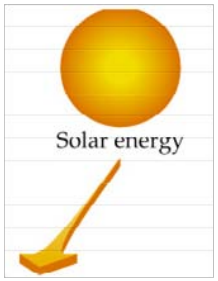
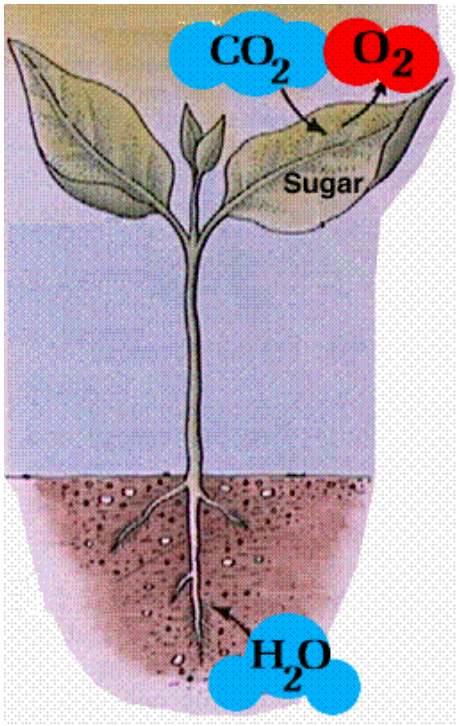
Oxygen Management, I. Storage and Transport Hemoglobin (Hb)/Hemerythrin/Hemocyanin

Readings:

Lippard + Berg: Chapter 11.1
Fenton Ch. 3 Dioxygen management—storage and transport
Kaim + Schwederski: Chapter 5.1-5.2

Life Chemistry: Oxygen Chemistry





$Fe^{2+} \rightarrow Fe^{3+}$

$Cu^{+} \rightarrow Cu^{2+}$

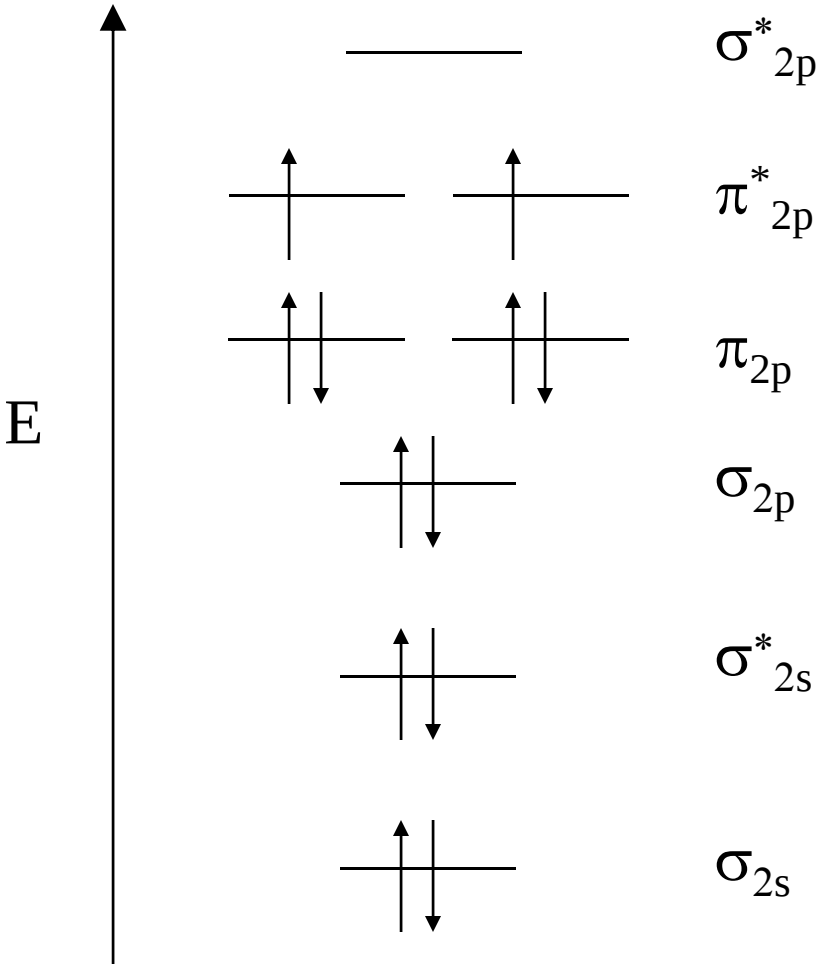
$S^{2-} \rightarrow SO_4^{2-}$

$H_2 \rightarrow H_2O$

$NH_3 \rightarrow NO_3^-$, NO_2^-, etc.

$6\ H_2O + 6\ CO_2$ ----------> $C_6H_{12}O_6 + 6\ O_2$

General Properties of O_2



	$^3O_2$	$^2O_2^{\cdot-}$	$^1O_2^{2-}$
Bond Order	2	1.5	1
Bond Length (pm)	121	128	149
Vibrational Frequency (cm^{-1})	1560	1150-1100	850-740

PLUS: Highly Reactive

$$\text{Bond Order} = \frac{\#\text{ bonding e}^-\text{s} - \#\text{ anti-bonding e}^-\text{s}}{2}$$

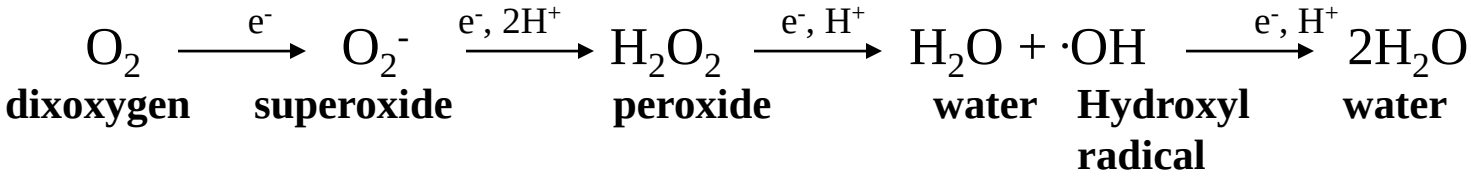
Table 11.2 Vibrational and geometrical properties of dioxygen species

Species	$\nu_{O-O}(cm^{-1})$	d_{O-O} (Å)
O_2^+	1,905	1.12
O_2	1,580	1.21
O_2^-	1,097	1.33
O_2^{2-}	802	1.49

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

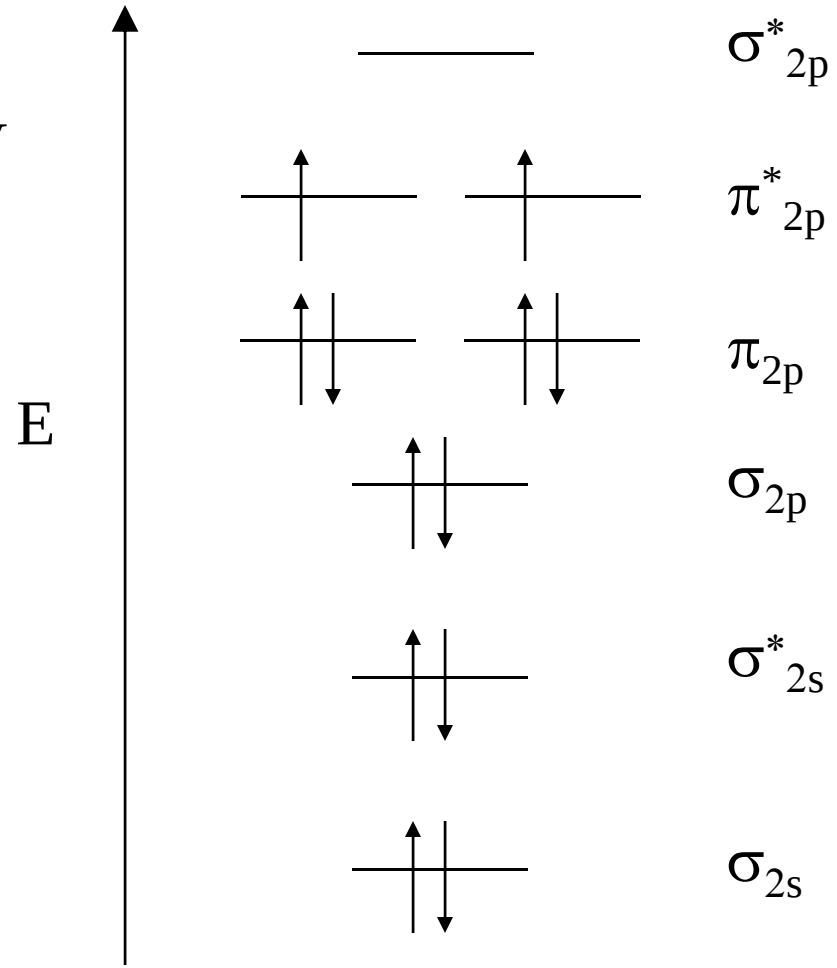
$O_2 + e^- \rightarrow O_2^-$ $\quad E° = - 0.33$ V

