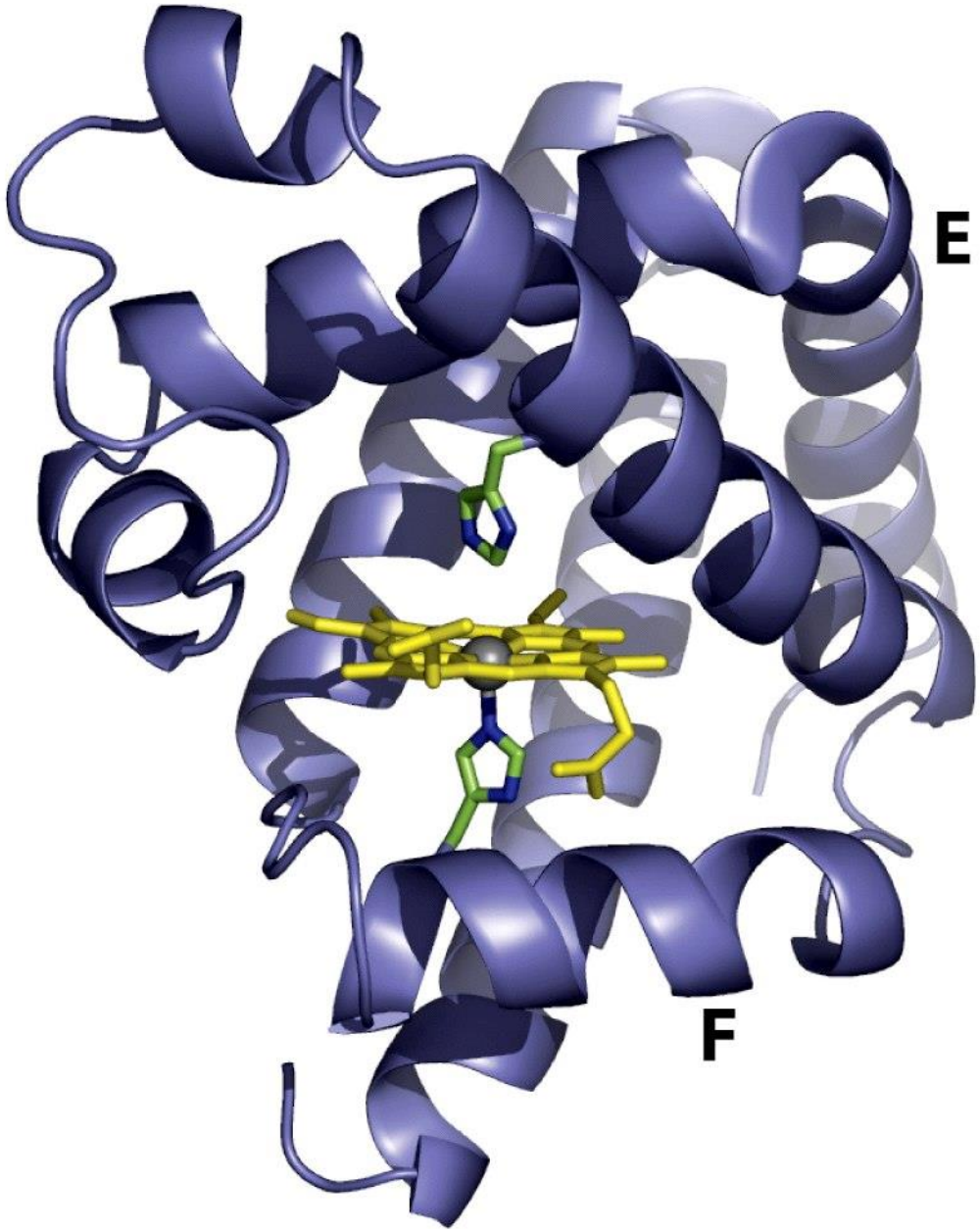
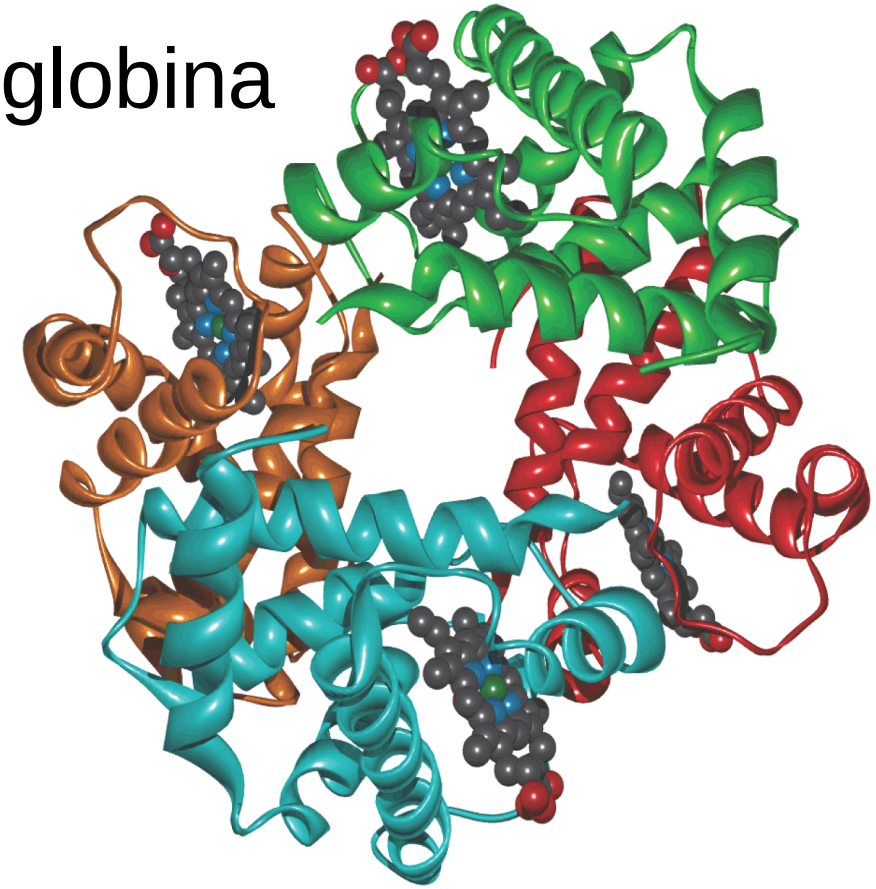
Mioglobina

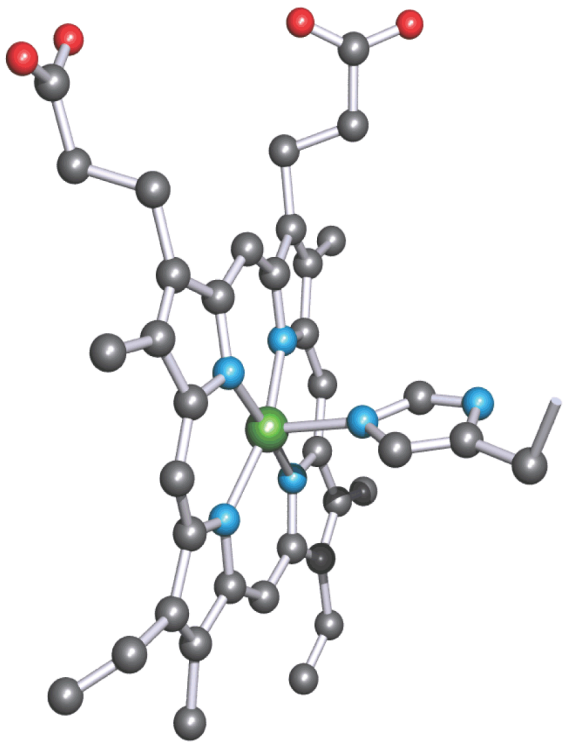
17.8 kDa



ca. 6% Fe_{tot}

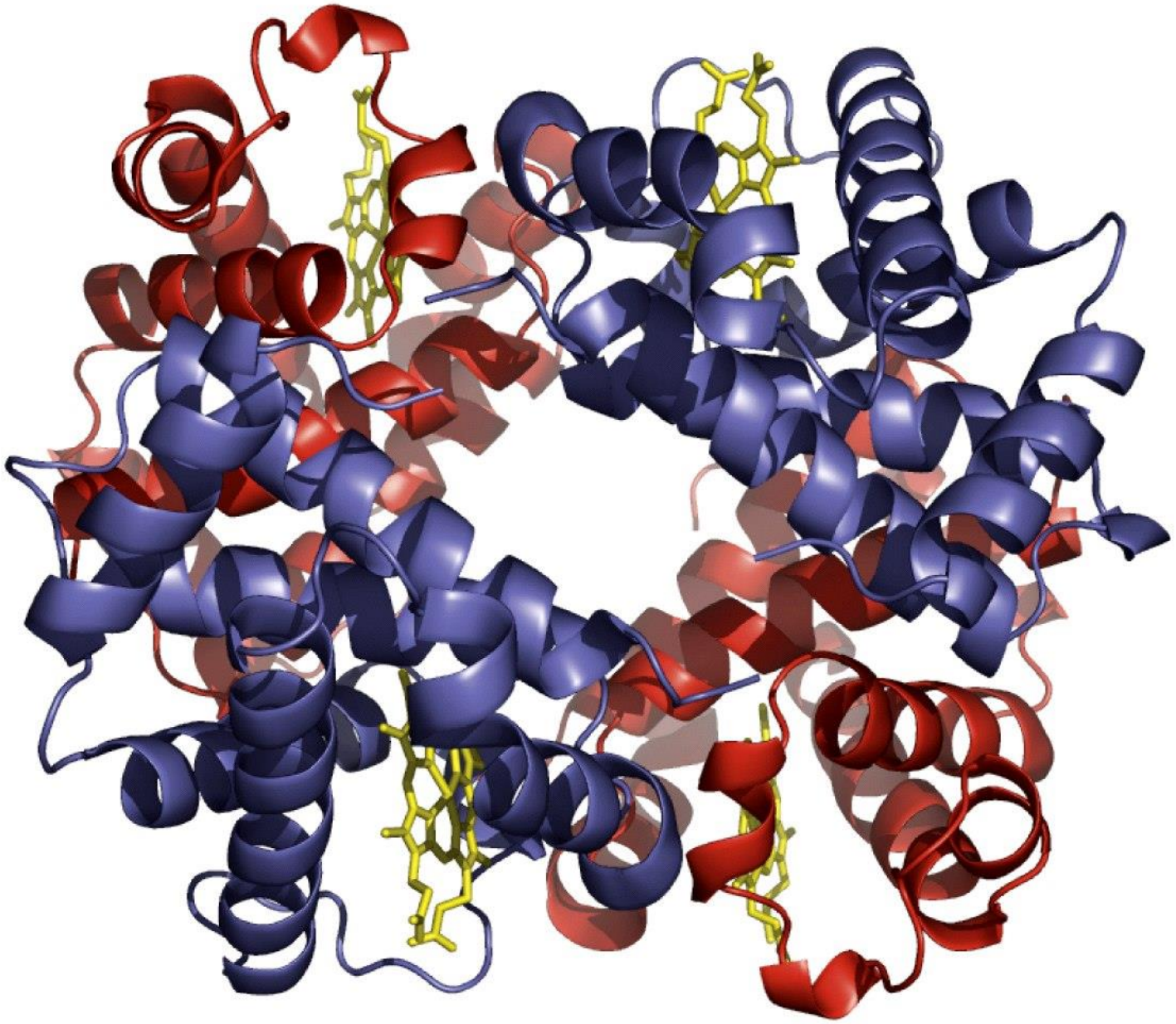
Emoglobina





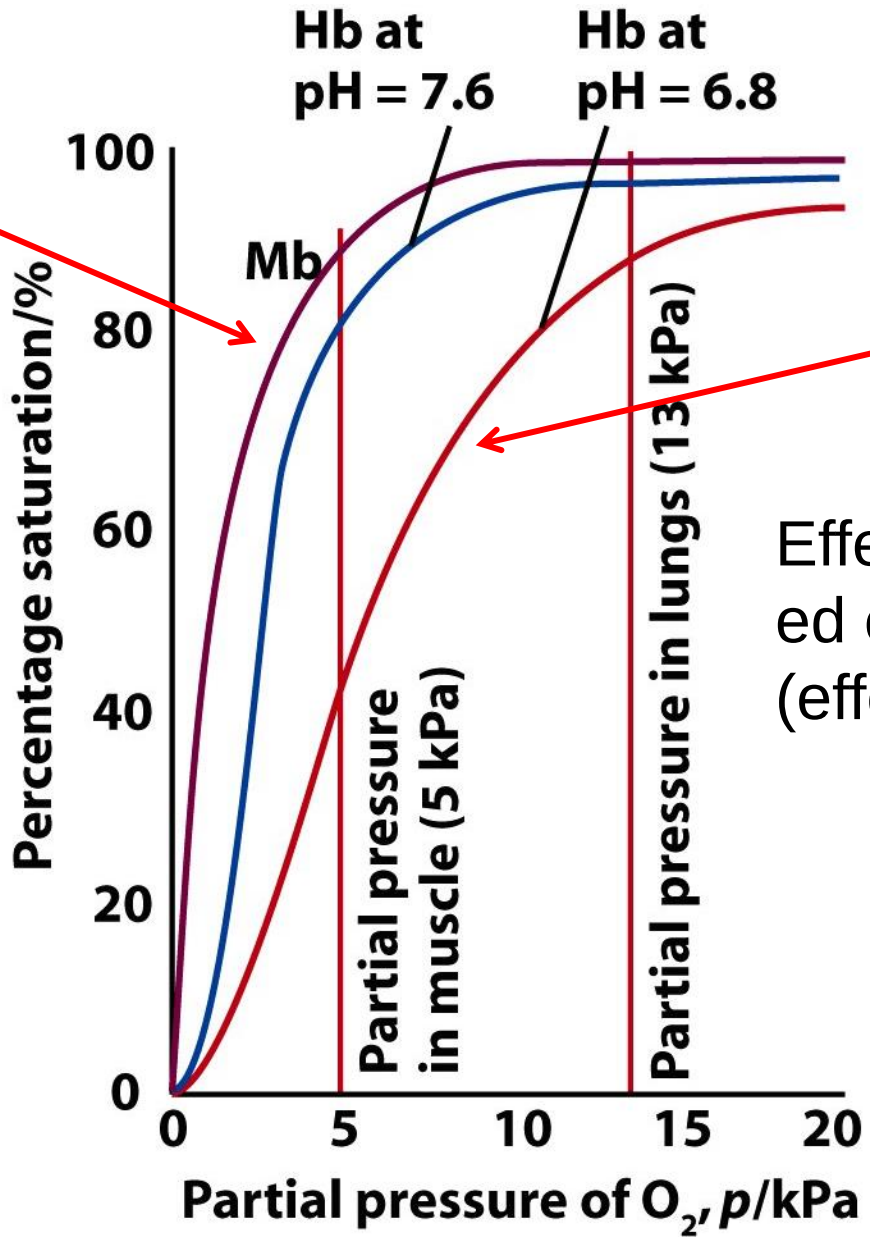
tetramero $\alpha_2\beta_2$ 141 e 146 a.a. 64.5 kDa

