Inorganic biochemistry

Average elemental composition of a human body (adult, 70 kg

element and symbol		mass (g)	year of discovery as an essential element
oxygen	O	45500	
carbon	C	12600	
hydrogen	H	7000	
nitrogen	N	2100	
calcium	Ca	1050	
phosphorus	P	700	
sulfur	S	175	
potassium	K	140	
chlorine	Cl	105	
sodium	Na	105	
magnesium	Mg	35	
iron	Fe	4.2	17th century
zinc	Zn	2.3	1896
silicon	Si	1.4	1972
rubidium[a]	Rb	1.1	
fluorine	F	0.8	1931
zirconium[a]	Zr	0.3	
bromine[b]	Br	0.2	
strontium[a]	Sr	0.14	
copper	Cu	0.11	1925
aluminum[a]	Al	0.10	
lead[b]	Pb	0.08	
antimony[a]	Sb	0.07	
cadmium[b]	Cd	0.03	(1977)
tin[b]	Sn	0.03	(1970)
iodine	I	0.03	1820
manganese	Mn	0.02	1931
vanadium[b]	V	0.02	(1971)
selenium	Se	0.02	1957
barium[a]	Ba	0.02	
arsenic[b]	As	0.01	1975
boron[b]	B	0.01	
nickel[b]	Ni	0.01	(1971)
chromium	Cr	0.005	1959
cobalt	Co	0.003	1935
molybdenum	Mo	<0.005	1953
lithium[b]	Li	0.002	

[a] Not essential. [b] Essentiality uncertain.

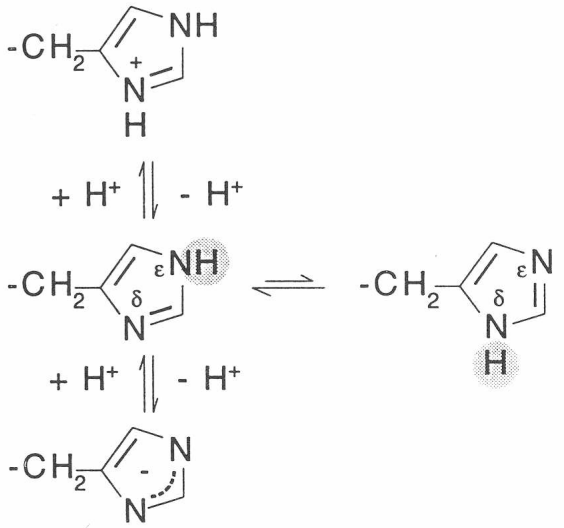
Biochemical roles of metals

Biological functions of selected metal ions

Metal	Function
Sodium	Charge carrier; osmotic balance
Potassium	Charge carrier; osmotic balance
Magnesium	Structure; hydrolase; isomerase
Calcium	Structure; trigger; charge carrier
Vanadium	Nitrogen fixation; oxidase
Chromium	Unknown, possible involvement in glucose tolerance
Molybdenum	Nitrogen fixation; oxidase; oxo transfer
Tungsten	Dehydrogenase
Manganese	Photosynthesis; oxidase; structure
Iron	Oxidase; dioxygen transport and storage; electron transfer; nitrogen fixation
Cobalt	Oxidase; alkyl group transfer
Nickel	Hydrogenase; hydrolase
Copper	Oxidase; dioxygen transport; electron transfer
Zinc	Structure; hydrolase

The most important metal-coordinating amino acids

Table 2.5 The most important metal-coordinating amino acids

α-amino acid R–$^{\alpha}$CH(NH_3^+)CO_2^-	side chain, R
histidine (His)	
methionine (Met)	$-CH_2CH_2SCH_3$
cysteine (Cys)	$-CH_2SH$
selenocysteine (SeCys)	$-CH_2SeH$
tyrosine (Tyr)	
aspartic acid (Asp)	$-CH_2COOH$
glutamic acid (Glu)	$-CH_2CH_2COOH$

pK_a = 6.5

pK_a = 14

sometimes named « the 21st amino-acid »

pK_a = 10.5

H : acidic protons which may be substituted by metal cations

(1) Introduction to aerobic life

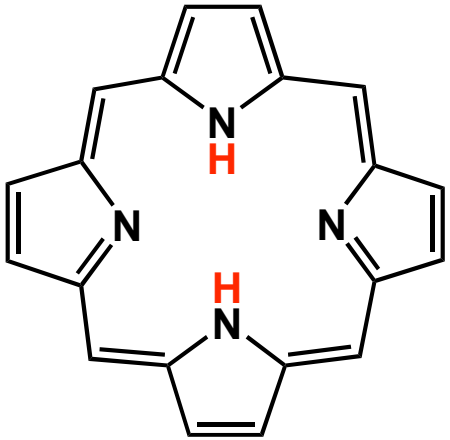
All aerobic living organisms utilize dioxygen as an essential component for three main roles which are necessary to maintain life: the production of energy, the oxidative metabolism of endogenous compounds and the defence of the organism against various kinds of infections.

O_2 activation by aerobic living organisms	Necessary roles for life	- Energy production - Oxidative steps of metabolism - Defence against infection
Formation of active species ($O_2^{\cdot -}$, H_2O_2, $\cdot OH$...)	Adverse effects	Oxidative stress and toxic effects
Control by protecting systems		

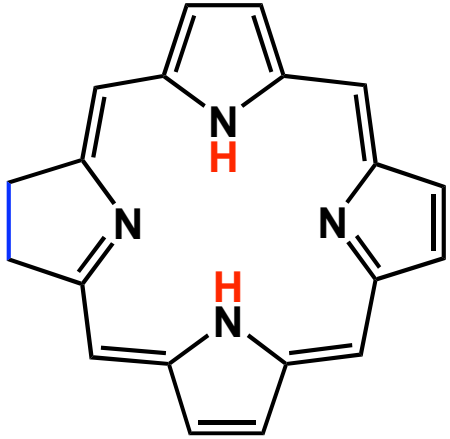
Useful and adverse effects of the activation of dioxygen by aerobic living organisms.

The most important ligating prosthetic groups

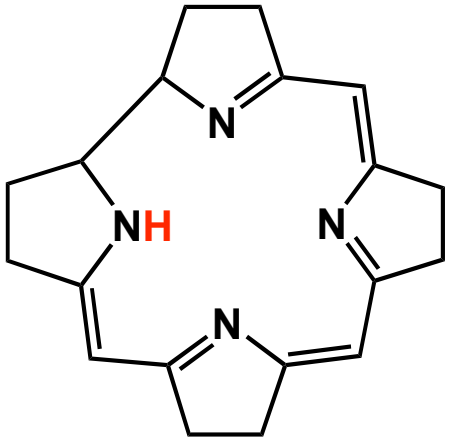
Porphyrins and related macrocycles:



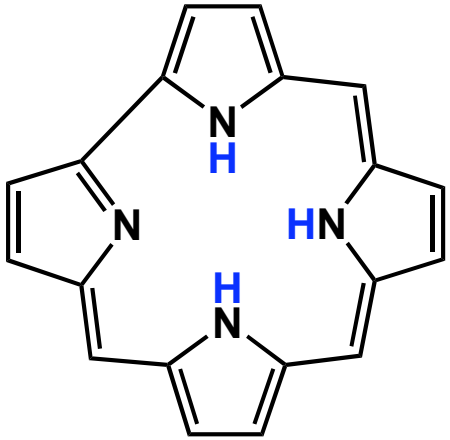
porphyrin



chlorin (1 C=C bond reduced)



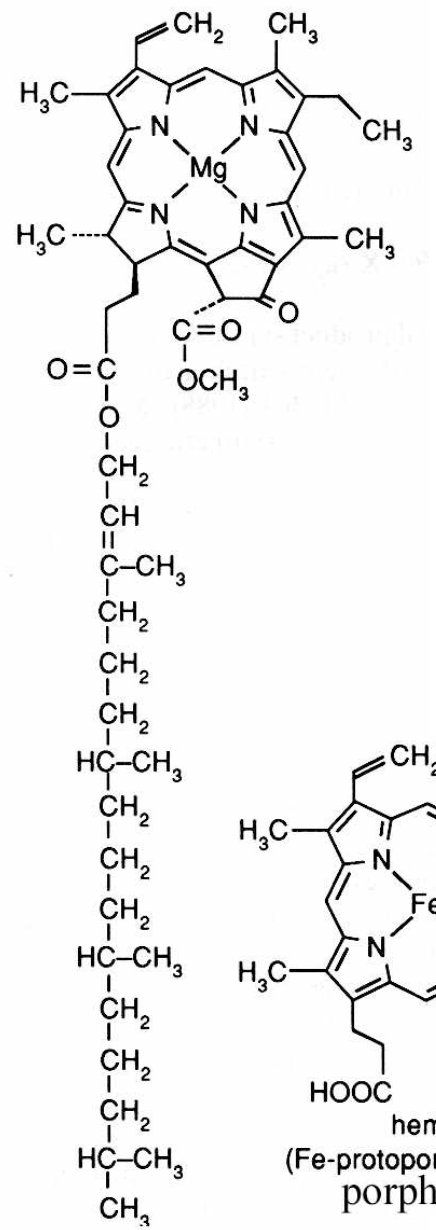
corrin (contrated cycle)



corrole (contrated cycle)

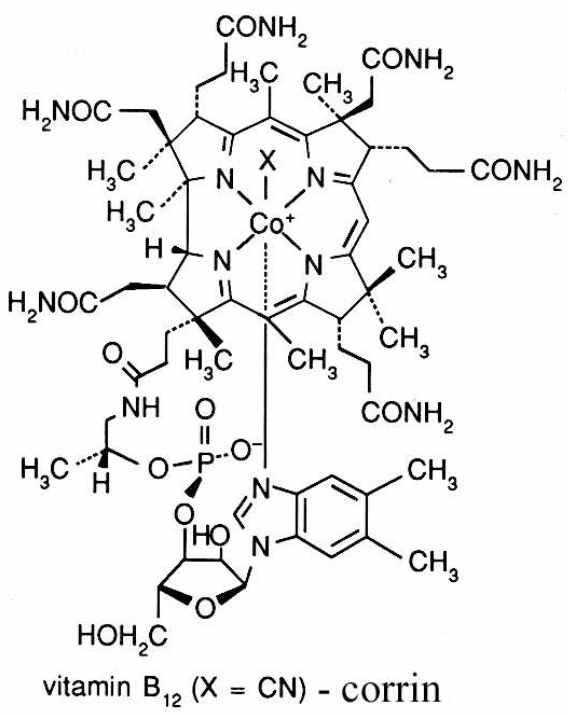
In organisms, only complexes of Fe, Co, Ni and Mg are known.
Porphyrins serve as tetradentate, rigid, square-planar ligands, forming very stable complexes (metalloporphyrins) with most metals and many non-metals or quasi-metals.

Examples of important porphyrinoid prosthetic groups

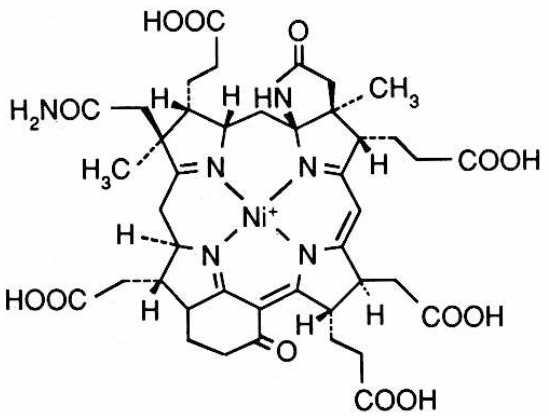


chlorophyll *a* - chlorin

heme
(Fe-protoporphyrin IX)
porphyrin



vitamin B_{12} (X = CN) - corrin



coenzyme F430

possible evolutionary precursor to modern porphyrinoid prosthetic groups

Important heme proteins

class	*axial ligands on Fe*	*biological function*
cytochromes b and c	2 imidazoles or imidazole/methionine	electron transport
myoglobin, hemoglobin	1 imidazole	O_2 transport/storage
cytochrome c oxidase	1 imidazole	O_2reduction (to H_2O)
cytochrome P-450	1 cysteine	oxygenation of organic substrates
peroxidases	1 imidazole or cysteine	oxygenation of organic substrates
catalases	1 phenolate (tyrosine)	H_2O_2 disproportionation

Heme groups participating in catalytic transformations must be 5 coordinate (open coordination site for substrate binding). Electron transfer hemes are 6 coordinate.

Exogenous ligands have different affinities for Fe^{II} or Fe^{III} state:

CO, O_2, NO, RNC prefer Fe^{II}
CN^-, N_3^-, F^- prefer Fe^{III}.

These molecules can be substrates or enzyme **inhibitors**:

- Heme enzymes whose catalytic cycles involve *5-coordinate complexes of both Fe^{II} and Fe^{III}* will be susceptible to inhibition by both types of exogenous ligands.

- Heme enzymes that operate only in the *5-coordinate Fe^{II} form* (Mb, Hb) are not inhibited by CN^-, N_3^-, F^-; heme enzymes that operate only in the *5-coordinate Fe^{III} form* are not inhibited by CO, O_2, etc.

Major inhibitors of the heme proteins

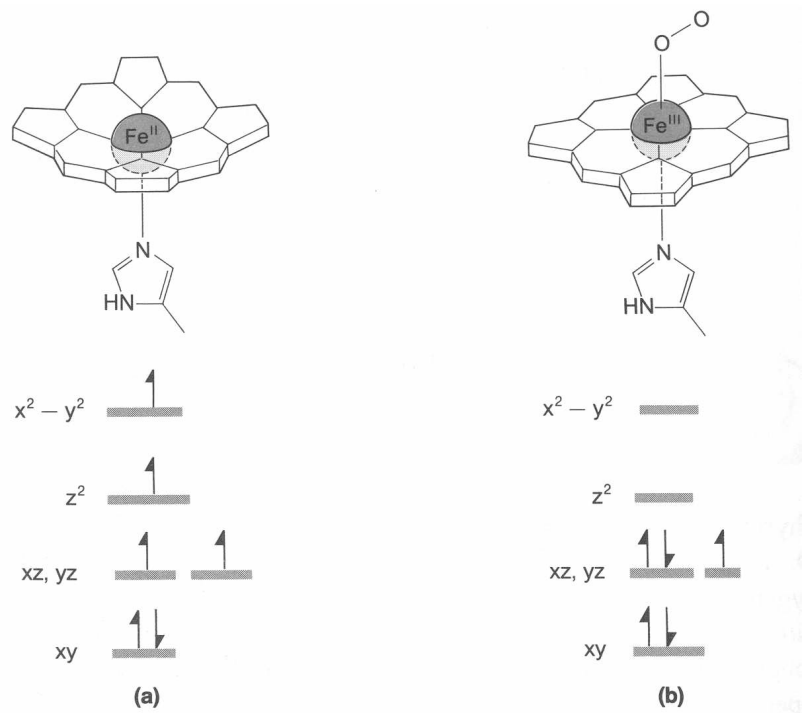
class	*axial ligands on Fe*	The resting and the substrate-binding forms	Inhibitor
cytochromes b and c	2 imidazoles or imidazole/methionine	6-coordinate Fe^{II}, 6-coordinate Fe^{III}	none
myoglobin, hemoglobin	1 imidazole	5-coordinate Fe^{II}	CO
cytochrome c oxidase	1 imidazole	5-coordinate Fe^{II} 5-coordinate Fe^{III}	CO CN^-, N_3^-, F^-
cytochrome P-450	1 cysteine	5-coordinate Fe^{II}	CO
peroxidases	1 imidazole or cysteine	5-coordinate Fe^{III}	CN^-
catalases	1 phenolate (tyrosine)	5-coordinate Fe^{III}	CN^-

Cytochromes b and c are not inhibited, since they are always 6 coordinate.

The major inhibitor for myoglobins and hemoglobins is CO not CN^- because the substrate-binding form is Fe(II).

The major inhibitor for peroxidases and catalases is CN^- because the substrate-binding form is Fe(III).

Electronic changes upon O_2 binding



The Fe-O_2 complex should be considered as an antiferromagnetically-coupled Fe^{III}-($O_2^{\cdot -}$) adduct.

ν(O-O) in MbO_2 = 1105 cm^{-1}, a value characteristic of a coordinated superoxide

Therefore, O_2 affinity correlates with the redox potential of the metal:

$M^{+n} + O_2 = M^{+(n+1)}$ ($O_2^{\cdot -}$) :

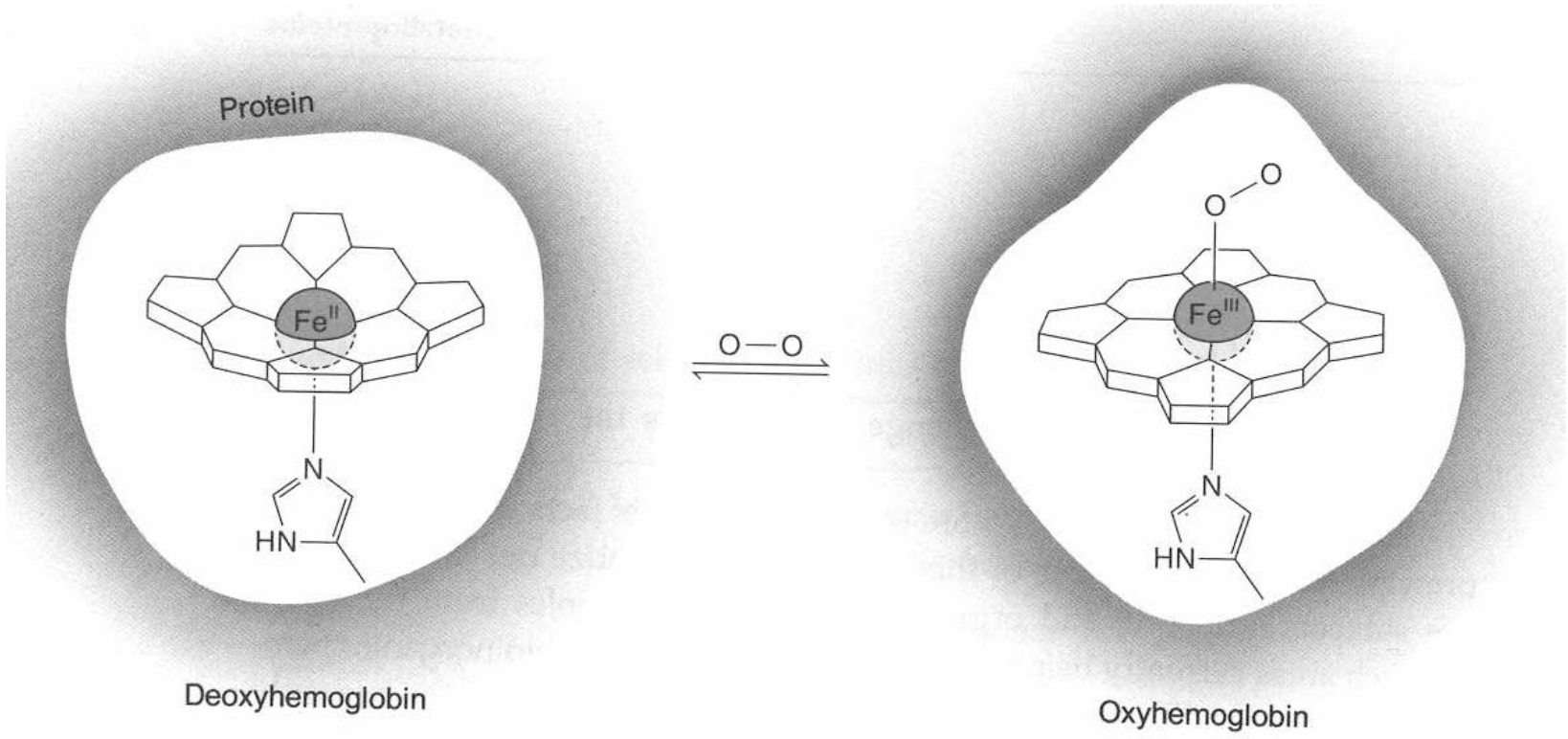
$O_2 + 1e^- = O_2^{\cdot -}$:	ΔE is metal-independant
$M^{+n} + 1e^- = M^{+(n+1)}$:	ΔE depends on metal
$M^{+(n+1)} + O_2^{\cdot -} = M^{+(n+1)}$:	ΔE is (almost) metal-independant

O_2 affinity: $Cr^{II} > Fe^{II} > Co^{II}$ (the same order as the $M^{II/III}$ potential).

