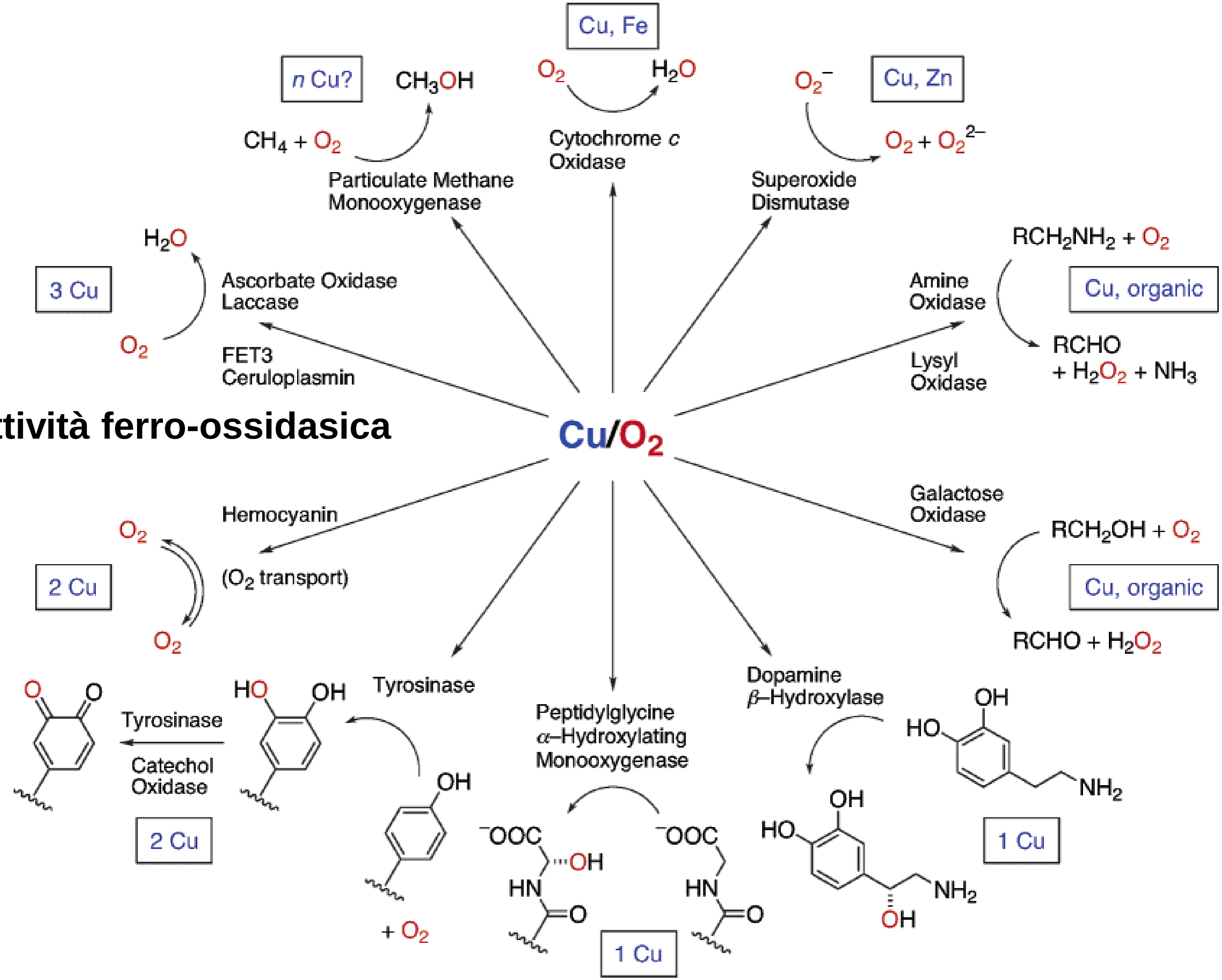
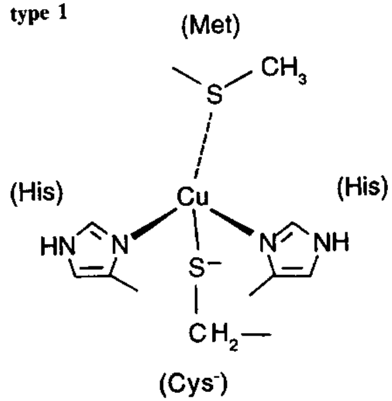
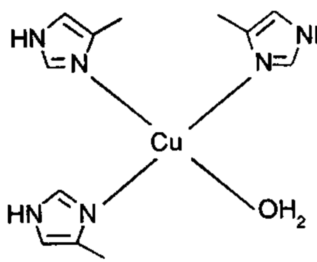
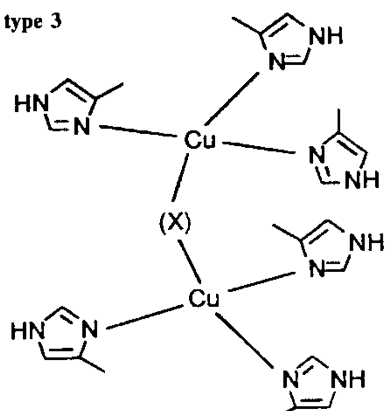
# Proteine al rame

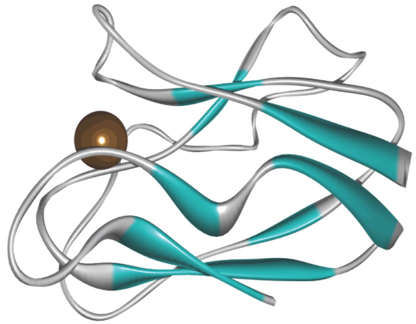


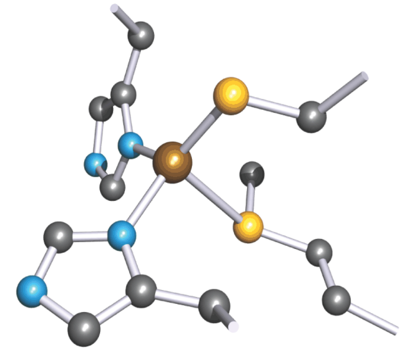

**reattività ferro-ossidasica**

**Table 10.2** Characteristics of 'classical' copper centers in protein

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

Plastocianina
(da spinaci)
10.5 kDa,
ca. 100 a.a.

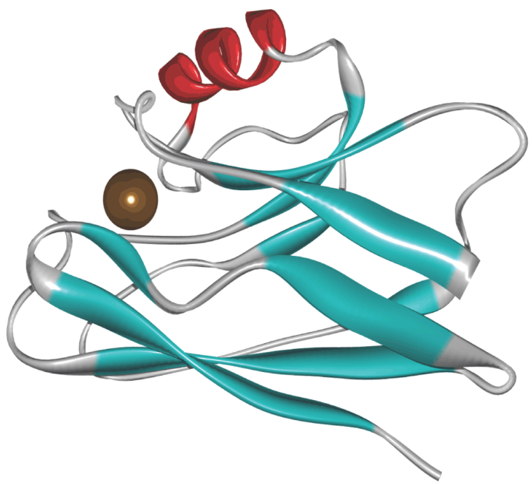




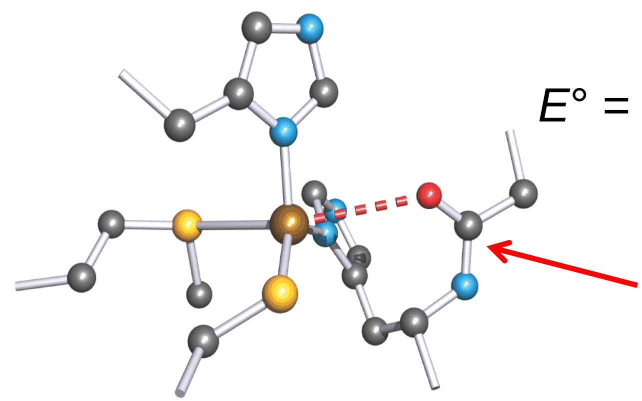
*E*° = + 370 mV

Coordinazione 3 + 1

Azurina
(da batteri)
14.5 kDa,
ca. 130 a.a.



*E*° = + 308 mV

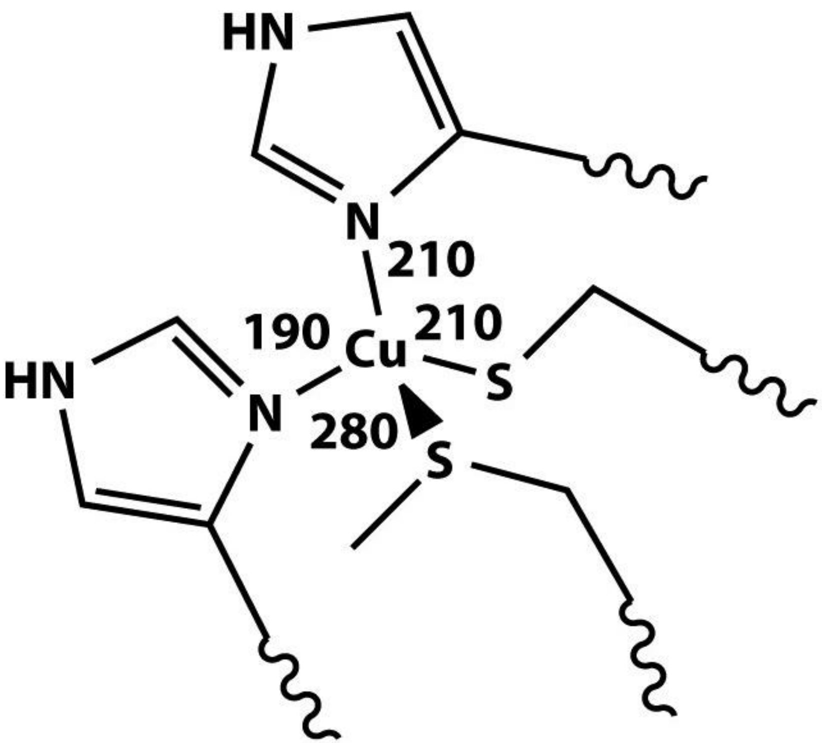



Coordinazione 3 + 1 + 1

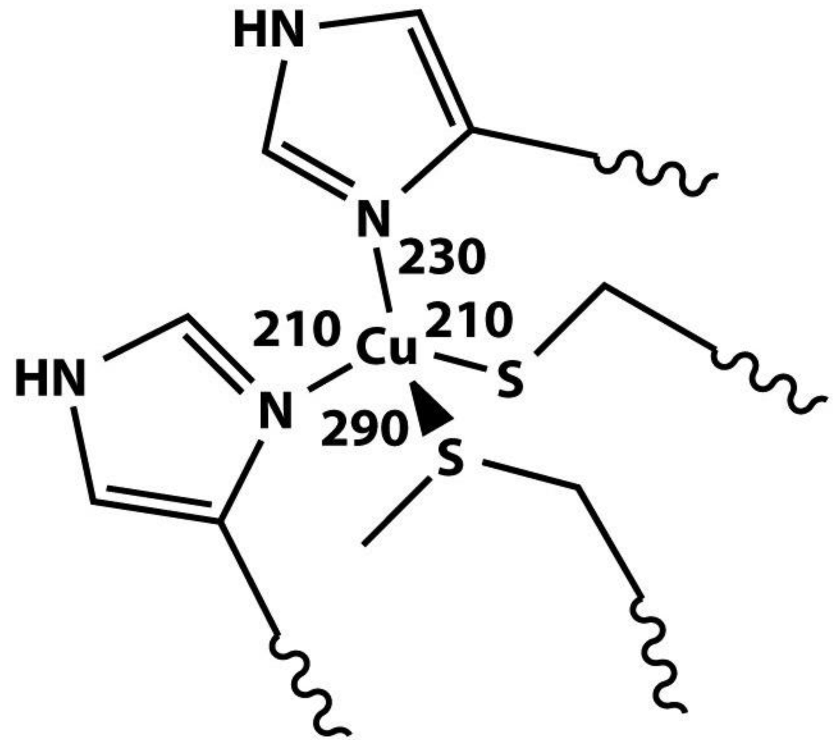
# *Blue copper proteins*

Forte assorbimento a ca. 600 nm, LMCT da $Cys^-$ a Cu(II)

