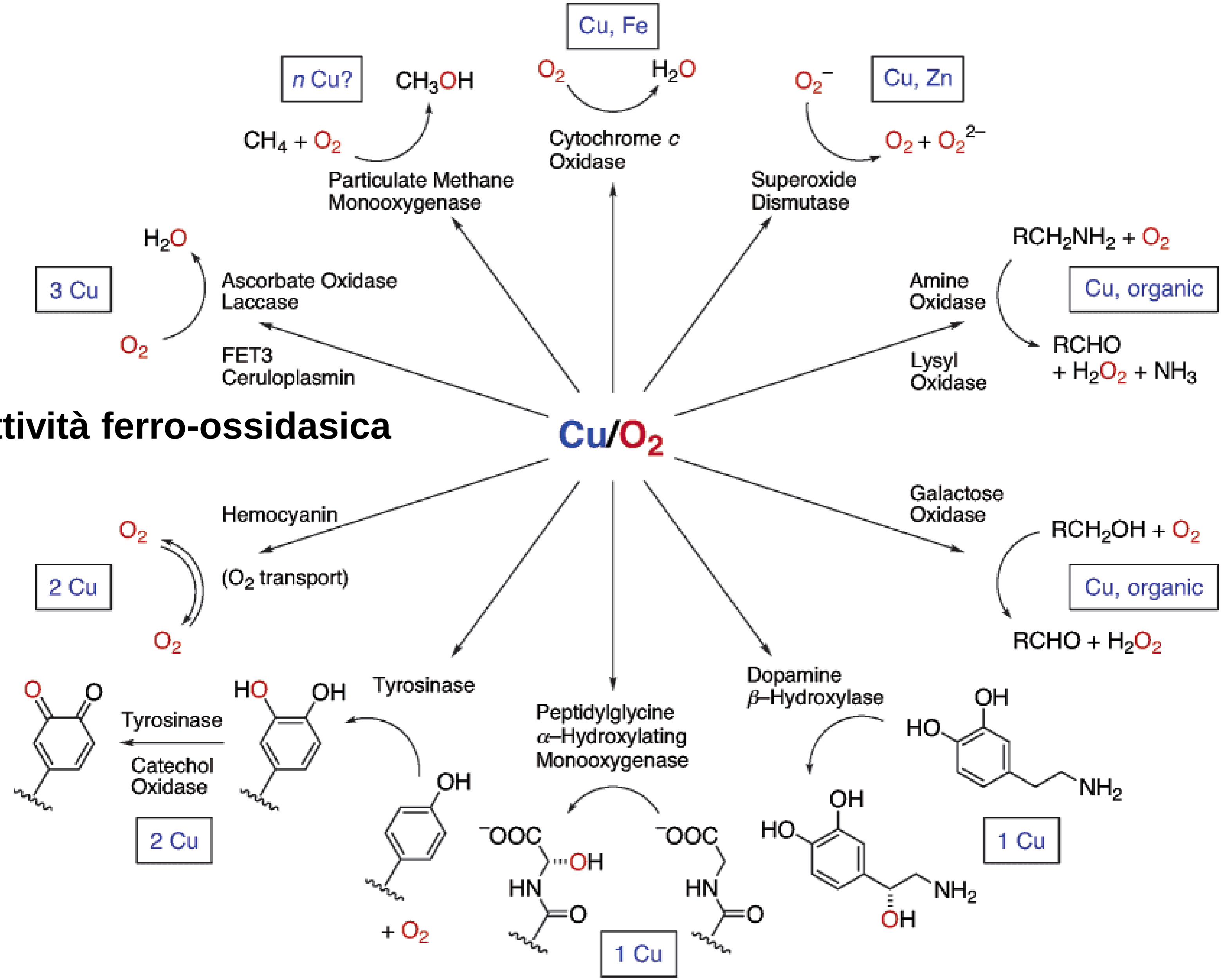
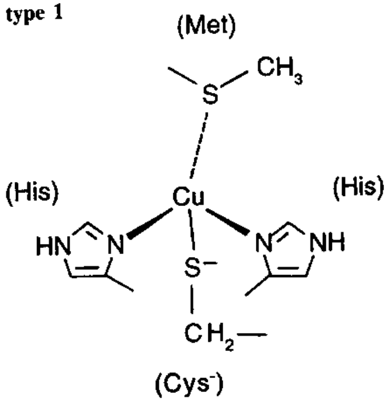
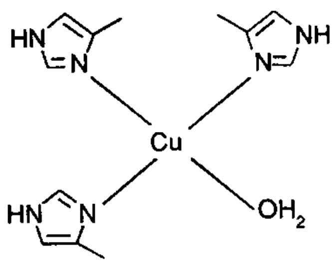
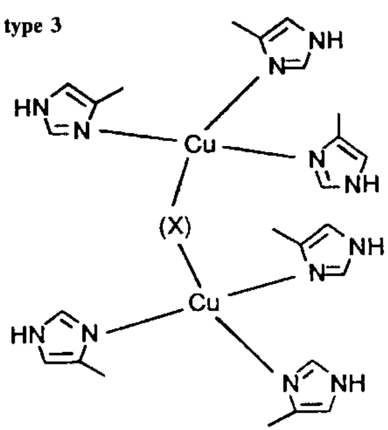
Proteine al rame



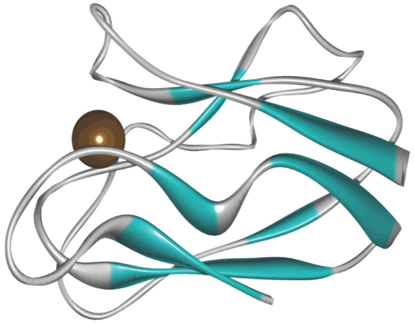
reattività ferro-ossidasica

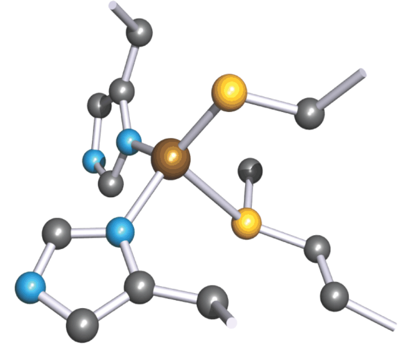
Table 10.2 Characteristics of 'classical' copper centers in protein

generalized coordination geometry	function, structure, characteristics
type 1  	**type 1**: 'blue' copper centers function: reversible electron transfer $Cu^{II} + e^- \rightleftharpoons Cu^I$ structure: strongly distorted, (3+1) coordination absorption of the copper(II) form at about 600 nm, molar extinction coefficient $\varepsilon > 2000\ M^{-1}cm^{-1}$; LMCT transition $S(Cys^-) \rightarrow Cu^{II}$ EPR/ENDOR of the oxidized form: small $^{63,65}Cu$ hyperfine coupling and g anisotropy, interaction of the electron spin with $-S-CH_2-$; $Cu^{II} \rightarrow S(Cys)$ spin delocalization
type 2  	**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}, $\varepsilon < 1000\ M^{-1}\ cm^{-1}$; ligand-field transitions (d → d) normal Cu^{II} EPR
type 3  	**type 3**: copper dimers function: O_2 uptake from the Cu^I-Cu^I state structure: (bridged) dimer, Cu–Cu distance about 360 pm after O_2 uptake intense absorptions around 350 and 600 nm, $\varepsilon \approx 20000$ and $1000\ M^{-1}cm^{-1}$; LMCT transitions $O_2^{2-} \rightarrow Cu^{II}$ EPR-inactive Cu^{II} form (antiferromagnetically coupled d^9 centers)

Plastocianina
(da spinaci)

10.5 kDa,
ca. 100 a.a.



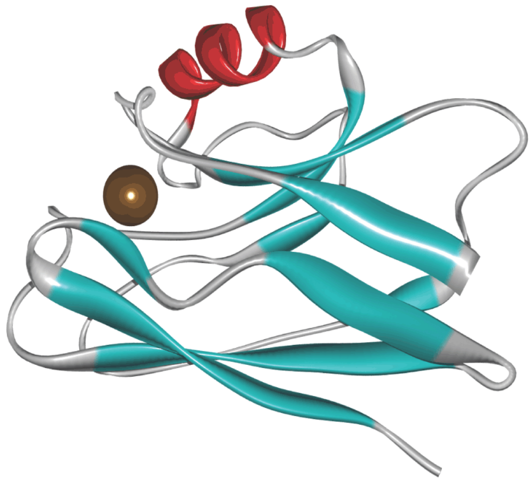


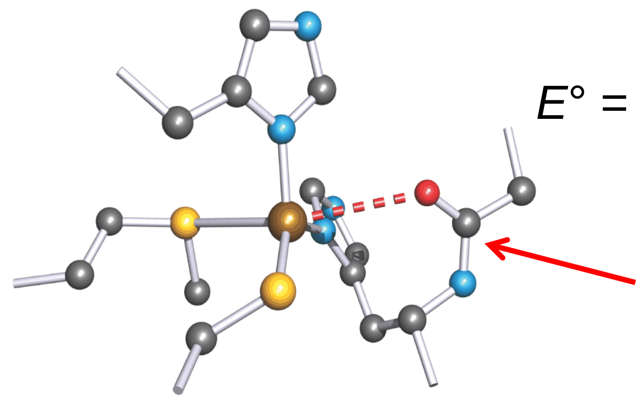
E° = + 370 mV

Coordinazione 3 + 1

Azurina
(da batteri)

14.5 kDa,
ca. 130 a.a.



