

Proteine al rame

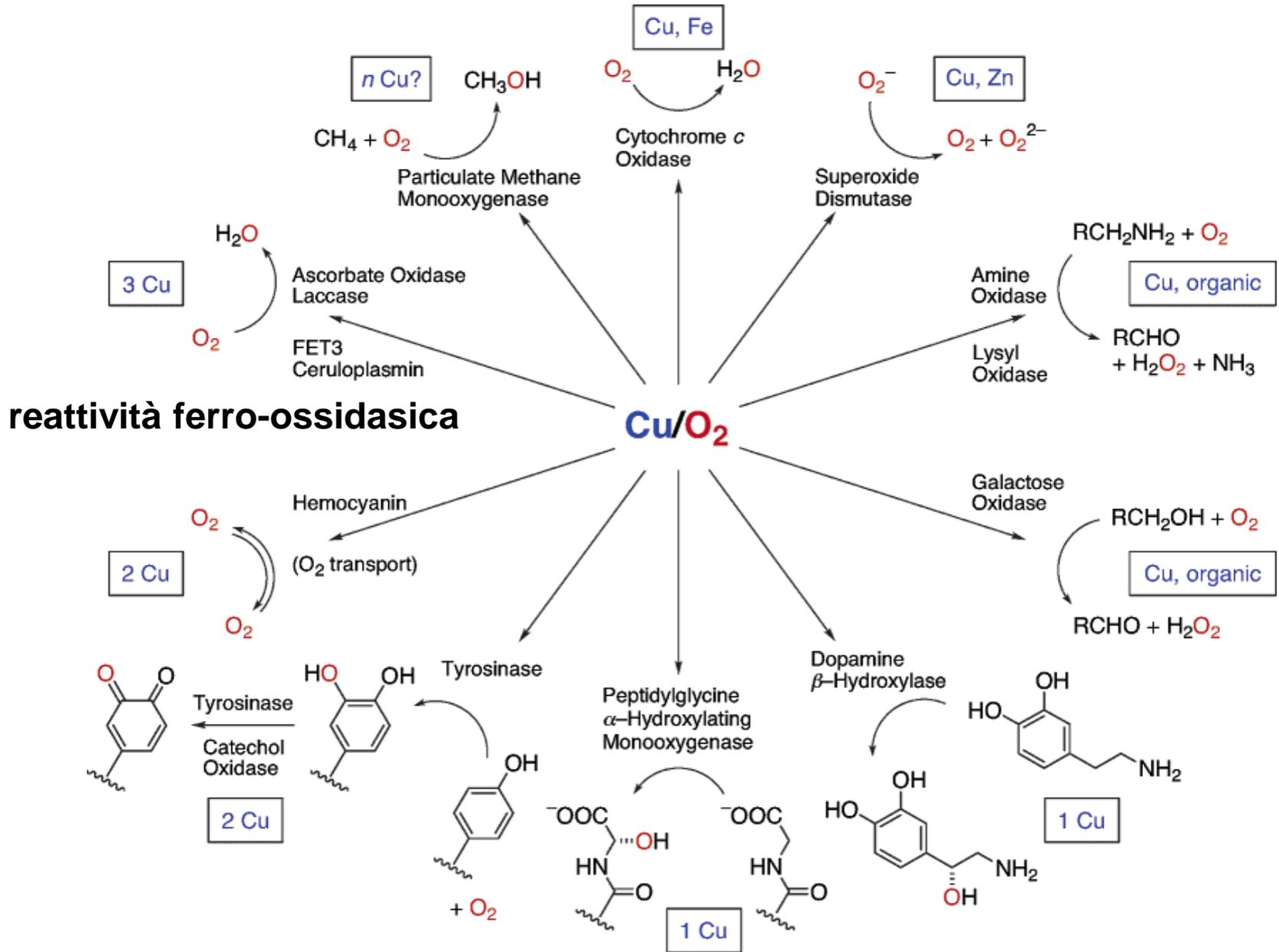
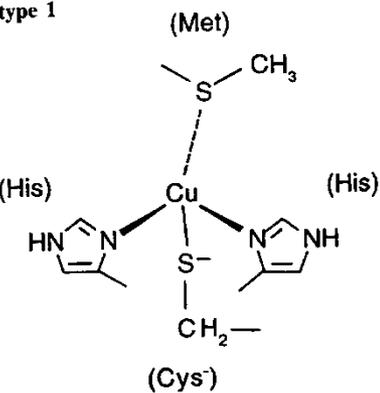
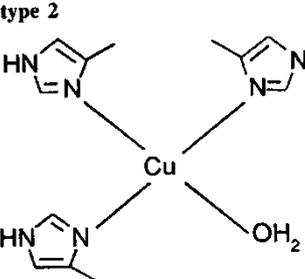
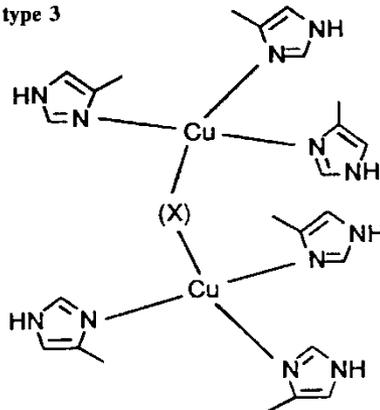


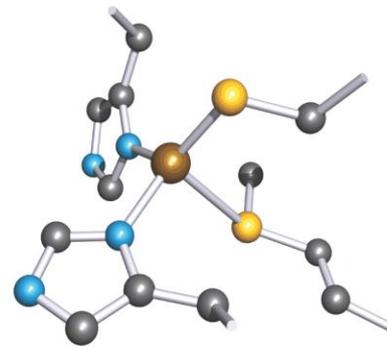
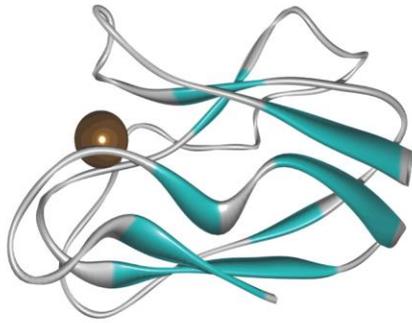
Table 10.2 Characteristics of 'classical' copper centers in protein

generalized coordination geometry	function, structure, characteristics
<p>type 1</p> 	<p>type 1: 'blue' copper centers function: reversible electron transfer $\text{Cu}^{\text{II}} + e^- \rightleftharpoons \text{Cu}^{\text{I}}$ structure: strongly distorted, (3+1) coordination absorption of the copper(II) form at about 600 nm, molar extinction coefficient $\epsilon > 2000 \text{ M}^{-1}\text{cm}^{-1}$; LMCT transition $\text{S}(\text{Cys}^-) \rightarrow \text{Cu}^{\text{II}}$ EPR/ENDOR of the oxidized form: small $^{63,65}\text{Cu}$ hyperfine coupling and g anisotropy, interaction of the electron spin with $-\text{S}-\text{CH}_2^-$; $\text{Cu}^{\text{II}} \rightarrow \text{S}(\text{Cys})$ spin delocalization</p>
<p>type 2</p> 	<p>type 2: normal, 'non-blue' copper function: O_2 activation from the Cu^{I} state in cooperation with organic coenzymes structure: essentially planar with weak additional coordination (Jahn-Teller effect for Cu^{II}) typically weak absorptions of Cu^{II}, $\epsilon < 1000 \text{ M}^{-1} \text{ cm}^{-1}$; ligand-field transitions ($d \rightarrow d$) normal Cu^{II} EPR</p>
<p>type 3</p> 	<p>type 3: copper dimers function: O_2 uptake from the $\text{Cu}^{\text{I}}\text{-Cu}^{\text{I}}$ state structure: (bridged) dimer, Cu-Cu distance about 360 pm after O_2 uptake intense absorptions around 350 and 600 nm, $\epsilon \approx 20000$ and $1000 \text{ M}^{-1}\text{cm}^{-1}$; LMCT transitions $\text{O}_2^{2-} \rightarrow \text{Cu}^{\text{II}}$ EPR-inactive Cu^{II} form (antiferromagnetically coupled d^9 centers)</p>

Plastocianina

(da spinaci)

10.5 kDa,
ca. 100 a.a.



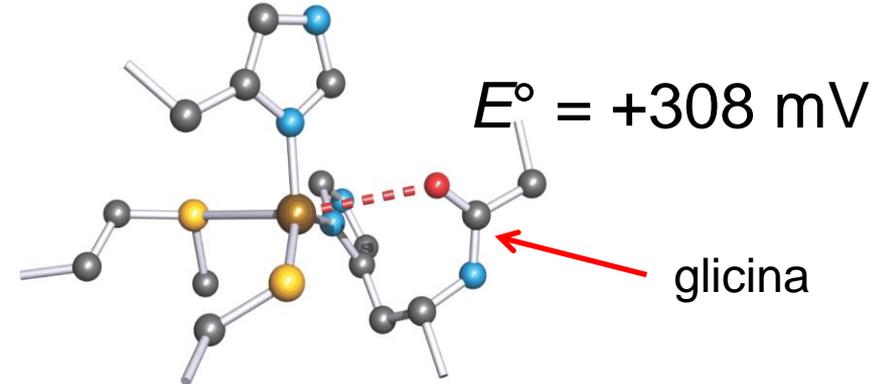
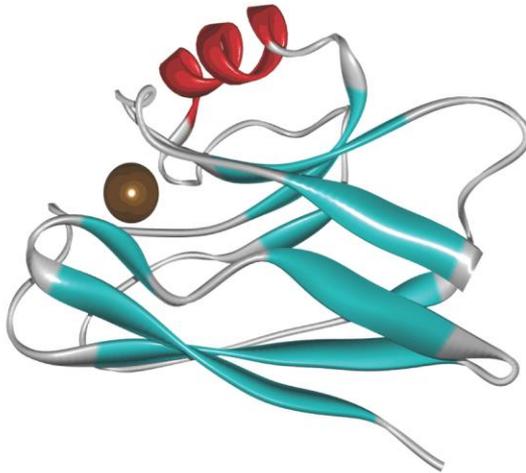
$$E^{\circ} = +370 \text{ mV}$$

Coordinazione 3 + 1

Azurina

(da batteri)

14.5 kDa,
ca. 130 a.a.