# Esempio di stato entatico



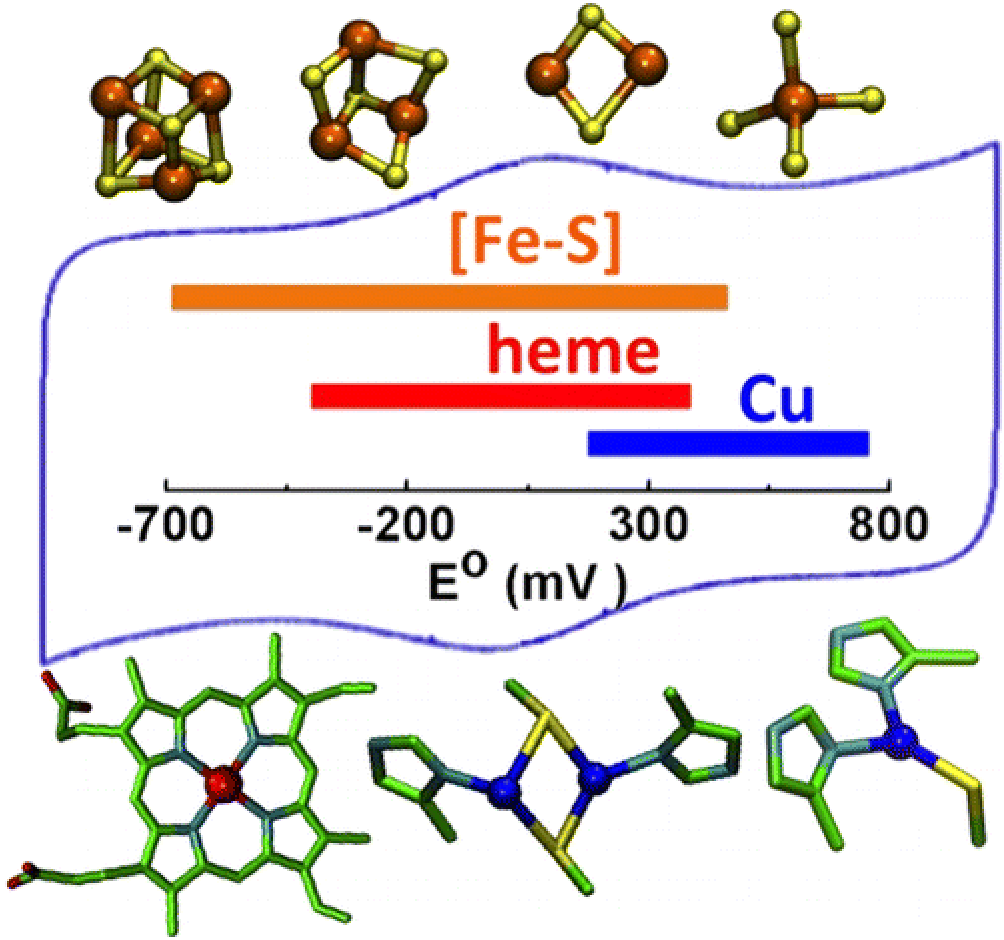

**Oxidized plastocyanin**




**Reduced plastocyanin**

*velocità di trasferimento elettronico dell'ordine $10^3$–$10^7$ $M^{-1}$ $s^{-1}$*
*(rispetto a $5\times10^{-7}$ $M^{-1}$ $s^{-1}$ per la coppia Cu(II)/Cu(I) acquosa)*

# Metallo-proteine per trasferimento elettronico

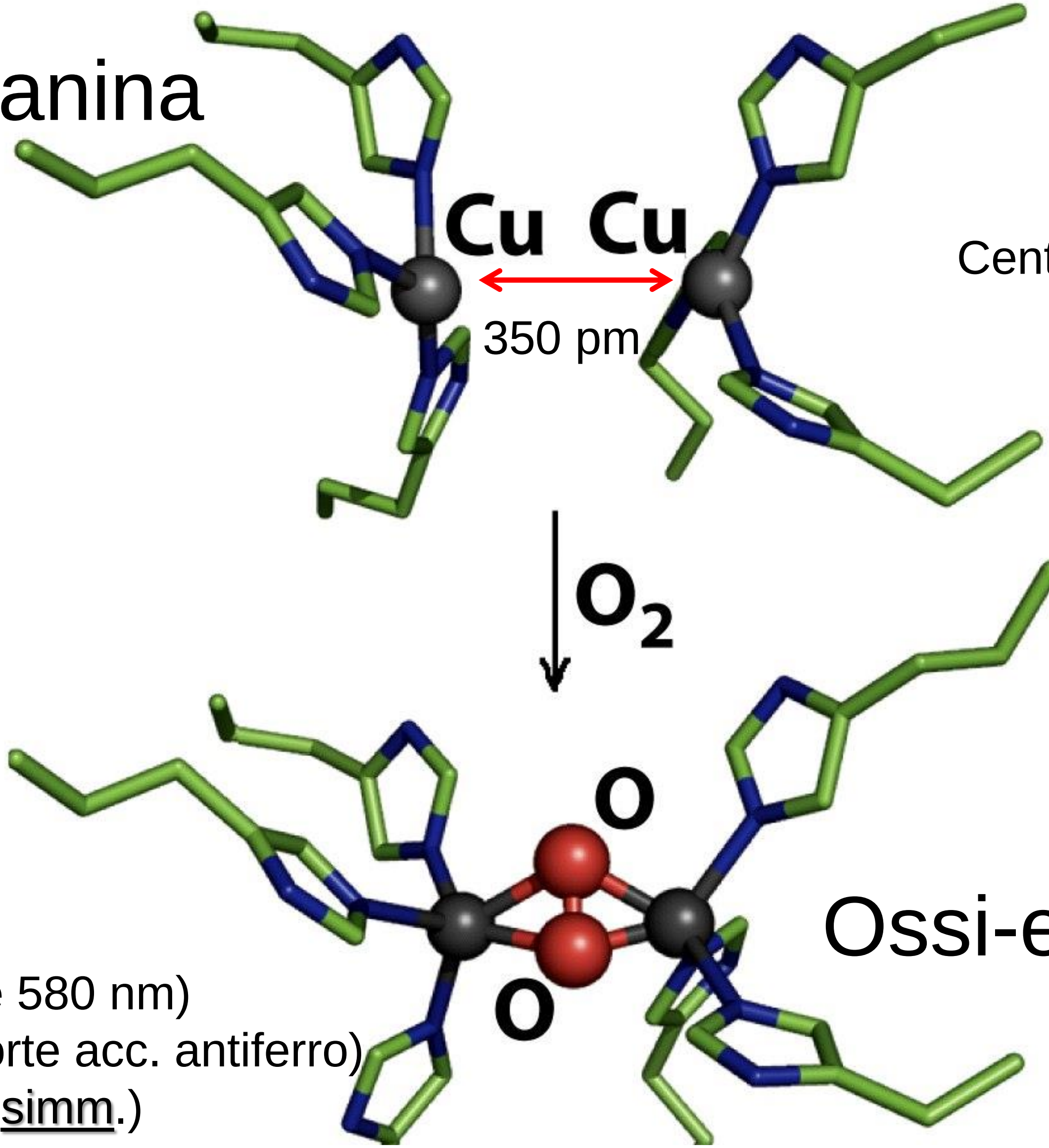


# Emocianina

fino a 1500 kDa,
ogni unità 75 kDa

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

Centro rame di tipo 3

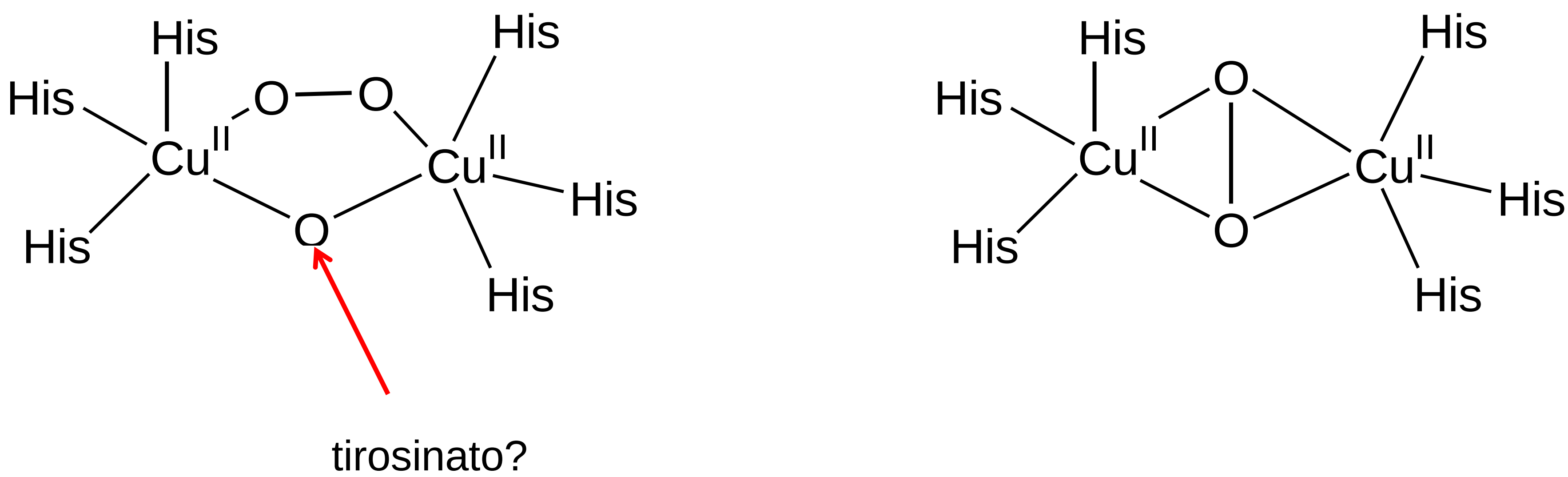



# Ossi-emocianina

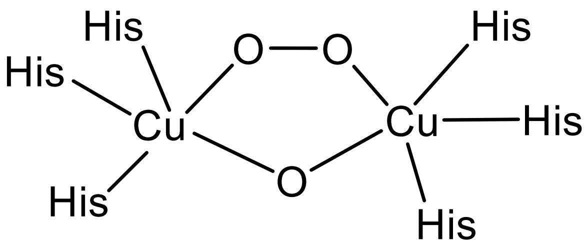
Viola (LMCT 350 e 580 nm)
2 Cu(II) (diamg., forte acc. antiferro)
IR: 755 cm$^{-1}$ ($O_2^{2-}$, simm.)