ca. 65% Fe_{tot}



tetramero $\alpha_2\beta_2$

Curva di saturazione iperbolica

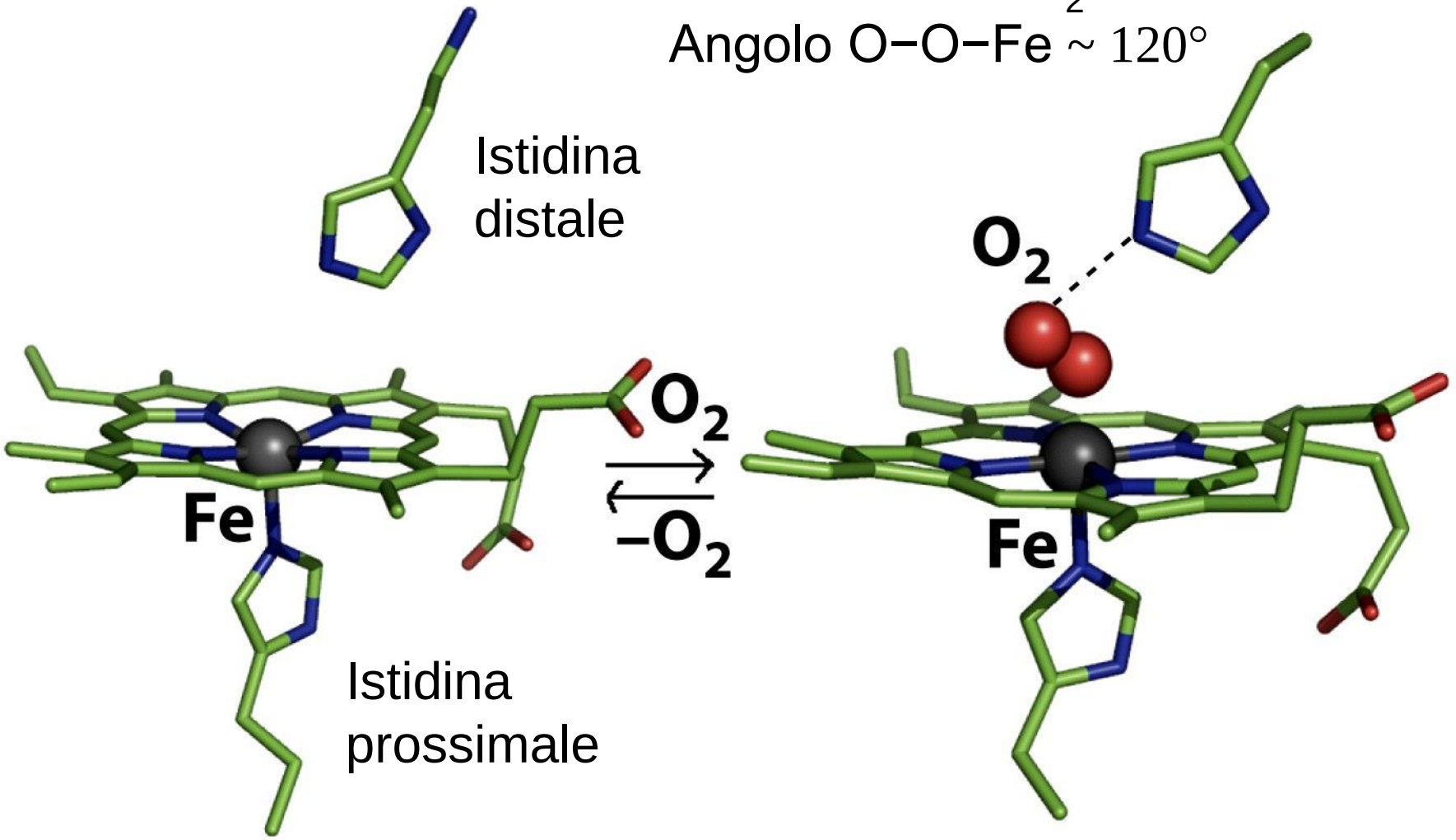


Curva di saturazione sigmoidale, pH dipendente

Effetto cooperativo ed effetto Bohr (effetto allosterico)

La coordinazione di CO_2 e protoni (pH) alla catena proteica dell'emoglobina causano variazioni conformazionali che influenzano l'affinità dell'eme per O_2

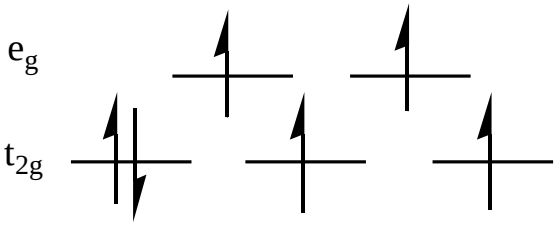
Coordinazione O_2 *end-on*
Angolo O−O−Fe ~ 120°



Fe(II), d^6, h.s.