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

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

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

2. Through excited singlet O_2

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

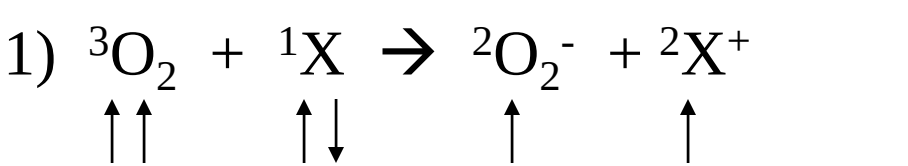
$E_{activation}$ > 22.5 kcal/mol

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

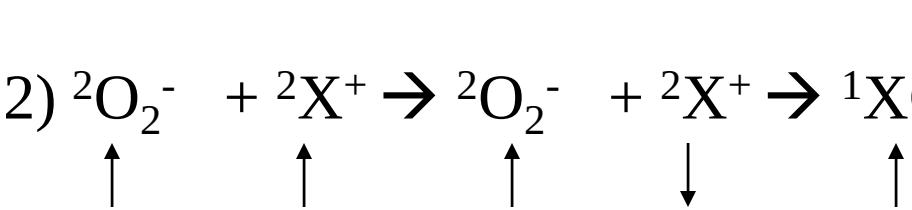
Solutions to increase O_2 reactivity

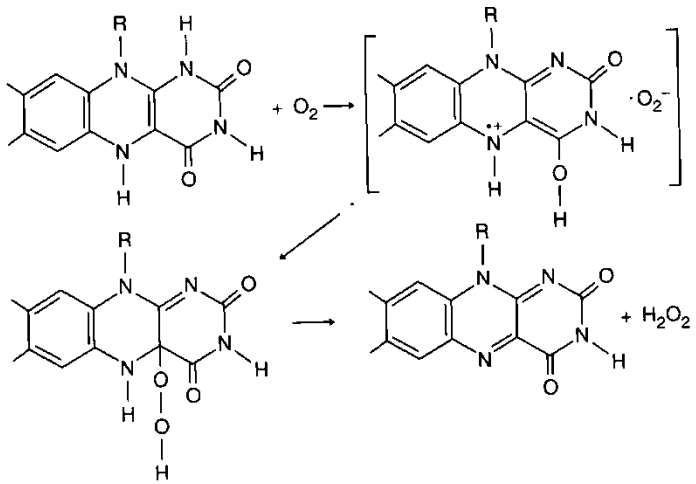
3. Through electron transfer (reduction)

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

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

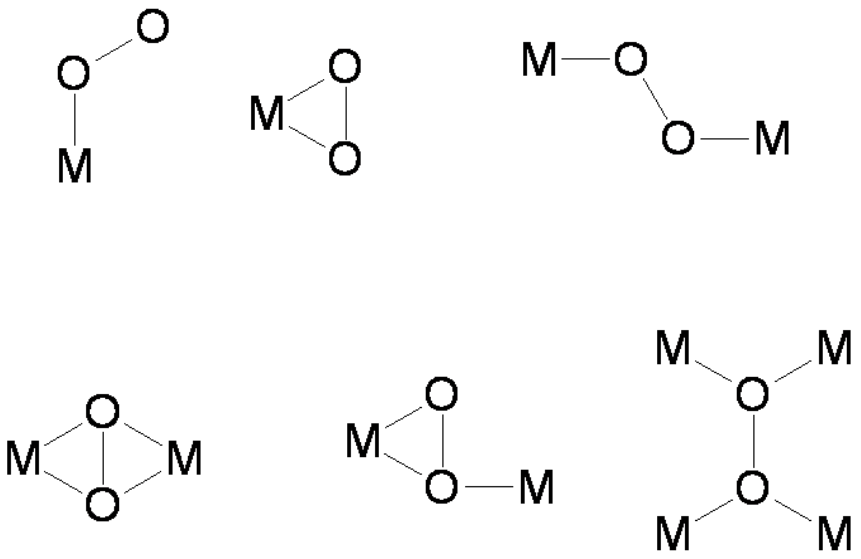
Termination: $R\cdot + ROO\cdot \rightarrow ROOR$

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

Needs initiators
Difficult to control selectivitiy
Can damage biomolecules

Solutions to increase O_2 reactivity

5. Through metal centers



Paramagnetic metal ions can overcome spin restriction

Mode of attachment to Metal ions

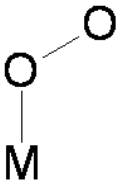
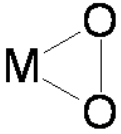
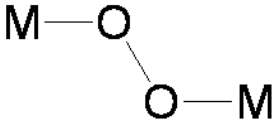
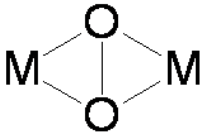
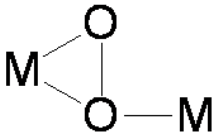
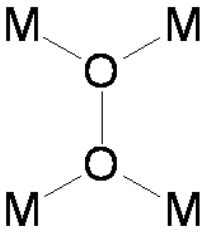
Structure	mode of coordination		biological example
	η^1	end-on	myoglobin
	η^2	side-on	
	μ–η^1: η^1	end-on bridging	
	μ–η^2: η^2	side-on bridging	hemocyanin
	μ–η^1: η^2	end-on/side-on bridging	
	μ_4–$(\eta^1)_4$	end-on fourfold bridging	

Table 11.1 Some properties of protein oxygen carriers

Property	Hemoglobin	Hemerythrin	Hemocyanin
Metal	Fe	Fe	Cu
Oxidation state of metal in deoxy protein	(II)	(II)	(I)
Metal: O_2	Fe^{III}: O_2^-	$2Fe^{III}$: O_2^-	$2Cu^{II}$: O_2
Color, oxygenated	Red	Violet-pink	Blue
Color, deoxygenated	Red-purple	Colorless	Colorless
Coordination of Fe	Porphyrin ring	Protein side chains	Protein side chains
Molecular weight	65,000	108,000	400,000 to 20,000,000
Number of subunits	4[a]	8	Many

[a]In some species (for example, *Glycera*), hemoglobins are monomeric; in others (for example, *Arenicola*), they are multisubunit oligomers with molecular weights in the millions.