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

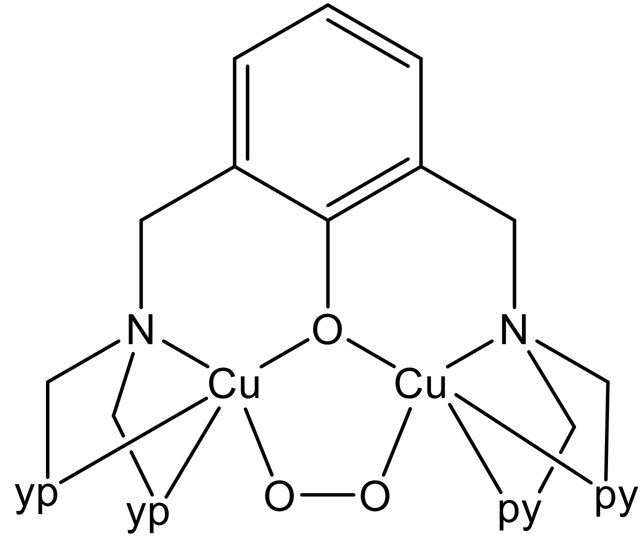
# Possibili coordinazioni simmetriche dello ione perossido

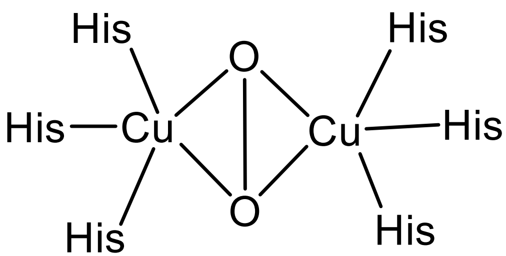


# Modelli per la coordinazione di $O_2$ alla emocianina

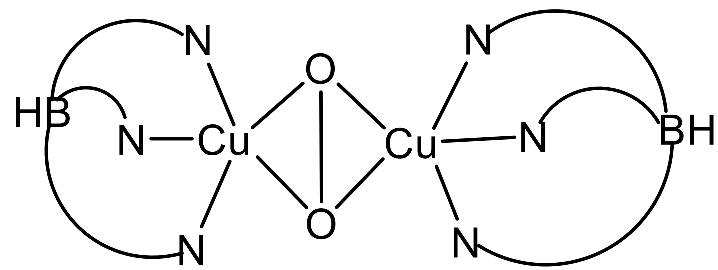


ipotesi sbagliata

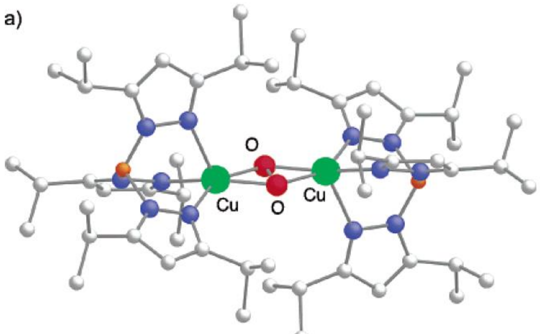




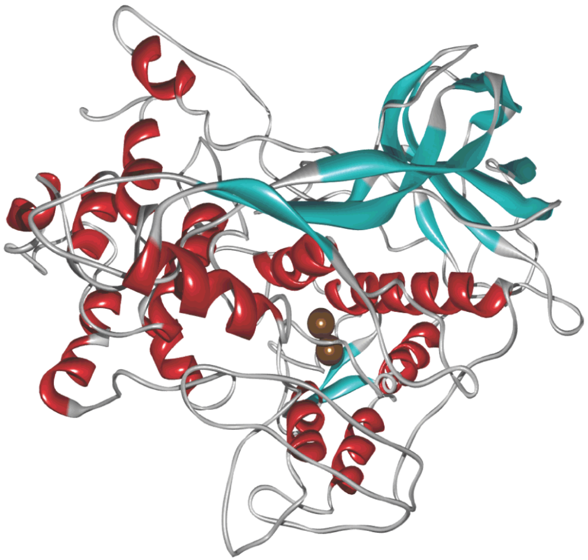
ipotesi corretta



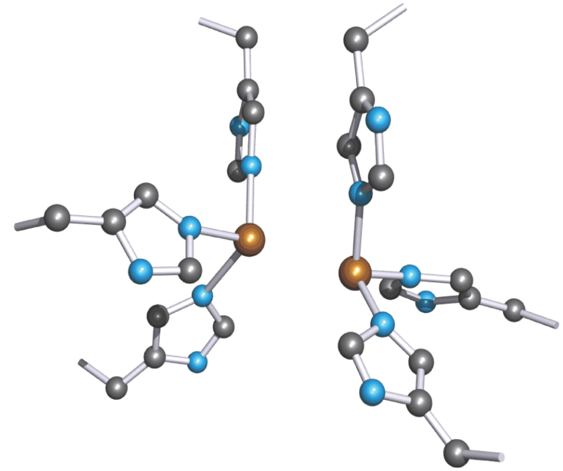
a)



# Emocianina

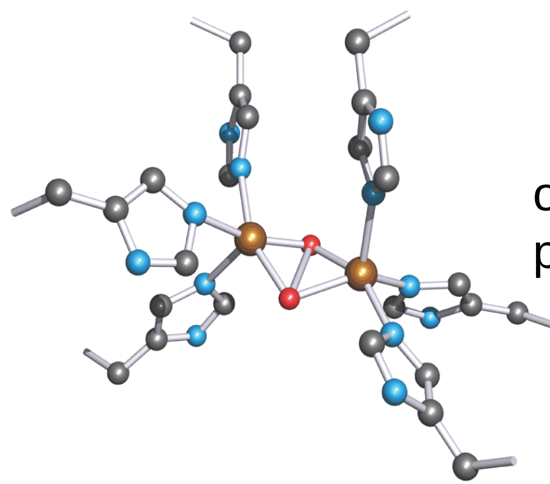


(a)



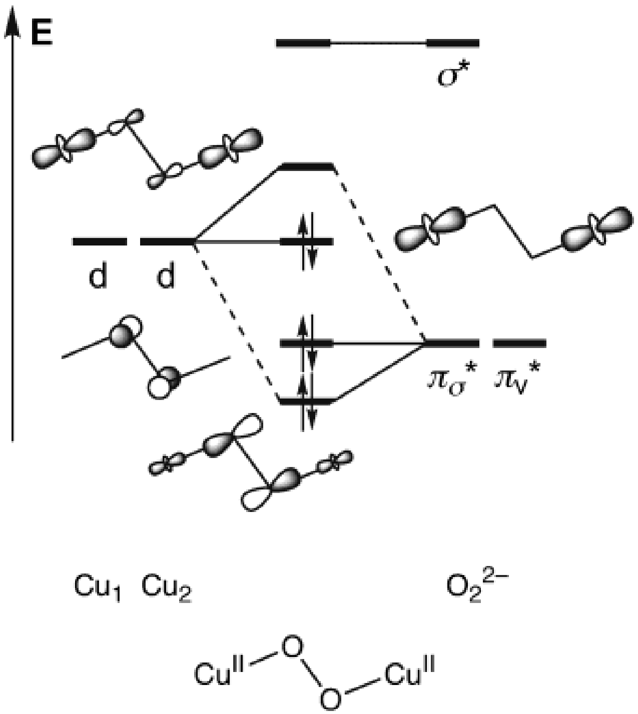
(b)

desossi,
tricoordinato

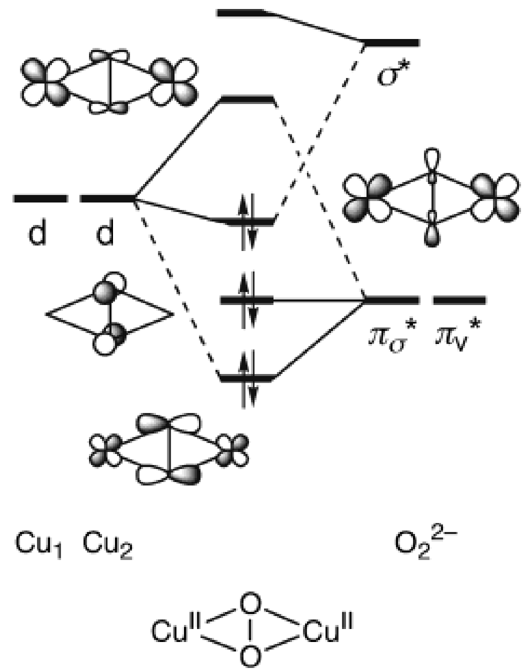


(c)

