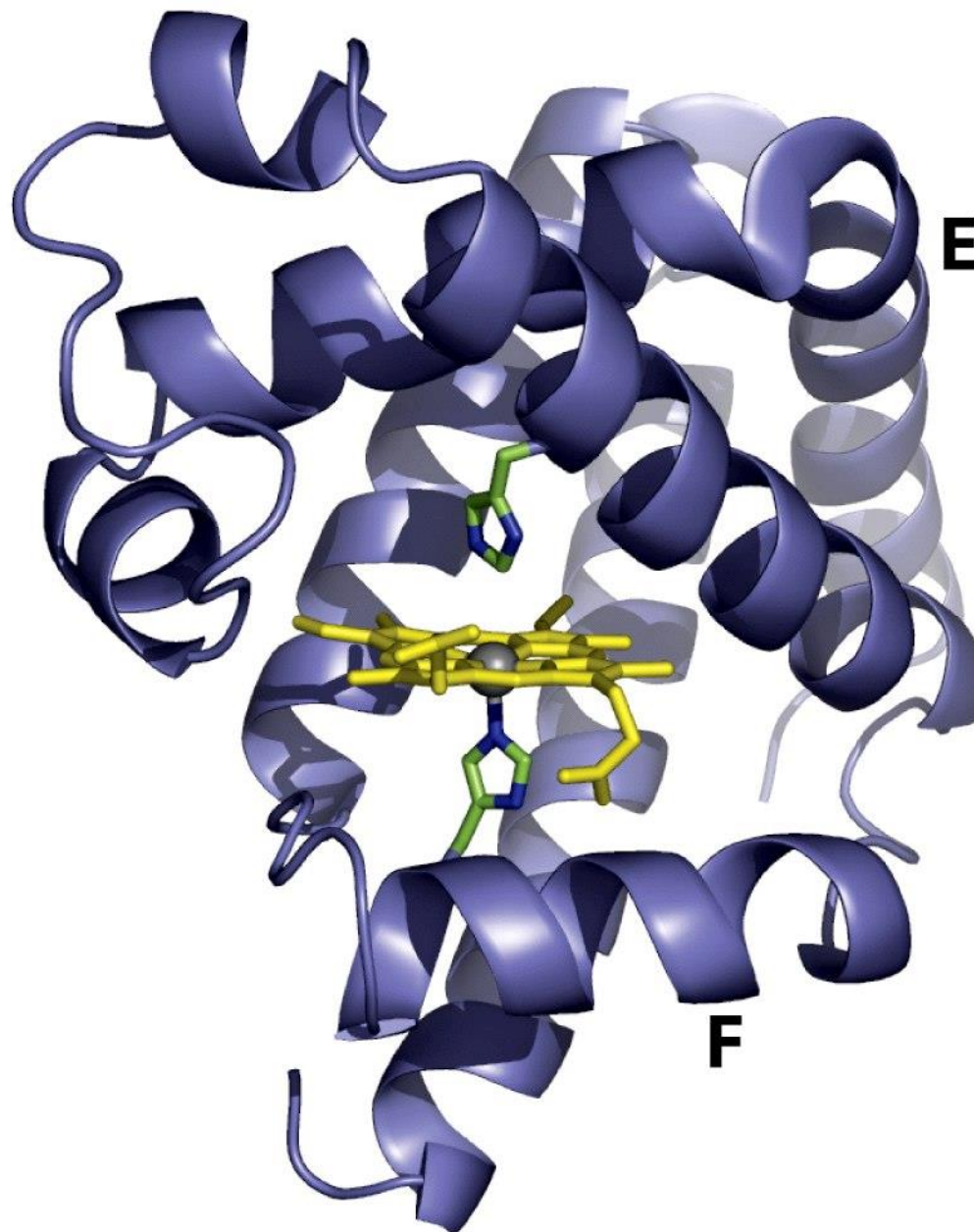


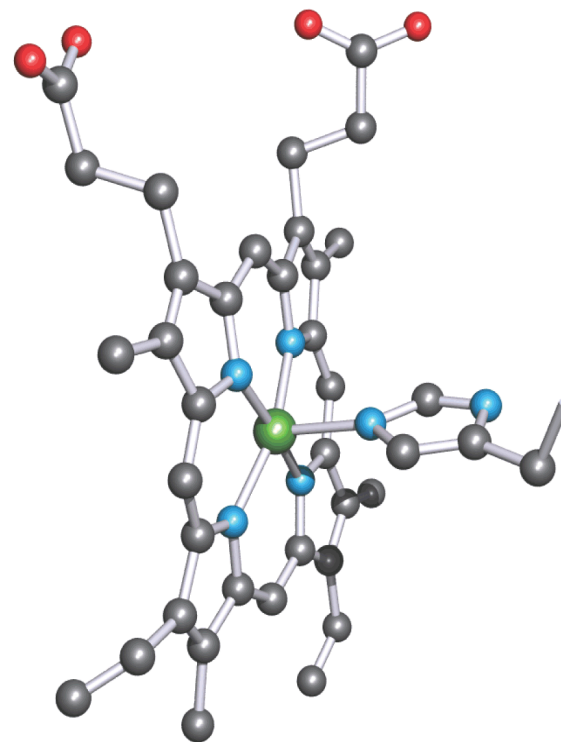
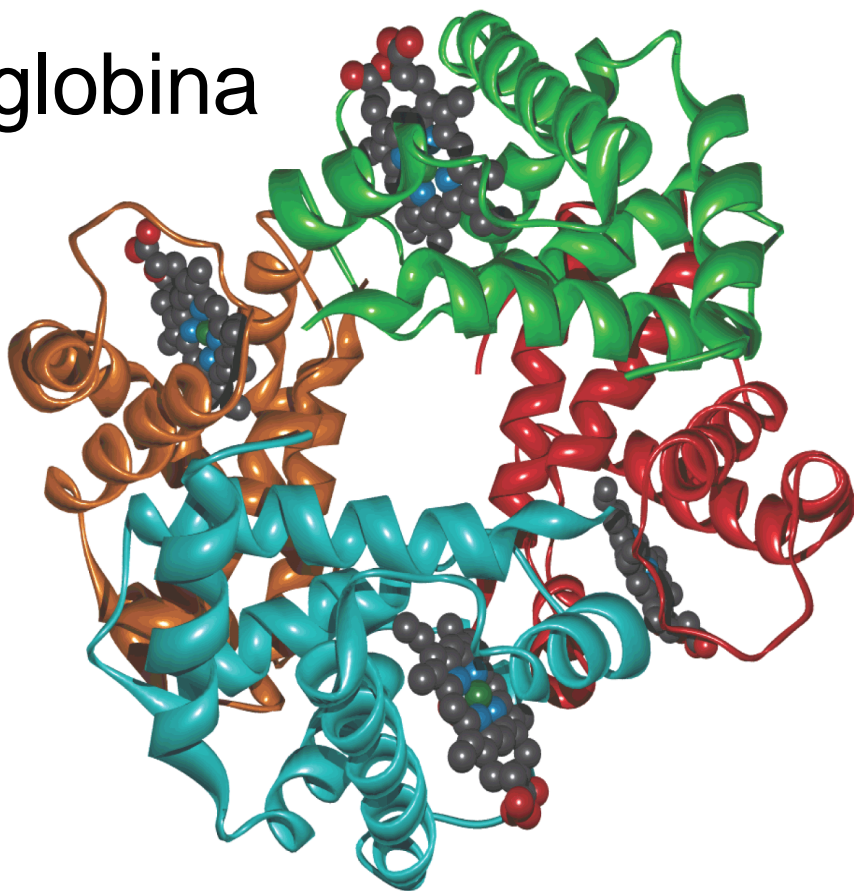
Mioglobina

17.8 kDa



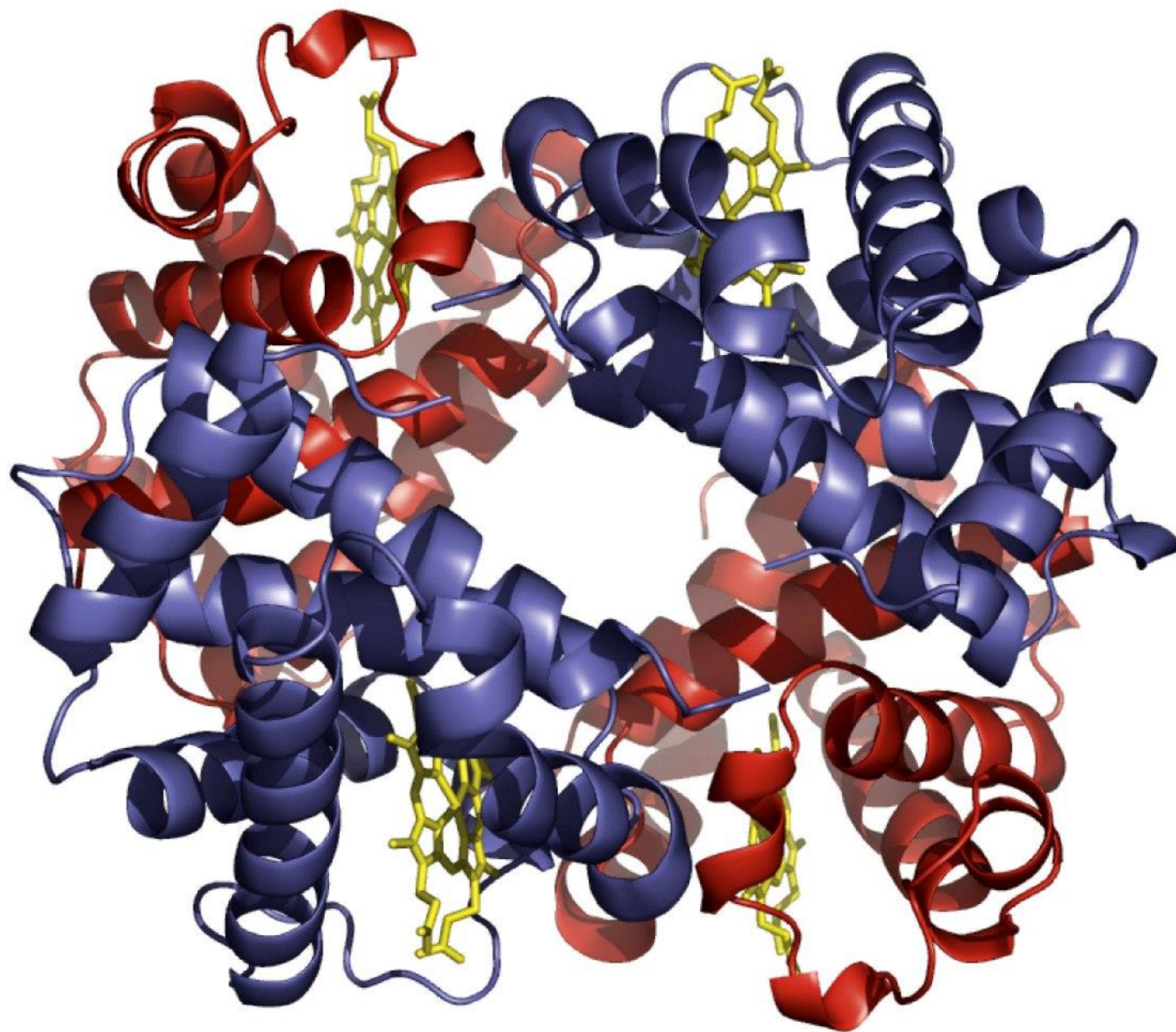
ca. 6% Fe_{tot}

Emoglobina



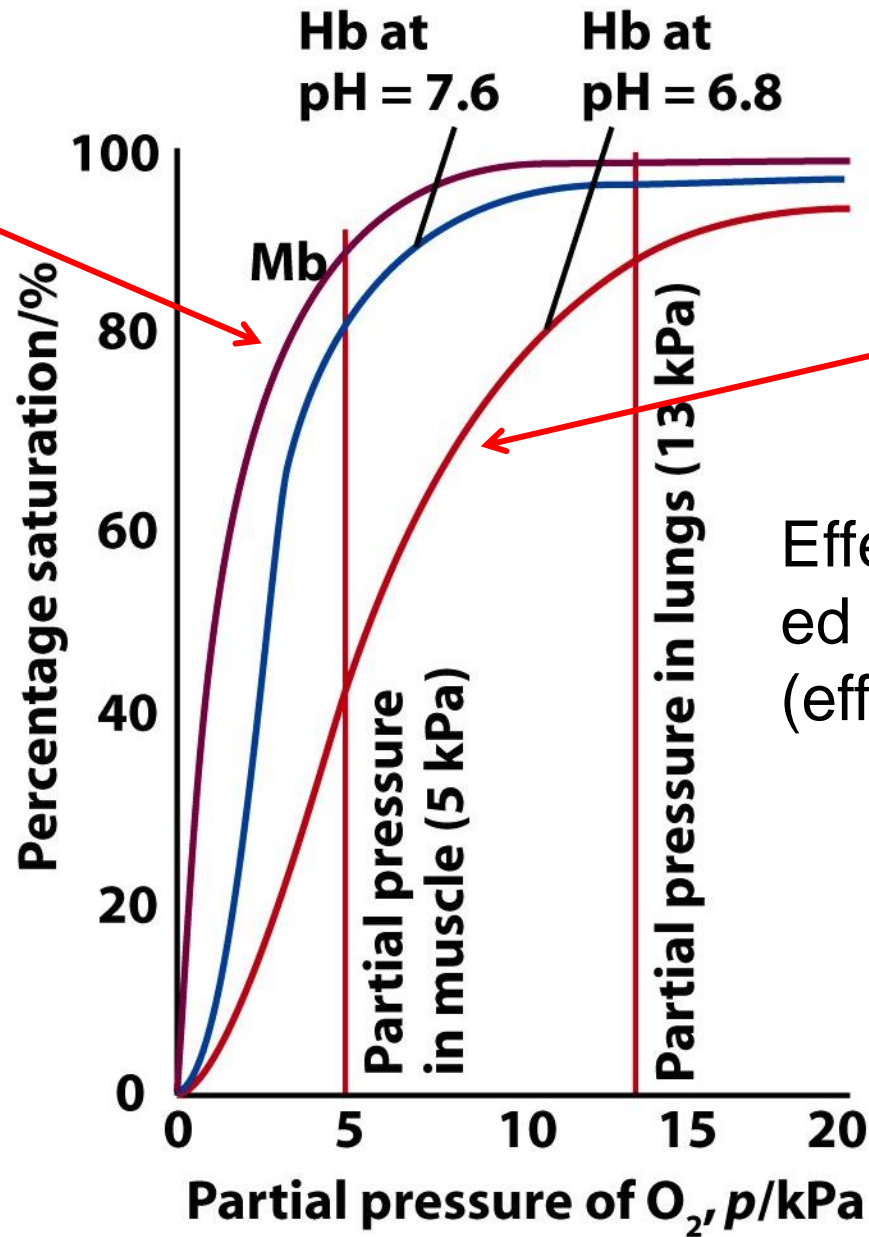
tetramero $\alpha_2\beta_2$ 141 e 146 a.a. 64.5 kDa

ca. 65% Fe_{tot}



tetramero $\alpha_2\beta_2$

Curva di saturazione iperbolica



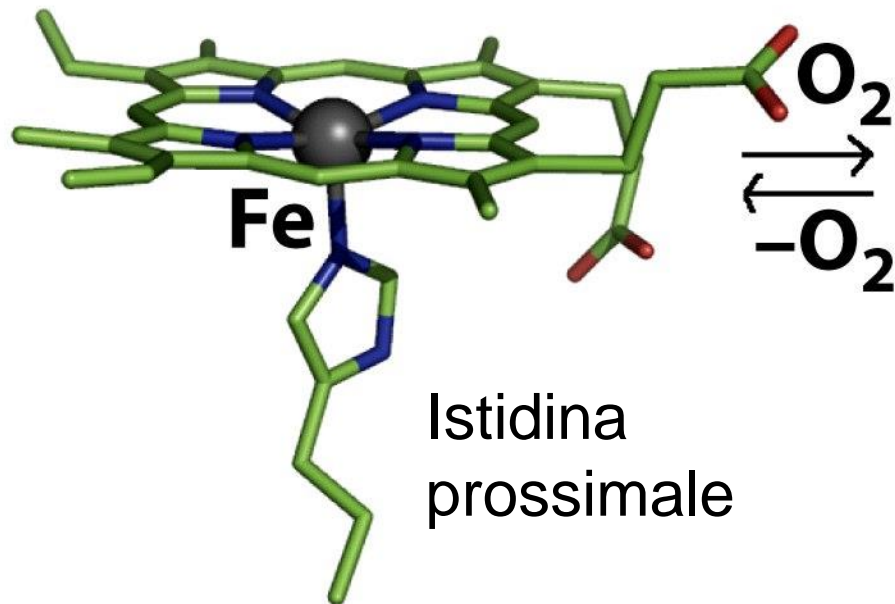
Curva di saturazione sigmoideale, pH dipendente

