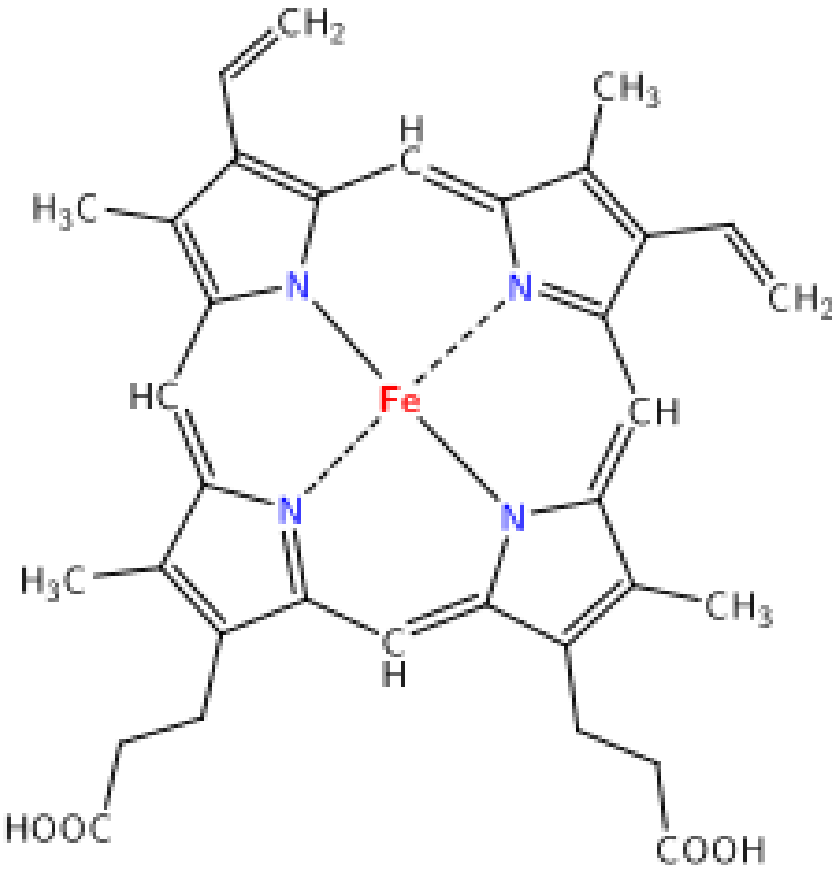
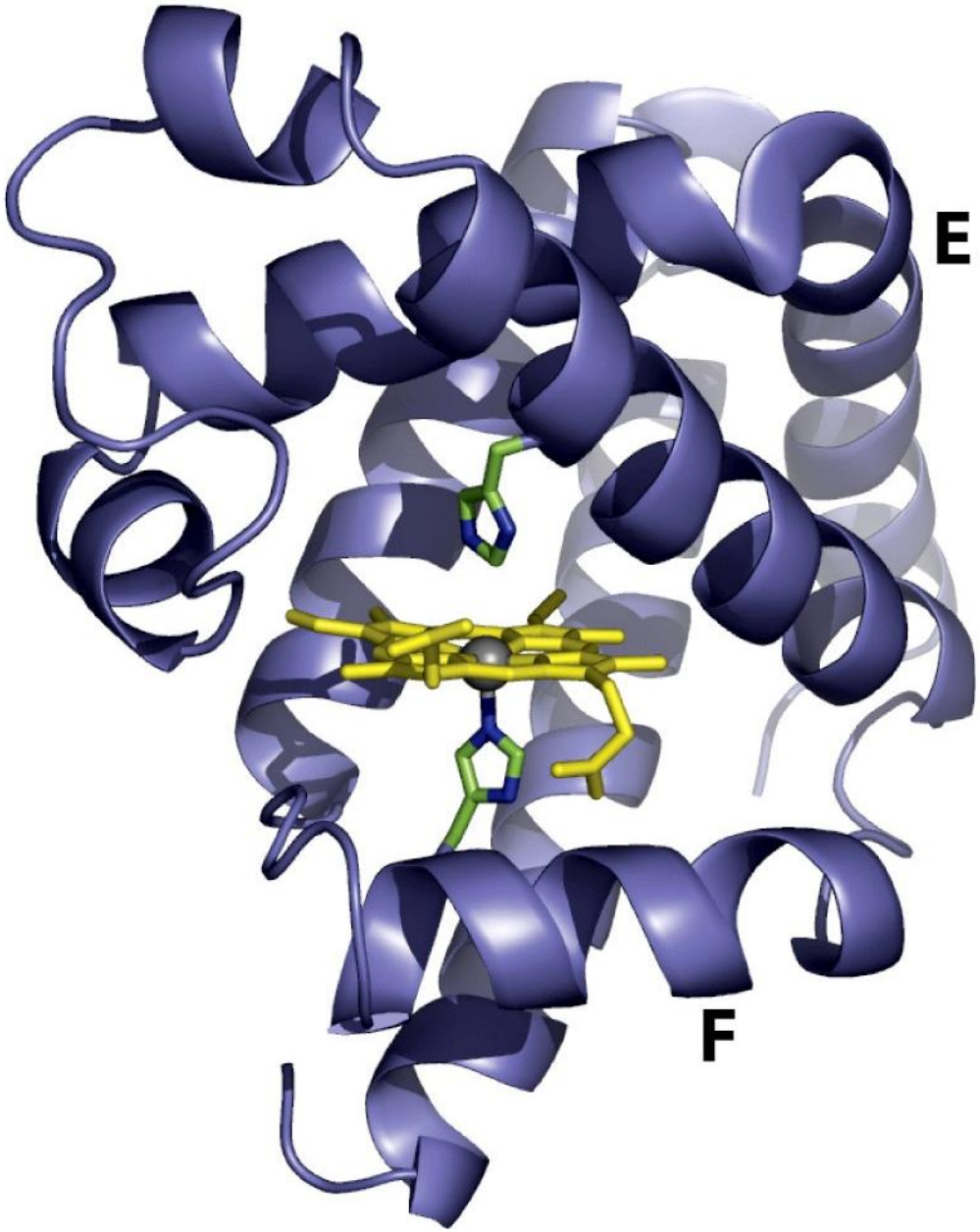
Heme-Fe



Oxygen uptake, transport and storage

Myoglobin
(storage)

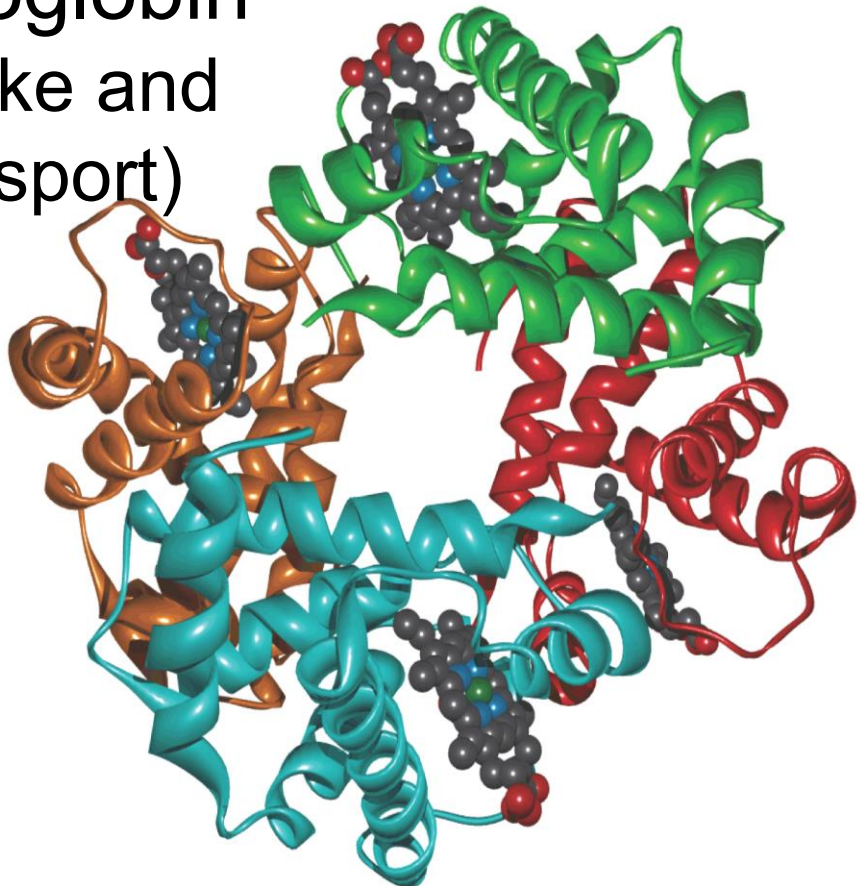
17.8 kDa

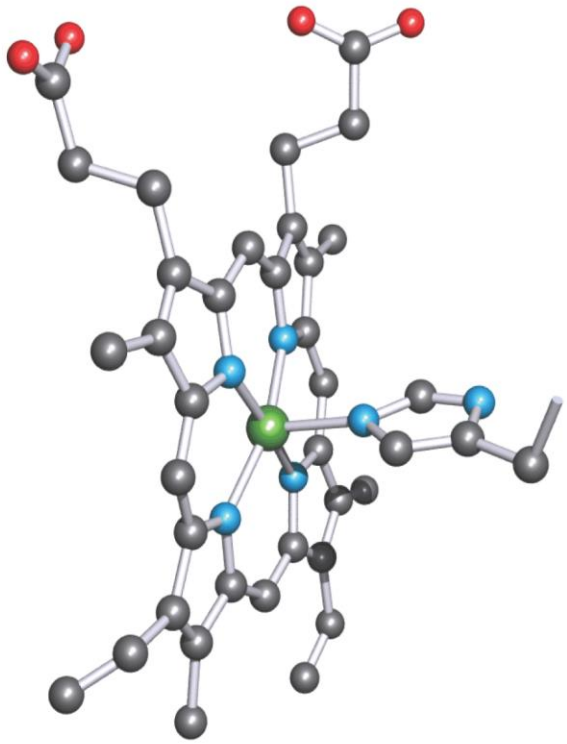


ca. 6% Fe_{tot}

Oxygen uptake, transport and storage

Hemoglobin
(uptake and transport)

