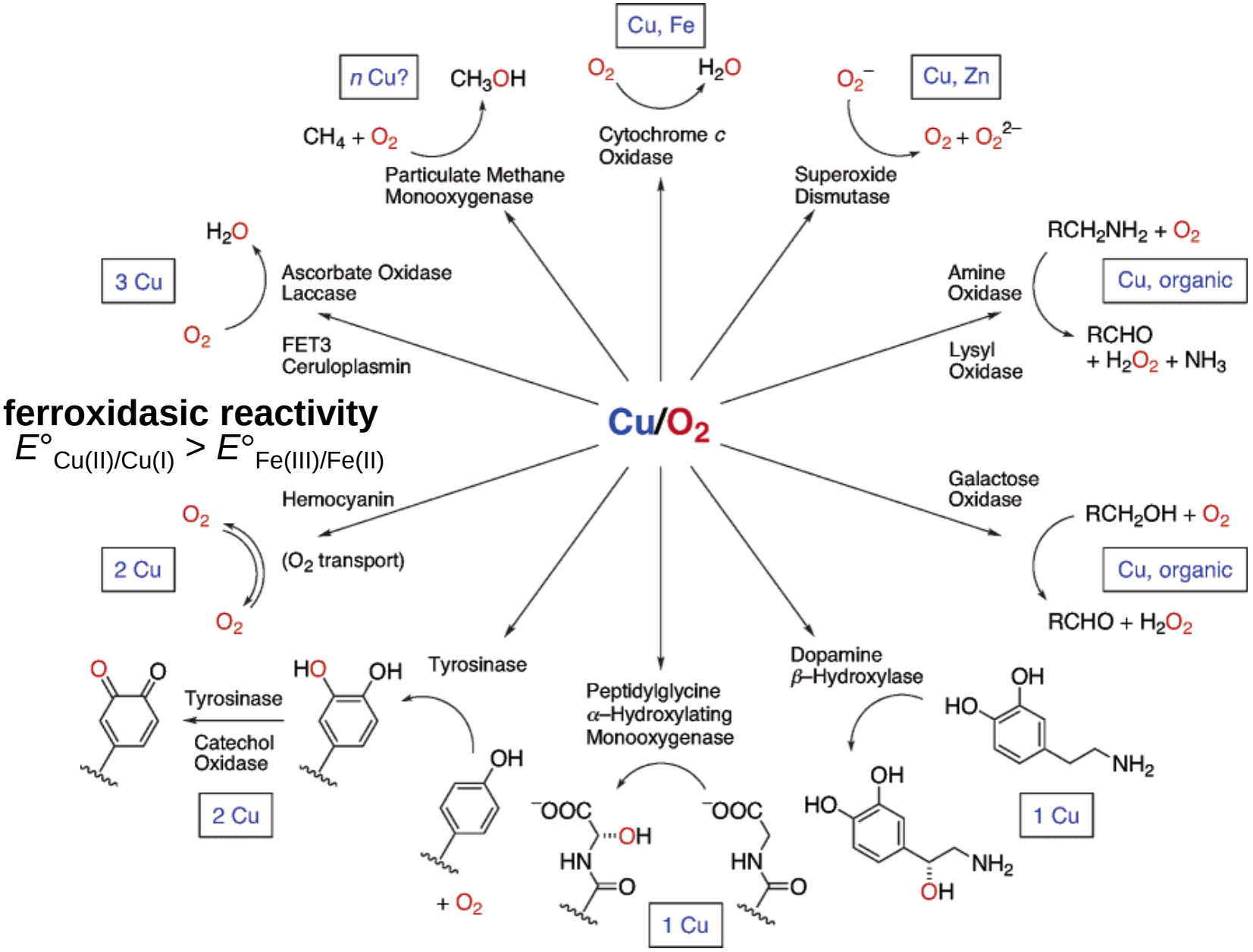
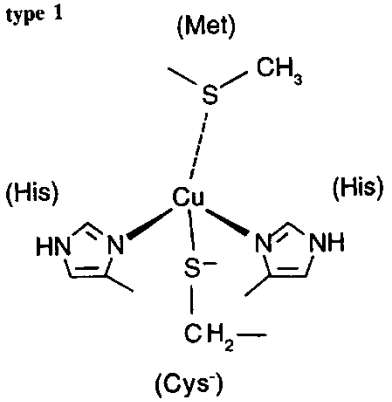
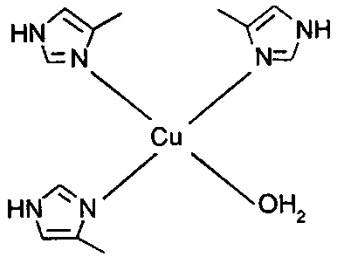
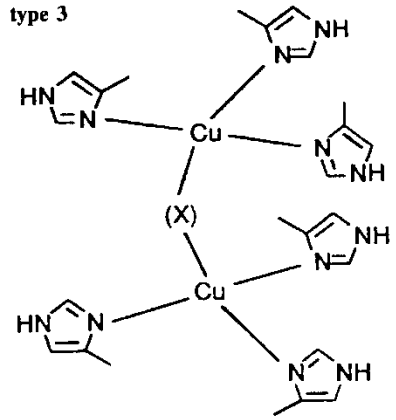
Copper proteins