Effetto cooperativo ed effetto Bohr (effetto allosterico)

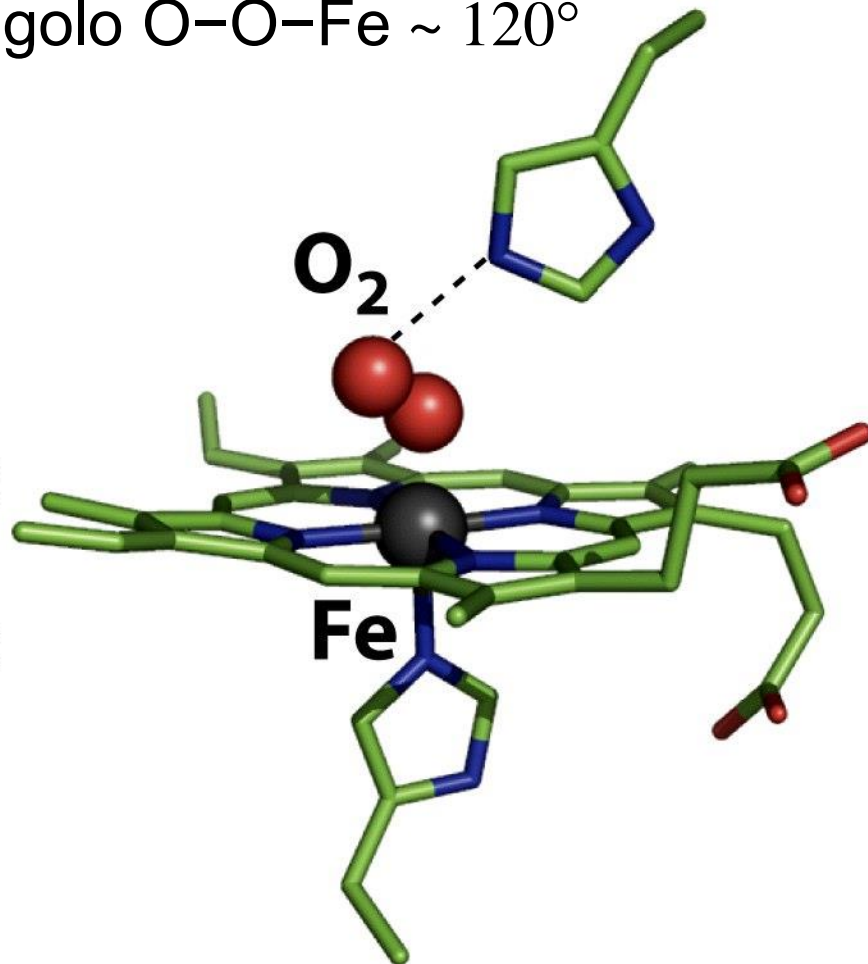
La coordinazione di CO₂ e protoni (pH) alla catena proteica dell'emoglobina causa variazioni conformazionali che influenzano l'affinità dell'eme per O₂

Coordinazione O_2 *end-on*
Angolo O-O-Fe $\sim 120^\circ$

Istidina
distale



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

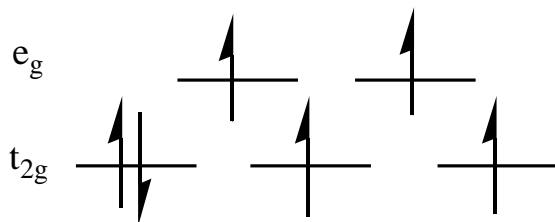


$S = 0$

desossi:

$S = 2$

paramagnetica



Fe(II), d^6 paramagnetico

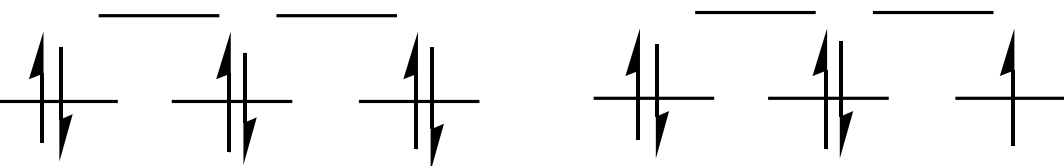
Pauling

Weiss

ossi:

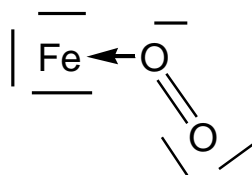
$S = 0$

diamagnetica



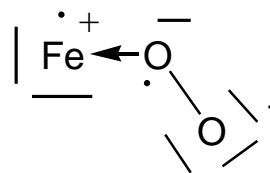
Fe(II) d^6 basso spin

+ $^1\text{O}_2$ legato



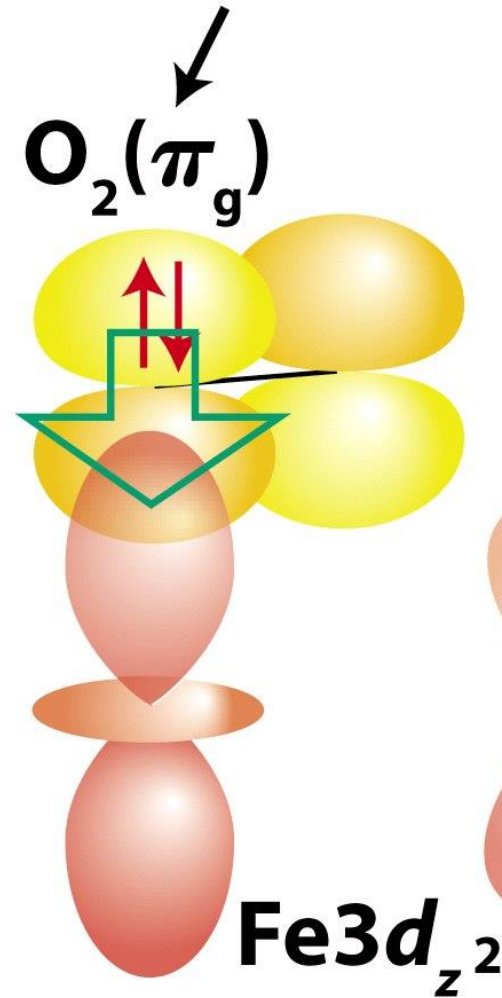
Fe(III) d^5 basso spin

+ $^2\text{O}_2^{\cdot-}$ legato

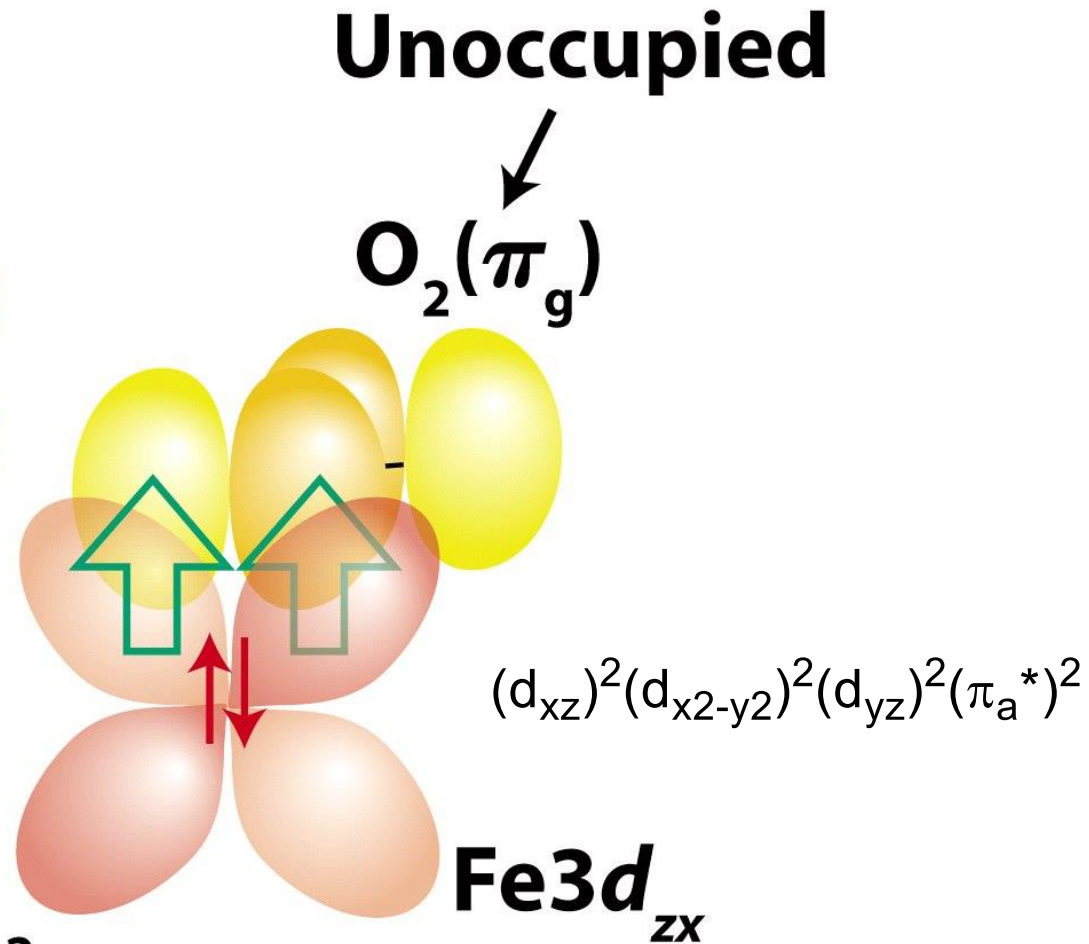


Modello di Pauling: Fe(II) I.s. + $^1\text{O}_2$

(a) Occupied



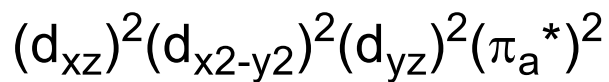
(b)



Pauling

Fe^{II} / O₂

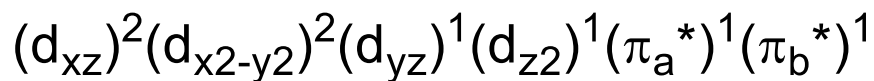
S = 0



McClure/Goddard

Fe^{II} / O₂

S = 1

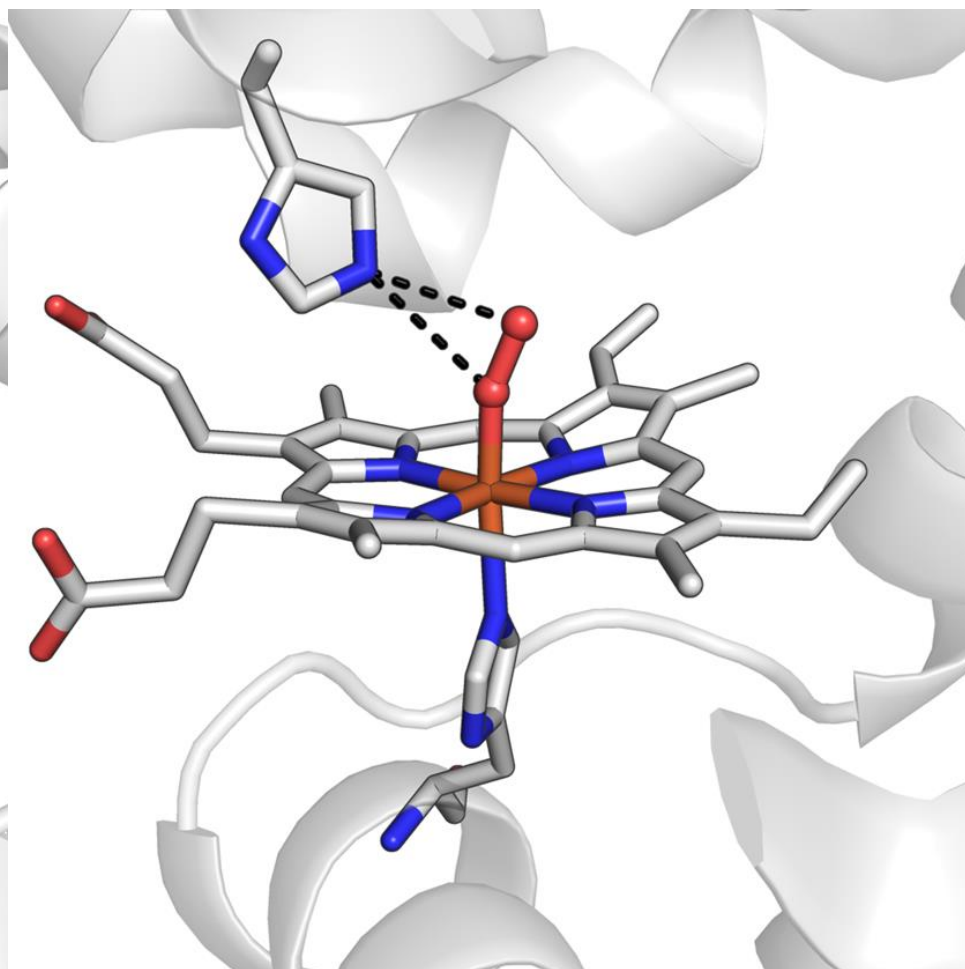
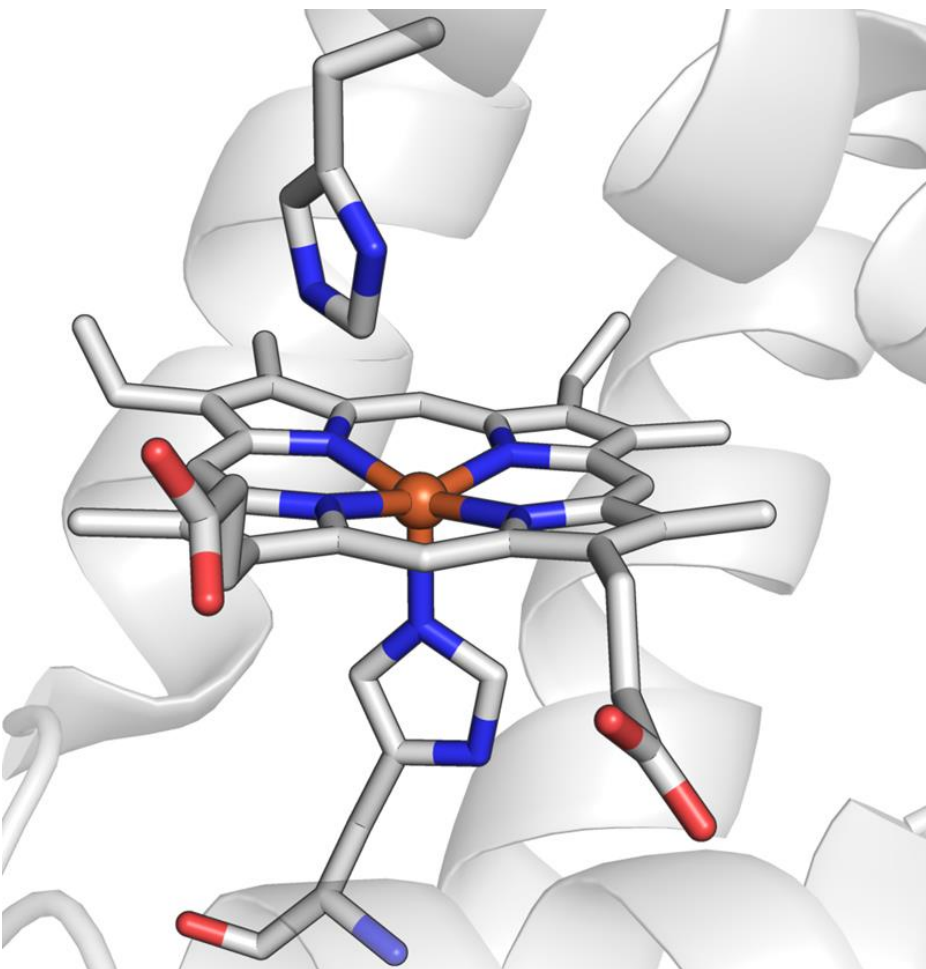