S = 0

desossi:
S = 2
paramagnetica

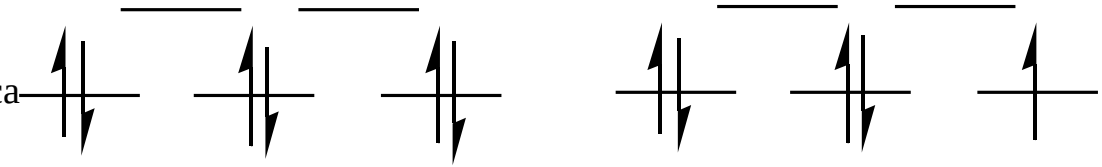


Fe(II), d^6 paramagnetico

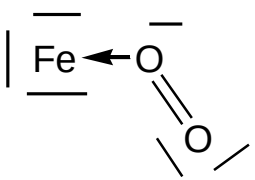
Pauling

Weiss

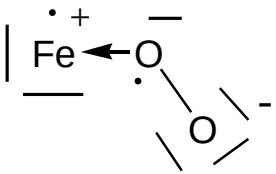
ossi:
S = 0
diamagnetica



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



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



Pauling

Fe^{II} / O_2

S = 0

McClure/Goddard

Fe^{II} / O_2

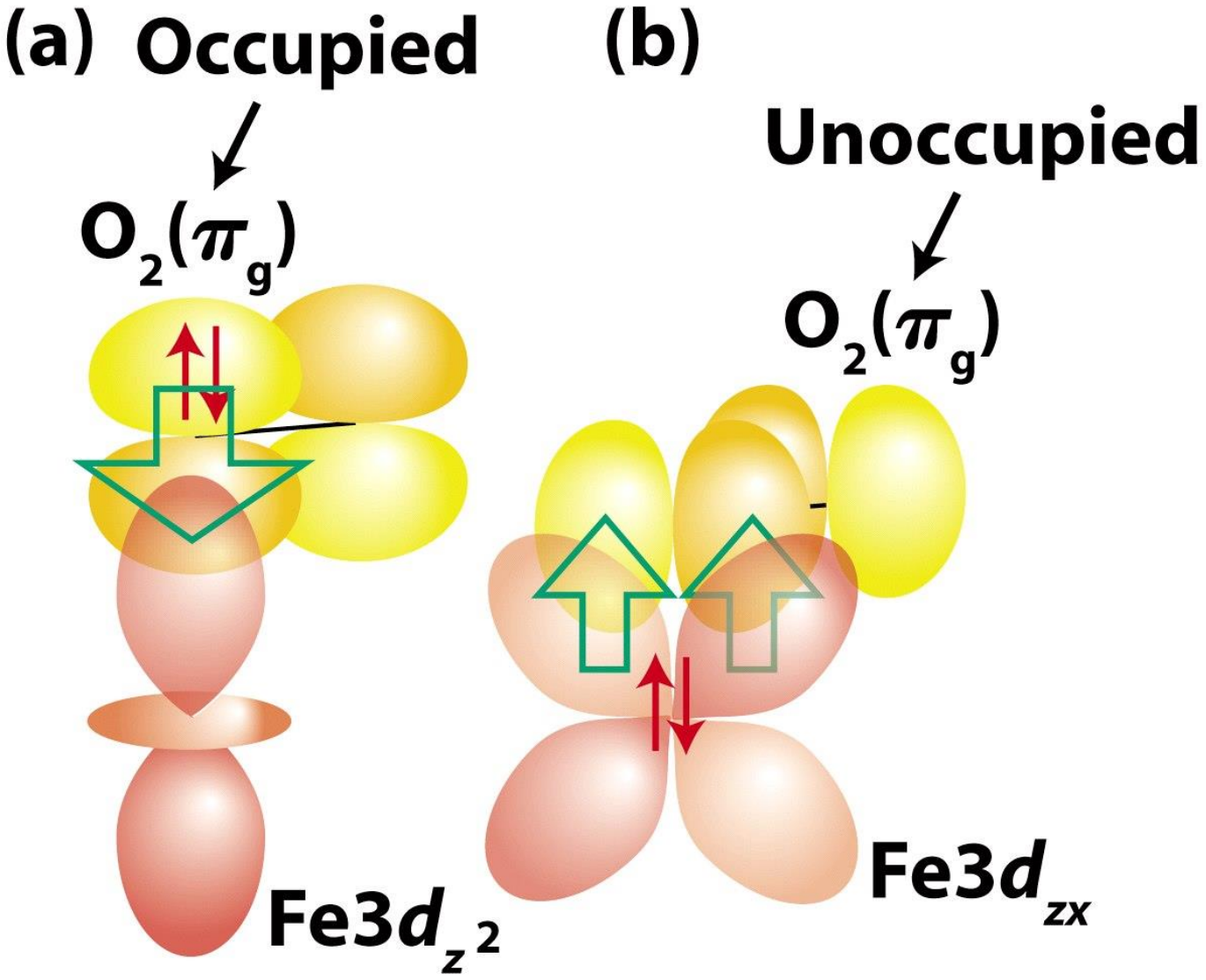
S = 1

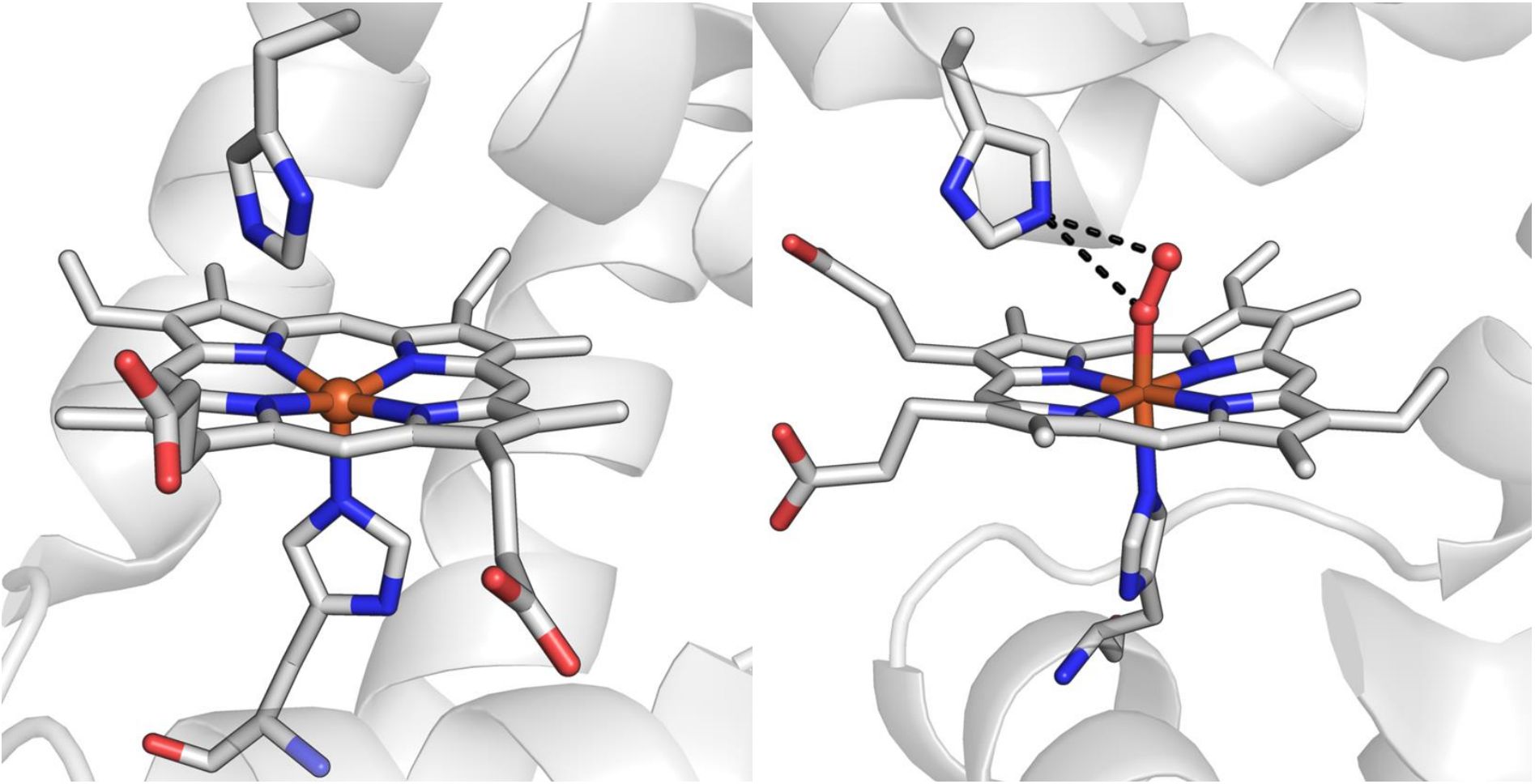
Weiss

Fe^{III} / O_2^-

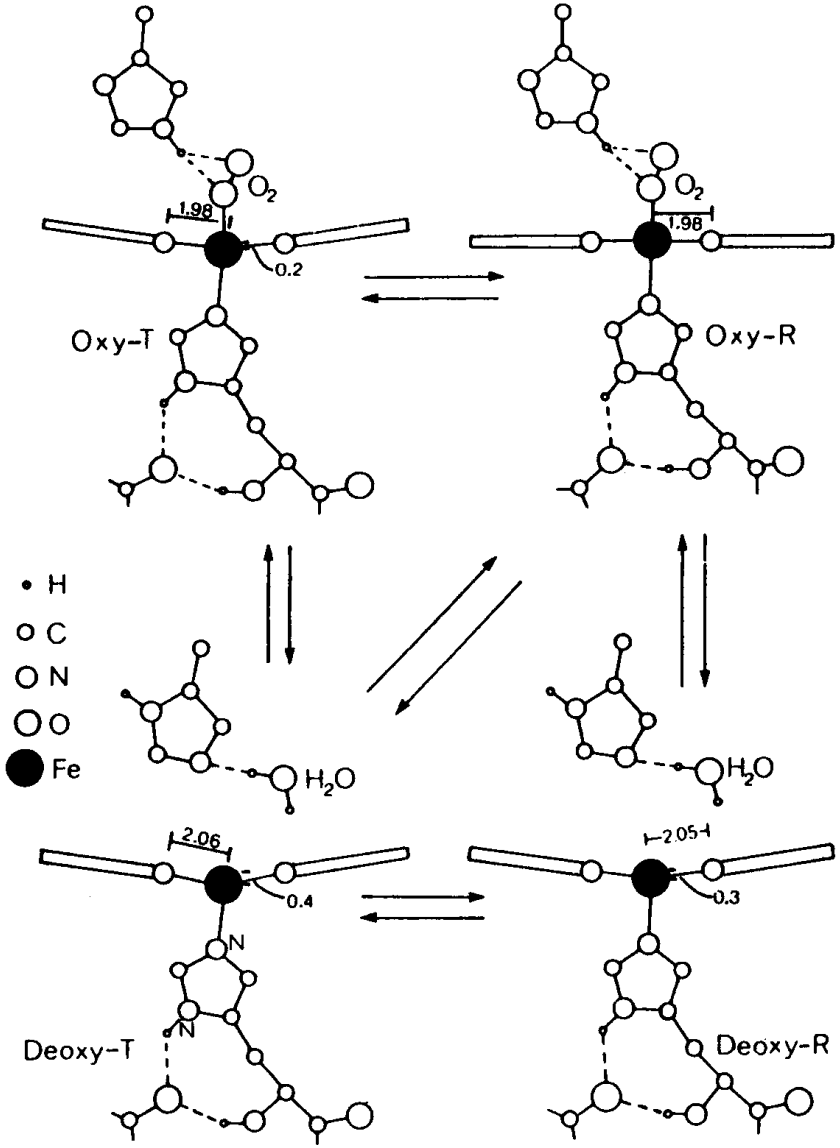
S = 1/2

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



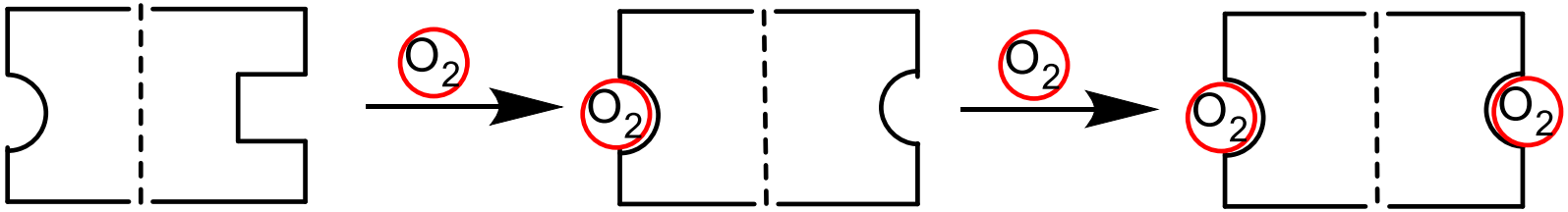


Fe(II) h.s.: 0.92 Å
Fe(II) l.s.: 0.75 Å
Fe(III) l.s.: 0.55 Å

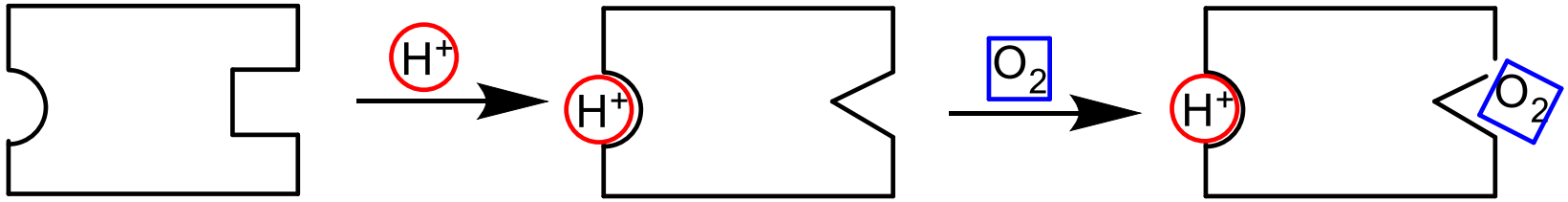


l'affinità della forma R per O_2 è circa la stessa delle catene α e β isolate, cioè della Mb, mentre quella della forma T è circa 12 – 14 kJ/mole inferiore

allosterismo = il cambio di attività e conformazione di un enzima in seguito al legame di un composto (*effettore*) ad un sito dell'enzima diverso da quello attivo

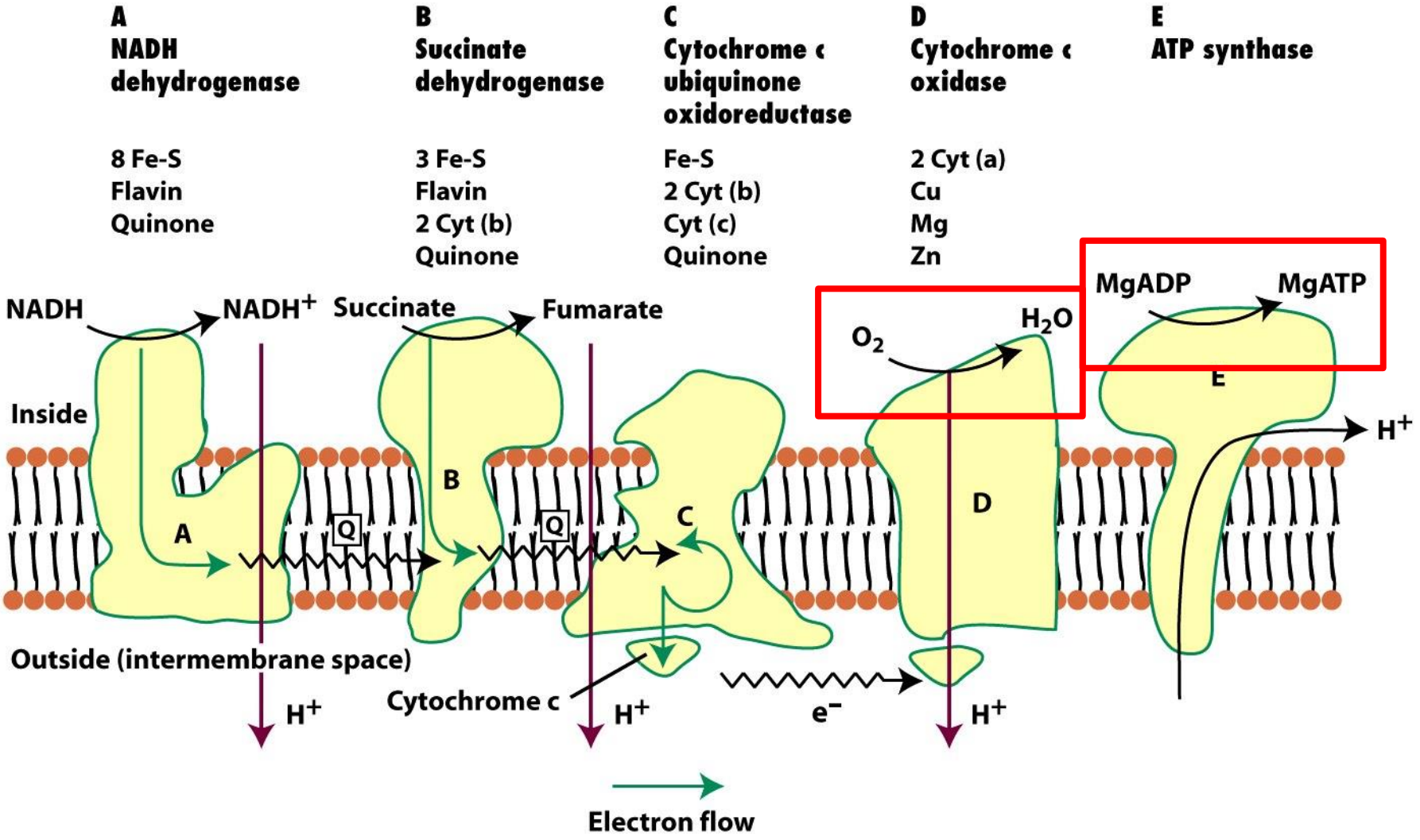


Allosterismo Omotropico



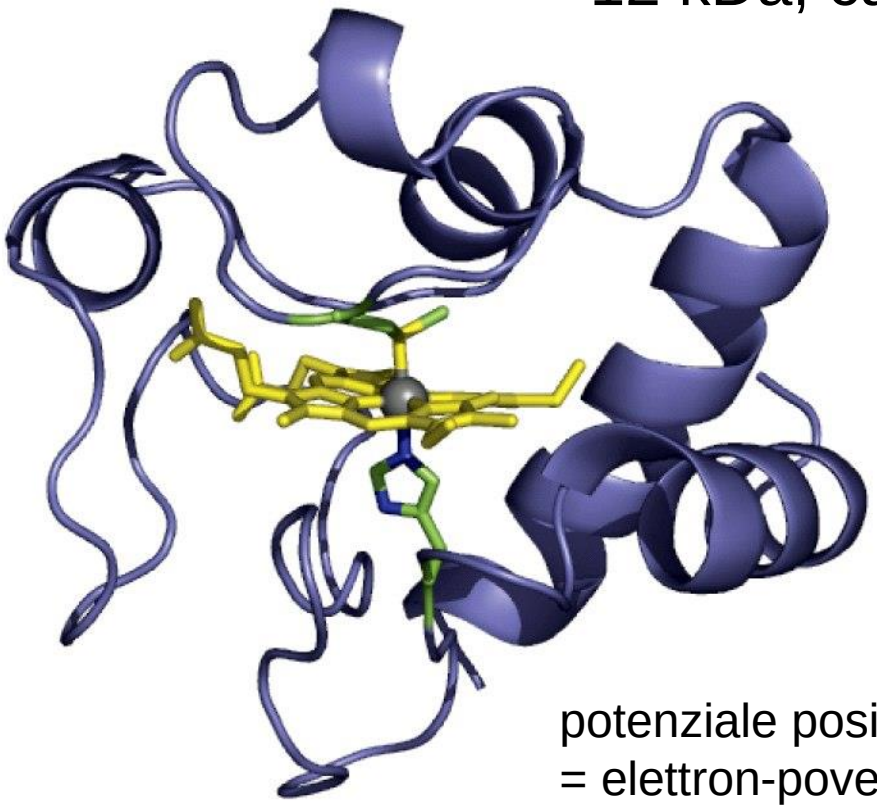
Allosterismo Eterotropico

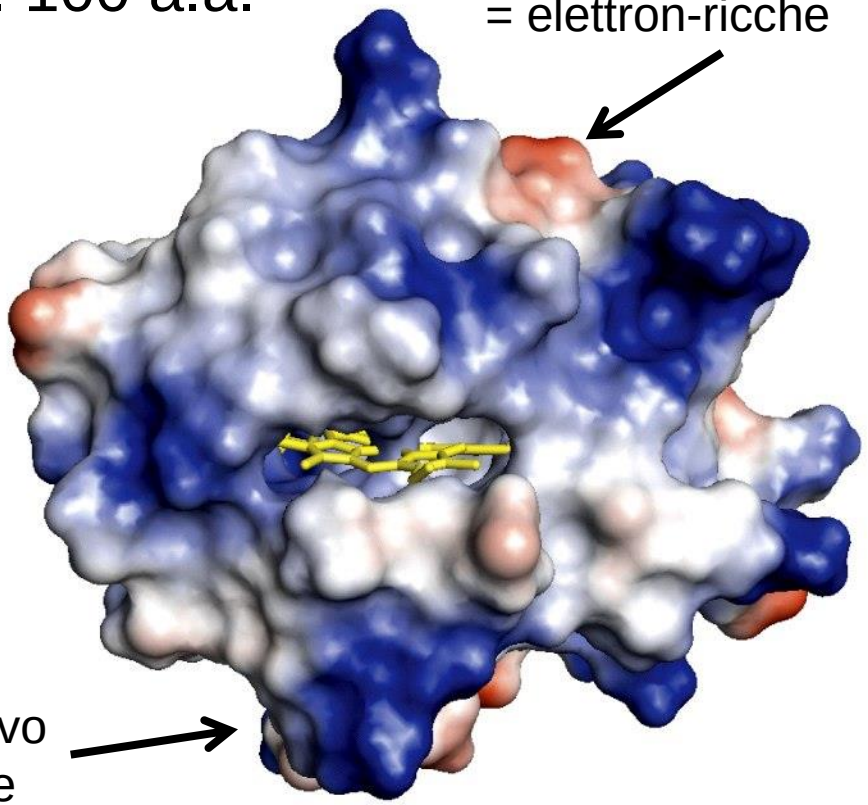
Catena della respirazione cellulare



Citocromo c (da cuore di cavallo)

12 kDa, ca. 100 a.a.