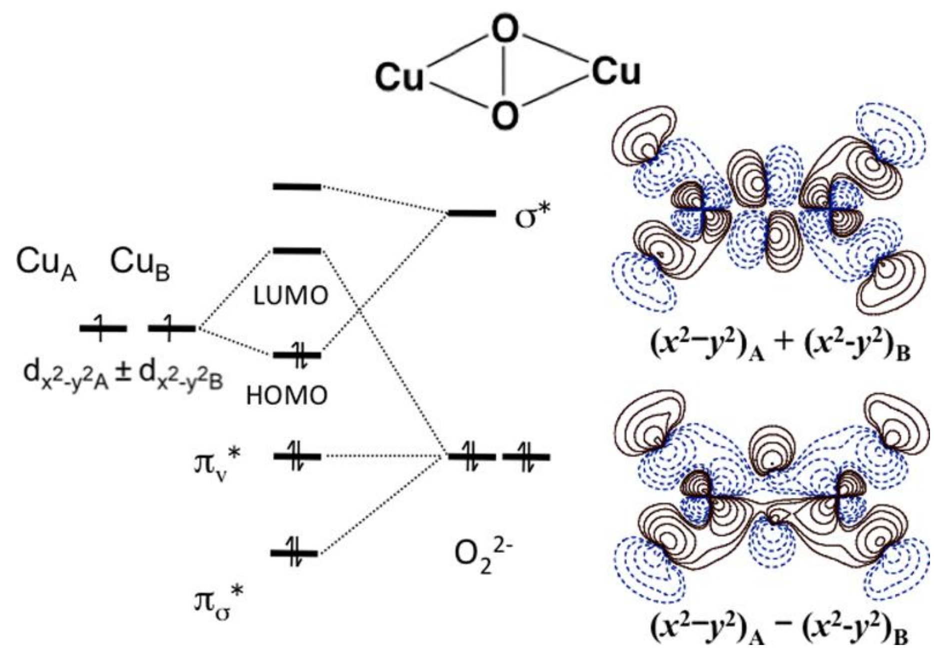
ossi,
pentacoordinato



E
σ*
d d
π_σ* π_v*
Cu1 Cu2
O2 2–
Cu^II–O–O–Cu^II

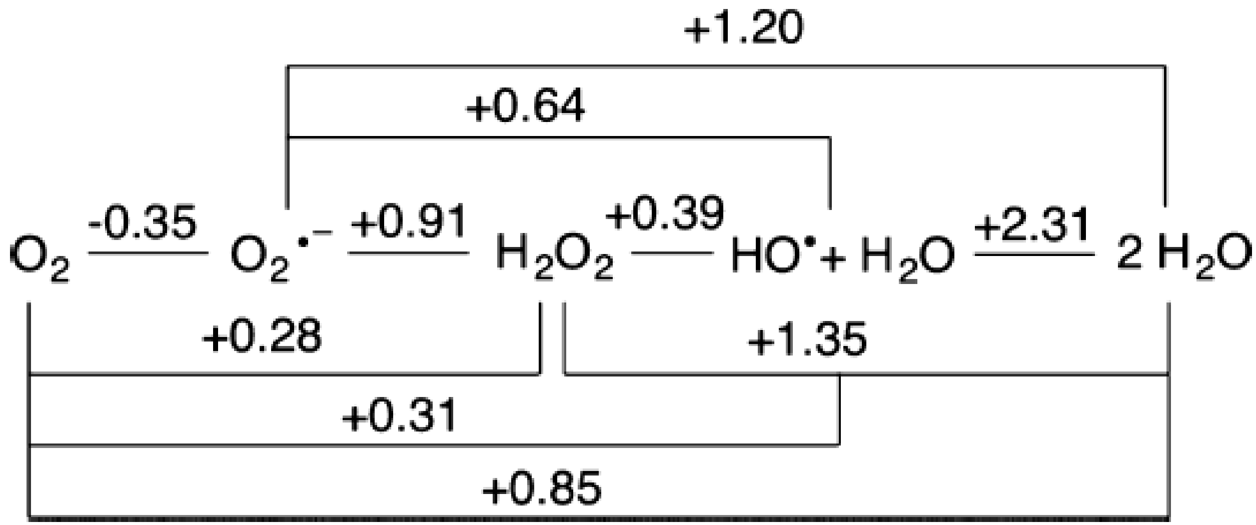



σ*
d d
π_σ* π_v*
Cu1 Cu2
O2 2–
Cu^II(O2)Cu^II

Cu O O Cu
σ*
CuA CuB
LUMO
HOMO
d_x2-y2A ± d_x2-y2B
π_v*
π_σ*
O2 2-
(x2−y2)A + (x2-y2)B
(x2−y2)A − (x2-y2)B

# Enzimi al Cu che attivano o riducono $O_2$



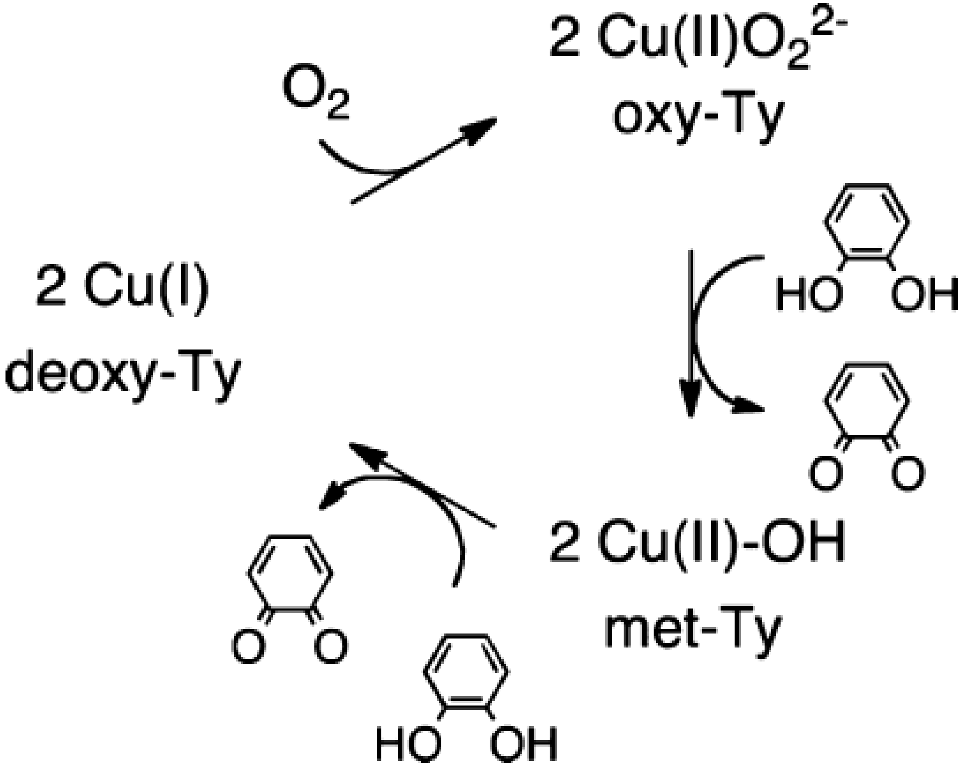

*...la riduzione mono-elettronica di $O_2$ a superossido è termodinamicamente sfavorita*

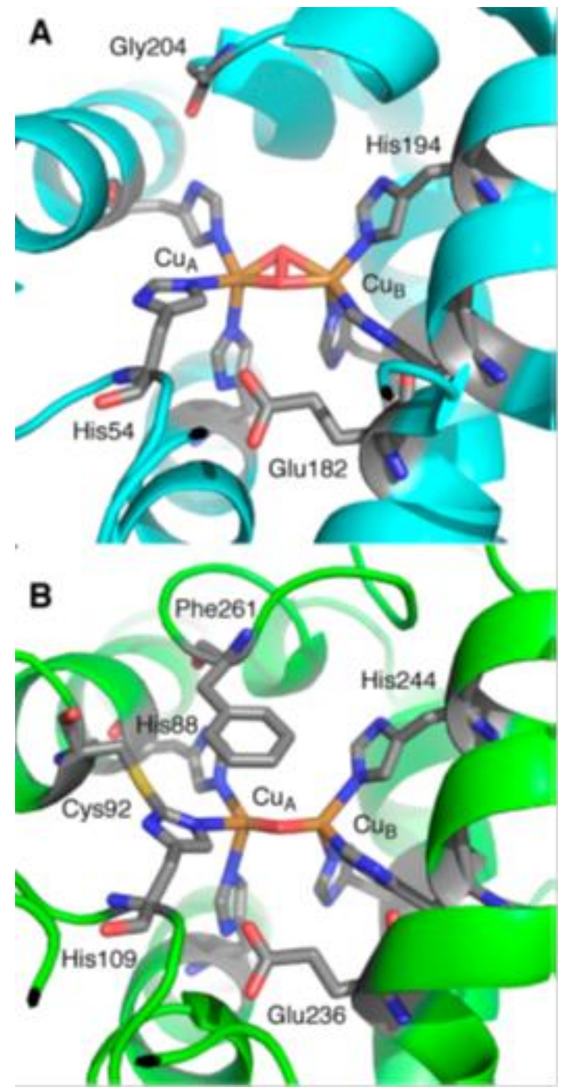
*fornire due elettroni richiede o la presenza di più ioni Cu oppure di un Cu e di un cofattore organico redox-attivo*