Fe, Zn, Cu: the most common transition metal ions in biology

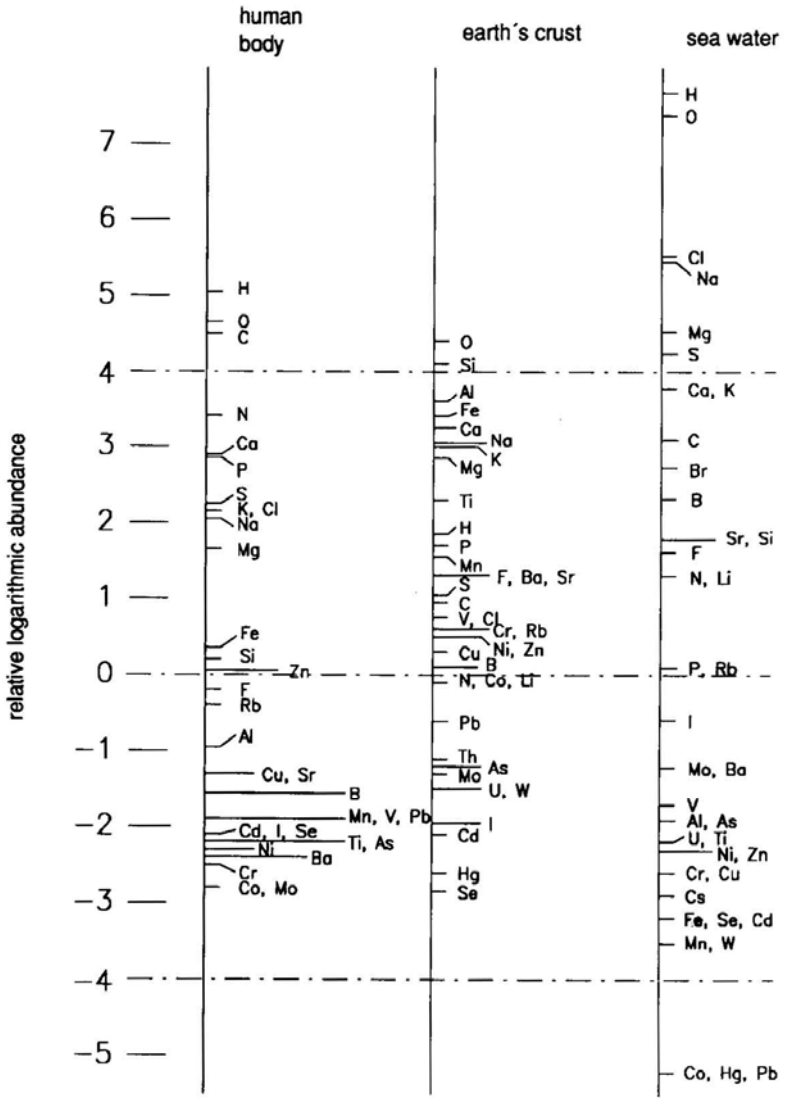


Figure 2.2
Logarithmic diagrams of relative molar concentrations of the elements in different environments (arbitrary units) (data from [1] and [4])

Element	Sea Water (M) x 10^{-8}	Human Plasma (M) x 10^{-8}
Fe	0.005-2	2230
Zn	8.0	1720
Cu	1.0	1650
Mo	10	1000
V	4.0	17.7
Mn	0.7	10.9
Cr	0.4	5.5
Ni	0.5	4.4
Co	0.7	0.0025

Bertini, I.; Gray, H. B.; Lippard, S. J.; Valentine, J. S. Bioinorganic Chemistry; University Science Books: Sausalito, CA, 1994.

1. Abundance

Fe: the most common transition metal ion in biology and used for O_2 management, chemistry, and e^--transfer

Table 5.1 Distribution of the major iron-containing proteins in an adult human (modified from [10])

protein	molecular mass of the protein (kDa)	amount of iron (g)	% of total body iron	type of iron: heme (*h*) or non-heme (*nh*)	number of iron atoms per molecule	function
hemoglobin	64.5	2.60	65	*h*	4	O_2 transport in blood
myoglobin	17.8	0.13	6	*h*	1	O_2 storage in muscle
transferrin	76	0.007	0.2	*nh*	2	iron transport
ferritin	444	0.52	13	*nh*	up to 4500	iron storage in cells
hemosiderin		0.48	12	*nh*		iron storage
catalase	260	0.004	0.1	*h*	4	metabolism of H_2O_2
peroxidases	variable	small	small	*h*	1	metabolism of H_2O_2
cytochrome *c*	12.5	0.004	0.1	*h*	1	electron transfer
cytochrome *c* oxidase	>100	<0.02	<0.5	*h*	2	terminal oxidation ($O_2 \rightarrow H_2O$)
flavoprotein oxygenases (e.g. P-450 system)	about 50	small	small	*h*	1	incorporation of molecular oxygen
iron–sulfur proteins	variable	about 0.04	about 1	*nh*	2-8	electron transfer
ribonucleotide reductase	260 (*E. coli*)	small	small	*nh*	4	transformation of ribonucleic acids to deoxyribonucleic acids

Fe: the most common transition metal ions in biology

Table 1.4
Fe redox potentials.

Complex	Coord. no., type	Fe^{3+}/Fe^{2+} E° (mV)
$Fe(OH_2)_6^{3+}$	6, aquo complex	770
Cytochrome a_3	6, heme	390
HIPIP	4, $Fe_4S_4(SR)_4^-$	350
Cytochrome c	6, heme	250
Rubredoxin	4, $Fe(SR)_4$	−60
Ferredoxins	4, $Fe_4S_4(SR)_4^{2-}$	−400

3. Thermodynamics: extensive range of redox potentials

Fe: the most common transition metal ions in biology

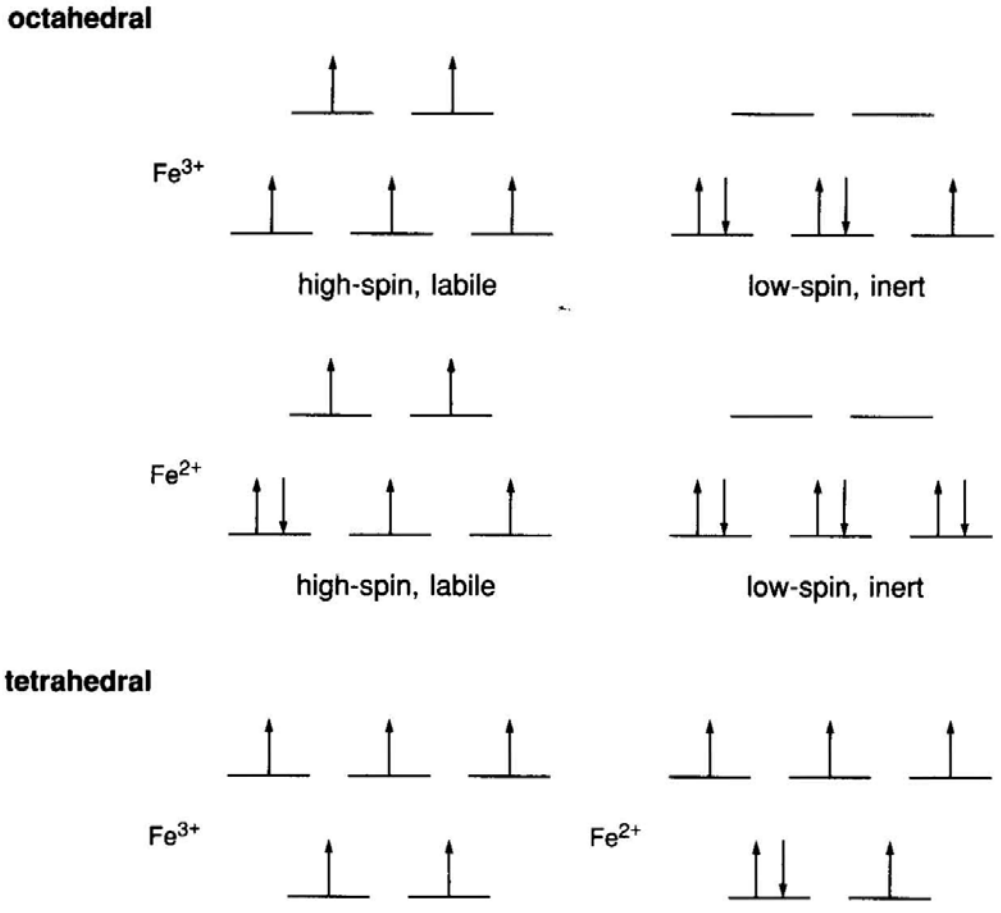


Figure 1.3
Versatility of Fe coordination complexes.