Oxygen transport (1)

O_2 is a poor Lewis base, its binding to a metal requires a redox change.

Three major types of O_2-transporting enzymes:

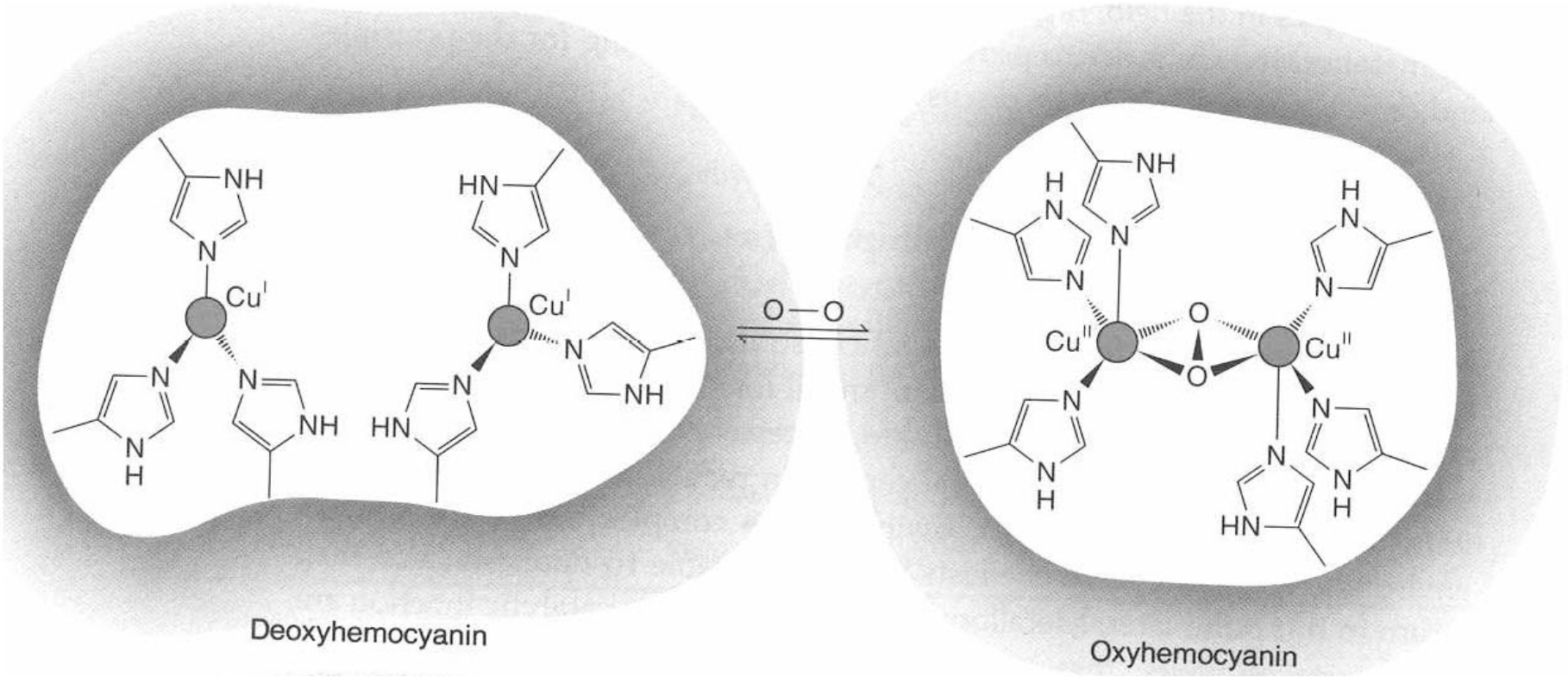


All O_2-carriers must bind O_2 reversibly and avoid the O-O bond cleavage and oxygenation chemistry

Oxygen transport (2)

O_2 is a poor Lewis base, its binding to a metal requires a redox change.

Three major types of O_2-transporting enzymes:

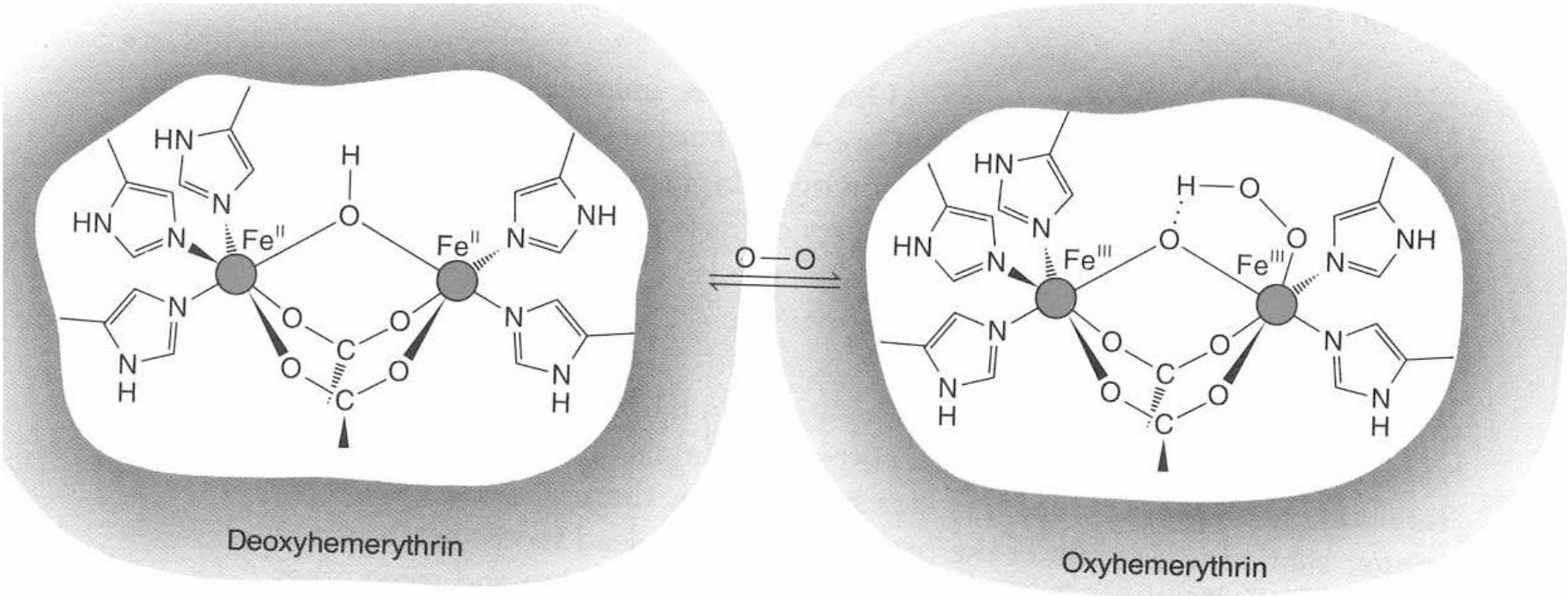


All O_2-carriers must bind O_2 reversibly and avoid the O-O bond cleavage and oxygenation chemistry

Oxygen transport (3)

O_2 is a poor Lewis base, its binding to a metal requires a redox change.

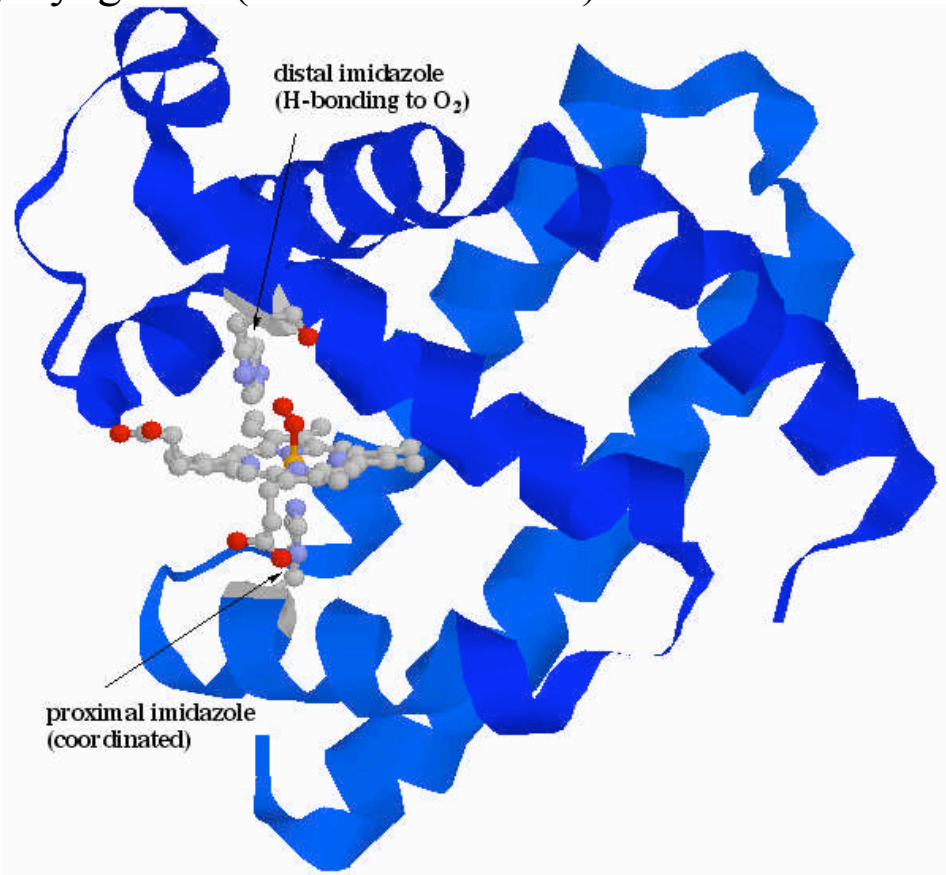
Three major types of O_2-transporting enzymes:



All O_2-carriers must bind O_2 reversibly and avoid the O-O bond cleavage and oxygenation chemistry

Myoglobin and Hemoglobin

Crystal structure of oxymyoglobin (PDB code 1A6M):

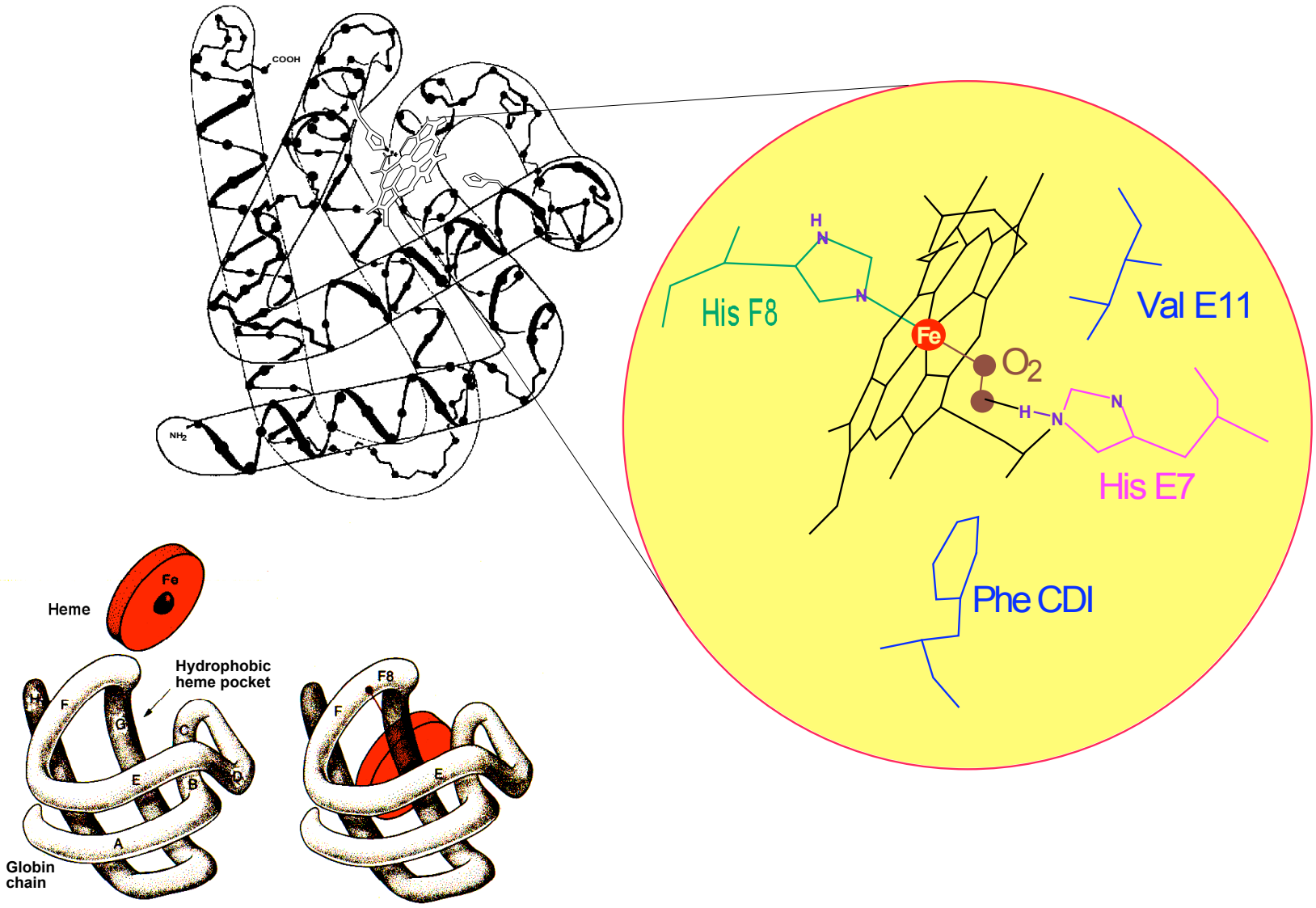


The heme group is bound to the protein backbone only via a coordinate Fe-N(histidine) bond.

A closely positioned distal imidazole stabilizes the O_2 adduct via hydrogen bonding.

Hemoglobin is a tetramer: 2 pairs of slightly different units, called α and β (overall composition: $\alpha_2\beta_2$); each unit has a structure similar to Mb.

A closer view to Myoglobin



Adapted from - Biochemistry, L. Stryer, Fourth edition, 1995, WW.H. Freeman and Company
- Biochemistry, G. Zubay, 1983, Addison-Wesley Publishing

O_2-binding (1)

$$Mb + O_2 \rightleftharpoons MbO_2$$

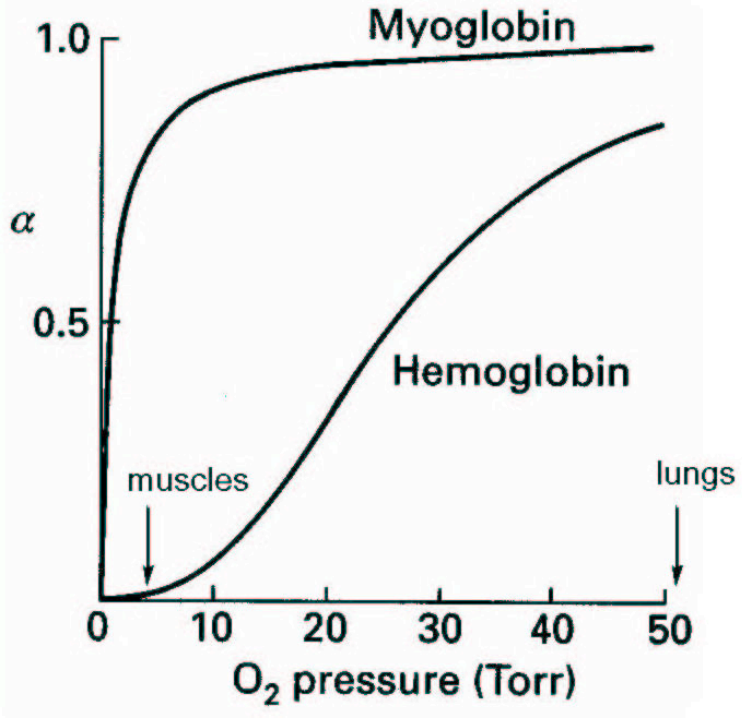
$$K = \frac{[MbO_2]}{[Mb]p_{O_2}} \qquad \textbf{(equation a)}$$

$p(O_2)$ - partial pressure of O_2.

The *fractional O_2 saturation*:

$$\alpha = \frac{[MbO_2]}{[Mb]+[MbO_2]}$$

(fraction of the oxygenated Mb).



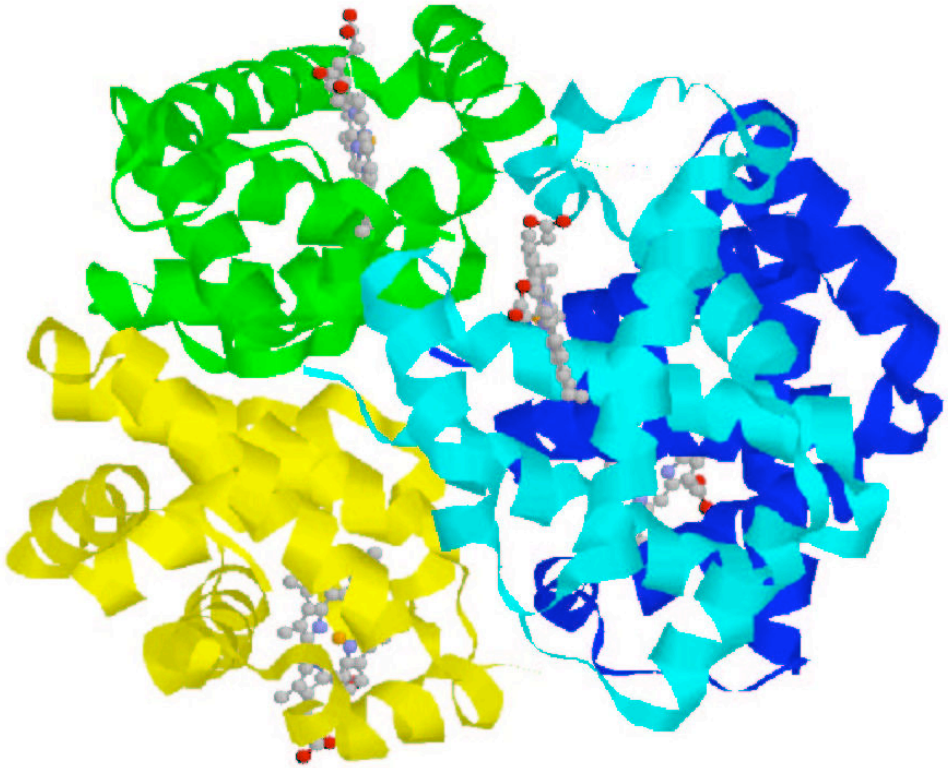
α is a function of $p(O_2)$ and equilibrium constant: $\alpha = \frac{Kp_{O_2}}{1+Kp_{O_2}}$.