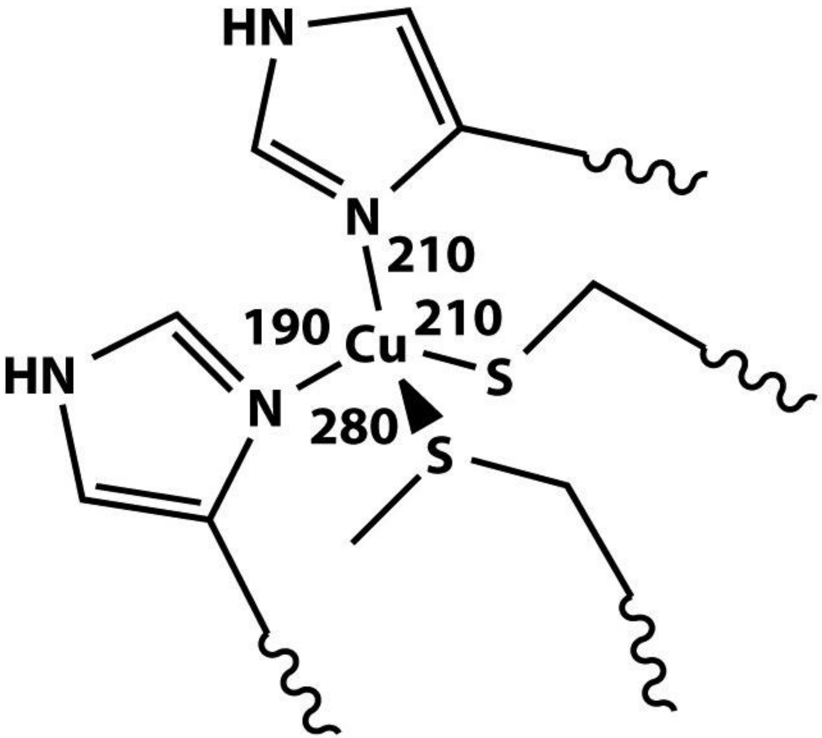


E° = + 308 mV

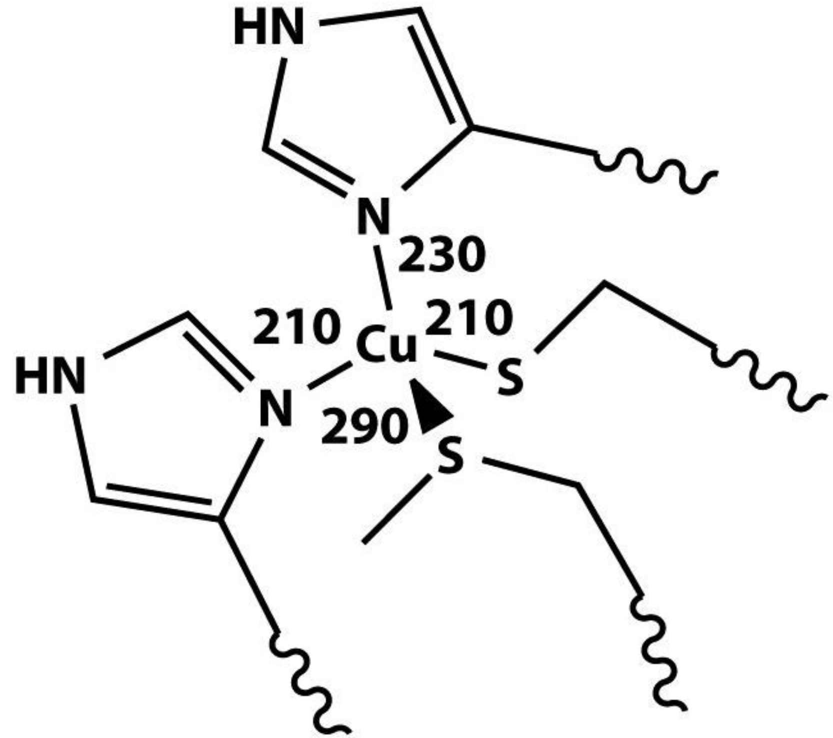
Coordinazione 3 + 1 + 1

Blue copper proteins

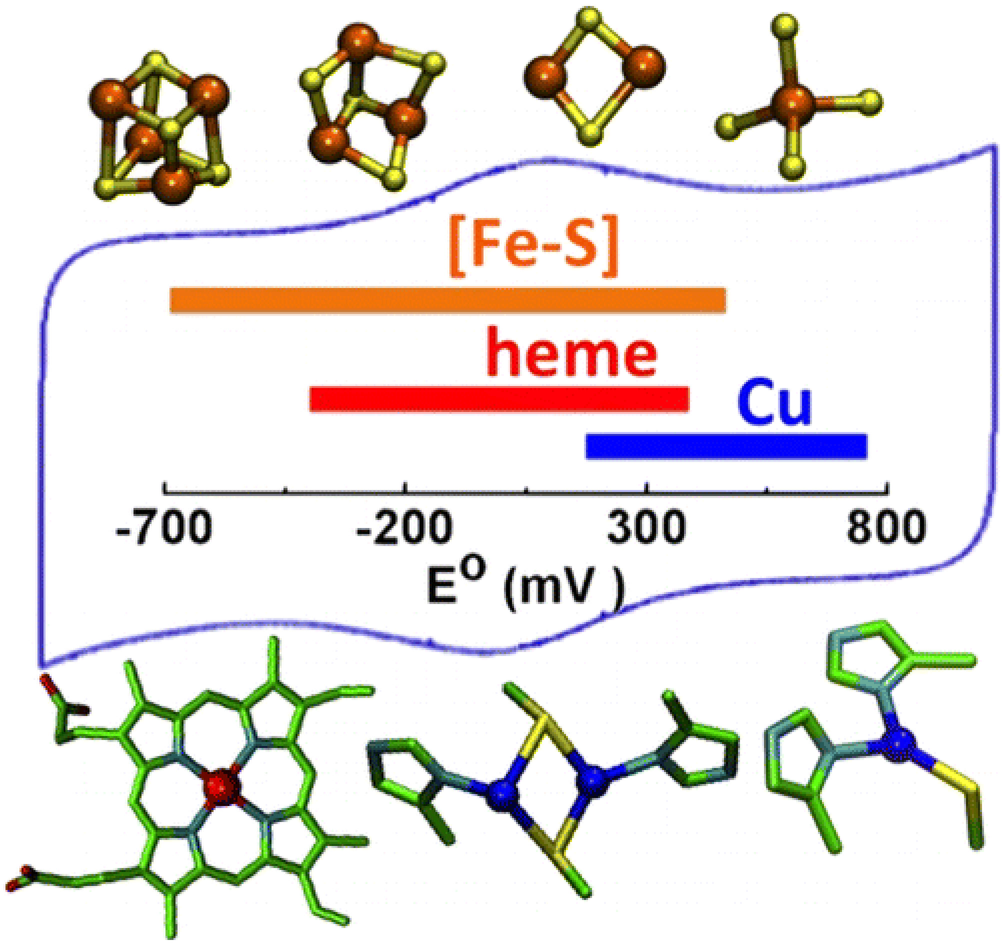
Esempio di stato entatico



Oxidized plastocyanin



Reduced plastocyanin



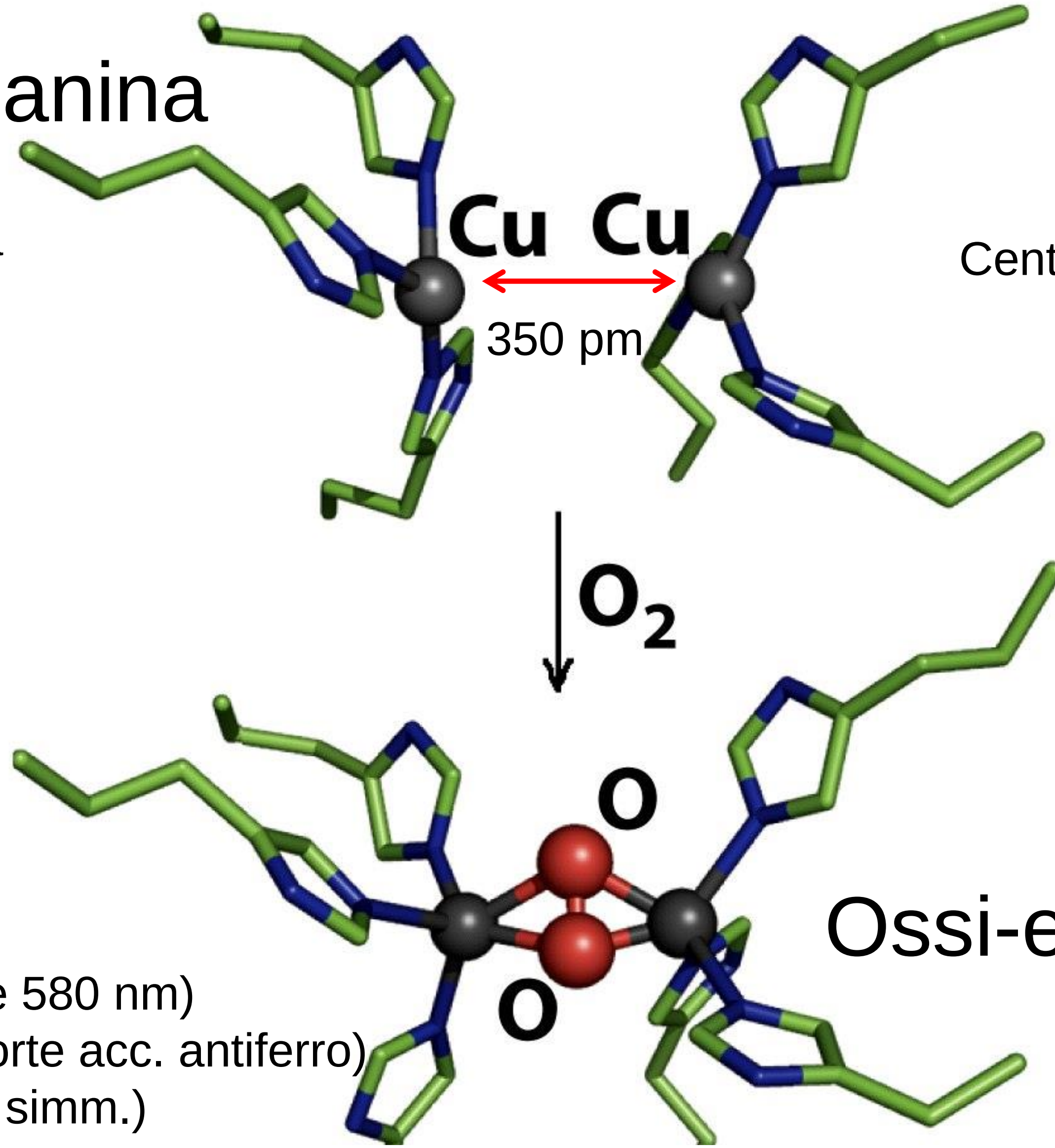
[Fe-S]
heme
Cu
-700
-200
300
800
E^{o} (mV)

Emocianina

fino a 1500 kDa,
ogni unità 75 kDa

Incolore
2 Cu(I) (S = 0)

Centro rame di tipo 3

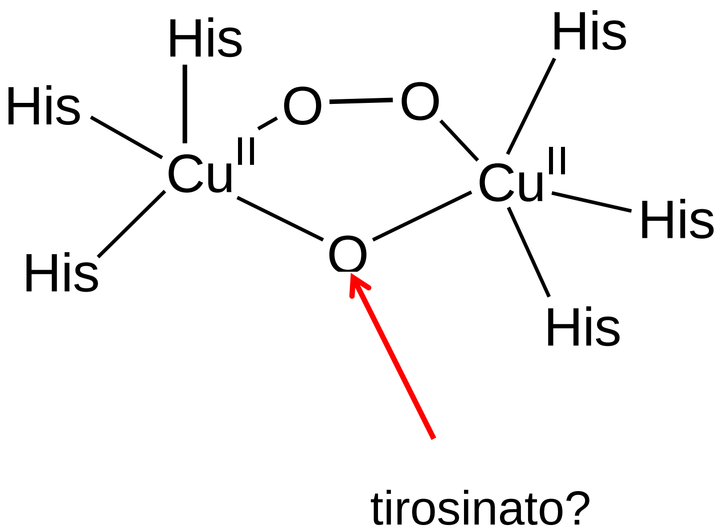


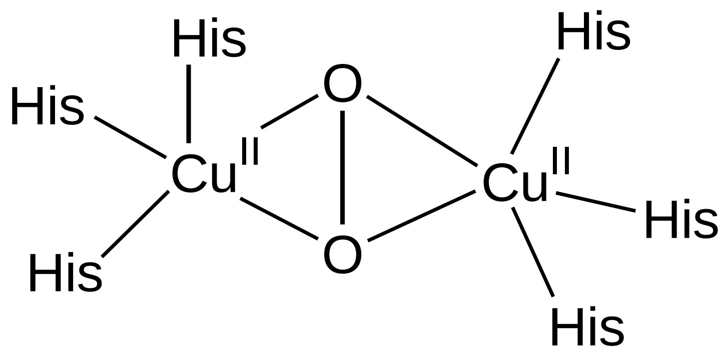
Ossi-emocianina

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

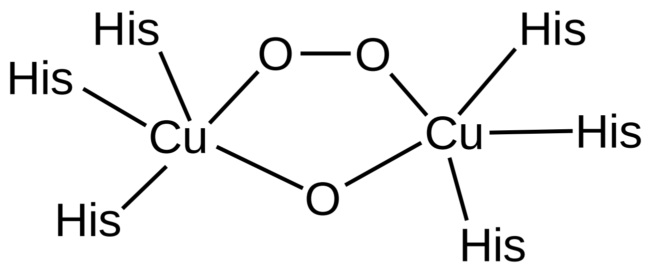
trasporto di O_2 di molluschi (lumache, calamari) e artropodi (granchi, aragoste, gamberi, scorpioni)