4. Kinetics: facile redox reactions (because of electronic structure)

Fe/O_2 Chemistry as carrier/storage

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

Problems:

1. Dimerization => μ-oxo species

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

$$Fe(III)\text{-}O_2^- + Fe(II) \rightleftharpoons Fe(III)\text{-}O_2^{2-}\text{-}Fe(III)$$

$$Fe(III)\text{-}O_2^{2-}\text{-}Fe(III) \longrightarrow 2Fe(IV)=O$$

$$Fe(IV)=O + Fe(II) \longrightarrow Fe(III)\text{-}O\text{-}Fe(III)$$

Fe/O_2 Chemistry

2. Auto-oxidation

$$Fe^{II} + O_2 \rightleftharpoons Fe^{III}\text{-}O_2^-$$

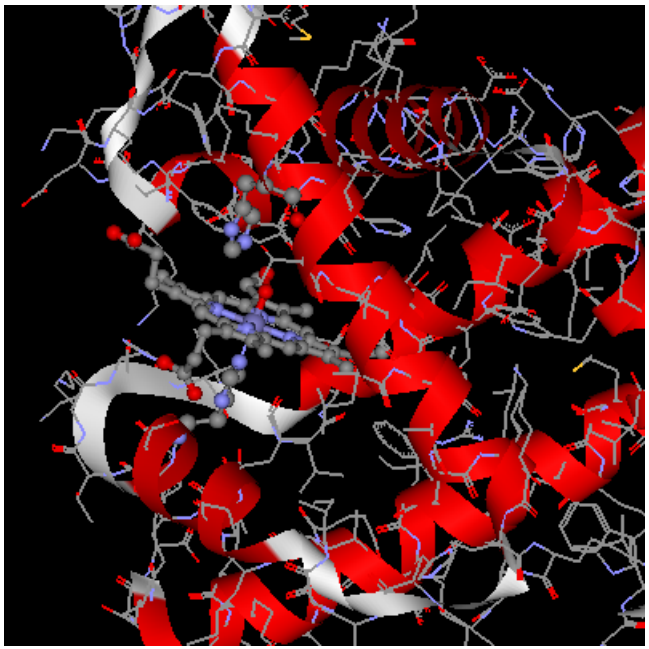
$$Fe^{III}\text{-}O_2^- \rightleftharpoons Fe^{III} + O_2^-$$

$$Fe^{III} + Cl^- \rightleftharpoons Fe^{III}\text{-}Cl$$

-- favorable in the presence of a nucleophile such as Cl^-

Role of protein scaffold

- Prevent dimerization
  - Through isolation of heme
- Prevent autooxidation
  - Prevent side reactions
  - Prevent binding of distal ligands
- Provide ligands for modulation of O_2 binding affinity
  - H-bond from distal His
  - H-bond to proximal His
  - Porphyrin doming
    - In deoxy-form, compression of the Fe-Im bond and decrease in the out-of-plane displacement
    - In the oxy form, unrestricted motion of the Fe-His to the porphyrin plane



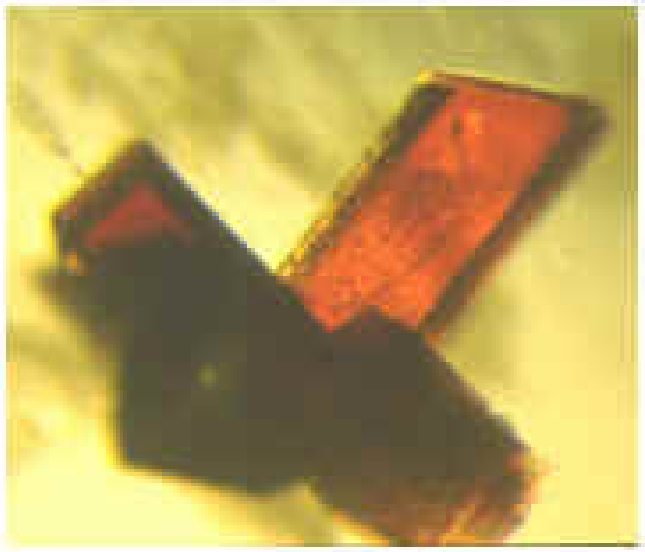
Myoglobin- Key Properties

- **An O_2 transport protein in muscle**
- **A globular soluble protein, 151 residues (16 kDa)**
- **8 α-helices (A,B,C,…..H)- first protein crystal structure!**
- **Contains a heme prosthetic group**

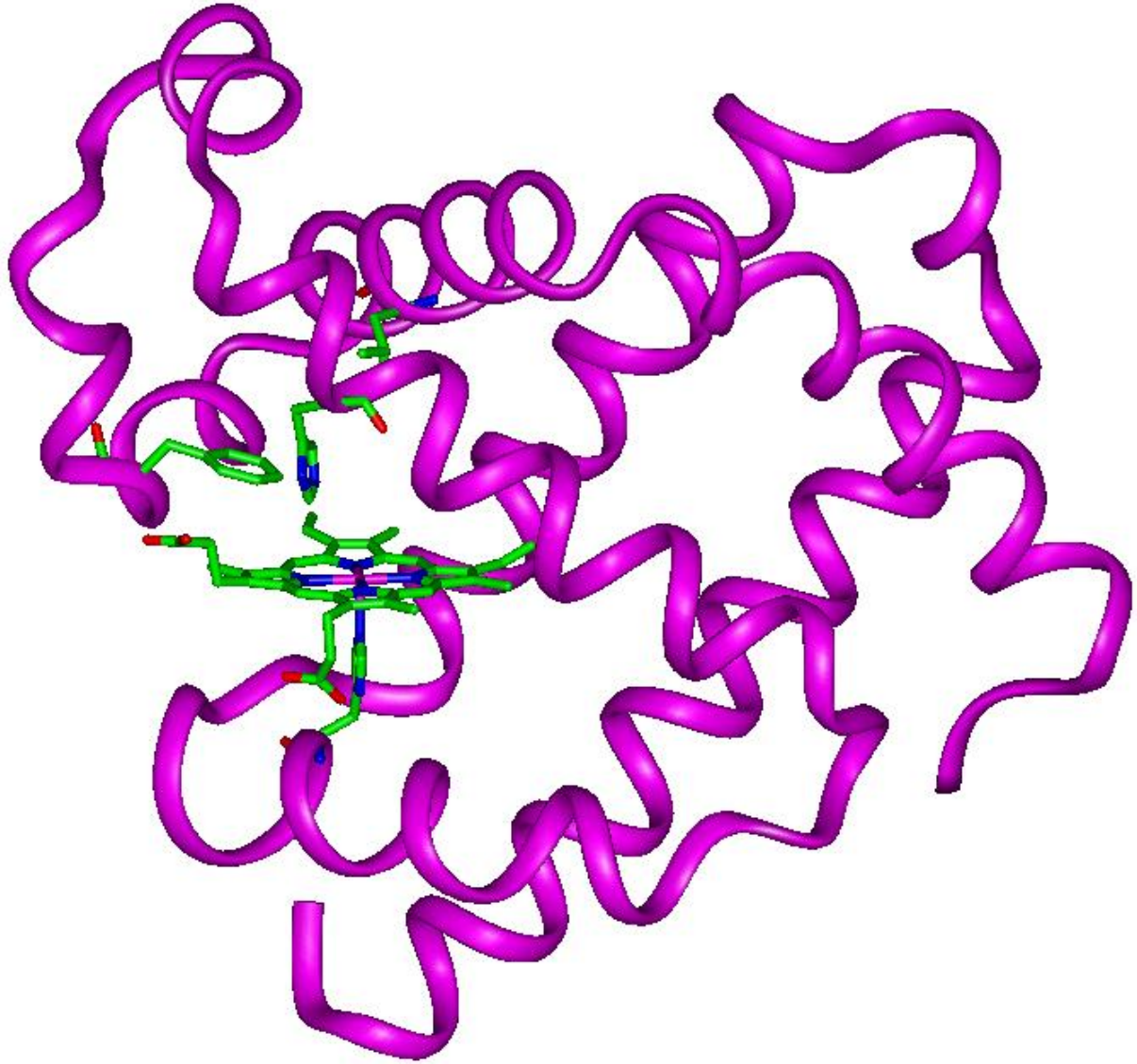
*structbio.vanderbilt.edu/chazin/classnotes/biochem.5012/Biochem-5012.6.**ppt***