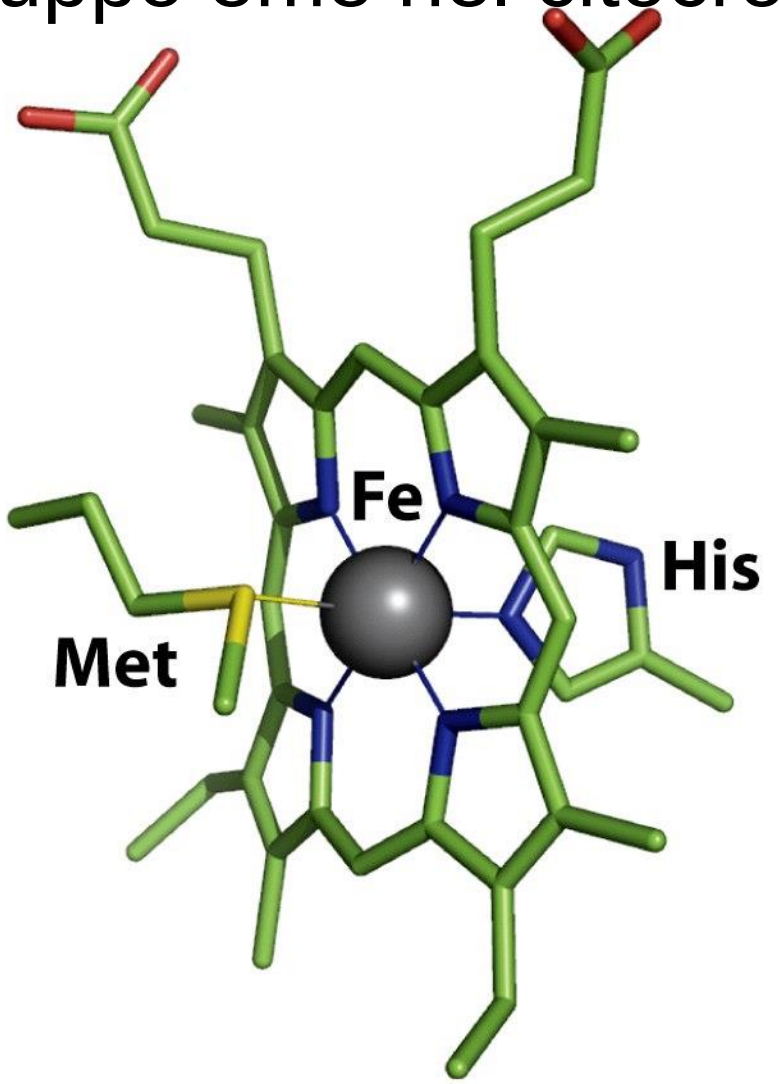




mappa del potenziale elettrostatico

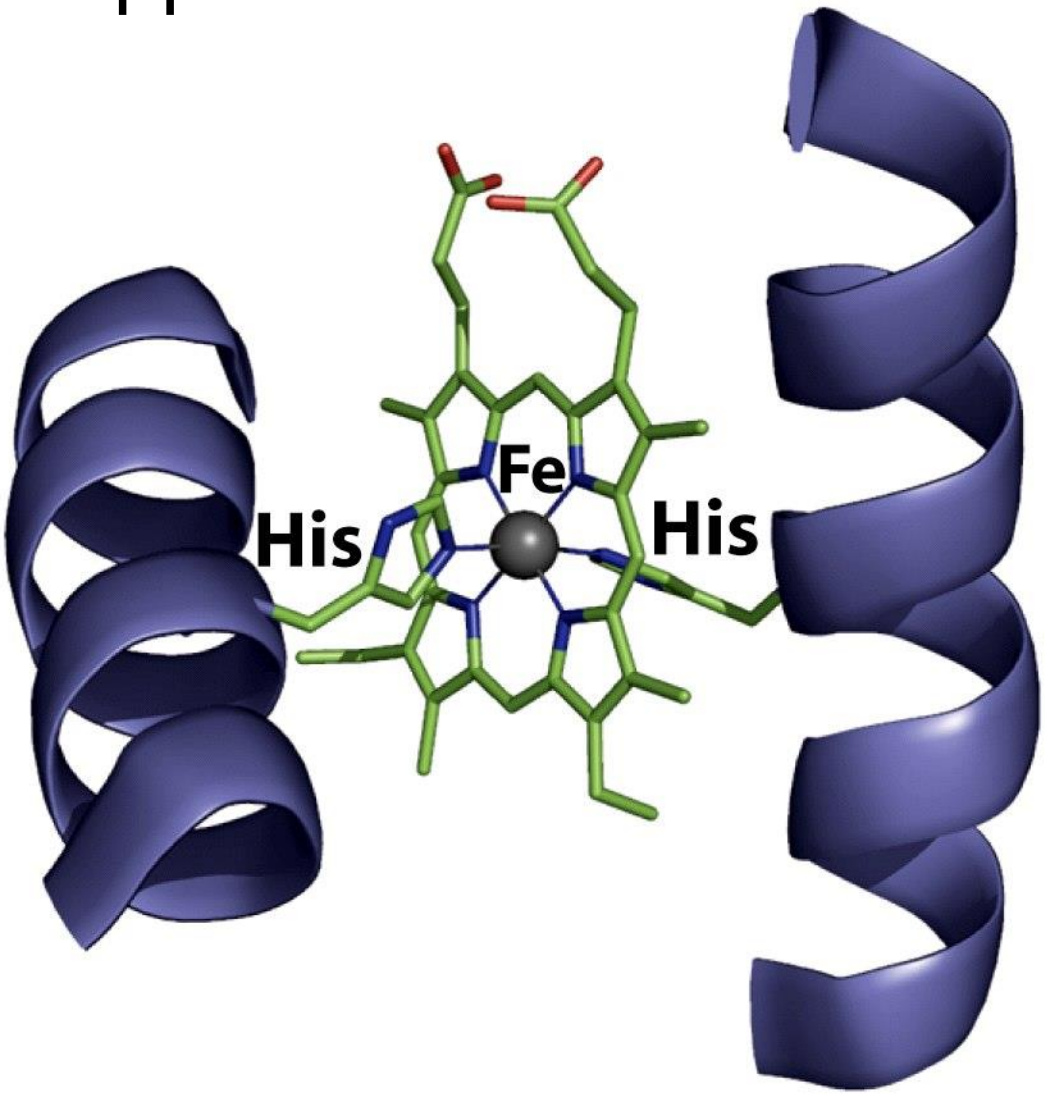
$E_{Fe(III)/Fe(II)}$ = +260 mV

Gruppo eme nel citocromo c



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

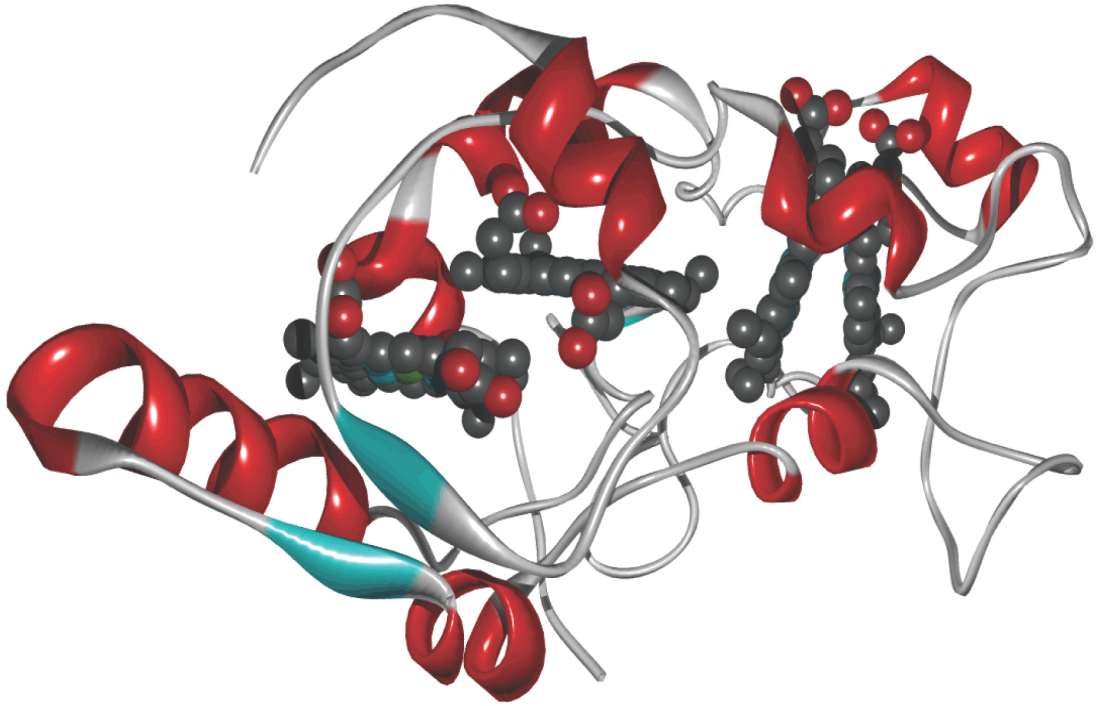
Gruppo eme nel citocromo b



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

Citocromo c554

(nitrificazione catalizzata da batteri)

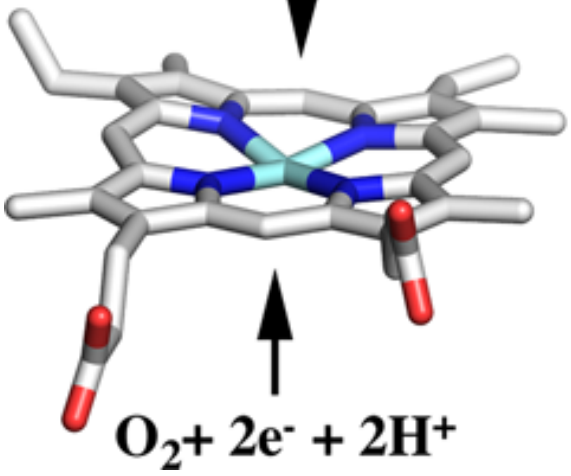


Distanze Fe···Fe ≈ 950, 1220, 920 pm

Ossidazioni catalizzate da eme-proteine

perossidasi

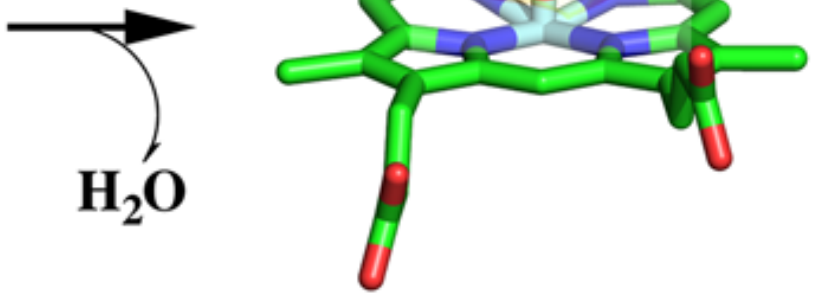
H_2O_2



$O_2 + 2e^- + 2H^+$

ossigenasi

H_2O_2 = intermedio indesiderato nell'ossidazione fotosintetica di H_2O o per riduzione incompleta di O_2 durante la respirazione



H_2O

ossidare non sempre implica anche ossigenare i substrati

Perossidasi e Catalasi

e.g. *lignina perossidasi, horse-radish perossidasi (HRP)*, *Citocromo c perossidasi, ascorbato perossidasi…*

sono anche enzimi detossificanti

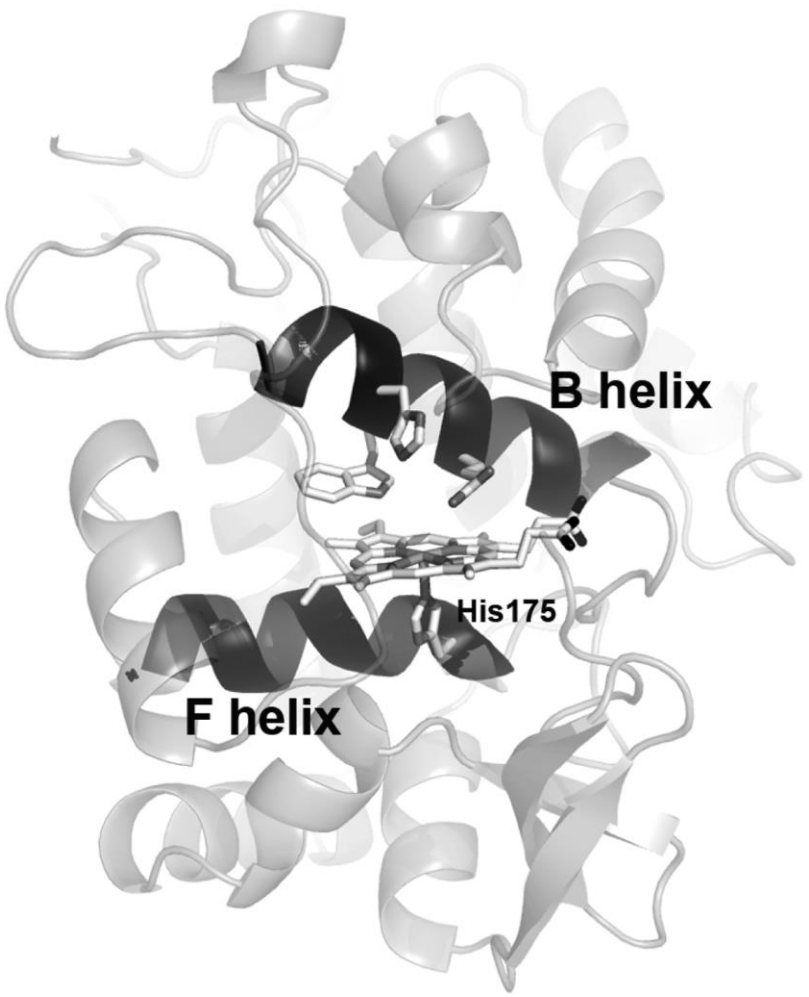
$$H_2O_2(aq) + AH_2 \rightarrow 2\ H_2O(l) + A$$

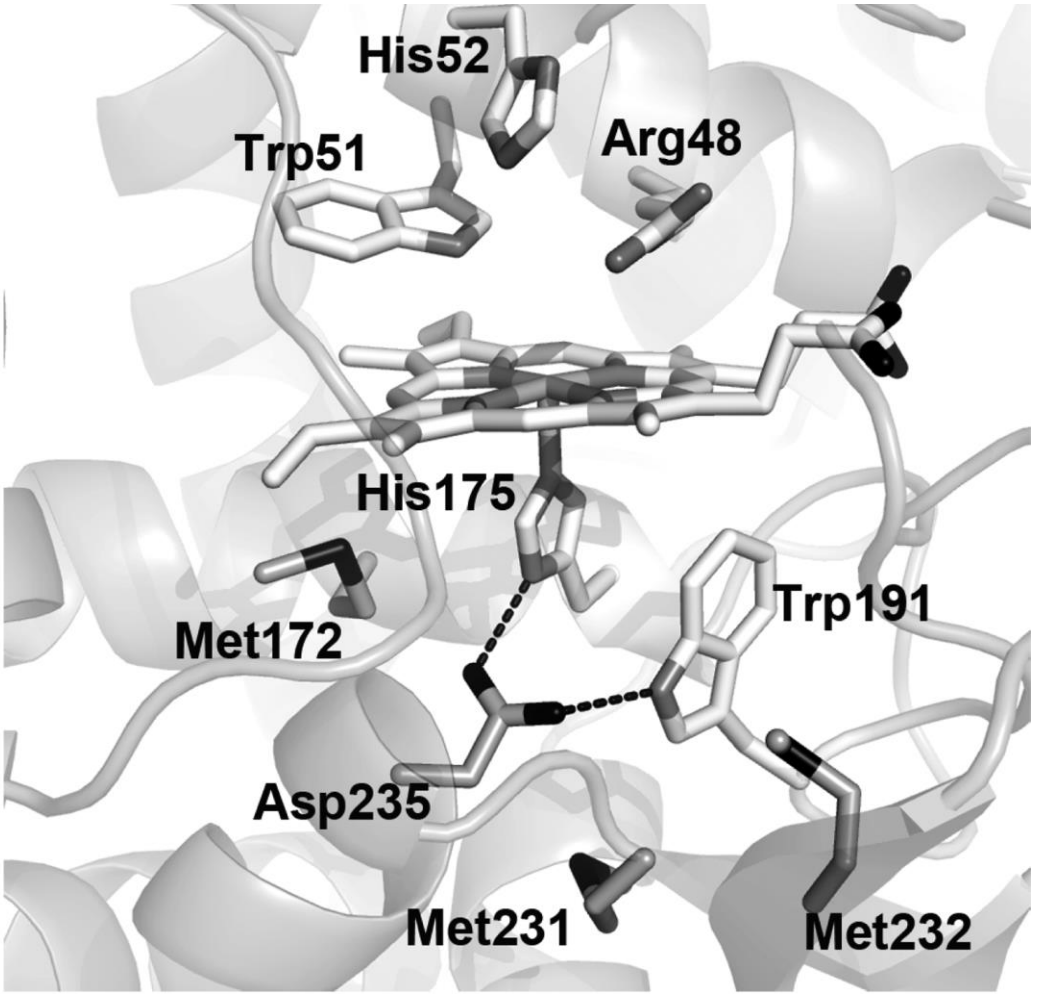
$$H_2O_2(aq) + H_2O_2(aq) \rightarrow 2\ H_2O(l) + O_2$$

Substrati: acidi grassi, ammine, fenoli, tossine xenobiotiche,….