Weiss

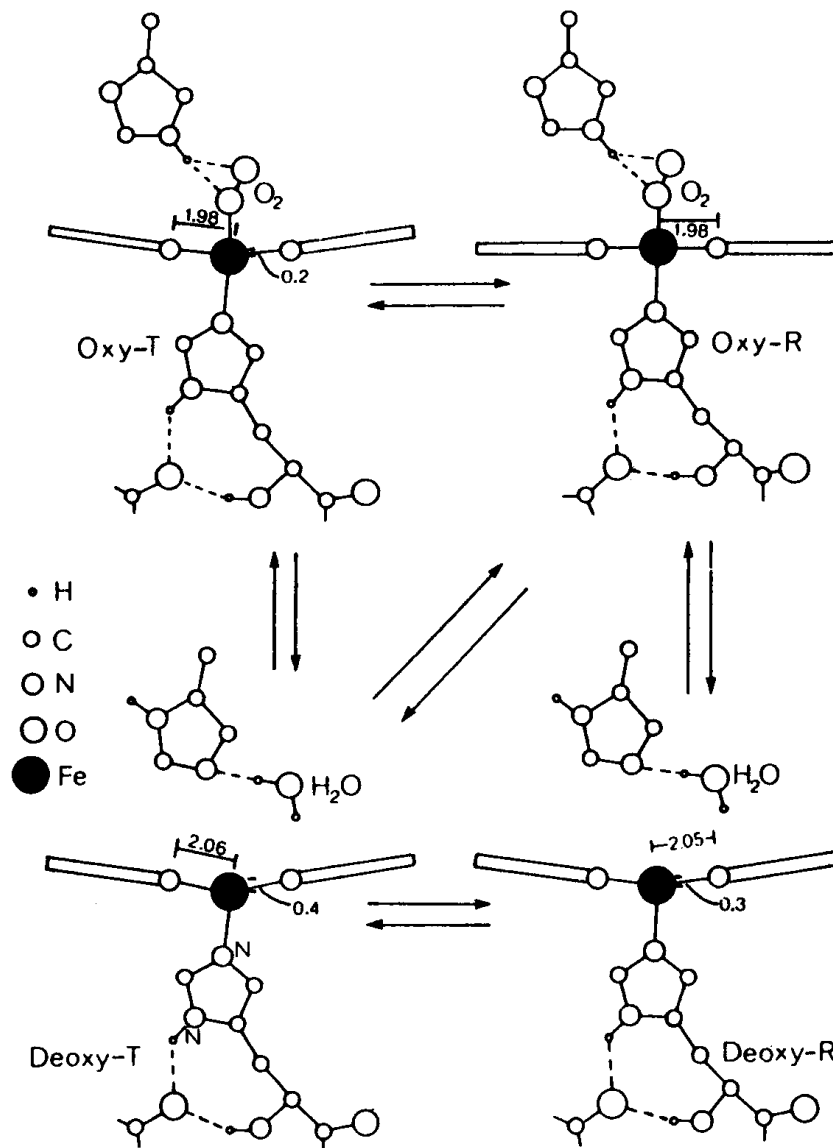
Fe^{III} / O₂⁻

S = 1/2



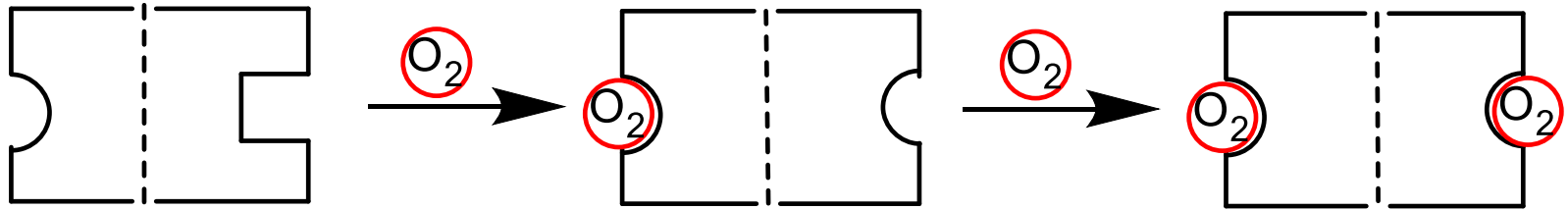


Fe(II) h.s.: 0.92 Å
Fe(II) l.s.: 0.75 Å
Fe(III) l.s.: 0.55 Å

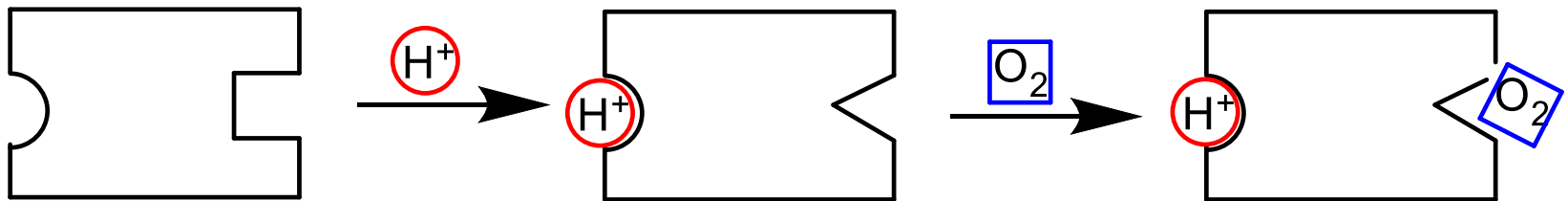


l'affinità della forma R per O₂ è circa la stessa delle catene α e β isolate, cioè della Mb, mentre quella della forma T è circa 12 – 14 kJ/mole inferiore

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

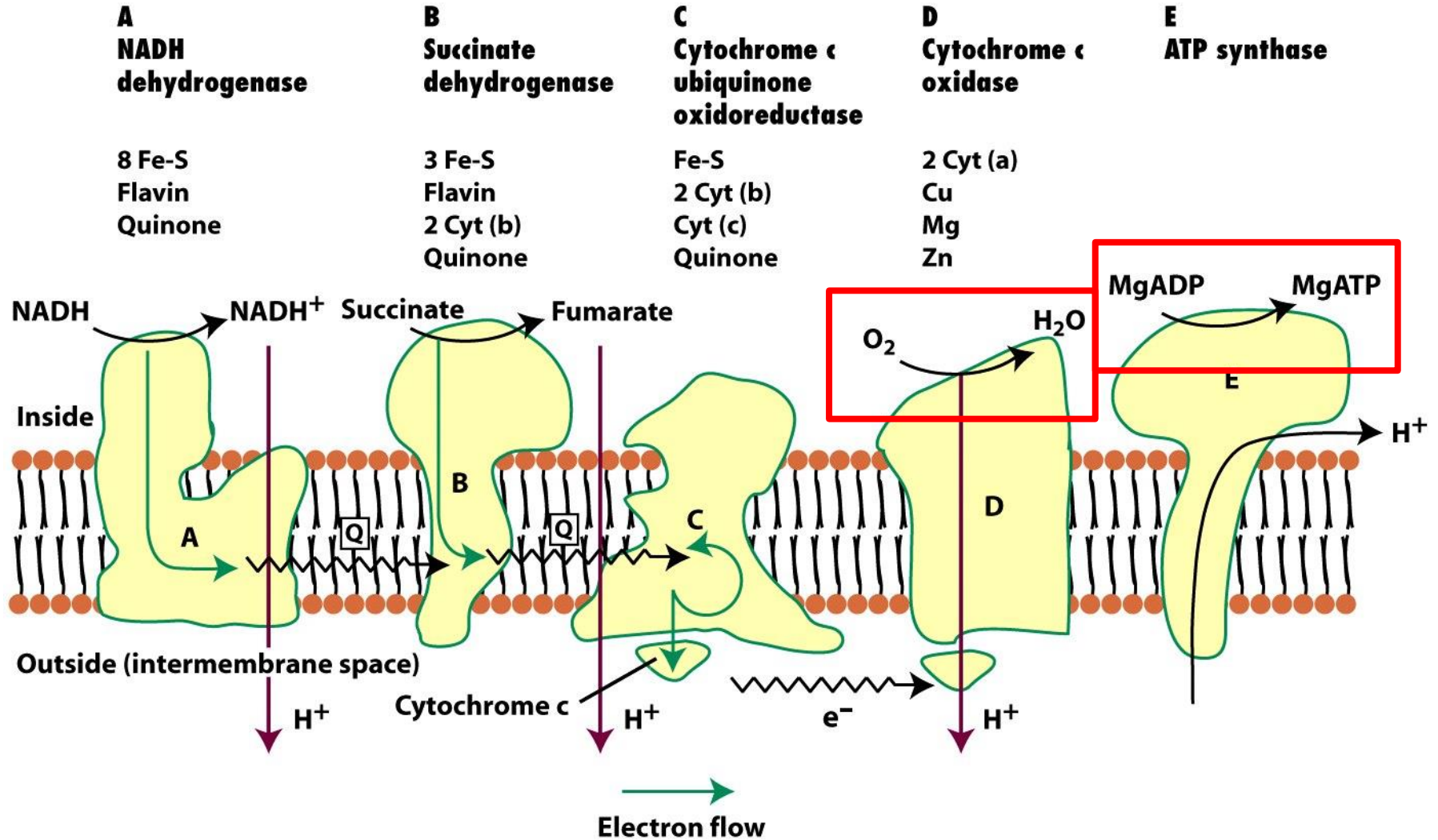


Allosterismo Omotropico



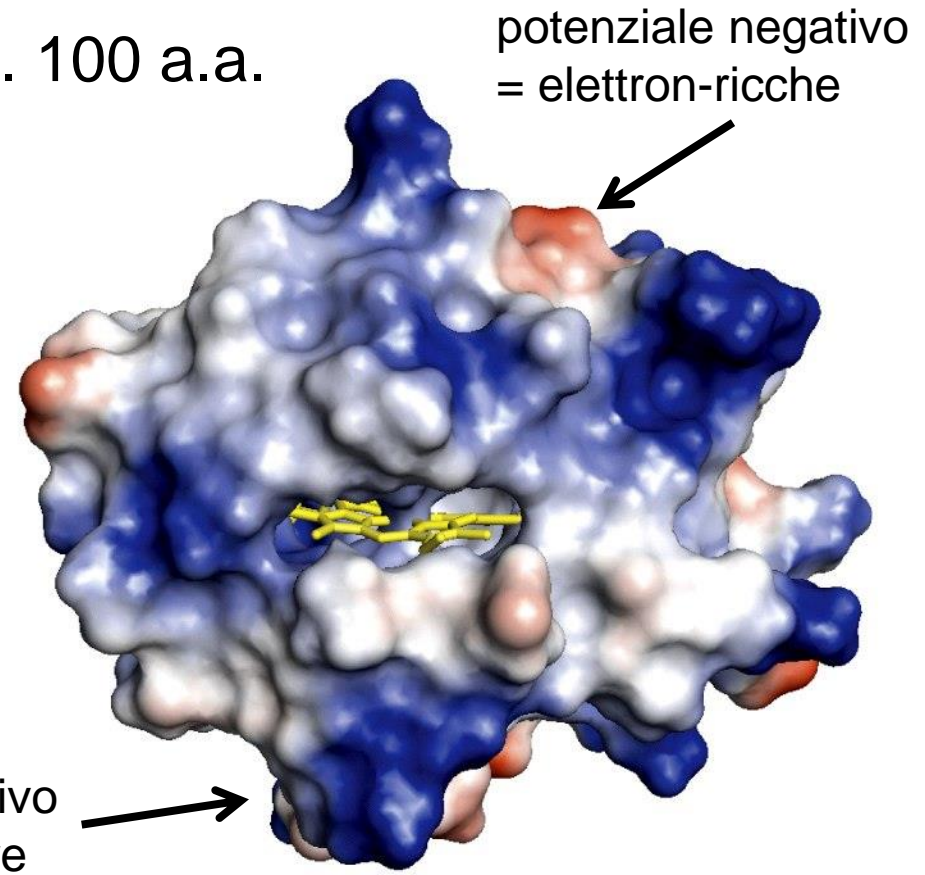
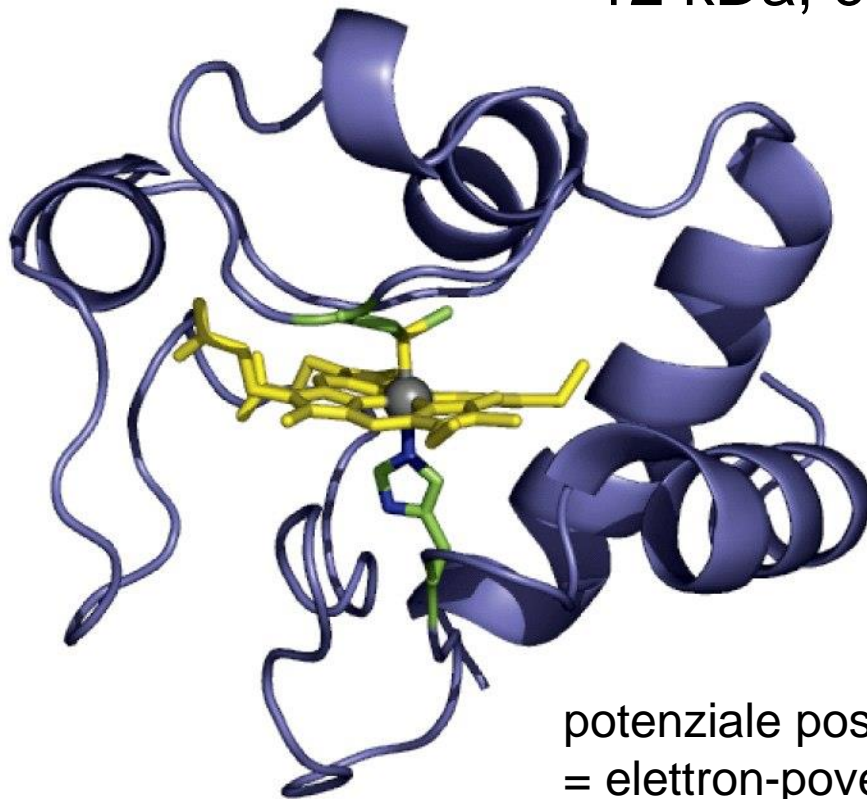
Allosterismo Eterotropico (Effetto Bohr)

Catena della respirazione cellulare



Citocromo c (da cuore di cavallo)

12 kDa, ca. 100 a.a.