Possibili coordinazioni simmetriche dello ione perossido

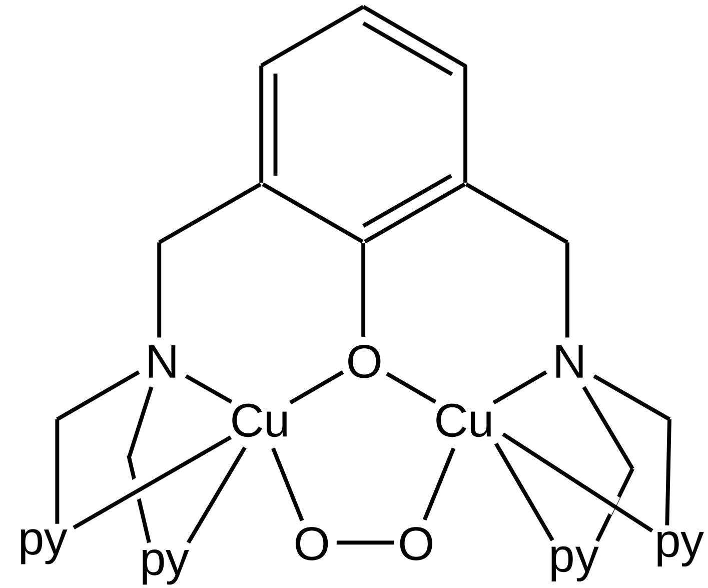


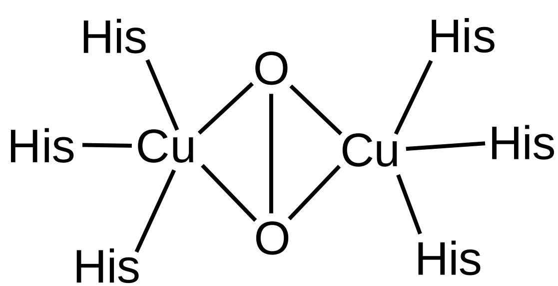


Modelli per la coordinazione di O_2 alla emocianina

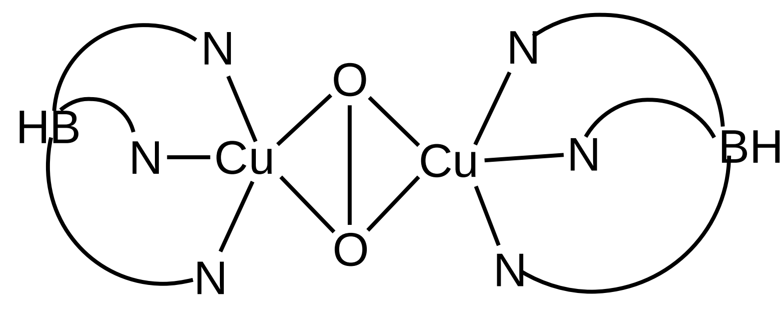


ipotesi sbagliata

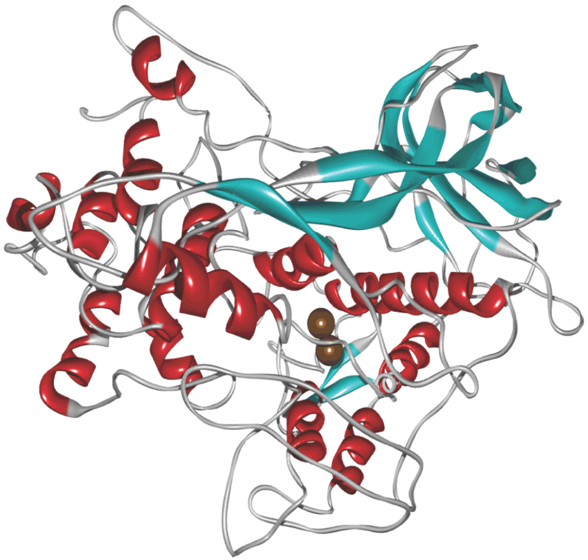




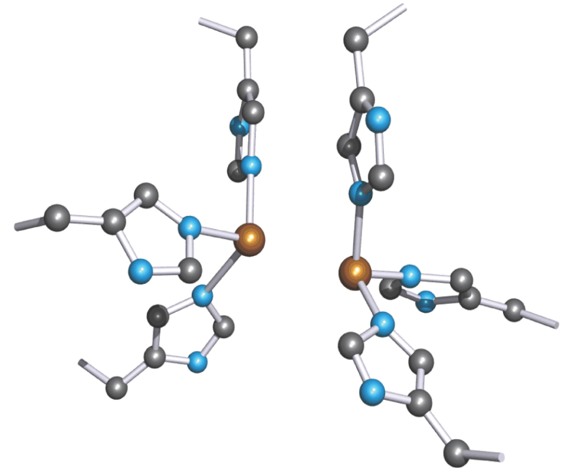
ipotesi corretta



Emocianina

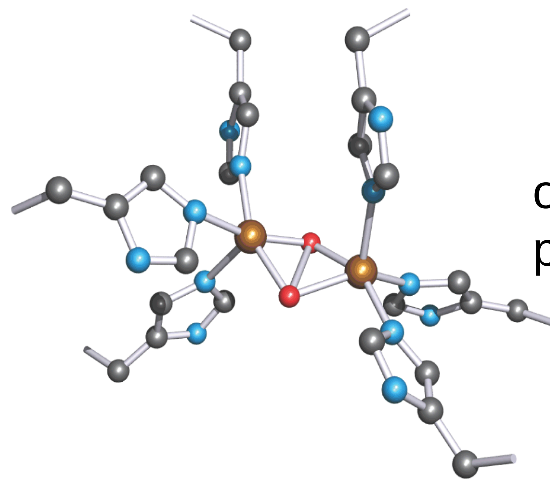


(a)



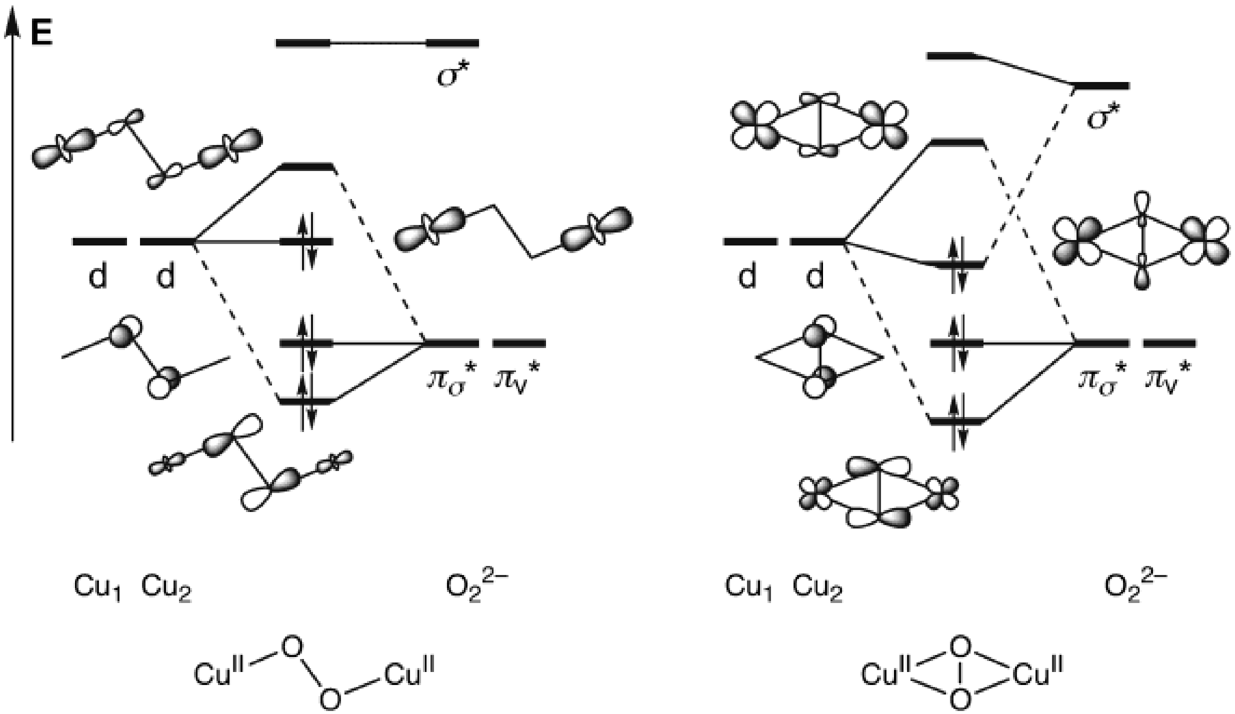
(b)

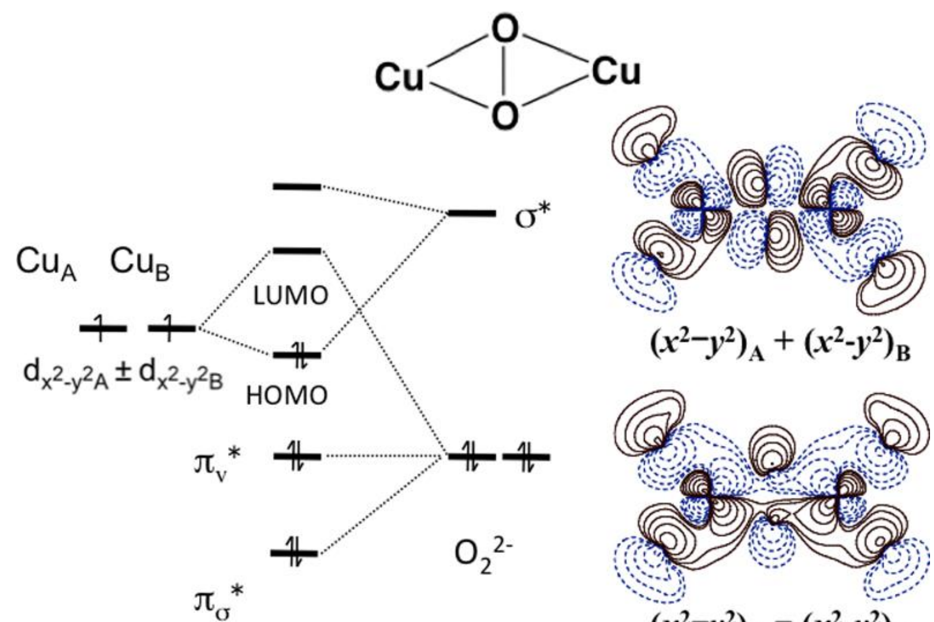
desossi,
tricoordinato

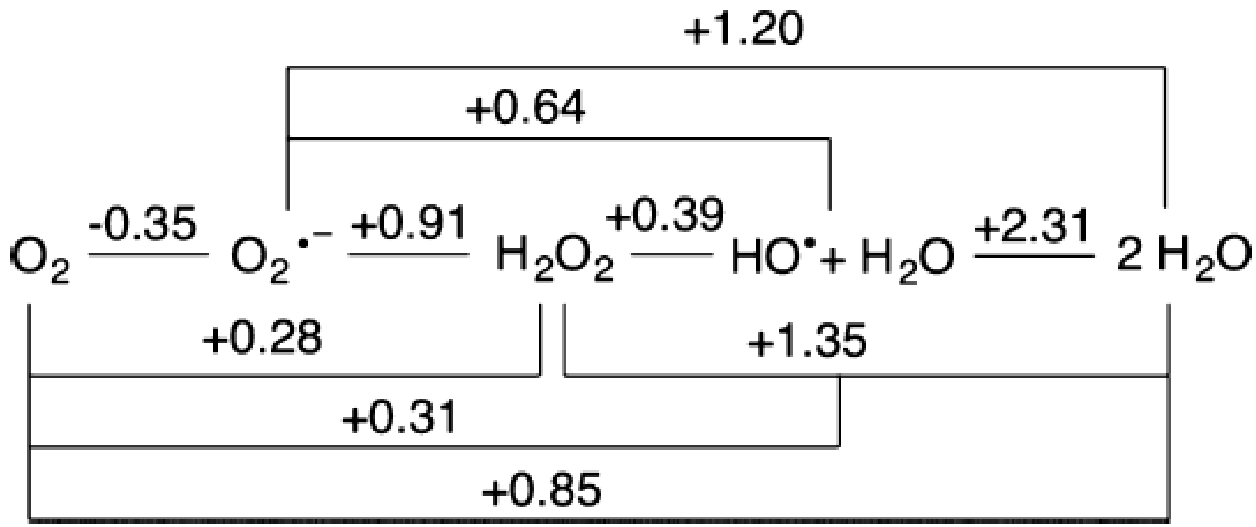


(c)

ossi,
pentacoordinato


E
σ*
d d
π_σ* π_v*
Cu1 Cu2
O2 2–
CuII O O CuII
σ*
d d
π_σ* π_v*
Cu1 Cu2
O2 2–
CuII O O CuII

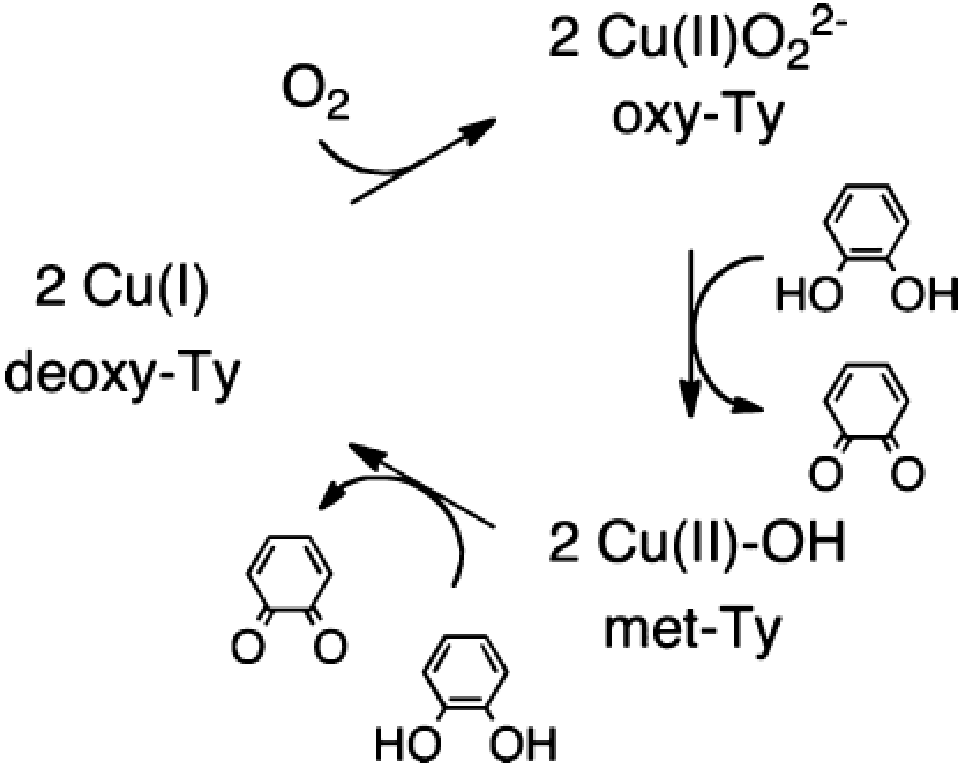

O
Cu Cu
O
σ*
CuA CuB
LUMO
$d_{x^2-y^2 A} \pm d_{x^2-y^2 B}$
HOMO
π_v*
π_σ*
O2 2-
$(x^2-y^2)_A + (x^2-y^2)_B$
$(x^2-y^2)_A - (x^2-y^2)_B$