# Polifenolo ossidasi

*Tirosinasi, Catecolo-ossidasi*

2 *o*-difenolo + $O_2 \rightarrow$ 2 *o*-chinone + $2H_2O$



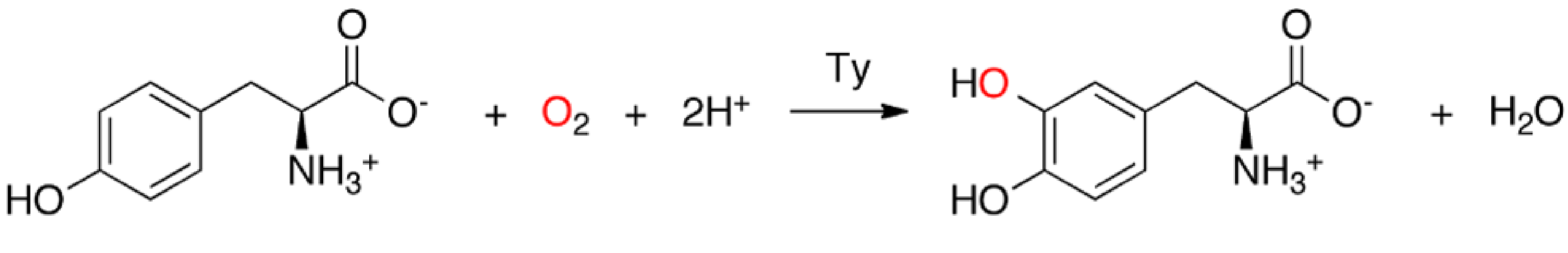




oxy-Ty

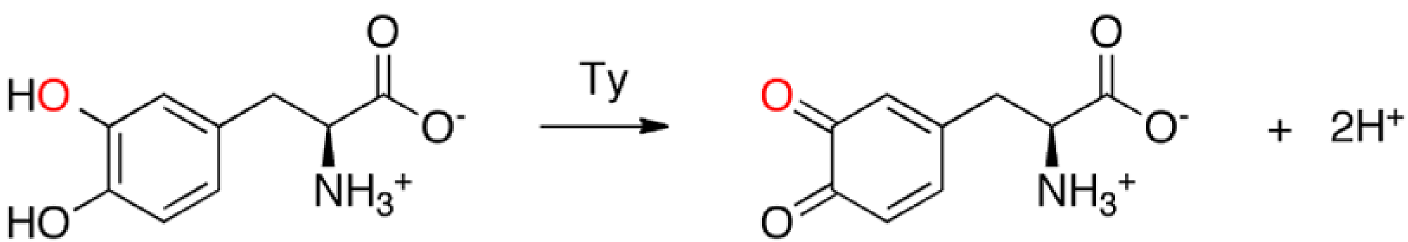
met-Ty

Nel ciclo vengono trasferiti 4 elettroni ai 2O

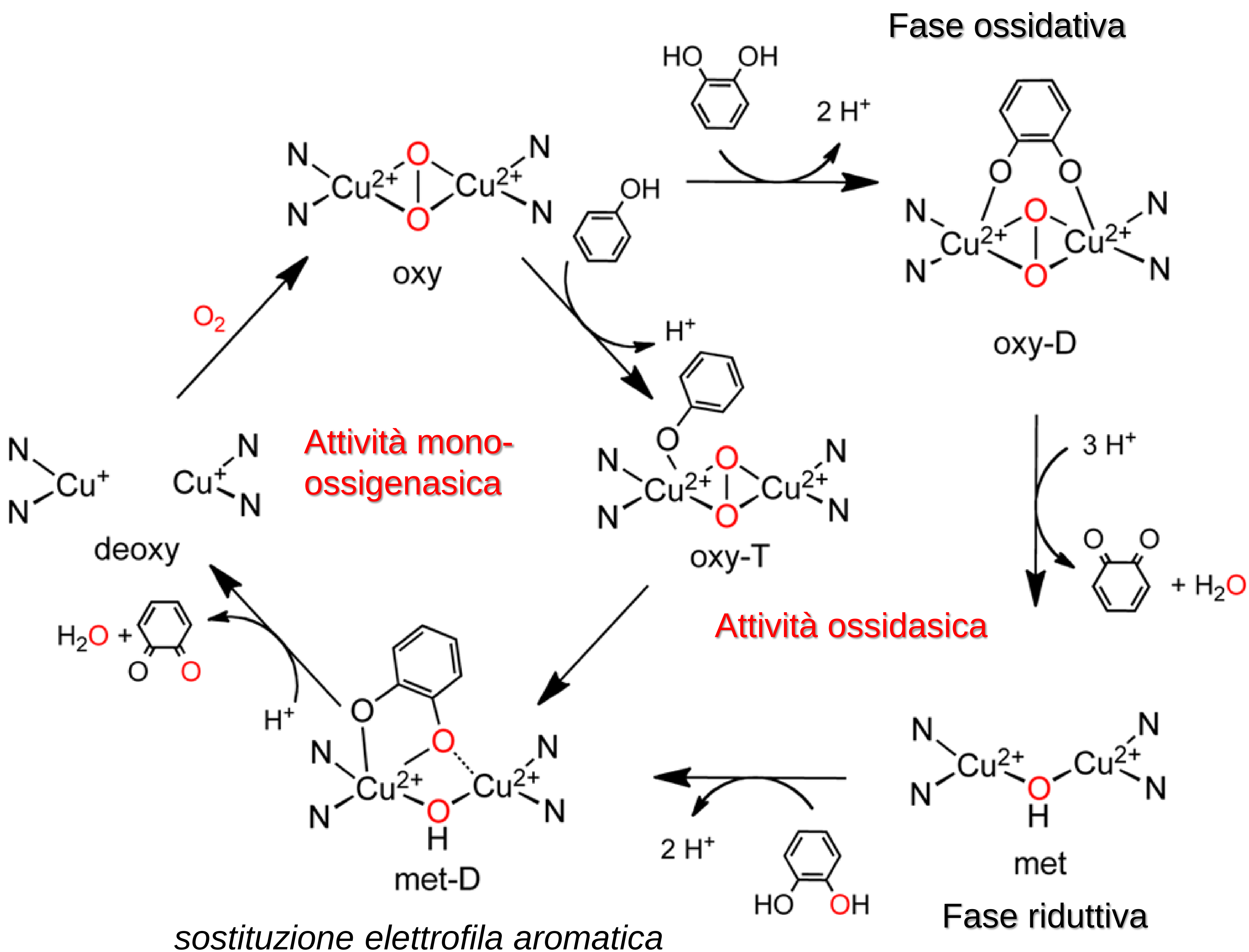
# Tirosinasi come mono-ossigenasi




L-tyrosine L-DOPA




L-DOPA *o*-L-DOPAquinone


Fase ossidativa
HO
OH
2 H+
O
O
N
Cu2+
O
O
Cu2+
N
N
N
oxy-D
3 H+
O
O
+ H2O
Attività ossidasica
N
Cu2+
O
H
Cu2+
N
N
N
met
Fase riduttiva
HO
OH
2 H+
met-D
H+
H2O +
O
O
deoxy
N
Cu+
N
Cu+
N
N
O2
oxy
OH
H+
oxy-T
Attività mono-
ossigenasica
sostituzione elettrofila aromatica

*Multicopper oxidases*, MCOs
(ossidasi azzurre)

Substrati organici

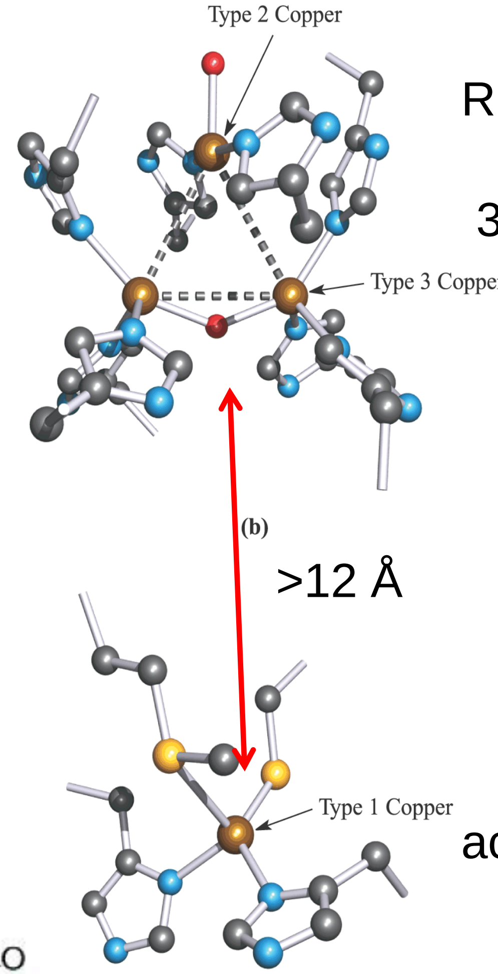
Ascorbato ossidasi
Laccasi

$$4\ RH + O_2 \rightarrow 4R\bullet + 2\ H_2O$$
(RH = fenolo)

Substrati «metallici»

Ceruloplasmina
Epestina
Fet3p
Rame-ossidasi (CueO)

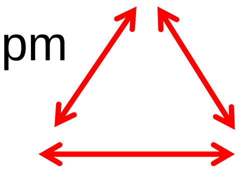
(a)




(b)

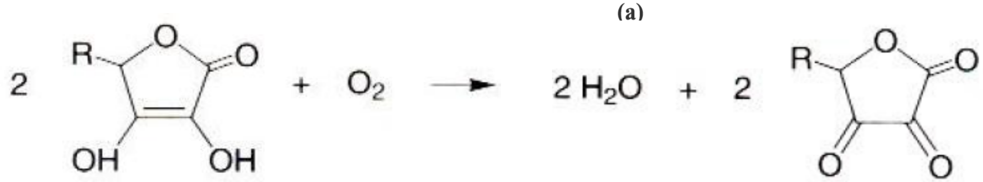
(c)

Riduzione $O_2$ ($4e^-$)




accettore di elettroni

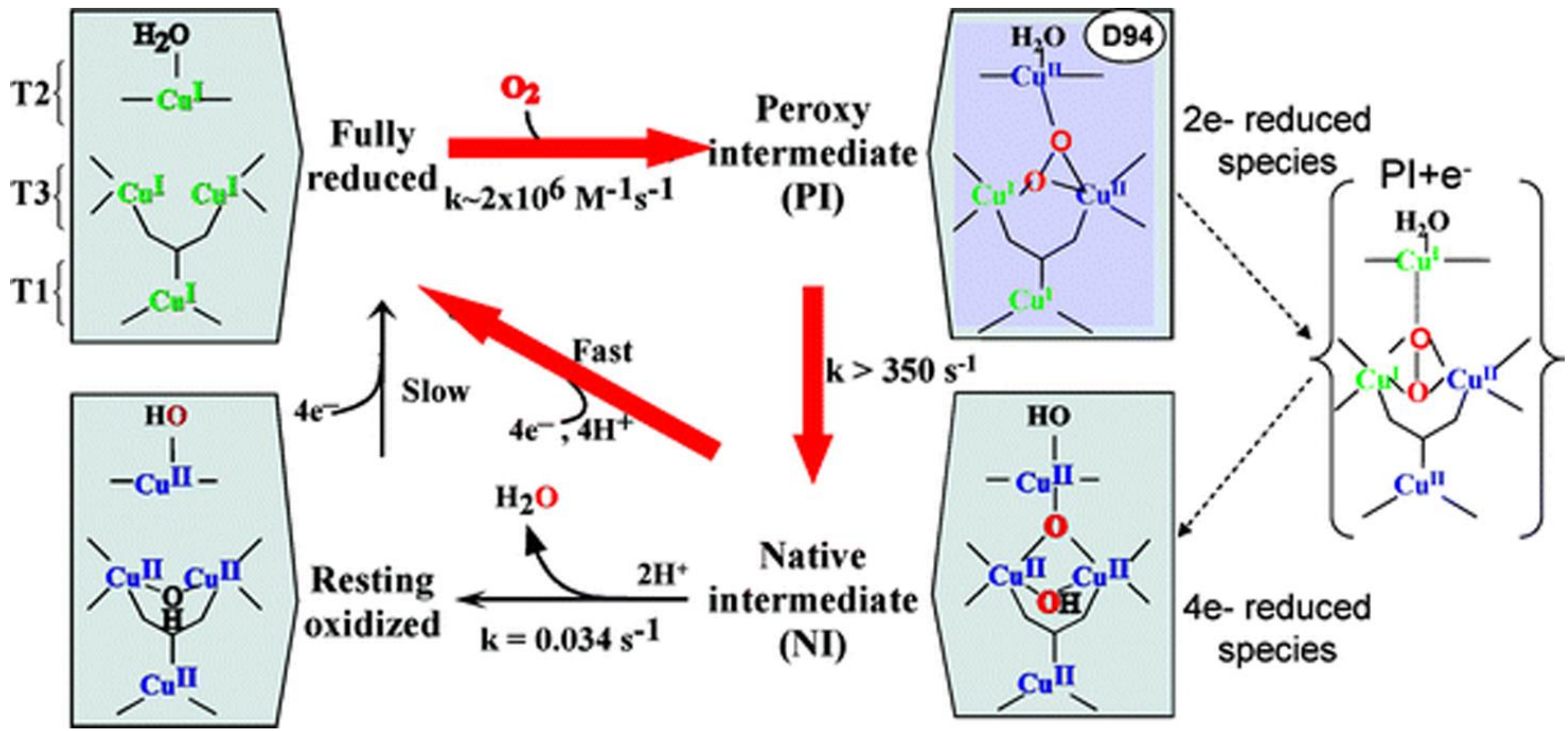
R = $CH(OH)CH_2OH$




# Ascorbato ossidasi (da zucchini)

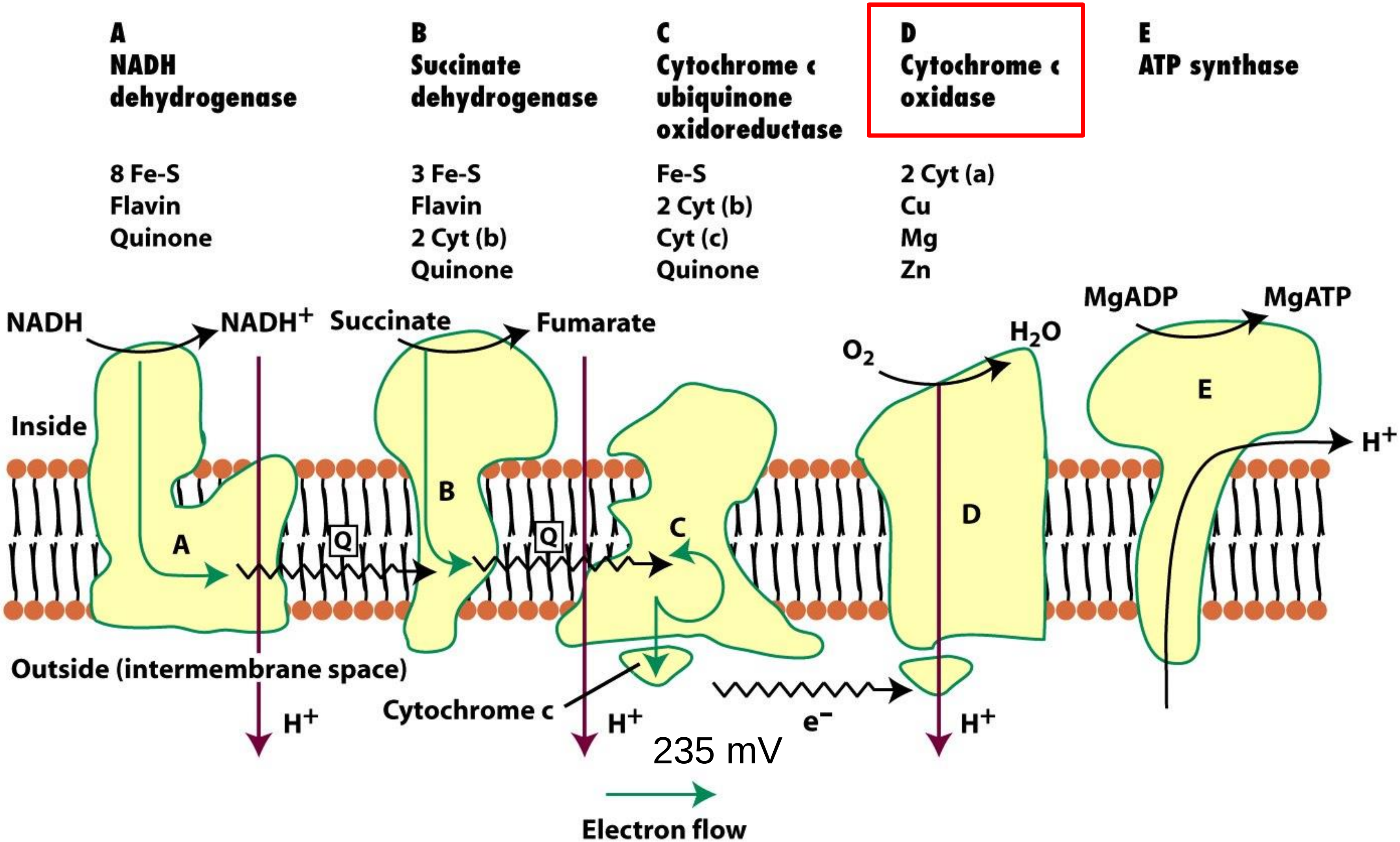
$$4\ RH + O_2 \rightarrow 4R\cdot + 2\ H_2O$$