$$E^{\circ} = +308 \text{ mV}$$

glicina

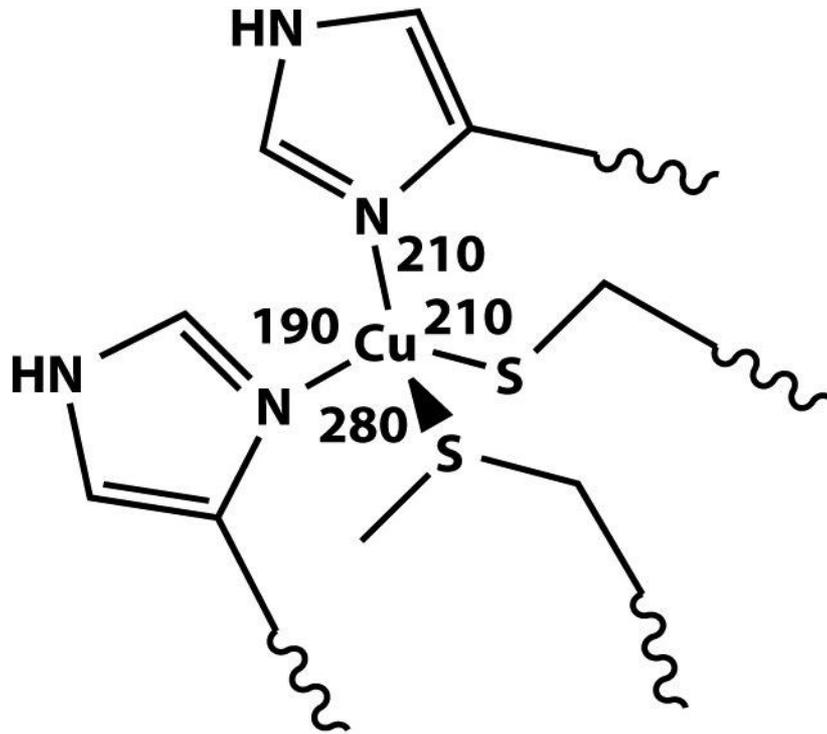
Coordinazione 3 + 1 + 1

$$E^{\circ}_{\text{Cu(II)/Cu(I)}} = +153 \text{ mV}$$

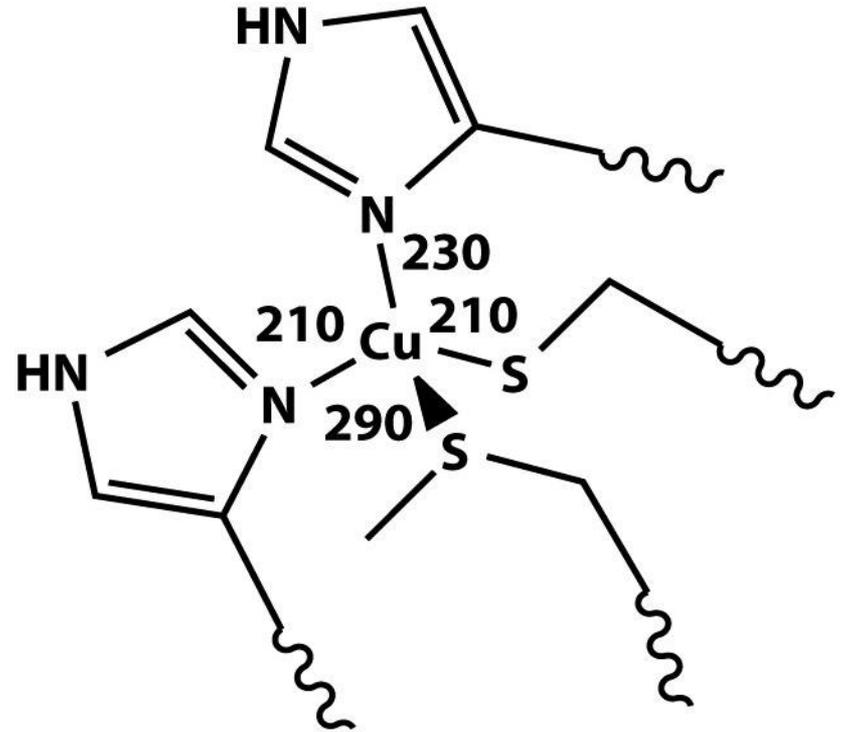
Blue copper proteins

Forte assorbimento a ca. 600 nm, LMCT da Cys⁻ a Cu(II)

Esempio di stato entatico



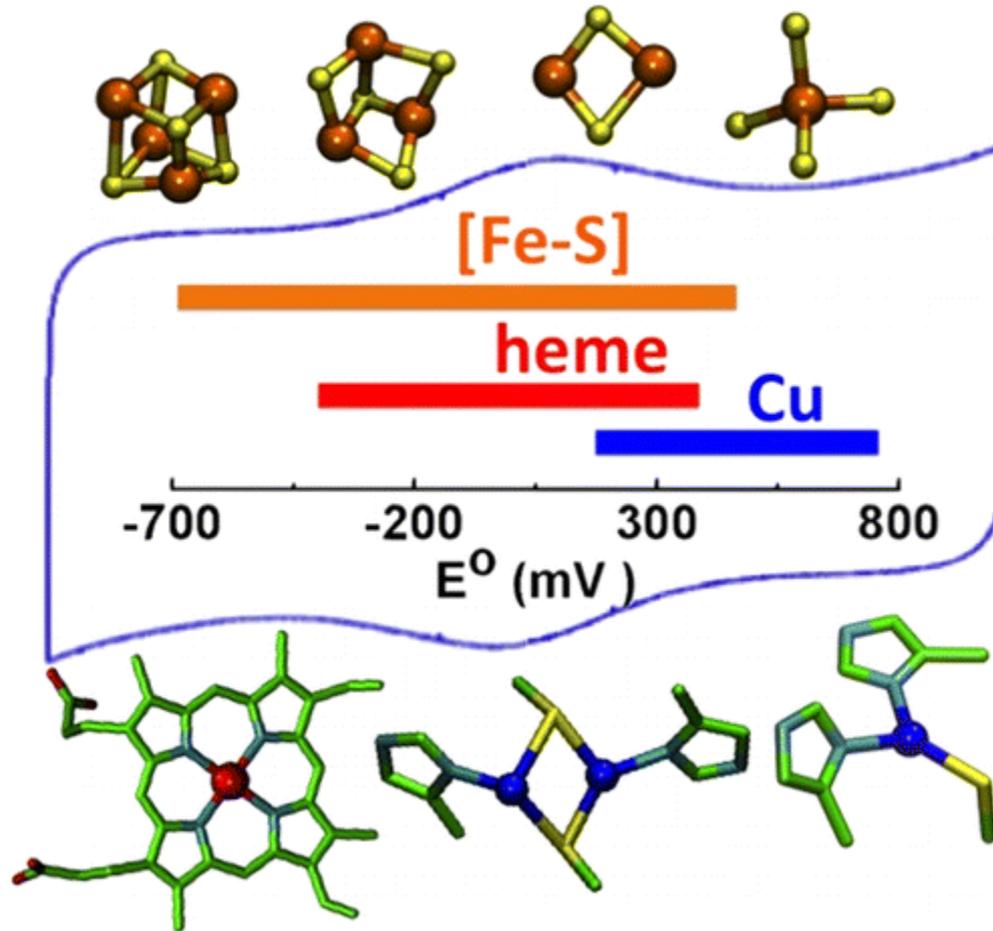
Oxidized plastocyanin



Reduced plastocyanin

*velocità di trasferimento elettronico dell'ordine 10^3 – 10^7 $M^{-1} s^{-1}$
(rispetto a $5 \times 10^{-7} M^{-1} s^{-1}$ per la coppia $Cu(II)/Cu(I)$ acquosa)*

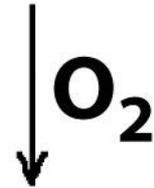
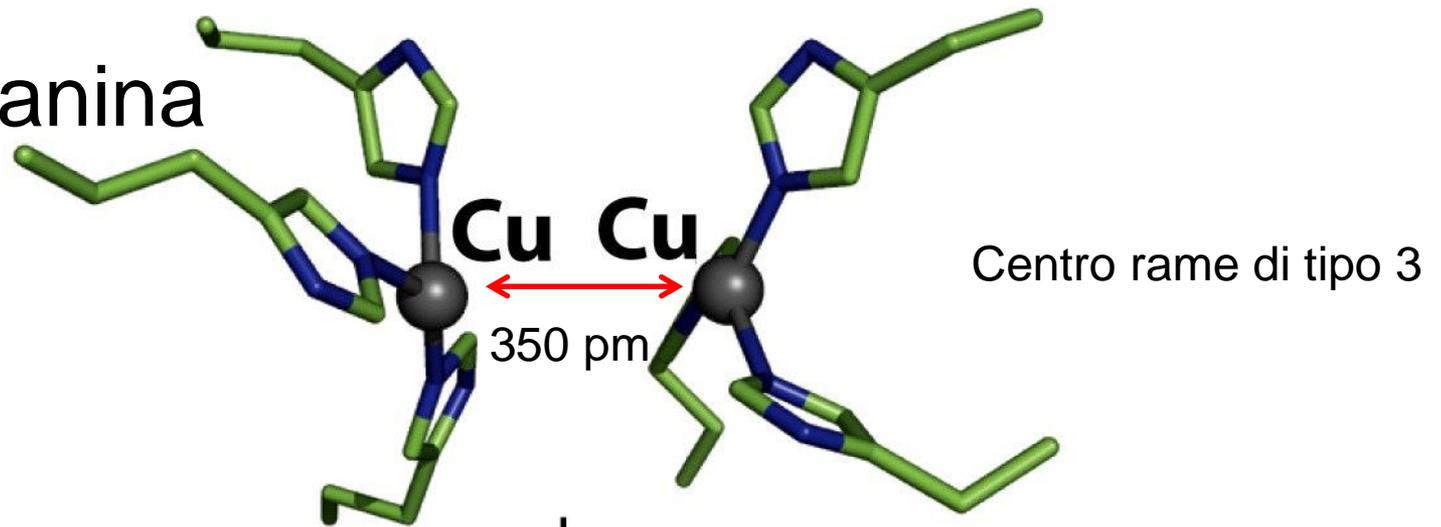
Metallo-proteine per trasferimento elettronico



Emocianina

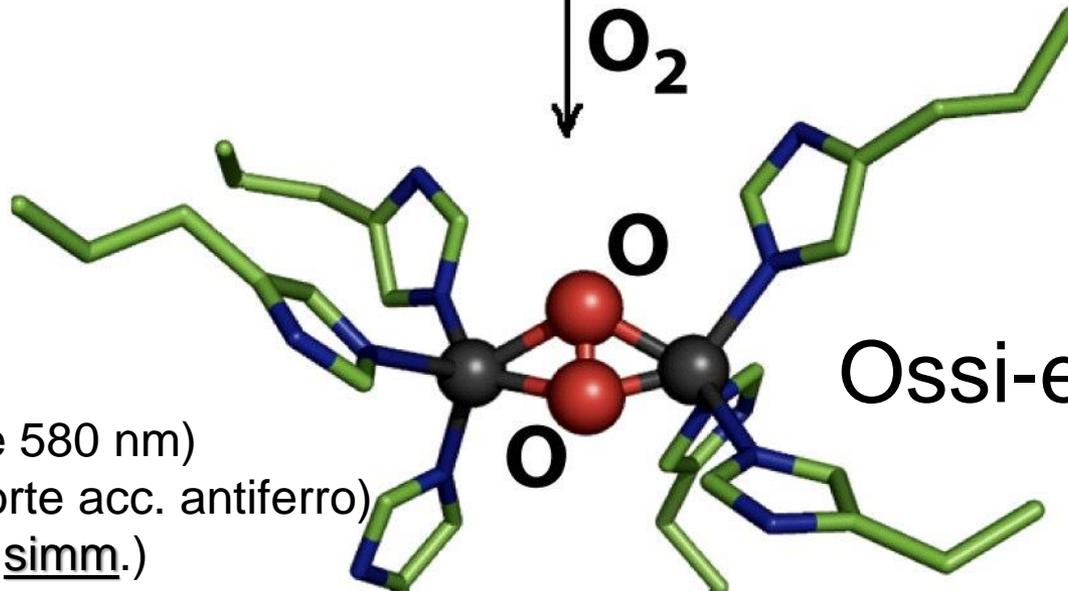
fino a 1500 kDa,
ogni unità 75 kDa

Incolore
2 Cu(I) ($S = 0$)



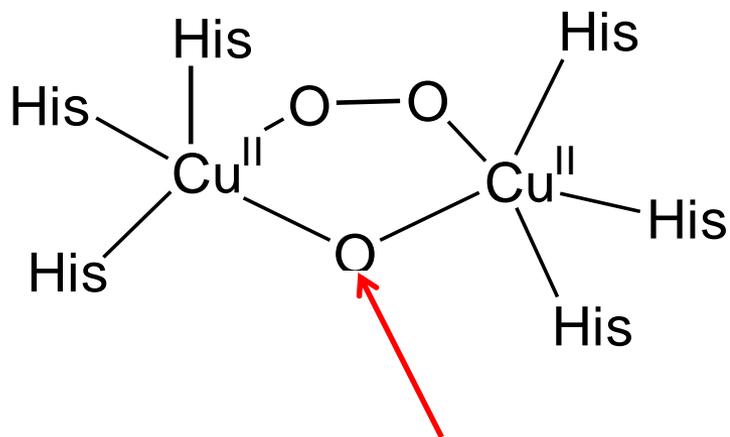
Ossi-emocianina

Viola (LMCT 350 e 580 nm)
2 Cu(II) (diamg., forte acc. antiferro)
IR: 755 cm^{-1} (O_2^{2-} , simm.)