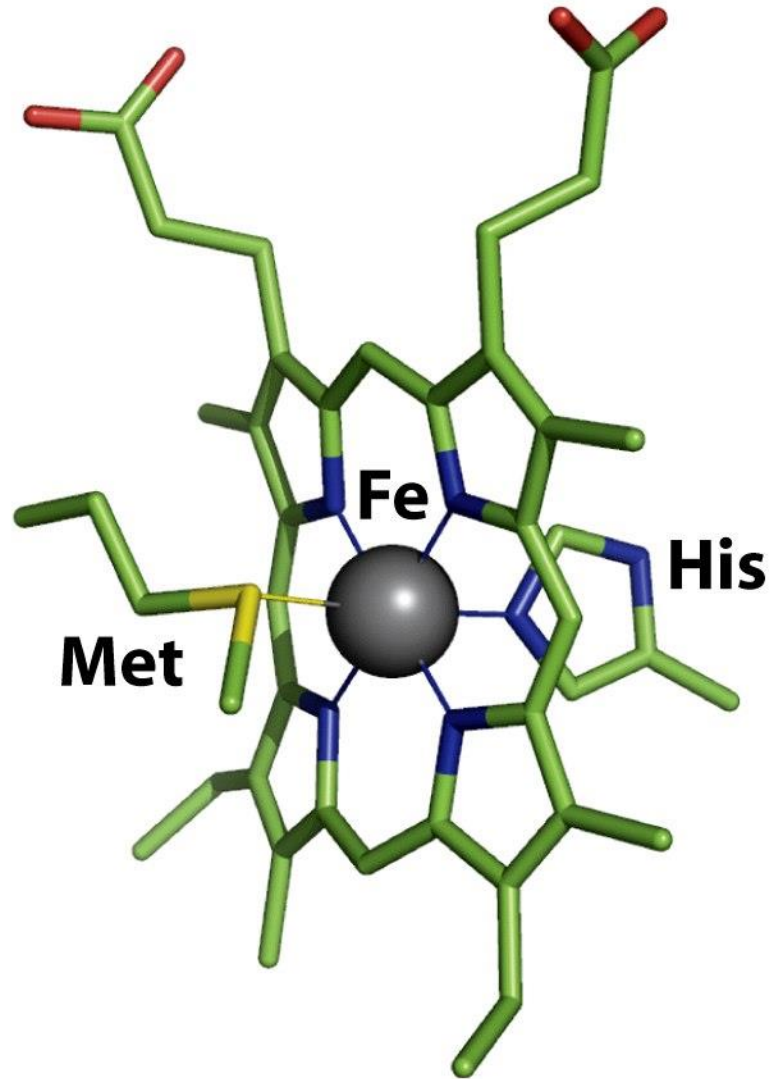


mappa del potenziale elettrostatico

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

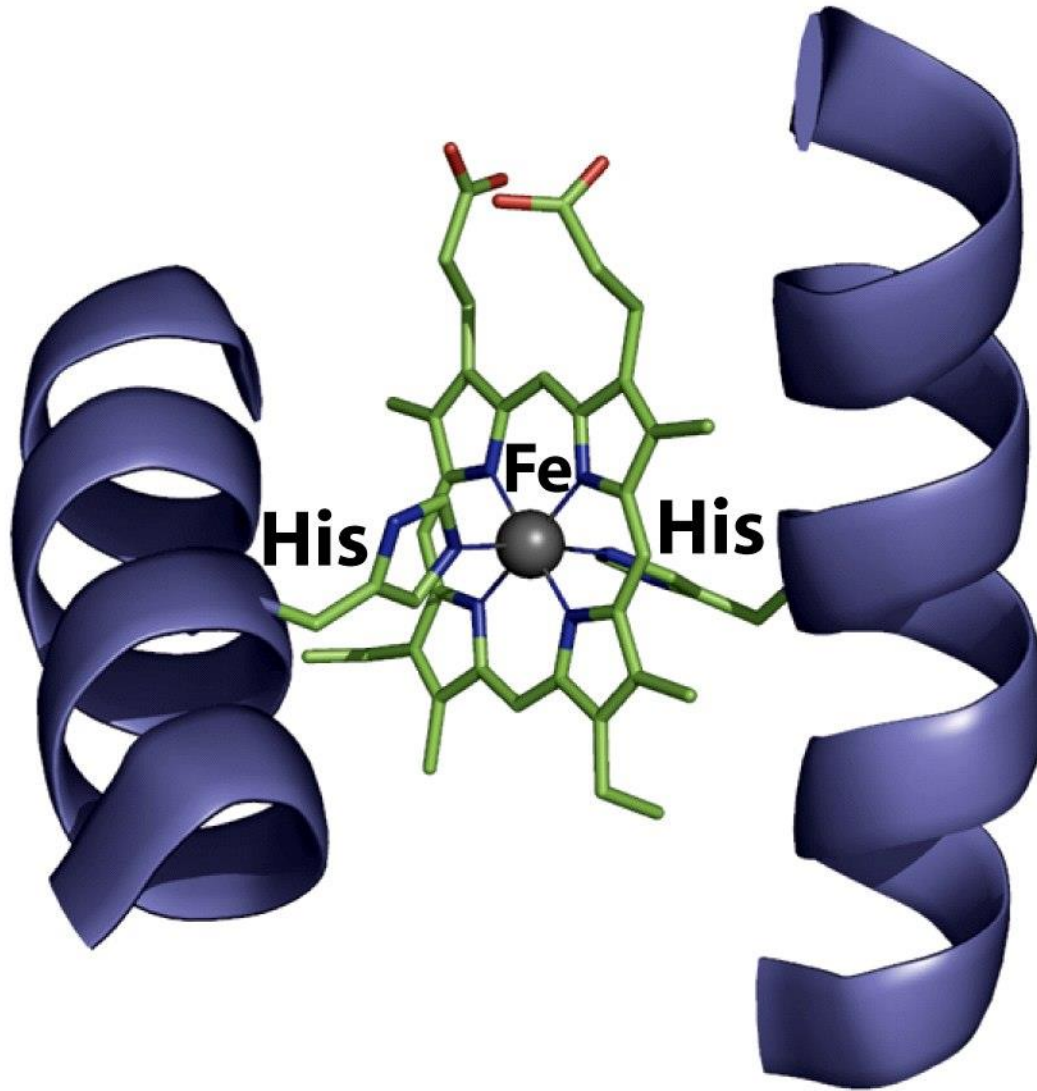
Il Fe è basso spin in entrambi gli stati di ossidazione

Gruppo eme nel Citocromo c



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

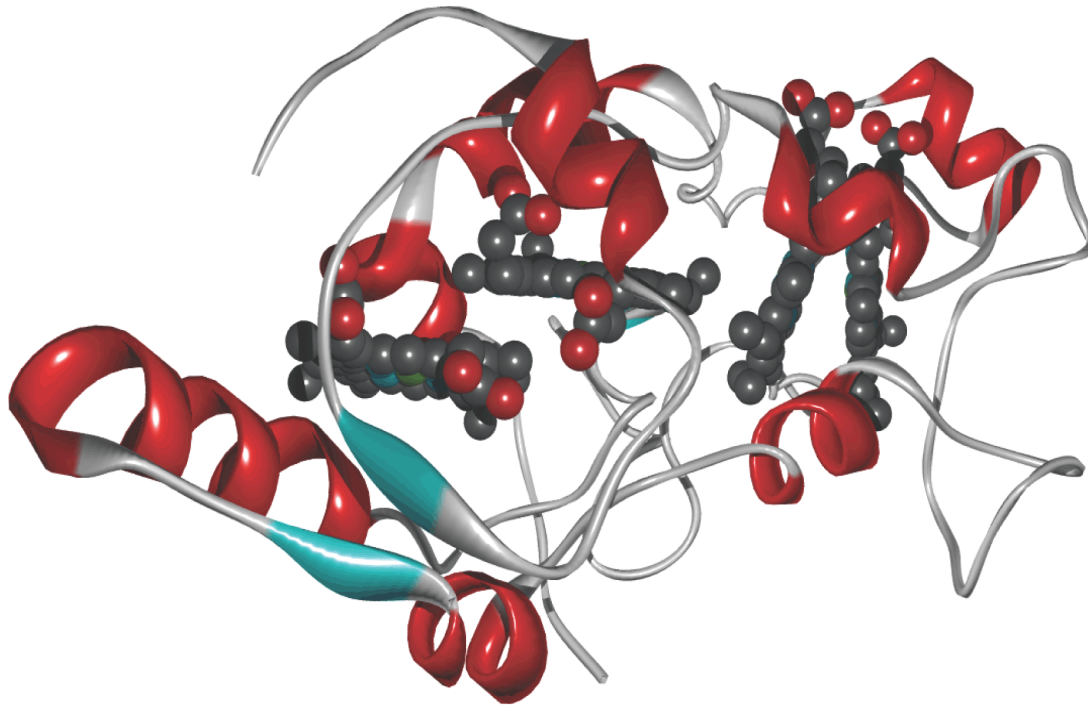
Gruppo eme nel Citocromo b



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

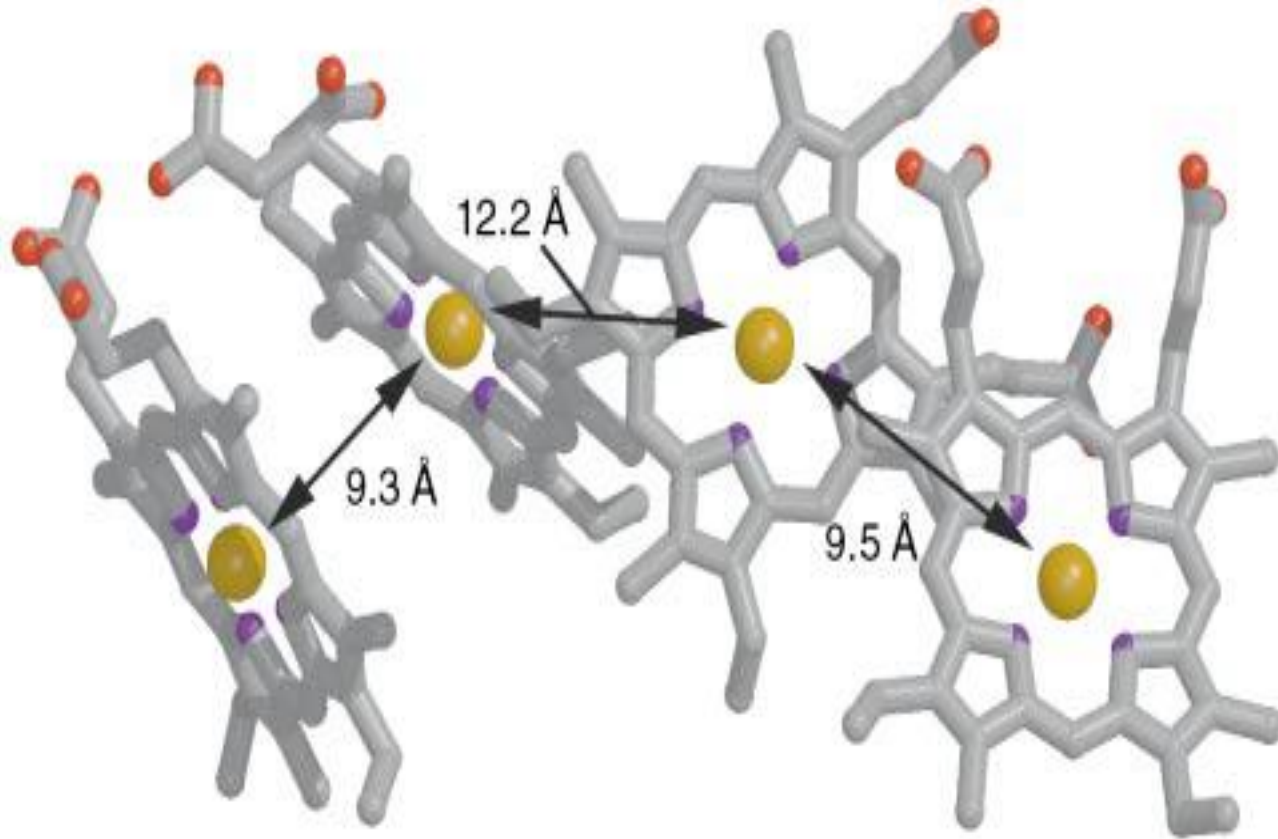
Citocromo c554

(nitrificazione catalizzata da batteri)