O_2-binding cooperativity in Hb

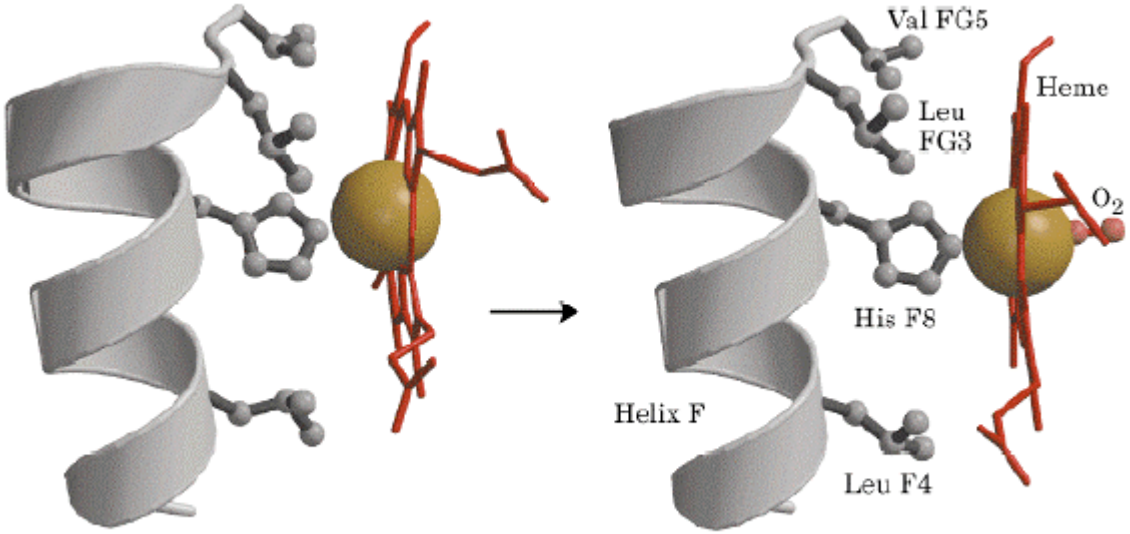
α_{Mb} follows this formula; however, α_{Hb} follows a modified law:

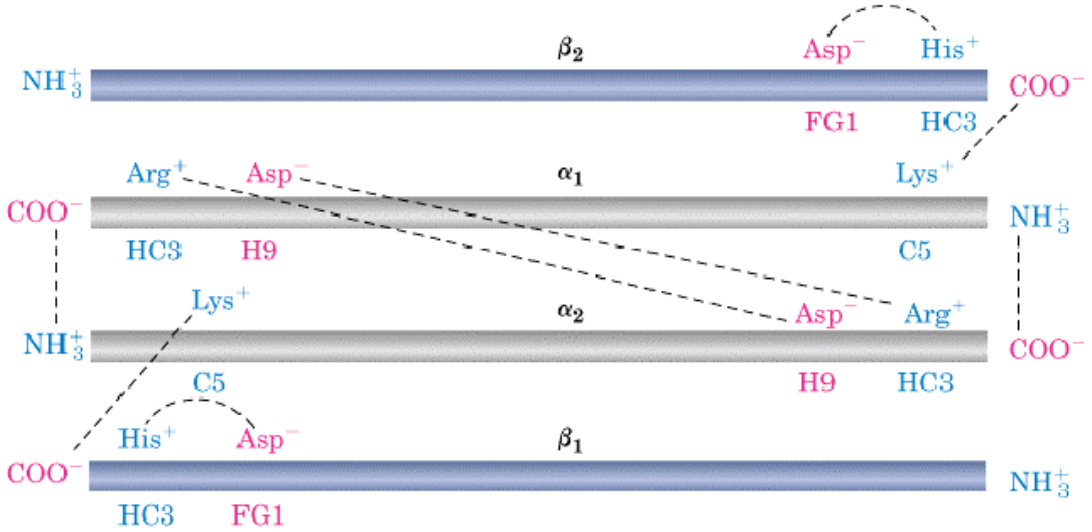
$$\alpha_{Hb} = \frac{Kp_{O_2}^n}{1 + Kp_{O_2}^n}$$, where n ≈ 2-3: Hb displays *cooperativity* in O_2 binding. Binding of the 2nd O_2 molecule is more favorable than the first.

Origin - interaction between the monomers (see picture - note the parallel alignment of pairs of the hemes).



Conformational changes in Hb upon O_2 binding





Schematic representation of some salt bridges in hemoglobin

O_2-binding (2)

Soit Co, la concentration totale en myoglobine :

- à t = 0, avant ajout de dioxygène : $Co = [Mb]$;
- à l'instant t, à la pression partielle $P(O_2)$, $Co = [Mb] + [MbO_2]$ **(équation b)**

En reportant dans l'**équation a**, on obtient alors **(équation c) :**

$$K = \frac{[MbO_2]}{(Co - [MbO_2]).\ P(O_2)}$$

Seules les espèces Mb et MbO_2 présentent une absorption par sepctroscopie UV-visible, la loi de Beer-Lambert est donc définie par (en prenant une cellule d'une largeur de 1 cm) :

- à t = 0, $Ao = \varepsilon_{Mb}Co = \varepsilon_{Mb}[Mb] + \varepsilon_{MbO2}[MbO_2]$ **(équation d)**
- à l'instant t, $Ao = \varepsilon_{Mb}[Mb] + \varepsilon_{MbO2}[MbO_2]$ **(équation e)**

Soit en retranchant les deux équations (**équation e – d)** :

$$A - Ao = (\varepsilon_{MbO2} - \varepsilon_{Mb}).[MbO_2]$$

ou encore (**équation f)** :

$$\Delta A = \Delta\varepsilon.[MbO_2]$$

avec $\Delta A = A - Ao$ et $\Delta\varepsilon = \varepsilon_{MbO2} - \varepsilon_{Mb}$

O_2-binding (3)

En reportant l'**équation f** dans l'**équation c**, on obtient alors (**équation g**) : $$K = \frac{\Delta A}{(Co\Delta\varepsilon - \Delta A).P(O_2)}$$

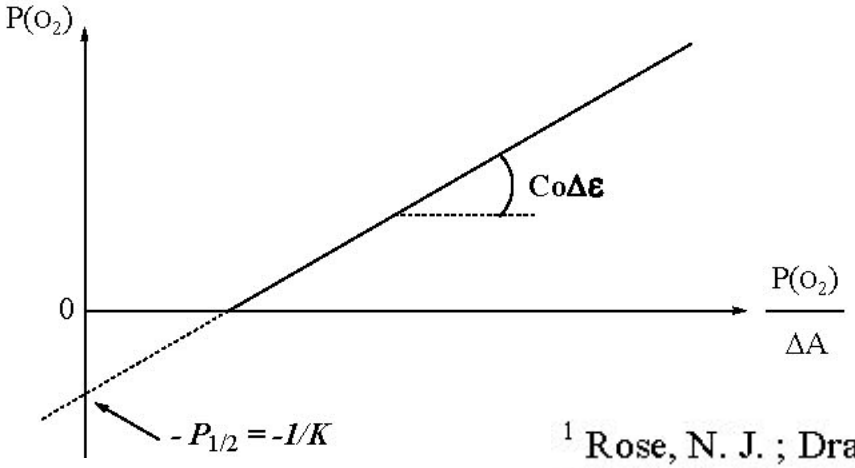
ou en prenant l'inverse de cette équation :

$$K^{-1} = \frac{Co\Delta\varepsilon}{\Delta A} . P(O_2) - P(O_2)$$

ou encore

$$P(O_2) = \frac{Co\Delta\varepsilon}{\Delta A} . P(O_2) - \frac{1}{K}$$

Il est donc possible d'exprimer la pression partielle du dioxygène $P(O_2)$ en fonction du rapport de cette même pression sur la différence d'absorbance ΔA observée entre le dérivé pentacoordiné de départ et l'absorbance à la même longueur d'onde au temps t après ajout d'une quantité connue de dioxygène (**équation h**) [1-3]:



[1] Rose, N. J. ; Drago, R. S., *J. Am. Chem. Soc.*, **1959**, *81*, 6138-6141.
[2] Beugelsdijk, T. J. ; Drago, R. S., *J. Am. Chem. Soc.*, **1975**, *97*, 6466-6472.
[3] Long, J. R. ; Drago, R. S., *J. Chem. Ed.*, **1982**, *59*, 1037-1039.

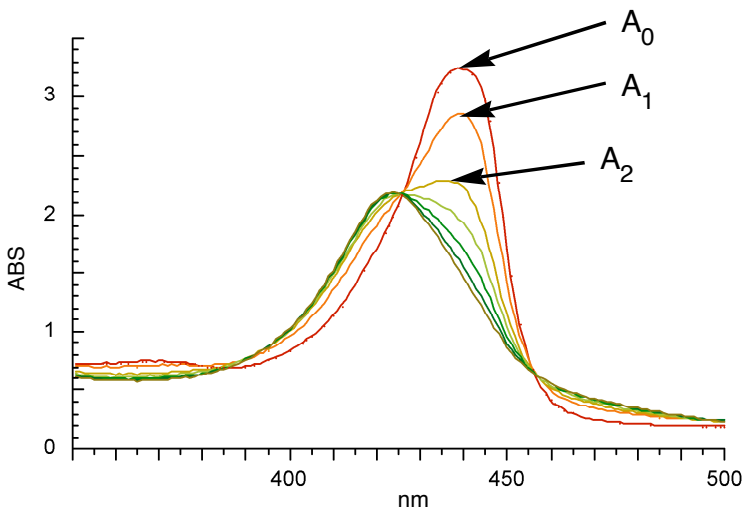
Measurement of $P_{1/2}$

$$B—Fe^{II} \underset{K}{\overset{O_2}{\rightleftharpoons}} B—Fe^{II}—O_2$$

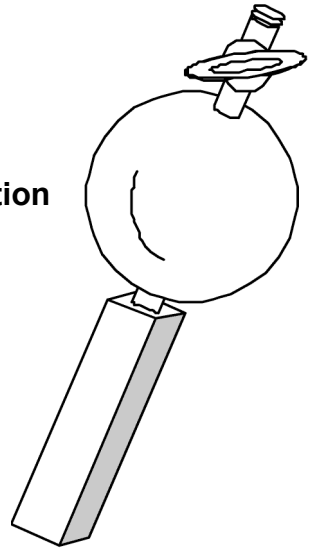
$$pO_2 = [BFe]_t.l.\Delta\varepsilon(pO_2/\Delta A) - 1/K$$

By plotting pO_2 vs $pO_2/\Delta A$, a straight line is obtained with an intercept of $-1/K$

pO_2 : partial pressure of dioxygen
$[BFe]_t$: total metalloporphyrin concentration
l : pathlength of the cell
$\Delta\varepsilon$: difference in the molar extinction coefficients between the oxygenated and deoxygenated forms
ΔA : difference between the absorptions at pO_2 and without dioxygen



Schematic representation of a tonometer



$$P_n = \frac{P_{n-1}(V - v) + 760v}{V}$$

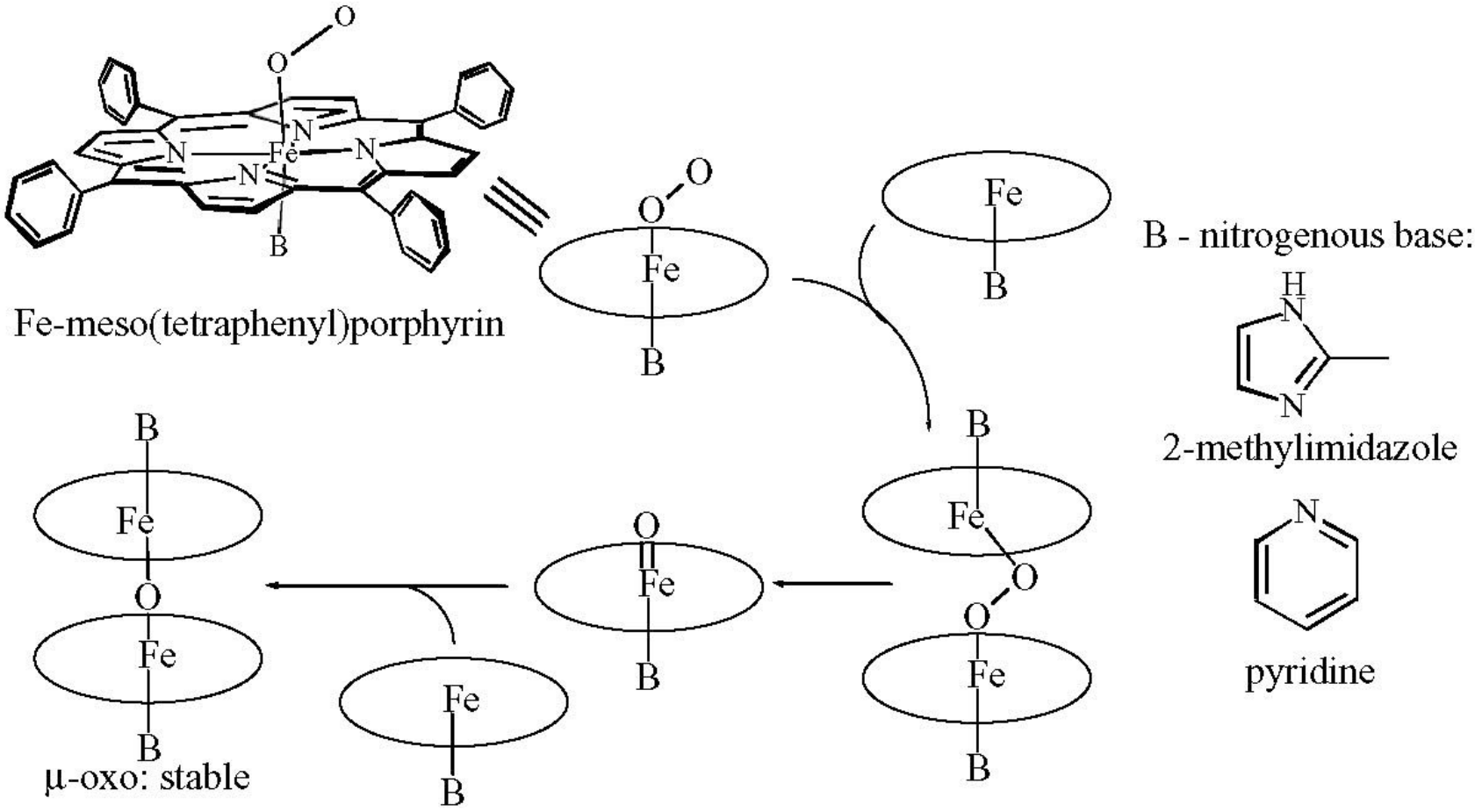
V : volume of the tonometer excluding the solution volume
v: volume of gas removed and oxygen added
(total pressure constant at 760 Torr)

According to Rose, N. J.; Drago, R. S. *J. Am. Chem. Soc.* **1959**, *81*, 6138-6141 and Collman, J. P. *et al. J. Am. Chem. Soc.* **1978**, *100*, 2761-2766.

Synthetic models of Mb

Can the reversible binding of O_2 in Mb and Hb be achieved with low molecular weight Fe-porphyrins?

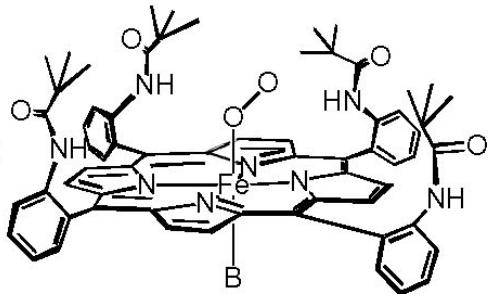
Simple Fe^{II}-porphyrins undergo irreversible oxygenation in solution:

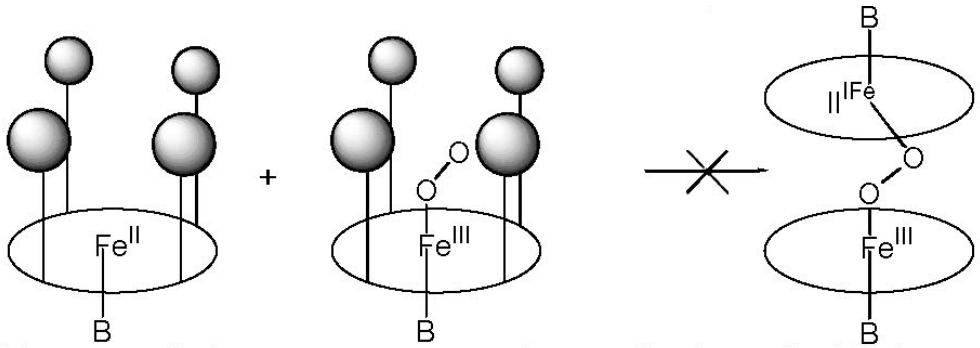


Oxidation of dioxygen carriers (1)

Solution: prevent formation of μ-oxo dimer using sterics:

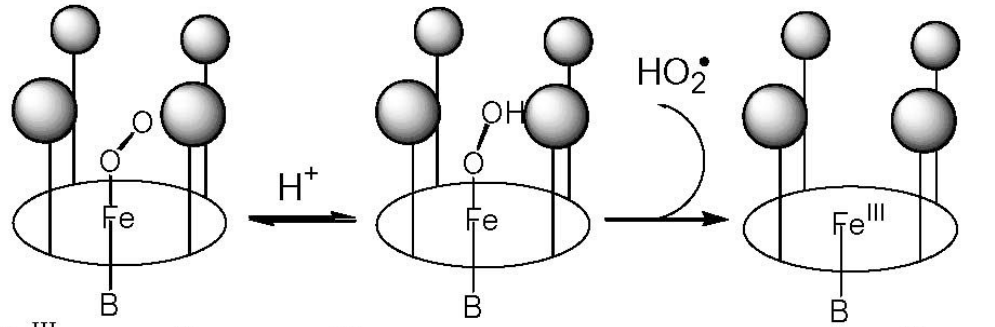
picket-fence porphyrin





This approach does not prevent another mechanism of oxidation:

This approach does not prevent another mechanism of oxidation:



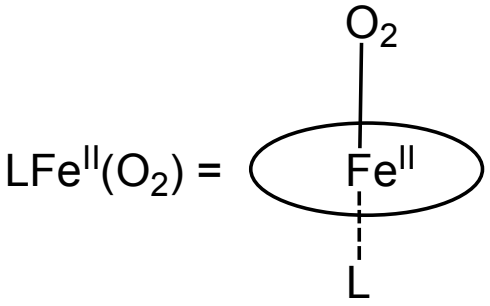
Fe^{III}-superoxido Fe^{III}-hydrosuperoxido met-form - Fe^{III}(por)

The same mechanism is responsible for autooxidation of Mb and Hb *in vivo*.