First Protein Structure

- Myoglobin.
- Protein purified from whale blood.
- Max Perutz 1958.
- Showed a 75% α-helical fold.
- 155 amino acids, ~ 17 kDa.



X-ray Structure of Sperm Whale Myoglobin

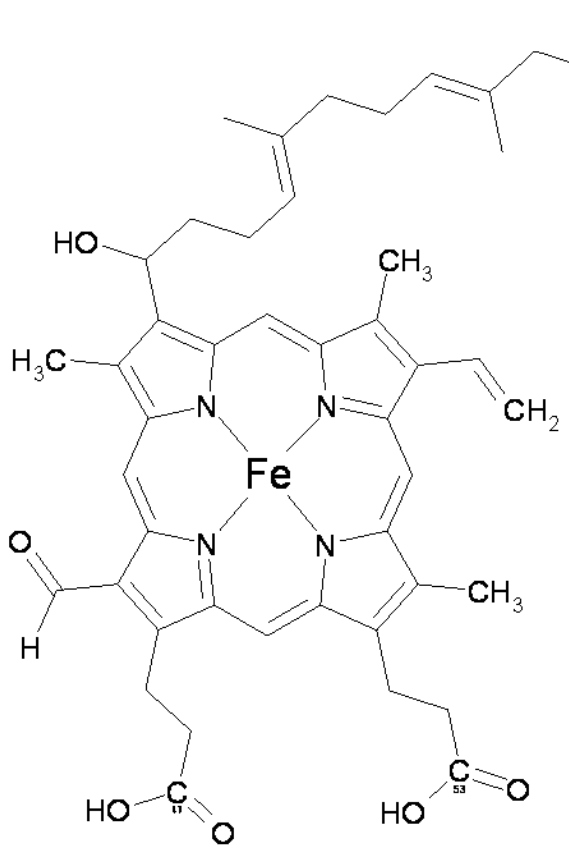


The Heme Prosthetic Group

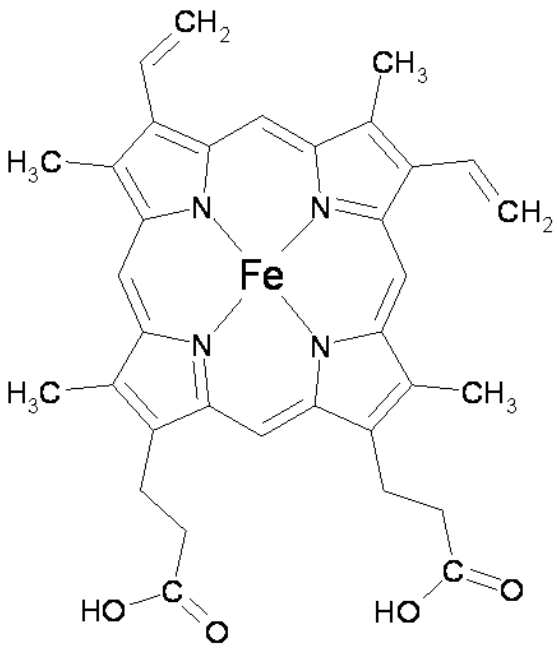
The O_2 carrier in Myoglobin and Hemoglobin

- **Protoporphyrin with Fe^{II}**
- **Covalent attachment of Fe via His F8 side chain**
- **Additional stabilization via hydrophobic interaction**
- **Fe^{II} state is active, Fe^{III} [oxidized]**
- **Fe^{II} atom in heme binds O_2**