Citocromo c Perossidasi

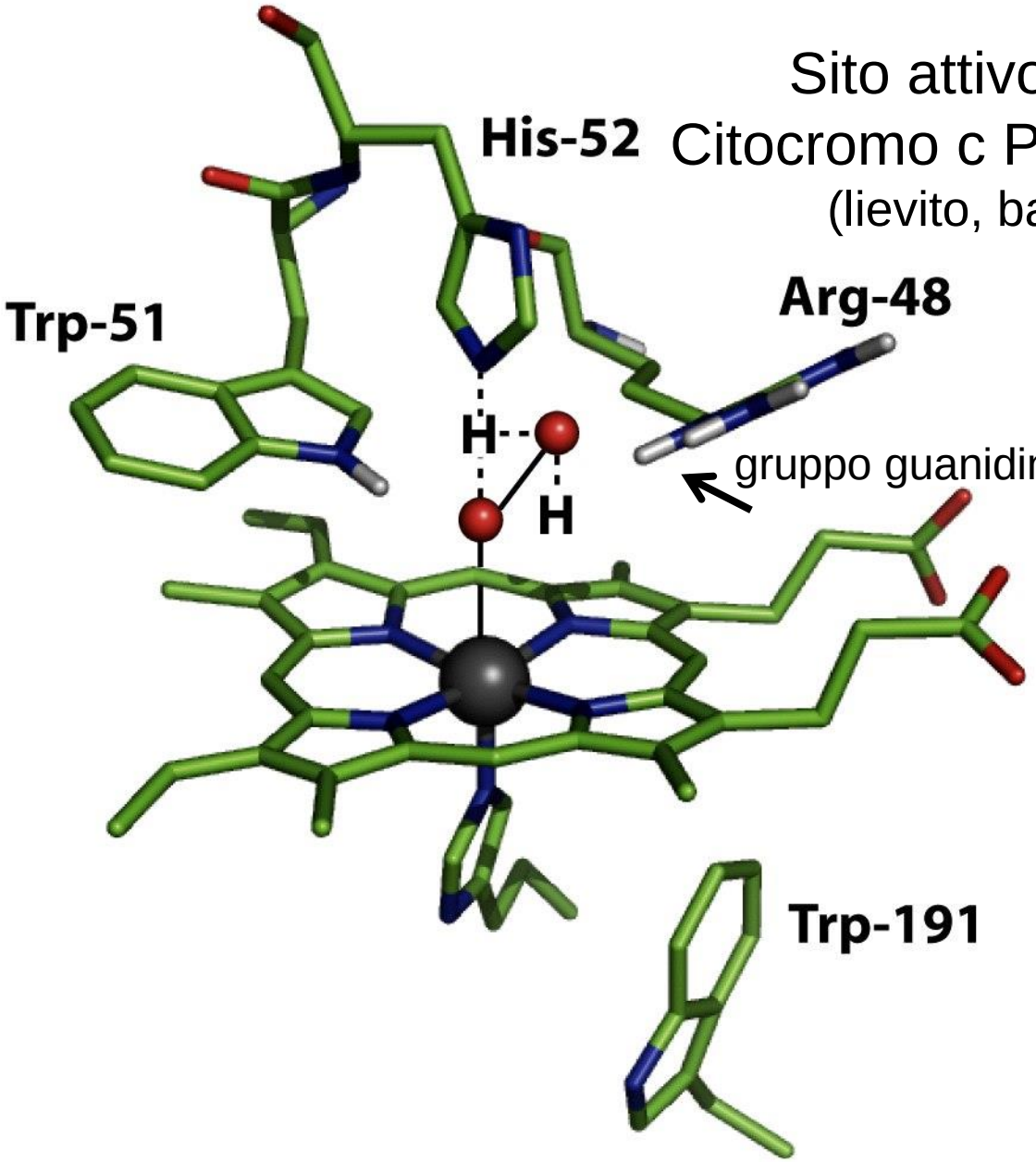
(lievito, batteri)





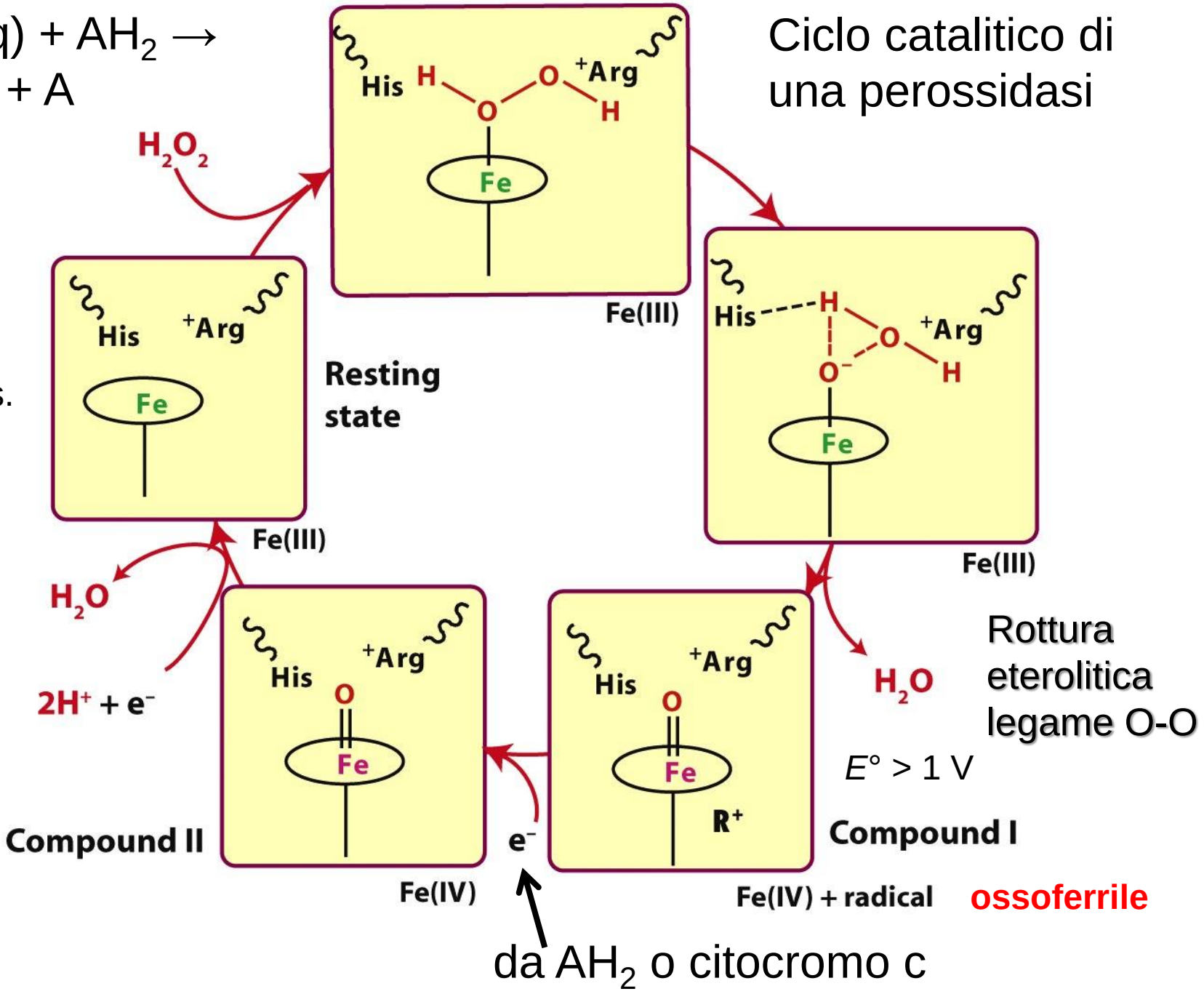
Sito attivo della Citocromo c Perossidasi

(lievito, batteri)