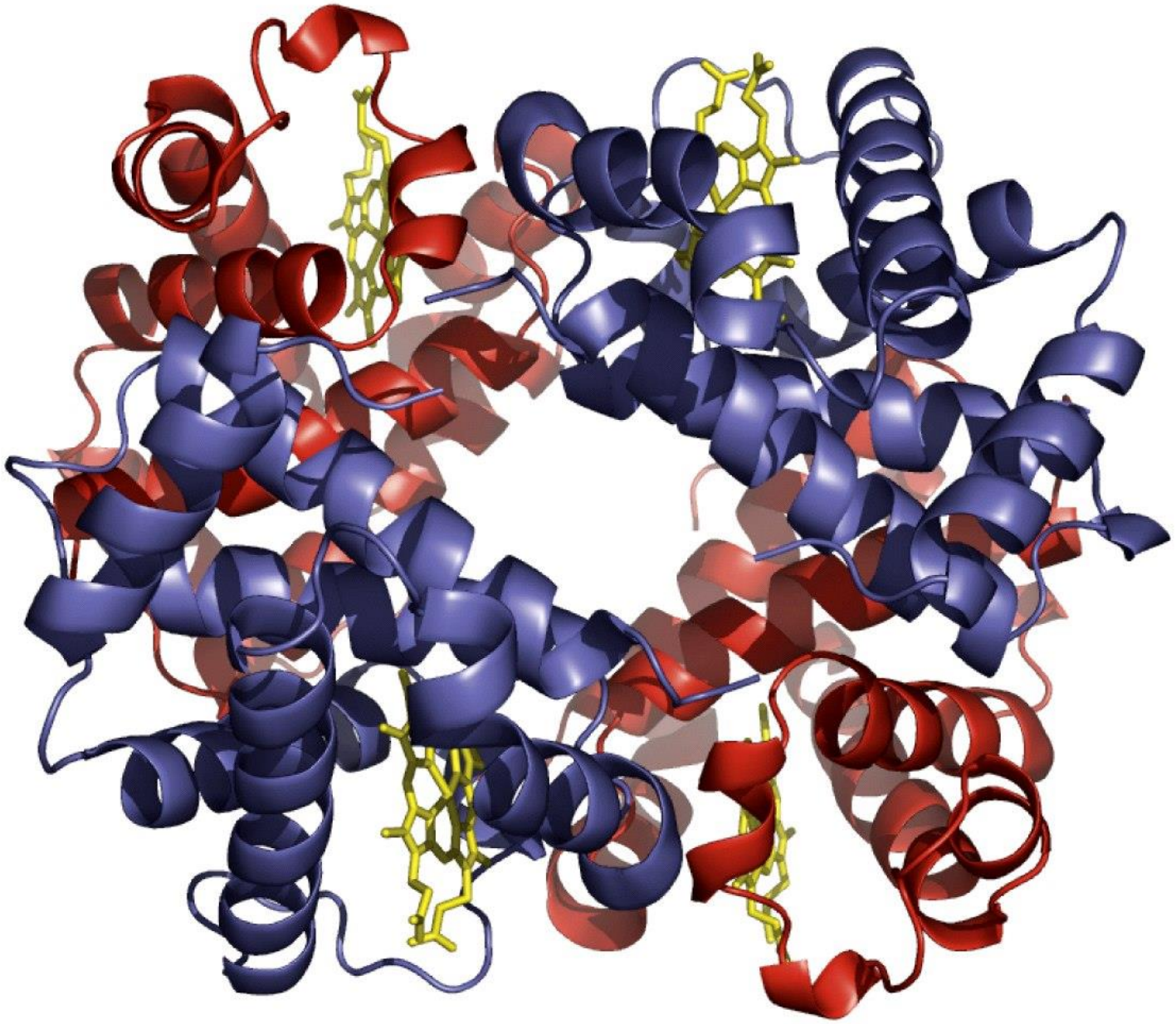




tetramer $\alpha_2\beta_2$

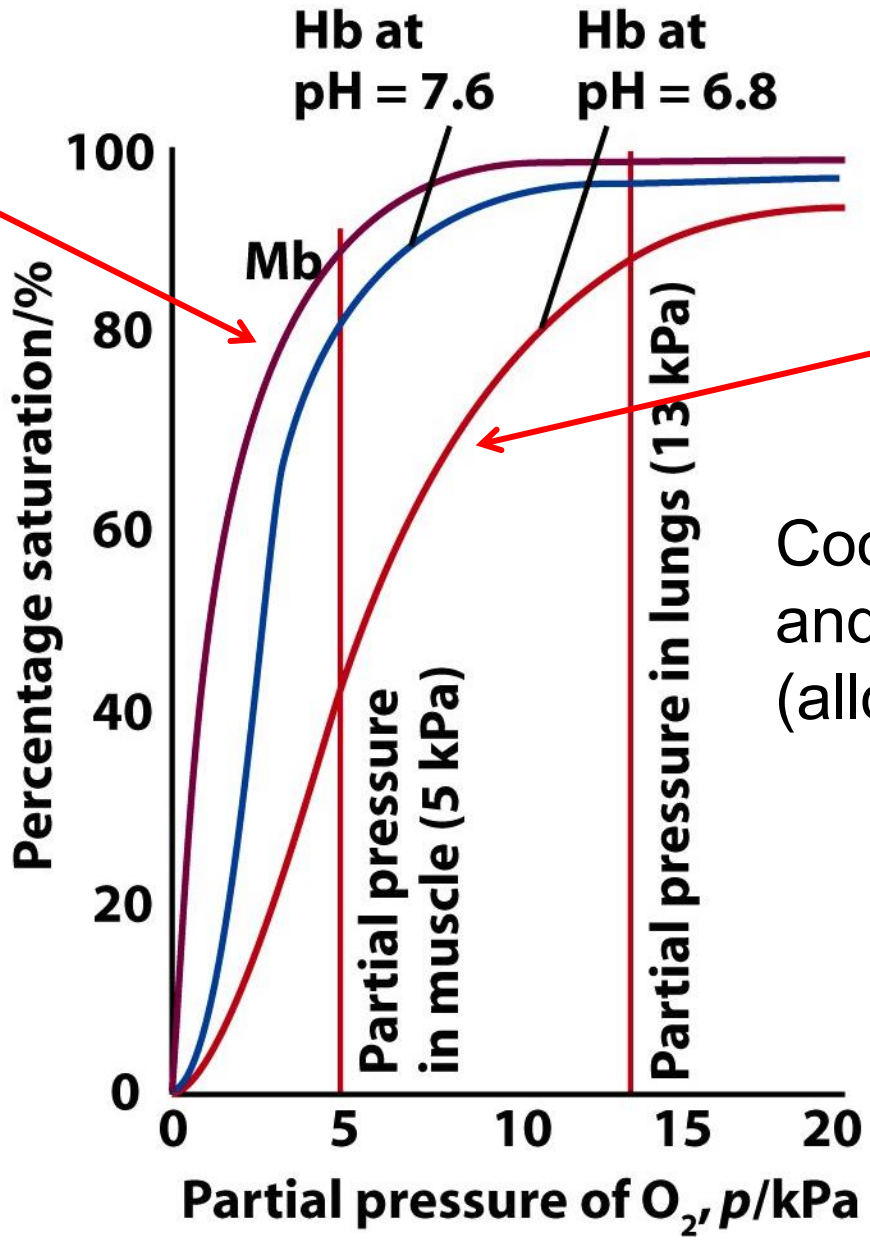
141 and 146 a.a. 64.5 kDa

ca. 65% Fe_{tot}



tetramer $\alpha_2\beta_2$

Hyperbolic
saturation curve

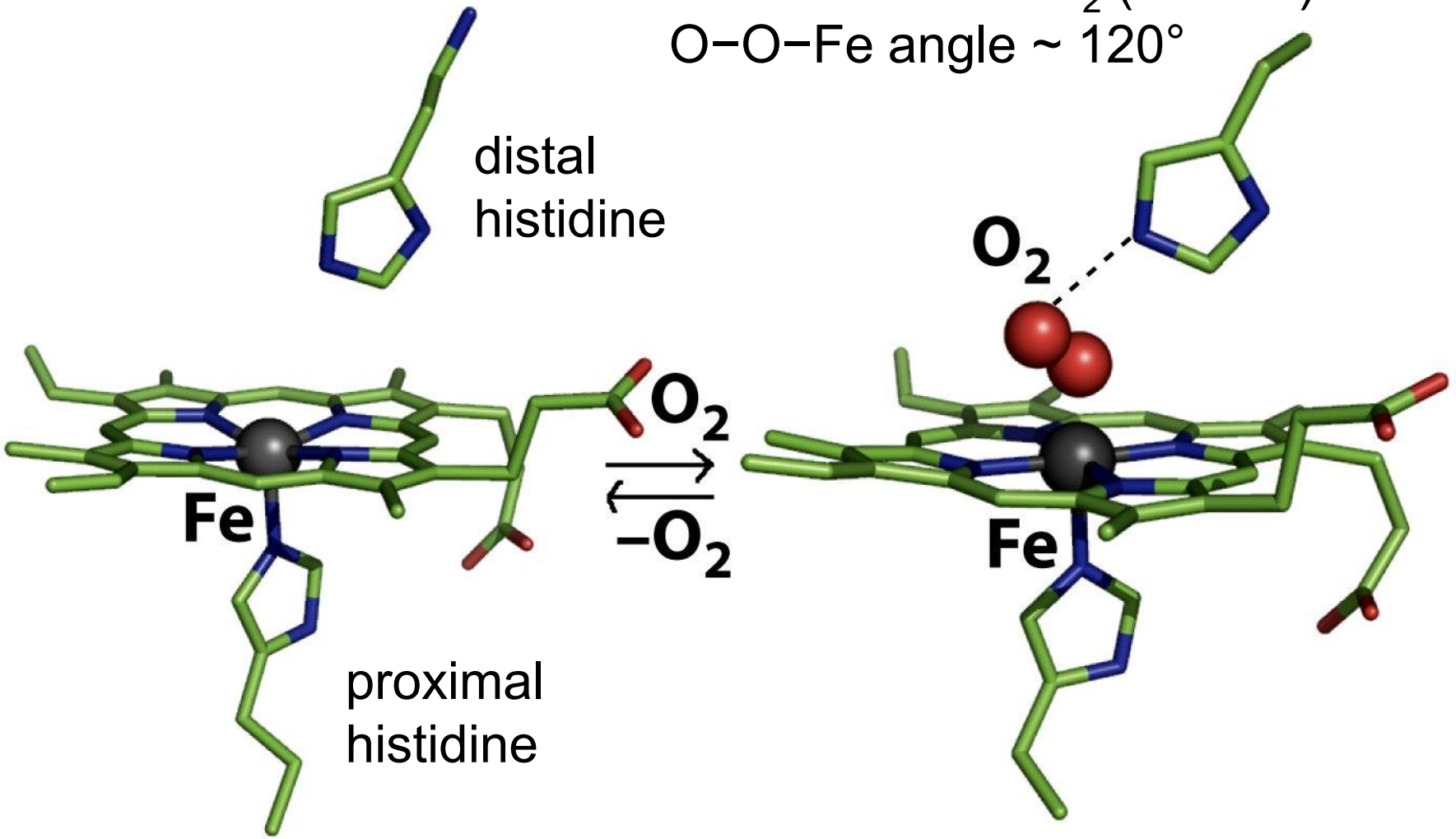


Sigmoidal
saturation curve, pH
dependant

Cooperative effect
and Bohr effect
(allosteric effect)

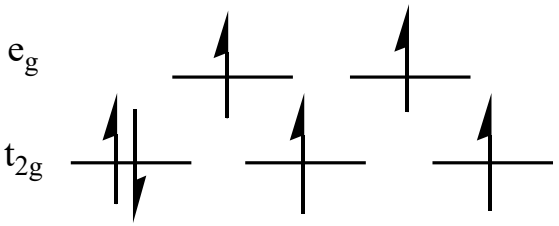
The coordination of CO_2 and protons (pH) to the proteic chain of hemoglobin induces conformational variations that affect the affinity of the heme for O_2

Coordination of O_2 (*end-on*)
O−O−Fe angle ~ 120°



Fe(II), d^6, h.s. four unpaired e^- $S = 0$

desossi:
S = 2
paramagnetica

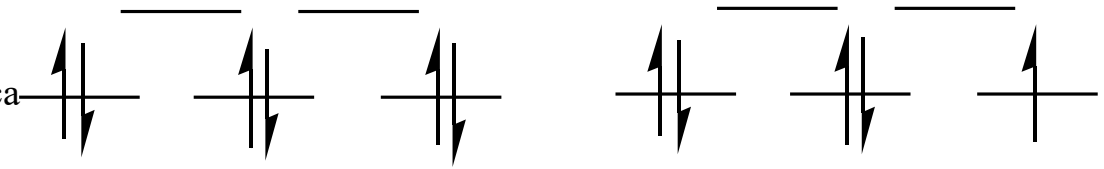


Fe(II), d^6 paramagnetico

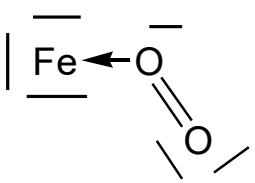
Pauling

Weiss

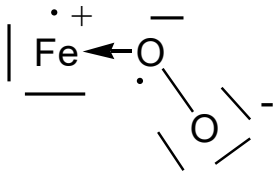
ossi:
S = 0
diamagnetica



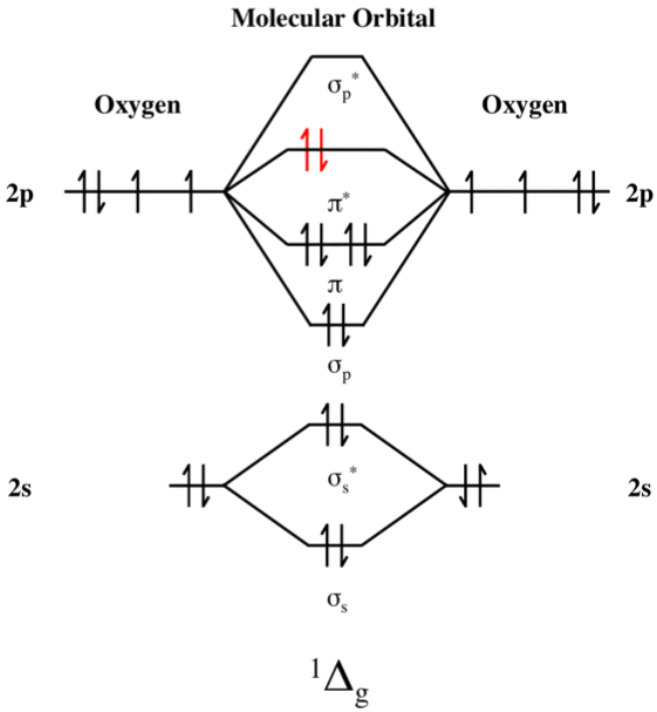
Fe(II) d^6 basso spin
+ $^1O_2$ legato



Fe(III) d^5 basso spin
+ $^2O_2^{\cdot -}$ legato

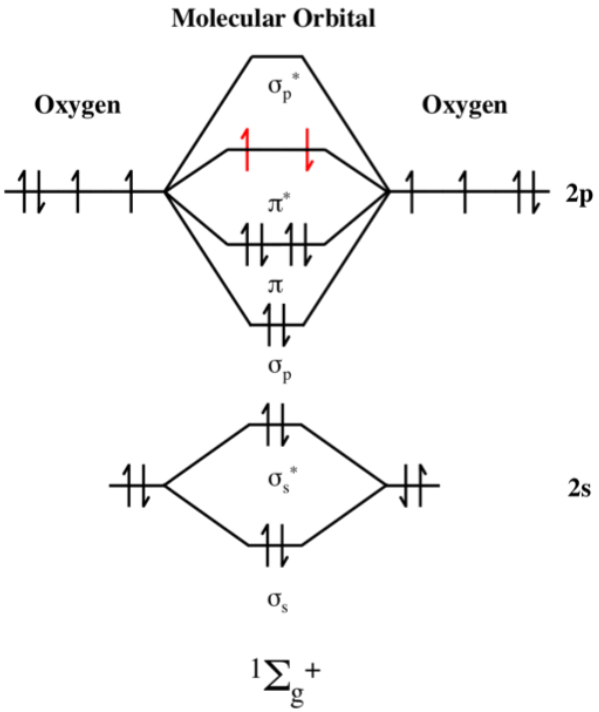


Triplet ground state and singlet excited states of O_2



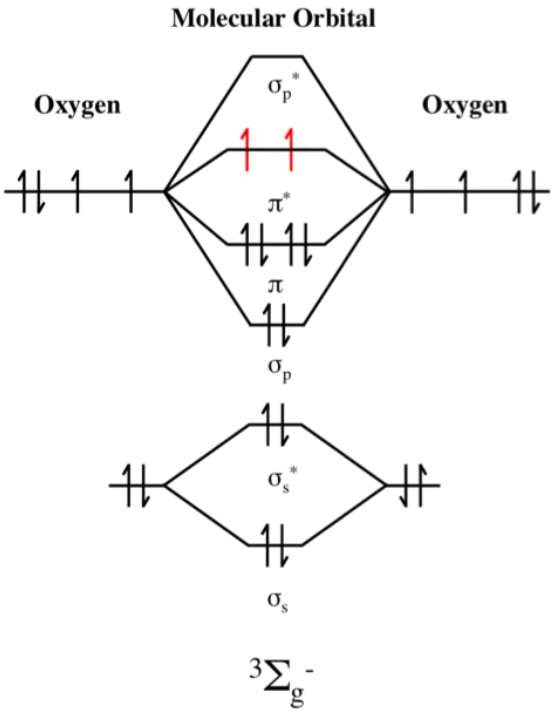
$^1\Delta_g$

+94 kJ mol^{-1}



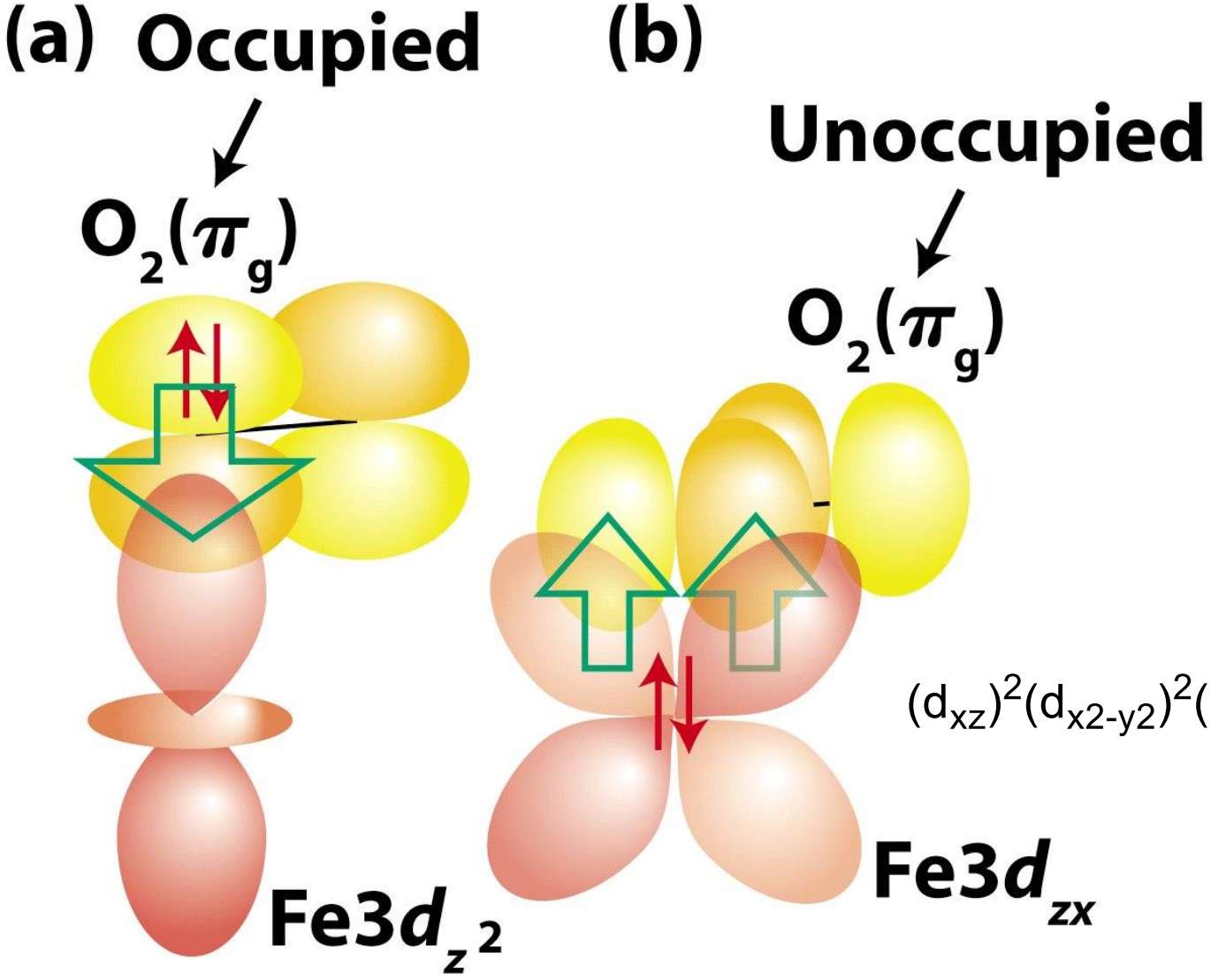
$^1\Sigma_g^+$

+158 kJ mol^{-1}



$^3\Sigma_g^-$

Pauling model: Fe(II) l.s. + $^1O_2$



Pauling

Fe^{II} / O_2

$S = 0$

$^1O_2$

$(d_{xz})^2(d_{x2-y2})^2(d_{yz})^2(\pi_a^*)^2$

McClure/Goddard

Fe^{II} / O_2

$S = 1$

Fe(II) in an intermediate spin state, with 2 unpaired electrons ($S = 1$)