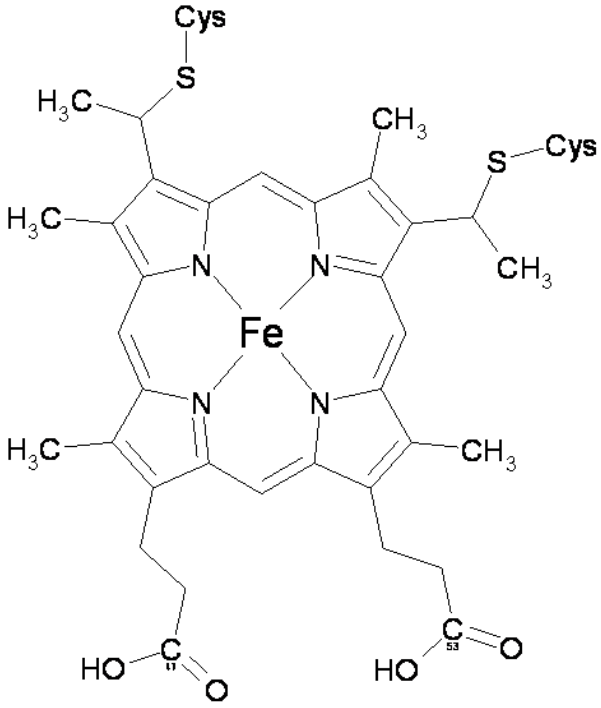
Different types of hemes



heme a



heme b



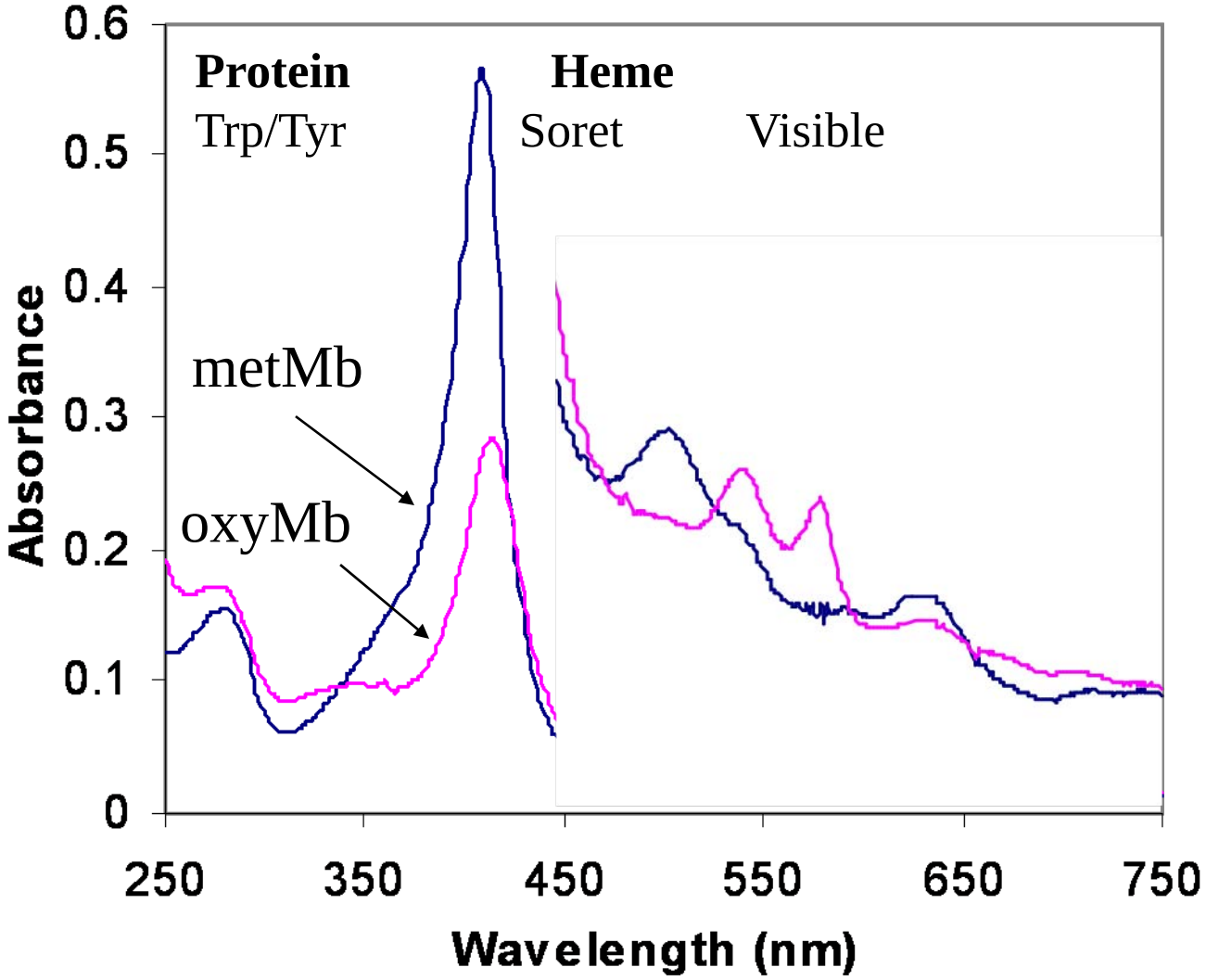
heme c

Soluble in different oxidation states

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 X 10^{-4} M) in water**
  - **Solubility problem overcome by binding to proteins**
  - **Also increases diffusion**
- **Binding of O_2 alters heme electronic structure**
  - **Causes changes in heme electronic spectrum (Vis)**

UV-vis Spectral Characteristics of Heme Proteins



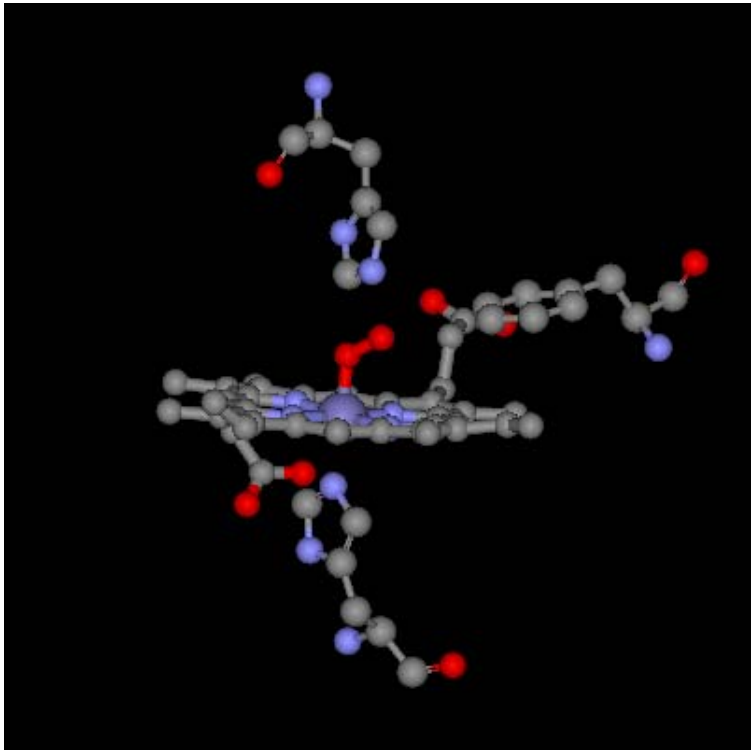
Carbon Monoxide Poisoning

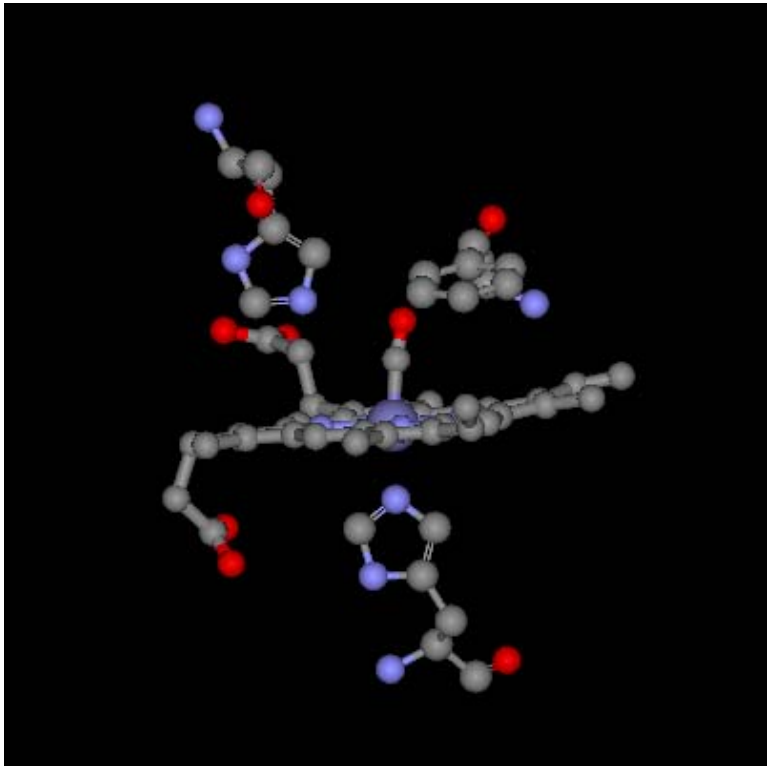
- **Heme Fe(II) binds many other small molecules with structures similar to O_2 including: CO, NO, H_2S**

- **O_2 is actually a fairly weak binder relative to these other molecules, particularly CO. [Essential for Biology]**

- **When exposed to CO, even at low concentrations, O_2 transport proteins will be filled with CO $\rightarrow$ limiting their vital O_2 capacity.**