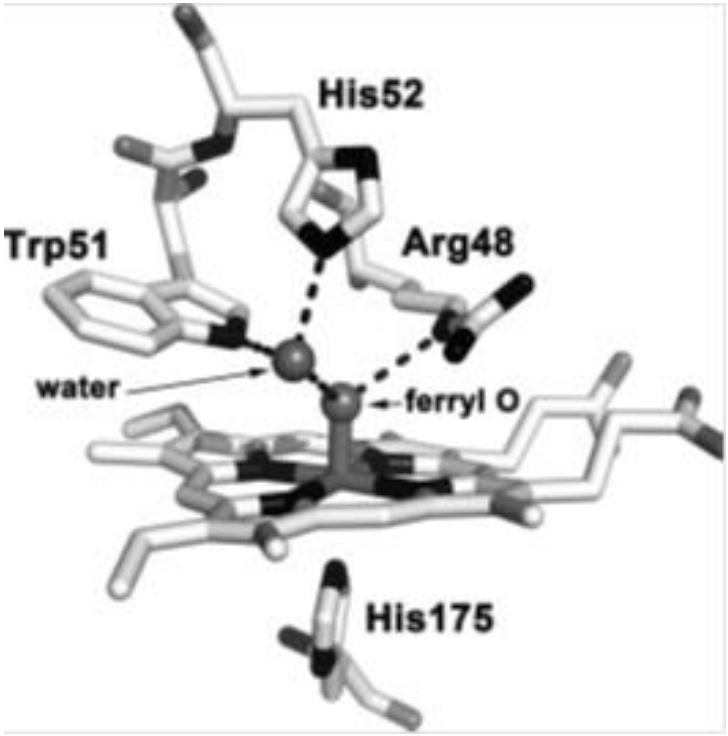


$H_2O_2(aq) + AH_2 \rightarrow 2H_2O(l) + A$

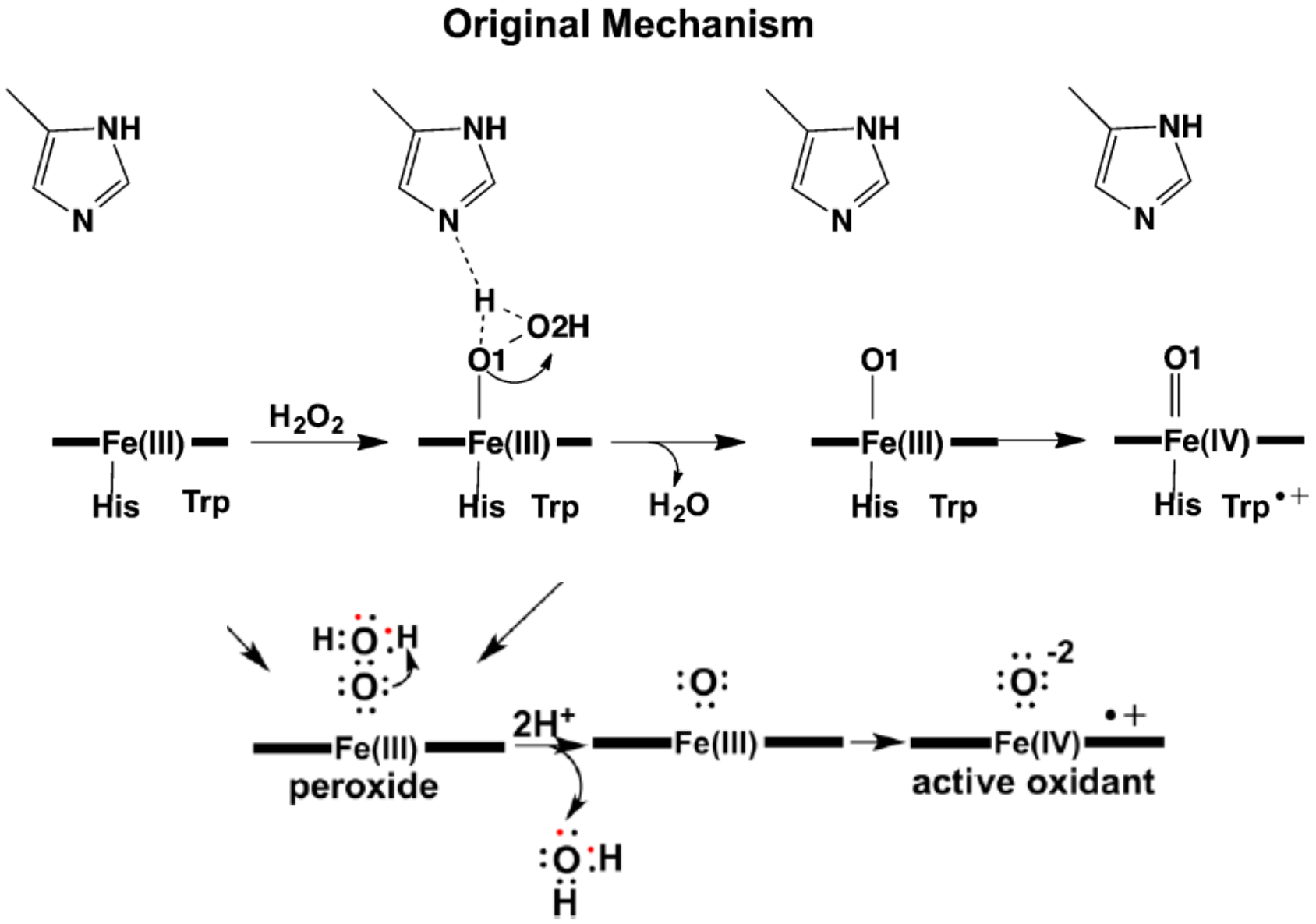
Ciclo catalitico di una perossidasi



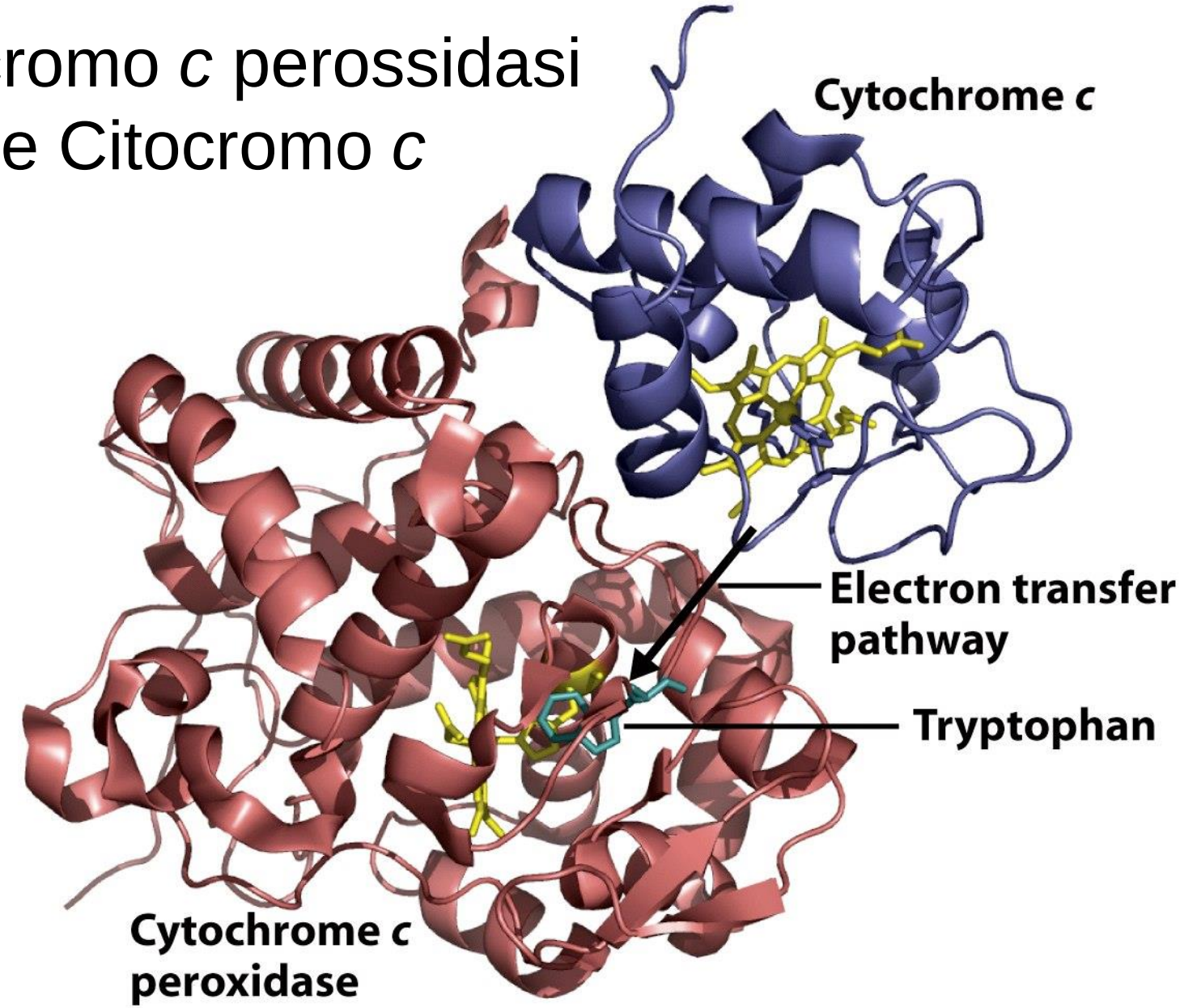
Struttura ai raggi X dell'osso-ferrile



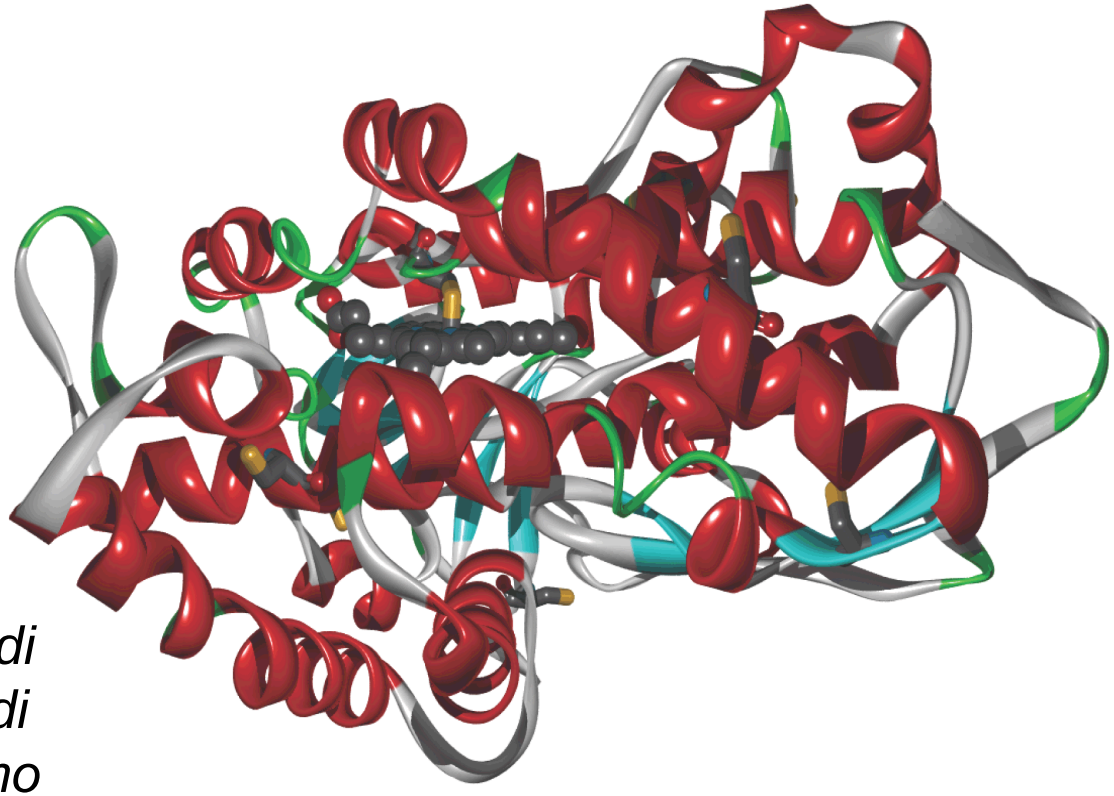
Formazione dell'osso-ferrile



Citocromo *c* perossidasi e Citocromo *c*



Monoossigenasi



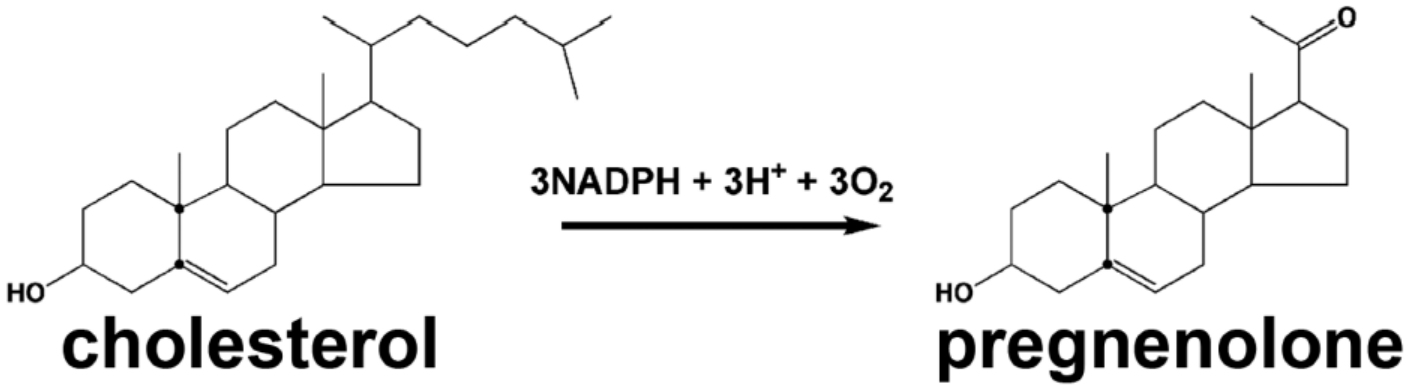
individuati più di 18.000 P450, di cui 57 nell'uomo

Citocromo P-450

(da *Pseudomonas putida*) 50 kDa

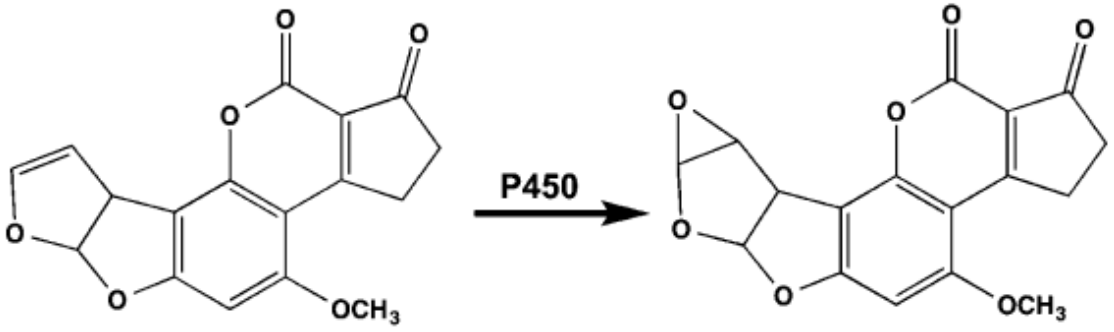
$$R\text{-}H + O_2 + 2e^- + 2H^+ \xrightarrow{\text{P-450}} R\text{-}OH + H_2O$$

Ossigenazione di substrati specifici da parte di P450

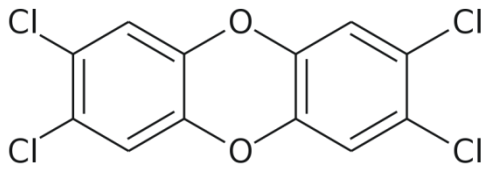


cholesterol **pregnenolone**

Attivazione (involontaria) di substrati da parte di P450



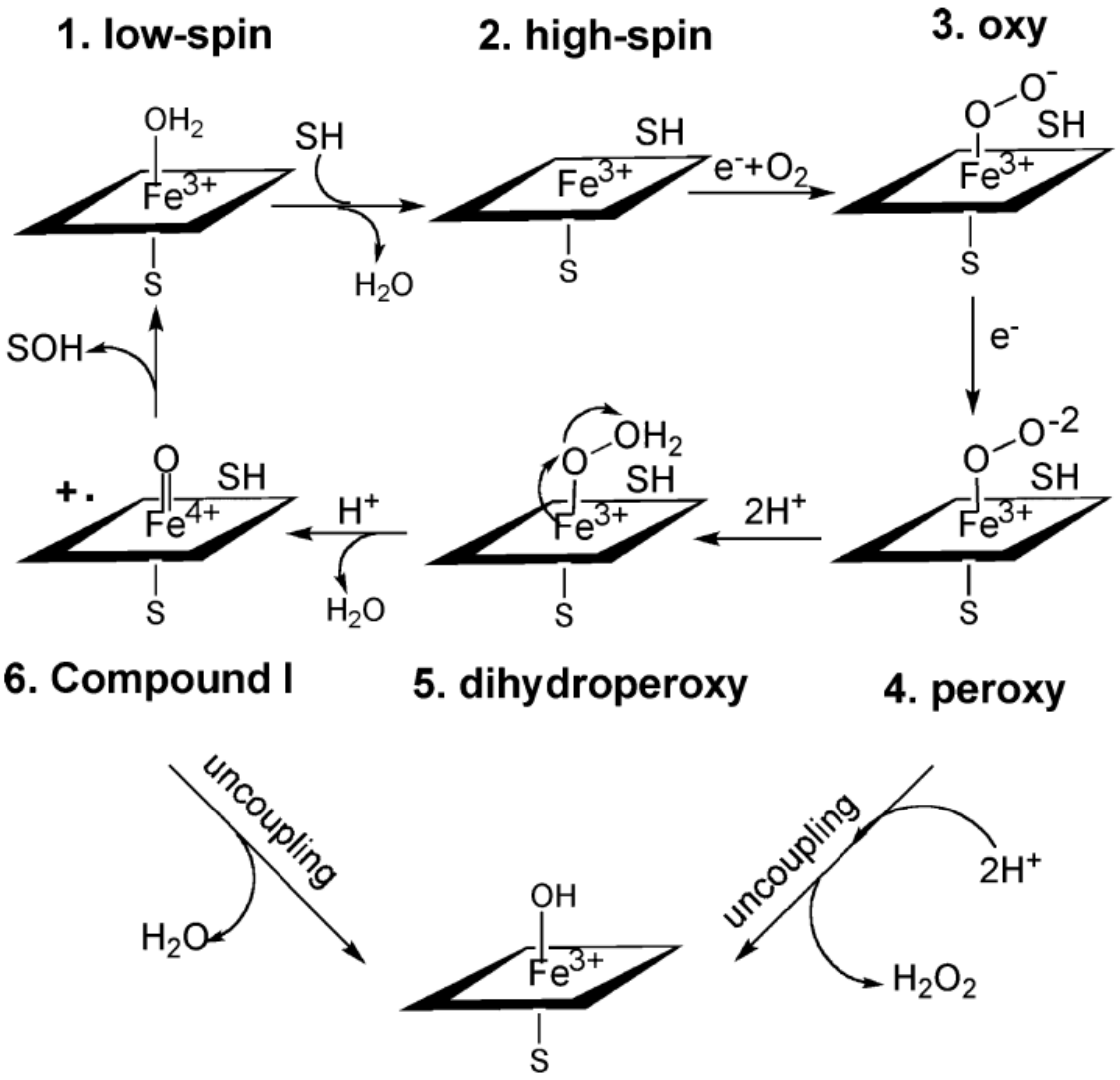
aflatoxin B_1 **aflatoxin B_1-9,9-*exo*-epoxide**