$^3O_2$

$(d_{xz})^2(d_{x2-y2})^2(d_{yz})^1(d_{z2})^1(\pi_a^*)^1(\pi_b^*)^1$

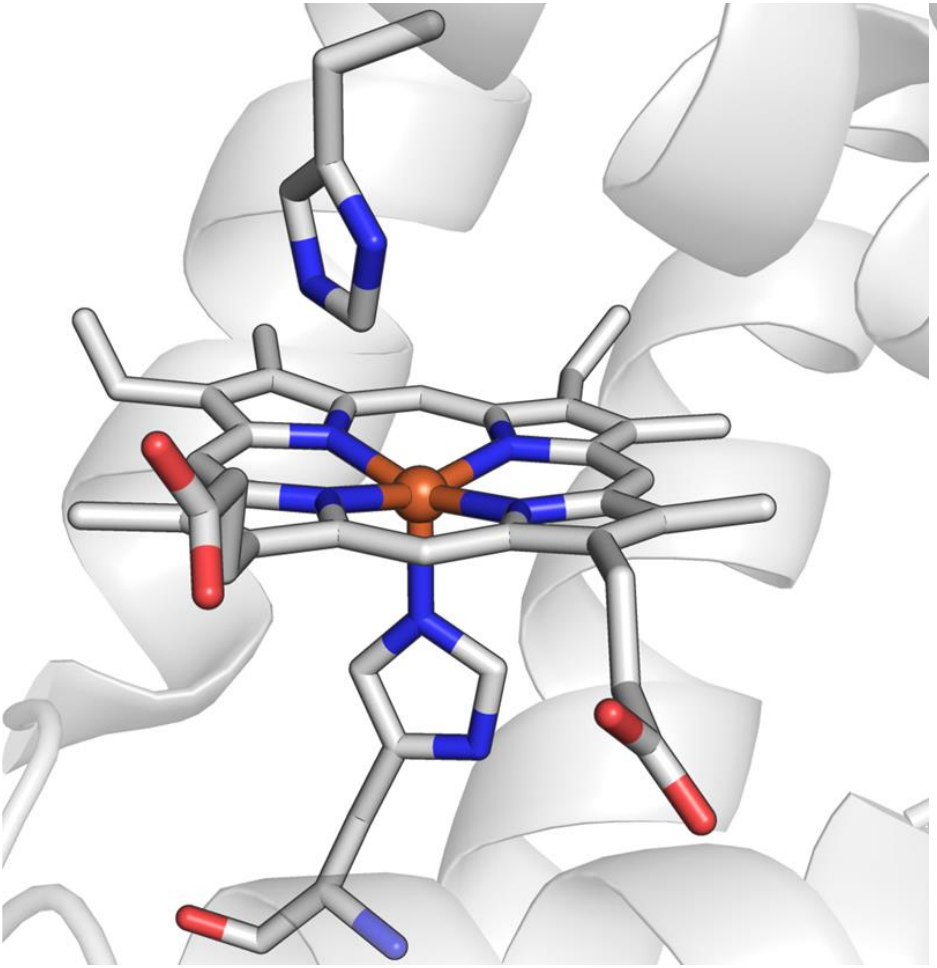
Weiss

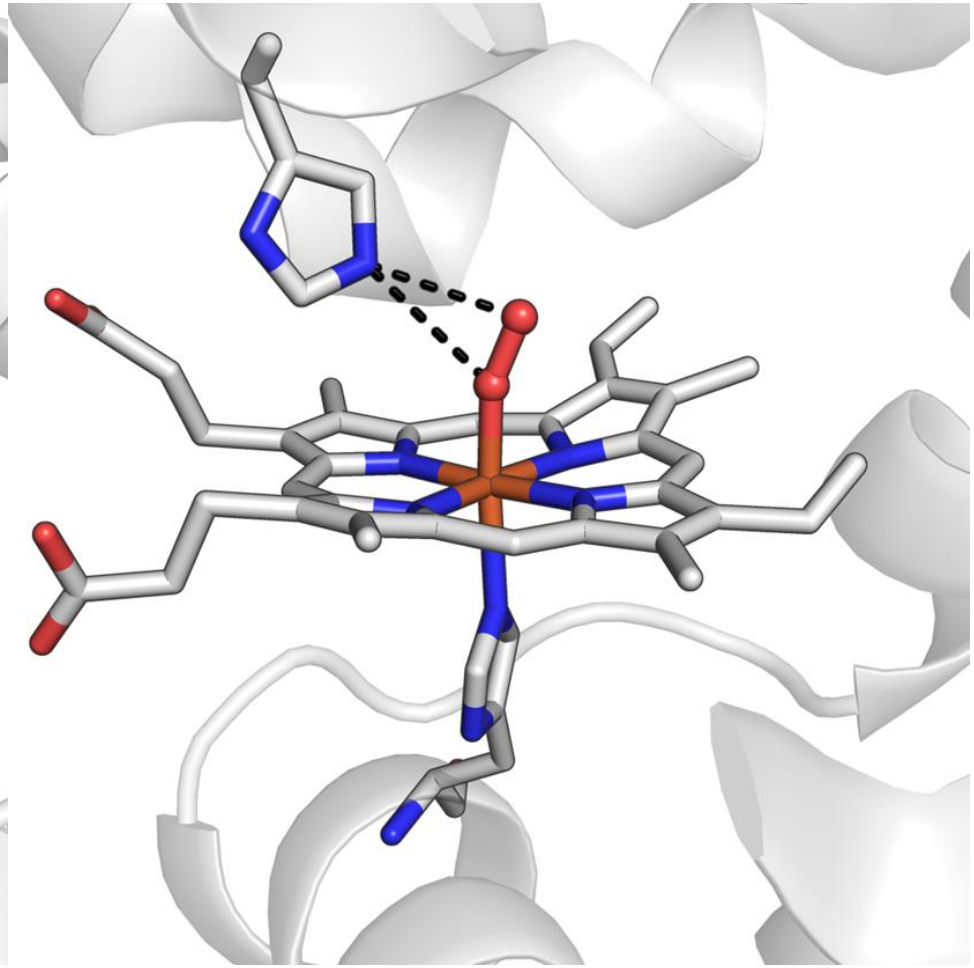
Fe^{III} / O_2^-

$S = 1/2$

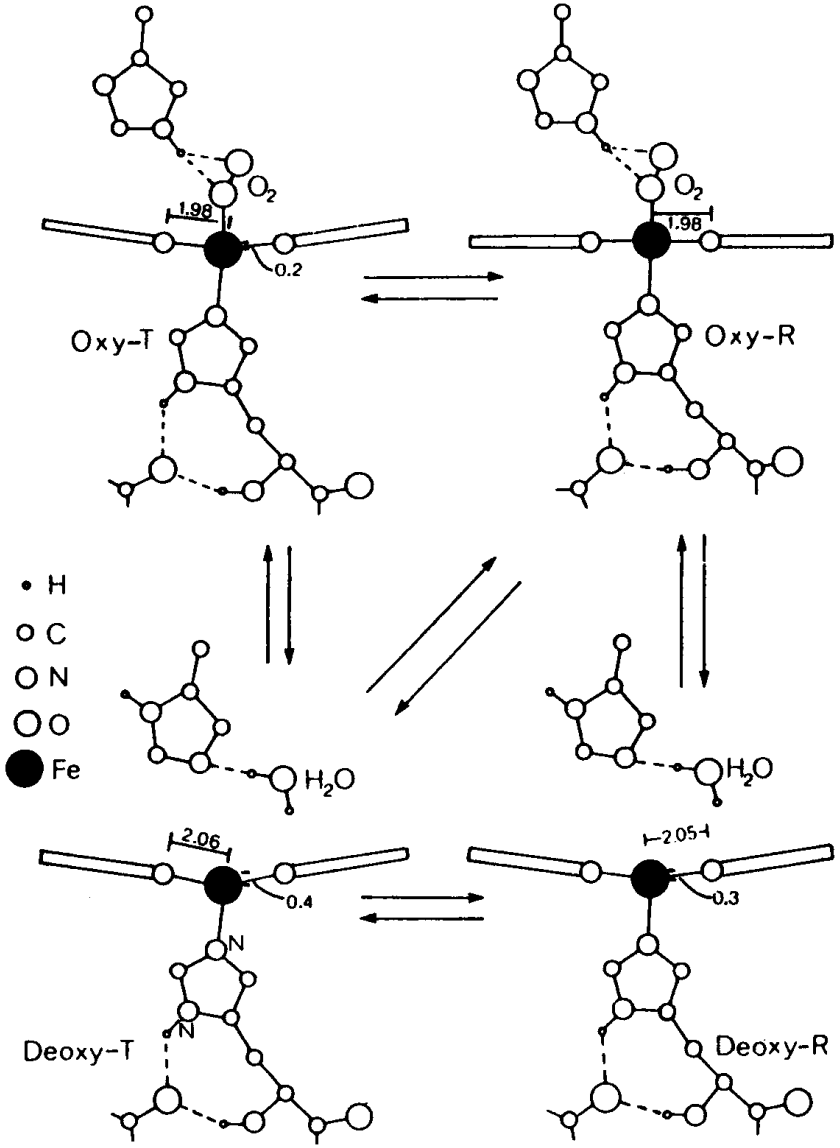
The emerging bonding situation includes an essentially ferrous iron center, little charge transfer to O_2 and minor superoxide character of the non-innocent ligand, significant double-bond properties of the Fe–O_2 interaction, and three-center ozone-like electron delocalization.

PNAS, **2017**, *114*, 8556-8561.



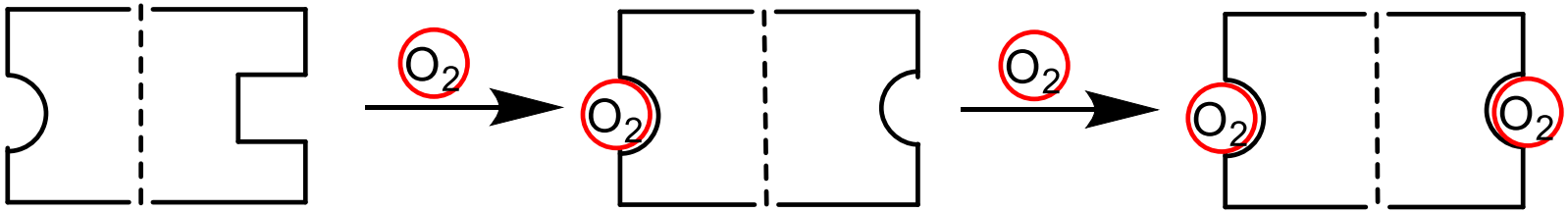


Fe(II) h.s.: 0.92 Å
Fe(II) l.s.: 0.75 Å
Fe(III) l.s.: 0.55 Å

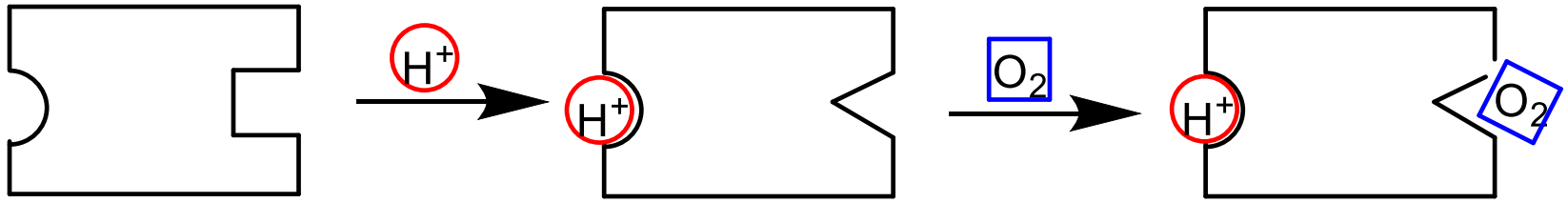


The affinity of the relaxed (R) form for O_2 is approximately equal to that of the isolated α and β chains, that is of Mb, whereas that of the tense (T) form is about 12 – 14 kJ/mol lower

allosterism = the change in activity and conformation of an enzyme as a result of the binding of a compound (*effector*) to a site of the enzyme other than the active one



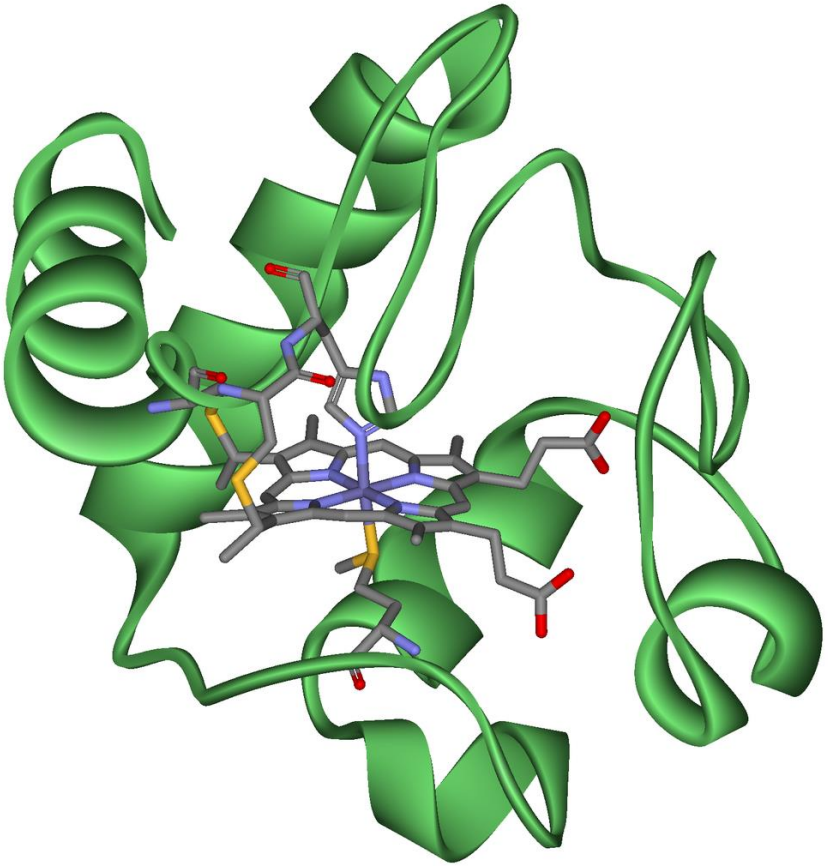
Homotropic Allosterism



Heterotropic Allosterism (Bohr Effect)

Cytochromes

Cytochromes are proteins for electron transfer containing a heme, with a central iron (Fe) atom at its core, as a cofactor.

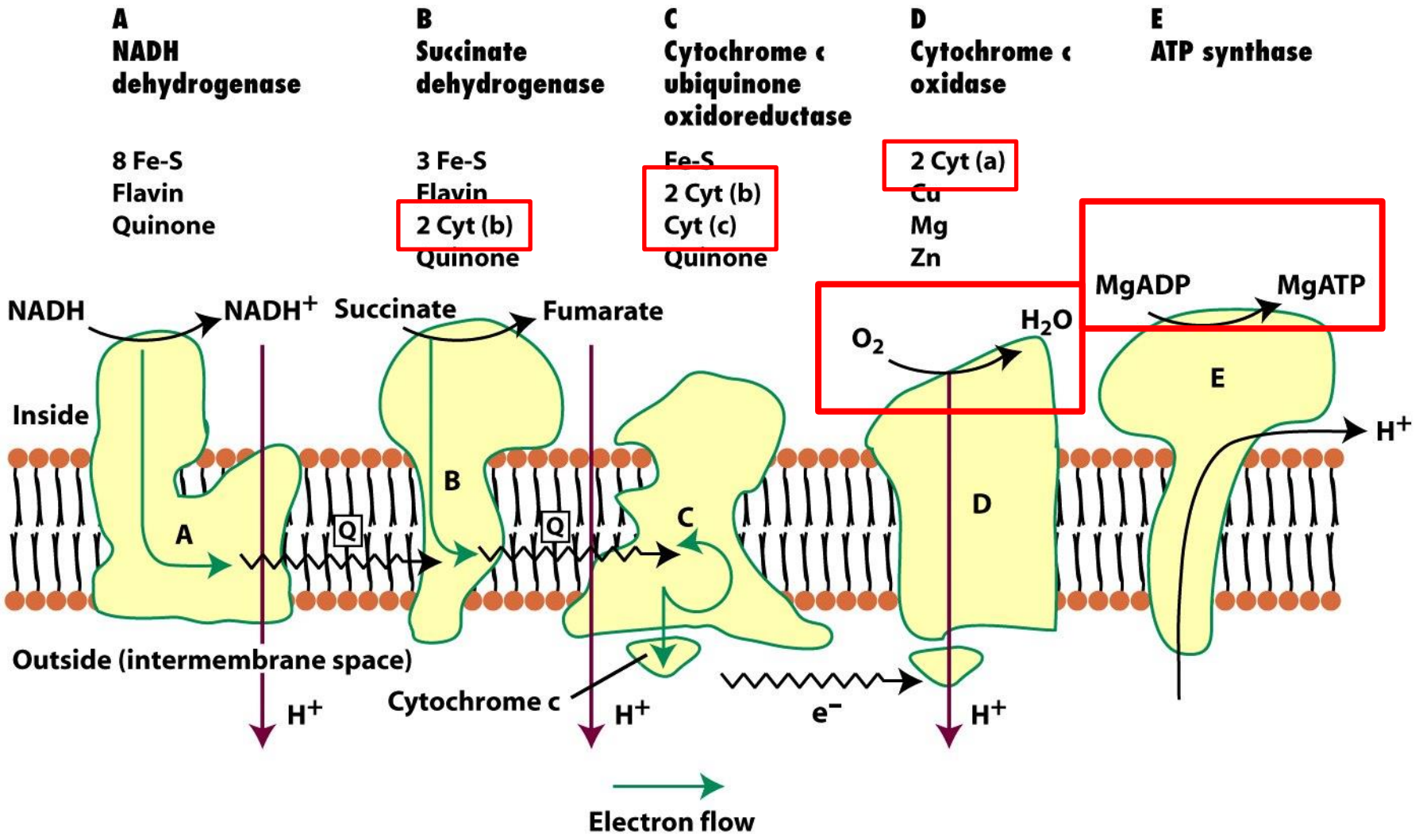


Electron transfer in biological systems

Although electron transfer is one of the simplest chemical reactions, at least three variables must be considered:

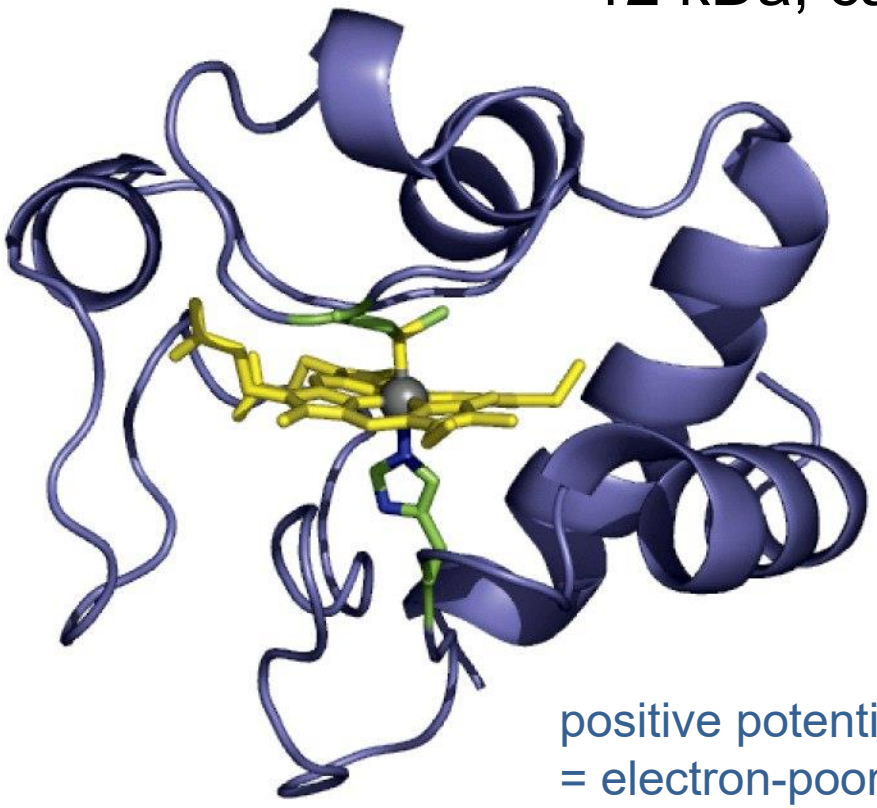
1. **energy**, i.e. the redox potential at which it occurs;
2. **space**, i.e. the directionality of the transfer;
3. **time**, i.e. the speed of the electron transfer (Marcus equation).