(RH = fenolo)



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

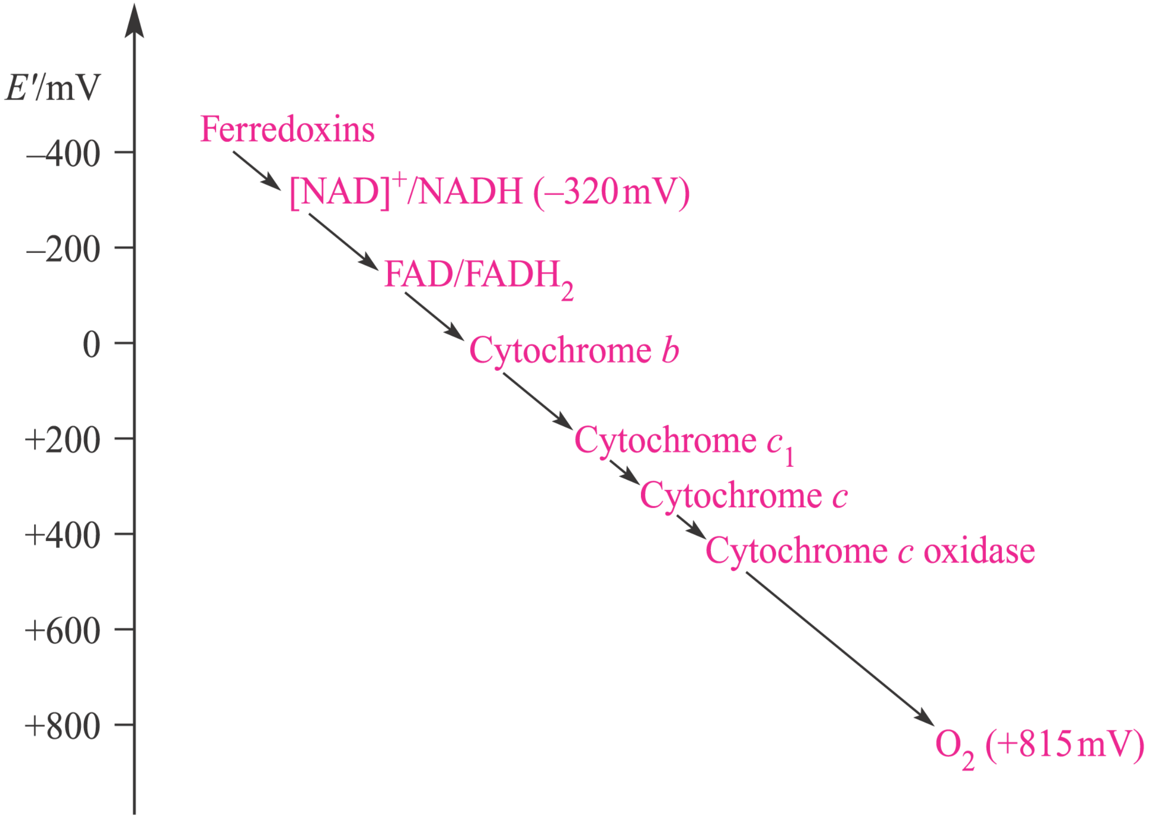
# Catena respiratoria (fosforilazione ossidativa)

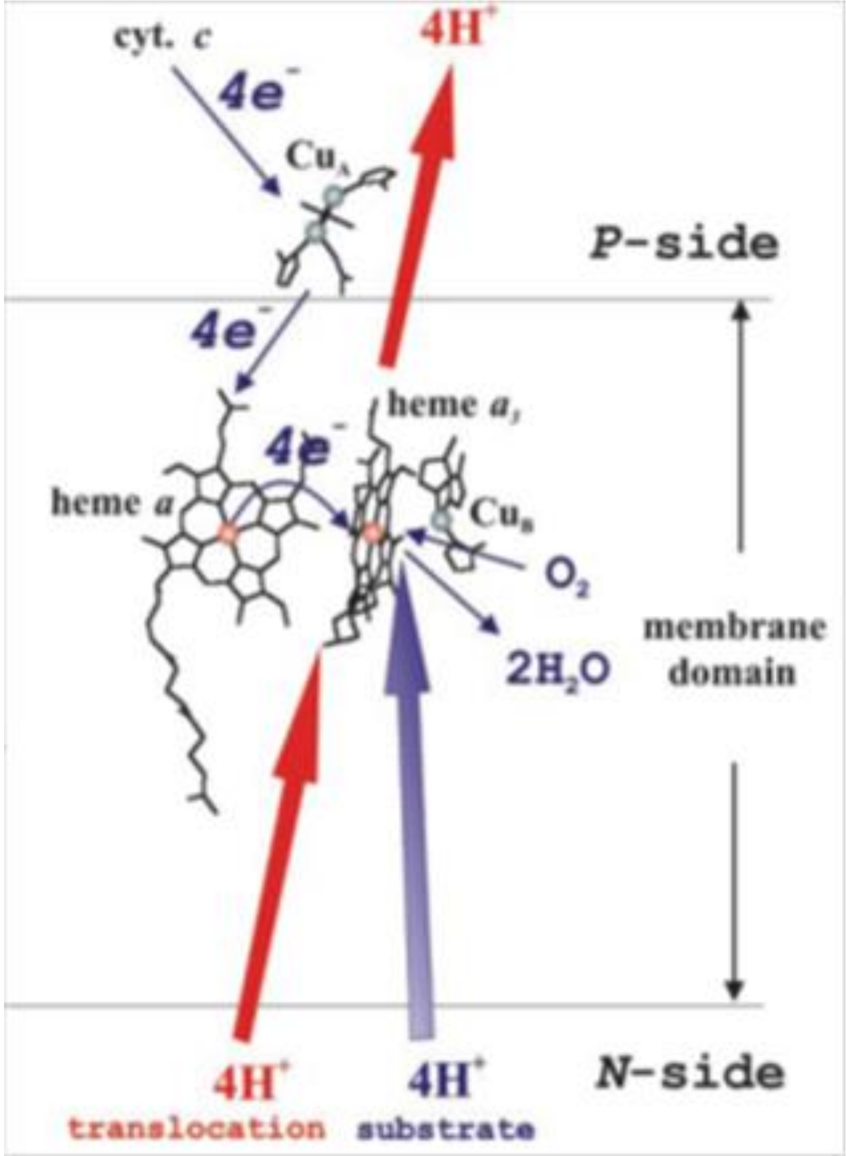



*Driving force* per elettrone:
815 – 235 = 580 mV (ca. 13 kcal/mol)

$O_2 + 4H^+ + 4e^- \leftrightarrows 2H_2O$
$\Delta E°$ (pH 7) = 815 mV; $\Delta G°$ = -80 kcal/mol

# La sequenza di trasferimenti elettronici nei mitocondri

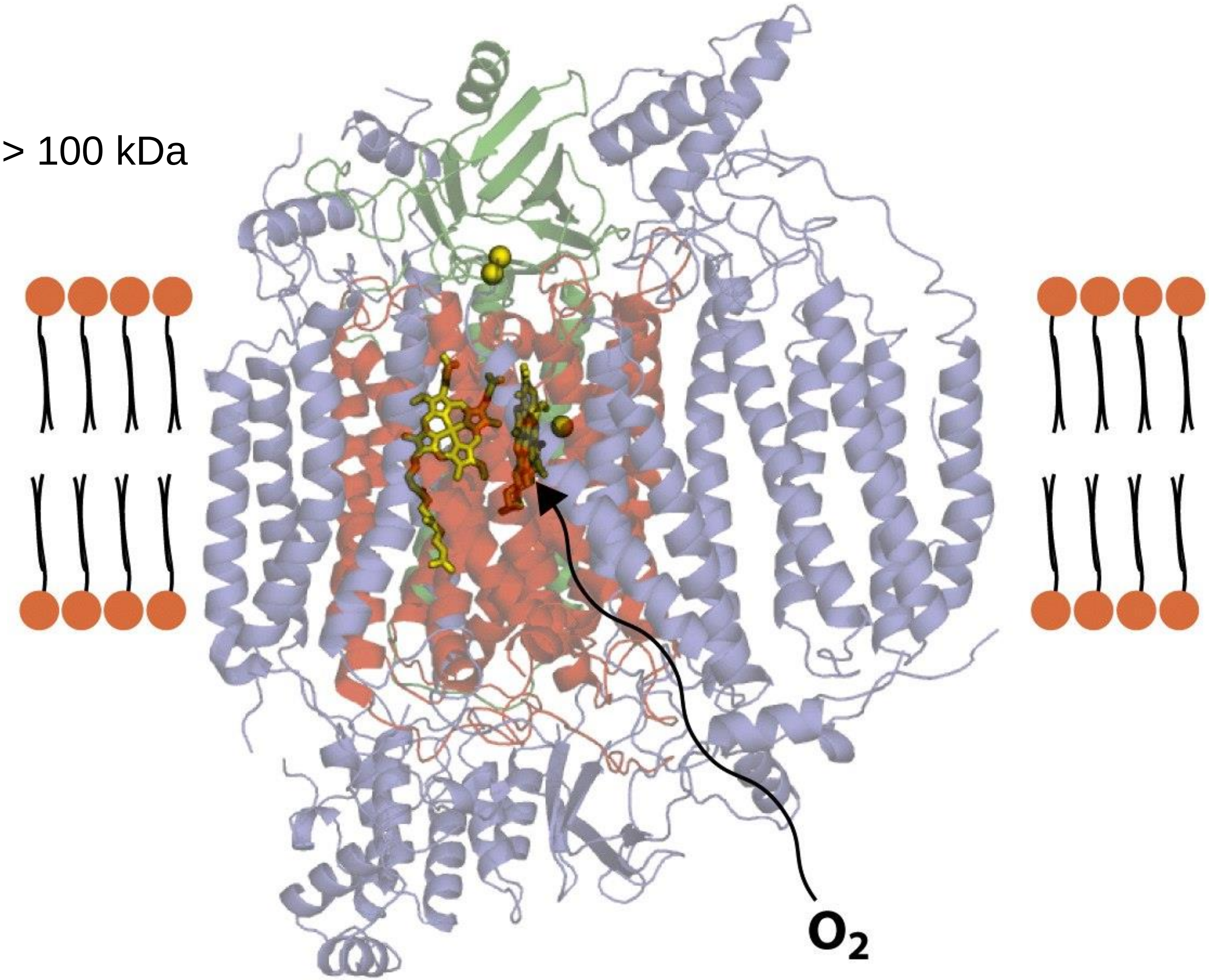




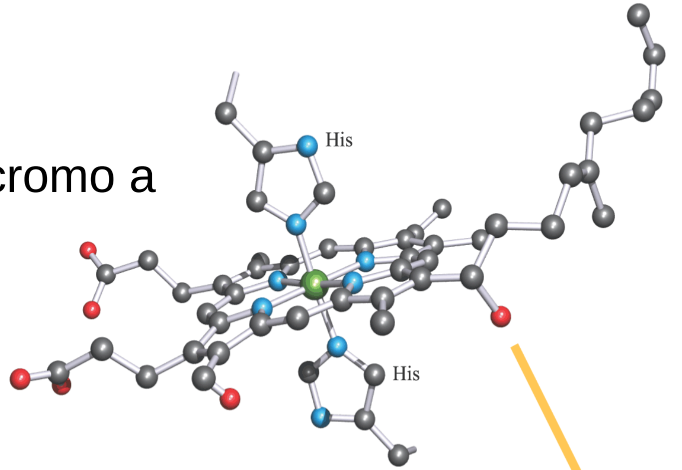

$O_2(g) + 4\ e^- + 8\ H^+(\text{inside}) \rightarrow 2\ H_2O(l) + 4\ H^+(\text{outside})$