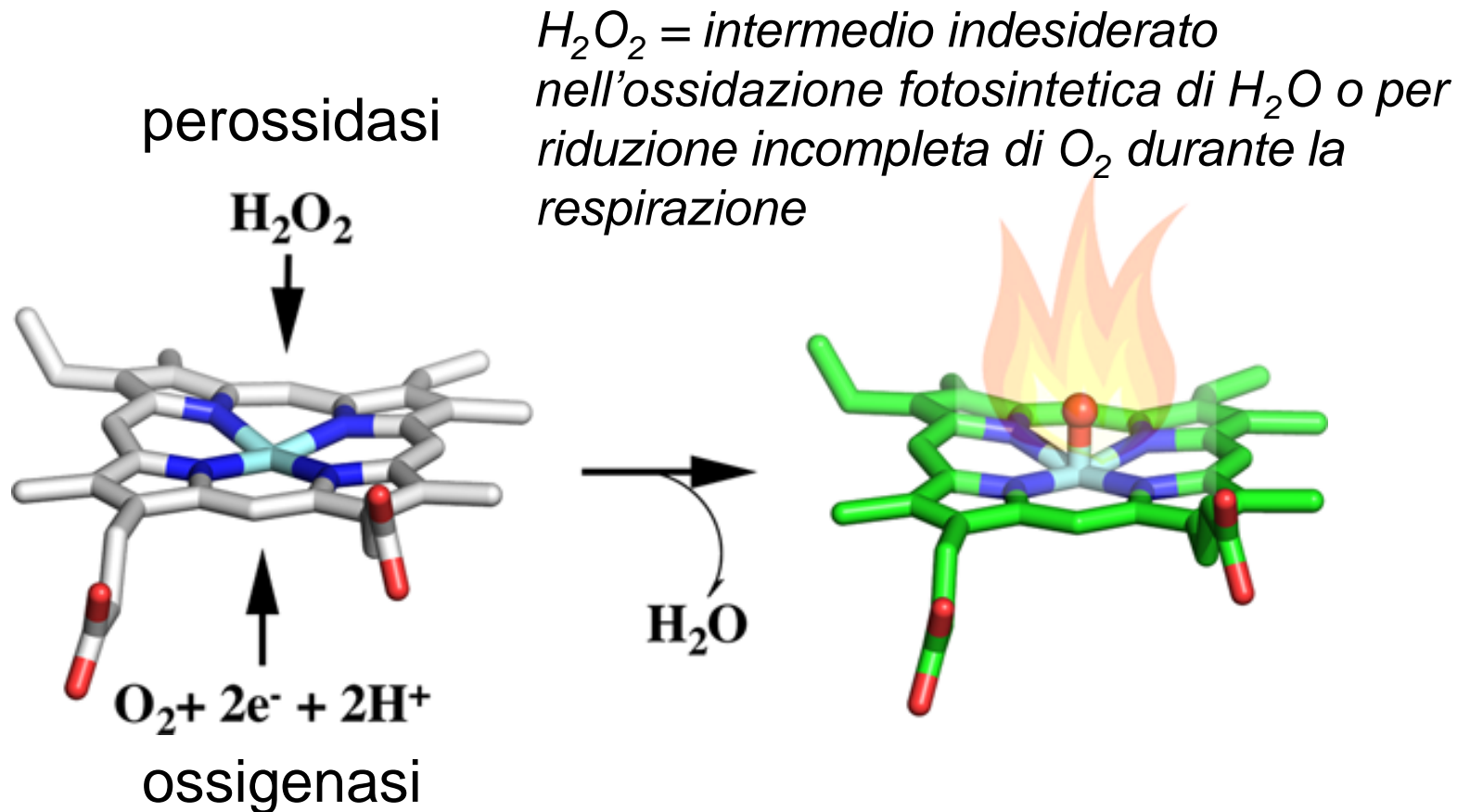


Distanze Fe...Fe \approx 950, 1220, 920 pm

Citocromo c554



Ossidazioni catalizzate da eme-proteine

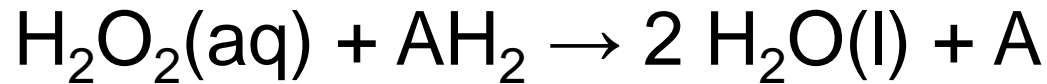


ossidare non sempre implica anche ossigenare i substrati

Perossidasi e Catalasi

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

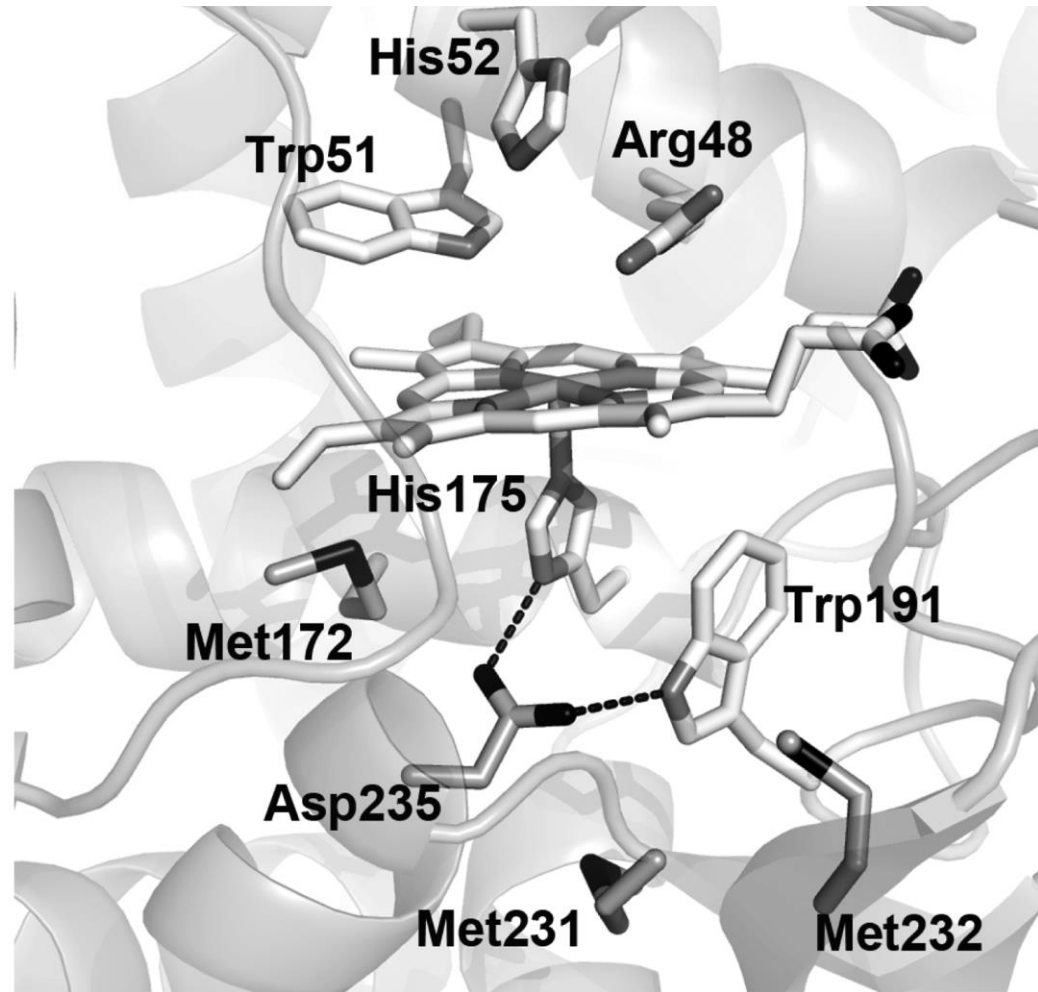
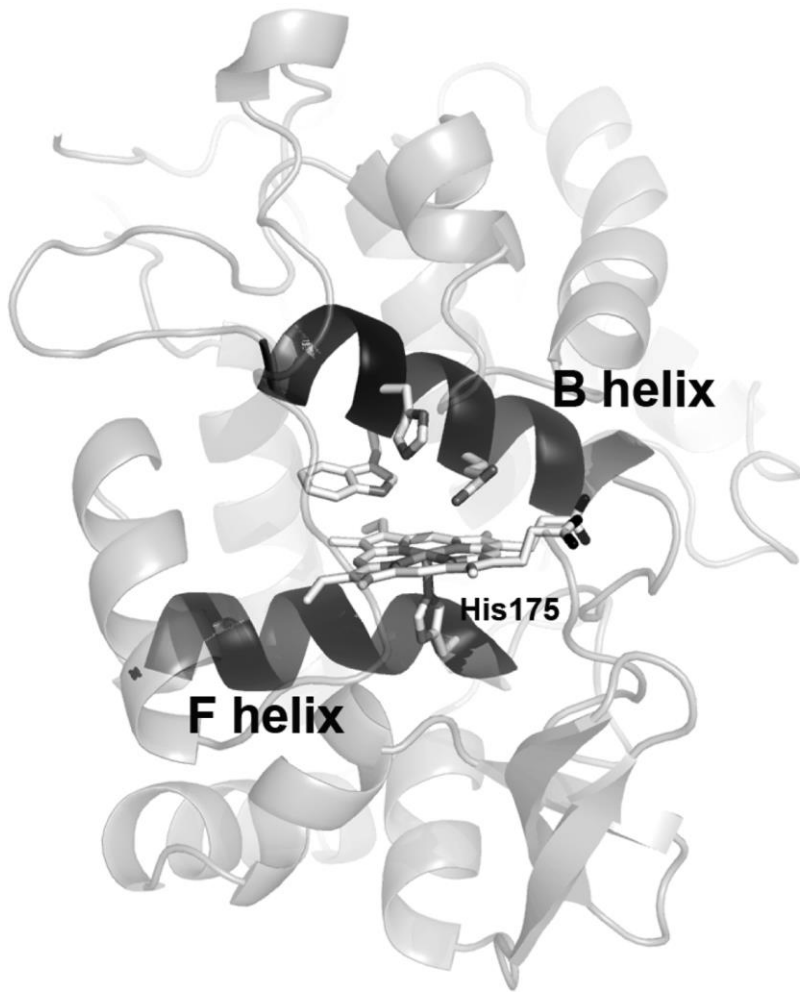
sono anche enzimi detossificanti



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

Citocromo c Perossidasi

(lievito, batteri)



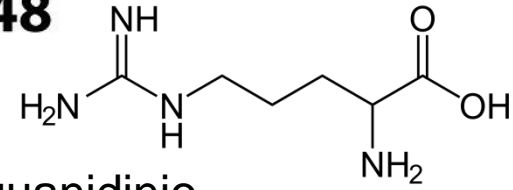
Il legame a idrogeno con un aspartato conferisce all'istidina maggior carattere di imidazolato (Im^-) e contribuisce ad abbassare il potenziale redox del ferro-eme

Sito attivo della
Citocromo c Perossidasi
(lievito, batteri)