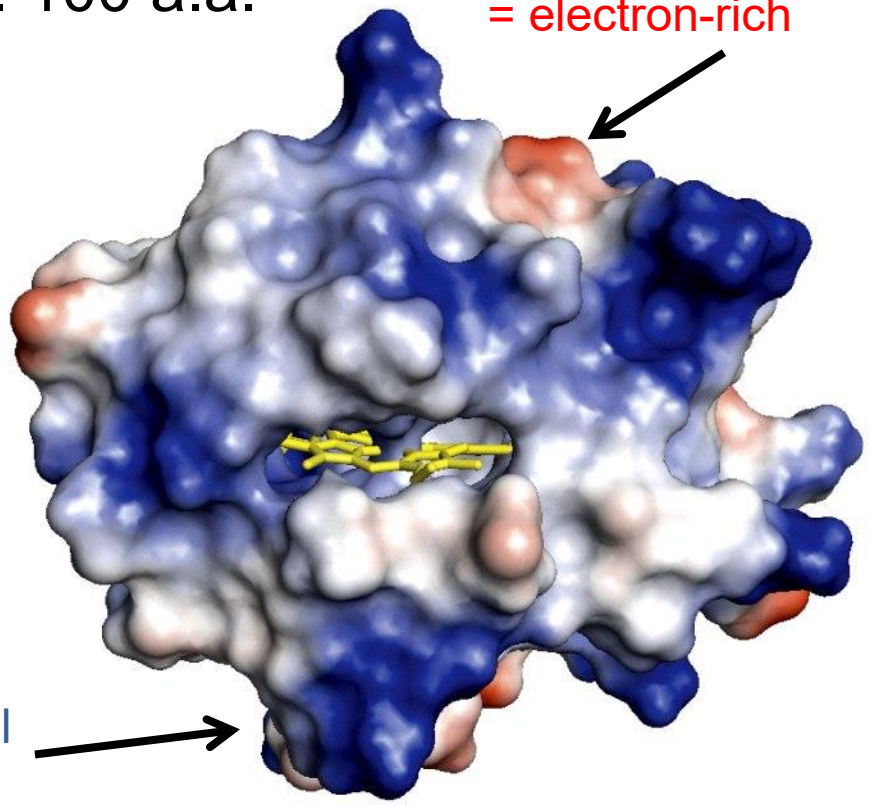
Cellular respiration chain



Cytochrome c (from horse heart)

12 kDa, ca. 100 a.a.



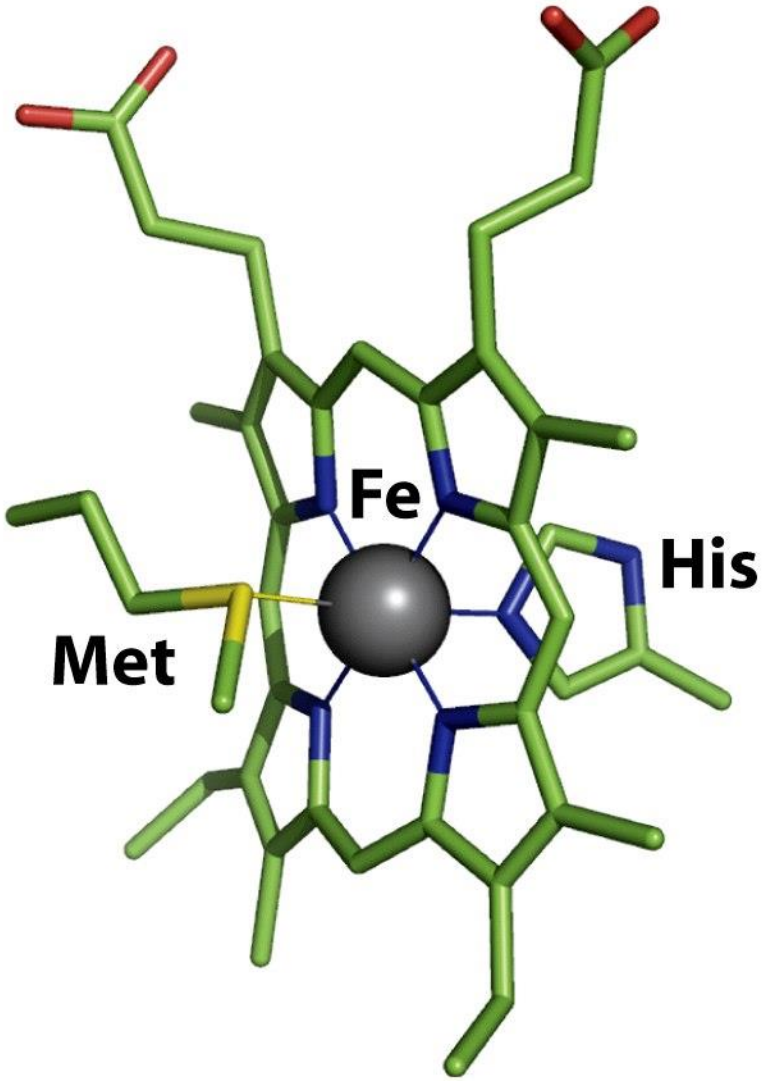


elettrostatic potential map

$E_{Fe(III)/Fe(II)}$ = +260 mV

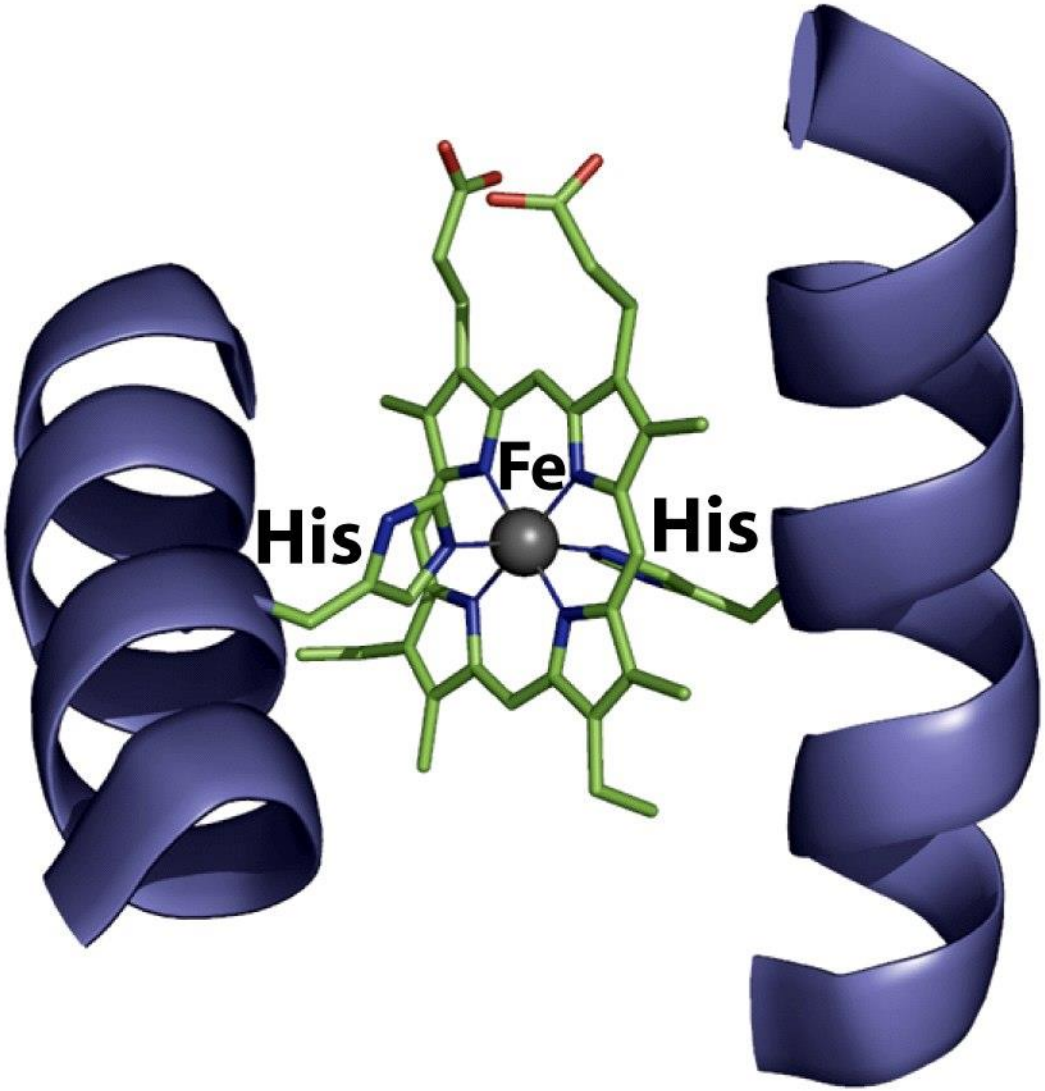
Fe low spin in both oxidation states → low reorganization energy

The heme group in Cytochrome c



$E_{Fe(III)/Fe(II)}$ = +260 mV

The heme group in Cytochrome b



$E_{Fe(III)/Fe(II)}$ = +20 mV

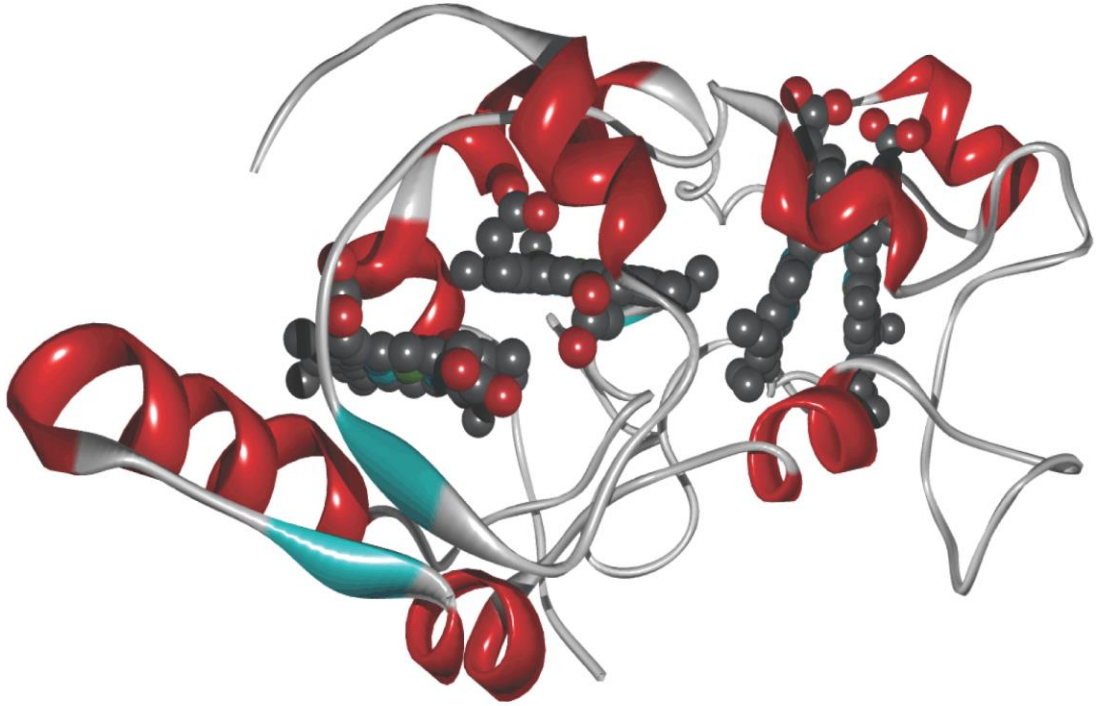
Influence of complexation on the standard potential of a metal ion M

- The formation of a thermodynamically more stable complex when the metal is in the lower oxidation state of a redox pair promotes its reduction, and the standard potential becomes **more positive**.
- The formation of a thermodynamically more stable complex when the metal is in the higher oxidation state of a redox pair promotes its oxidation and makes the standard potential more negative.

Thus, the change in redox potential from cytochrome b (+20 mV) to cytochrome c (+260 mV) is consistent with the soft Met axial ligand having an higher affinity for Fe(II)

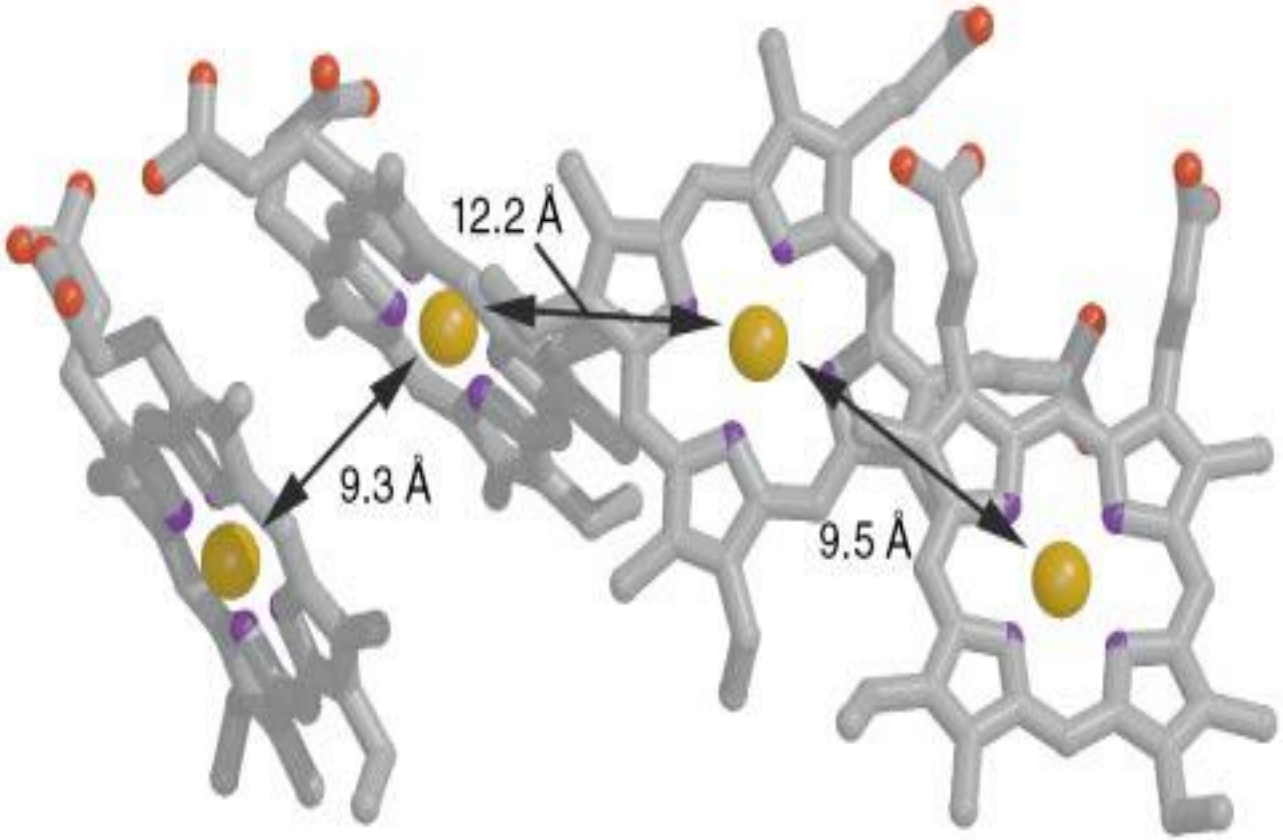
Cytochrome c554

(in bacteria catalyzes the nitrification process)



Fe···Fe distances ≈ 950, 1220, 920 pm

Cytochrome c554

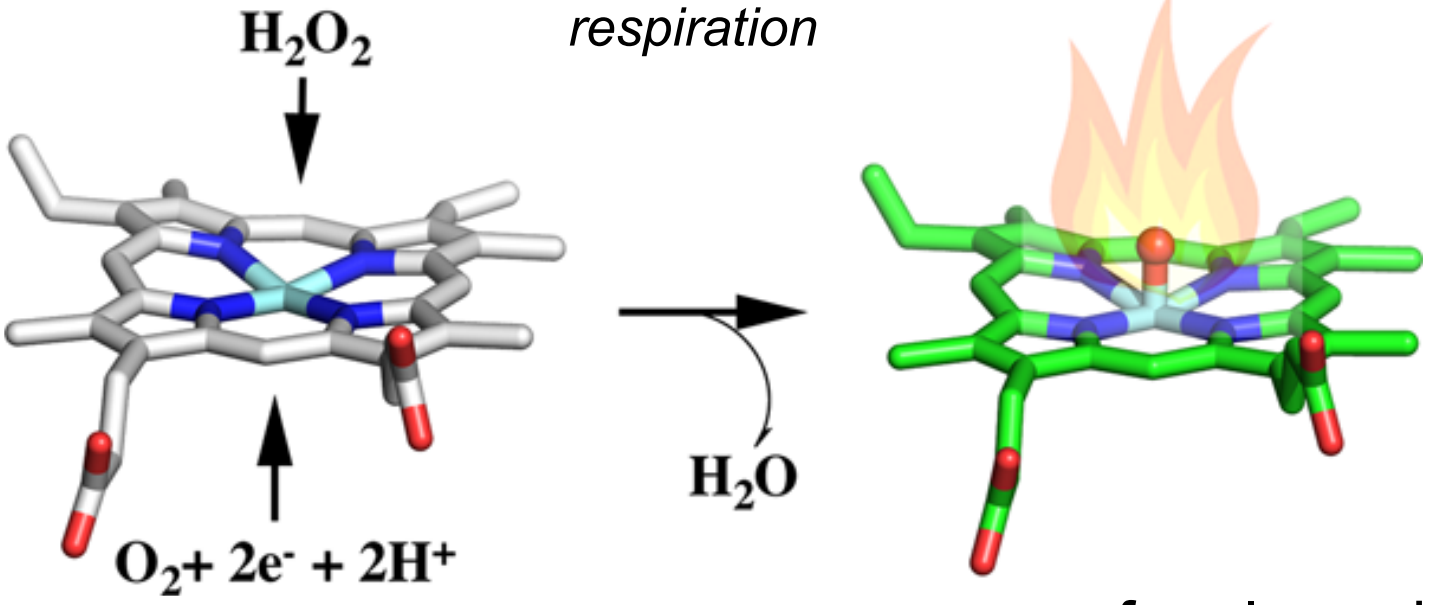


Peroxidases and Oxygenases

Oxidations catalyzed by heme-proteins

H_2O_2 = unwanted intermediate in the photosynthetic oxidation of H_2O or generated by incomplete reduction of O_2 during respiration

peroxidase



oxygenase

oxoferryl species

Oxidizing does not always also imply oxygenating a substrate

Peroxidases and Catalases

e.g. *lignin peroxidase, horse-radish peroxidase (HRP)*, *Cytochrome c peroxidase, ascorbate peroxidase…*