ferroxidasic reactivity

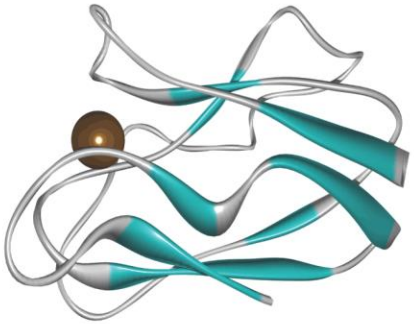
$E°_{Cu(II)/Cu(I)} > E°_{Fe(III)/Fe(II)}$

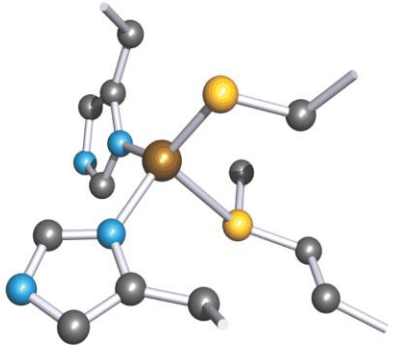
Table 10.2 Characteristics of 'classical' copper centers in protein

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 $S\text{-}(Cys^-) \rightarrow Cu^{II}$ EPR/ENDOR of the oxidized form: small $^{63,65}Cu$ hyperfine coupling and g anisotropy, interaction of the electron spin with $-S\text{-}CH_2-$; $Cu^{II} \rightarrow S(Cys)$ 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)

Plastocyanin
(from spinach)

10.5 kDa,
ca. 100 a.a.



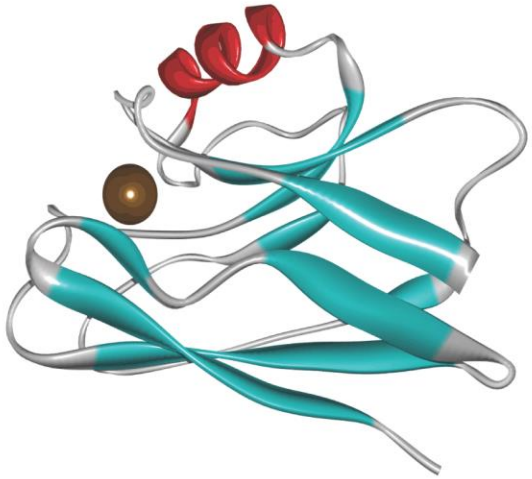


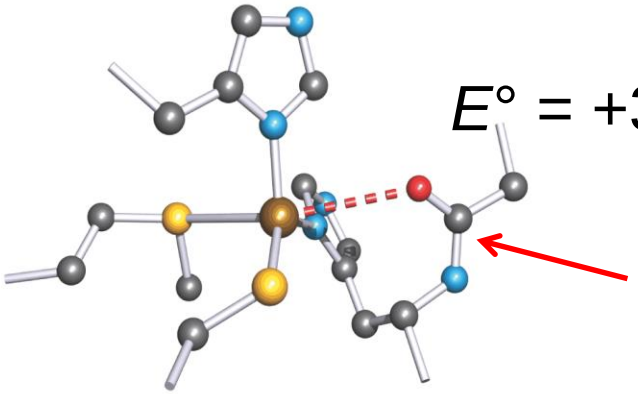
$E°$ = +370 mV

3 + 1 coordination

Azurin
(from bacteria)

14.5 kDa,
ca. 130 a.a.