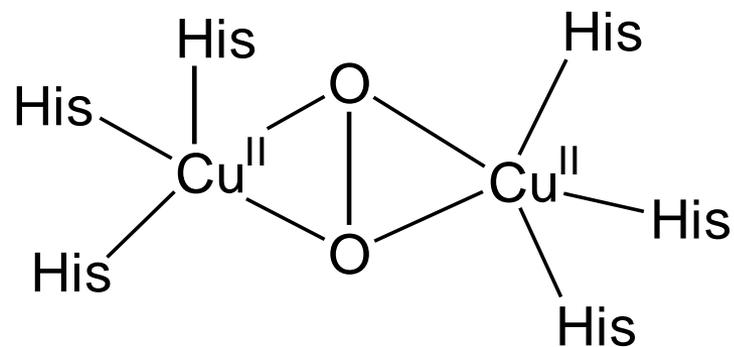


trasporto di O_2 di molluschi (lumache, calamari) e artropodi
(granchi, aragoste, gamberi, scorpioni)

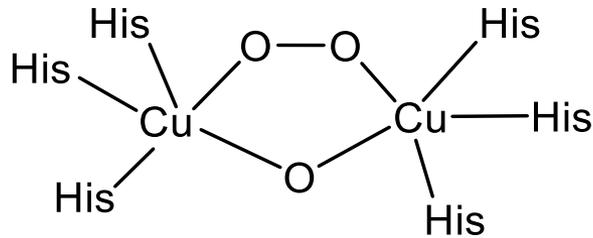
Possibili coordinazioni simmetriche dello ione perossido



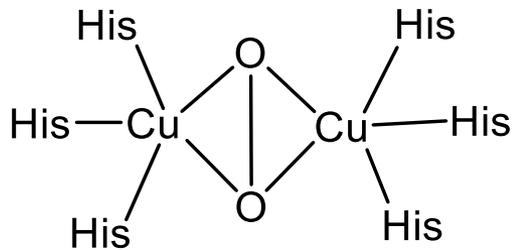
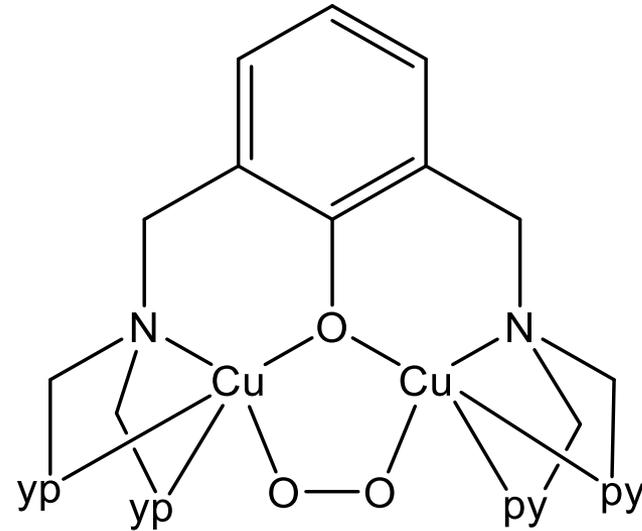
tirosinato?



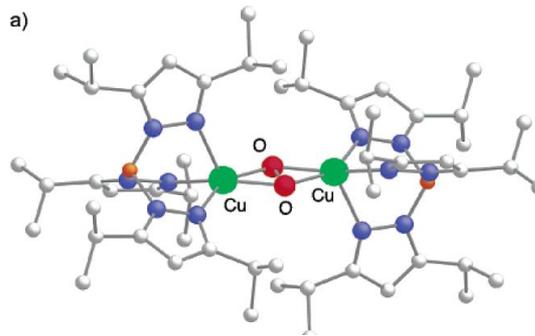
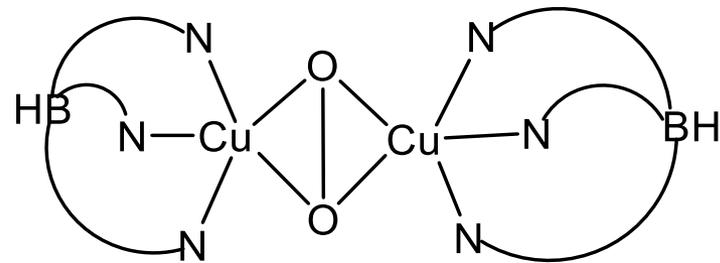
Modelli per la coordinazione di O₂ alla emocianina



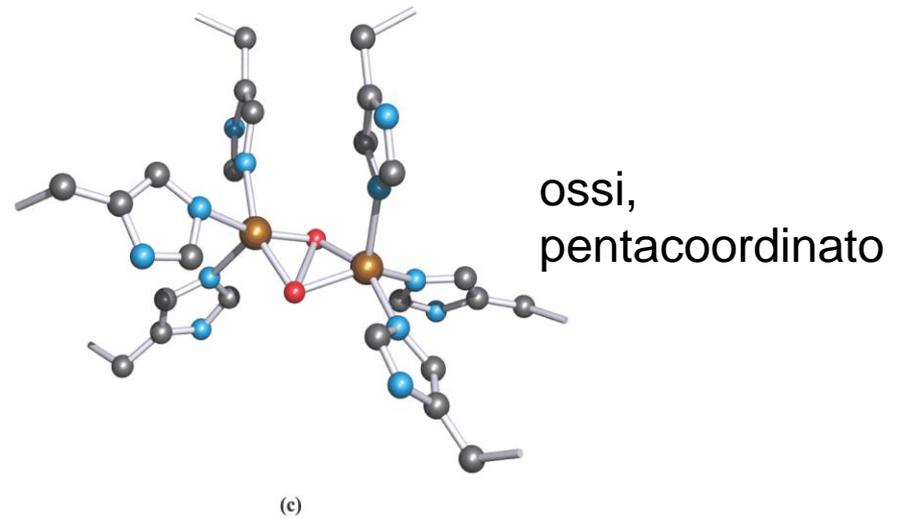
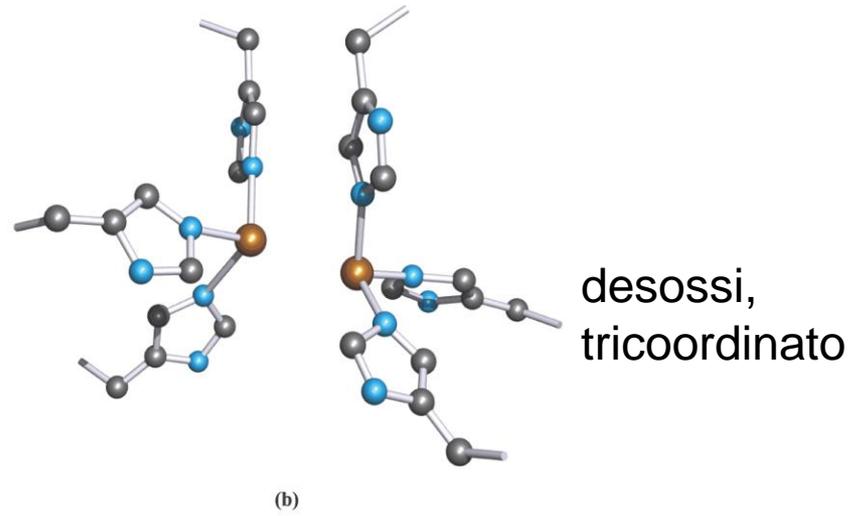
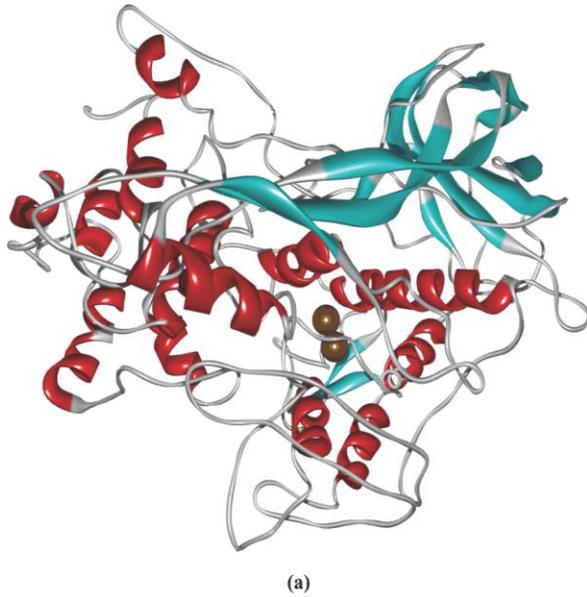
ipotesi sbagliata

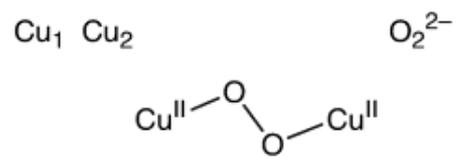
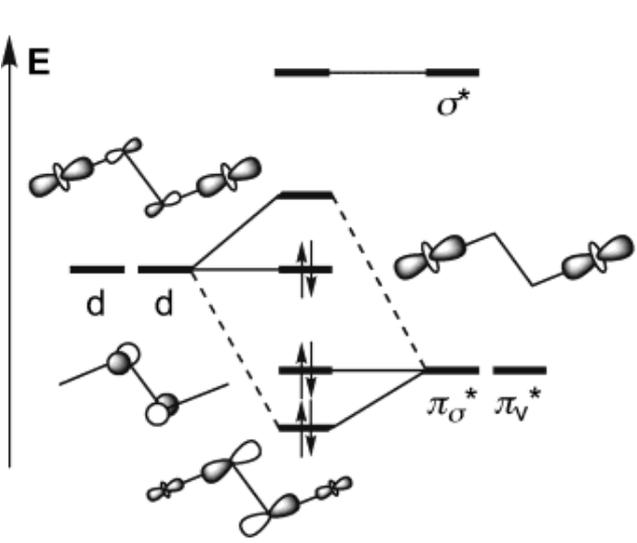


ipotesi corretta ^{a)}

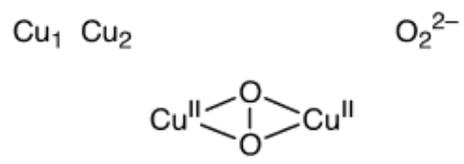
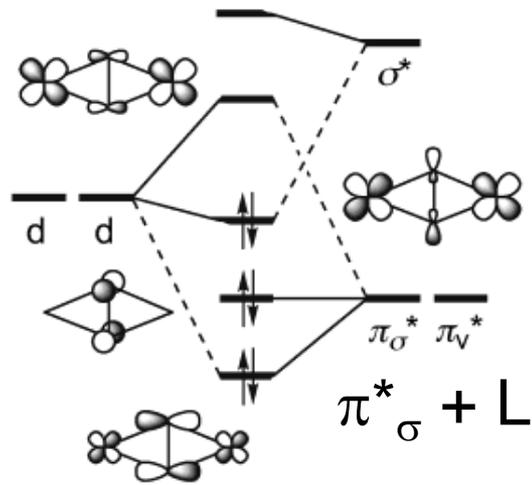
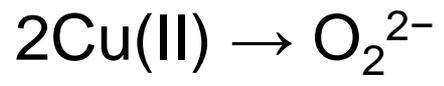


Emocianina

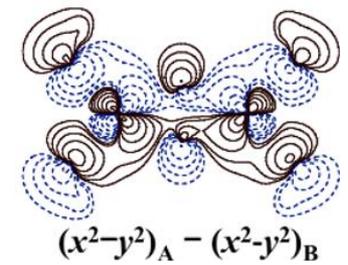
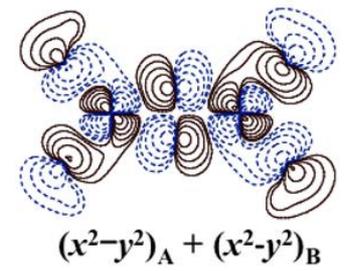
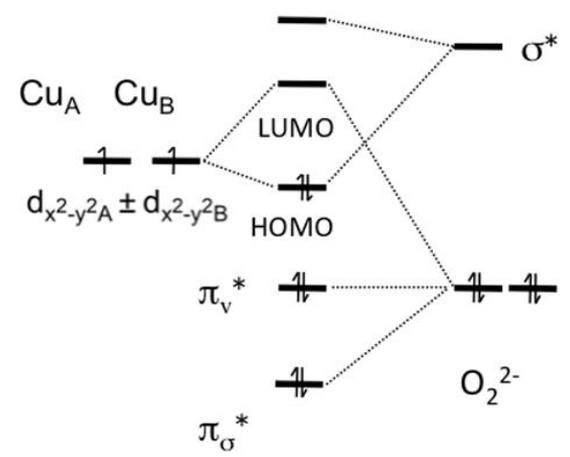
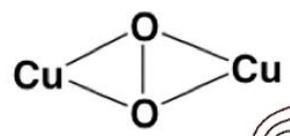
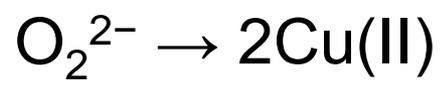