They are also **detoxifying** enzymes

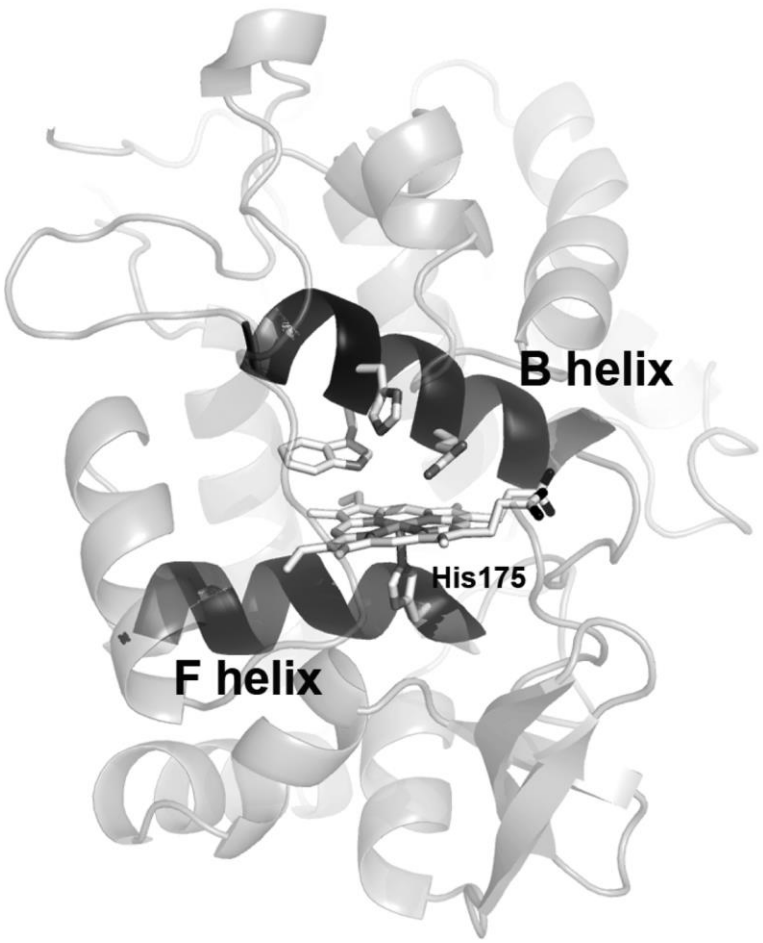
$$H_2O_2(aq) + AH_2 \rightarrow 2\ H_2O(l) + A$$

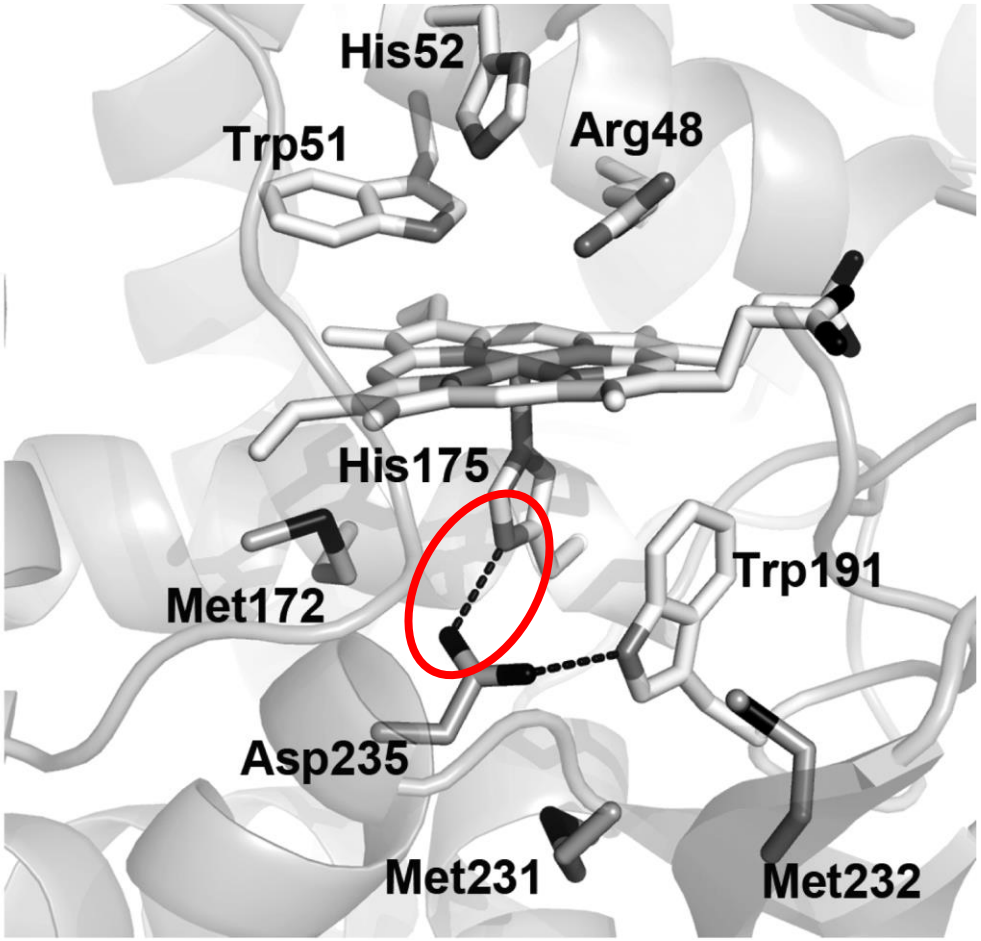
$$H_2O_2(aq) + H_2O_2(aq) \rightarrow 2\ H_2O(l) + O_2$$

Substrates AH_2: fatty acids, amines, phenols, xenobiotic toxins,..

Cytochrome c Peroxidase (CcP)

(yeast, bacteria)

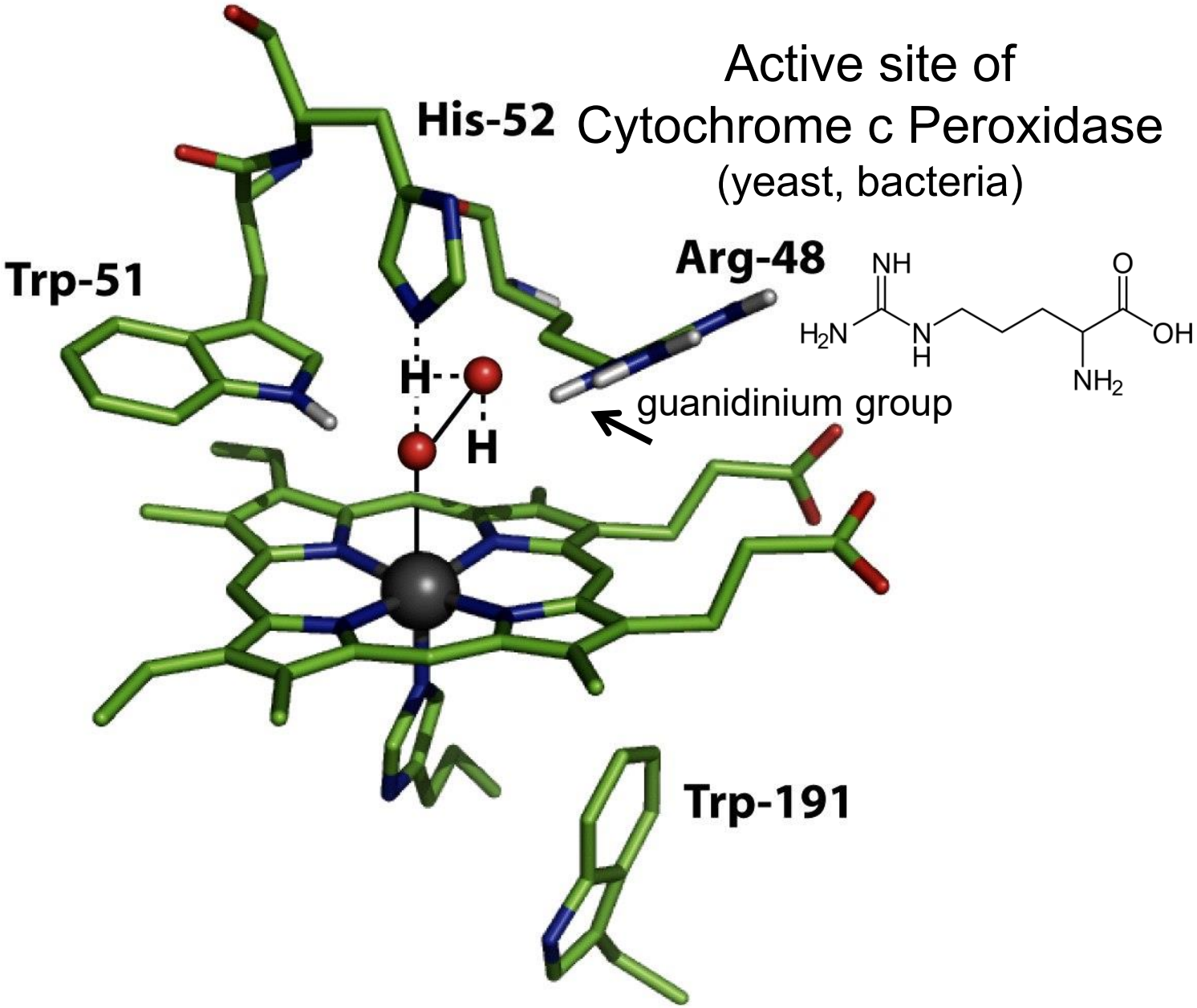




The hydrogen bond of proximal histidine (His175) with an aspartate (Asp235) gives it more **imidazolate character** (Im^-) and contributes to lowering the redox potential of iron-heme (stabilizes Fe(III))

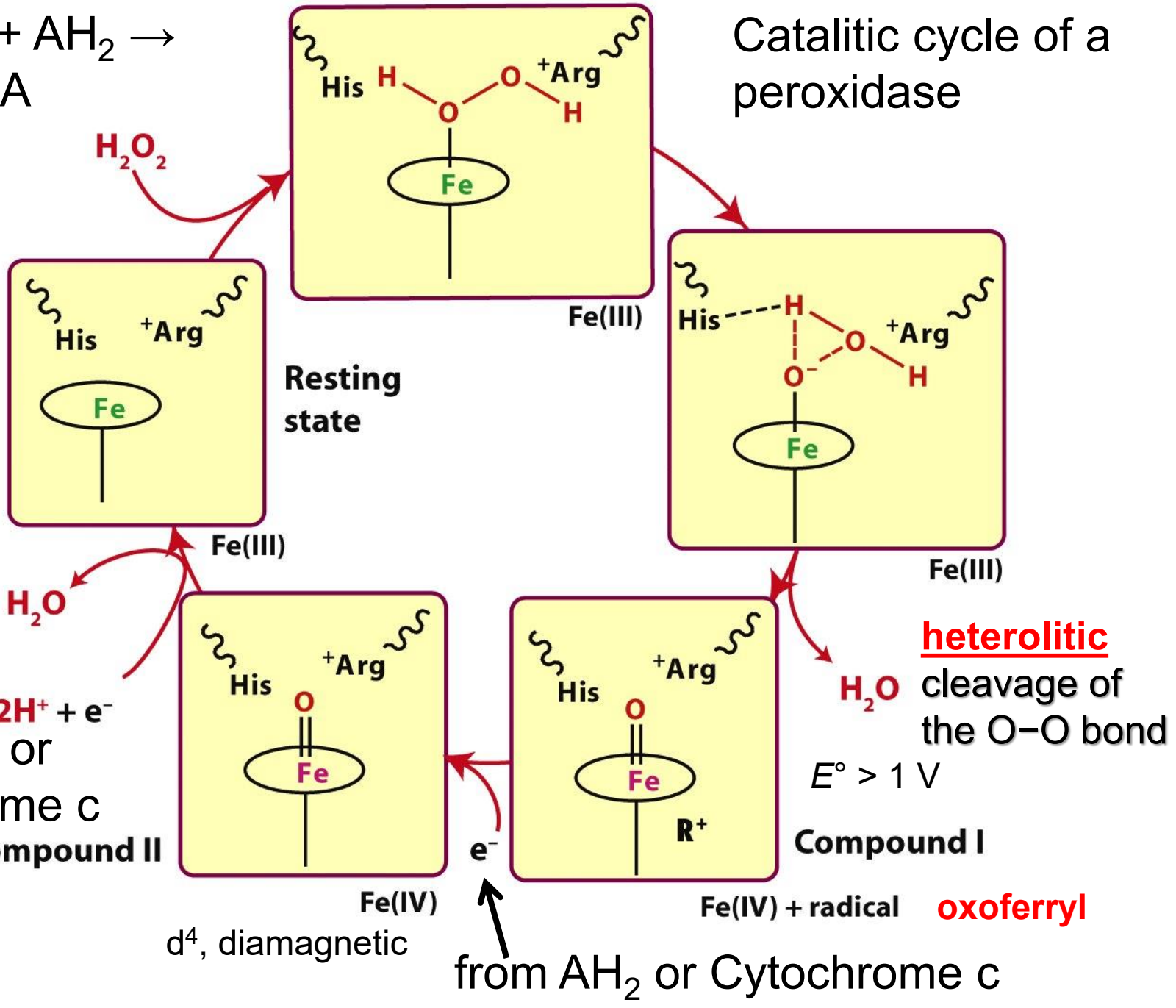
Active site of Cytochrome c Peroxidase

(yeast, bacteria)