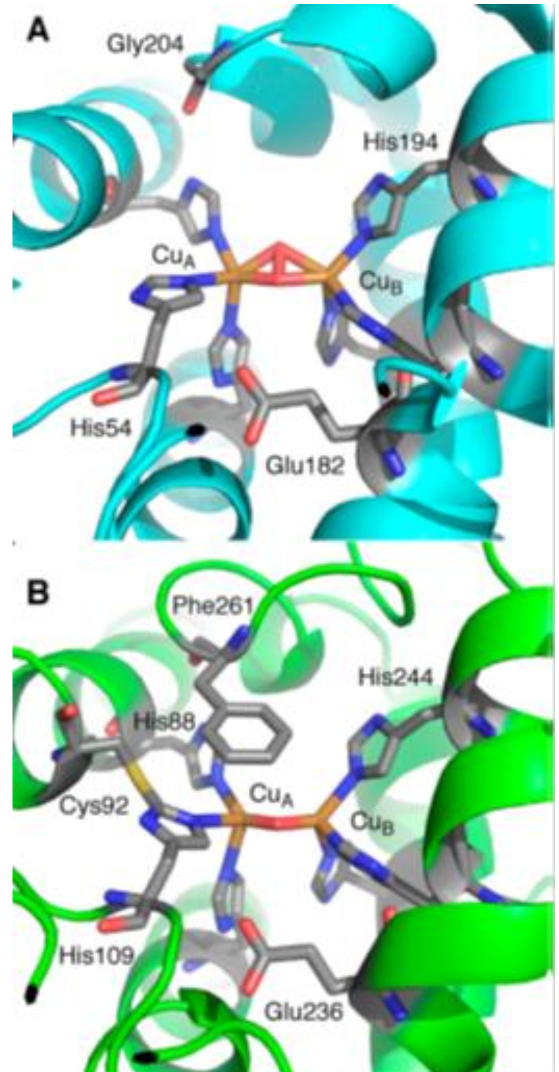

+1.20
+0.64
O_2 -0.35 $O_2^{\bullet -}$ +0.91 H_2O_2 +0.39 $HO^{\bullet} + H_2O$ +2.31 $2\,H_2O$
+0.28
+1.35
+0.31
+0.85