$\sigma^* + \text{HOMO Cu } (d_{x^2-y^2} - d_{x^2-y^2})$

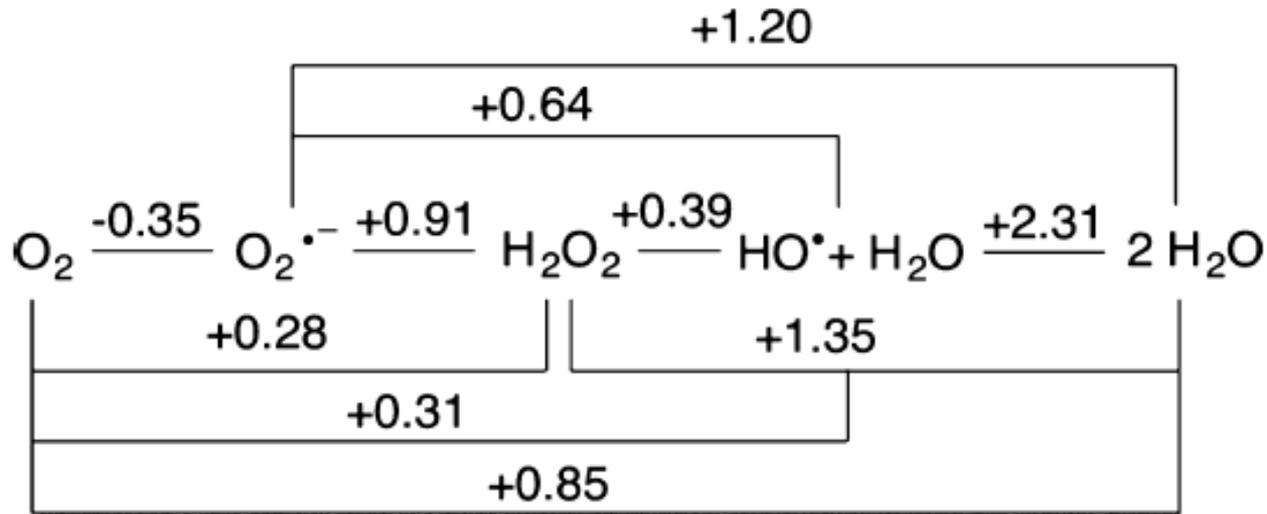


$\pi^*_\sigma + \text{LUMO Cu } (d_{x^2-y^2} + d_{x^2-y^2})$



Enzimi al Cu che attivano o riducono O₂

pH = 7

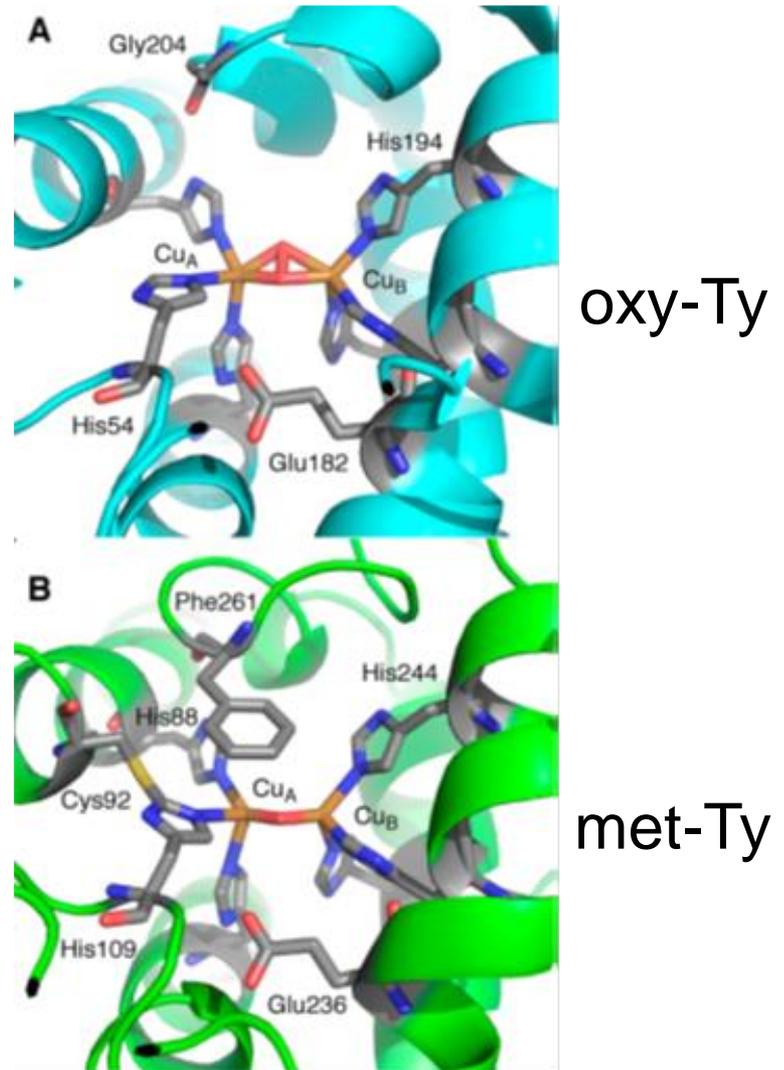
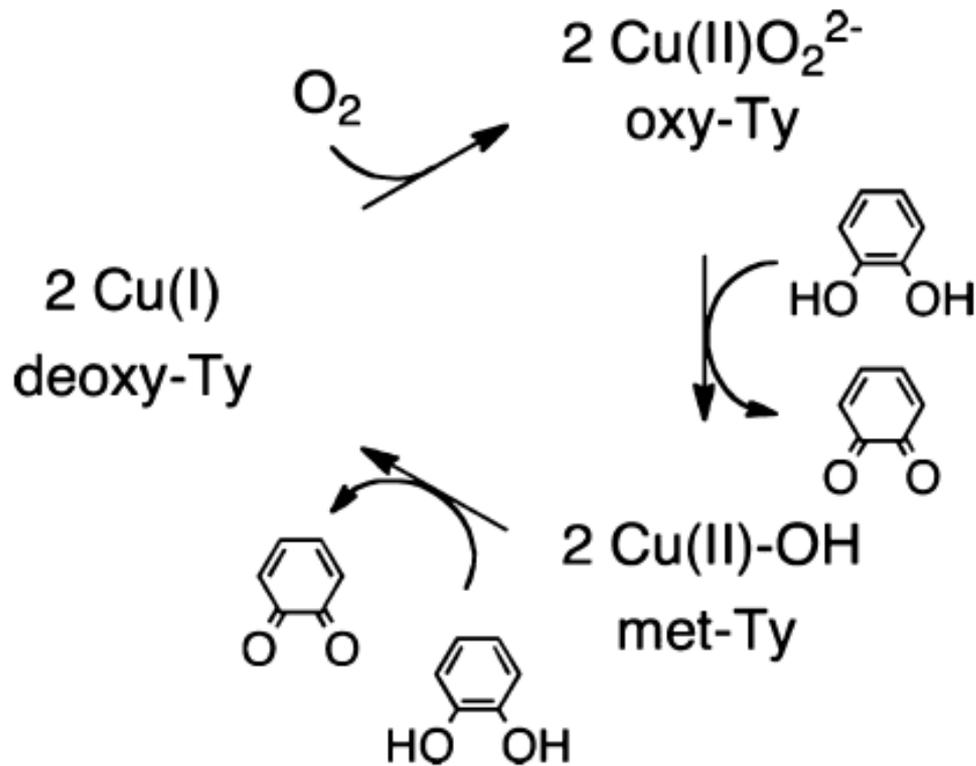
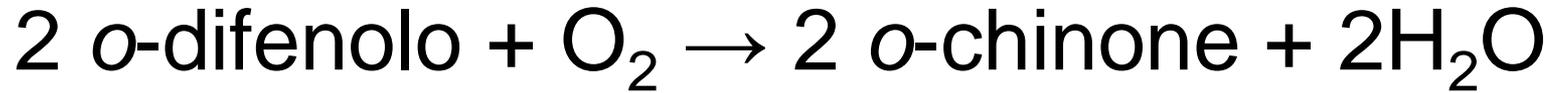


...la riduzione mono-elettronica di O₂ a superossido è termodinamicamente sfavorita

fornire due elettroni richiede o la presenza di almeno due ioni Cu oppure di un Cu e di un cofattore organico redox-attivo

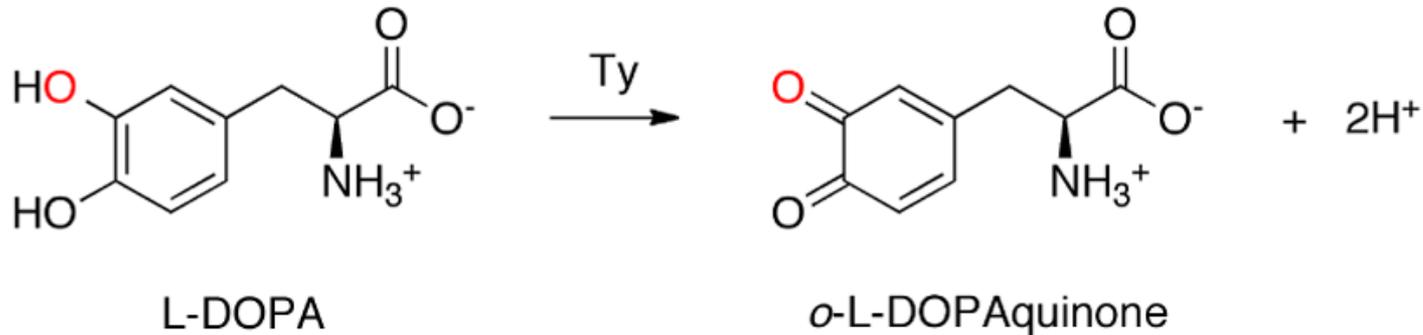
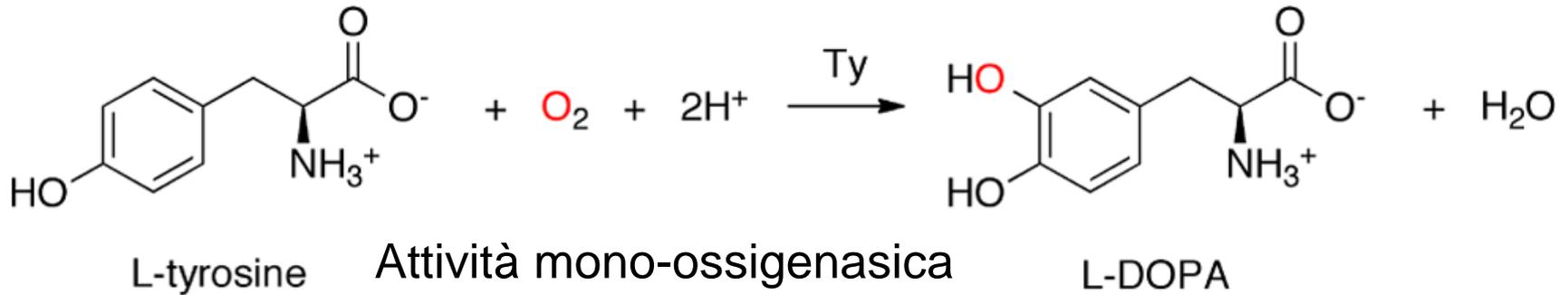
Polifenolo ossidasi