Momenteau & Reed, Chem. Rev, **1994**

Oxidation of dioxygen carriers (2)

In summary, where two mechanisms exist for the oxydation of synthetic oxygen carriers, only one occurs for those natural



$$LFe^{II}(O_2) + H_2O \longrightarrow LFe^{III}(H_2O) + O_2^{\bullet -} \quad \text{(eq. 1)}$$

$$LFe^{II}(O_2) + H^+ + H_2O \longrightarrow LFe^{III}(H_2O) + {}^{\bullet}O_2H \quad \text{(eq. 2)}$$

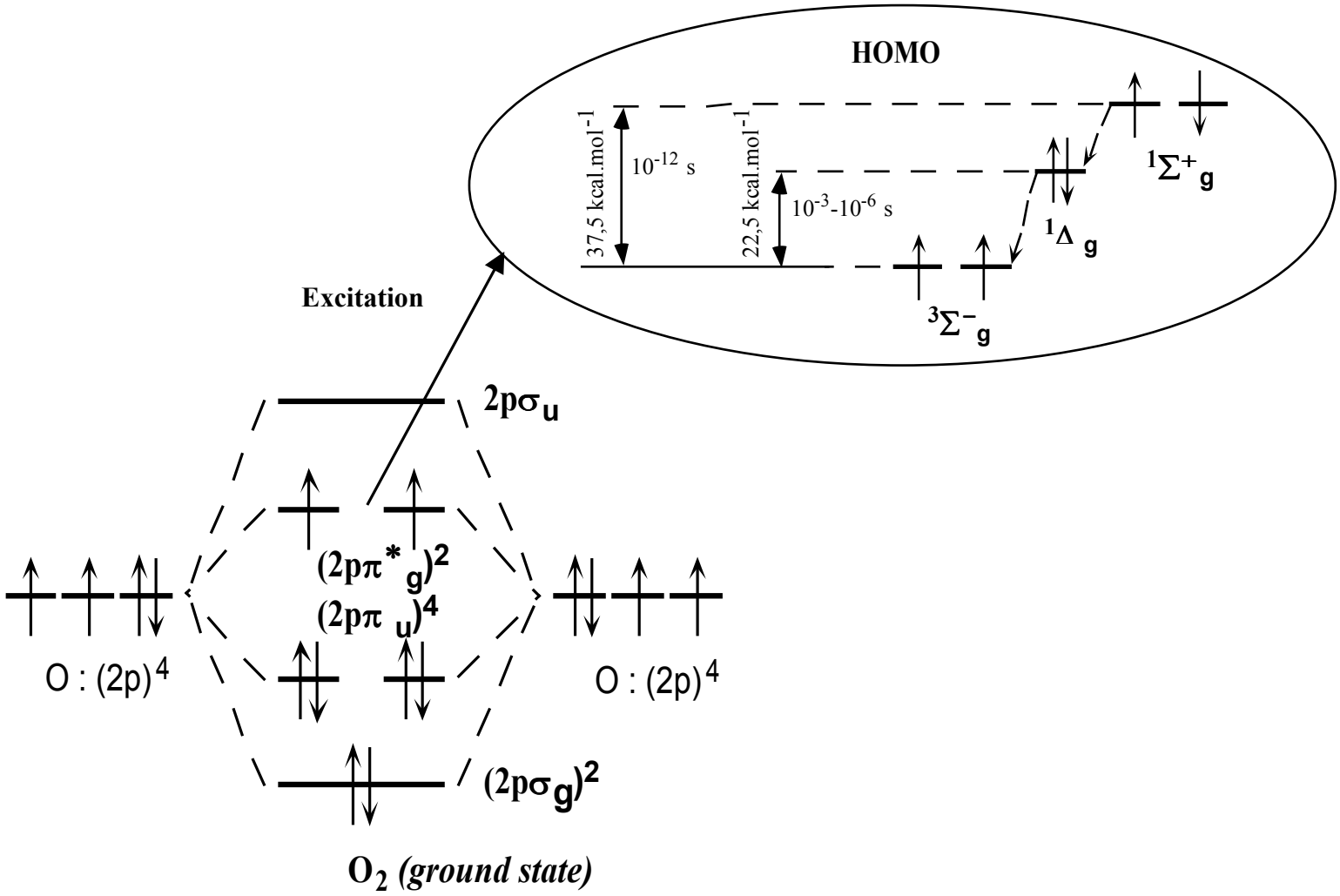
$$LFe^{II}(O_2) + HO^- \longrightarrow LFe^{III}(HO^-) + O_2^{\bullet -} \quad \text{(eq. 3)}$$

$$LFe^{II}(O_2) + LFe^{II} \longrightarrow LFe^{III}\text{-}O_2\text{-}Fe^{III}L \longrightarrow 2\ LFe^{IV}{=}O \quad \text{(eq. 4)}$$

$$LFe^{IV}{=}O + LFe^{II} \longrightarrow LFe^{III}\text{-}O\text{-}Fe^{III}L \quad \text{(eq. 5)}$$

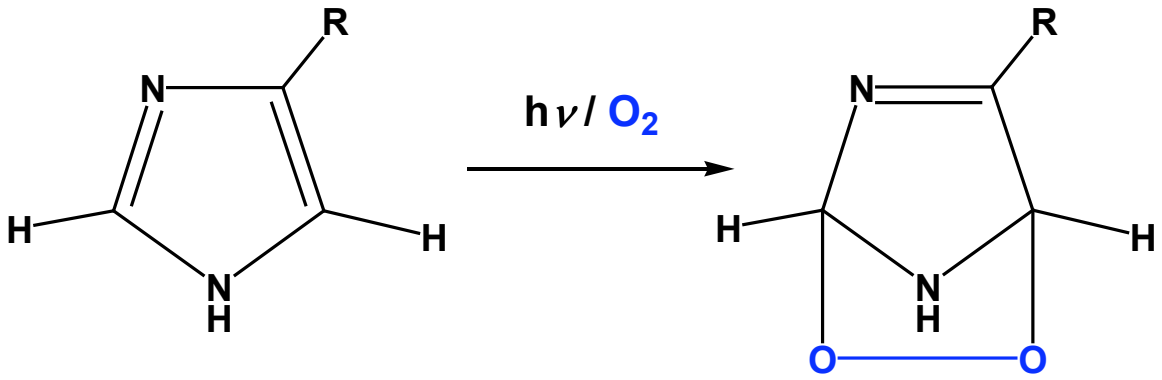
Momenteau & Reed, Chem. Rev, **1994**

Molecular Orbitals of Dioxygen



O_2 : $(1\sigma_g)^2\ (1\sigma_u^*)^2\ (2\sigma_g)^2\ (2\sigma_u^*)^2\ (2p\sigma_g)^2\ (2p\pi_u)^4\ (2p\pi_g^*)^2$

Photosensitized Oxidations of Imidazoles

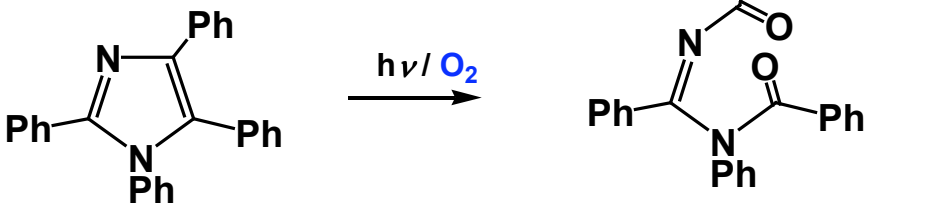


^{1}sensitizer + hν ---------> ^{3}sensitizer

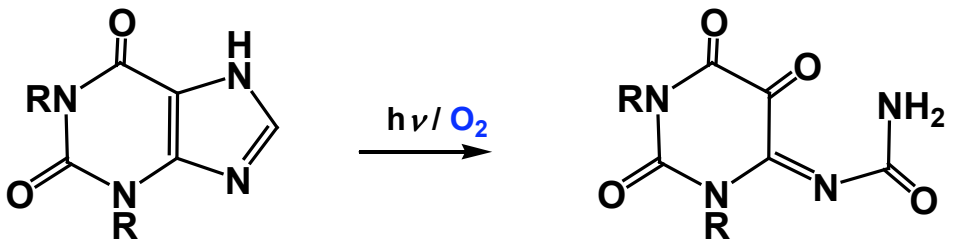
^{3}sensitizer + $^3\Sigma_g^-$ O_2 ---------> $^1\Delta_g^-$ O_2 + ^{1}sensitizer

$^1\Delta_g^-$ O_2 + acceptor ---------> acceptor-O_2

e.g.



N,N'-Diaroylbenzamidines



H. H. Wasserman, K. Stiller, M. B. Floyd, *Tetrahedron Lett.* **1968**, *29*, 3277-3280.
M. Kaplan, *Chem. Tech.* **1971**, 621-626.
T. Matsuura, I. Saito, J. Chem. Soc. Chem. Commun., **1967**, 693-694

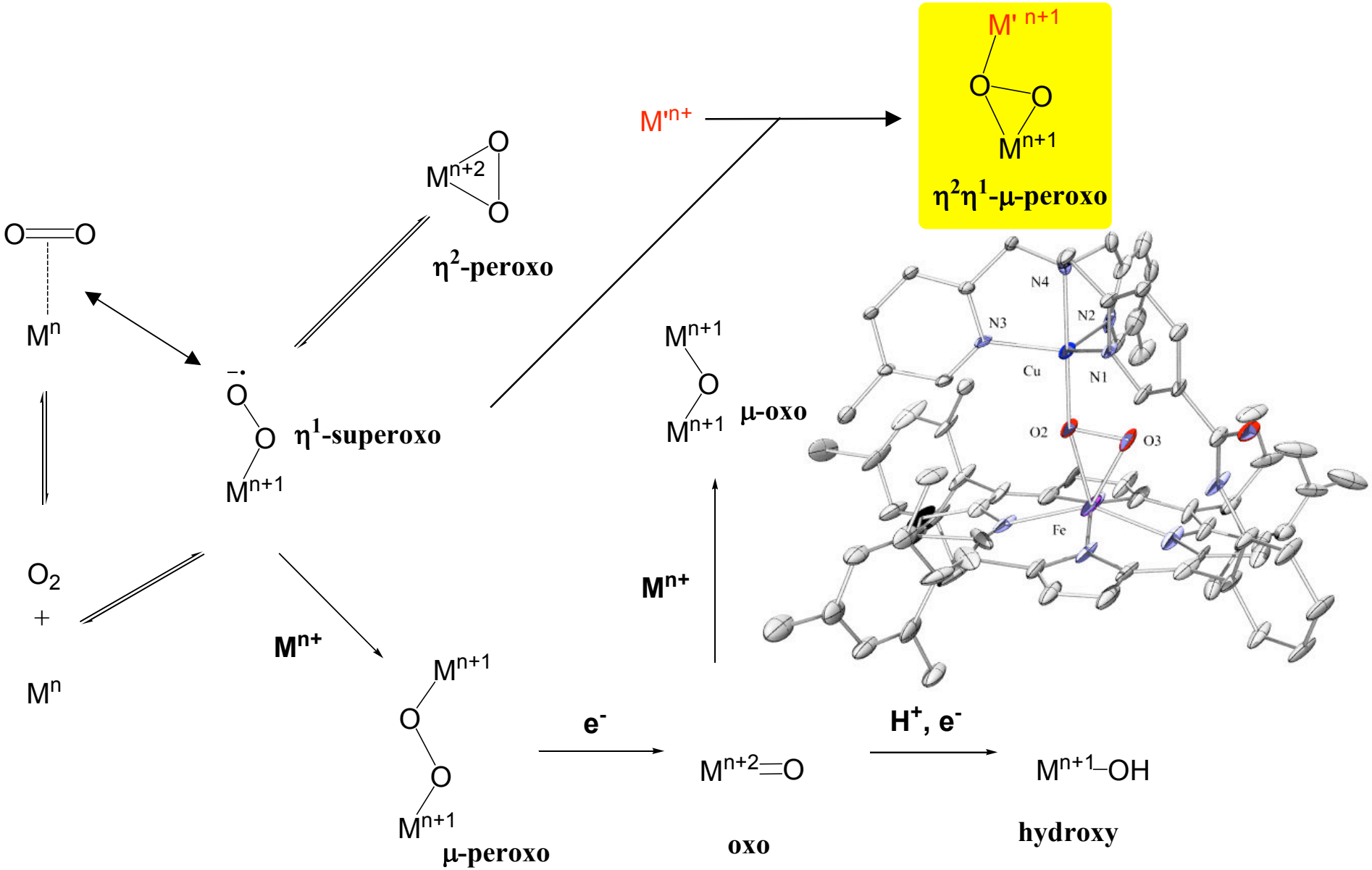
The various oxidation states of dioxygen

Species	Typical compounds	Bond order (O-O)	Bond length (Å)	Bond length (kcal.mol^{-1})	ν_{O-O} (cm^{-1})
O_2^+	O_2AsF_6	2.5	1.12	149.4	1858
O_2 ($^3\Sigma^-_g$)	O_2	2	1.21	117.2	1555
O_2 ($^1\Delta_g$)	O_2	2	1.22	94.7	1484
O_2^-	KO_2	1.5	1.33		1145
O_2^{2-}	Na_2O_2	1	1.49	48.8	842

ν(O-O) in MbO_2 = 1105 cm^{-1}, a value characteristic of a coordinated superoxide

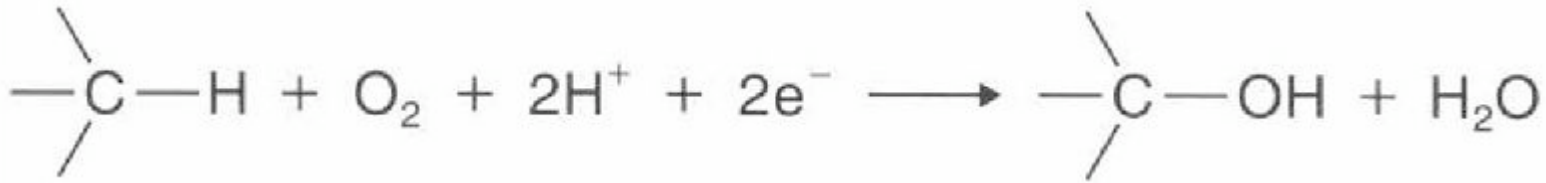
The various coordinations of dioxygen to a metal M

Y. Naruta et al. *Angew. Chem., Int. Ed.* **2003**, *42*, 2788 – 2791

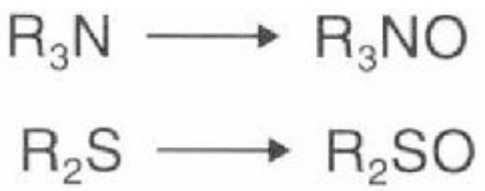


Cytochromes P-450

Oxygenation of organic substrates using O_2:



Also:



Biological role: conversion of lipophilic substances into water-Soluble forms for excretion as a part of detoxification.

(MONO)OXYGENATION ACTIVITY
(oxygen atom insertion)

Cytochromes P-450

Biological role: conversion of lipophilic substances into water-soluble forms for excretion as a part of detoxification.

Biologically relevant substrates in humans:

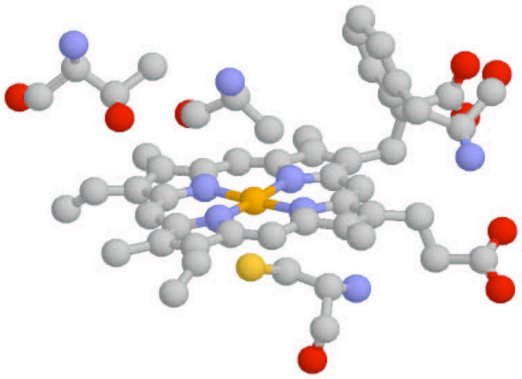
Major types of reactions catalyzed by cytochromes P-450

Reaction type	Simplified example	Typical substrate
Aliphatic hydroxylation	Cyclohexane → cyclohexanol	Pentobarbital
Aromatic hydroxylation	Benzene → phenol	Phenobarbital
Alkene epoxidation	Cyclohexene → cyclohexene oxide	Aldrin
N-dealkylation	$CH_3N(H)CH_3 \rightarrow CH_3NH_2 + H_2C{=}O$	Methadone
O-dealkylation	$C_6H_5OCH_3 \rightarrow C_6H_5OH + H_2C{=}O$	Codeine
Oxidative deamination	$(CH_3)_2CHNH_2 \rightarrow (CH_3)_2C{=}O + NH_3$	Amphetamine
S-oxidation	$CH_3SCH_3 \rightarrow (CH_3)_2S{=}O$	Chlorpromazine
Reductive dehalognation	$C_6H_5CH_2Br \rightarrow C_6H_5CH_3$	Halothane

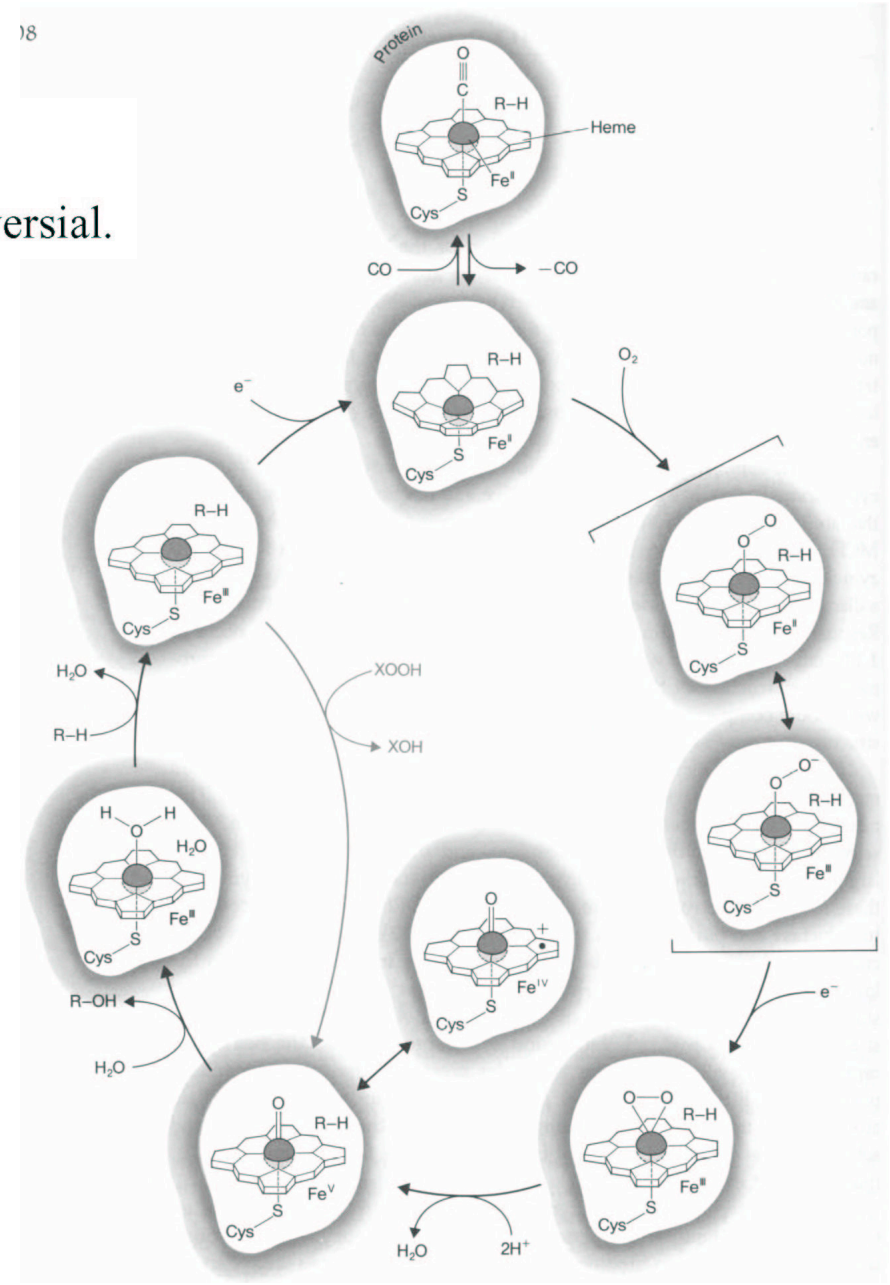
Cytochromes P-450: mechanism

5-coordinate Fe-porphyrin;
the enzyme shuttles between Fe^{II} and Fe^{III} states.
Precise mechanism of oxygenation is very controversial.

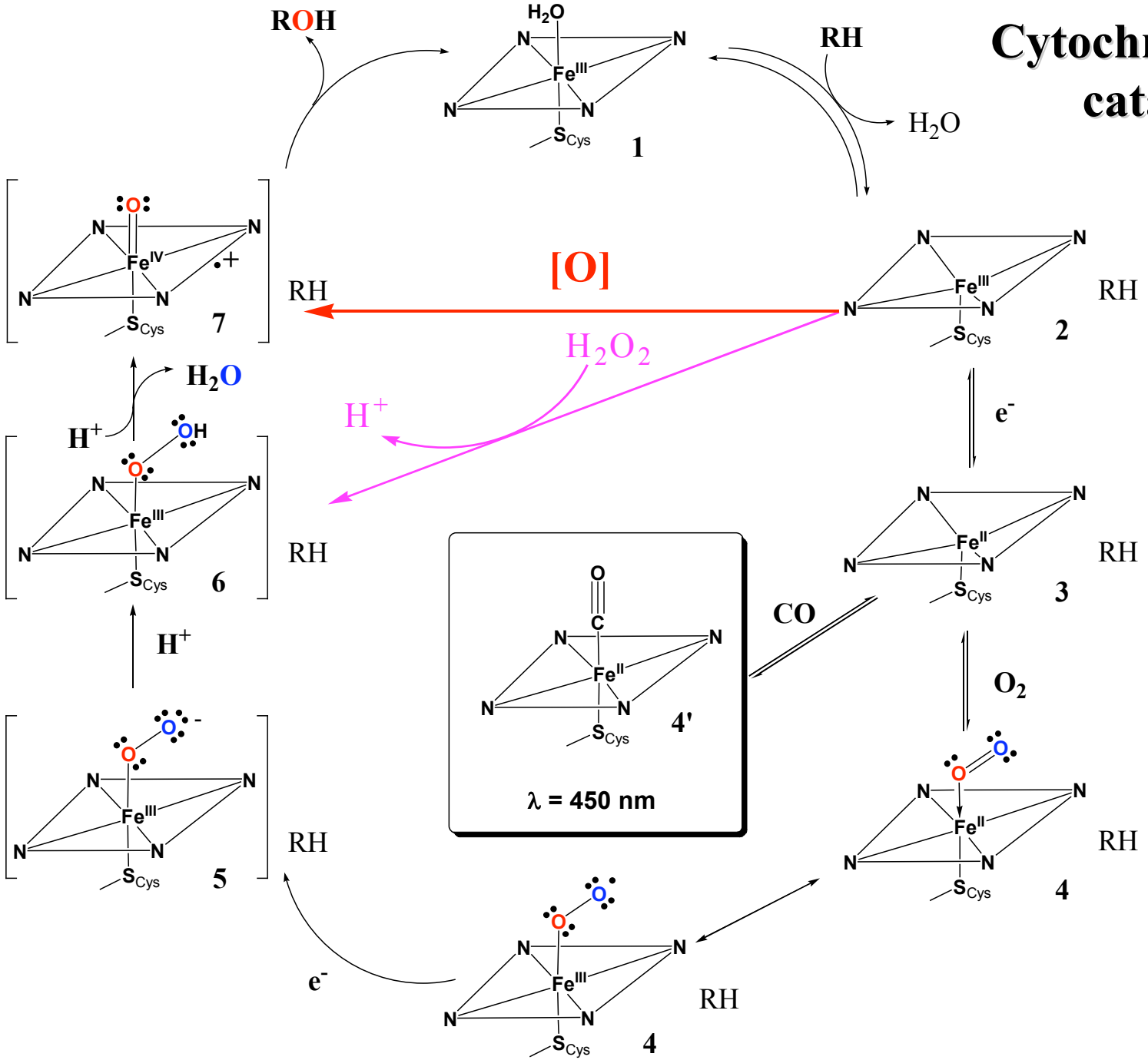
Simplified mechanism:



active site of a
cytochrome P-450.