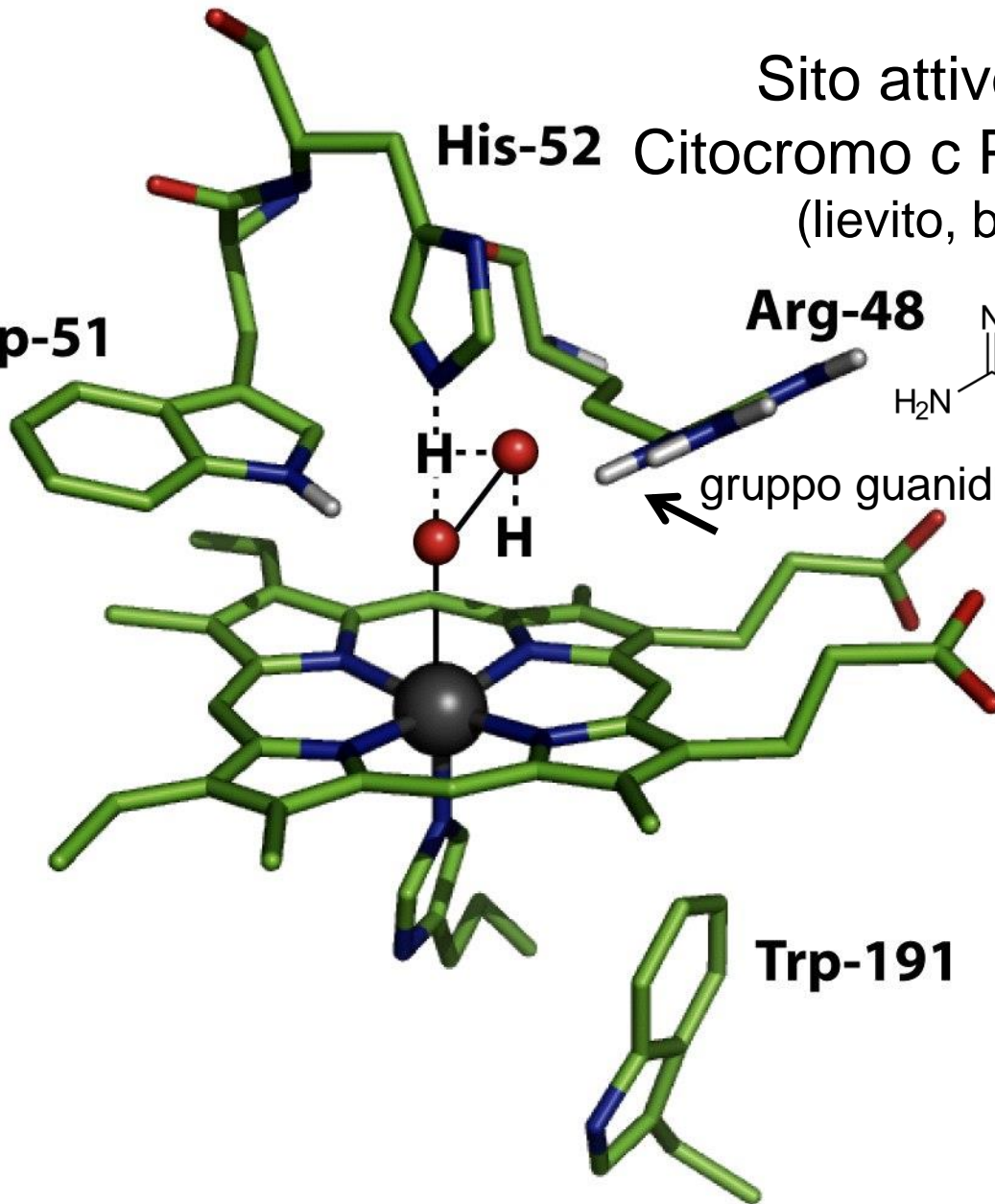
Trp-51

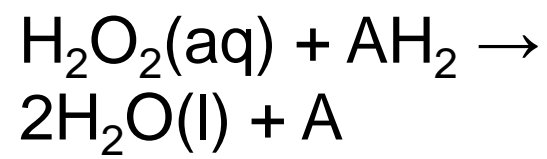
His-52

Arg-48



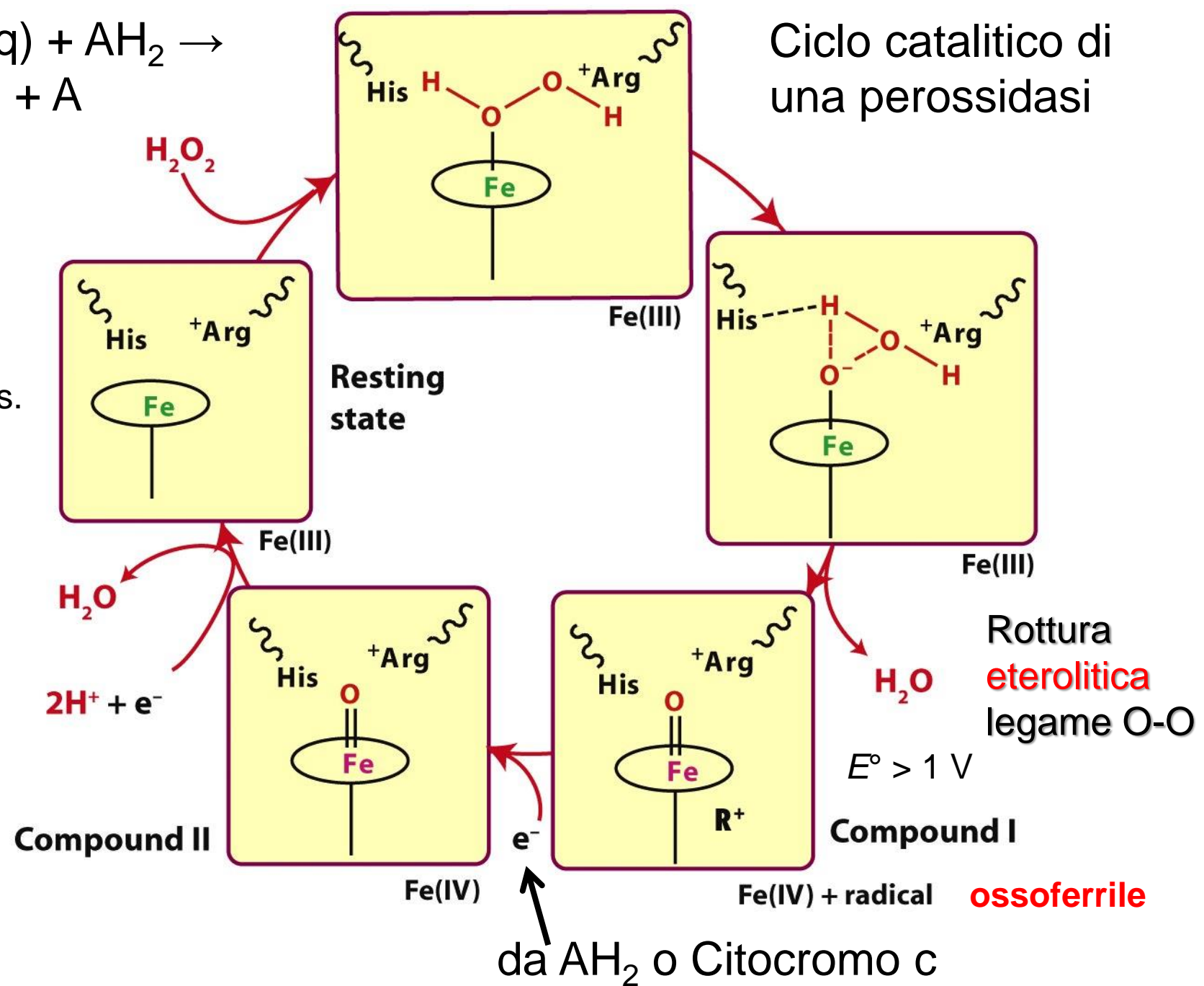
Trp-191





Ciclo catalitico di una perossidasi

Fe(III) h.s.
S = 5/2



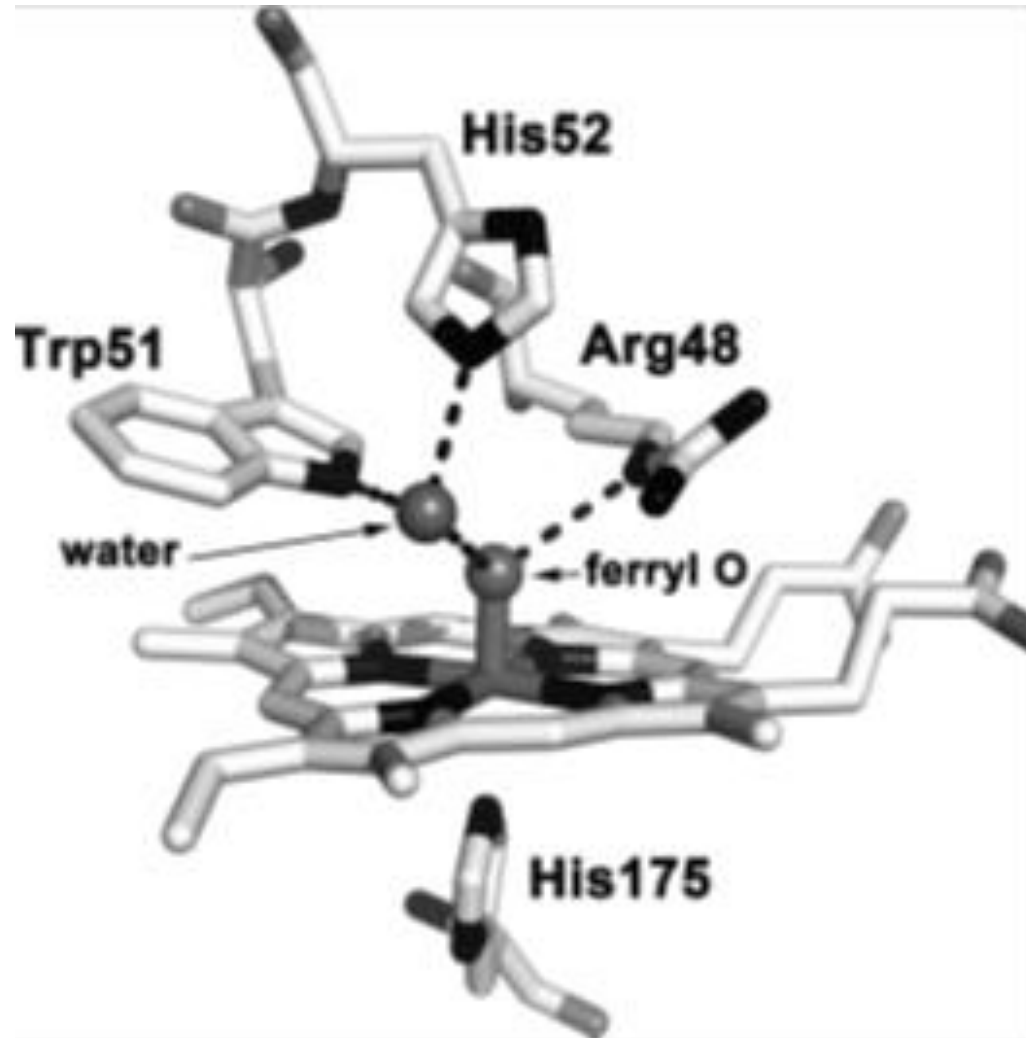
Rottura eterolitica legame O-O

E° > 1 V

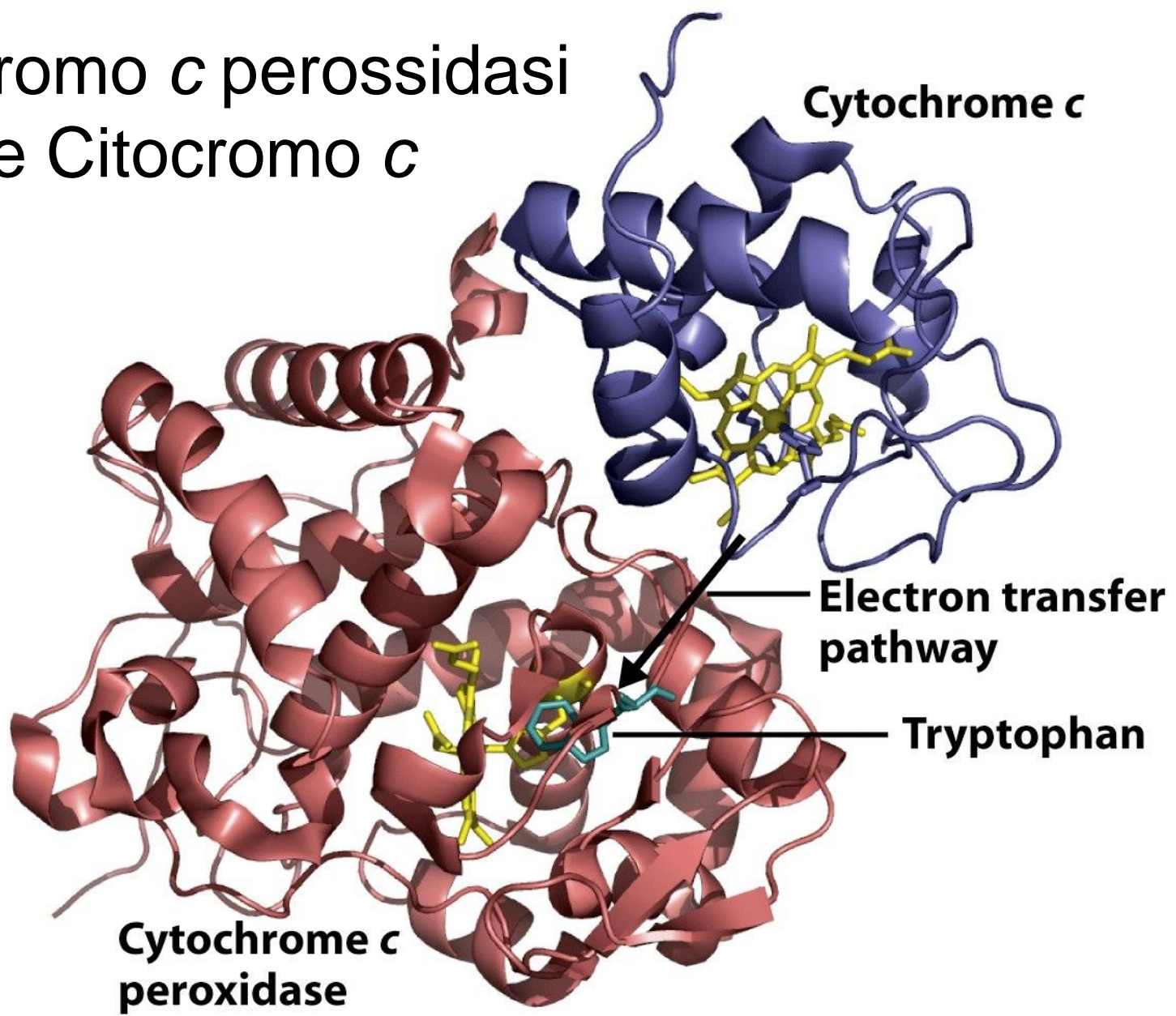
ossoferrile

da AH₂ o Citocromo c

Struttura ai raggi X dell'osso-ferrile nella CcP

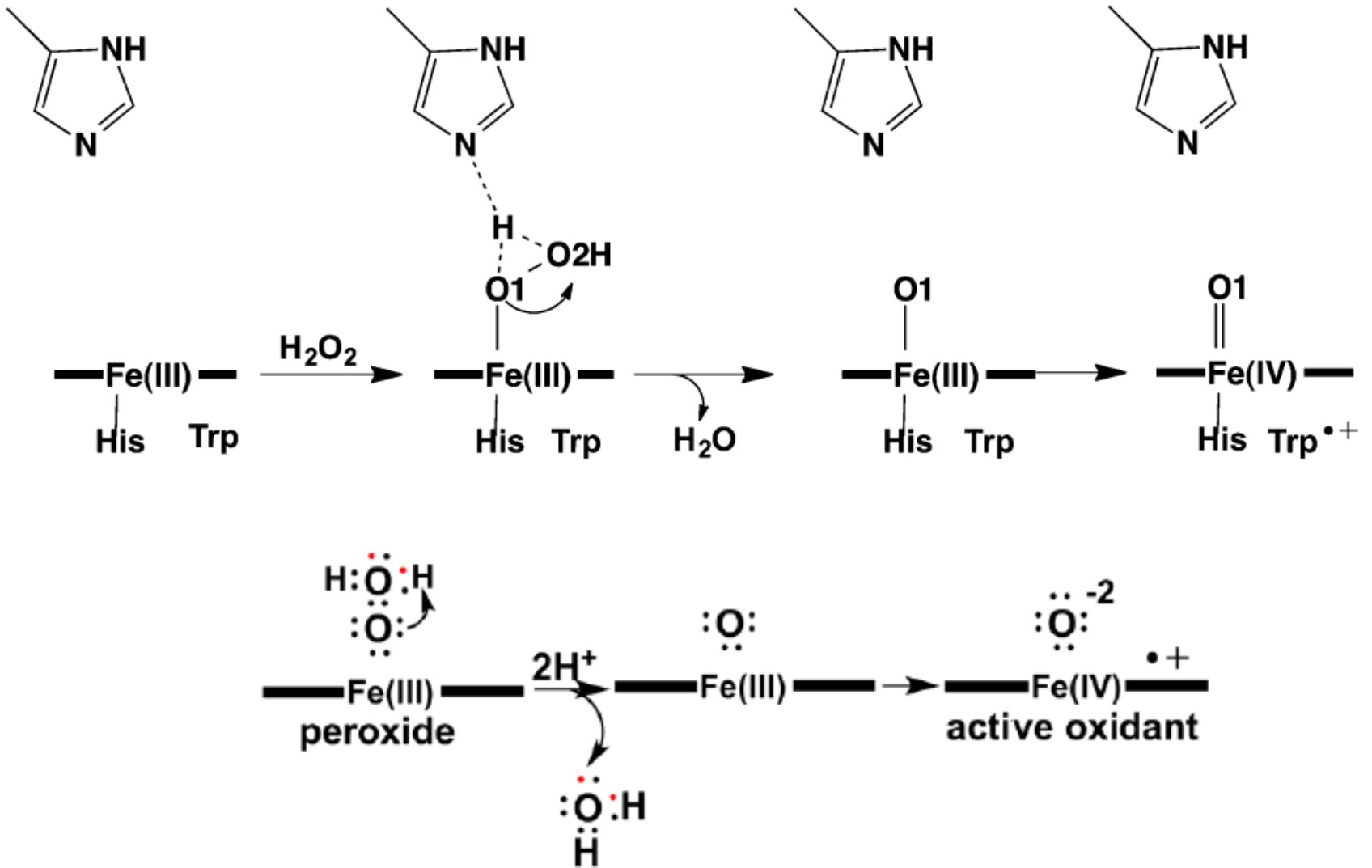


Citocromo c perossidasi e Citocromo c

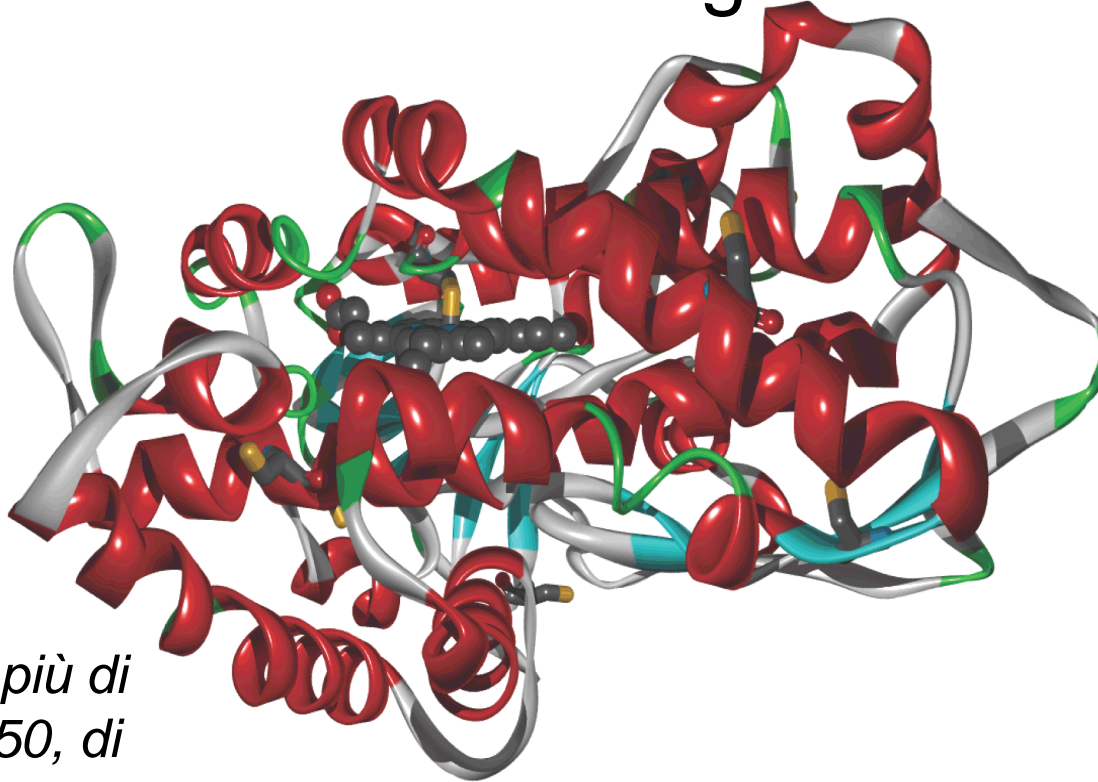


Formazione dell'osso-ferrile

Original Mechanism



Monoossigenasi

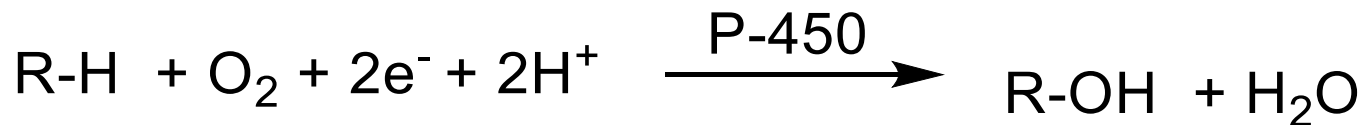


Un solo legante
assiale al ferro,
Cys⁻

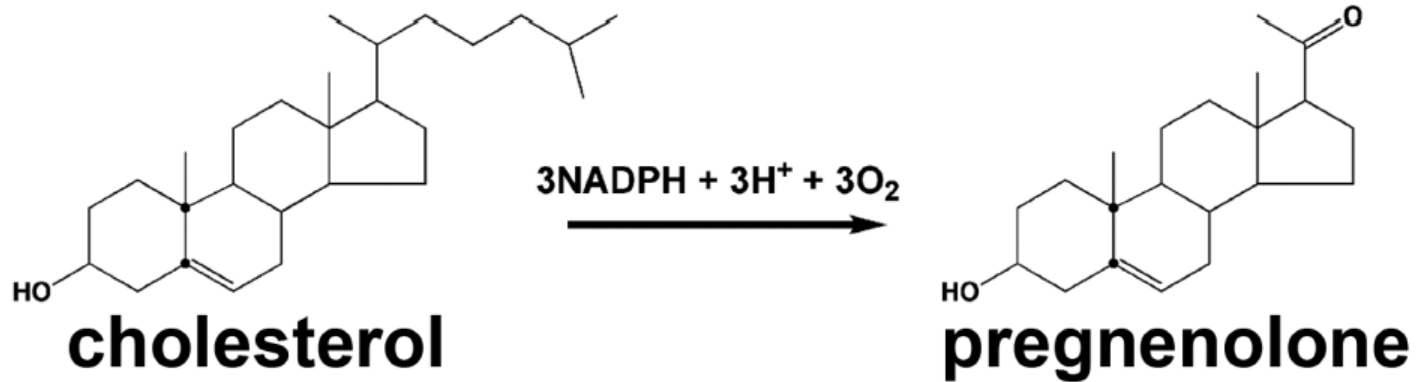
*individuati più di
18.000 P450, di
cui 57 nell'uomo*