Tirosinasi, Catecolo-ossidasi



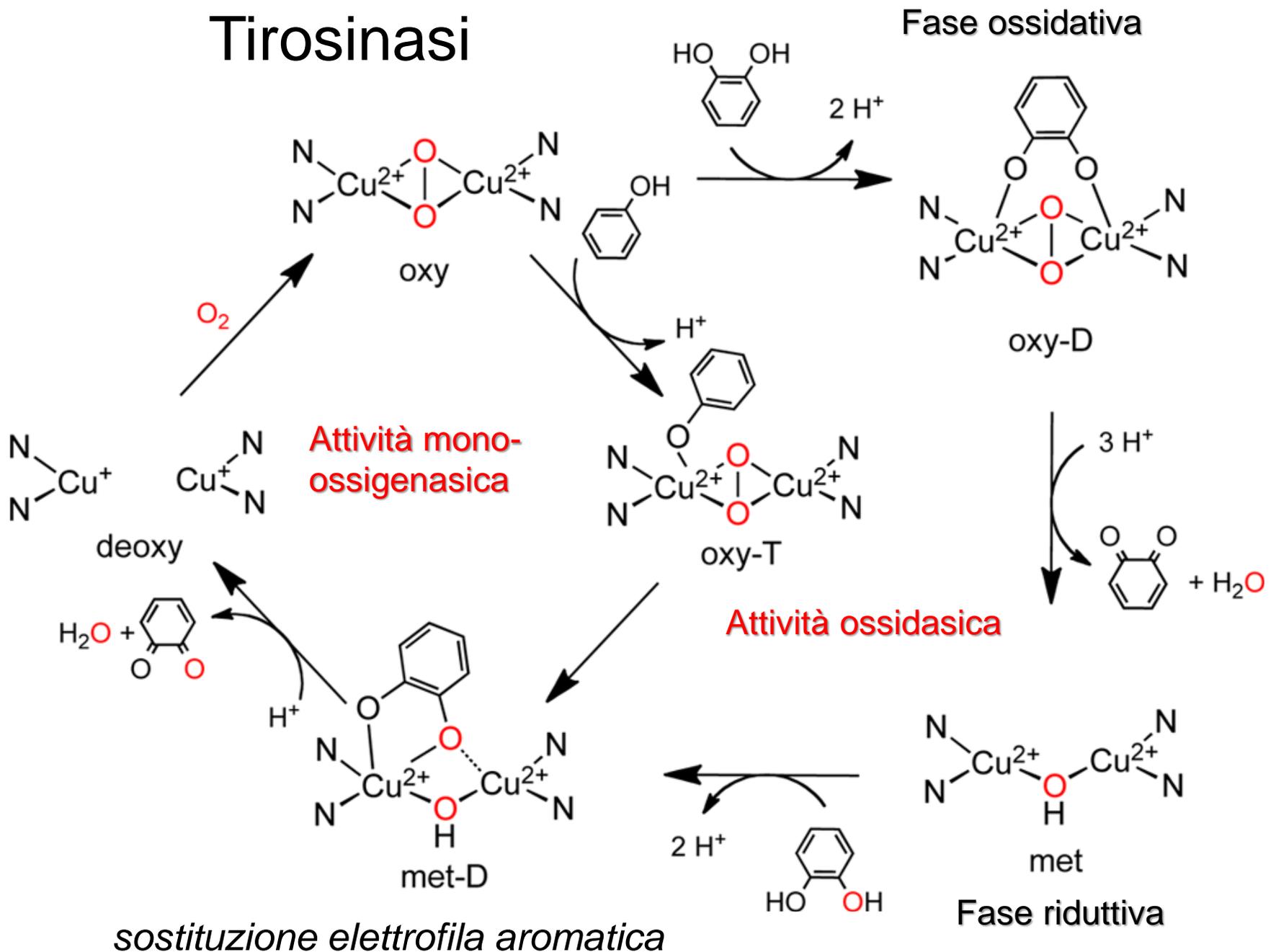
Nel ciclo vengono trasferiti 4 elettroni ai 2O

Tirosinasi come mono-ossigenasi



Attività ossidasica

Tirosinasi



Multicopper oxidases, MCOs (ossidasi azzurre)

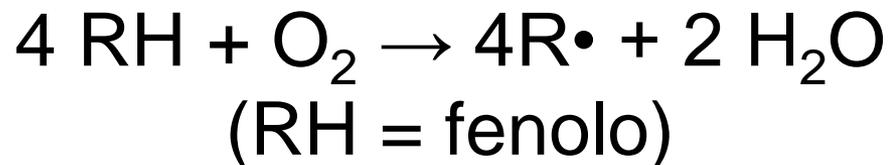
Almeno 4 Cu

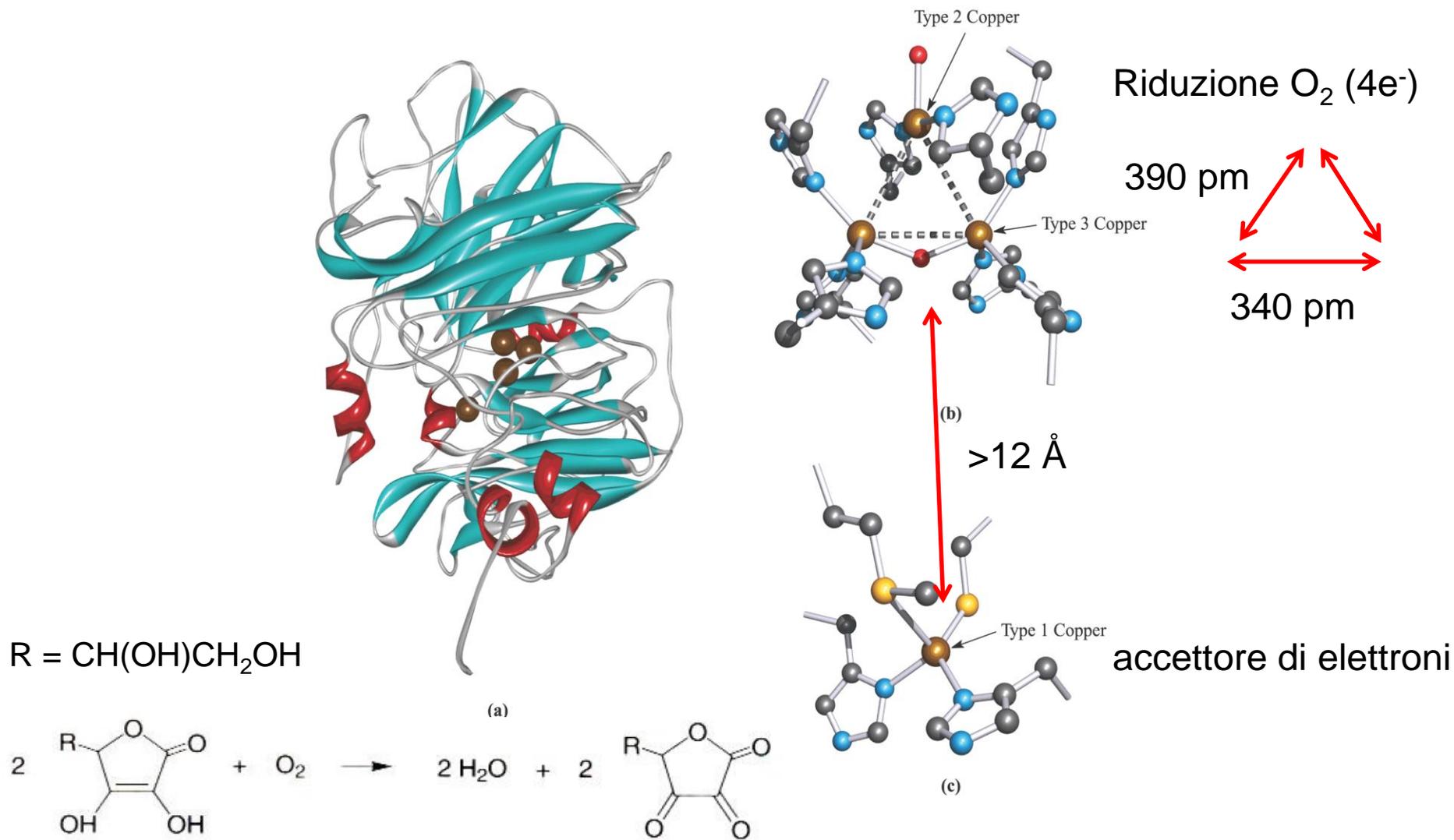
Substrati organici

Ascorbato ossidasi
Laccasi
+500 – +800 mV

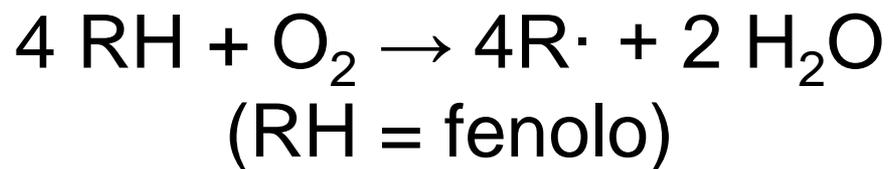
Substrati «metallici»

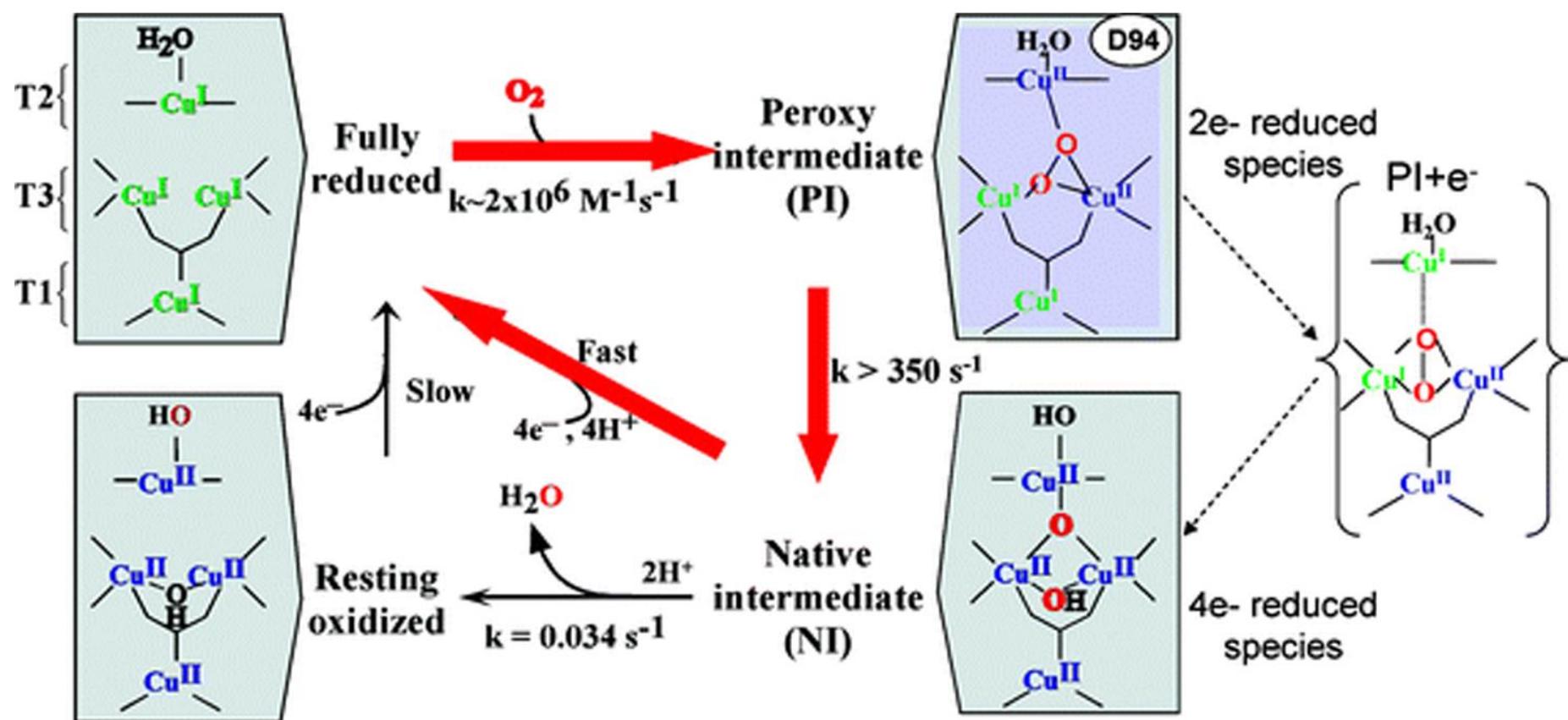
Ceruloplasmina
Epeptina
Fet3p
Rame-ossidasi (CueO)