Cytochromes P-450: catalytic cycle



Adapted from

Sono, M.; Roach, M. P.; Coulter, E. D.; Dawson, J. H., *Chem. Rev.* **1996**, 2841-2887.

Cytochrome P-450$_{cam}$: Active site X-ray

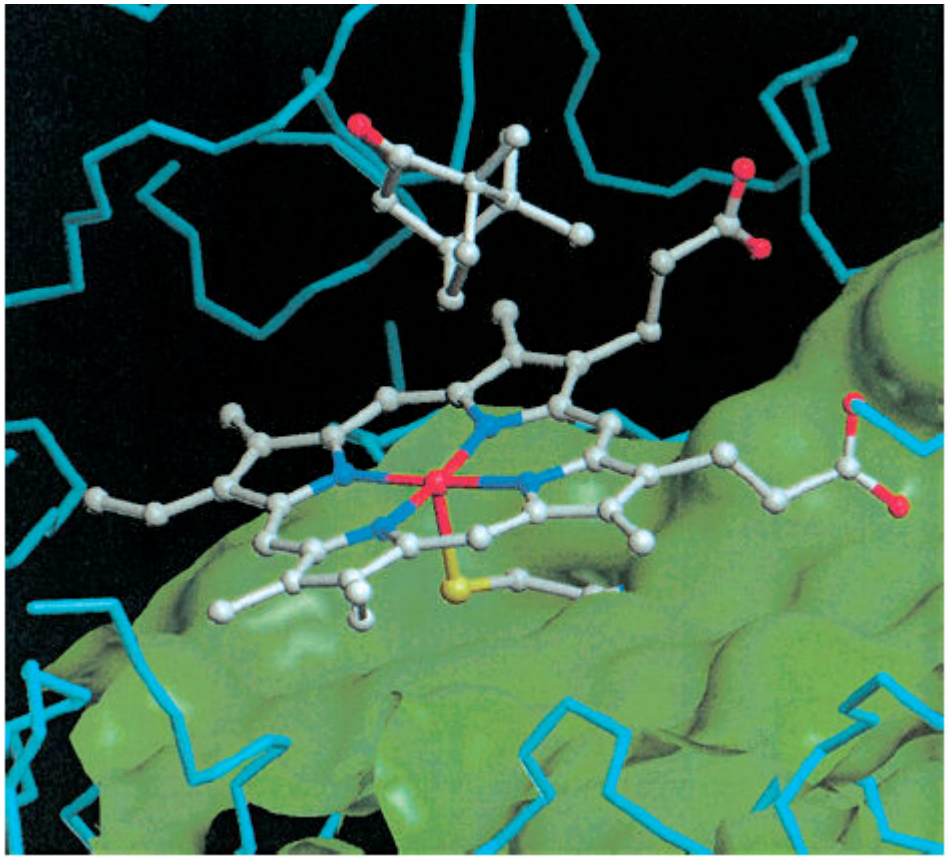
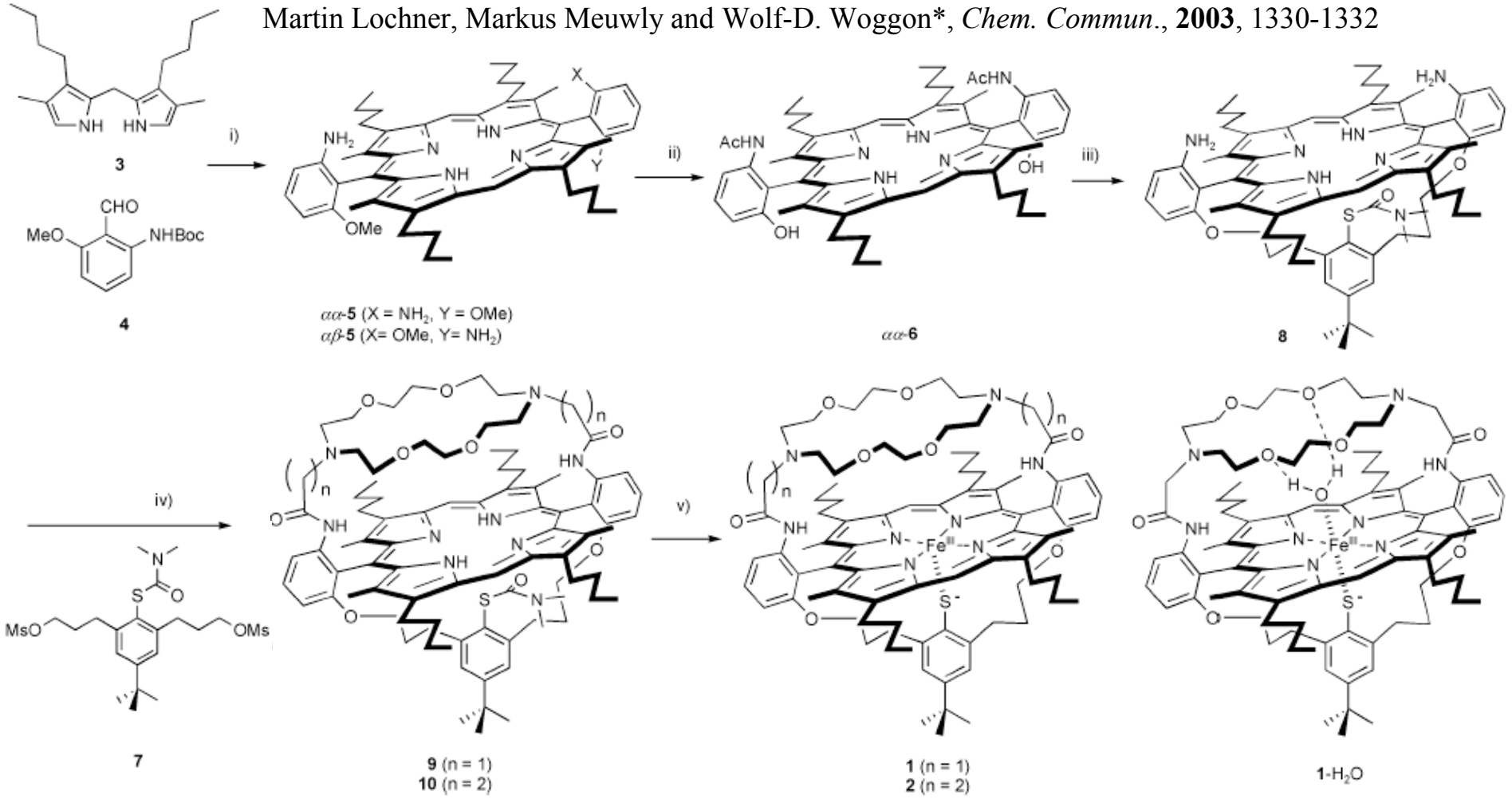


FIGURE 1. Portion of the crystal structure of P450$_{cam}$ with bound substrate showing the heme and protein cysteine ligation of iron; from ref 3, PDB file 1AKD.

Schlichting, I.; Jung, C.; Schulze, H. Crystal Structure of Cytochrome P-450cam Complexed with the (1S)-Camphor Enantiomer. *FEBS Lett.* **1997**, *415*, 253–257.

The origin of the low-spin character of the resting state of cytochrome P450cam investigated by means of active site analogues

Martin Lochner, Markus Meuwly and Wolf-D. Woggon*, *Chem. Commun*., **2003**, 1330-1332



Reagents and conditions: **(i)** (1) cat. PTSA, MeCN, rt, (2) DDQ, MeCN-THF, rt, (3) TFA, CH_2Cl_2, rt, 38% (3steps); (3) **(ii)** (1) AcCl, DMAP, pyridine, CH_2Cl_2, rt, (2) flash chromatography, (3) $AlCl_3$, EtSH-CH_2Cl_2 3:2, rt, 36% (3 steps); **(iii)** (1) Cs_2CO_3, DMF, 60°C, **7**, high-dilution, (2) 6M HCl, MeOH, reflux, 77% (2 steps); **(iv)** for n =1 (1) chloroacetyl chloride, $CHCl_3$, rt, (2) 1,10-diaza-18-crown-6, EtOH, reflux, 44% (2 steps); for n =2 (1) acryloyl chloride, Et_3N, CH_2Cl_2, rt, (2) 1,10-diaza-18-crown-6, MeOH-CH_2Cl_2, 80°C, 46% (2 steps);(v) (1) KOMe, dioxane, reflux, (2) $FeBr_2$, 2,6-lutidine, toluene, reflux, 44% for n = 1 (2 steps), 49% for n = 2 (2 steps)

(1) Energy metabolism

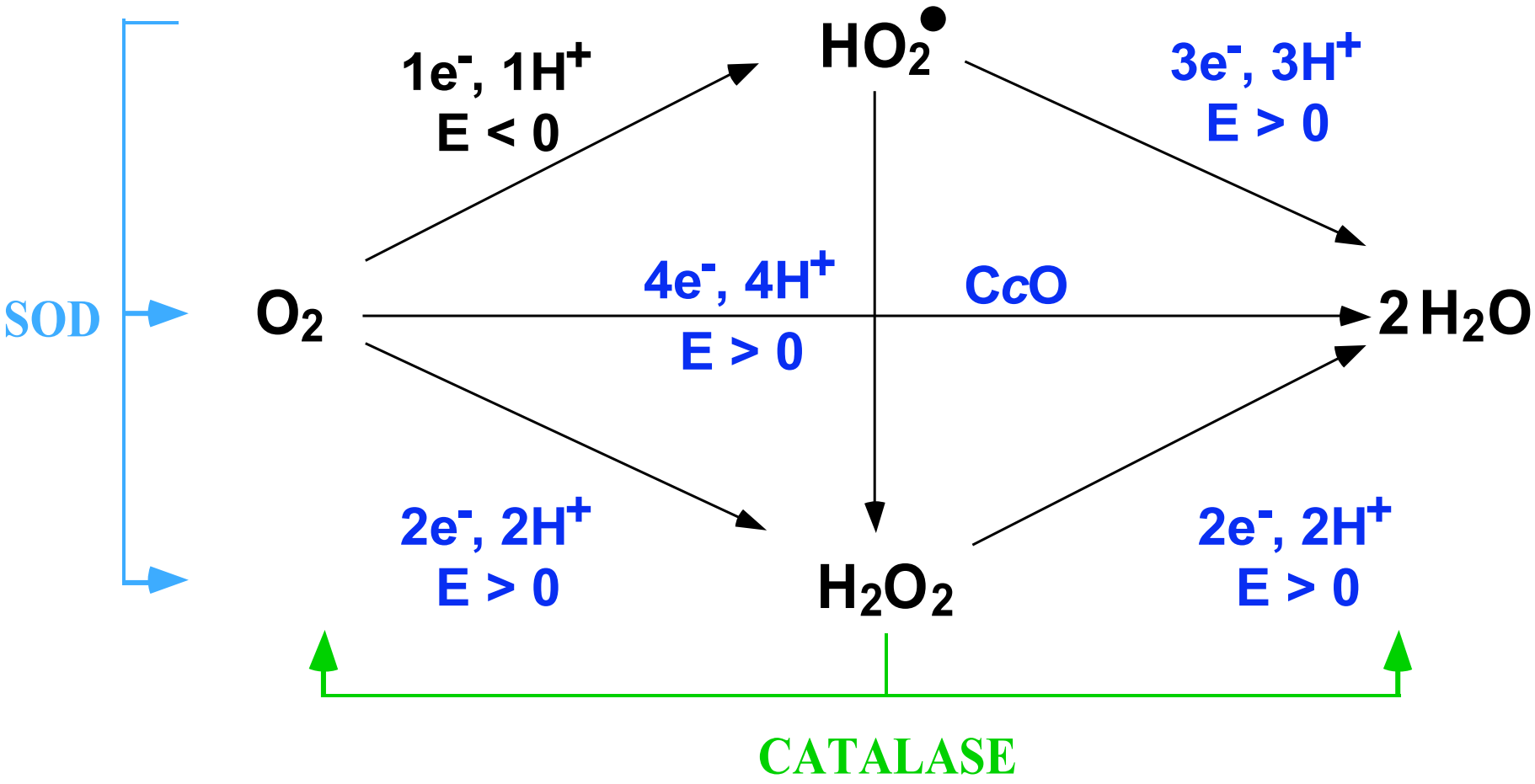
The only way a living organism can convert chemical energy stored in food into useful work, required to sustain life, is to use redox processes. Heat is a useful byproduct of this process.

Electrons and H^+ are stripped off foods by enzymes and participate in a series of spontaneous redox reactions, which are coupled to uphill processes, such as tissue growth.

In all aerobic species, this redox cycle has to operate in the presence of O_2 and in most it utilizes O_2 as the ultimate acceptor of the electrons and H^+.

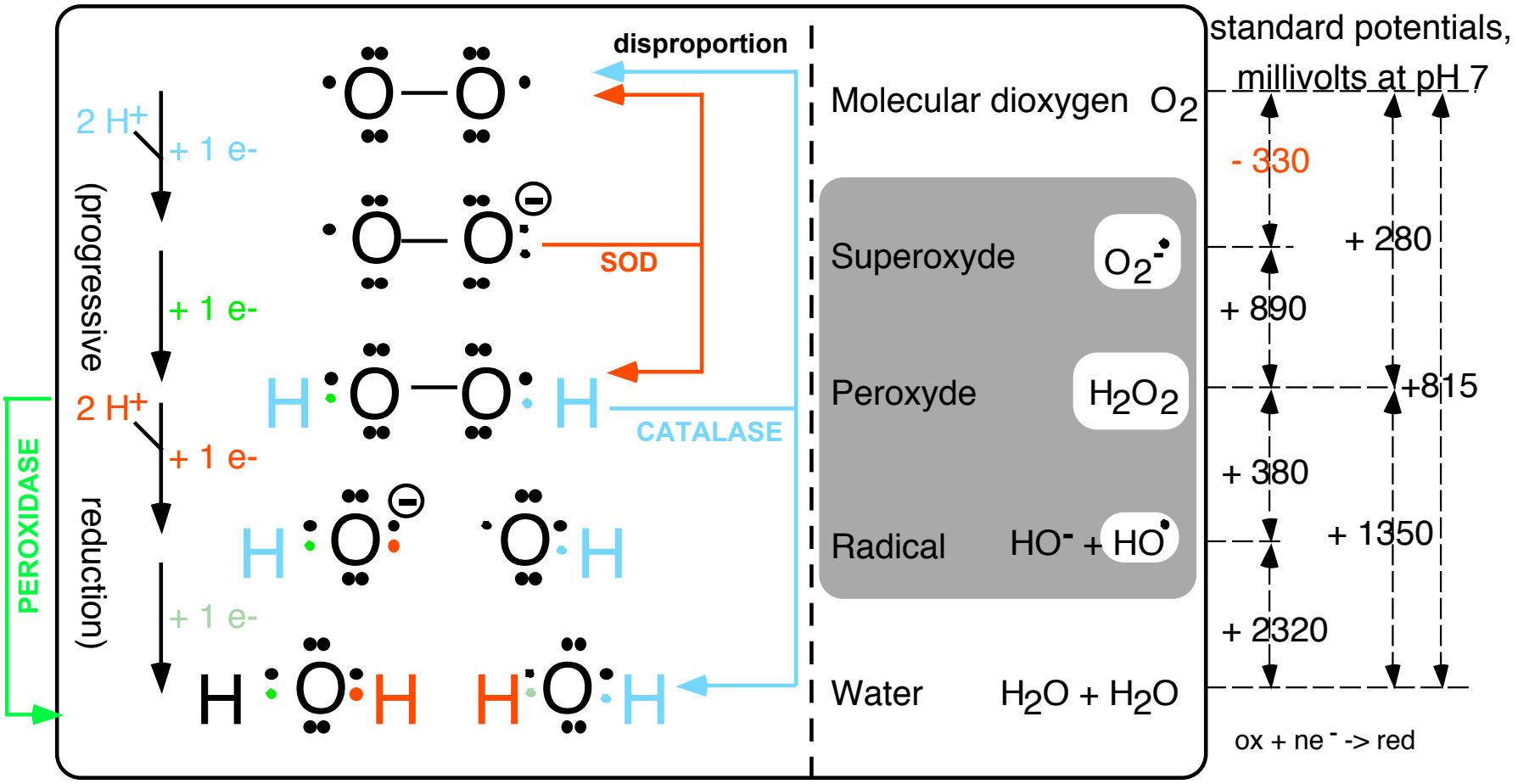
Understanding the redox chemistry of O_2 becomes important in understanding the energy metabolism in general and O_2 metabolism in particular.

Reduction of dioxygen



Fontecave, M.*Bull. Chem. Soc. Chim. Fr.*, **1991**, *128*, 505-520
Wood, P.M. *Biochem. J.* **1988**, *235*, 287-289.