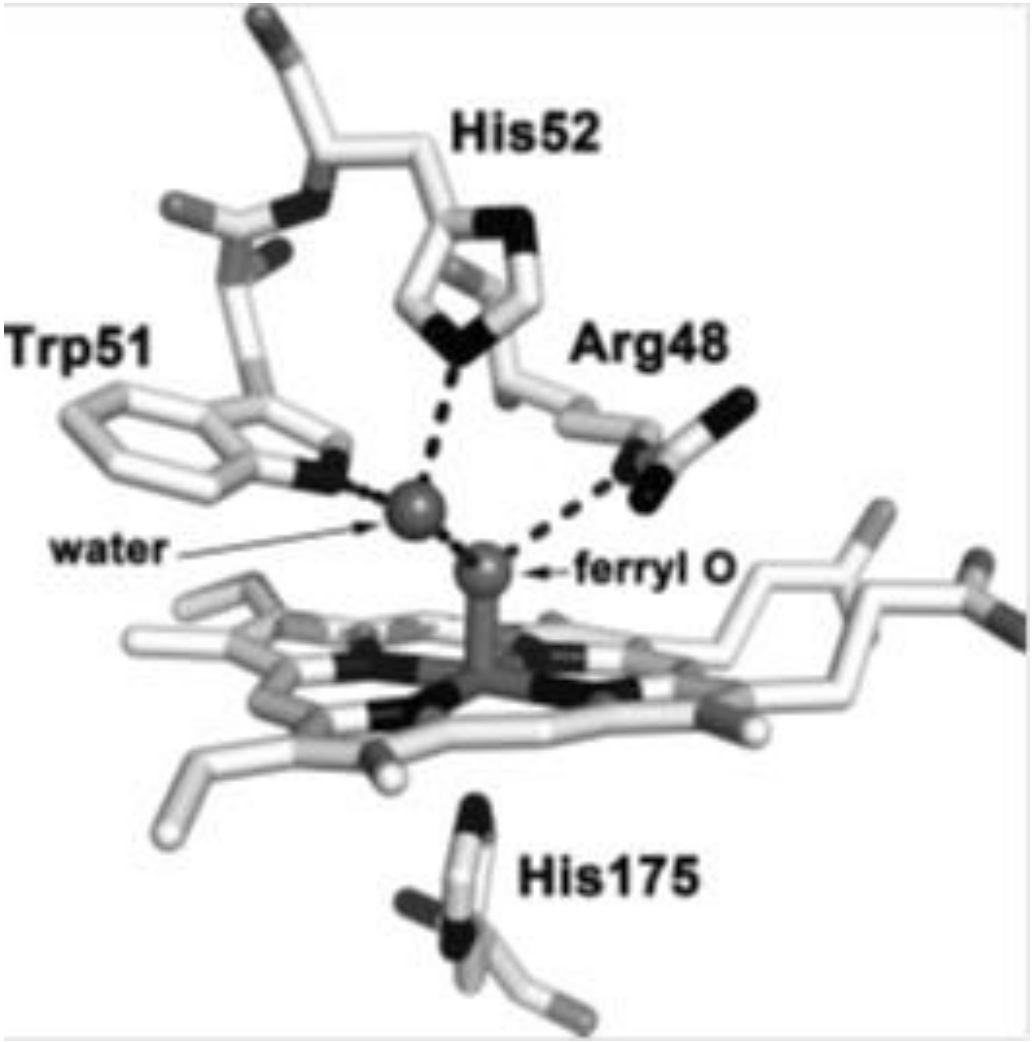


$H_2O_2(aq) + AH_2 \rightarrow 2H_2O(l) + A$

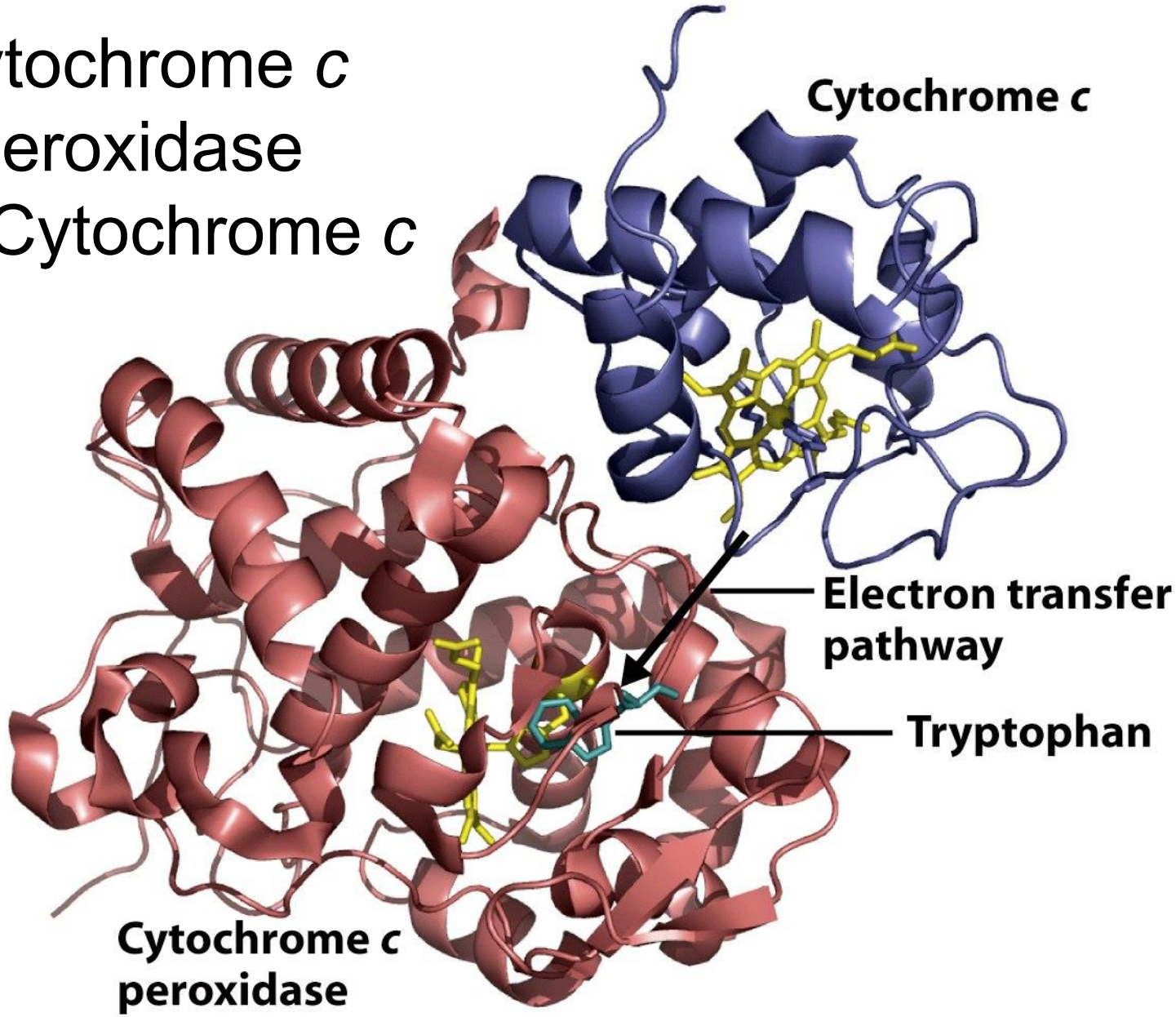
Catalitic cycle of a peroxidase



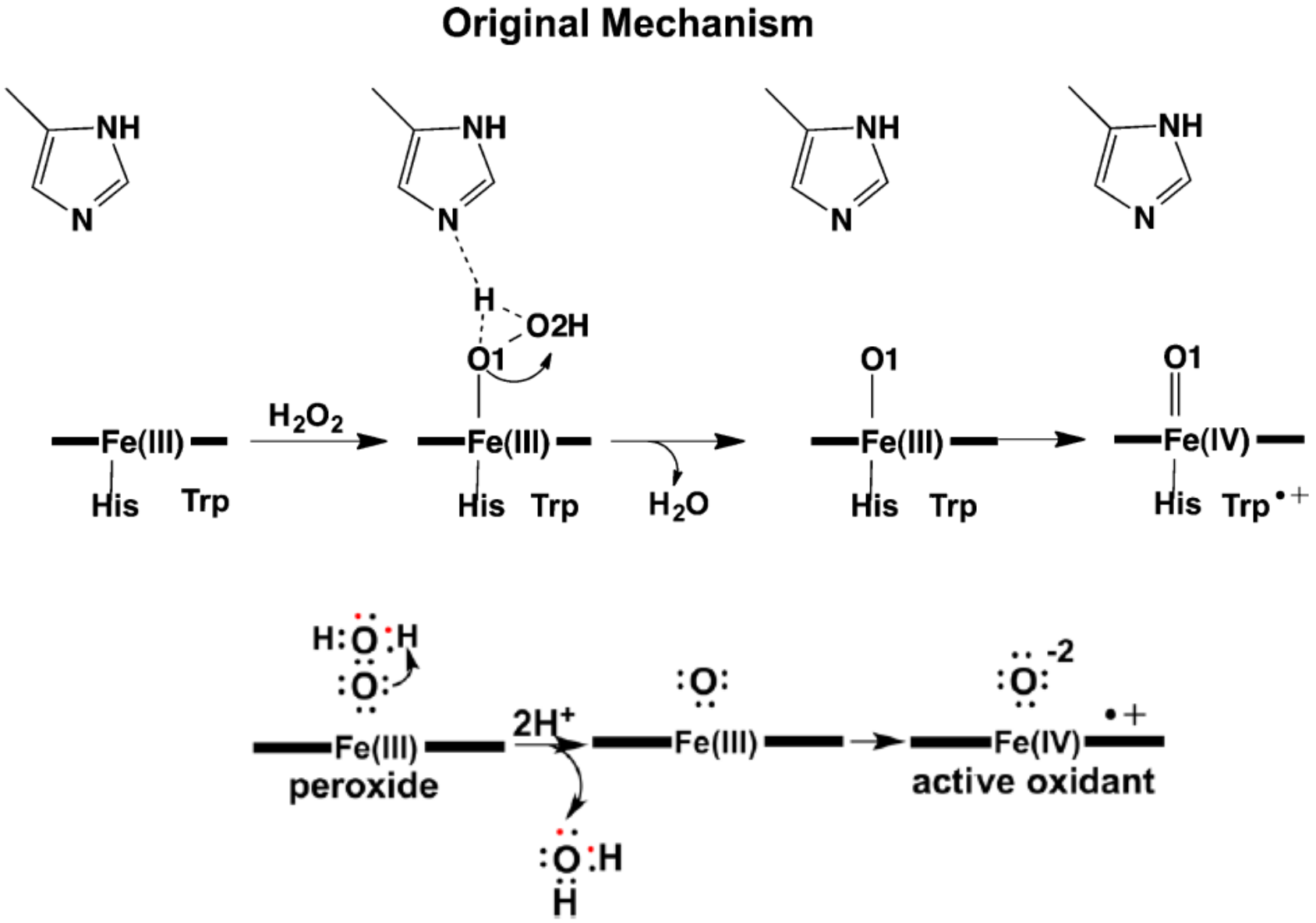
X structure of the oxoferryl intermediate in CcP



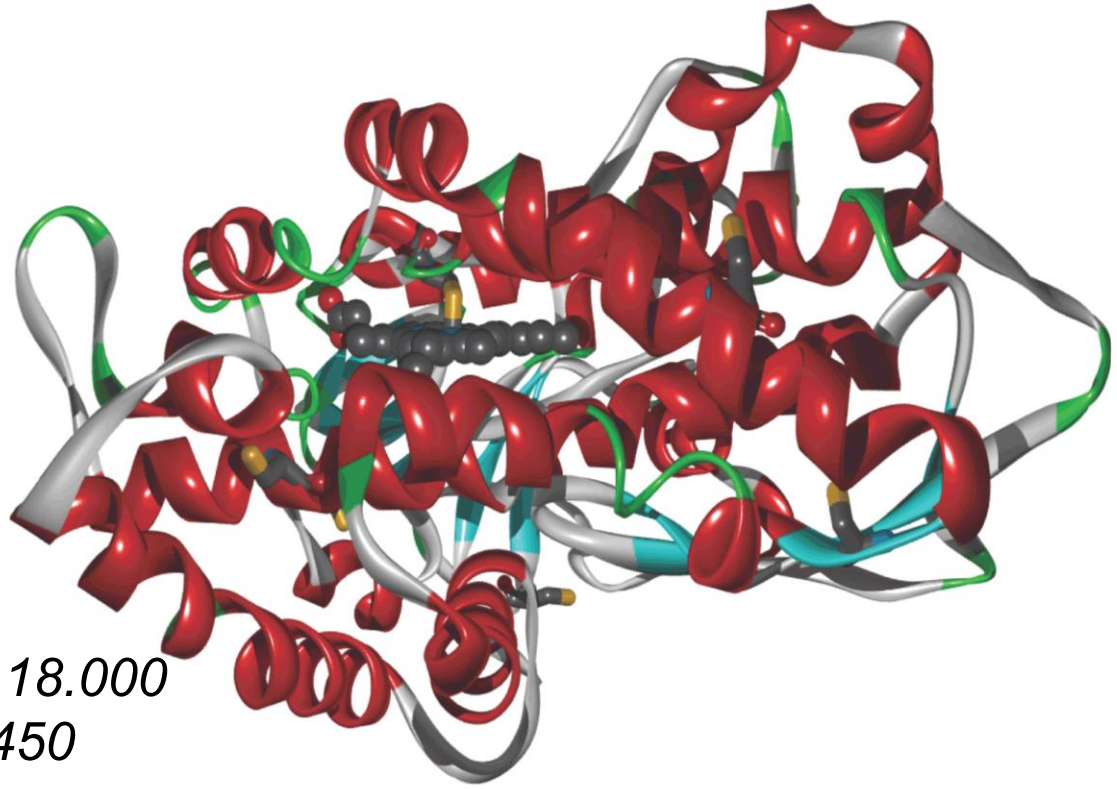
Cytochrome *c* peroxidase and Cytochrome *c*



Formation of the oxoferryl intermediate



Mono-oxygenases



Iron has a single axial ligand, Cys^-

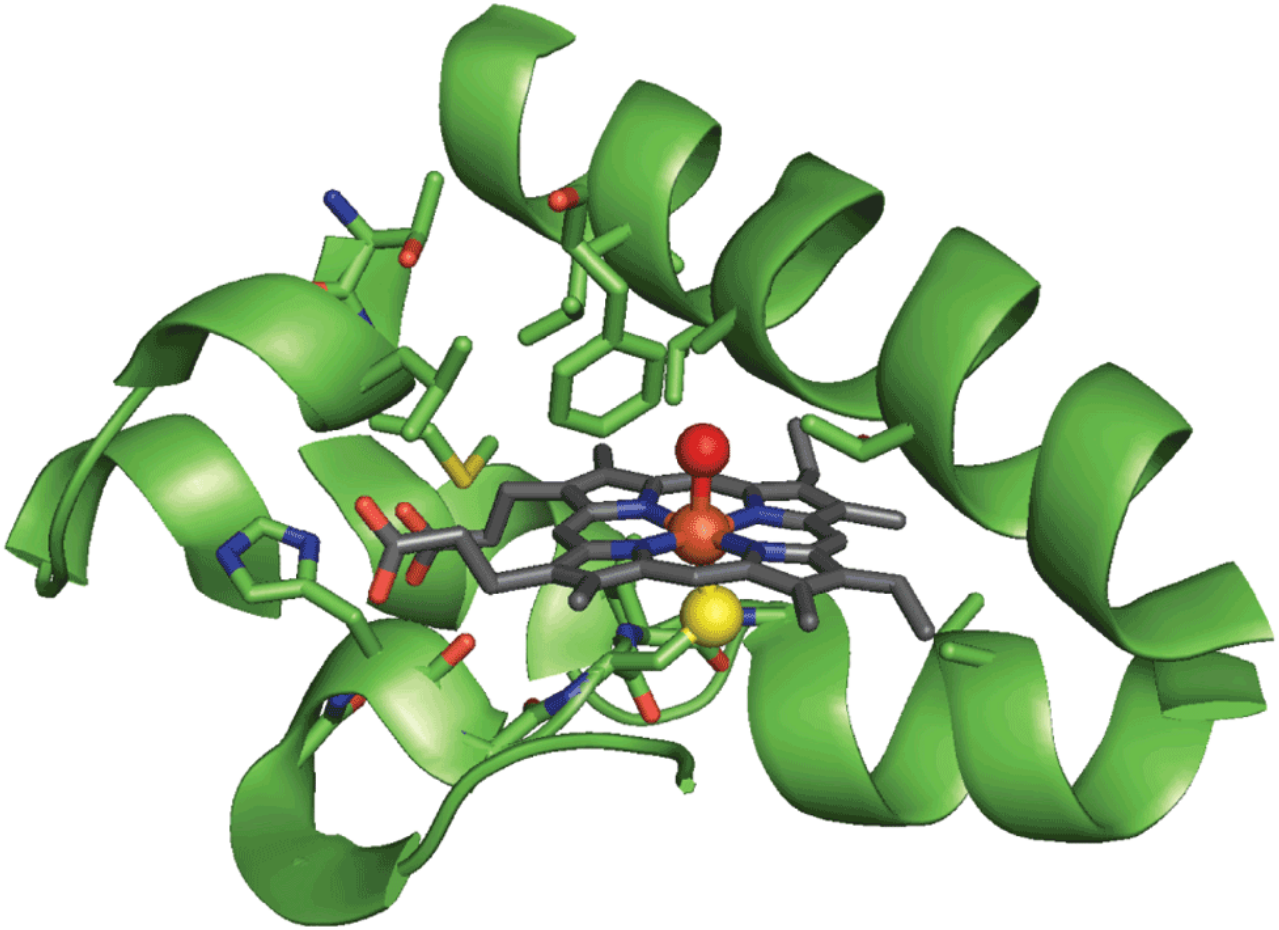
more than 18.000 types of P450 mono-oxygenases have been found, 57 of which in man

Cytochrome P-450

(from *Pseudomonas putida*) 50 kDa

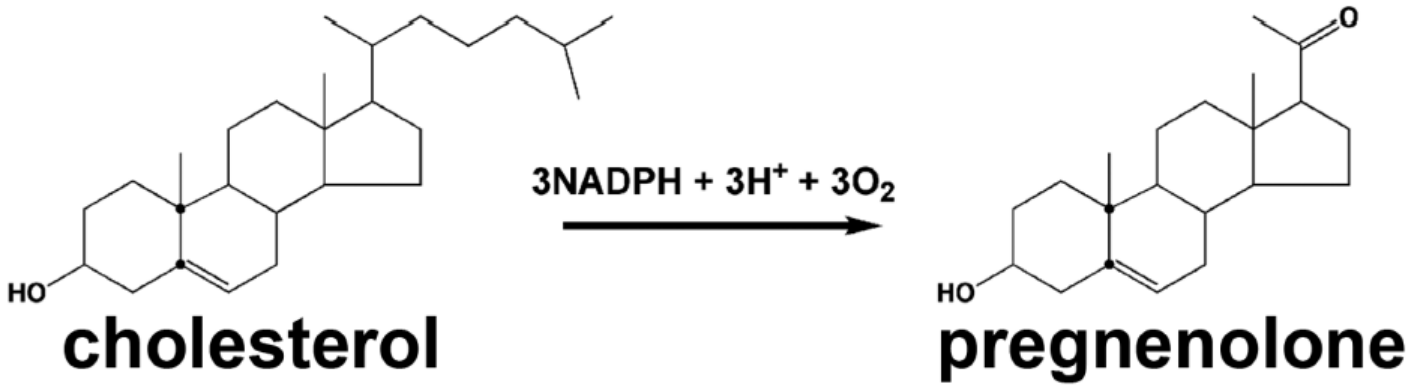
$$R\text{-}H + O_2 + 2e^- + 2H^+ \xrightarrow{\text{P-450}} R\text{-}OH + H_2O$$

Cytochrome P-450



The hydrogen bond between cysteine and the NH group from a peptide tunes (increases) the redox potential of iron. Cys^- is a strong σ and π donor, and thus stabilizes Fe(III).