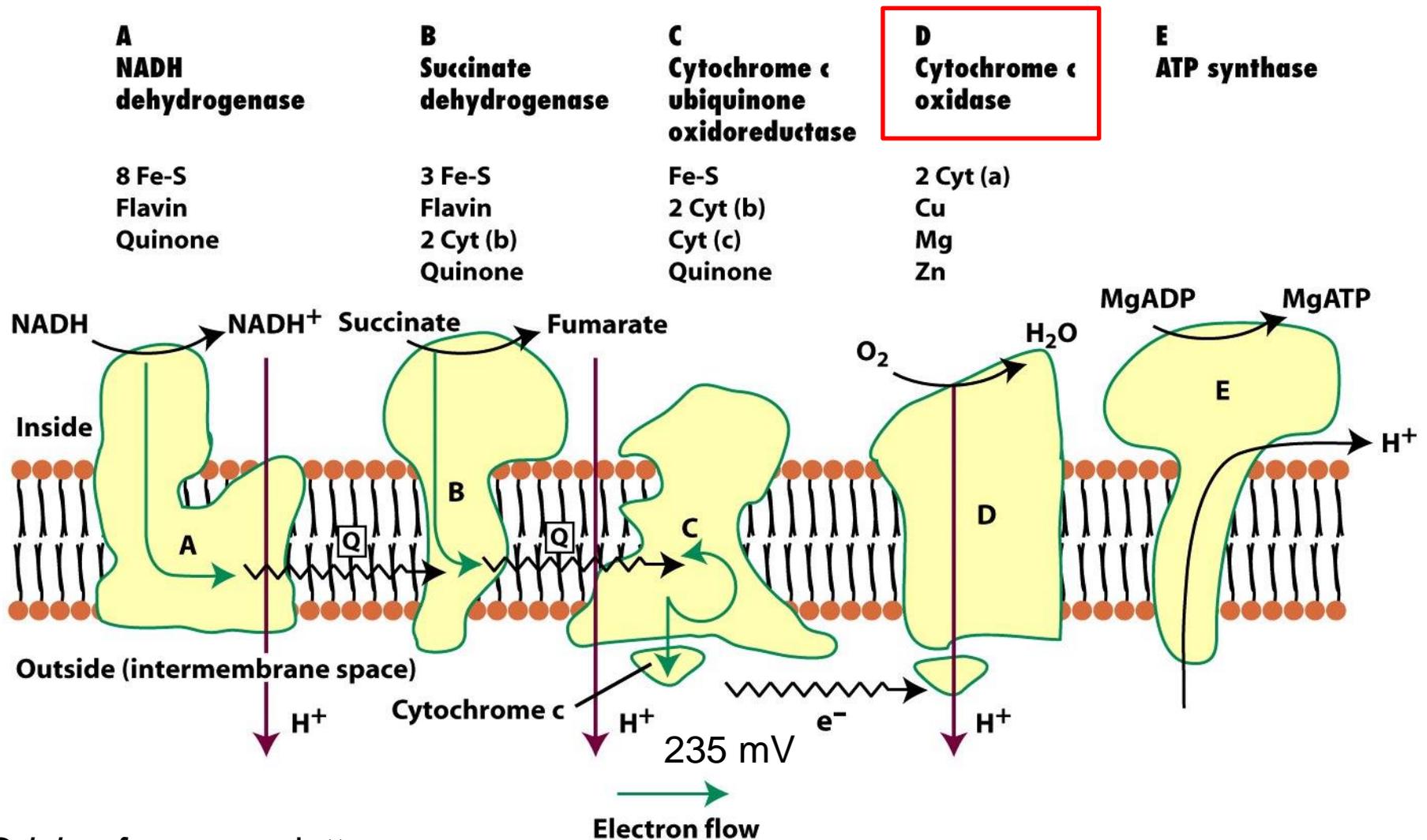


Ascorbato ossidasi (da zucchini)

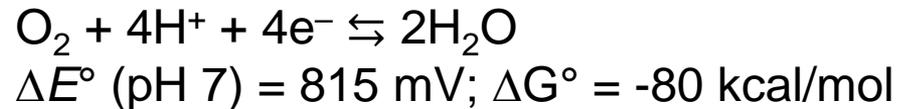




Catena respiratoria (fosforilazione ossidativa)

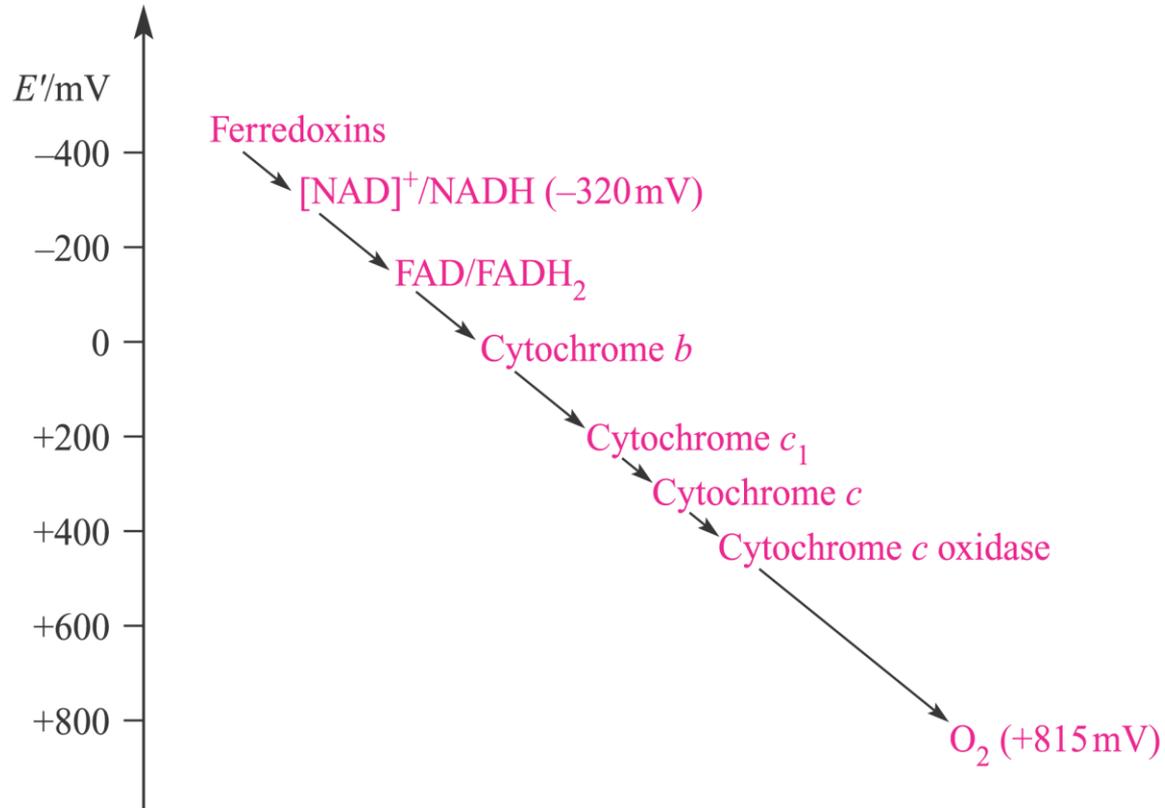


Driving force per electrone:
 $815 - 235 = 580 \text{ mV}$ (ca. 13 kcal/mol)

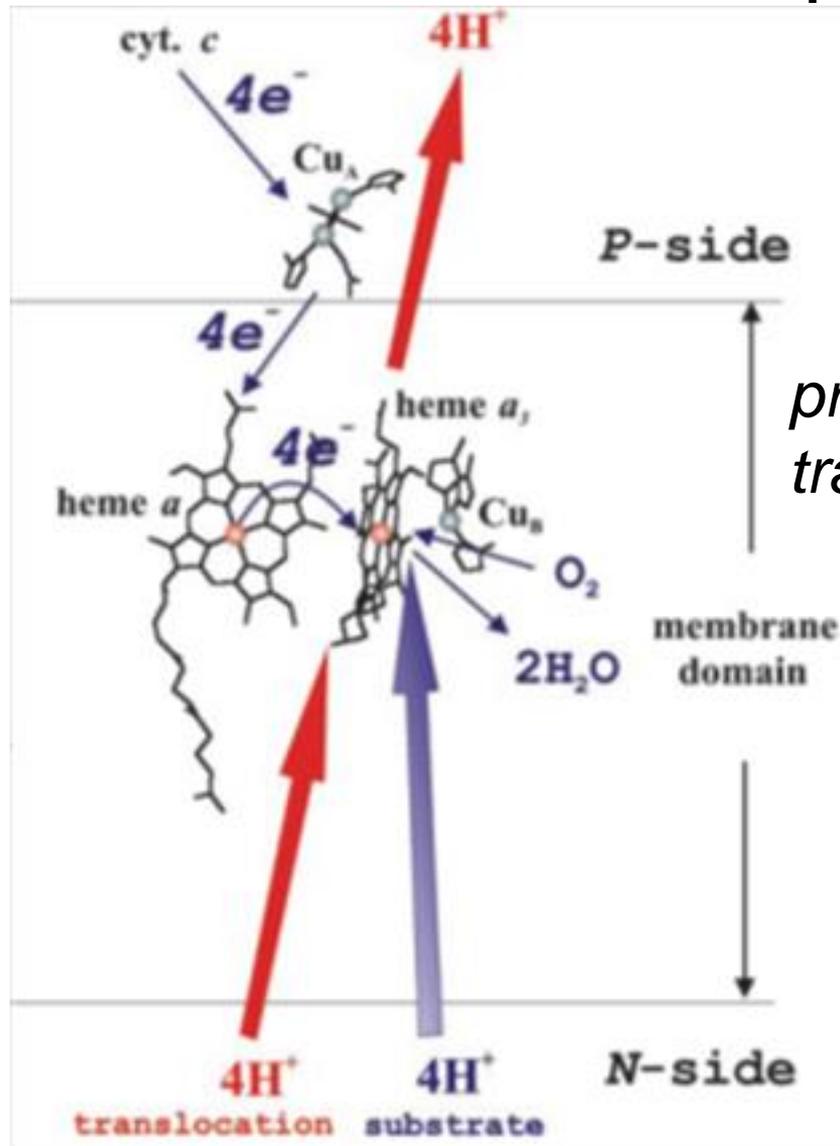


Ossidazione del Fe²⁺ in Cyt c

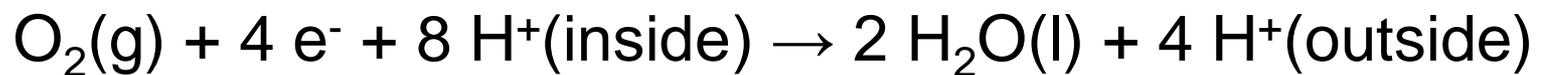
La sequenza di trasferimenti elettronici nei mitocondri