Citocromo P-450

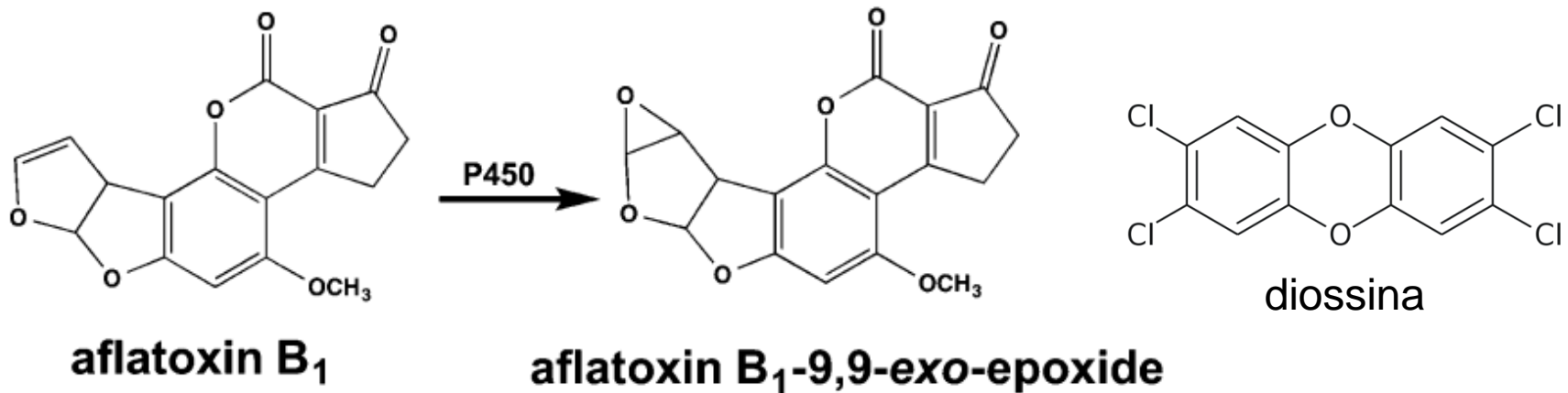
(da *Pseudomonas putida*) 50 kDa



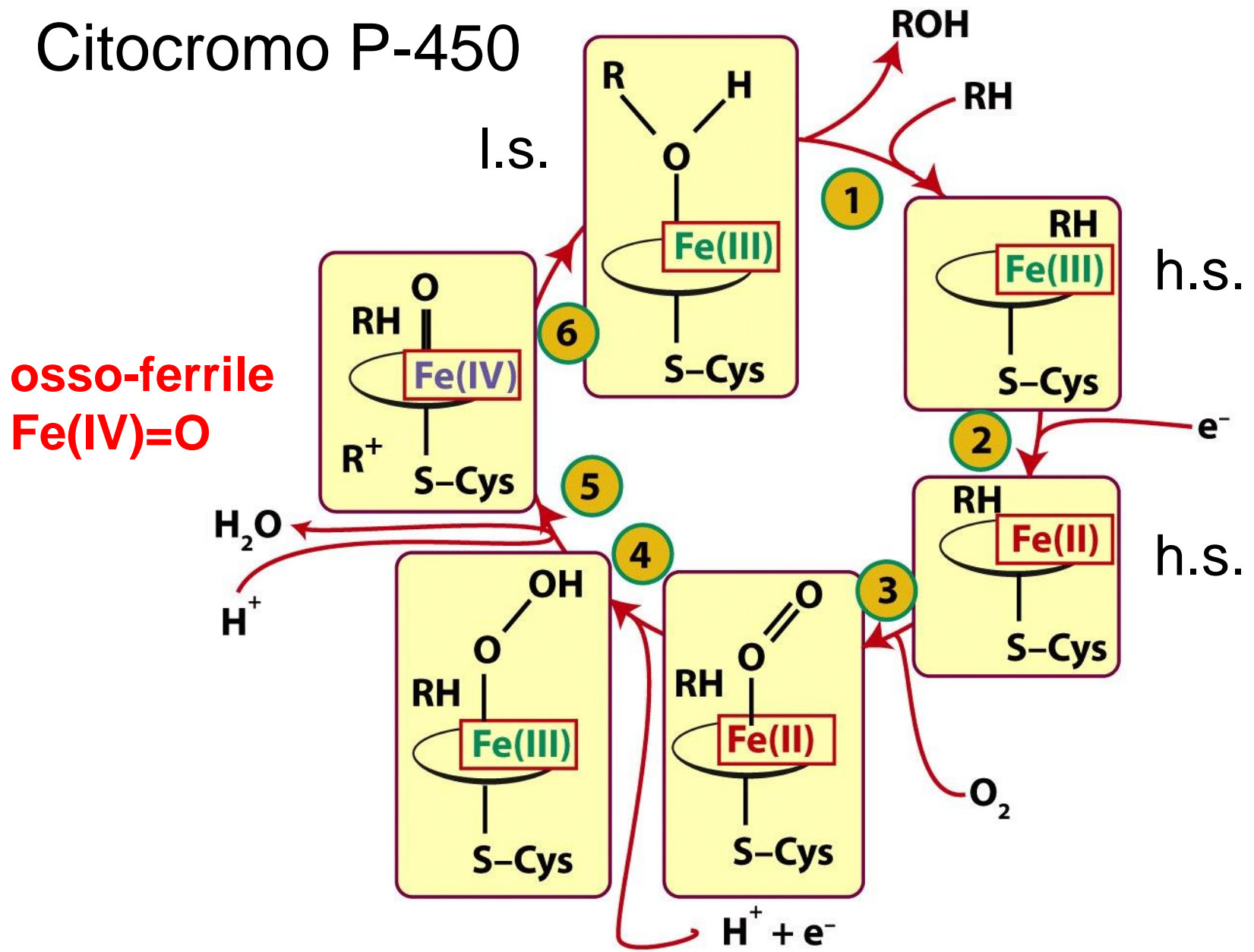
Ossigenazione di substrati specifici da parte di P450



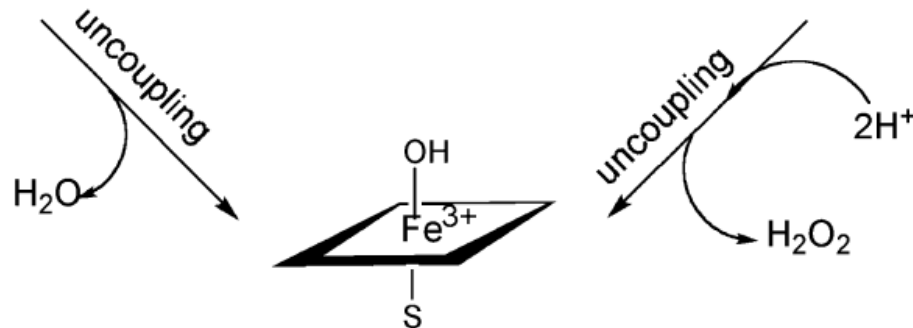
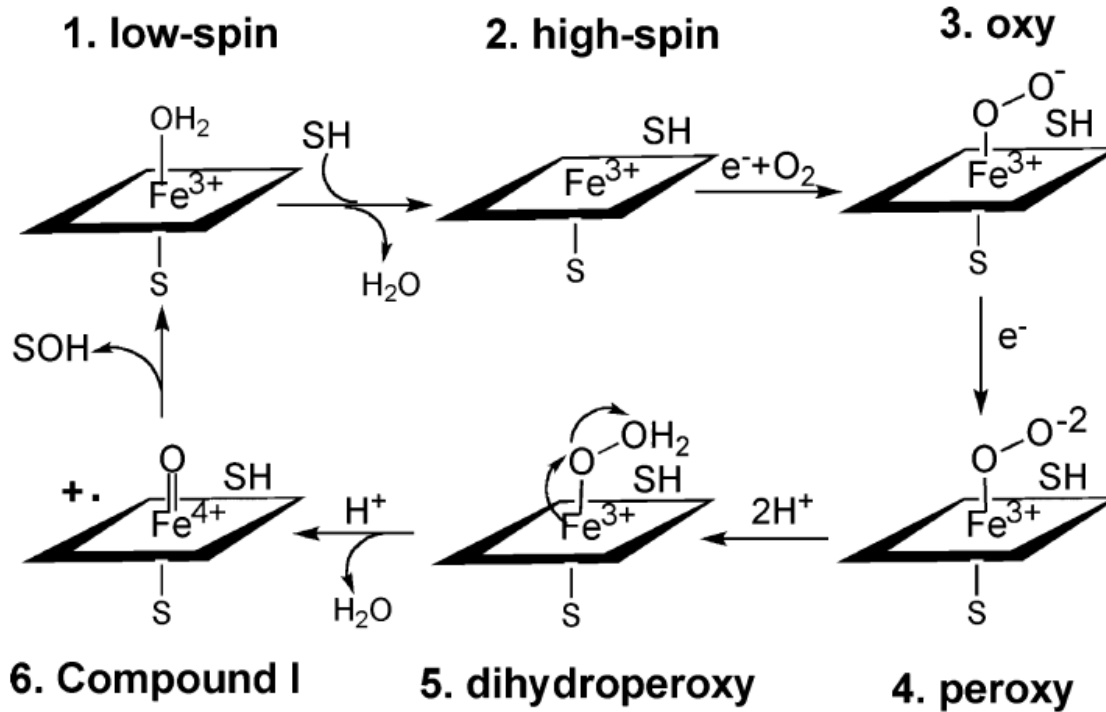
Attivazione (involontaria) di substrati da parte di P450



Citocromo P-450

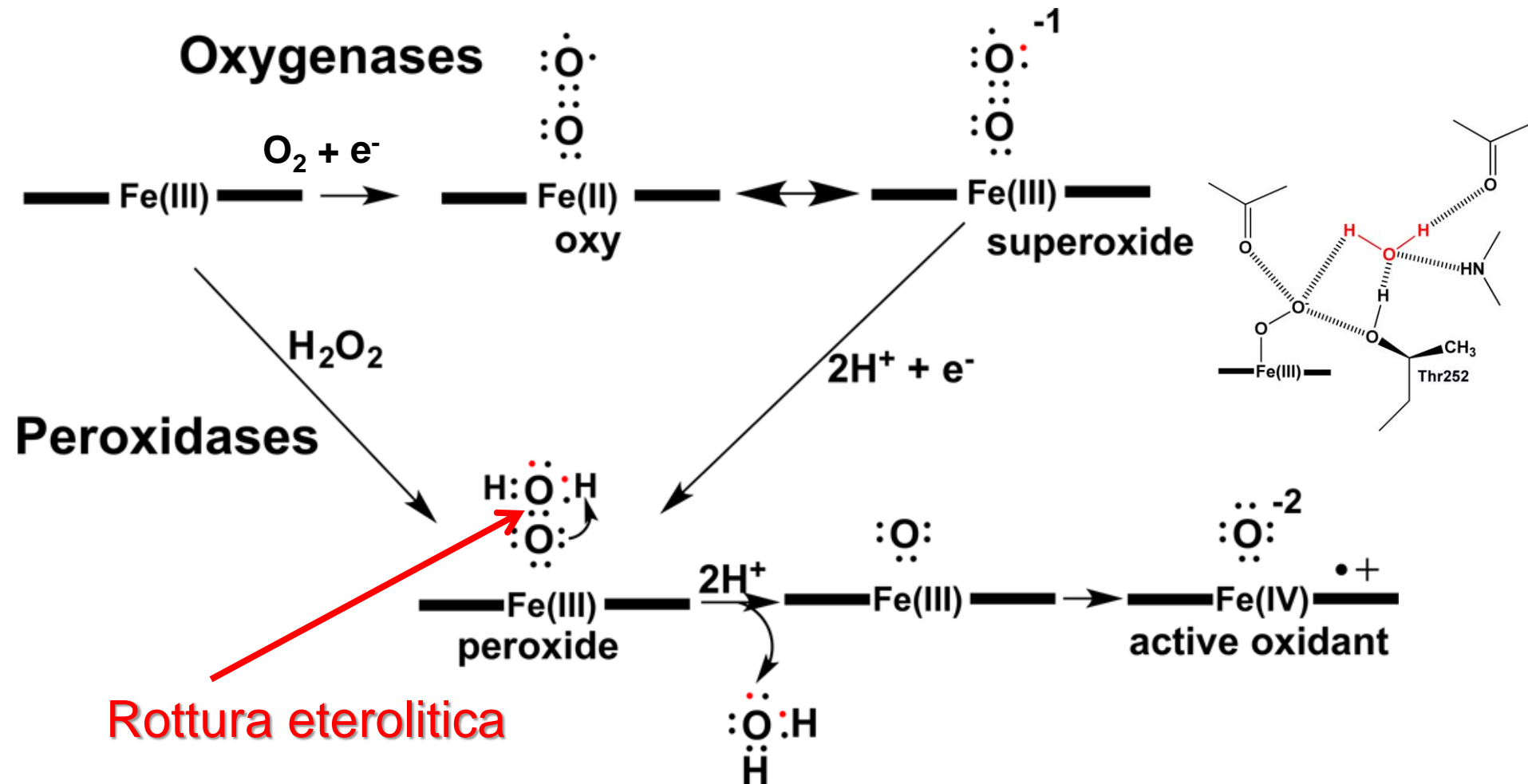


Citocromo P-450



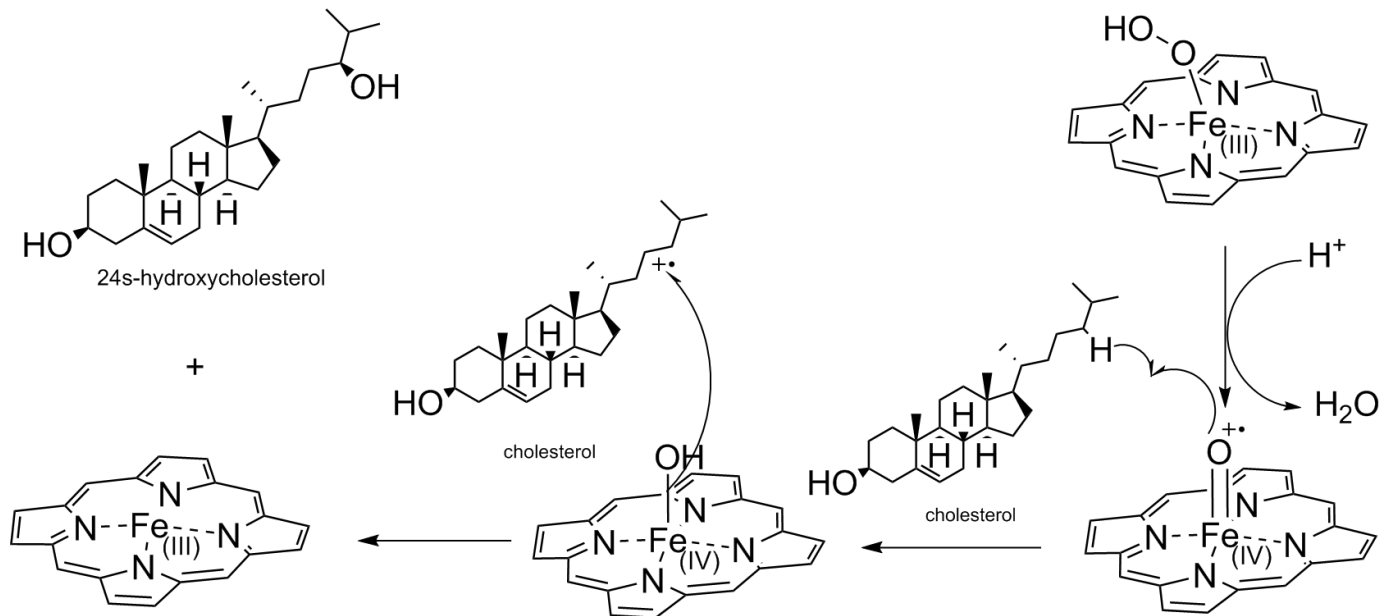
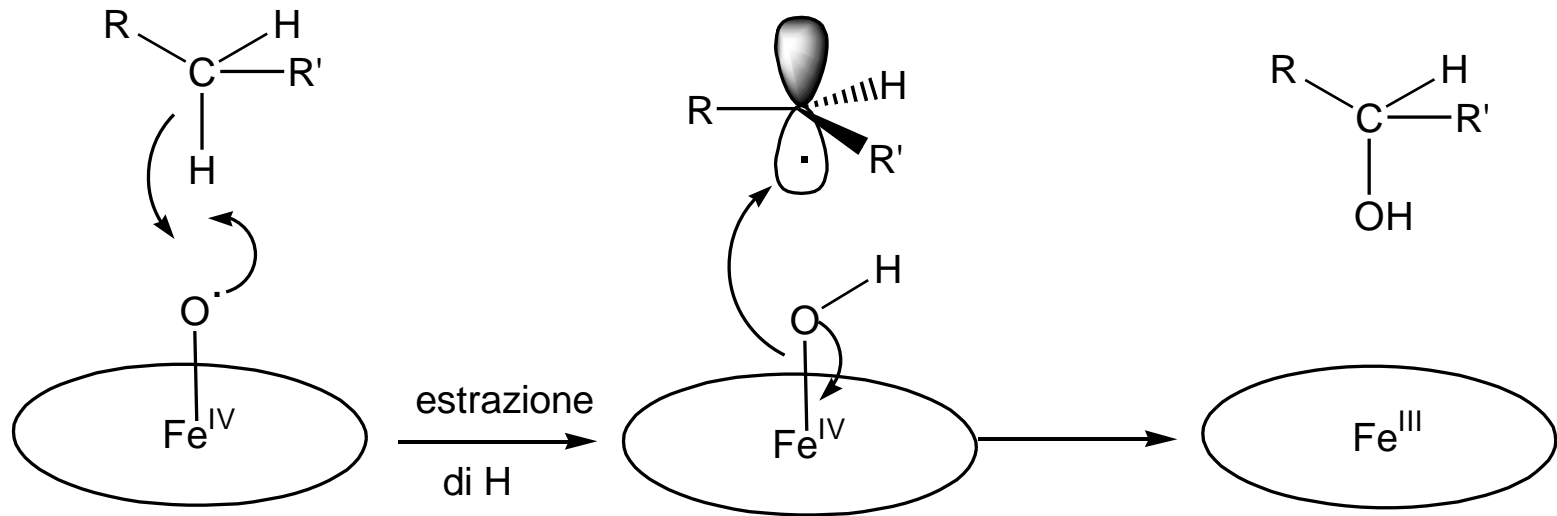
osso-ferrile
Fe(IV)=O