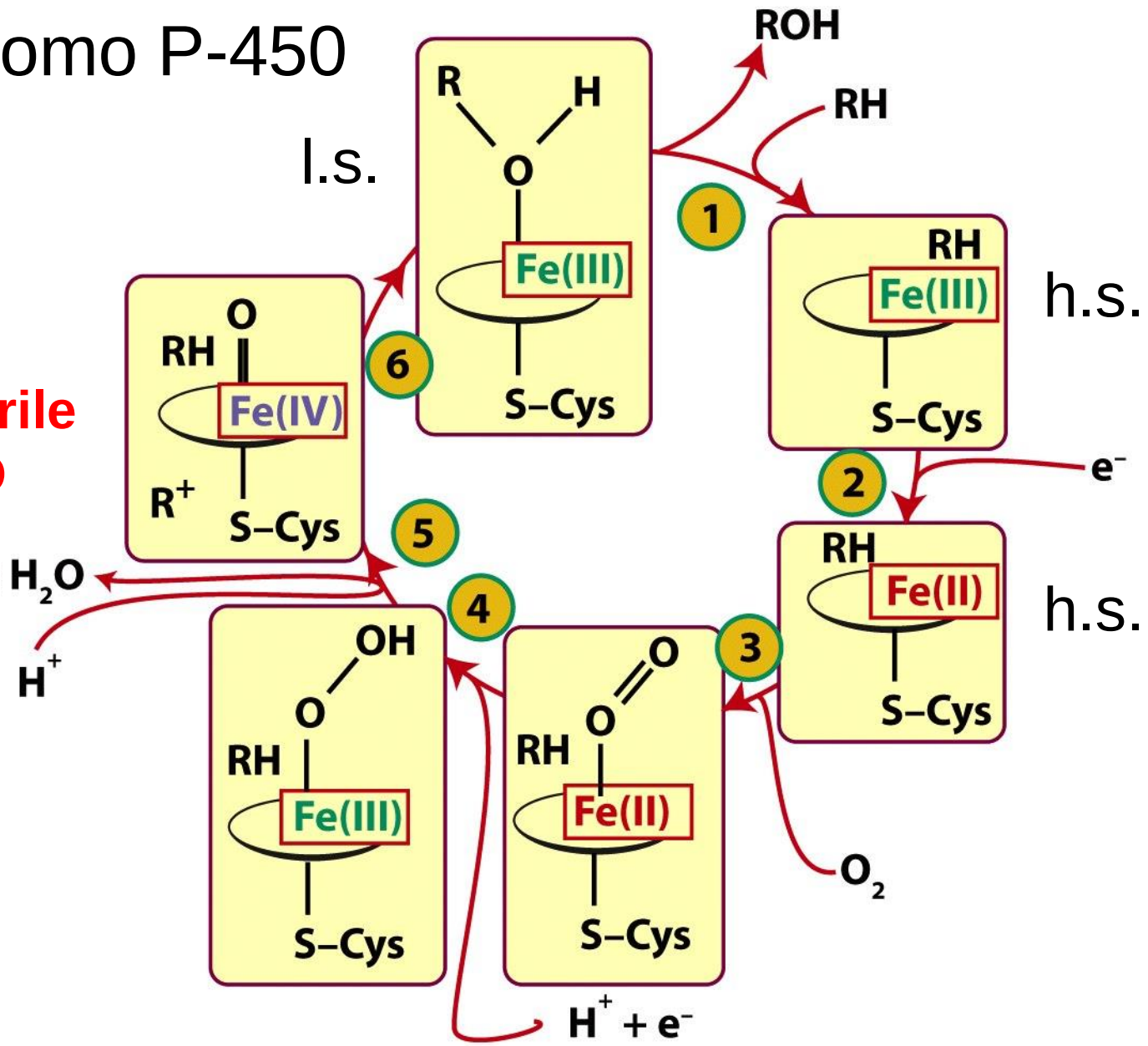
diossina

Citocromo P-450

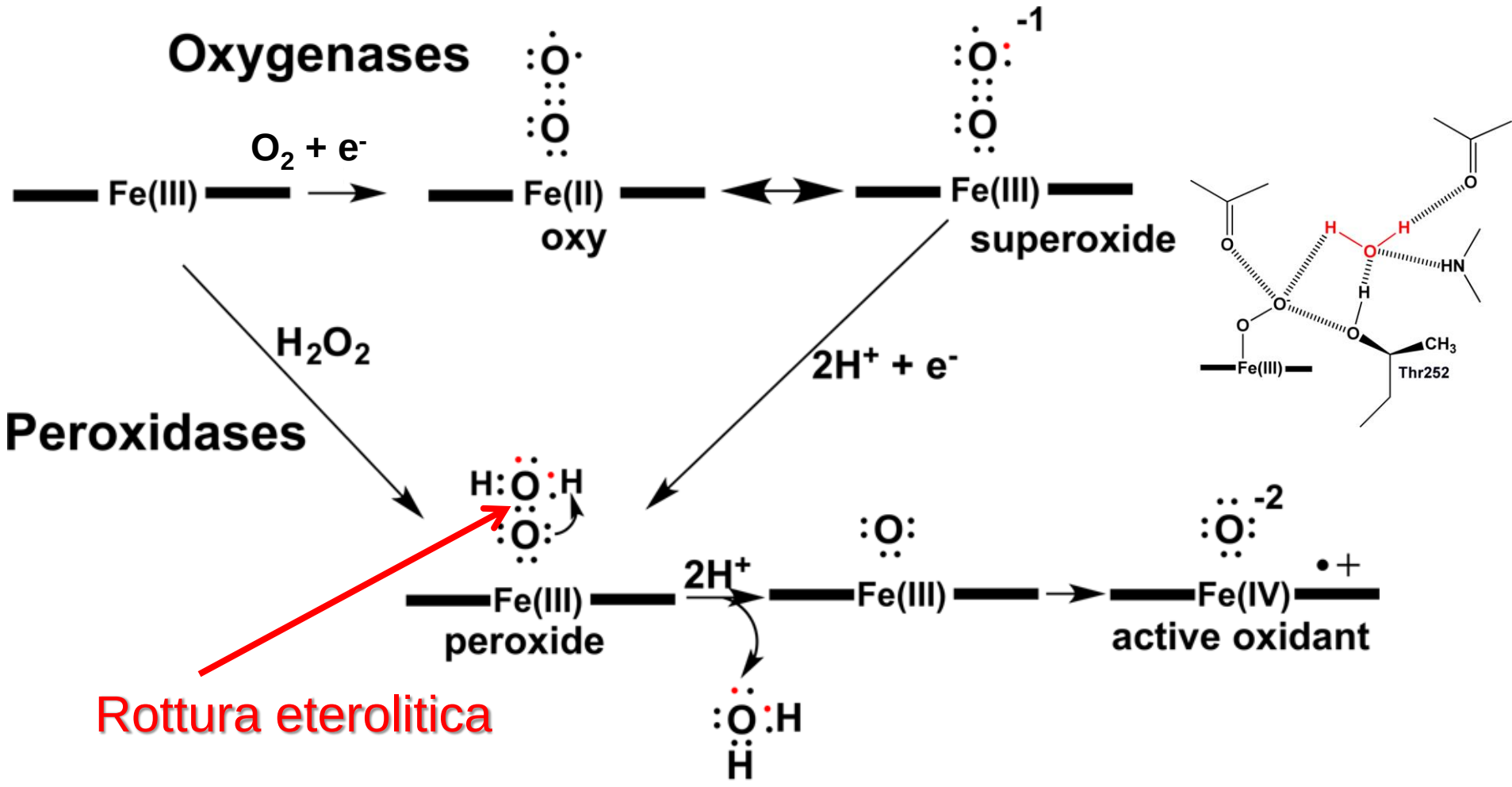
osso-ferrile
Fe(IV)=O



Citocromo P-450

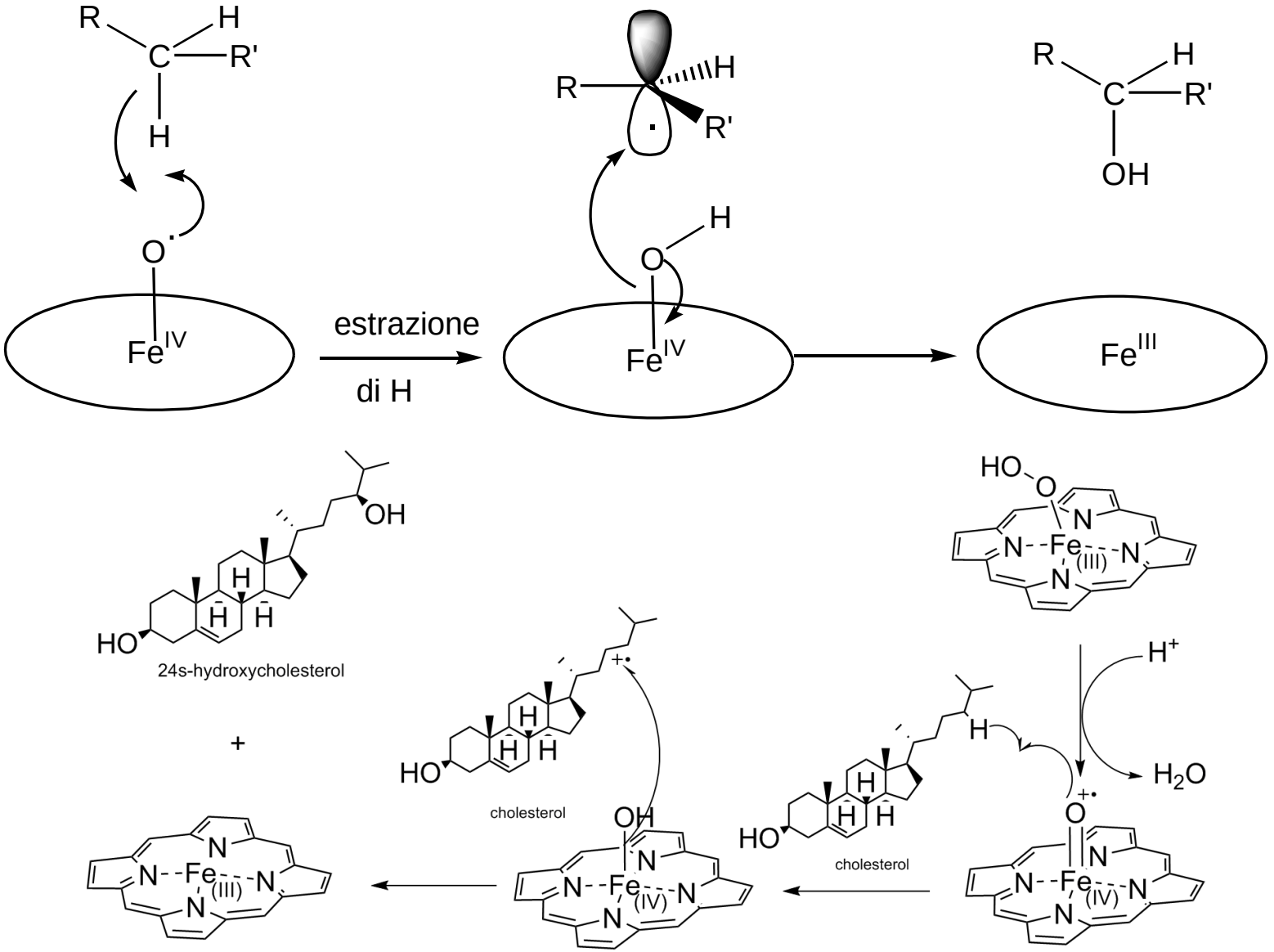


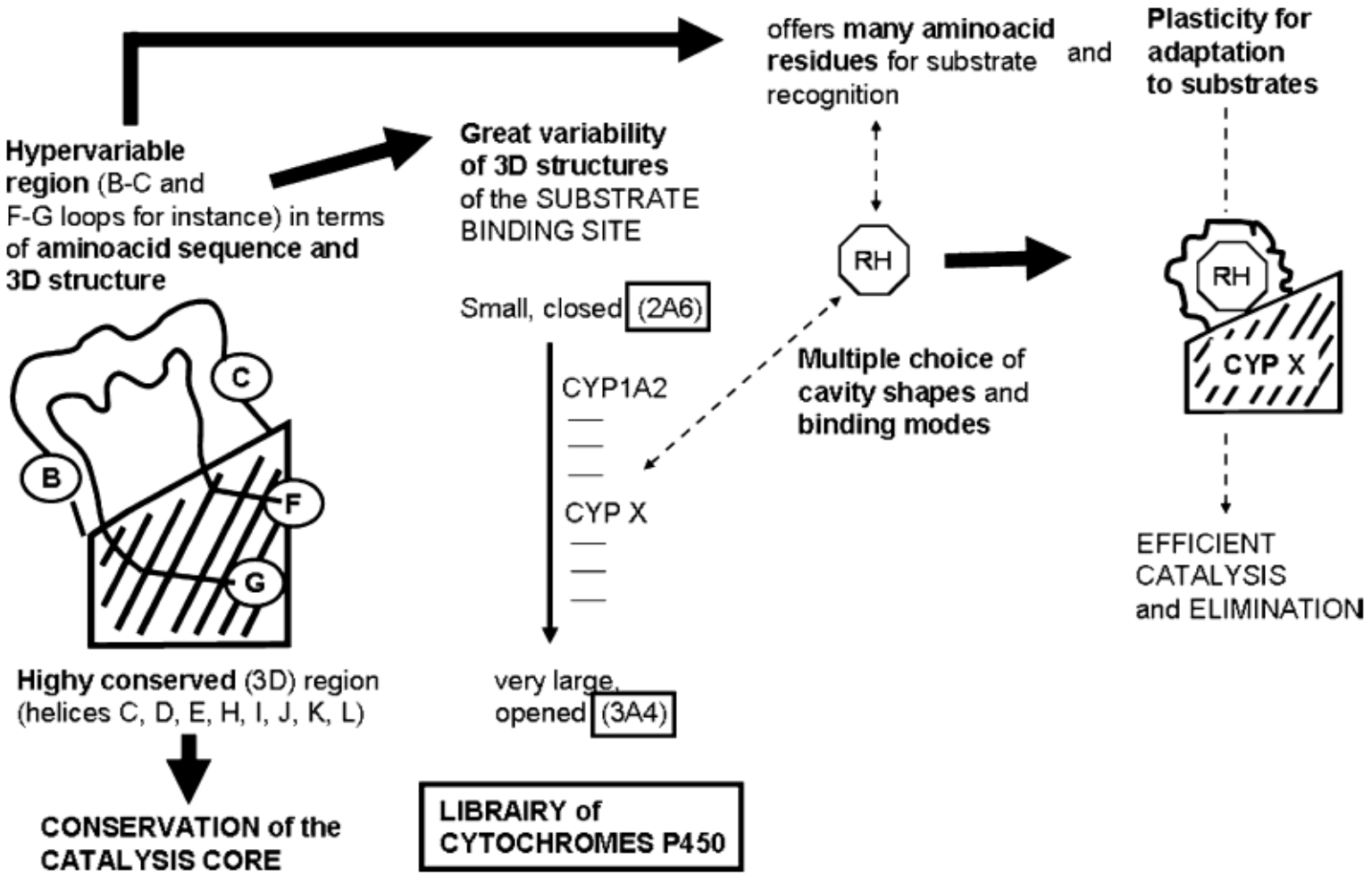
Rottura eterolitica del legame O–O e protonazione dell'ossigeno distale