La CcO è anche una pompa di protoni

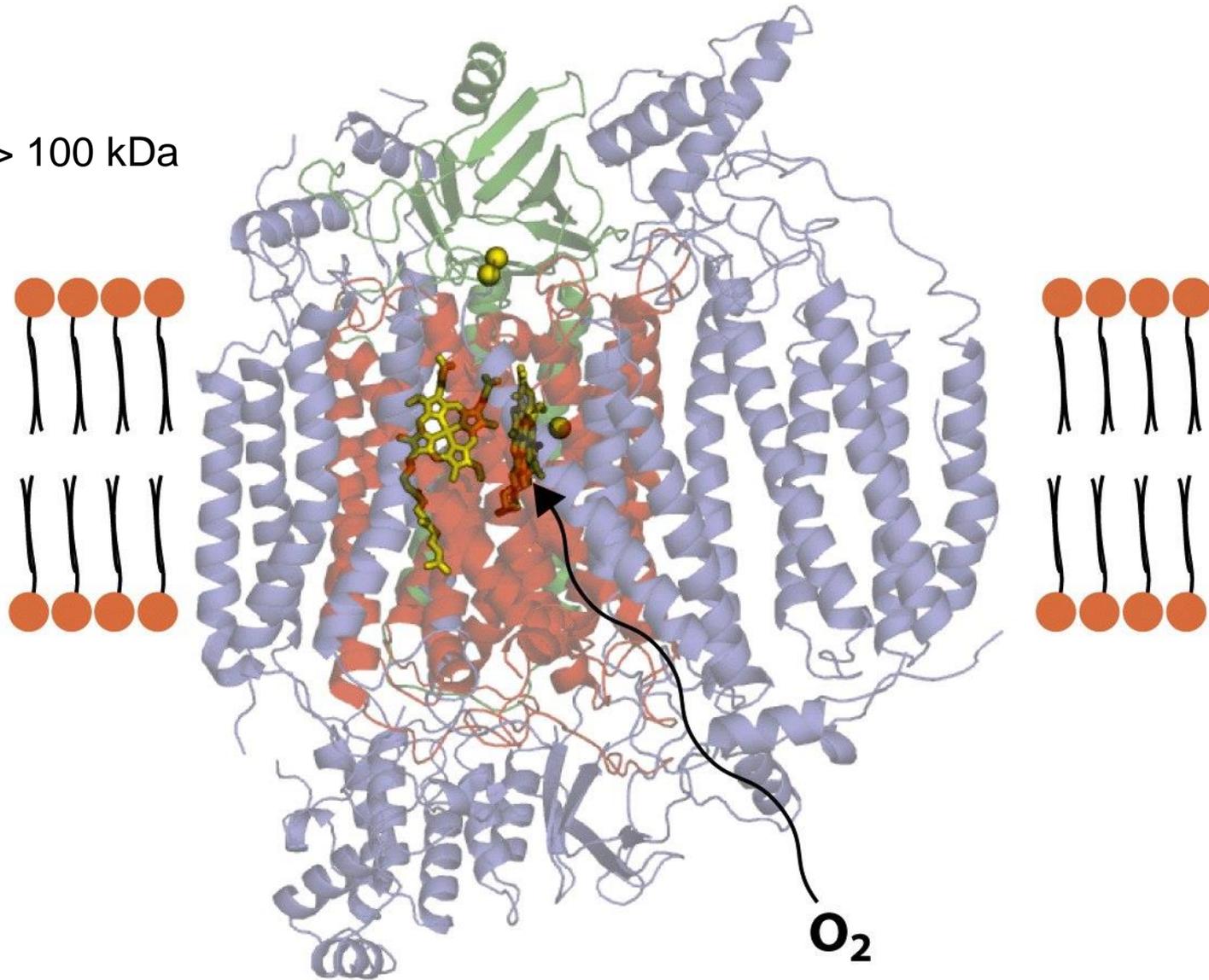


proton coupled electron transfer, PCET

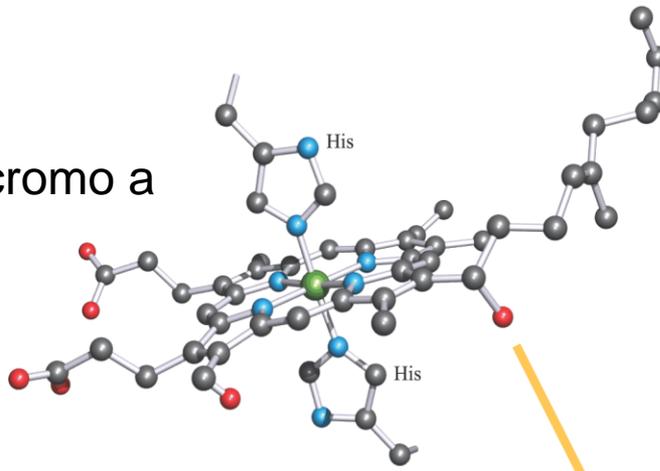


Reacts with cytochrome C

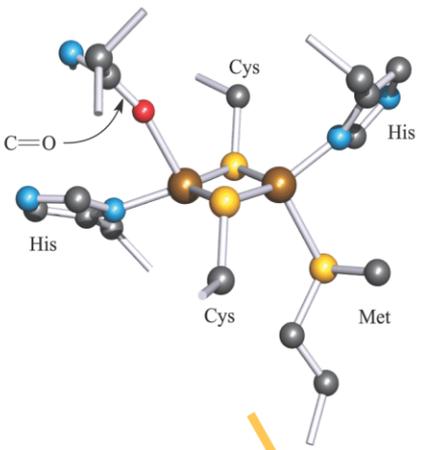
> 100 kDa



citocromo a

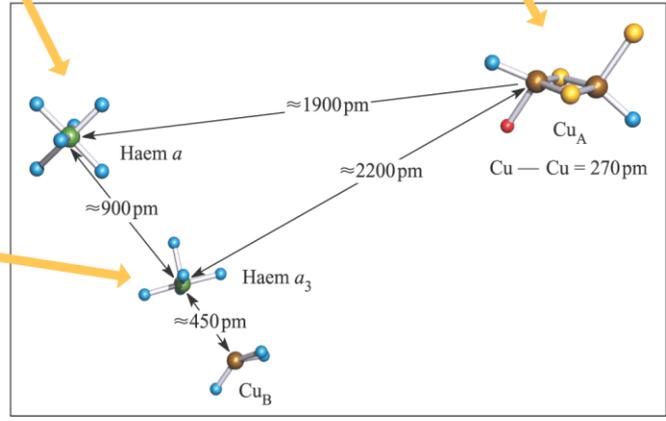
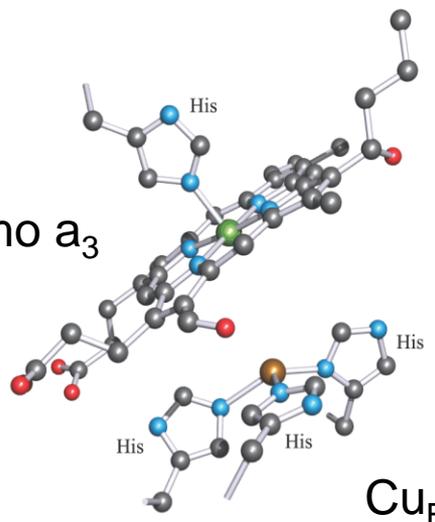