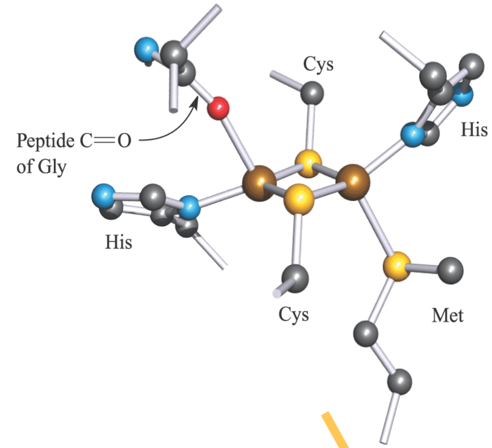
# Reacts with cytochrome C

> 100 kDa



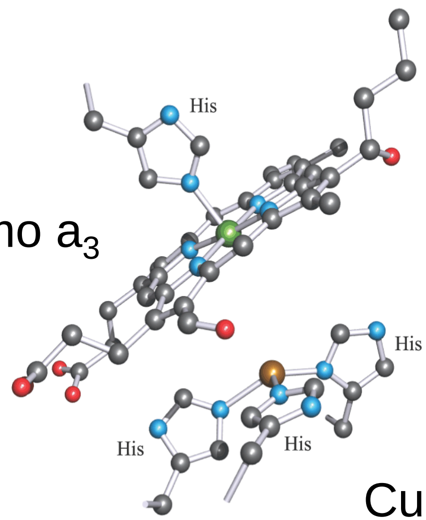
citocromo a

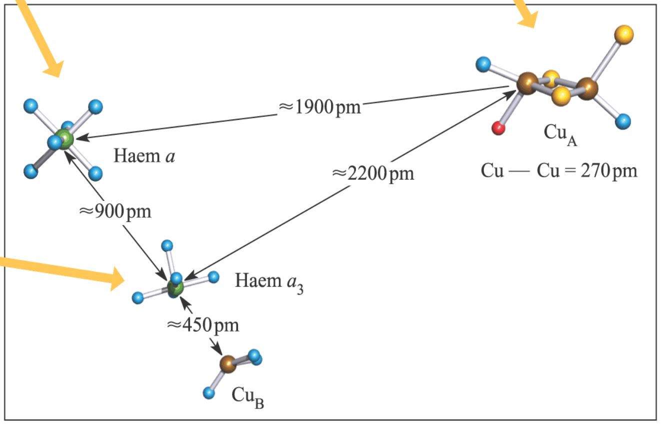


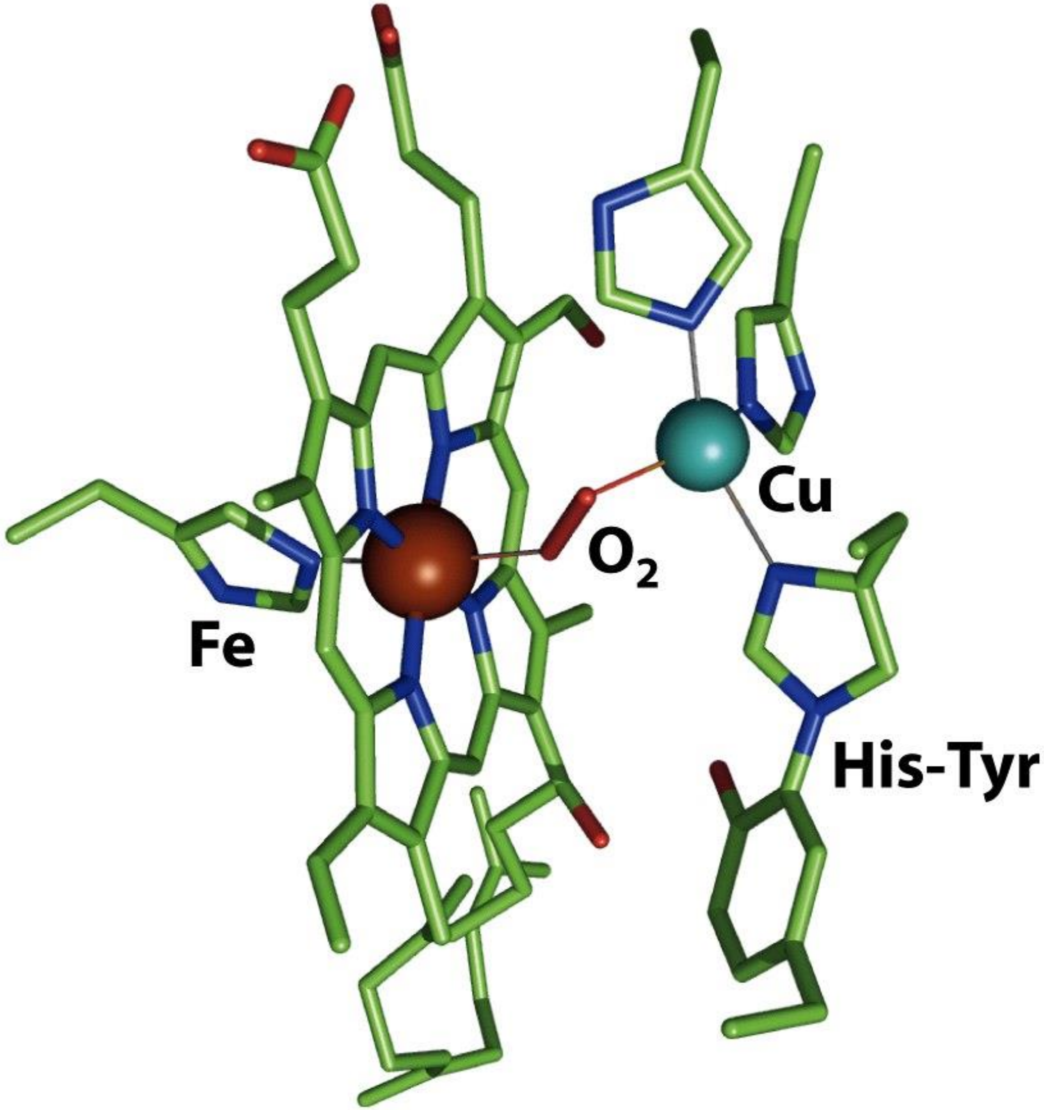

$Cu_A$

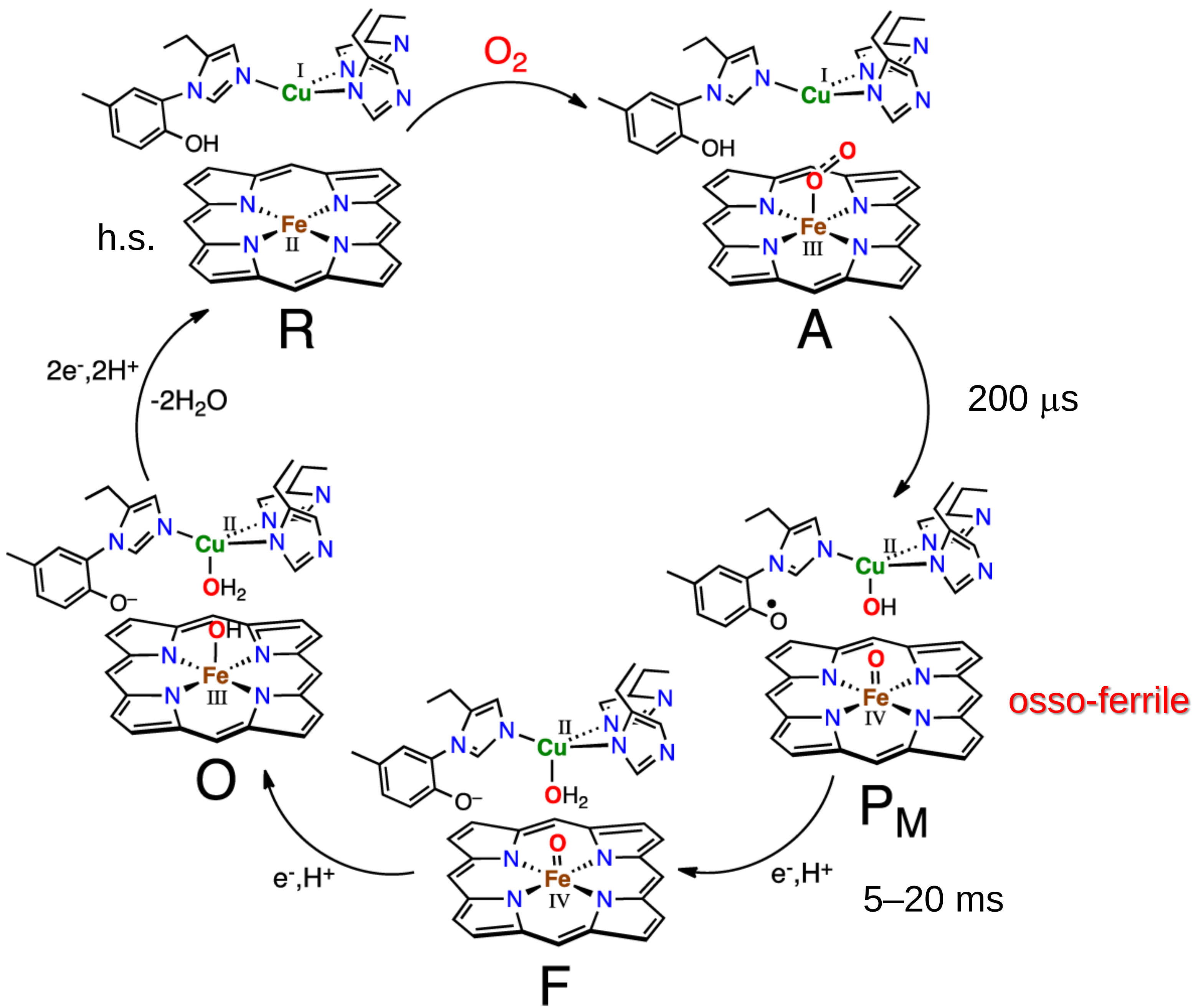
citocromo $a_3$



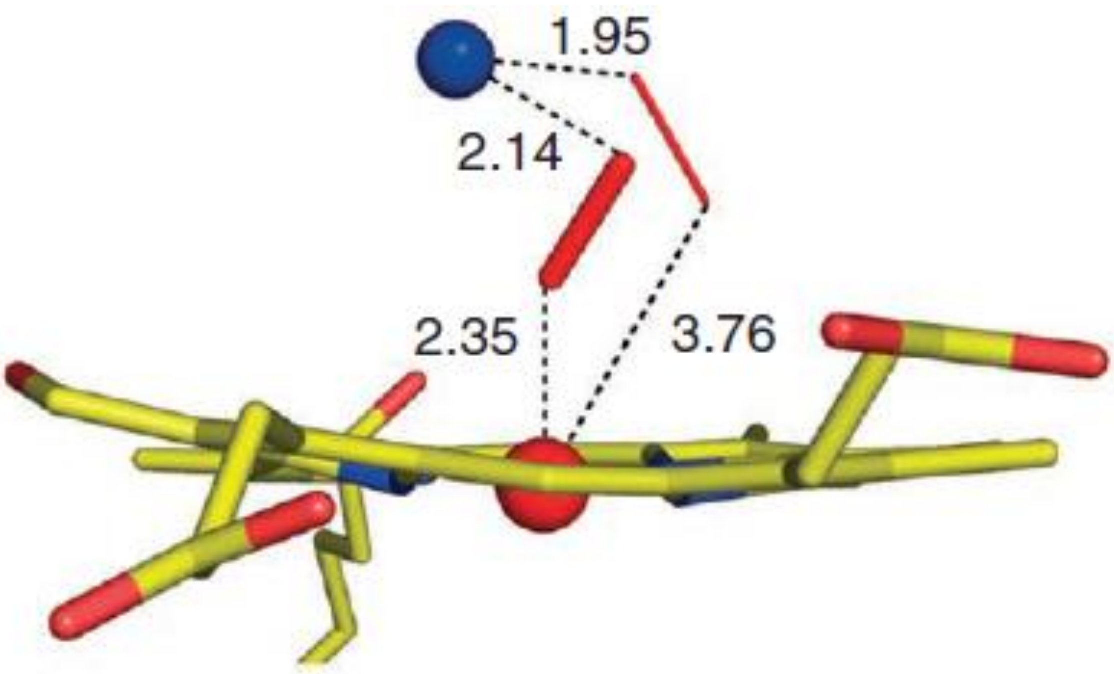

$Cu_B$ (rame tipo 2)


Cu
$O_2$
Fe
His-Tyr



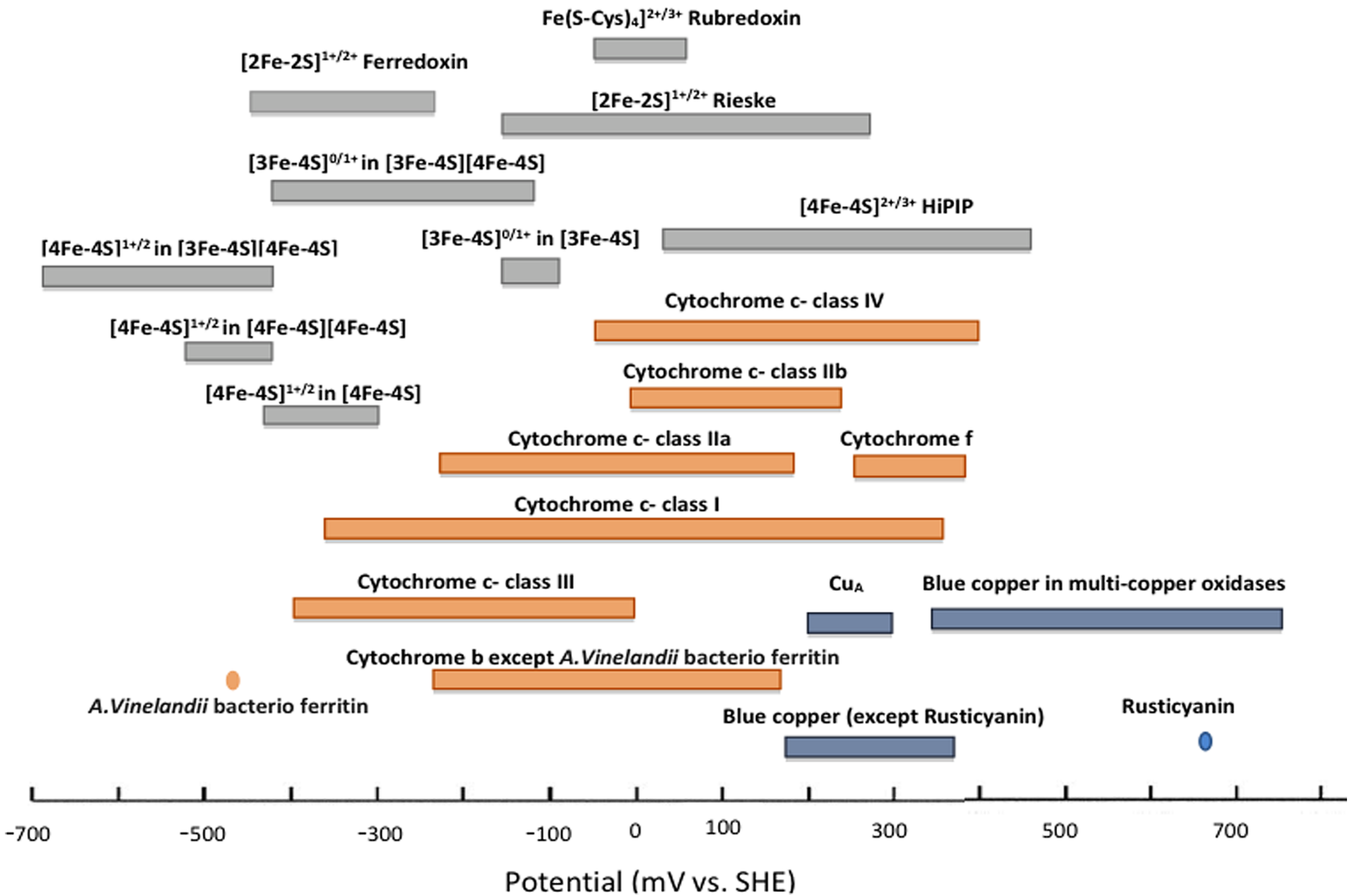
O2
R
A
h.s.
200 μs
osso-ferrile
PM
5–20 ms
e-,H+
F
e-,H+
O
2e-,2H+
-2H2O

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




*Nature*, 2014

# Metallo-proteine per trasferimento elettronico



# *Reactive Oxygen Species* (ROS)

Reazione di Haber-Weiss