Polifenolo ossidasi

Tirosinasi, Catecolo-ossidasi

$$2\ o\text{-difenolo} + O_2 \rightarrow 2\ o\text{-chinone} + 2H_2O$$

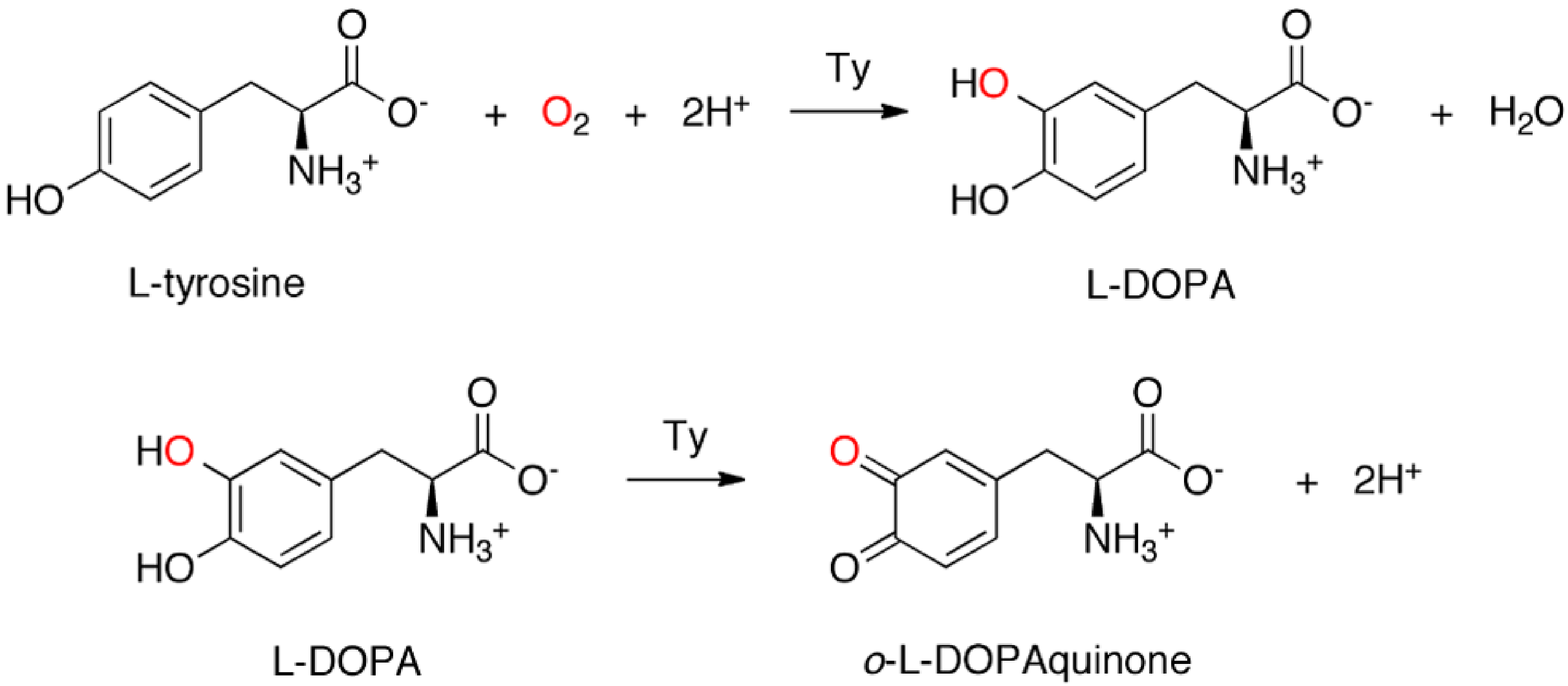


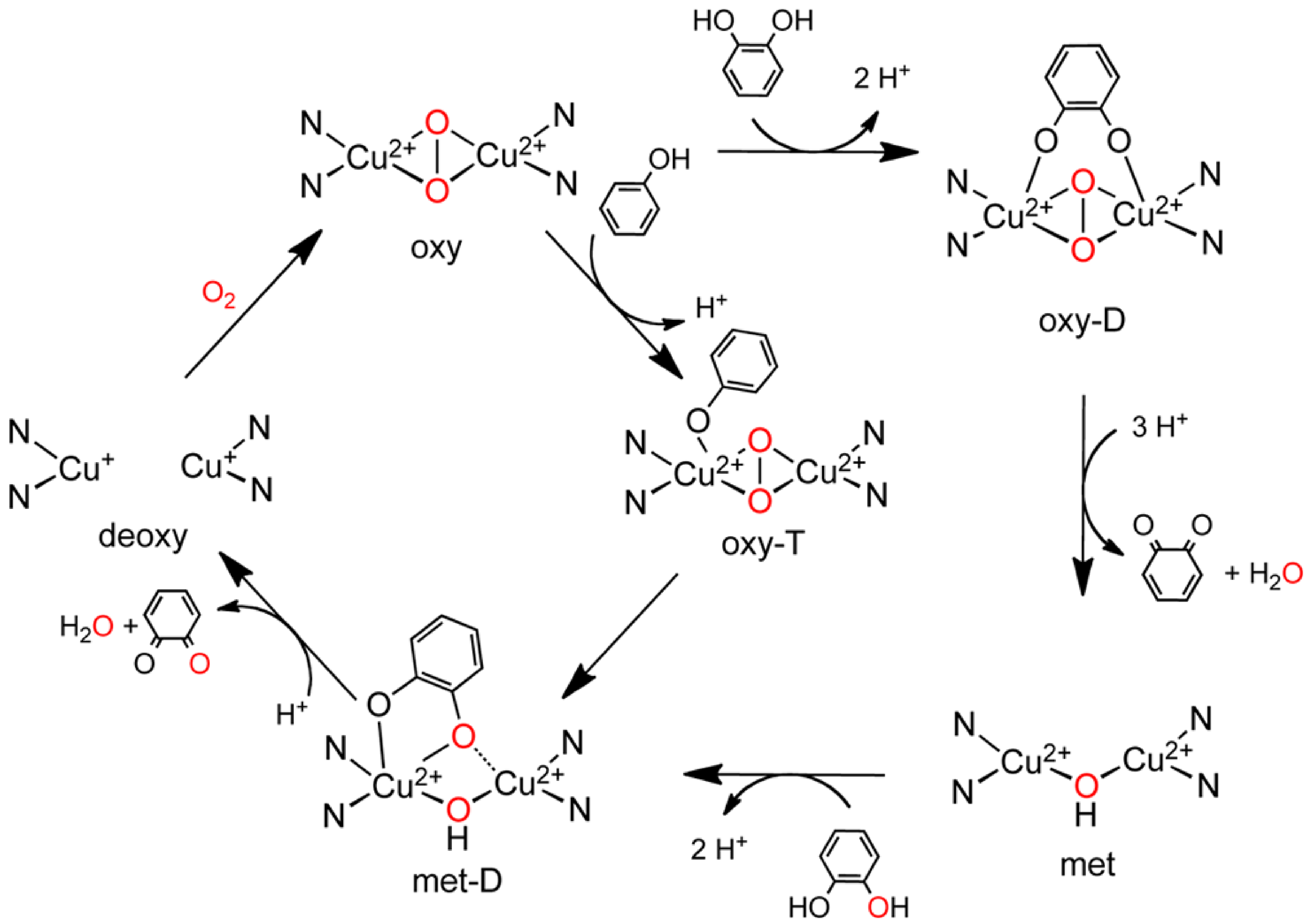


oxy-Ty

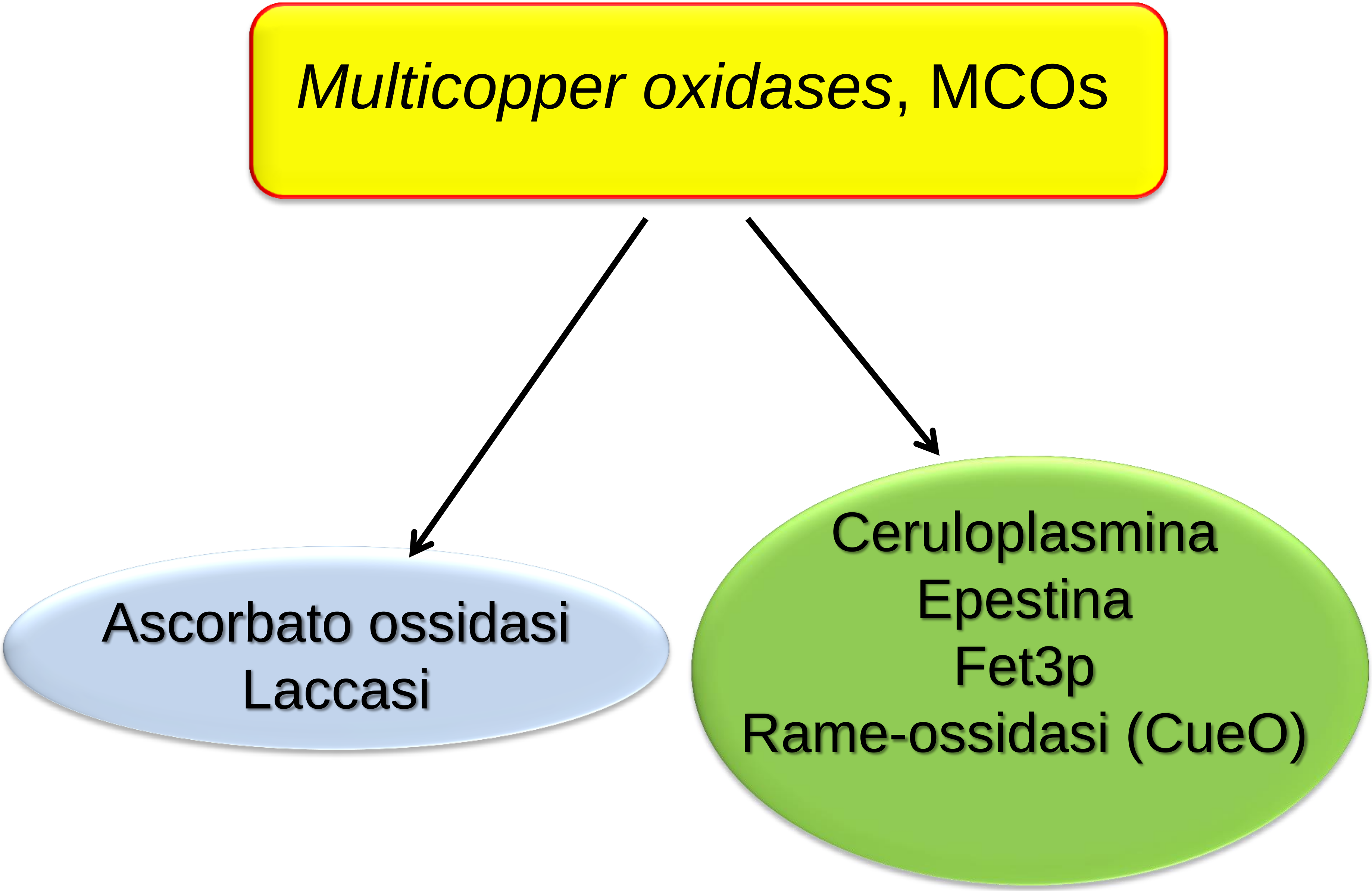
met-Ty

Tirosinasi come mono-ossigenasi

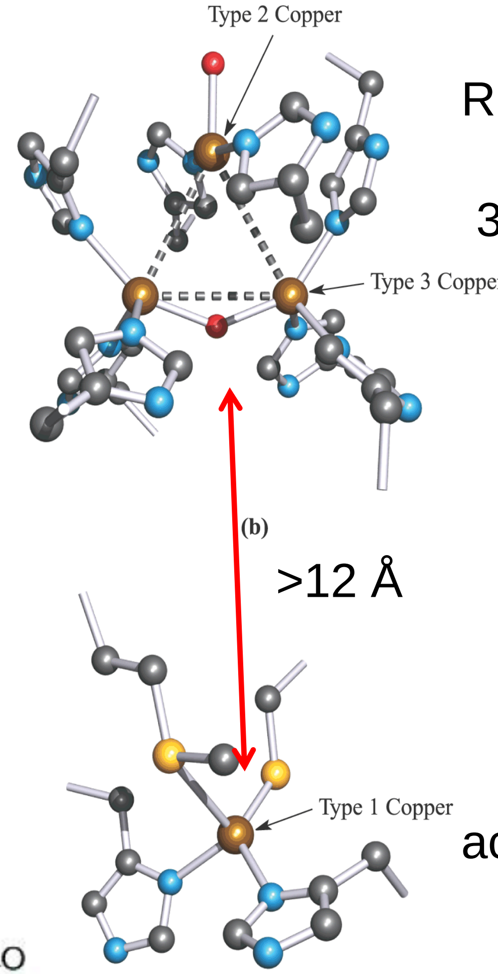




oxy
oxy-D
oxy-T
deoxy
met
met-D
O2
H+
2 H+
3 H+
+ H2O
H2O +


Multicopper oxidases, MCOs
Ascorbato ossidasi
Laccasi
Ceruloplasmina
Epestina
Fet3p
Rame-ossidasi (CueO)

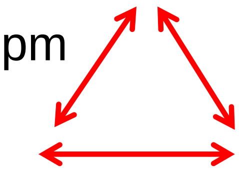
(a)



(c)

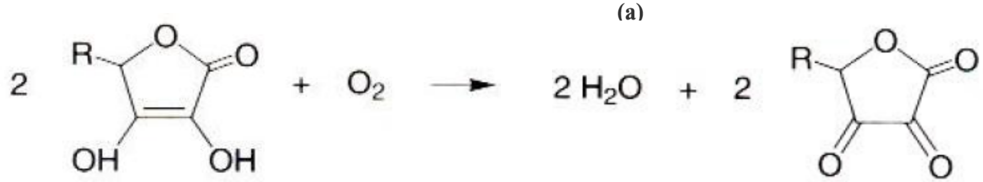
Riduzione O_2

390 pm



340 pm

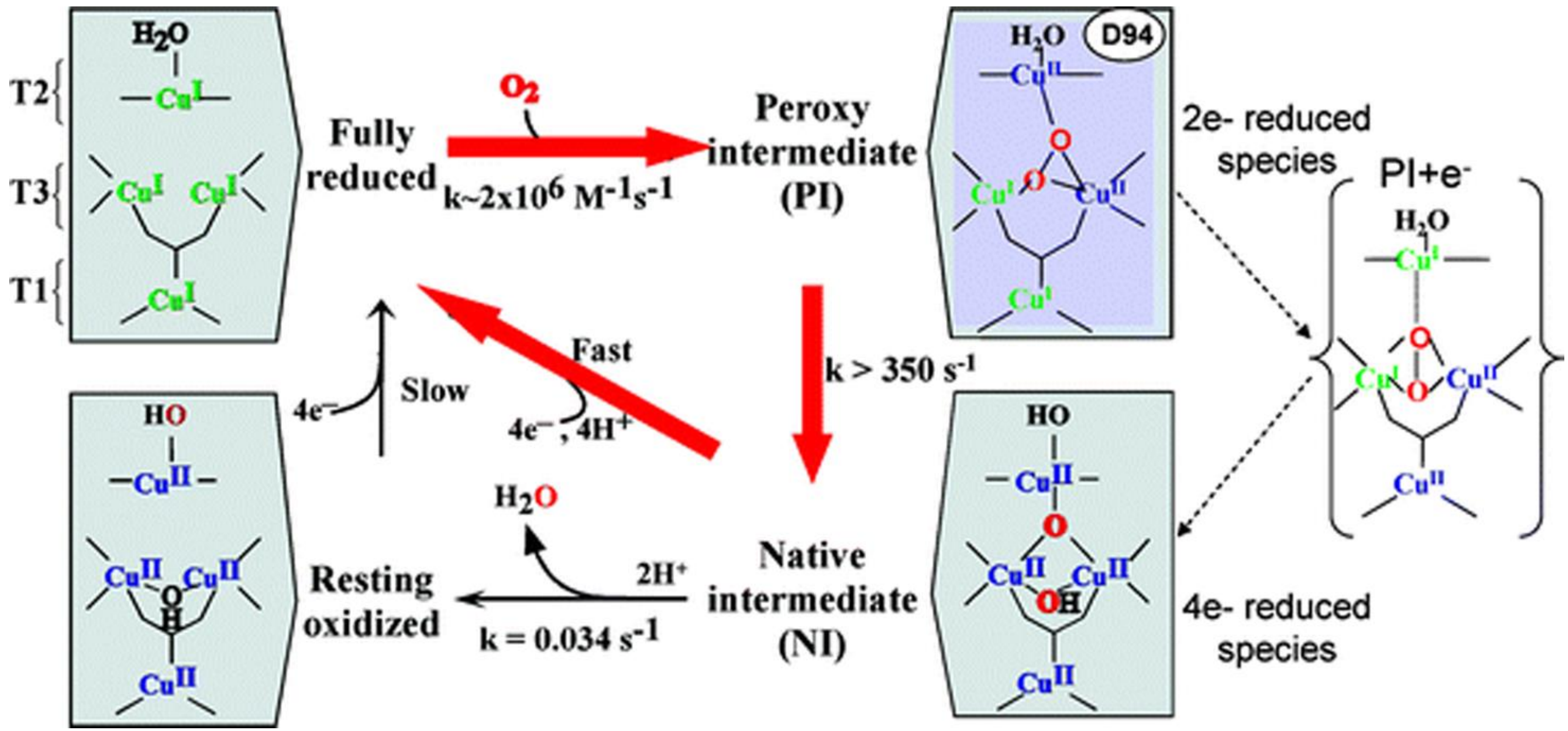
accettore di elettroni



Ascorbato ossidasi (da zucchini)

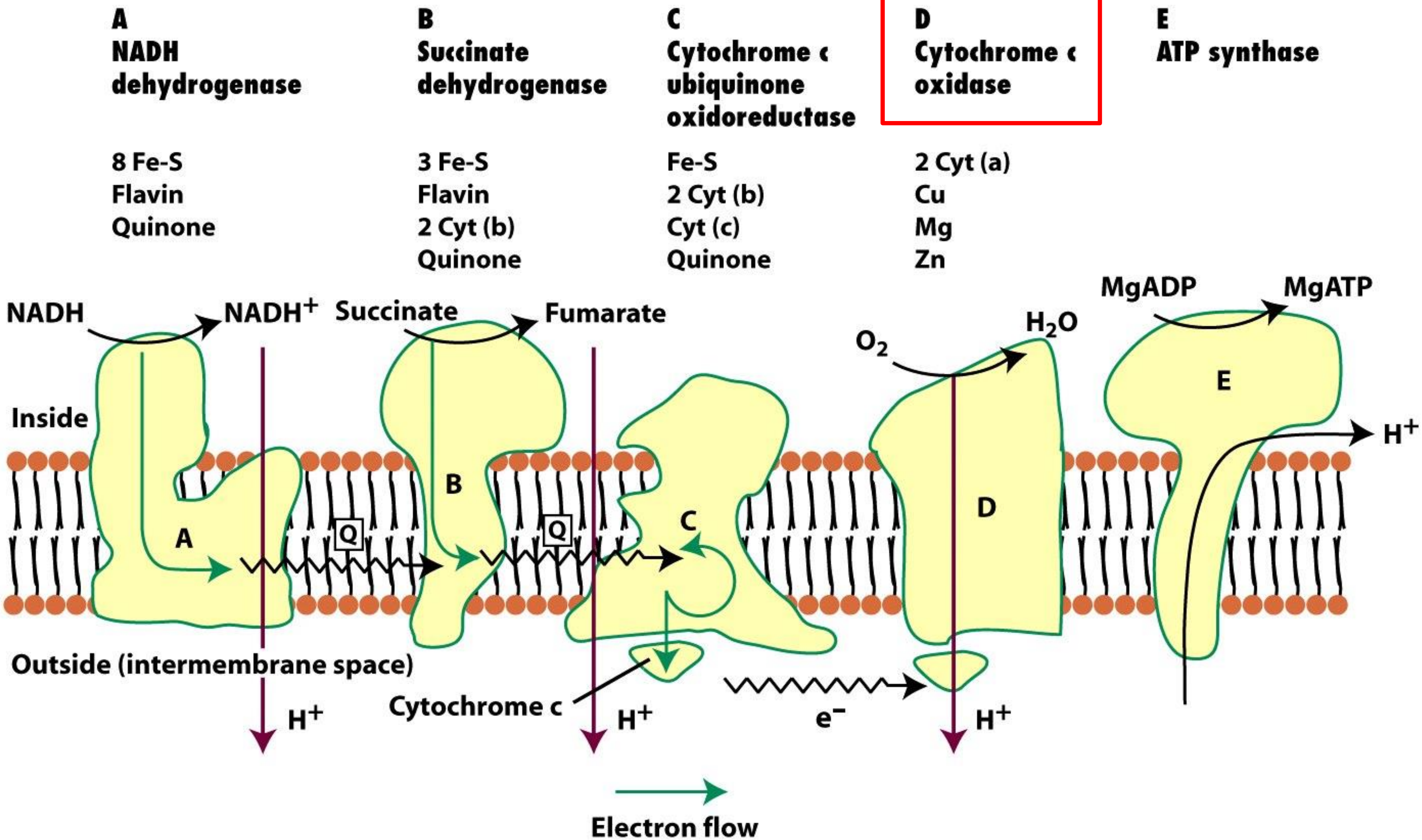
$$4\ RH + O_2 \rightarrow 4R\cdot + 2\ H_2O$$

(RH = fenolo)