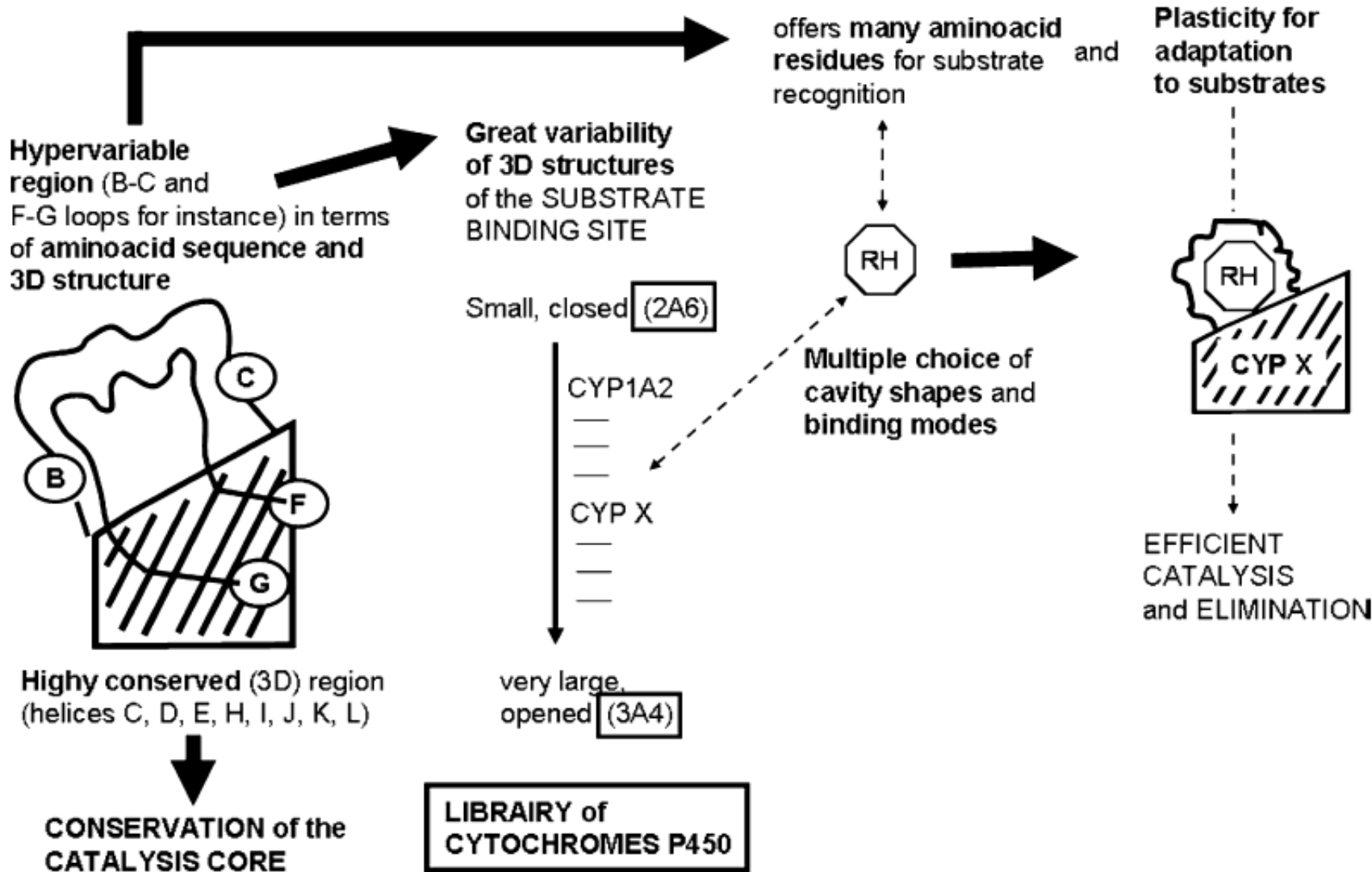
Rottura eterolitica del legame O–O e protonazione dell'ossigeno distale



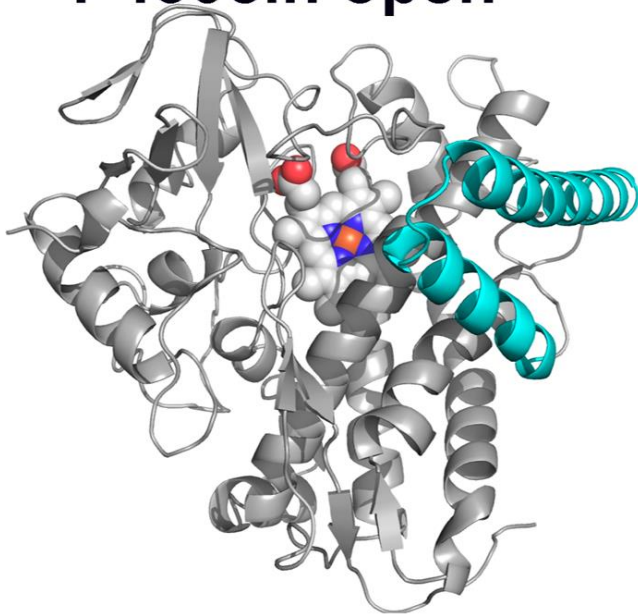
Meccanismo di ossigenazione del substrato

Oxygen Rebound Mechanism

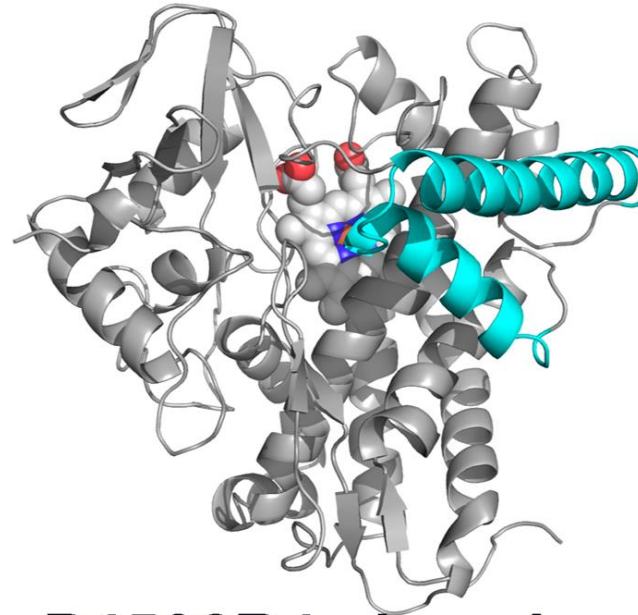




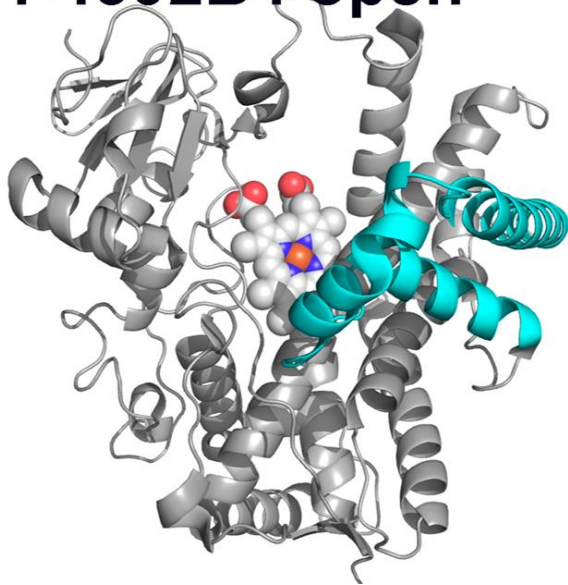
P450cin open



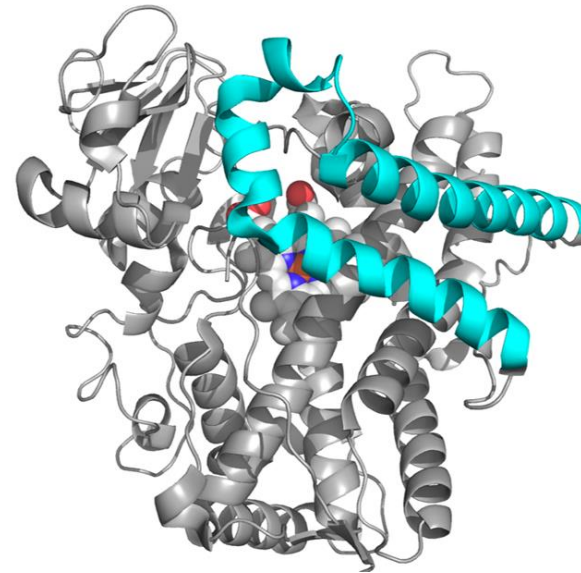
P450cin closed



P4502B4 open



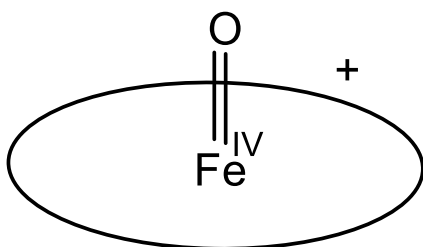
P4502B4 closed



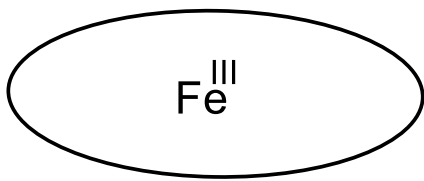
P450

cage

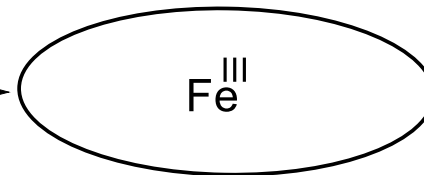
escape



+ Sub

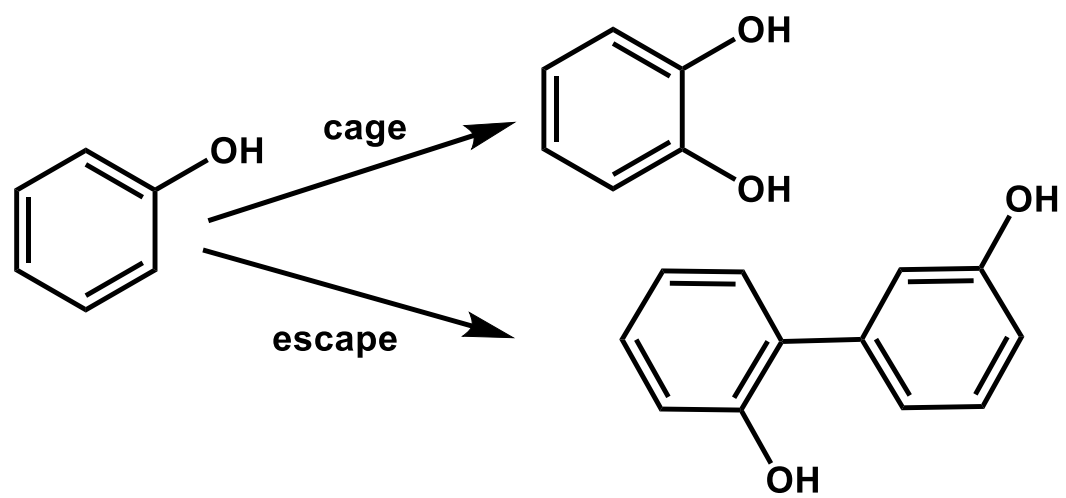
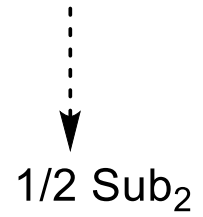


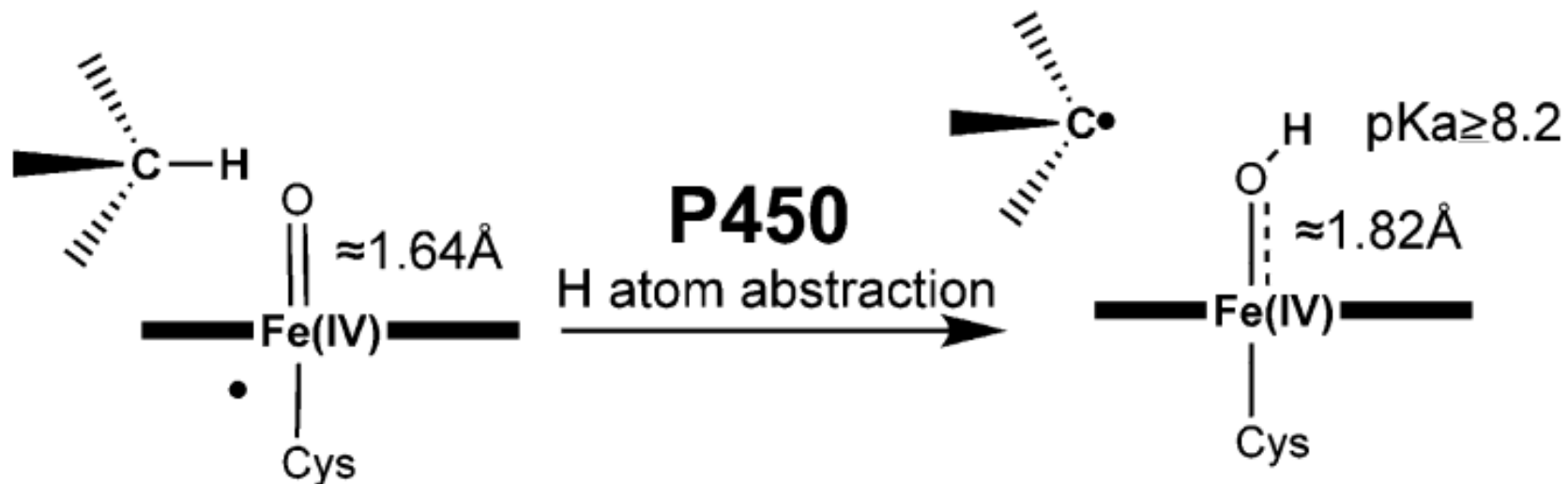
+ SubO



+ H₂O + Sub^{••}

perossidasi

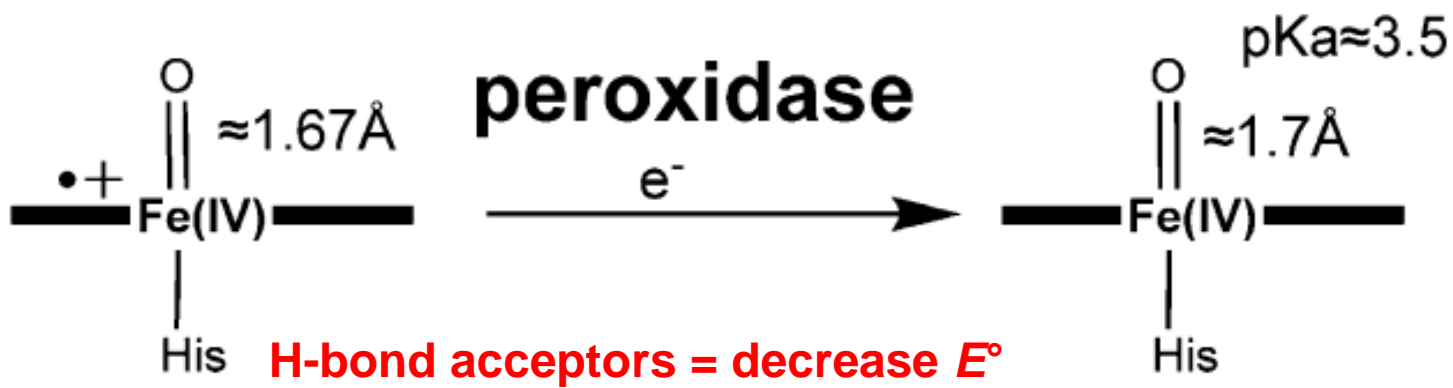




H-bond donors = increase E°

Compound I

Compound II



H-bond acceptors = decrease E°