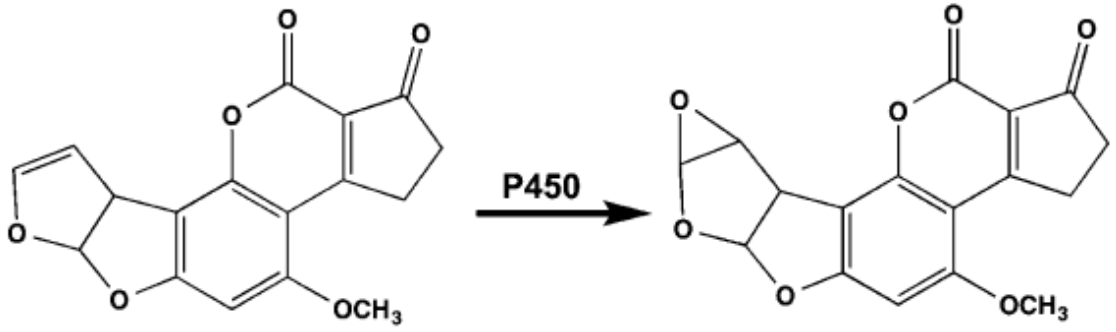
Oxygenation of specific substrates catalyzed by P450



cholesterol

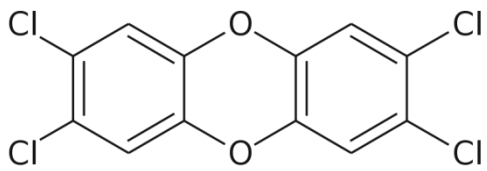
pregnenolone

(Unwanted) activation of substrates by P450



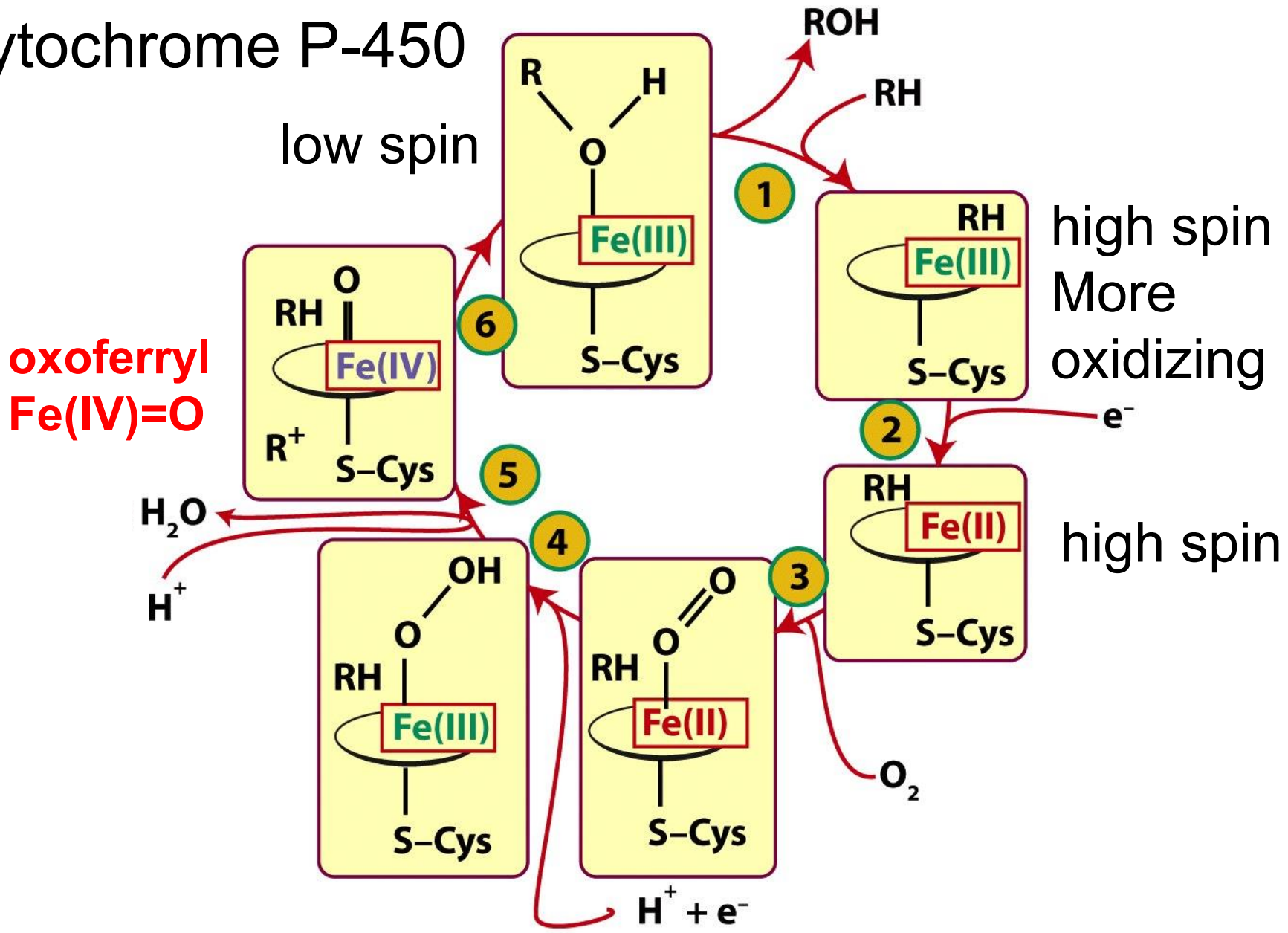
aflatoxin B_1

aflatoxin B_1-9,9-*exo*-epoxide



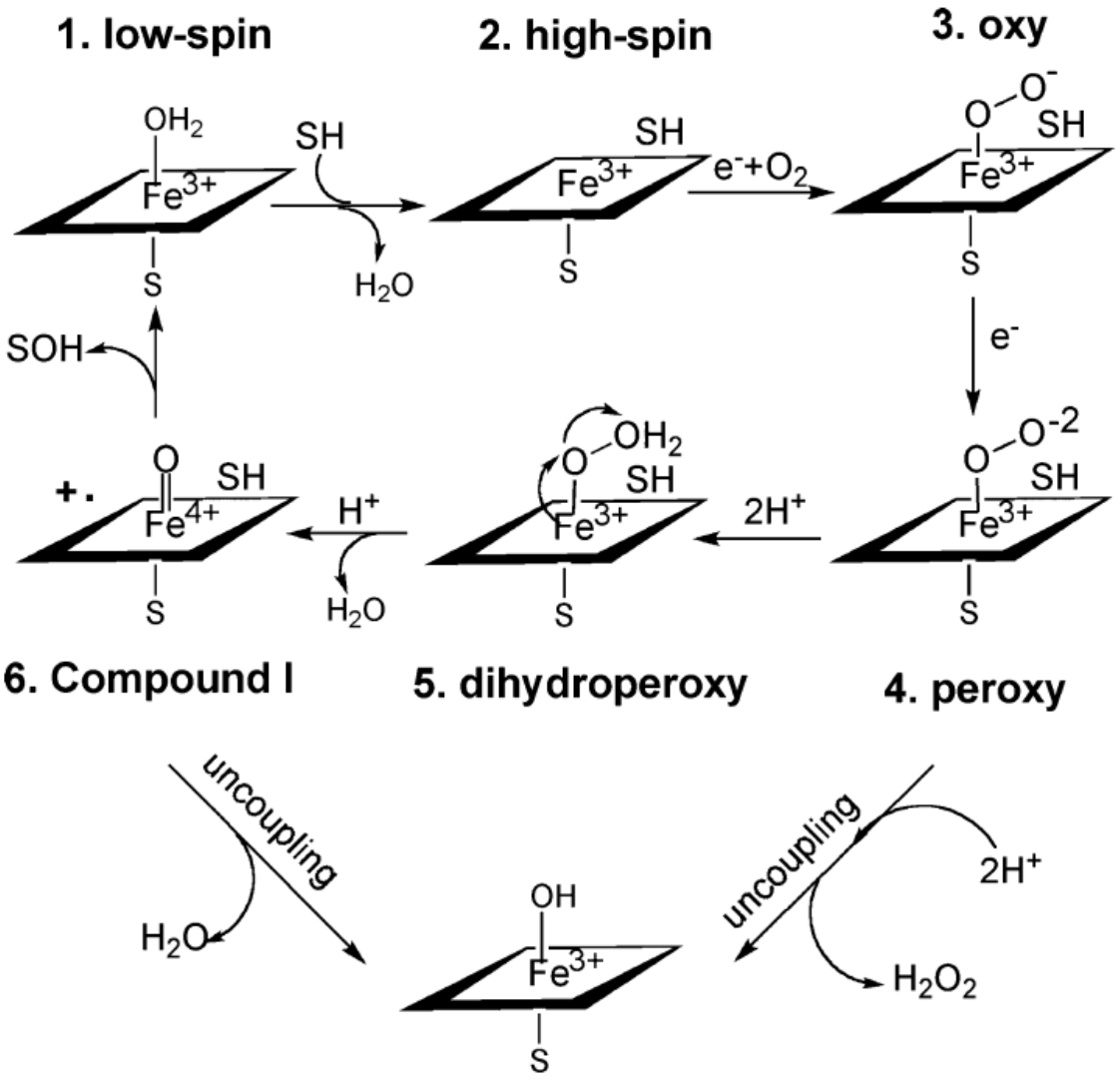
dioxine

Cytochrome P-450

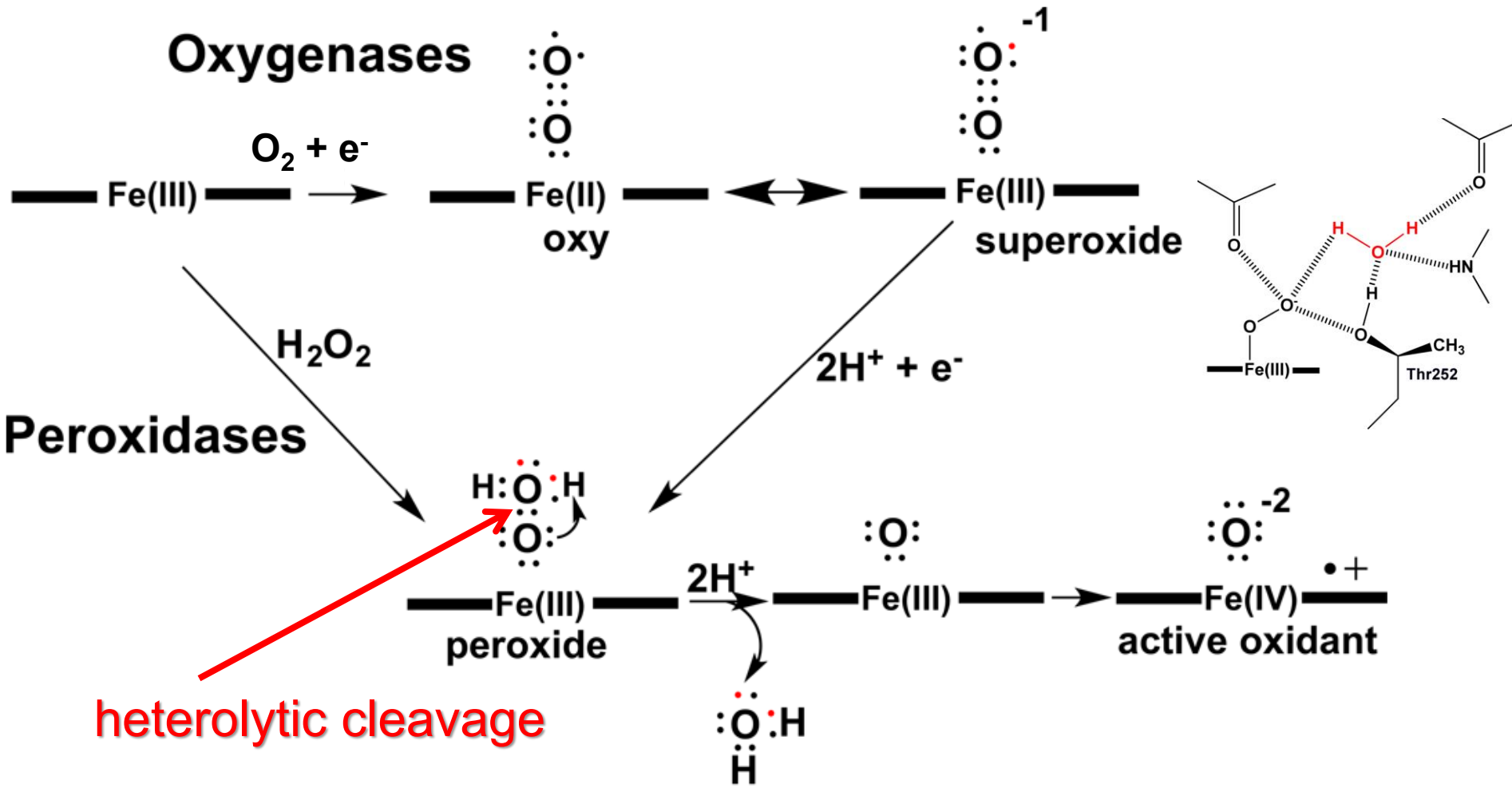


Cytochrome P-450

oxoferryl
Fe(IV)=O

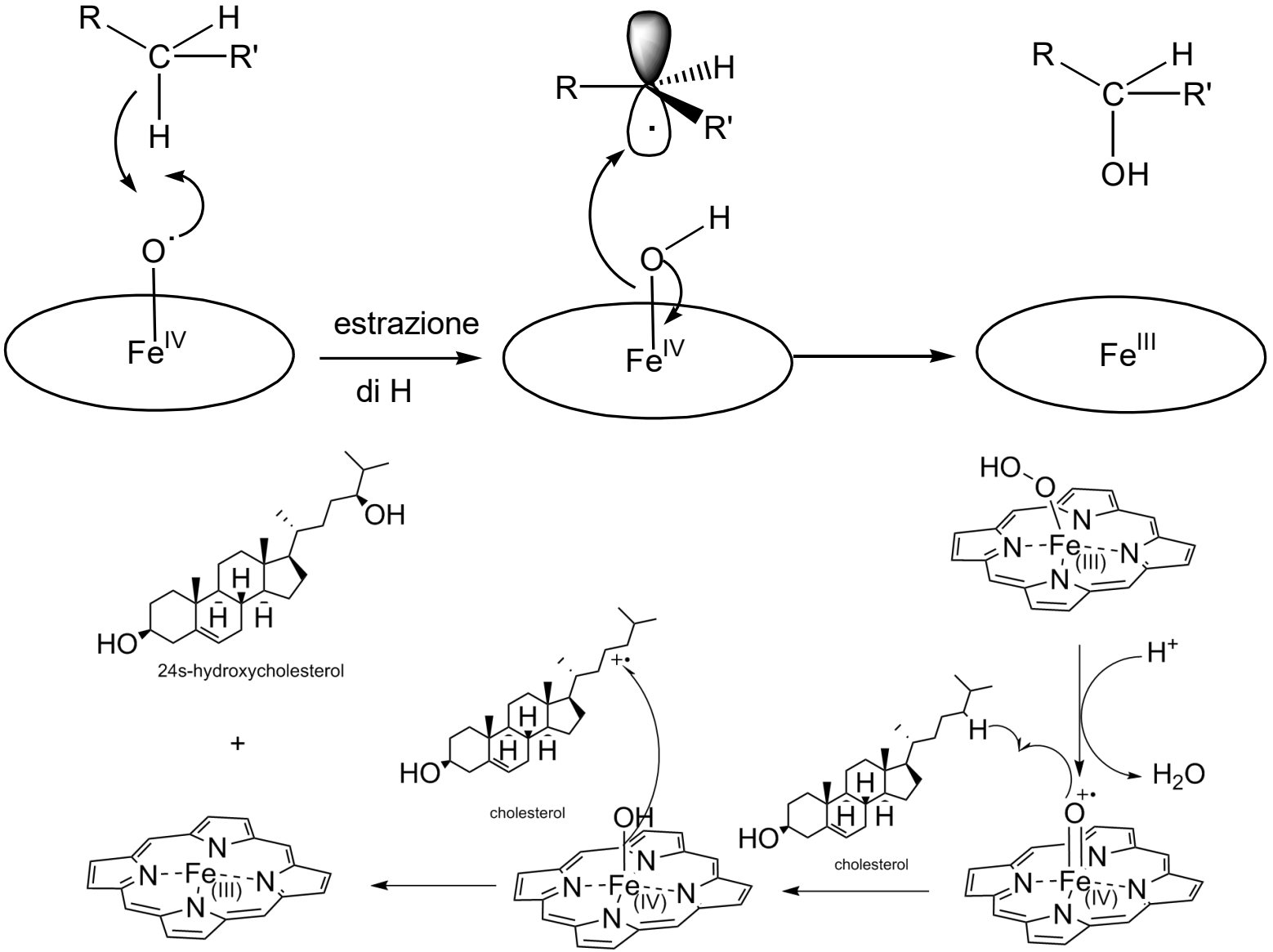


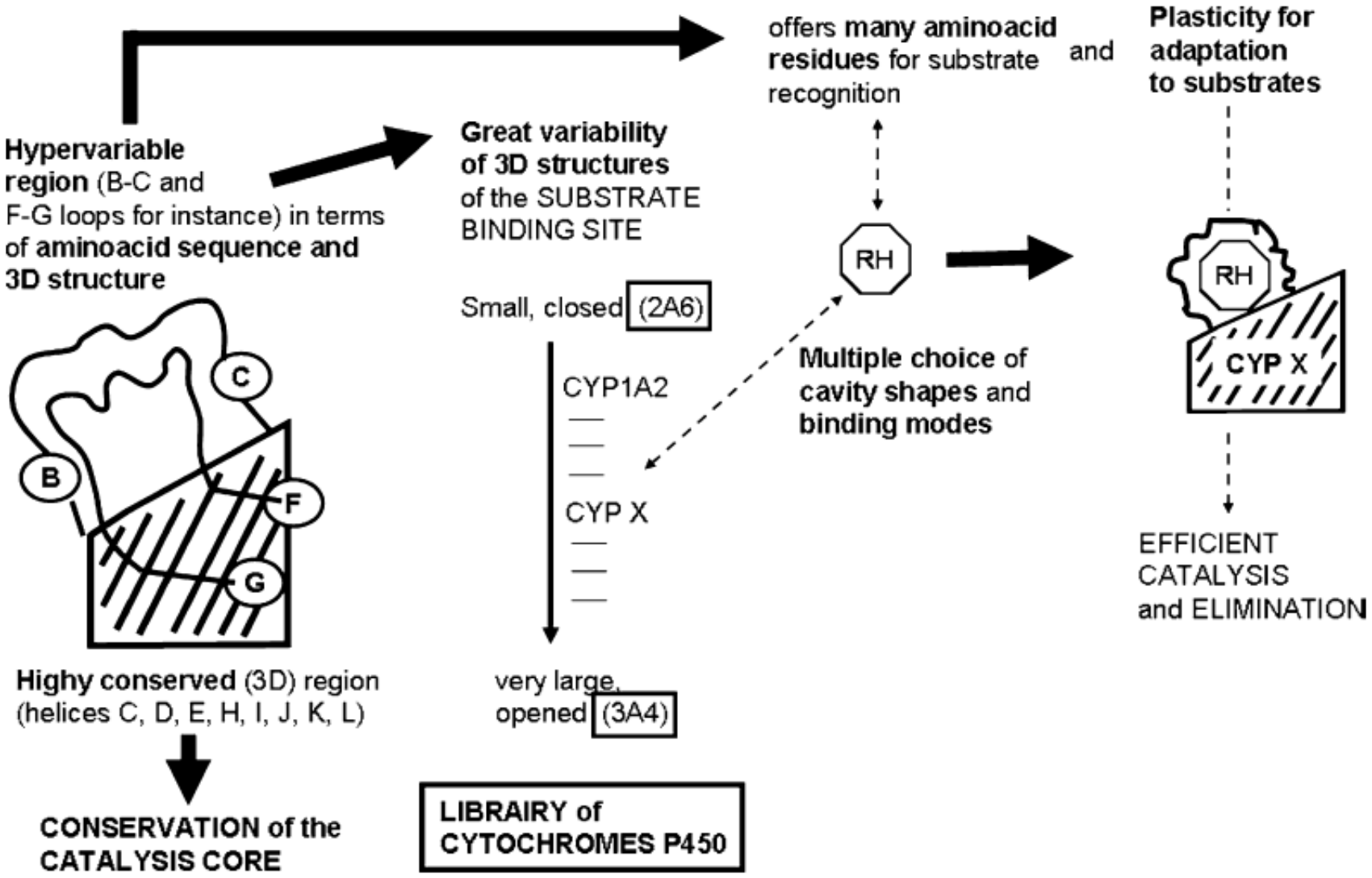
Heterolytic cleavage of the O–O bond and protonation of the distal oxygen atom



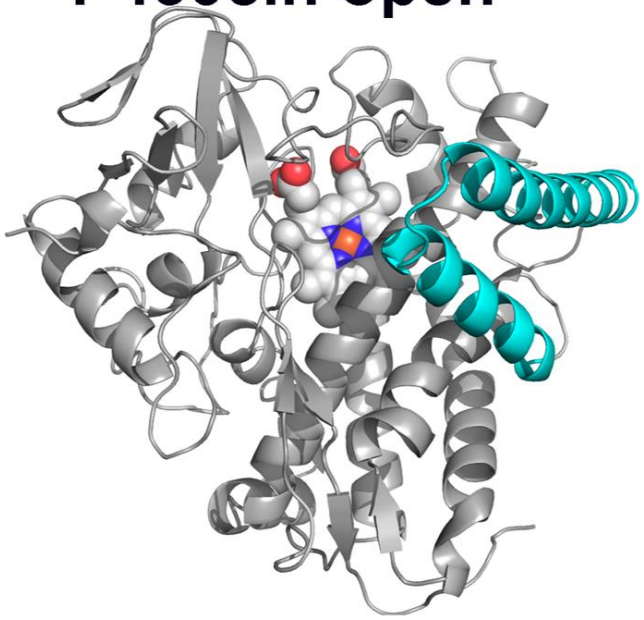
Substrate oxygenation mechanism

Oxygen Rebound Mechanism

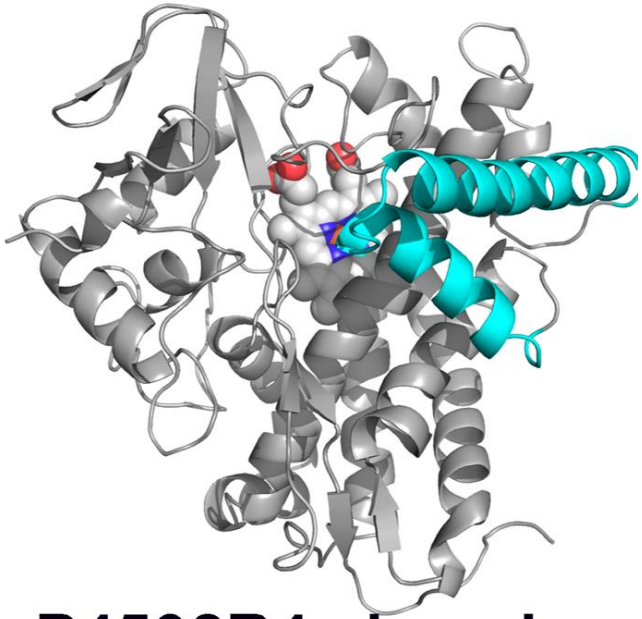



offers many aminoacid residues for substrate recognition
and
Plasticity for adaptation to substrates