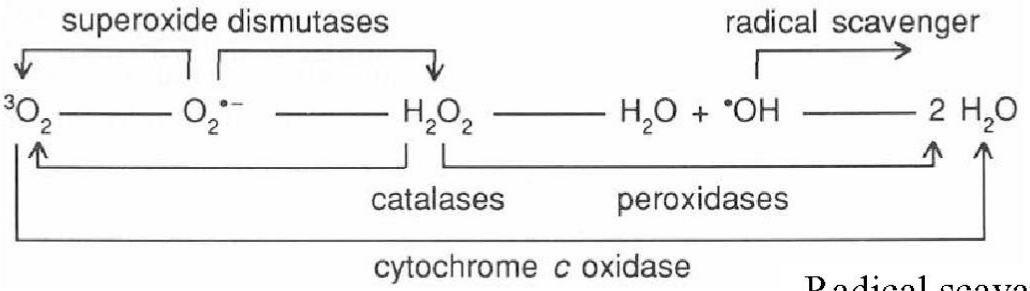
Monoelectronic reductions of dioxygen



couples	$O_2^{\cdot -}$ / $HO_2^{\cdot}$	O_2^{2-} / HO_2^-	HO_2^- / H_2O_2	$O^{\cdot -}$ / $HO^{\cdot}$	H_2O / HO^-
pKa	4,8	> 14	11,8	11,9	14

Dioxygen metabolism

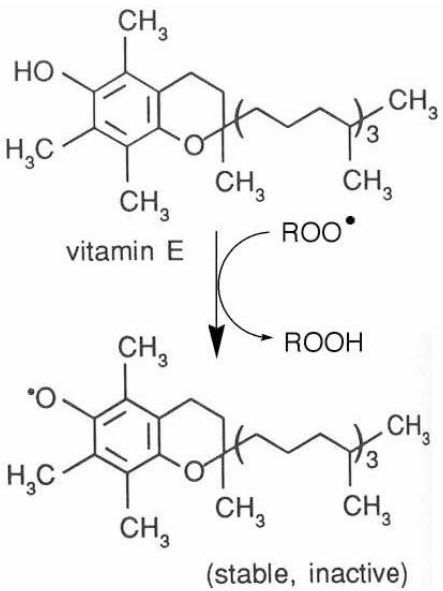
- Cytochrome c oxidase converts O_2 into H_2O thereby removing reducing equivalents from the organism.
- Some O_2 becomes only partially reduced (for example, via a redox reaction between O_2 and highly reduced cofactors).
- These partially reduced "reactive oxygen species" (ROS) are highly cytotoxic. Living organisms developed multiple mechanisms to deal with ROS:



Radical scavangers:

antioxidatively active compounds	targets
vitamin C (ascorbate) ceruloplasmin (in plasma)	$^\bullet OH$
vitamin E (α-tocopherol) β-carotene (in membranes)	$ROO^\bullet$
transferrin	$^\bullet OH$
S-rich compounds, e.g. metallothionein	$^\bullet OH$
uric acid	$^\bullet OH$
gold-containing compounds (therapeutical use)	$^1O_2$ (hypothetical)

Vitamin E at work:



Chemistry of ROS (1)

The hydroxyl radical represents the most powerful $1e^-$ oxidizing agent. It can be formed by reductive cleavage of hydrogen peroxide ($H_2O_2 + H^+ + e^- \rightarrow H_2O + HO^\bullet$) or produced according to Haber-Weiss reaction (equation 3) in an iron-containing environment.

$$O_2^- + Fe^{3+} \rightarrow Fe^{2+} + O_2 \quad \textbf{Eq. 1}$$

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + HO^\bullet + HO^- \quad \textbf{Eq. 2} \text{ (Fenton reaction)}$$

The global reaction being : $O_2^- + H_2O_2 \rightarrow HO^- + HO^\bullet + O_2$ **Eq. 3**

The hydroxyl radical can oxidize all kinds of substrates, even the phopholipidic membrane :

$$RH + HO^\bullet \rightarrow R^\bullet + H_2O \quad \textbf{Eq. 4}$$

$$R^\bullet + O_2 \rightarrow ROO^\bullet \quad \textbf{Eq. 5}$$

$$ROO^\bullet + RH \rightarrow ROOH + R^\bullet \quad \textbf{Eq. 6}$$

Chemistry of ROS (2)

Hydrogen peroxide can be produced by two different patways:

-By the 1e- reduction of superoxide:

$$O_2^- + 2H^+ + e^- \text{ ---> } H_2O_2 \quad \textbf{Eq. 7}$$

-By the 2e- reduction of molecular dioxygen by an oxidase: (example)

$$O_2 + 2H^+ + 2e^- \text{ ---> } H_2O_2 \quad \textbf{Eq. 8}$$

This reaction is used for the catabolism of fatty acids in peroxisomes.

Protection enzymes include catalases and peroxidases (glutathion peroxidases, cytochrome c peroxidase, thioredoxine reductase, NADPH peroxydase…).

Catalases are particularly concentrated in liver and red corpuscules. These enzymes are tetrameric and contain one heme molecule as well as one NADPH molecule. The reaction is catalyzed according to a two-step process: (axial ligand is tyrosinate)

$$H_2O_2 + Fe^{3+} \text{ ---> } Fe^{5+}\text{-}\ H_2O_2 + H_2O \quad \textbf{Eq. 9}$$

$$Fe^{5+}\text{-}\ H_2O_2 + H_2O_2 \text{ ---> } Fe^{3+} + H_2O + O_2 \quad \textbf{Eq. 10}$$

Chemistry of ROS (3)

(AH_2 being an organic substrate)

Back on peroxydase activity:

$$H_2O_2 + 2\ AH_2 \longrightarrow 2\ A\dot{H} + 2\ H_2O$$

$$A\dot{H} + A\dot{H} \longrightarrow A + AH_2$$

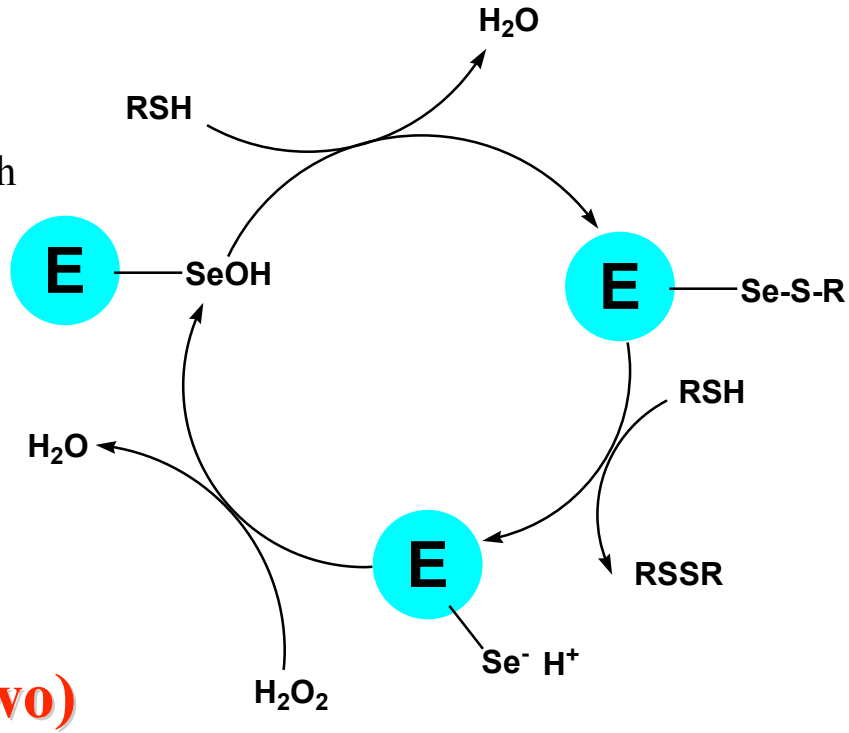
$$H_2O_2 + AH_2 \longrightarrow A + 2\ H_2O$$

Glutathion peroxidase : is not a hemoprotein as the other peroxidases !
Contains selenocystein and the catalyzed reaction is:

$$2\ R\text{-}SH + H_2O_2 \rightarrow R\text{-}S\text{-}S\text{-}R + 2\ H_2O$$

Active on a lot of thiols but particularly efficient with glutathion, which is oxidized 100 times faster than mercapoethanol.

Selenium goes through 3 different stages:
-selenolate (analoguous of cysteinate)
-seleninic acid (E-SeOH)
-selenenyl sulfur (E-Se-SR)



OXIDATION ACTIVITY
(electrons abstraction, usually two)

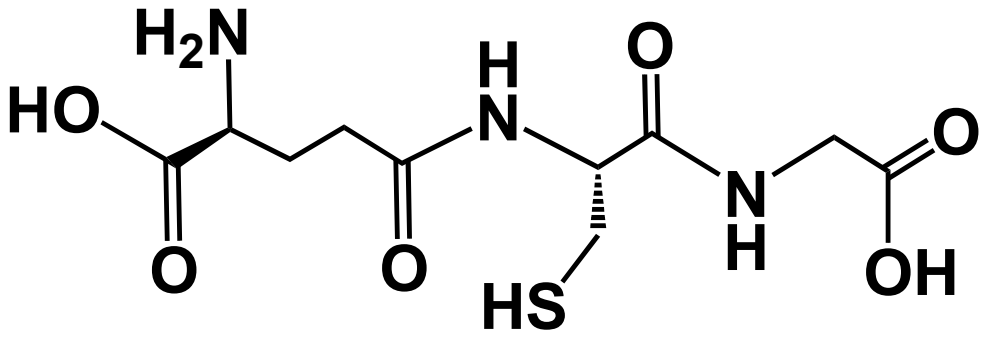
Chemistry of ROS (4)

Glutathion peroxidase: is not a hemoprotein as the other peroxidases !
Contains selenocystein and the catalyzed reaction in the case of GSH is:

$$2\ GSH + H_2O_2 \rightarrow GS\text{-}SG + 2\ H_2O$$

•Glutathion is actually:

N-[N-L-gamma-glutamyl-L-cystéinyl]glycine (or GSH) is the intracellular thiol non proteic the more abondant

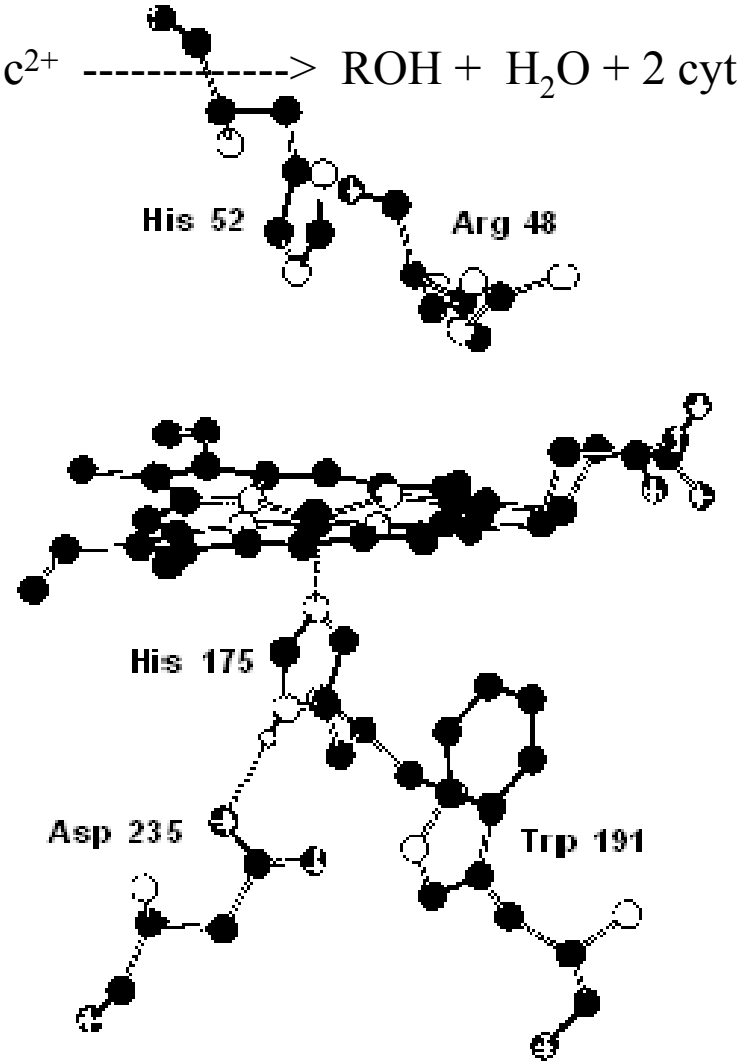


Described for the first time in 1888 by Rey-Pailhade who named it "Philothion"

Chemistry of ROS (5)

Another example of peroxidase activity is cytochrome *c* peroxidase, that reduces hydrogen (or organic) peroxide(s) according to :

$ROOH + 2\ H^+ + 2\ cyt\ c^{2+}$ ----------> $ROH + H_2O + 2\ cyt\ c^{3+}$ (R = H or alkyle)

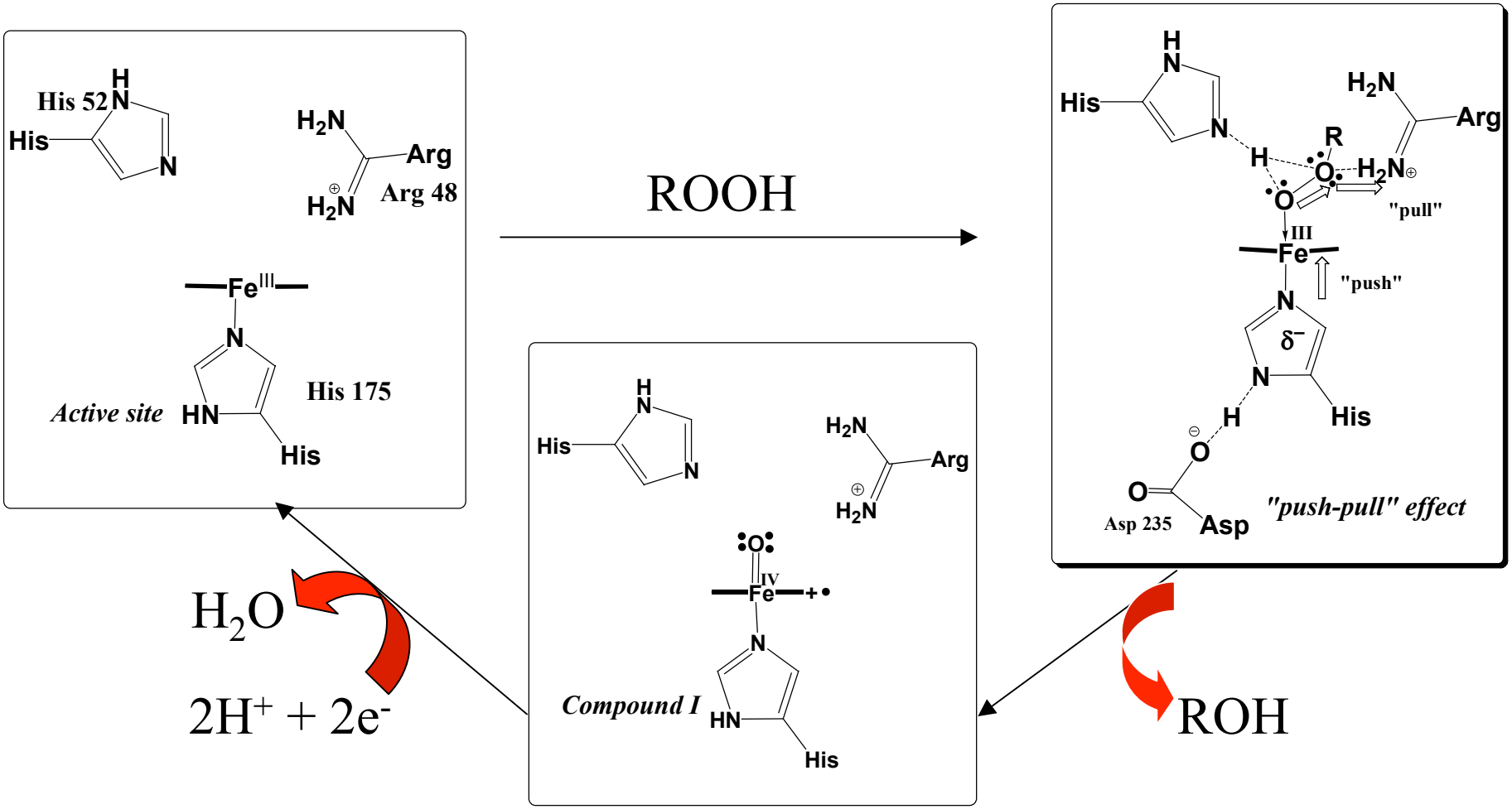


Chemistry of ROS (6)

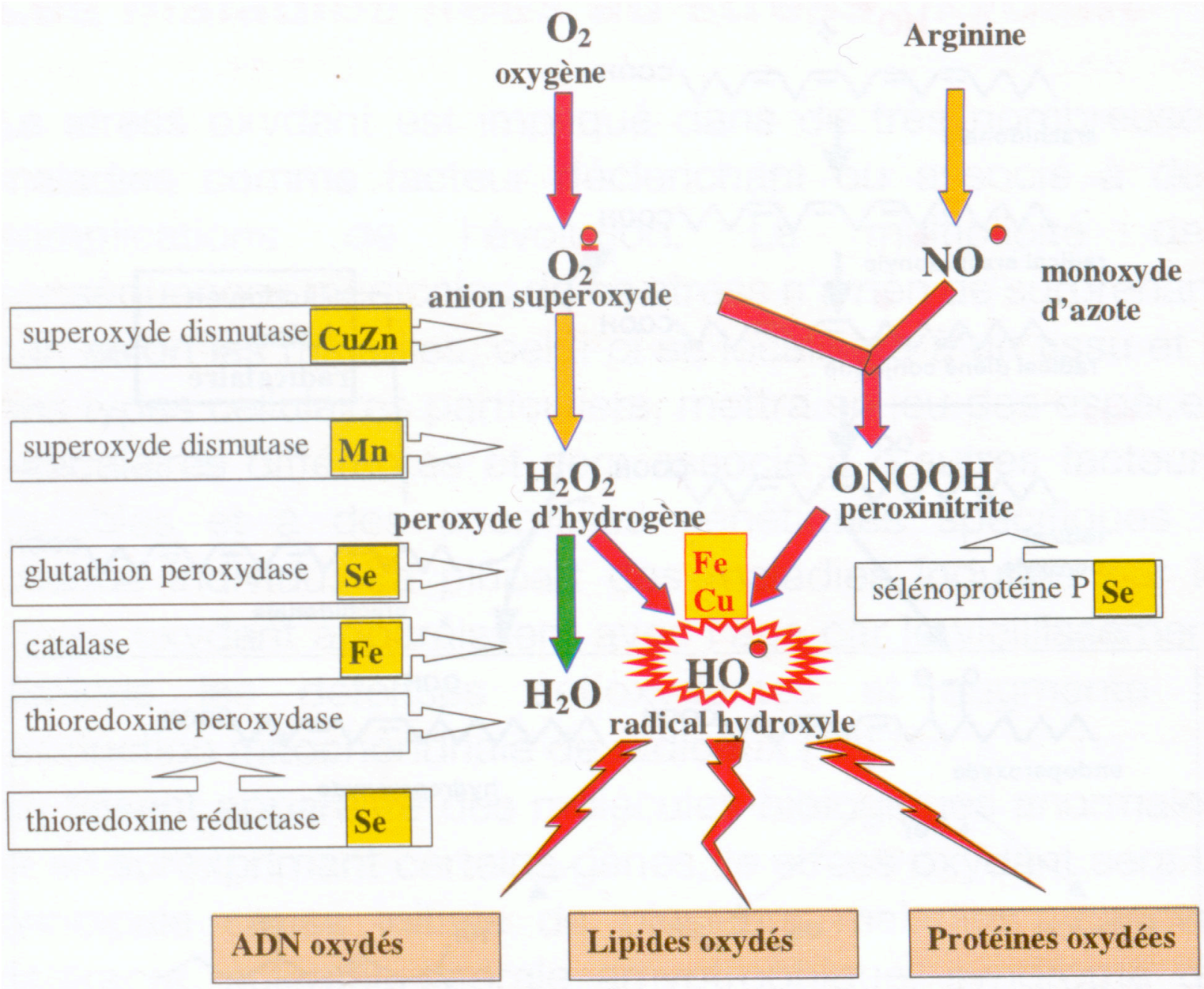
Another example of peroxidase activity is cytochrome *c* peroxidase, that reduces hydrogen (or organic) peroxide according to :

please, pay attention not to mix it up with cytochrome c oxidase !!!!!

