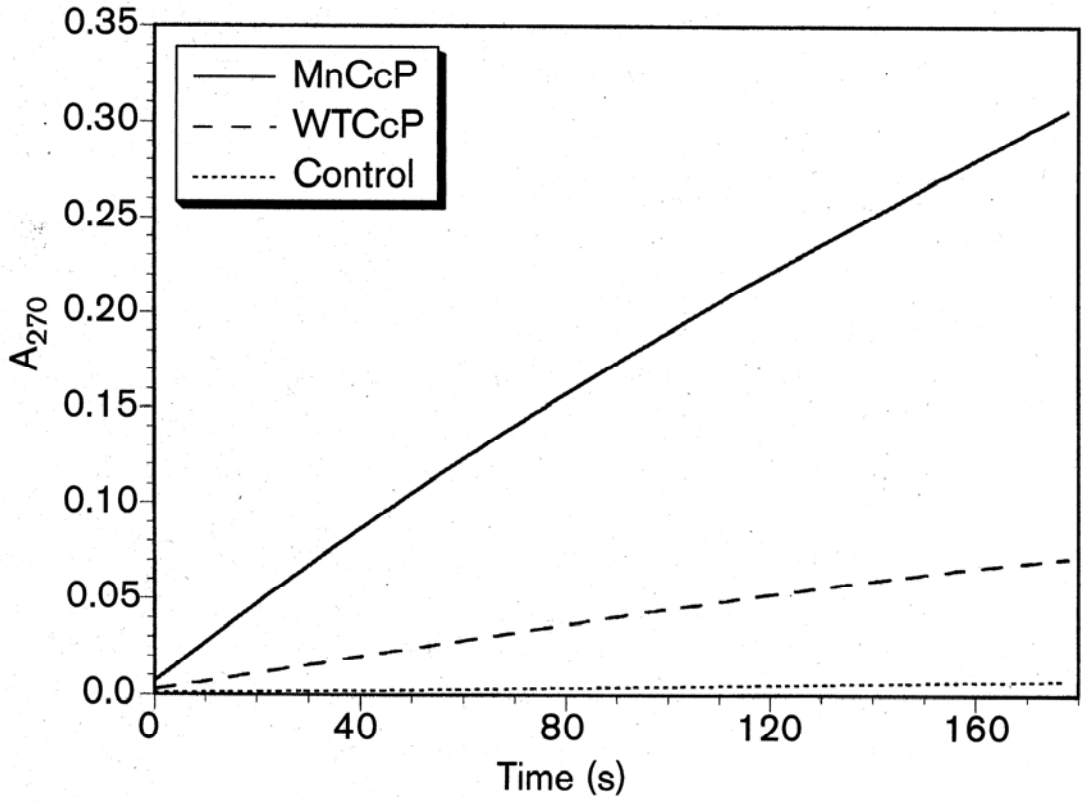
-Classical Peroxidase compound I – porphyrin π-cation radical

-C*c*P compound I - Fe(IV)-oxo (ferryl), Trp 191•+

Engineering the Mn(II)-binding site



From CcP to MnP



The engineered MnCcP displays new MnP activity

B. KS Yeung, X. Wang, J. A. Sigman, P. A. Petillo, and Y. Lu *Chem. & Biol. 4,* 215 (1996).