Hypervariable region (B-C and F-G loops for instance) in terms of aminoacid sequence and 3D structure
Great variability of 3D structures of the SUBSTRATE BINDING SITE
RH
RH
CYP X
Small, closed (2A6)
CYP1A2
CYP X
Multiple choice of cavity shapes and binding modes
C
B
F
G
EFFICIENT CATALYSIS and ELIMINATION
Highy conserved (3D) region (helices C, D, E, H, I, J, K, L)
very large, opened (3A4)
CONSERVATION of the CATALYSIS CORE
LIBRAIRY of CYTOCHROMES P450

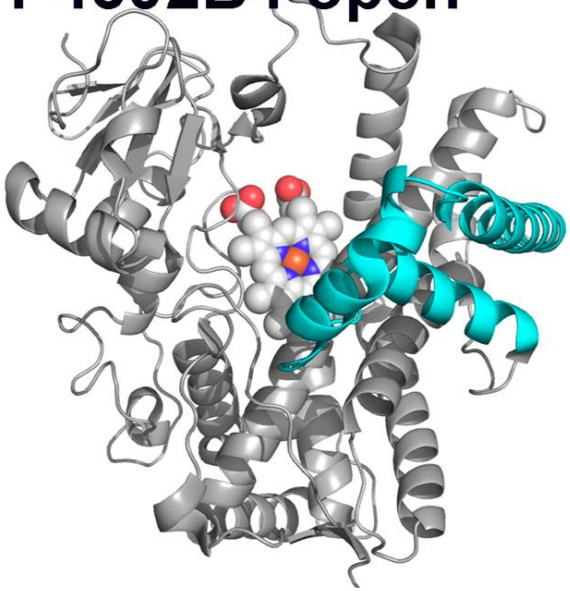
P450cin open



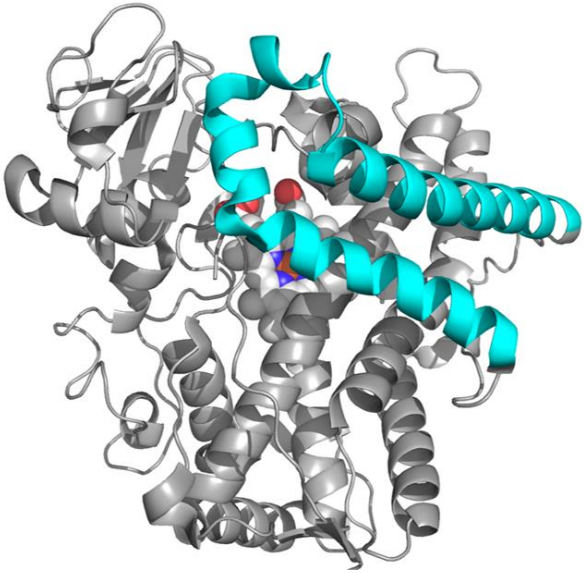
P450cin closed

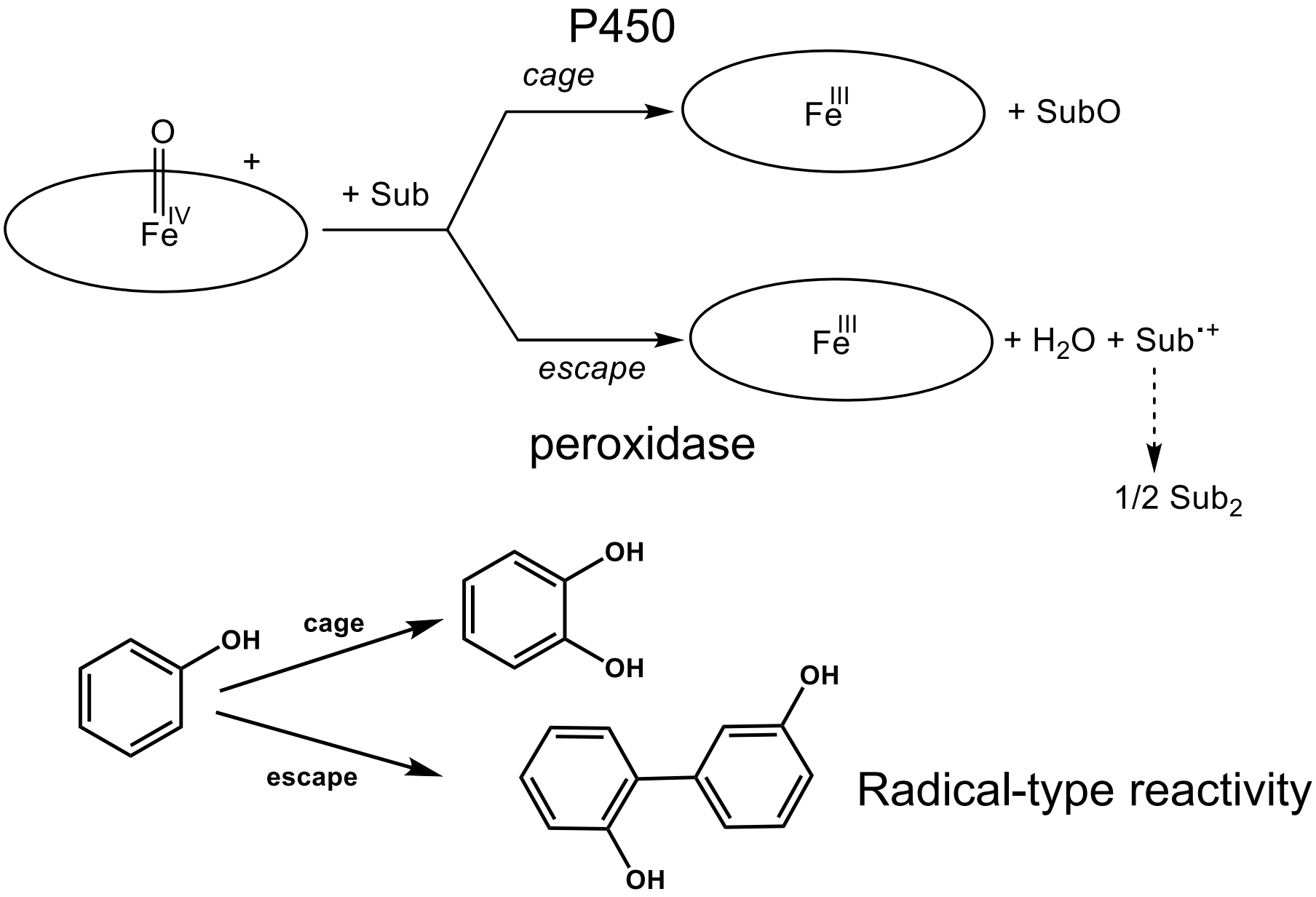


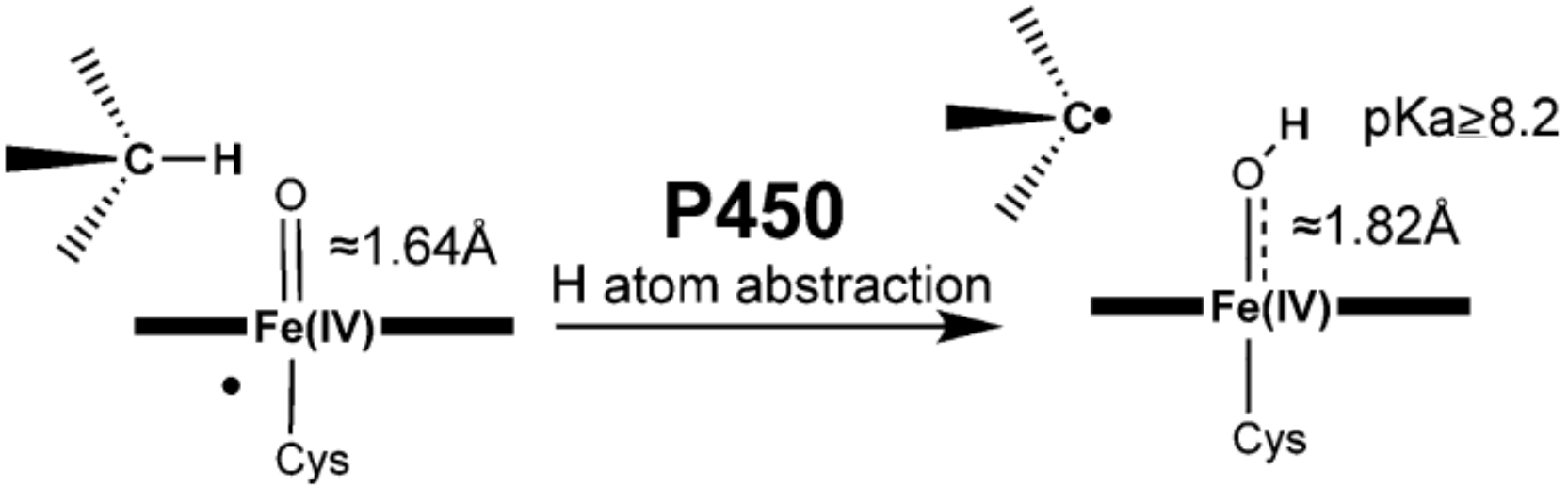
P4502B4 open



P4502B4 closed



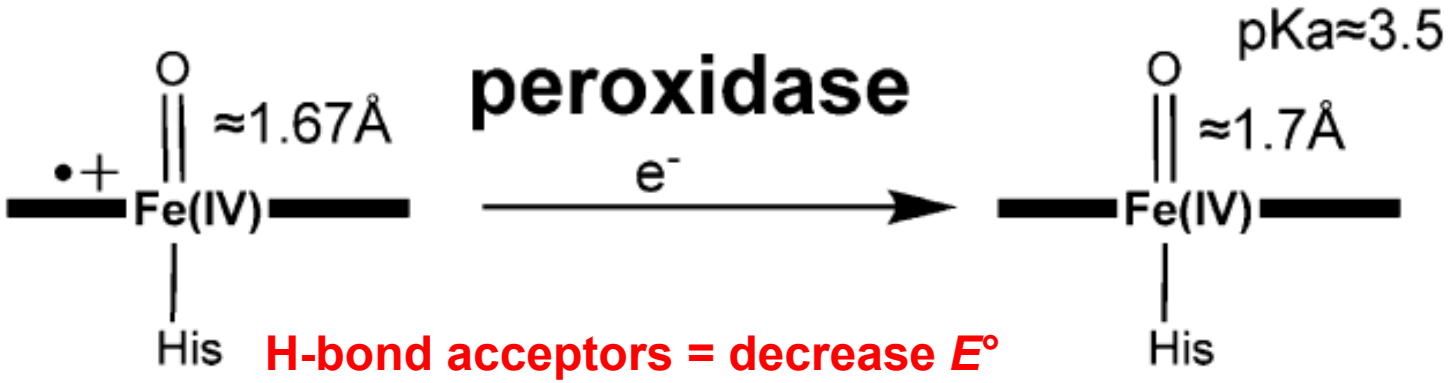

P450
cage
O
Fe IV
+
+ Sub
Fe III
+ SubO
Fe III
+ H2O + Sub•+
escape
peroxidase
1/2 Sub2
OH
cage
OH
OH
escape
OH
OH
Radical-type reactivity



H-bond donors = increase *E*°

Compound I

Compound II



H-bond acceptors = decrease *E*°