O_2 Compared to CO Adduct





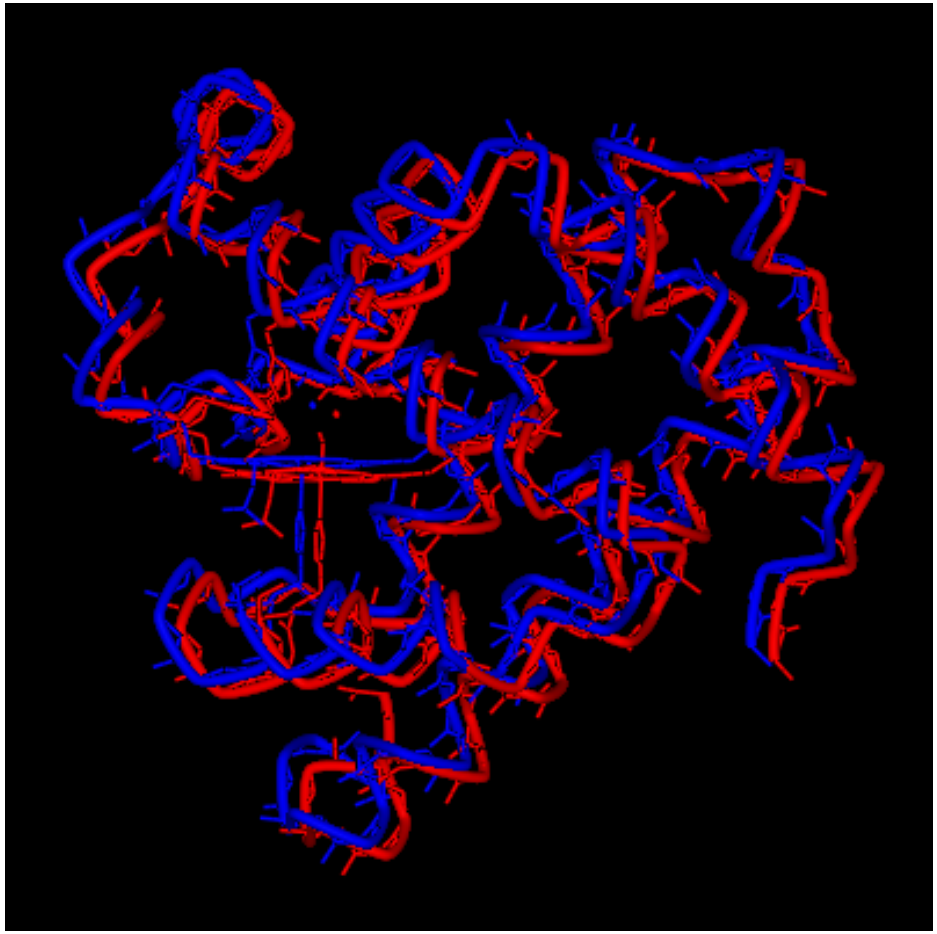
Fe/O_2 Chemistry

3. Binding affinity CO >> O_2

Table 4.6
Relative affinities (M) of iron-porphyrinato systems for O_2 and CO, and relative affinities (*N*) for O_2 of iron and cobalt-porphyrinato systems.

Compound	$P_{1/2}$(Fe—CO) Torr	$P_{1/2}$(Fe—O_2) Torr	M $P_{1/2}$(Fe—O_2)/$P_{1/2}$(Fe—CO)	$P_{1/2}$(Co—O_2) Torr	N $P_{1/2}$(Co—O_2)/$P_{1/2}$(Fe—O_2)
H_2O, pH 7					
Whale Mb	0.018	0.51	28	57	110
Whale Mb (E7His→Gly)	0.0049	6.2	1,300	—	—
Aplysia Mb	0.013	2.7	200	50 × CoMb	>1,000
Glycera Mb	0.00089	5.2	5,800	50 × CoMb	>1,000
Fe(PPIX-Im)	0.002	1.0	500	—	—
Toluene/Benzene					
Fe(PF-Im)/ Co(PF) (1-MeIm)	0.000022	0.58	27,000	140	240
M(PF)(1, 2-Me_2Im)	0.0089	38	4,300	900	24
M(Bis-Poc)- (1, 2-Me_2Im)	0.0091	508	55,800	—	—
Fe(PPIX-Im)/ Co(PPIX) (1-MeIm)	0.00025	5.6	22,000	18,000	3,200
M(C_2-Cap)(1-MeIm)	0.0054	23	4,200	140,000	6,100

Deoxy- and oxyMb



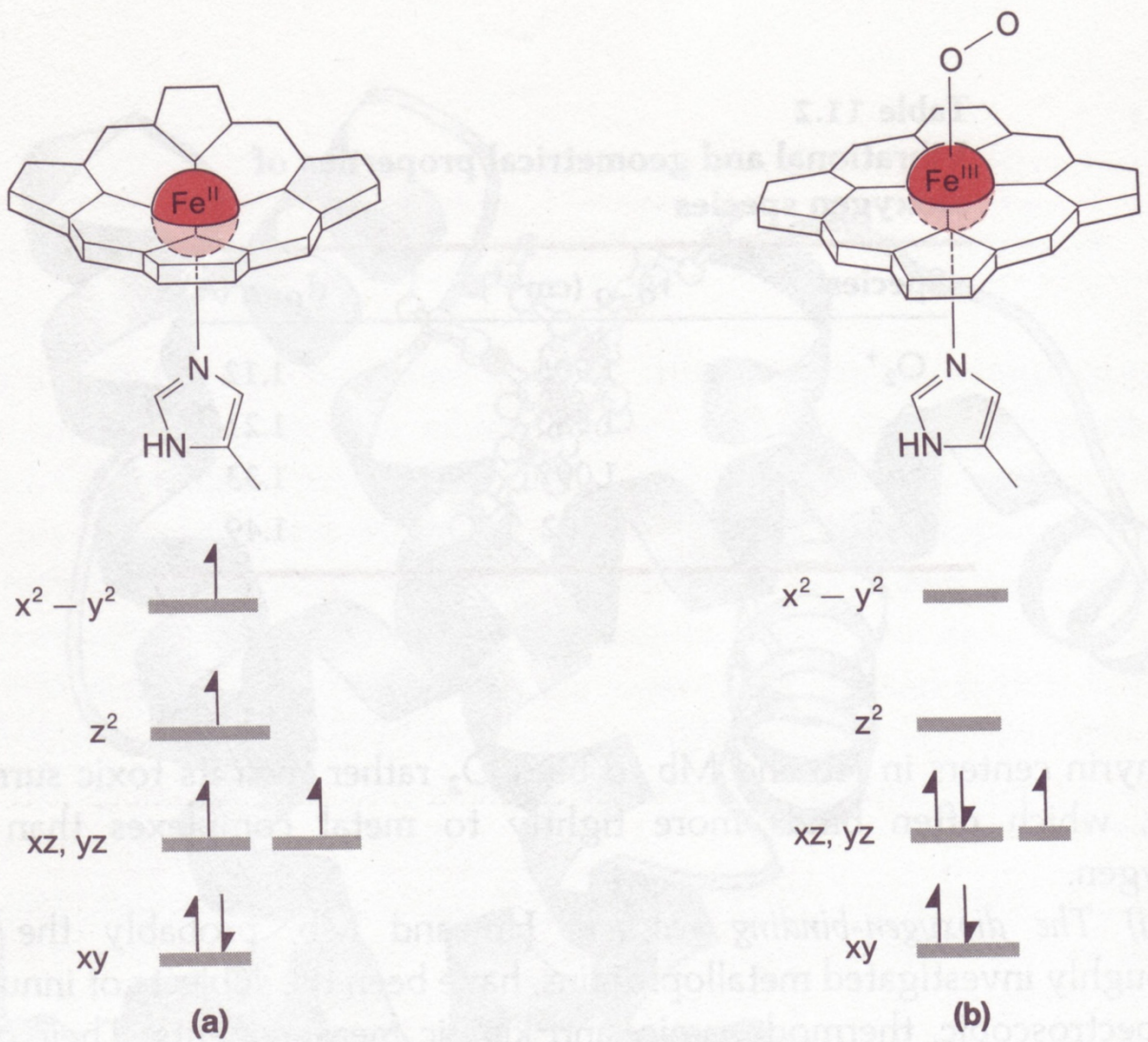
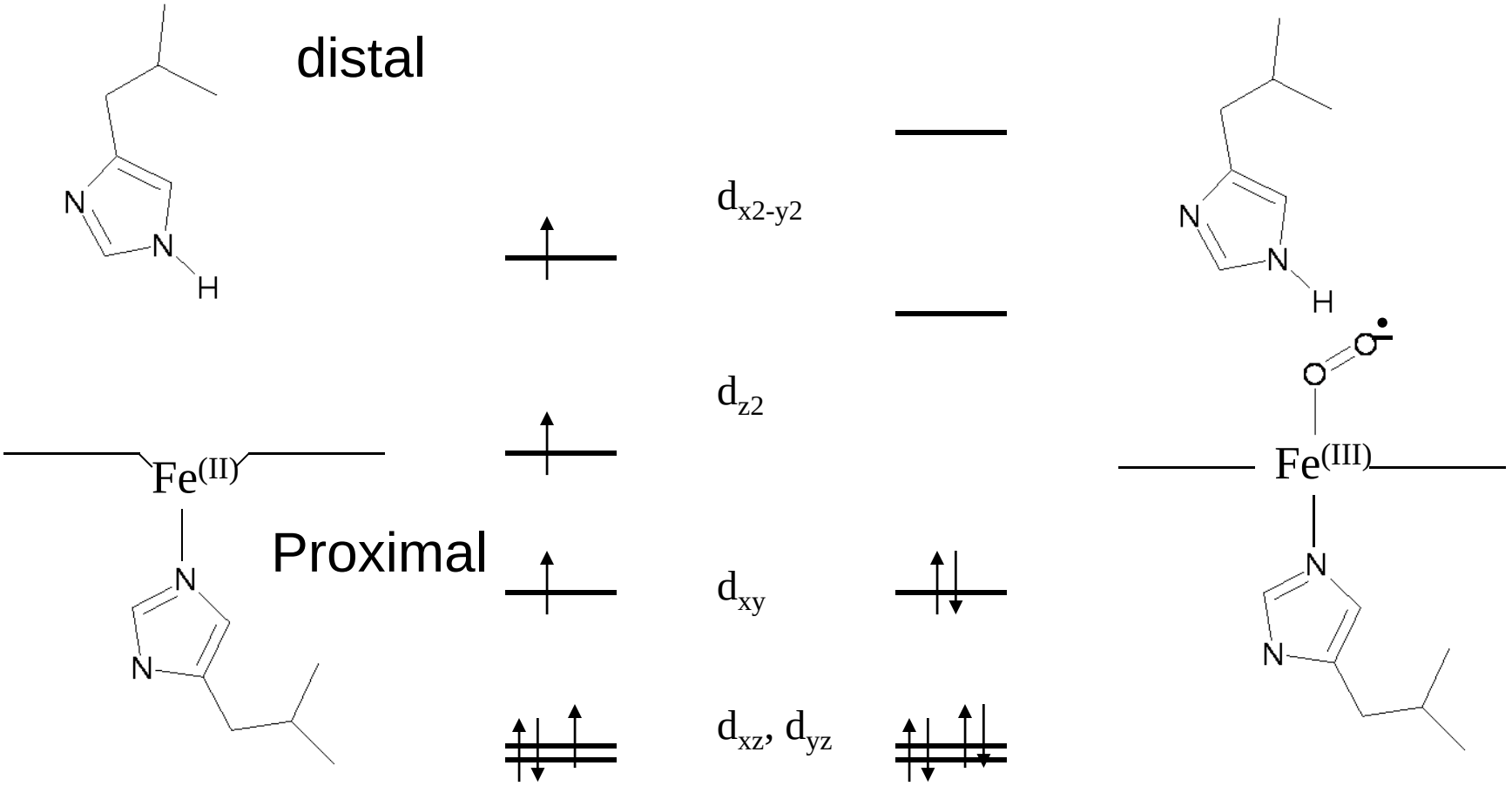