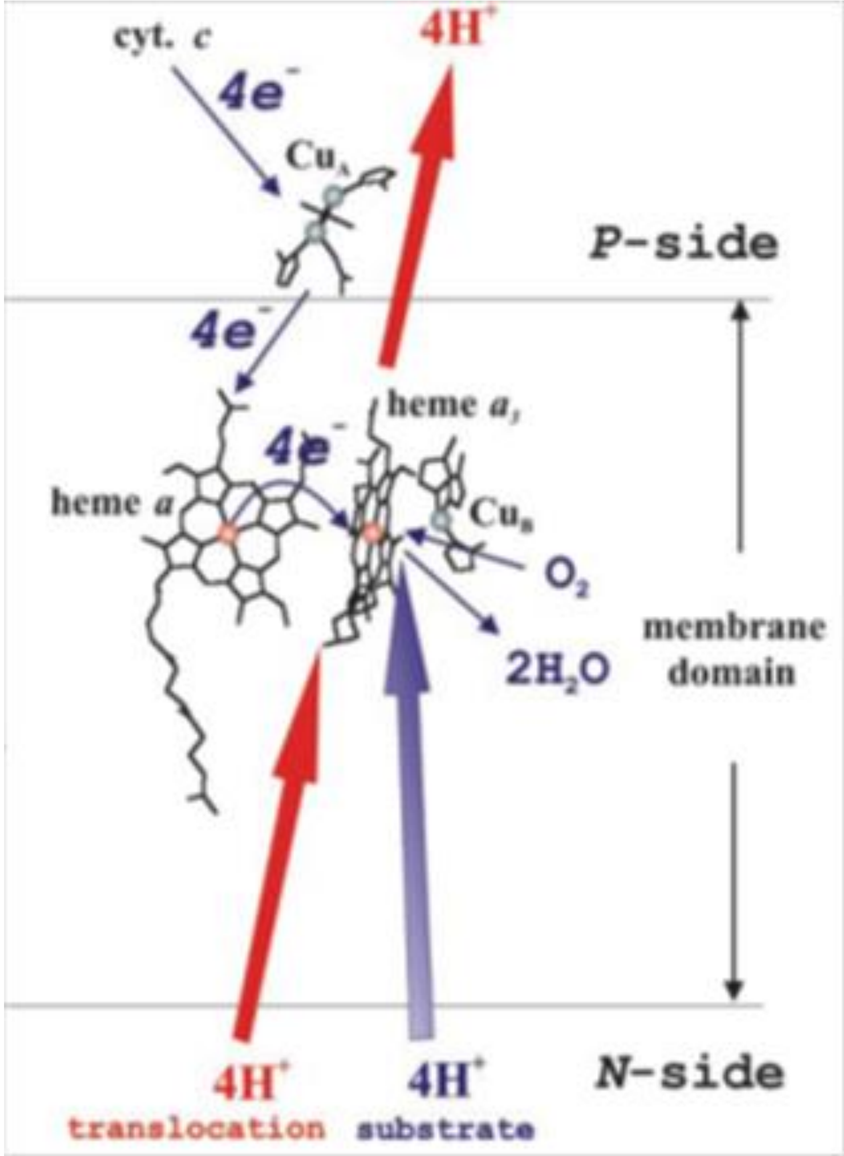
T2
T3
T1
H2O
CuI
Fully reduced
O2
k~2x10^6 M^-1 s^-1
Peroxy intermediate (PI)
D94
2e- reduced species
PI+e-
k > 350 s^-1
Fast
4e−, 4H+
Slow
4e−
HO
CuII
H2O
2H+
Resting oxidized
k = 0.034 s^-1
Native intermediate (NI)
4e- reduced species

Catena respiratoria (fosforilazione ossidativa)



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

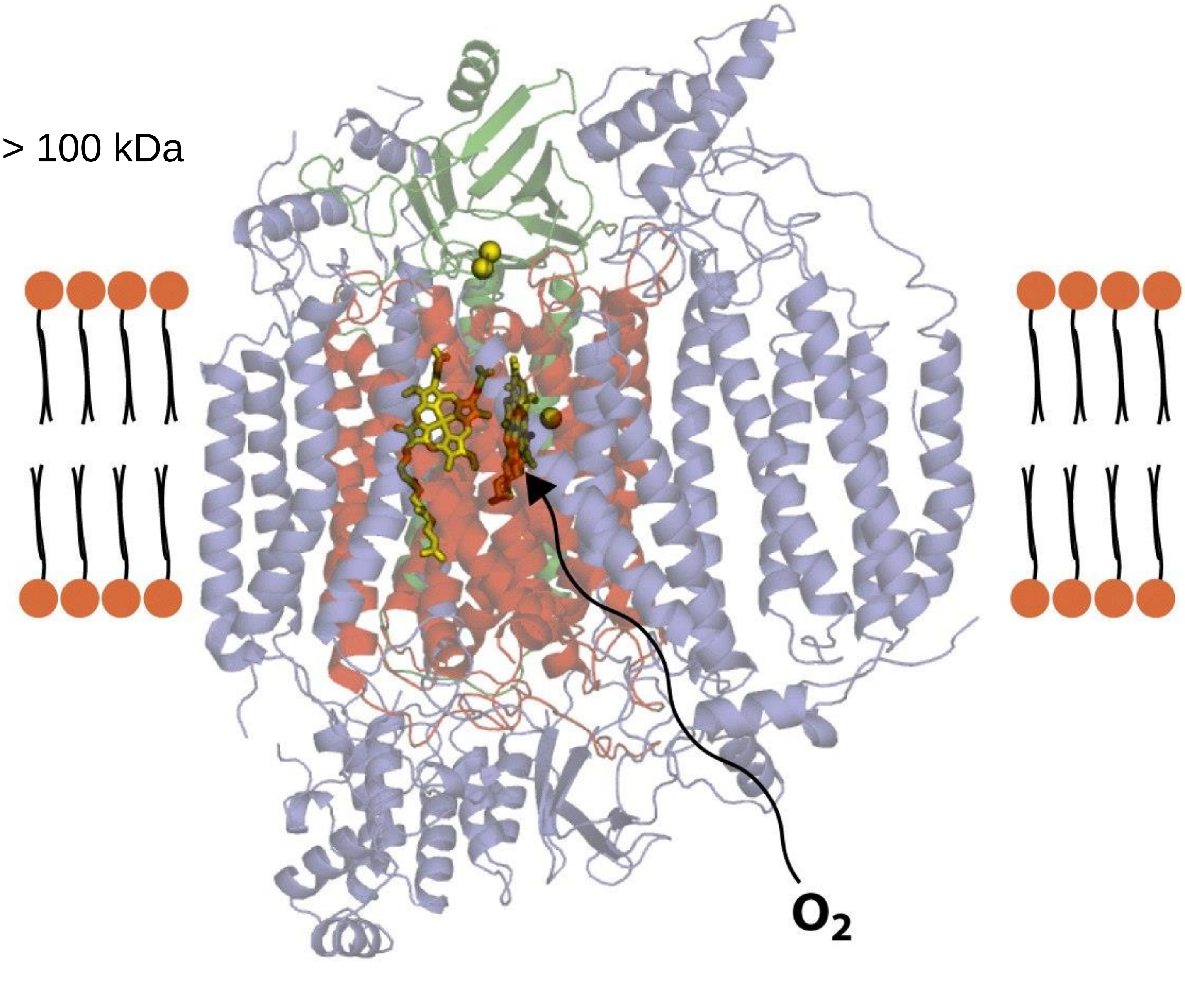
$\Delta E°$ (pH 7) = 815 mV; $\Delta G°$ = -80 kcal/mol



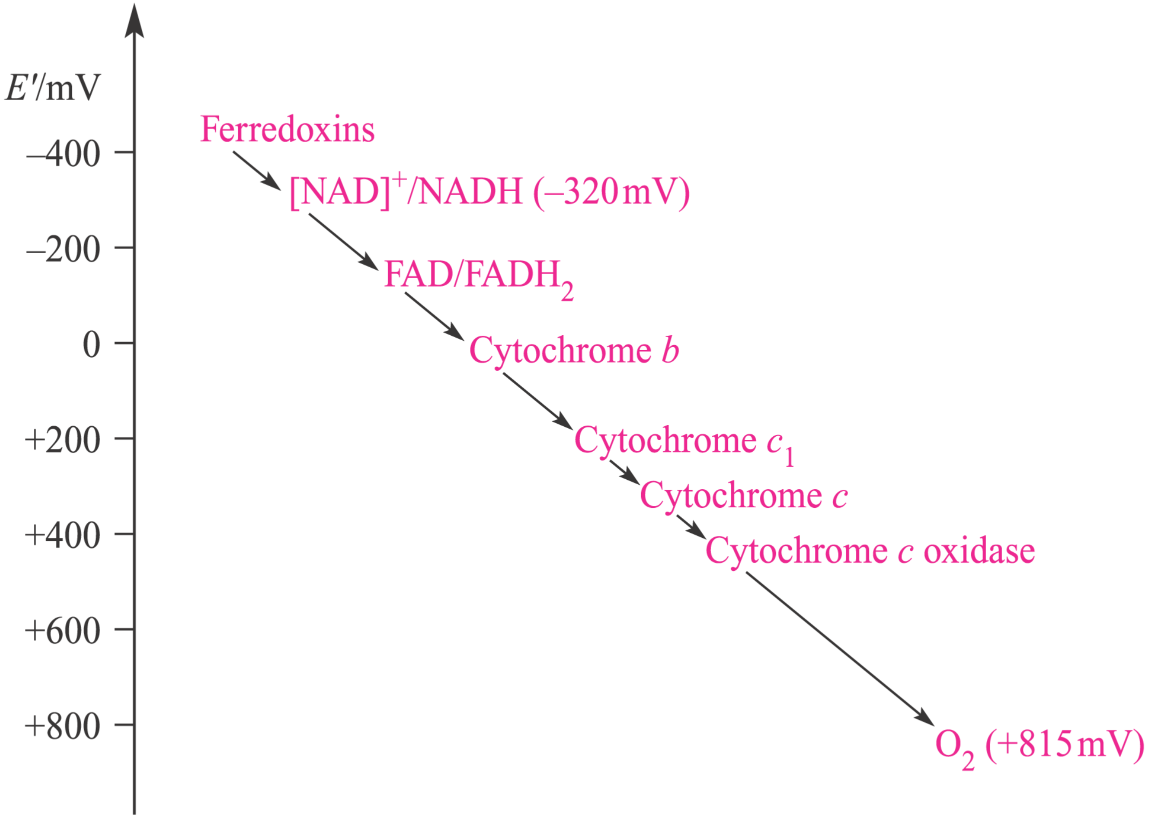
$$O_2(g) + 4\ e^- + 8\ H^+(\text{inside}) \rightarrow 2\ H_2O(l) + 4\ H^+(\text{outside})$$