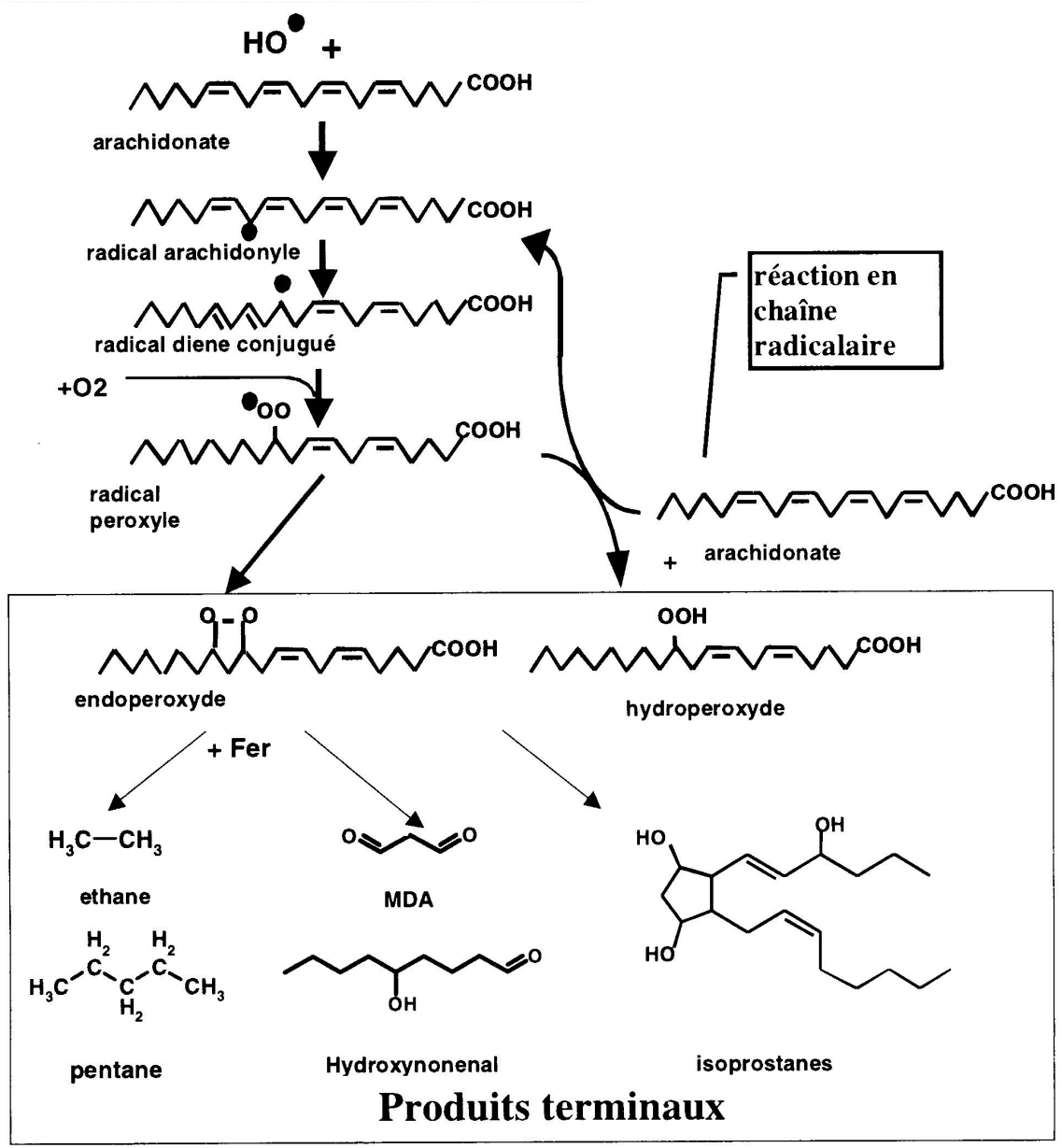
$ROOH + 2\ H^+ + 2\ cyt\ c^{2+}$ ----------> $ROH + H_2O + 2\ cyt\ c^{3+}$ (R = H or alkyle)



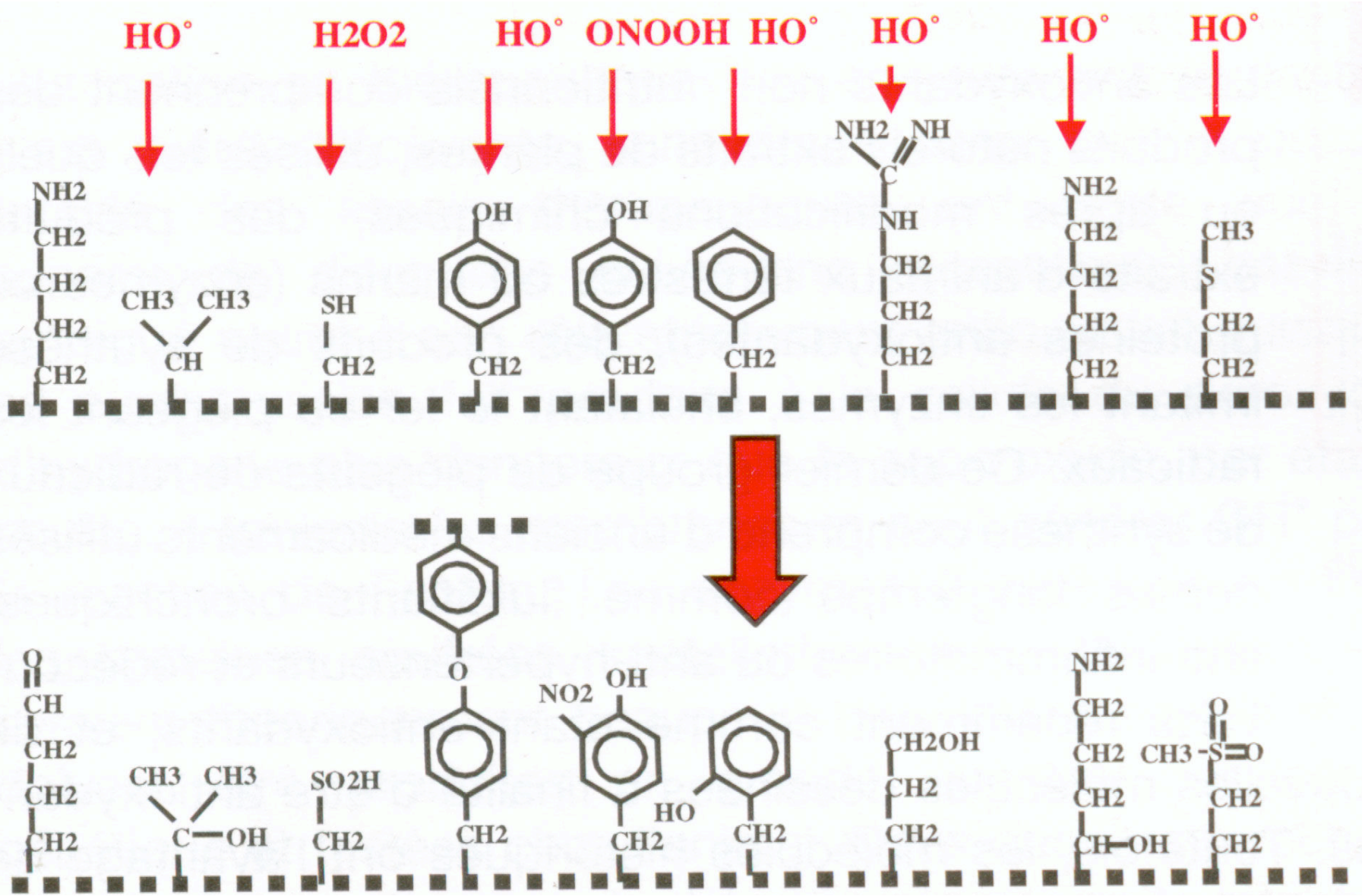
Oxidative stress or how dioxygen becomes toxic (1)



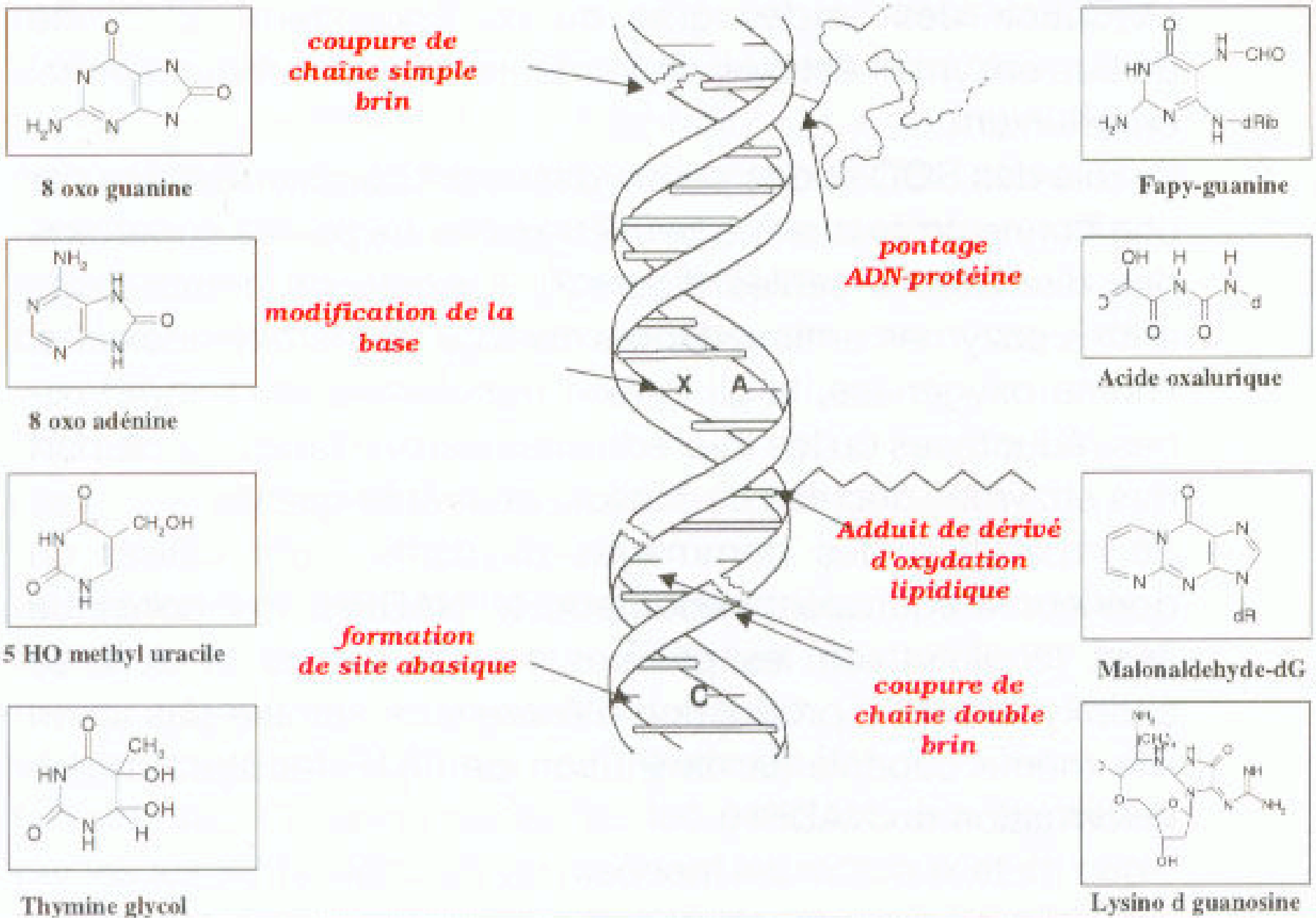
Oxidative stress : peroxidation of insaturated fatty acids



Oxidative stress : modifications of the side chain of amino acids



Oxidative stress : modifications of DNA



Chemistry of ROS (6)

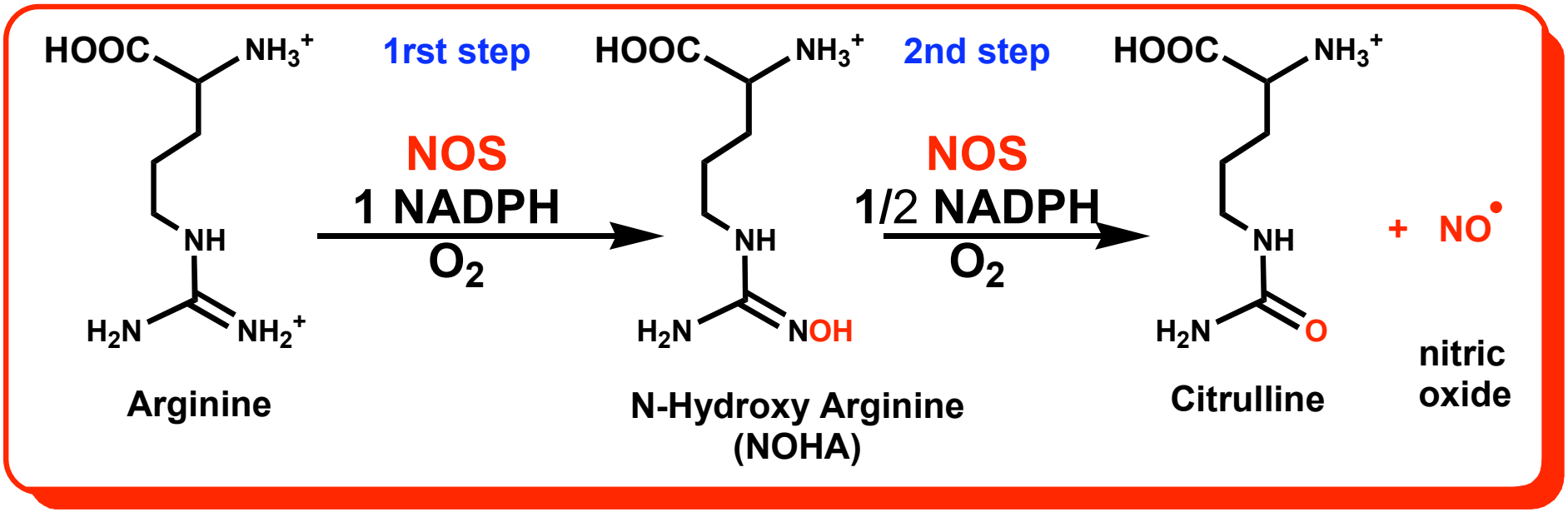
NO synthase: biosynthesis of NO from L-Arginine

$$\text{L-arginine} + 2O_2 + 3e^- + 2H^+ \rightarrow \text{L-citrulline} + 2\ H_2O + NO^\circ$$

The **NO synthase** oxidizes L-Arginine with dioxygen and with NADPH as the electron source
It acts as a double mono-oxygenation, but with an odd number of electrons.

It is considered as a P-450-like enzyme, hence a thiolate as the axial ligand.

The mechanism is still not clear but can be considered in a two-step process.

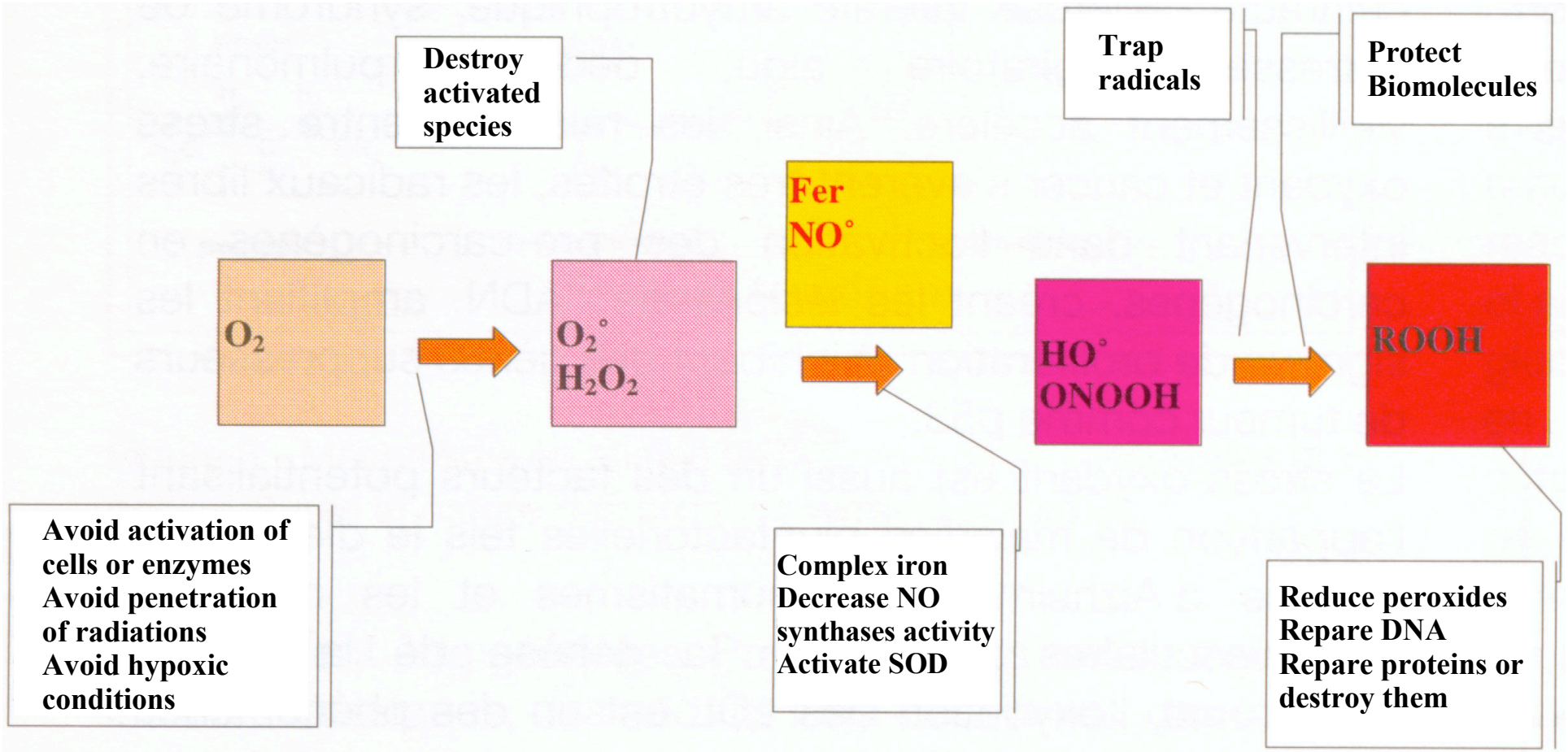


First step: L-Arginine hydroxylation

Second step: second oxygenation

Chemistry of ROS (7)

Implications in possible therapeutic pathways :



Reducing O_2 to H_2O: cytochrome c oxidase

$$4H^+ + 4e^- + O_2 \longrightarrow 2H_2O$$

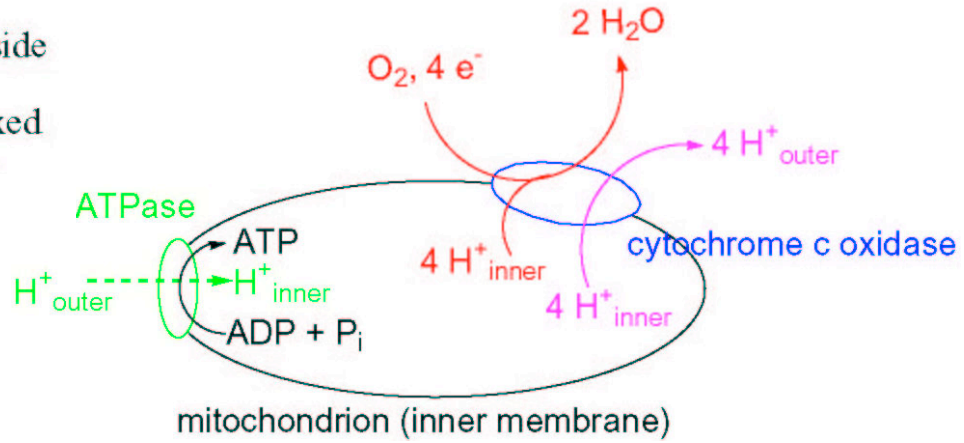
ΔE(pH=7, air saturated) = 0.8 V; ΔG = -77 kcal/mol.