Figure 11.2
Diagram illustrating the structural and spin state changes that occur upon binding of dioxygen to an iron porphyrin. Shown are (a) the high-spin ferrous deoxy form and (b) the low-spin ferric oxy form.

Mode of O_2 Binding



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

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

Modulation of Heme Activity

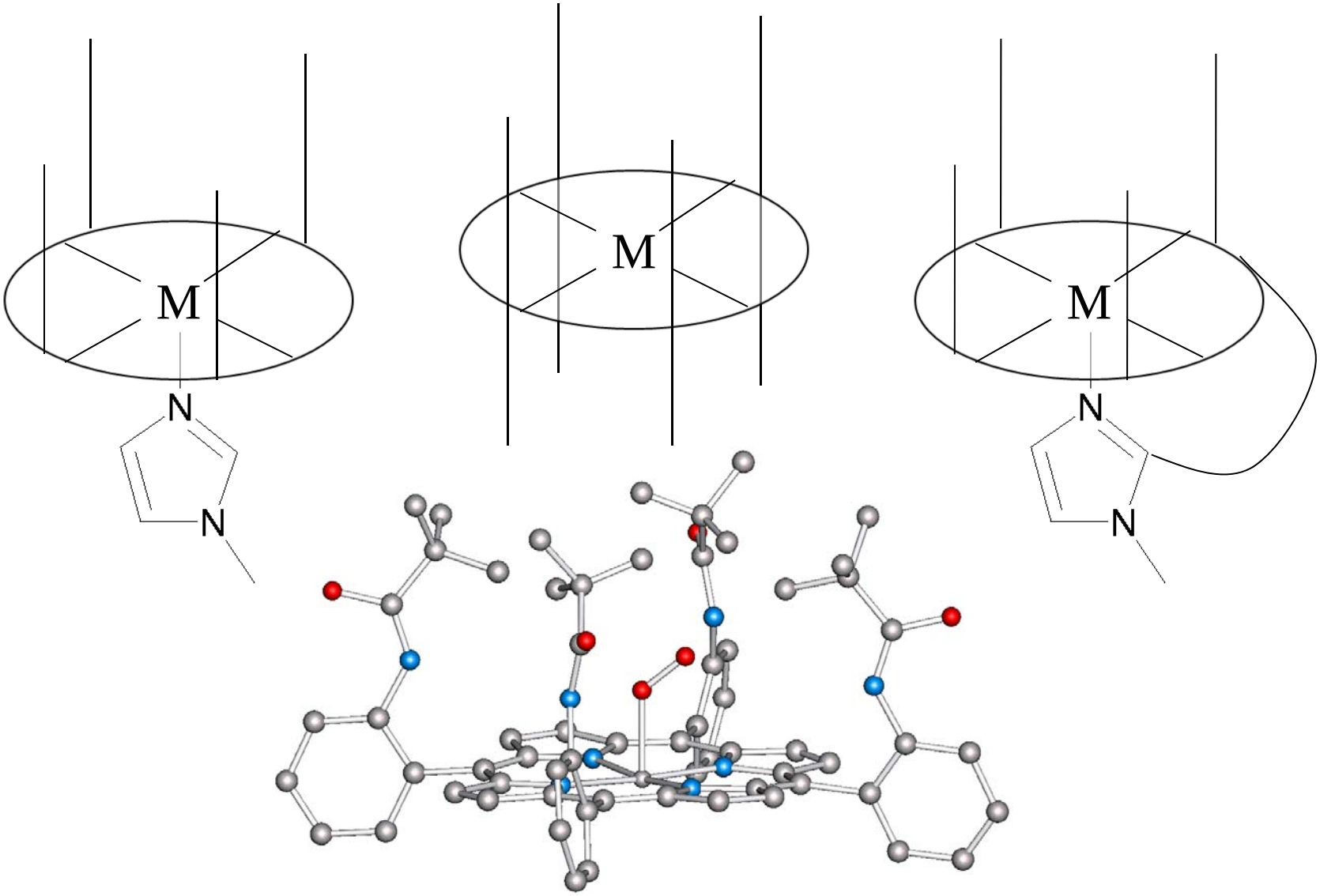
Distal Side

- substrate-binding
- metal-binding
- axial ligand
- H-bonding interactions
- local charge distribution

Proximal Side

- type of proximal ligand
- H-bonding to proximal ligand
- local charge distribution

Synthetic analogs of Hb and Mb



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