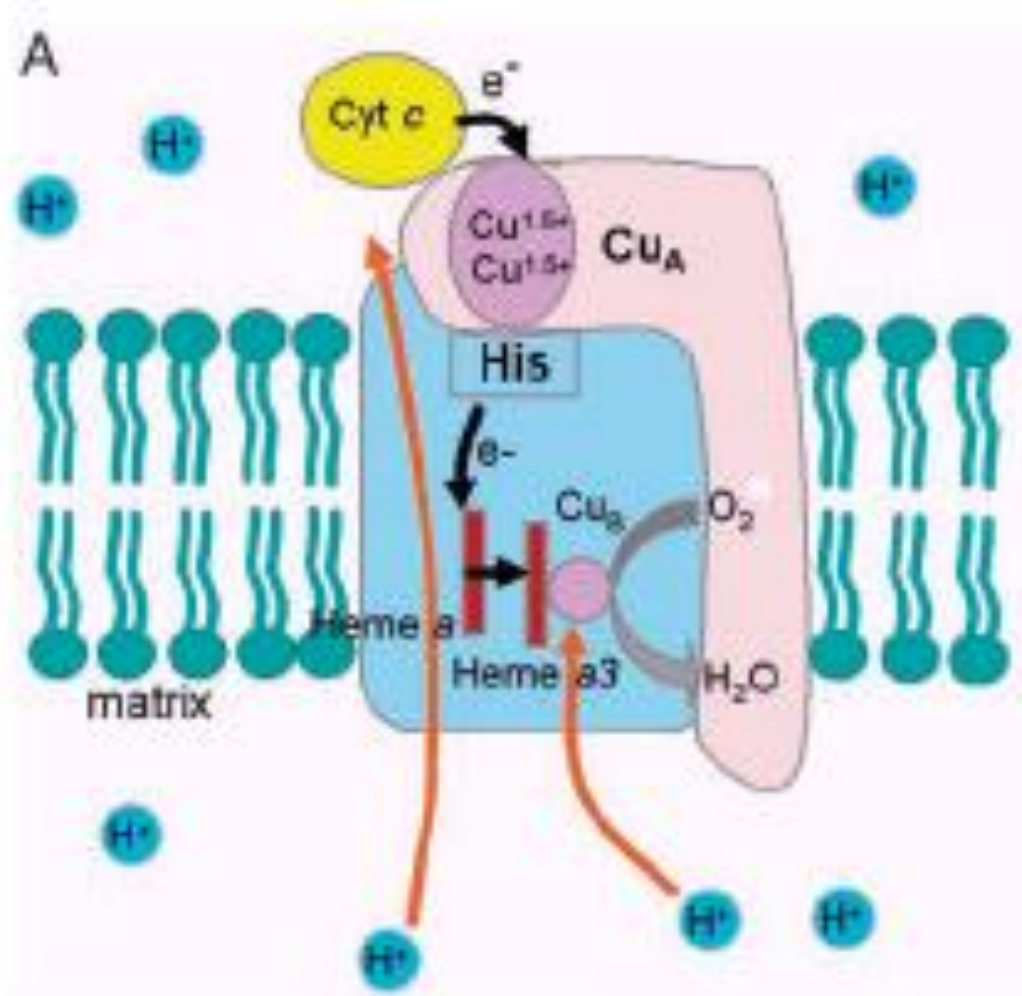
Reacts with cytochrome C

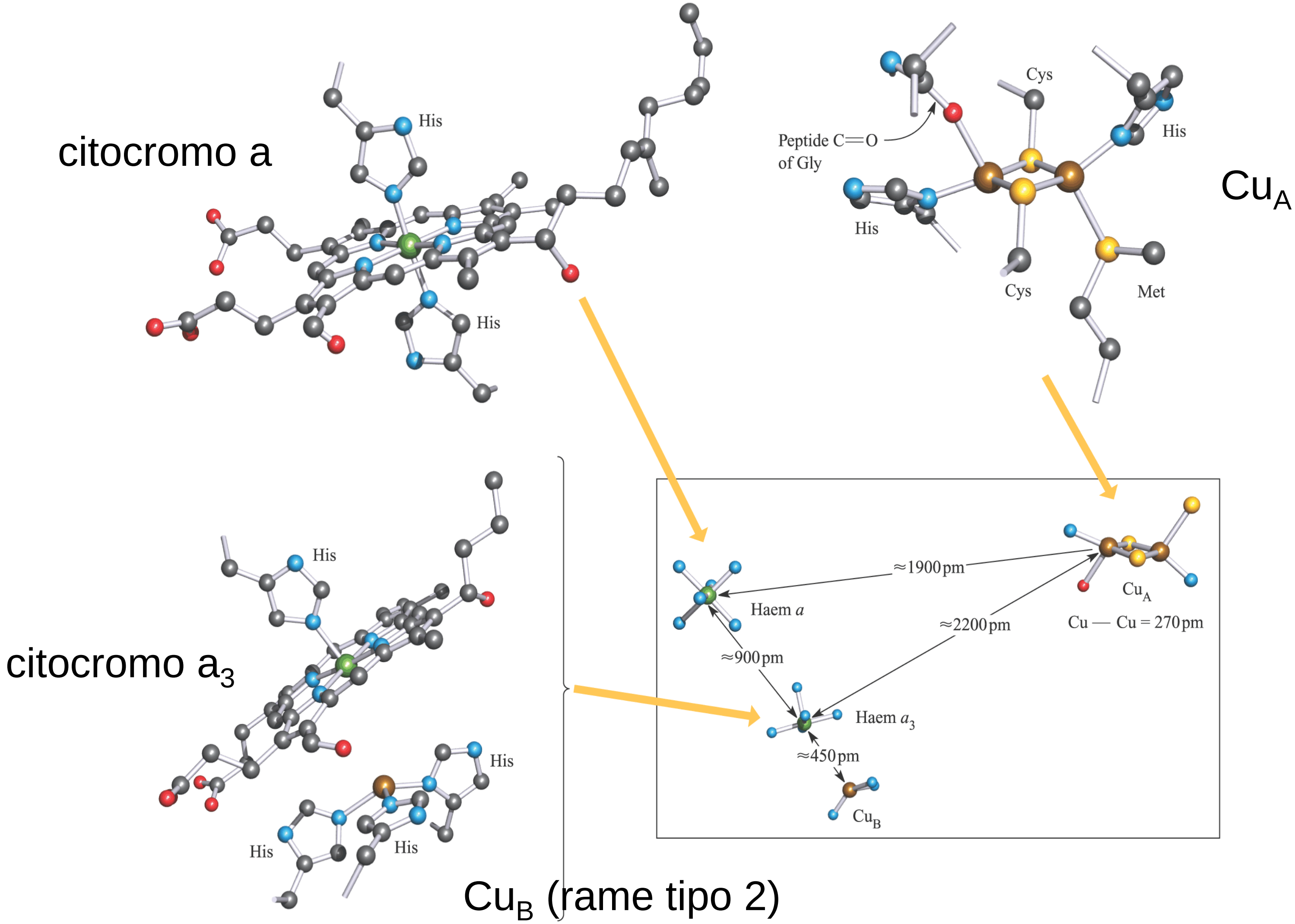
> 100 kDa

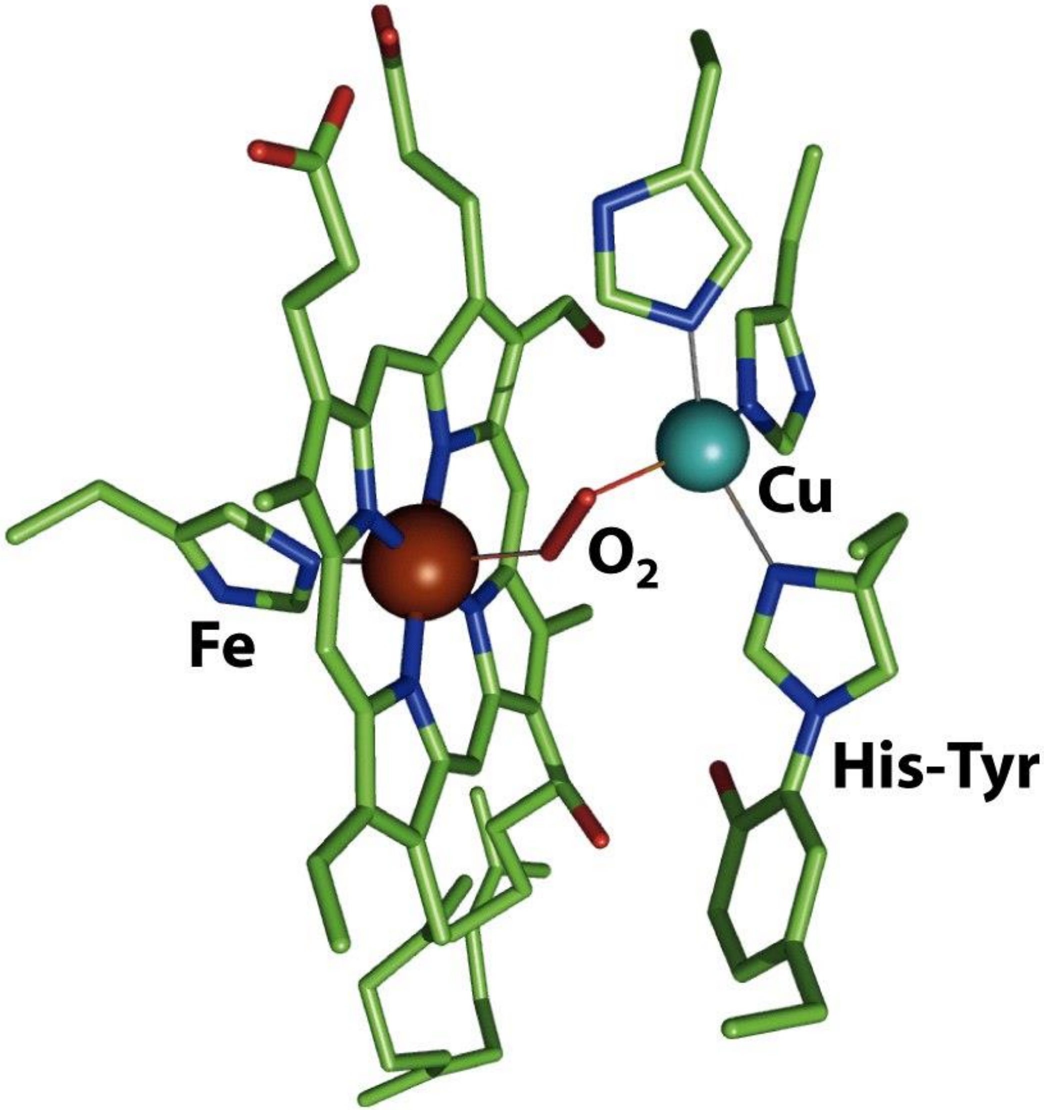


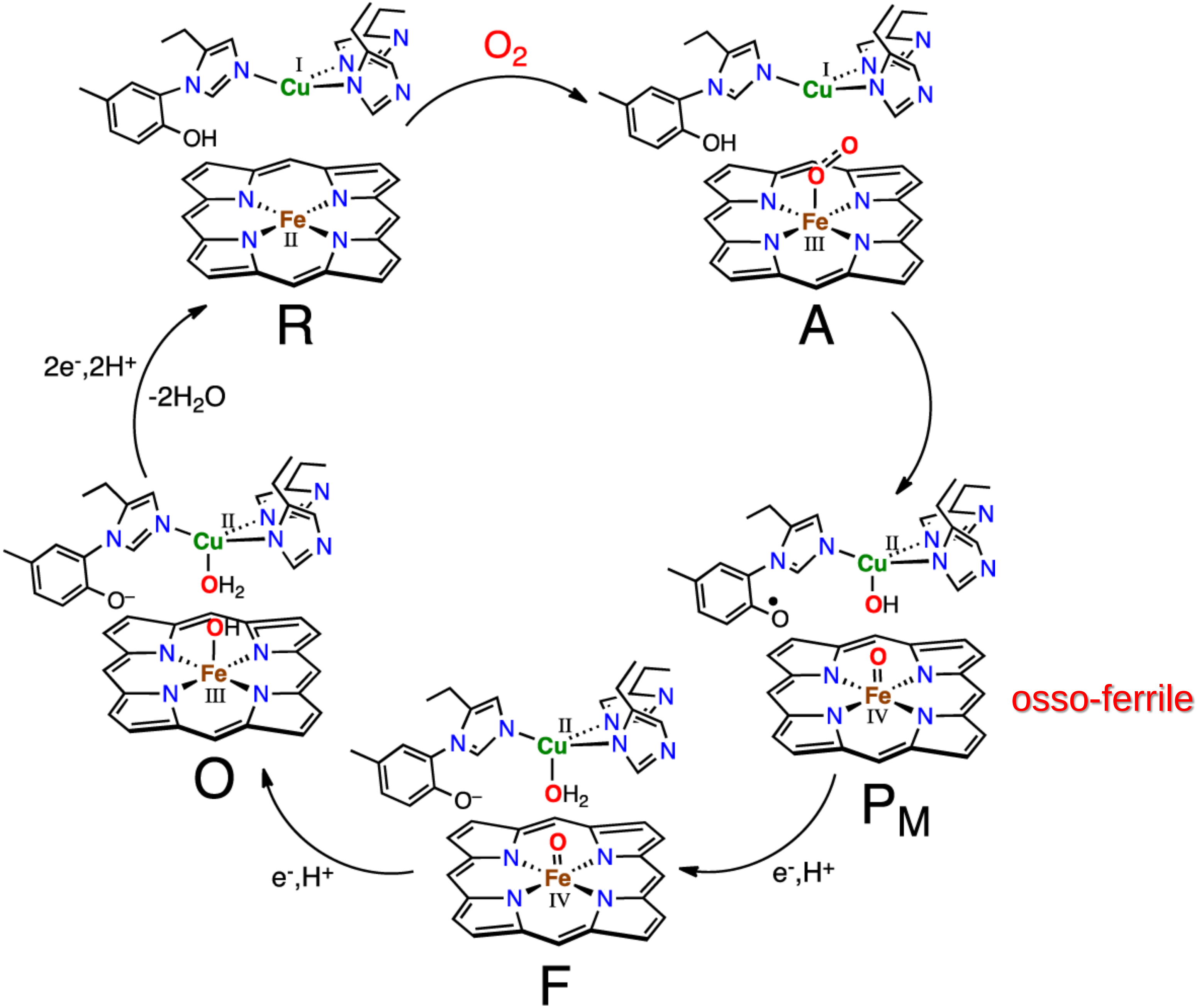
La sequenza di trasferimenti elettronici nei mitocondri



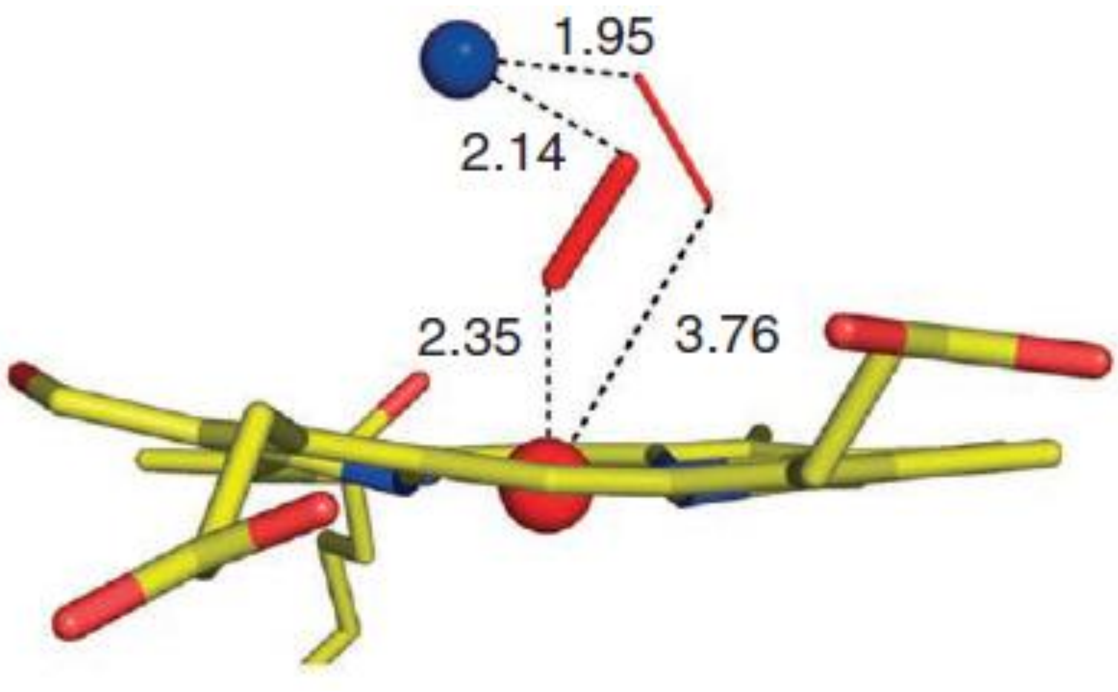

A
Cyt c
e-
Cu1.5+
Cu1.5+
CuA
His
e-
CuB
O2
Heme a
Heme a3
H2O
matrix
H+
H+
H+
H+
H+
H+
H+


citocromo a
His
His
Cys
Peptide C=O
of Gly
His
His
Cys
Met
Cu_A
citocromo a_3
His
His
His
His
Cu_B (rame tipo 2)
≈1900 pm
Haem *a*
≈2200 pm
Cu_A
Cu — Cu = 270 pm
≈900 pm
Haem a_3
≈450 pm
Cu_B