Thermodynamically, O_2 is a very powerful but kinetically a very slow oxidant

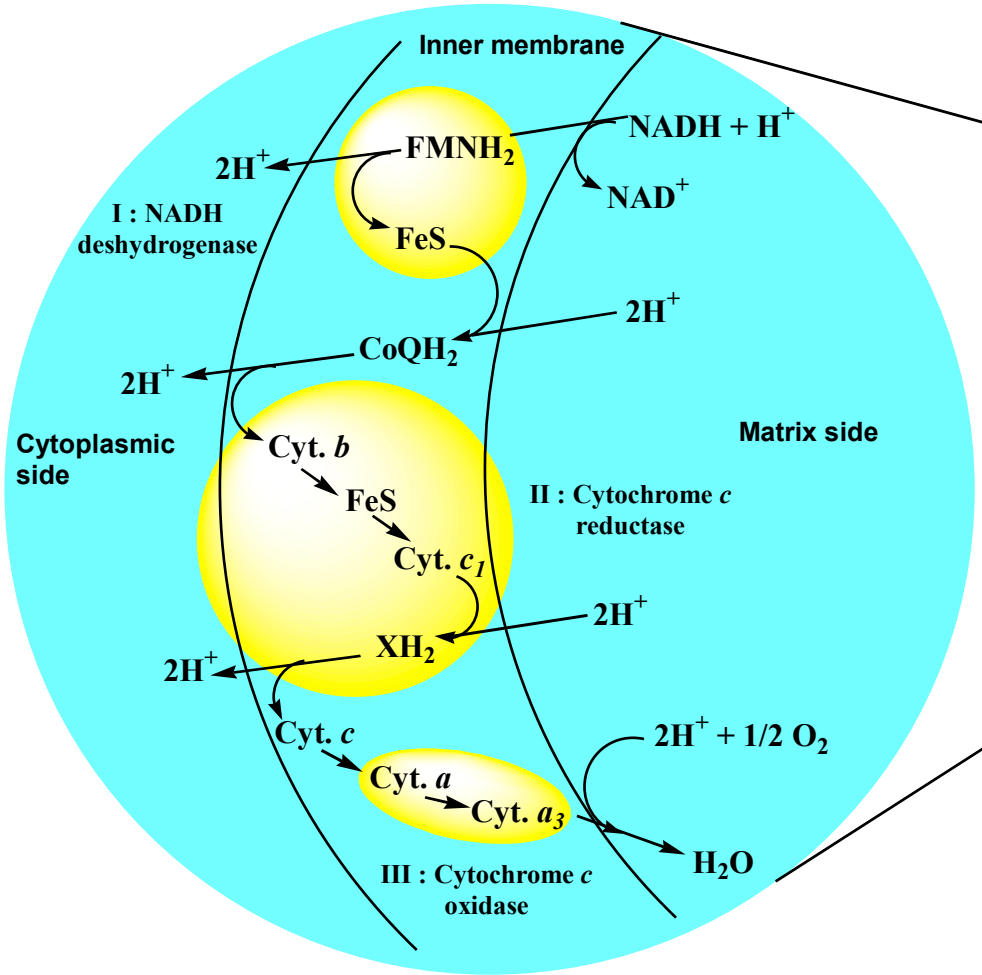
Simply adding 1 electron at a time means loss of energy and production of cytotoxic intermediates (O_2^-, H_2O_2, etc).

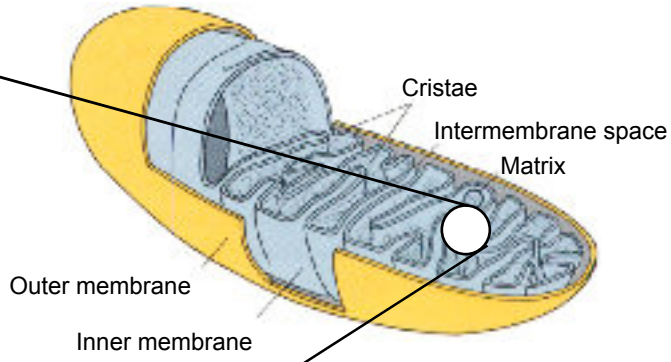
A catalyst is required to reduce O_2 to H_2O and extract energy

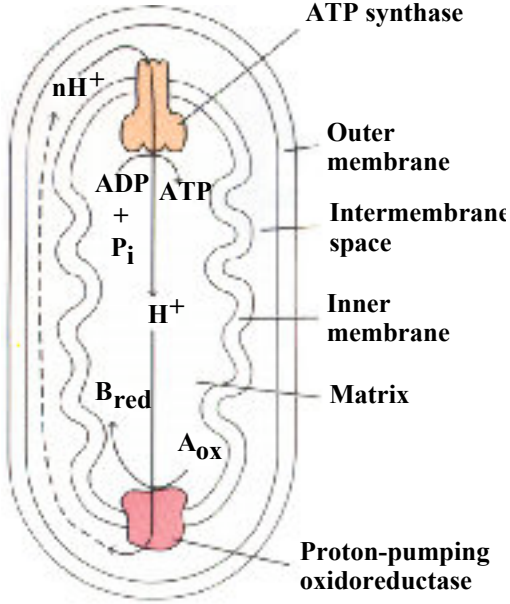
- Cytochrome c oxidase resides in a membrane of the mitochondrion.
- It takes 4 H^+ from the inside of the mitochondrion and O_2 and 4 e^- from the outside and combines them into H_2O.
- Concomitantly, it transfers 4 H^+ from the inside to the outside of the membrane.
- This generates a proton and charge gradient, which is relaxed via a membrane-bound ATPase, generating ATP and thus utilizing the ΔG of the O_2/H_2O reaction:



Oxidative phosphorylation and cytochrome c oxidase

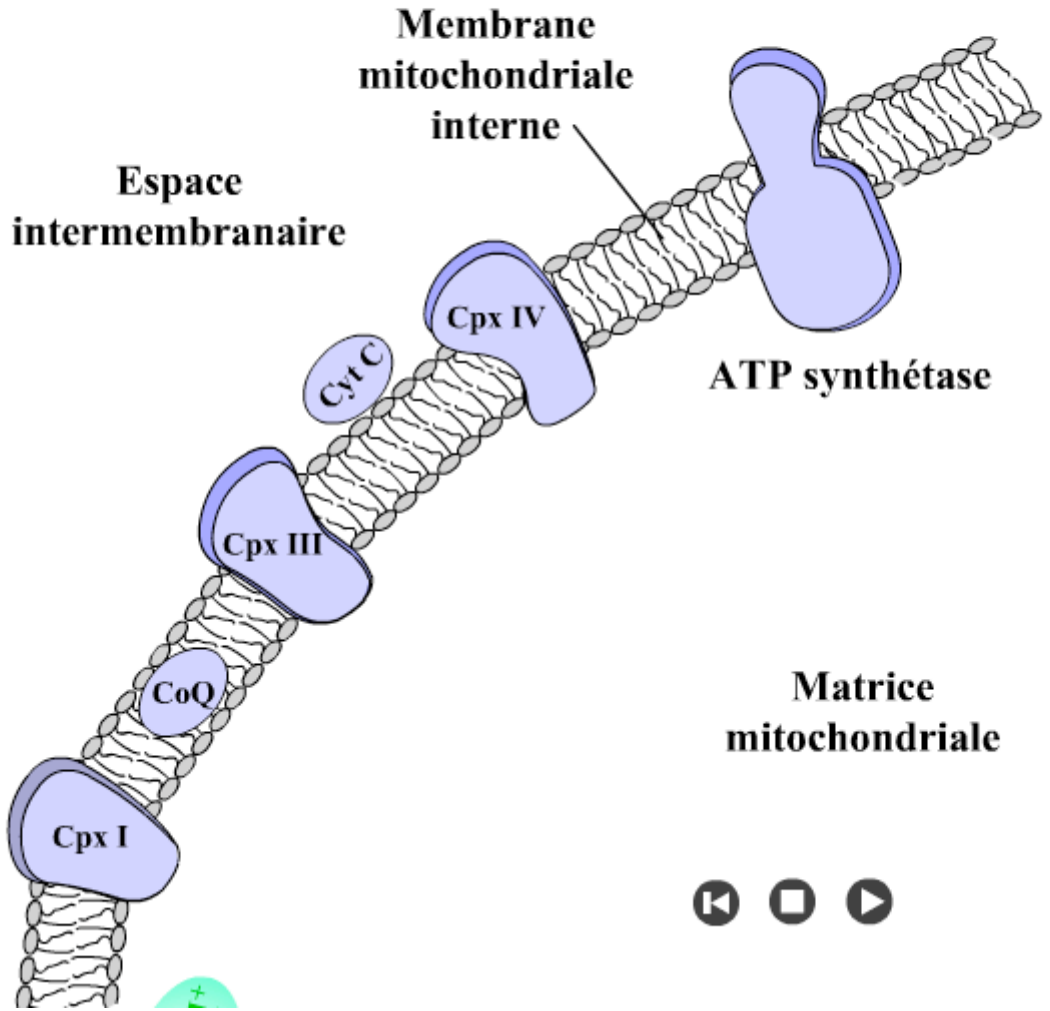






Adapted from *Biochemistry*, J.D. Rawn, International edition, **1989**, Neil Patterson Publishers

Oxidative phosphorylation and cytochrome c oxidase



Cytochrome c oxidase: the molecular structure

Stucturally, one of the most complex proteins.

The structural features are determined by the need to:
(a) store more than one reducing equivalent:

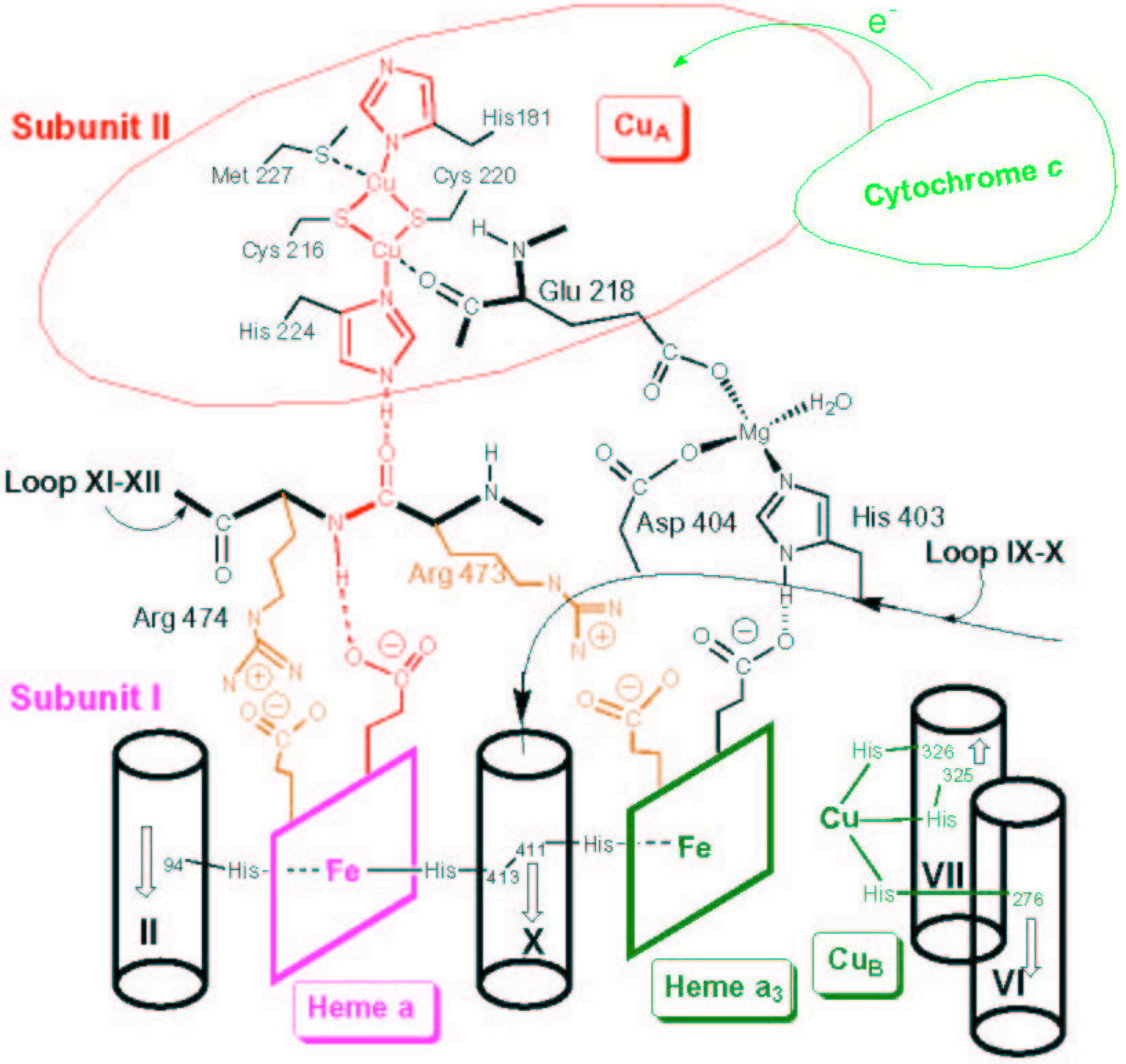
- several redox-active centers;

(b) redistribute the reducing equivalents (electrons) fast among the redox active centers:

- several electron transfer pathways;

(c) translocate H^+ and charge through the dielectric of the membrane:

- the catalytic site is half-way through the membrane.



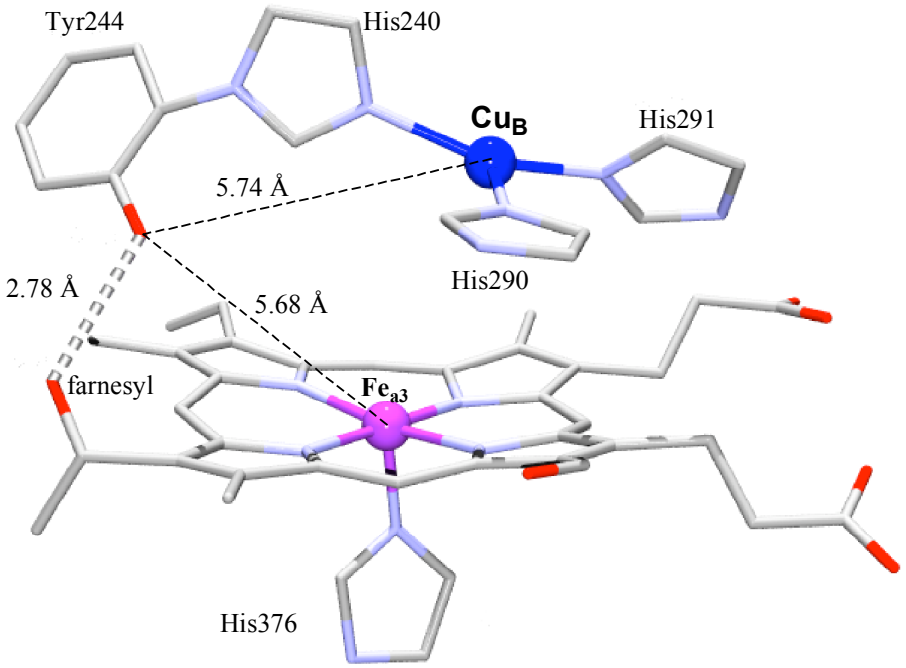
Cytochrome c oxidase: the catalytic site

The catalytic site is composed of

(a) 5-coordinate myoglobin-like heme (heme a_3);+

(b) Cu (Cu_b) is coordinated to 3 imidazoles:
- the remaining open coordination site is for substrate/intermediate/product binding
- the flexible coordination environment facilitates Cu^{I}/Cu^{II} conversion;

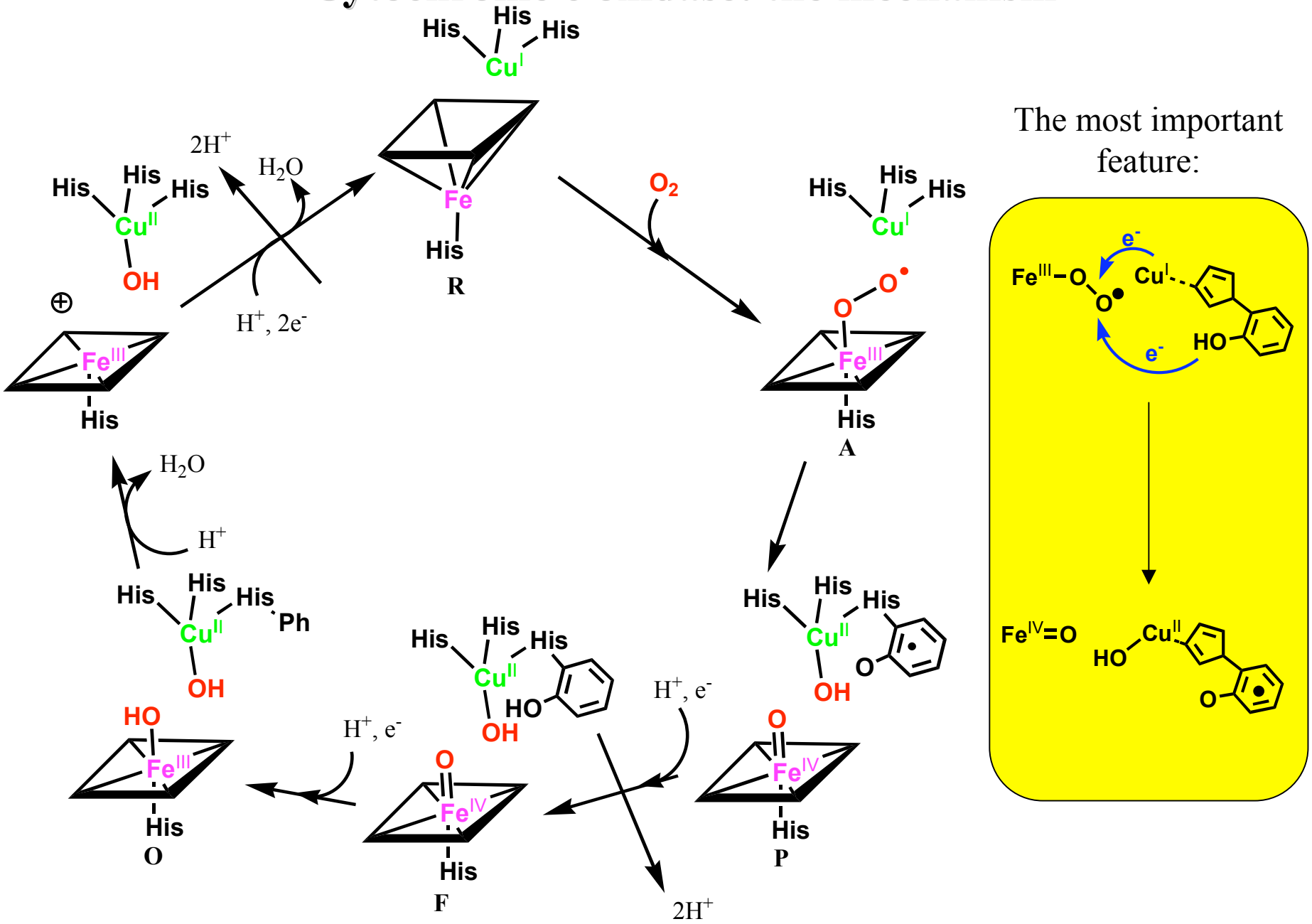
(c) a phenolate residue:
- an organic equivalent of a redox-active metal: capable of delivering both a reducing equivalent (electron) and an H^+ to the substrate.



The catalytic site is designed to have enough readily available reducing equivalents for a complete (4-e^-) reduction of O_2 thus avoiding a build-up of the potentially deleterious partially reduced O_2 derivatives.

Atom coordinates from the **Protein Data Bank**, *Brookhaven*, ID PDB 10CR

Cytochrome c oxidase: the mechanism



The most important feature:

Why models?

« Models do not yield information on the actual working of the enzyme, but they can verify or eliminate the possibility of suggested mechanisms » Wooley

« Reproducing complex biological reactivity within a simple synthetic molecule is a challenging endeavor with both intellectual and aesthetic goals. The sequence of examining biological reactivity, creating similar chemical architectures, and determining functional reaction conditions for model systems is a process that allows the biological code of reactivity to be deciphered... Functional models can provide an opportunity to examine a biological reactivity at a small-molecule level of detail through systematic and comparative studies. Although one goal of modeling is reproduction of reactivity, extension of this reactivity beyond the scope of the inspiring system is perhaps an even more important objective. » Mahadevan, Gebbink et Stack

Mahadevan, V. ; Gebbink, R. J. M. K. ; Stack, T. D. P., *Curr. Opin. Chem. Bio.,* **2000**, *4*, 228-234

Wooley, P. *Biophysics and Physiology of Carbon Dioxide* ; Bauer, C., Gros, G. and Bartels, H., Ed.s ; Springer-Verlag : Berlin, **1980**, pp 216