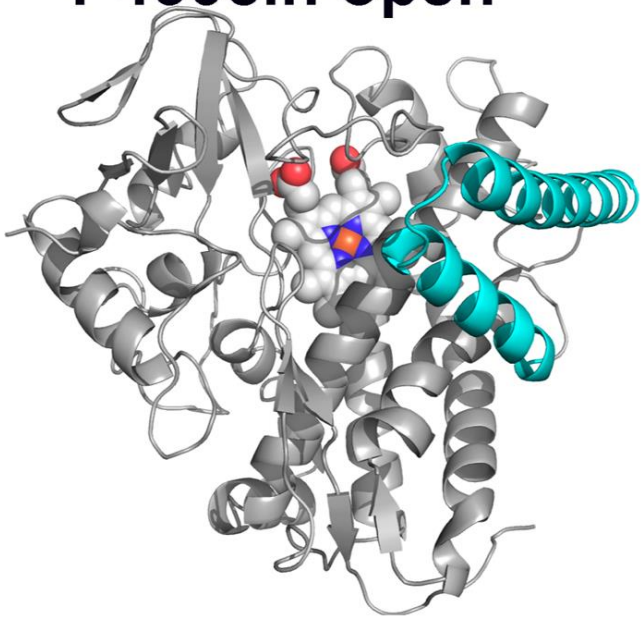
Meccanismo di ossidazione del substrato

Oxygen Rebound Mechanism

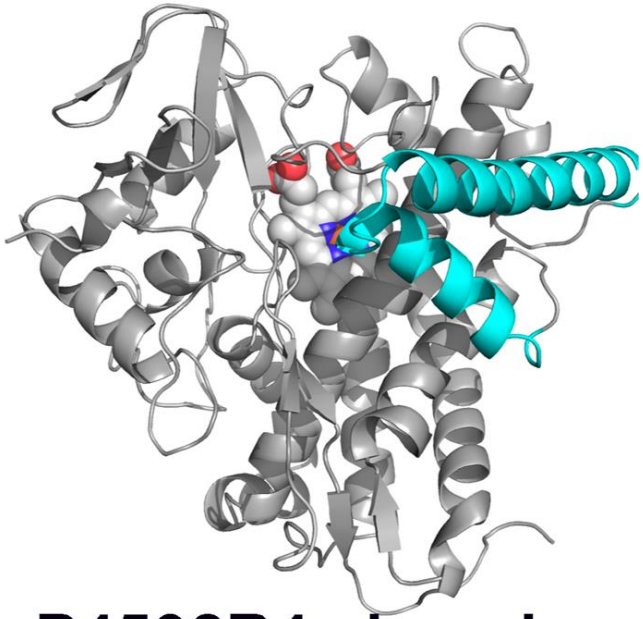



Hypervariable region (B-C and F-G loops for instance) in terms of aminoacid sequence and 3D structure
Great variability of 3D structures of the SUBSTRATE BINDING SITE
offers many aminoacid residues for substrate recognition
and
Plasticity for adaptation to substrates
RH
RH
CYP X
Multiple choice of cavity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

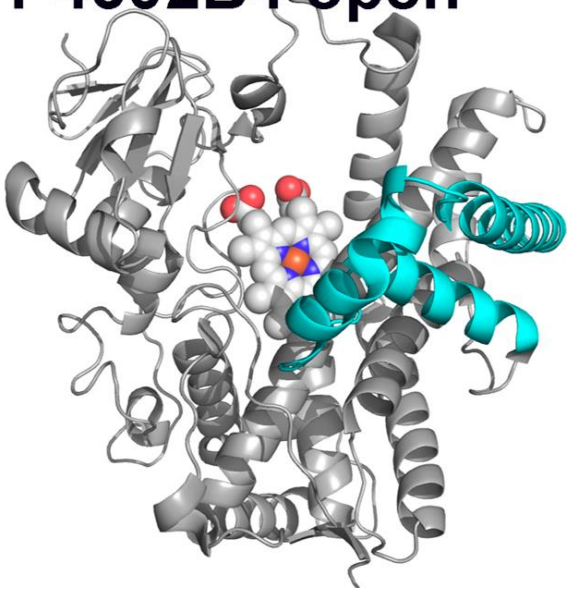
P450cin open



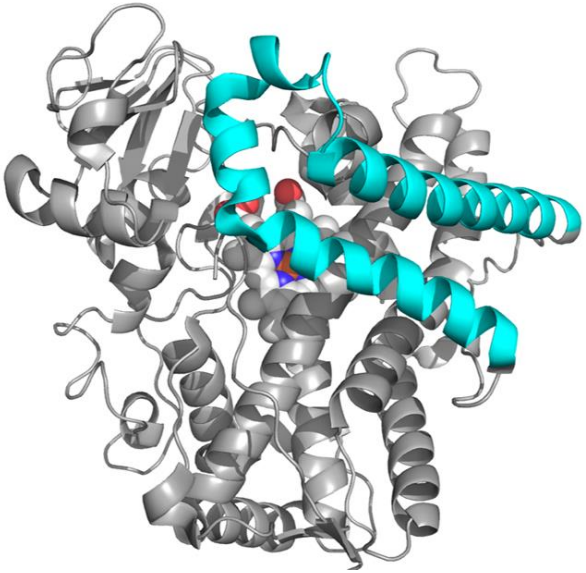
P450cin closed

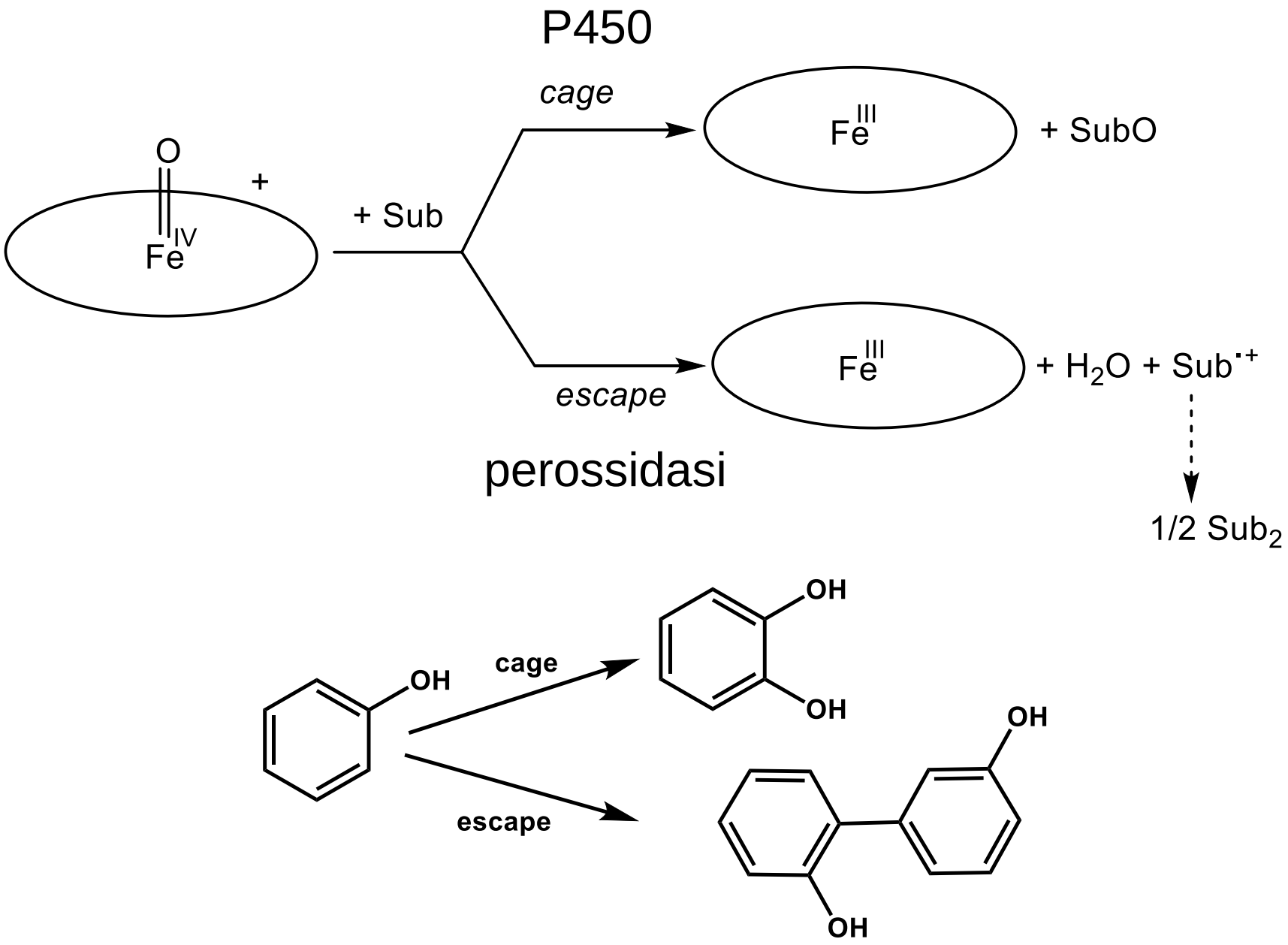


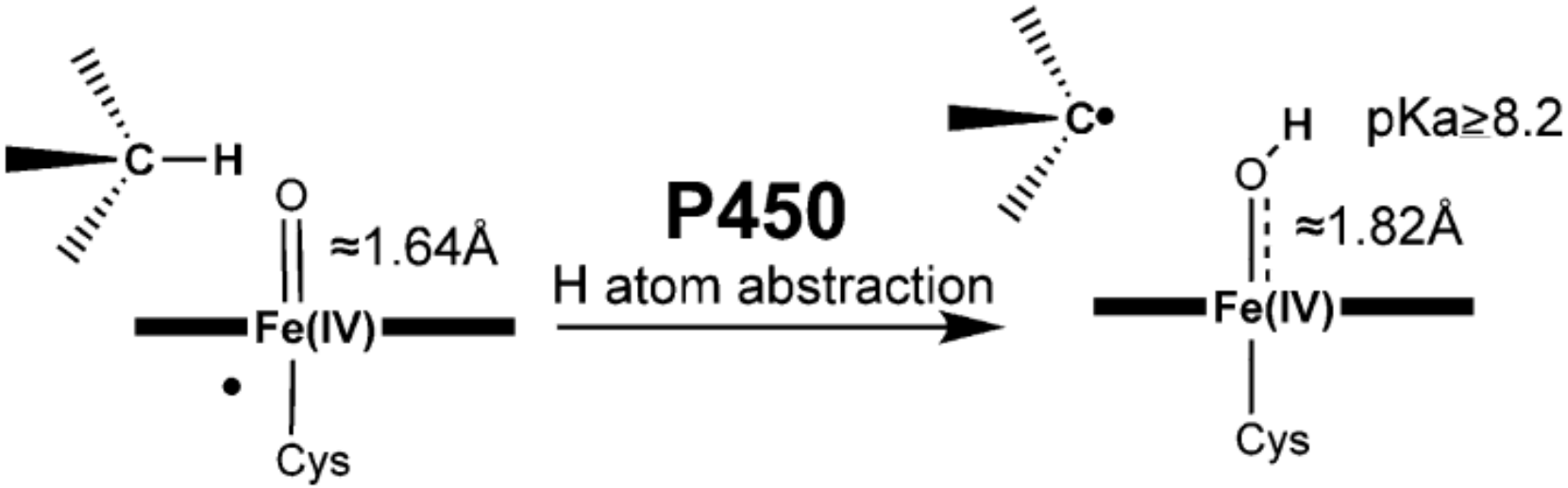
P4502B4 open



P4502B4 closed



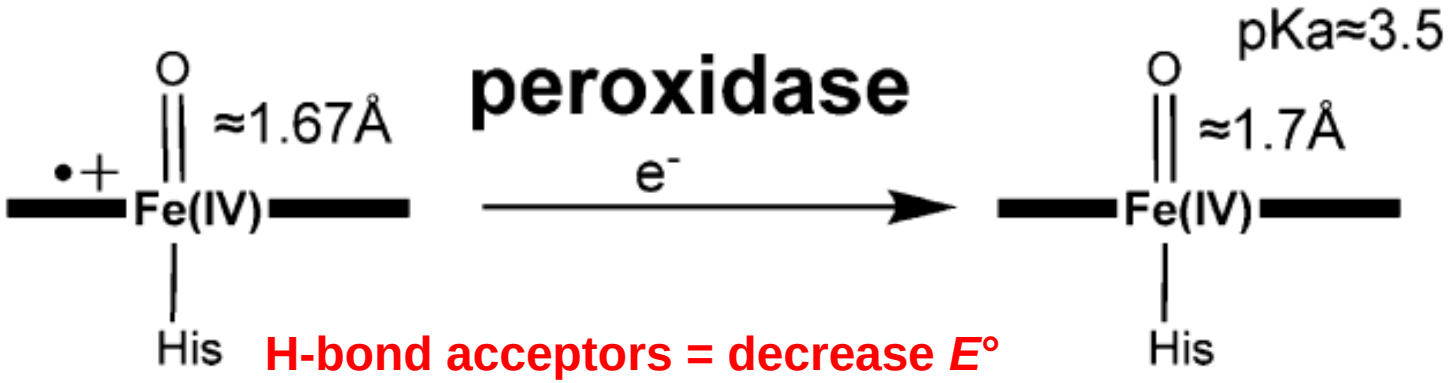

P450
cage
O
+
Fe IV
+ Sub
Fe III
+ SubO
Fe III
+ H2O + Sub·+
escape
perossidasi
1/2 Sub2
OH
cage
OH
OH
OH
escape
OH



H-bond donors = increase *E*°

Compound I

Compound II



H-bond acceptors = decrease *E*°