Cu
O_2
Fe
His-Tyr


O2
R
A
PM
F
O
osso-ferrile
e-,H+
e-,H+
2e-,2H+
-2H2O

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



Nature, 2014

Reactive Oxygen Species (ROS)

Reazione di Haber-Weiss

$$O_2^- + H_2O_2 \xrightarrow{Fe^{3+}/Fe^{2+} \text{ o } Cu^{+}/Cu^{2+}} O_2 + OH\bullet + OH^-$$

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet}$$

Reazione di Fenton

The net reaction:

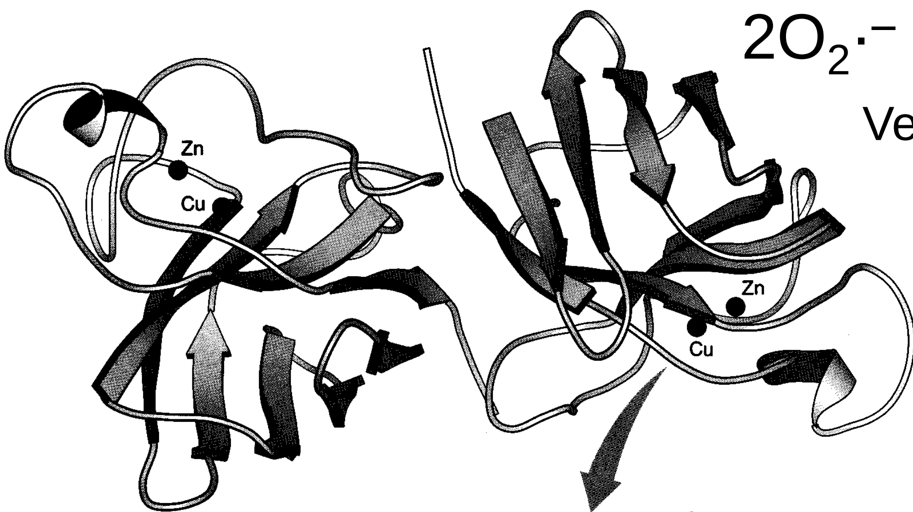
$$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + OH^- + OH^{\bullet}$$

$$Fe(II) + {}^3O_2 \rightarrow Fe(III) + O_2\bullet^-$$

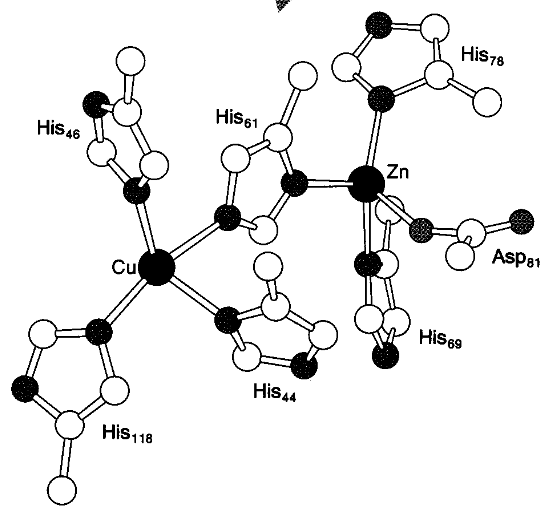
Cu-Zn superossi dismutasi

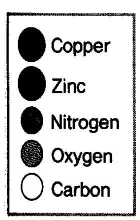
$$2O_2\cdot^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Velocità quasi-diffusiva

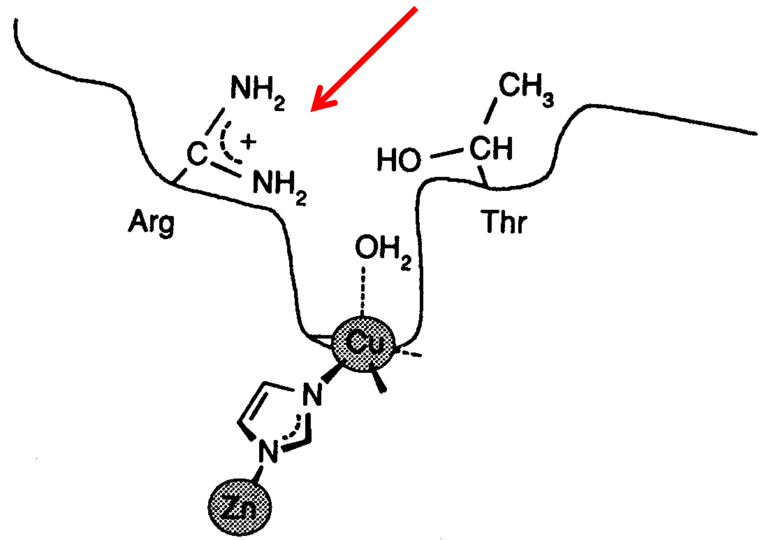


16 kDa

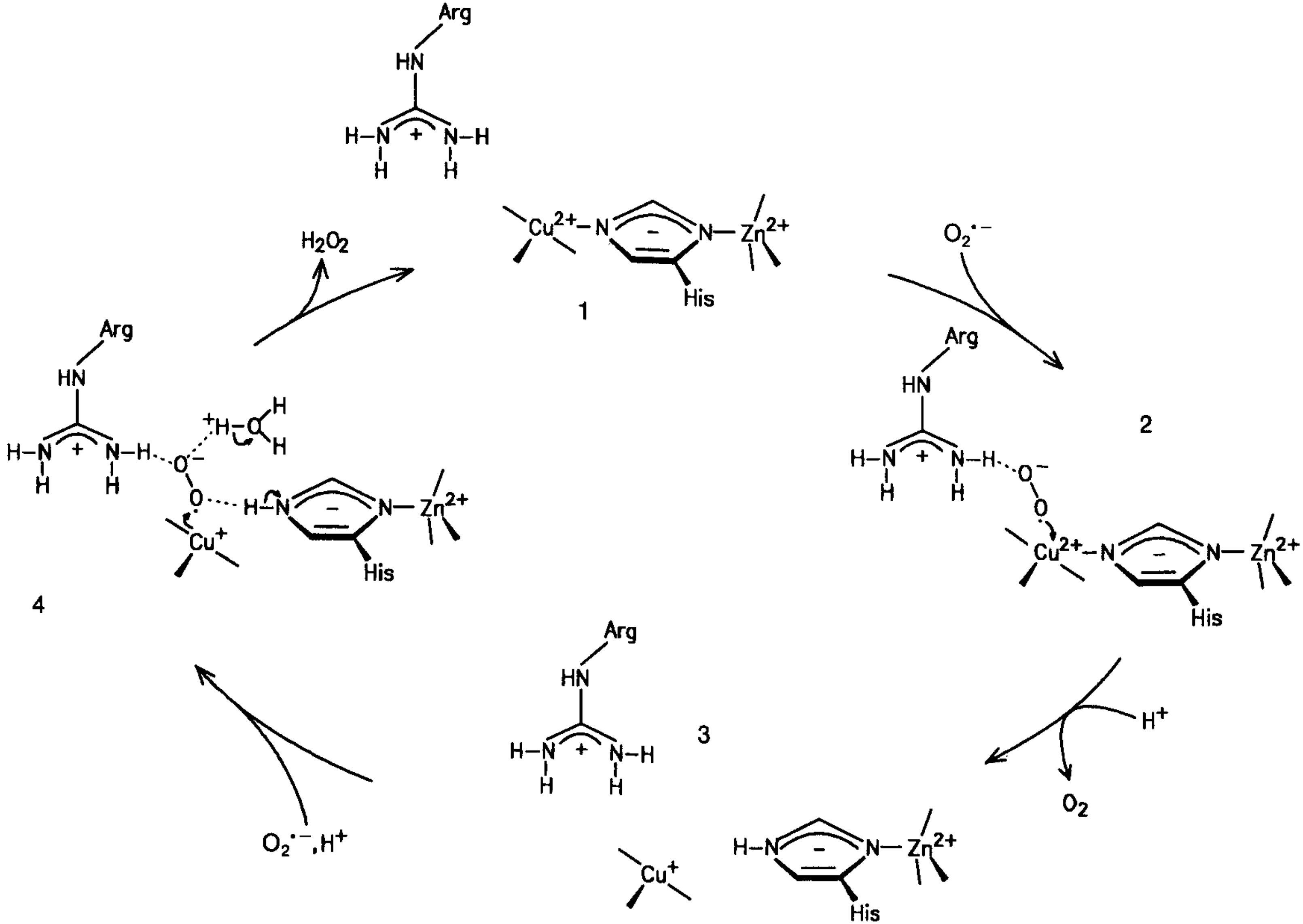


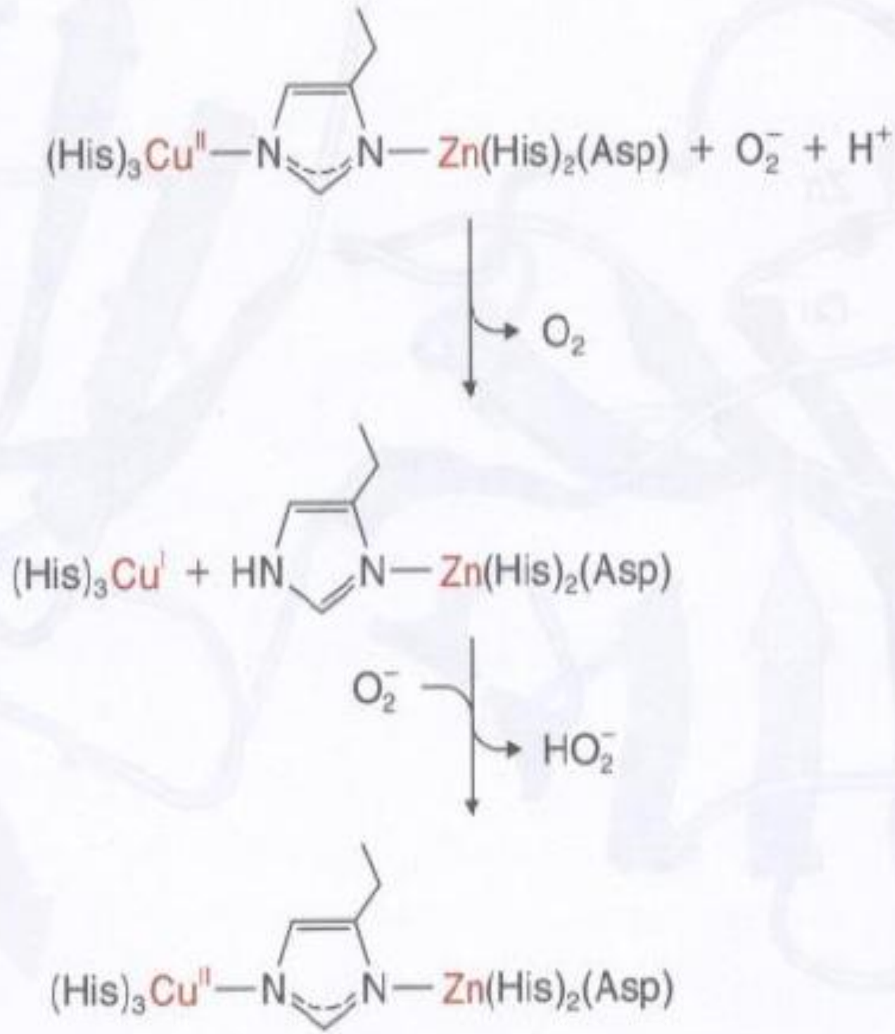


gruppo guanidinio



Ciclo catalitico della superossi dismutasi





(His)3CuII—N N—Zn(His)2(Asp) + O2− + H+
O2
(His)3CuI + HN N—Zn(His)2(Asp)
O2−
HO2−
(His)3CuII—N N—Zn(His)2(Asp)