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

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

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

Reazione di Fenton

The net reaction:

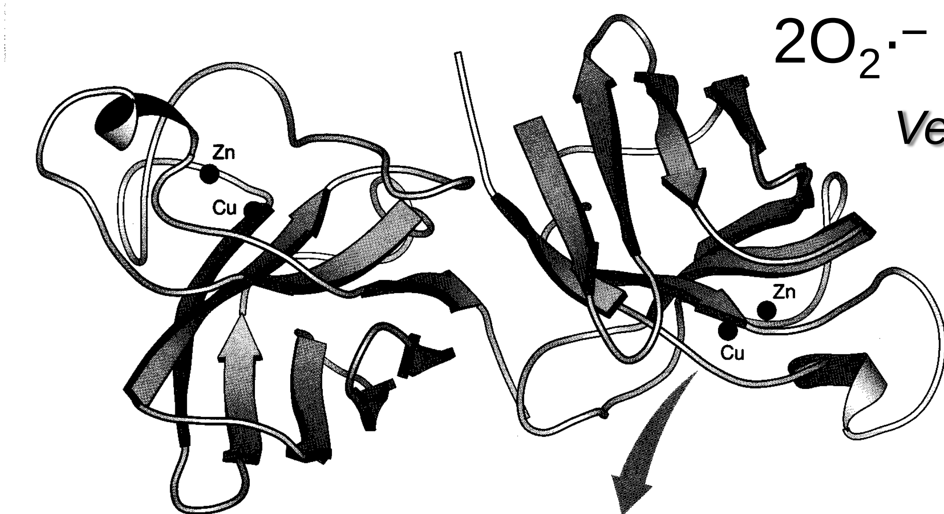
$$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + OH^- + OH^{\bullet}$$

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

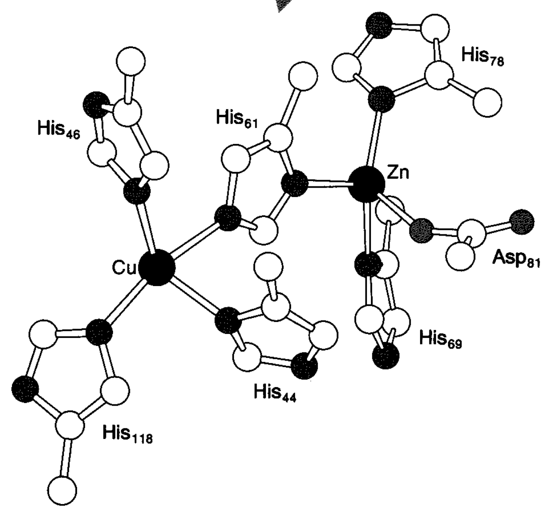
# Cu-Zn superossido dismutasi

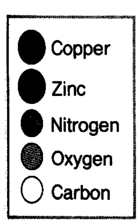
$$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$$

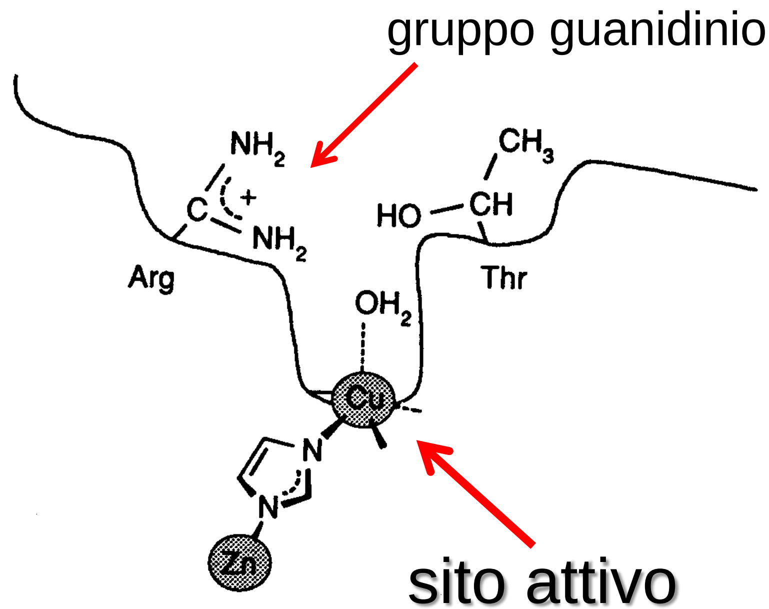
*Velocità quasi-diffusiva*




16 kDa



# Ciclo catalitico della superossido dismutasi