Peptide C=O
of Gly

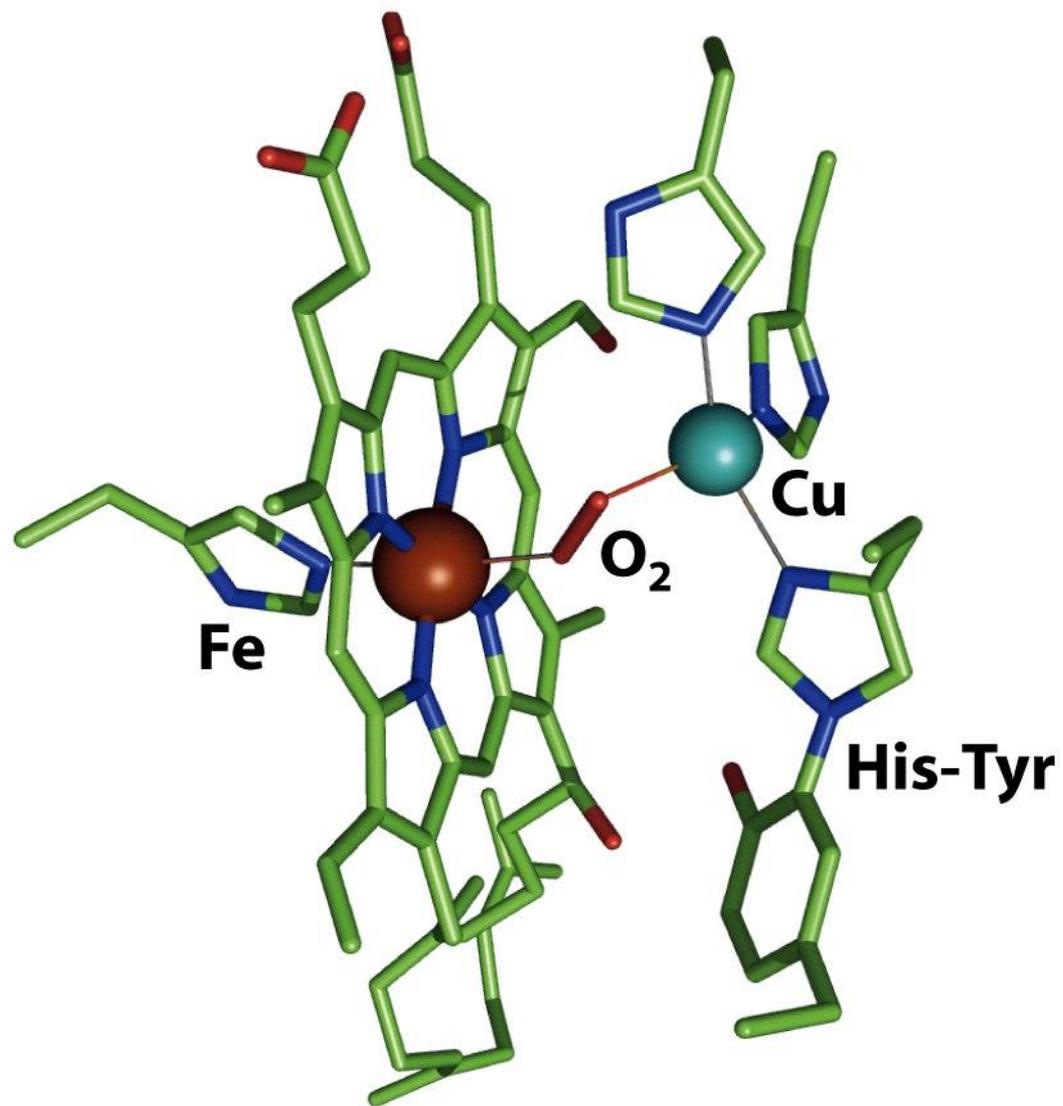


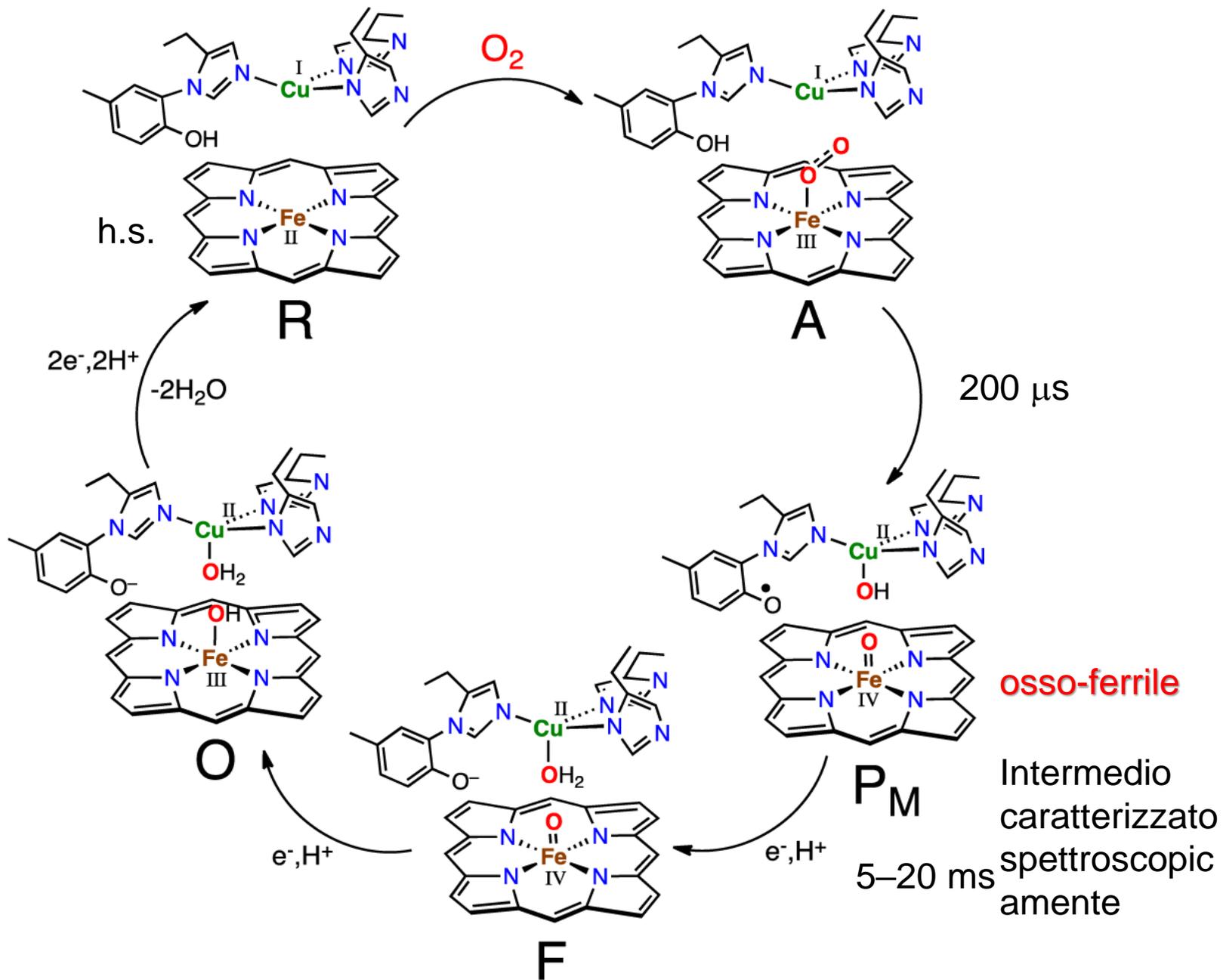
Cu_A

citocromo a₃

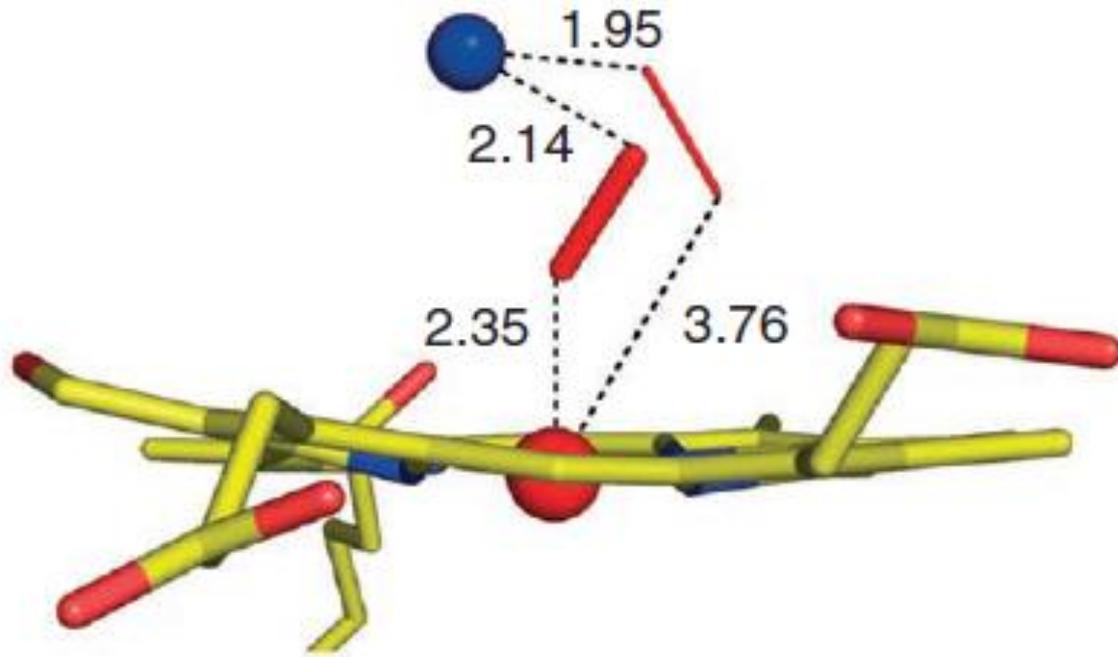


Cu_B (rame tipo 2)

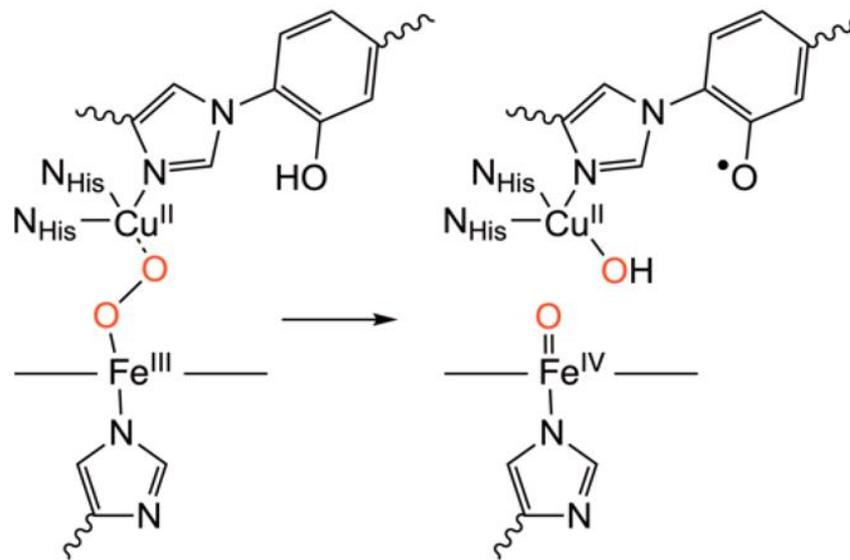




Intermedio perossidico *X-ray free-electron laser (XFEL)*

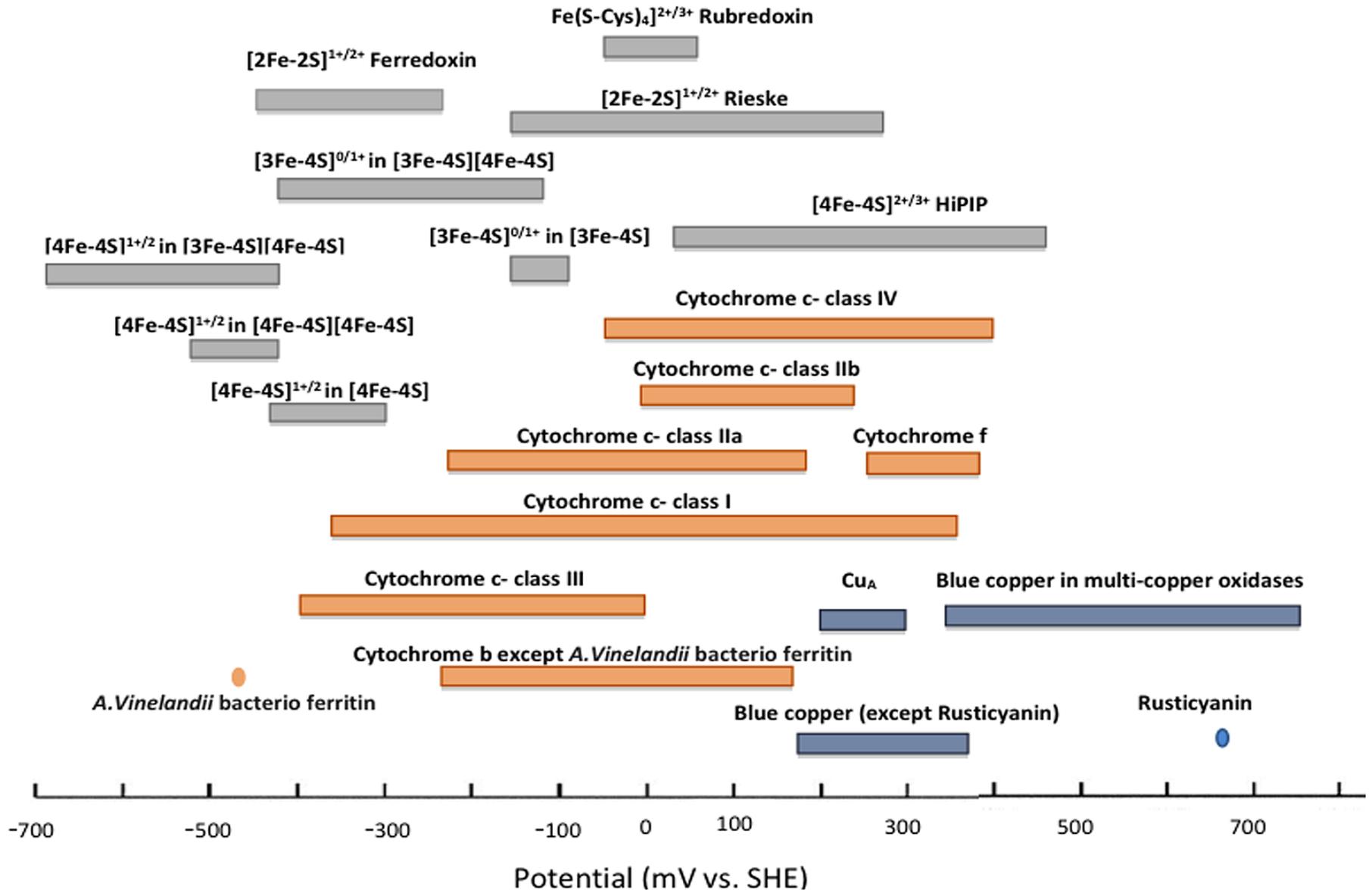


Nature, 2014



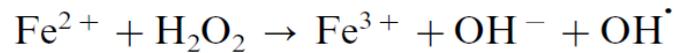
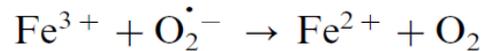
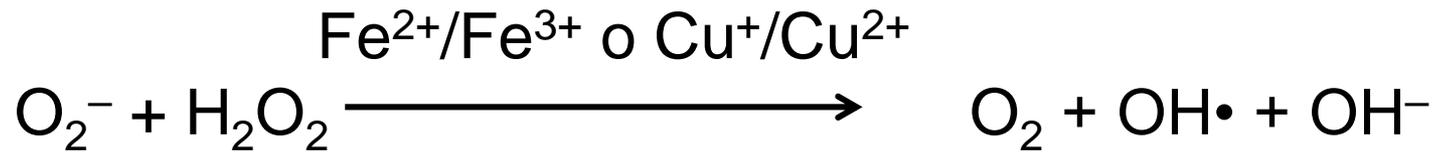
(a) Proposed O-O cleavage mechanism in CcO

Metallo-proteine per trasferimento elettronico



Reactive Oxygen Species (ROS)

Reazione di Haber-Weiss

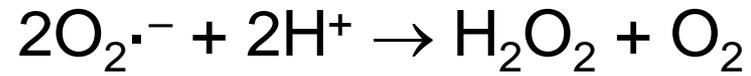


Reazione di Fenton

The net reaction:



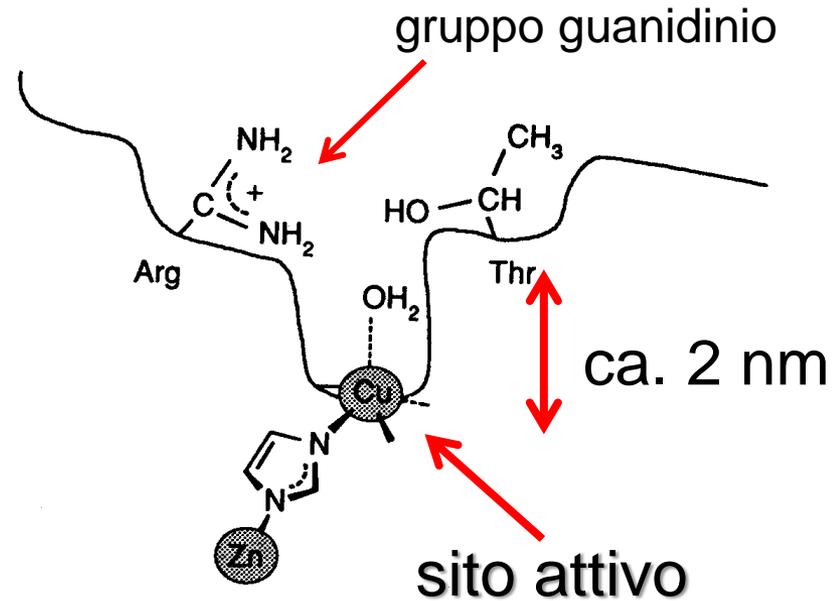
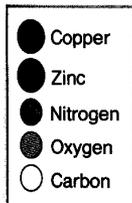
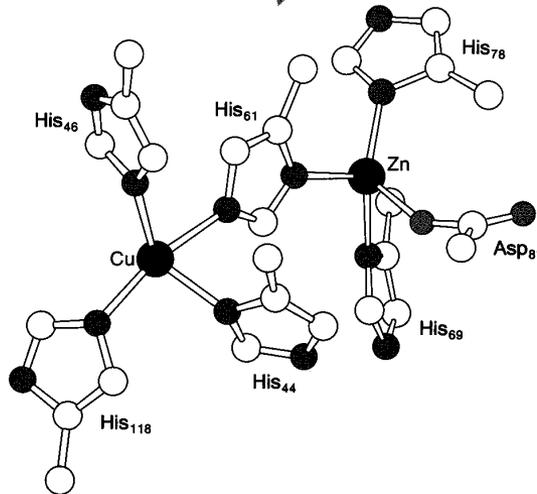
Cu-Zn superossido dismutasi



Velocità quasi-diffusiva



16 kDa



Ciclo catalitico della superossido dismutasi