$E°$ = +308 mV

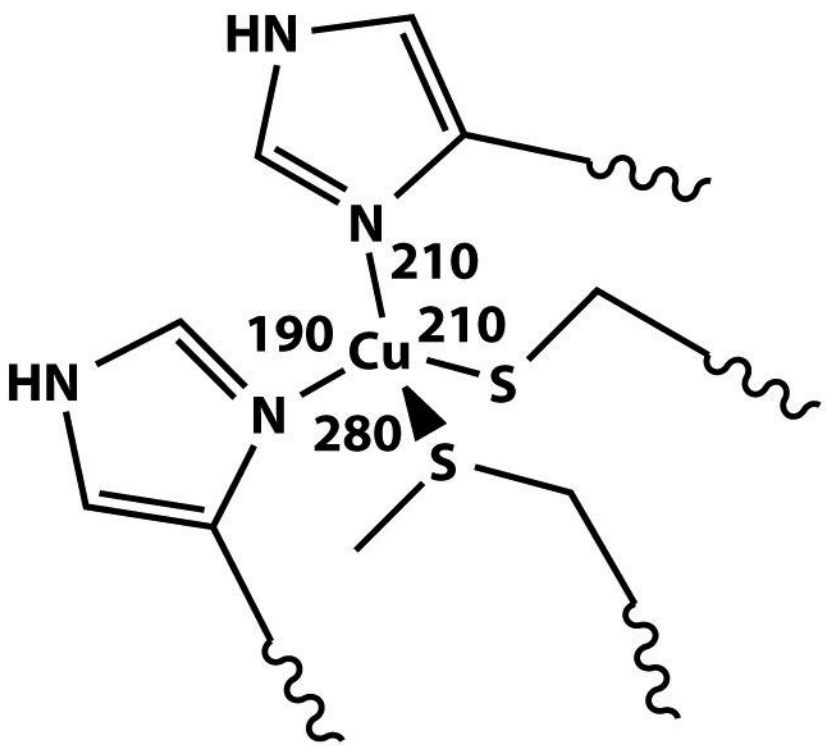
3 + 1 + 1 coordination

$E°_{Cu(II)/Cu(I)}$ (aquaion) = +153 mV

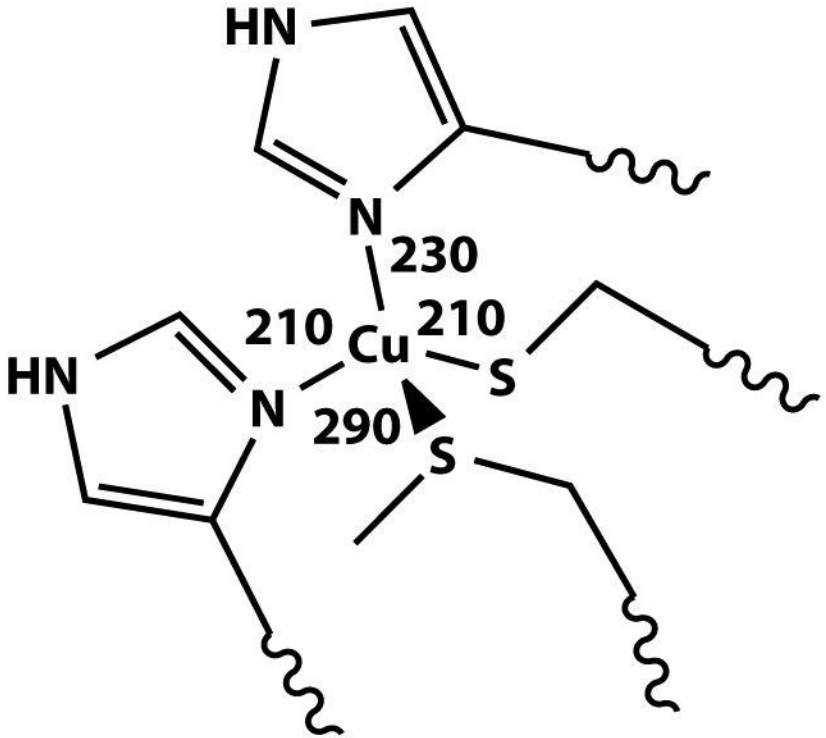
Blue copper proteins

Strong absorption at ca. 600 nm, LMCT from Cys^- to Cu(II)

Example of entatic state



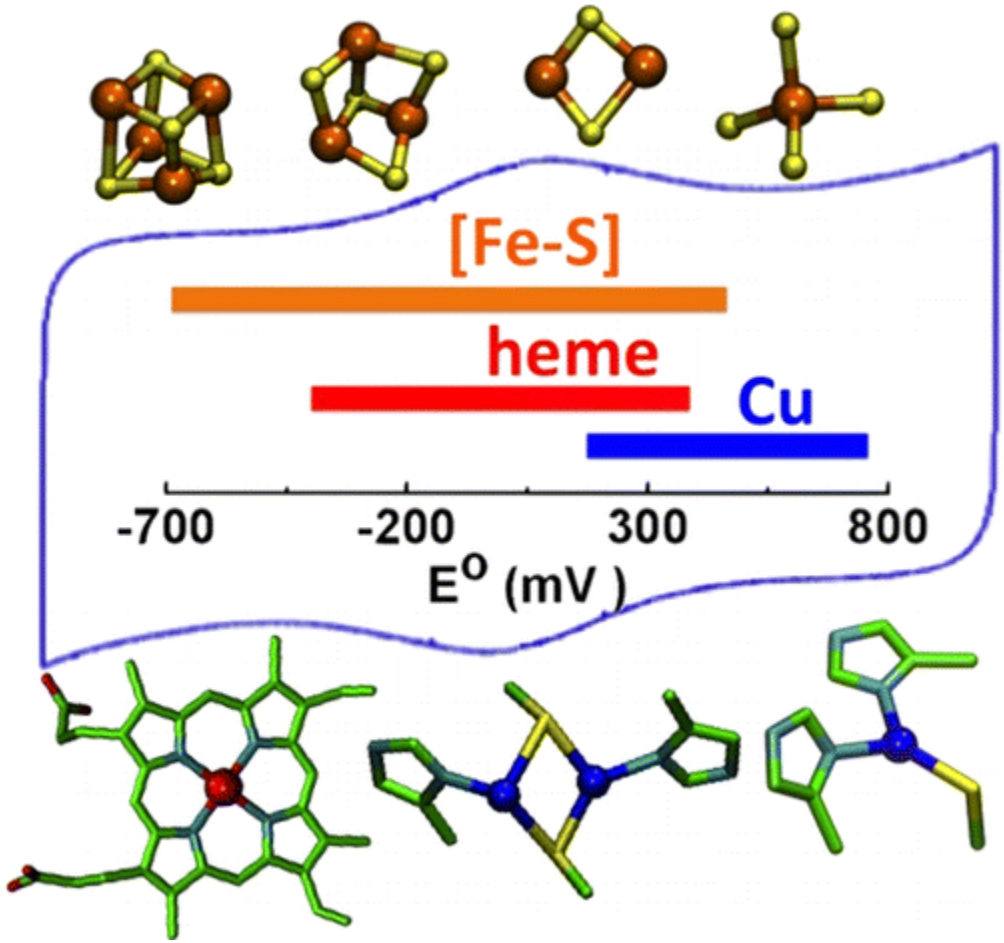
Oxidized plastocyanin



Reduced plastocyanin

*Blue-copper proteins are **very efficient electron transfer enzymes**. The rate for electron transfer is in the range 10^3–10^7 M^{-1} s^{-1} (compared to 5×10^{-7} M^{-1} s^{-1} for the aquo redox couple Cu(II)/Cu(I))*

Metallo-proteins for electron transfer

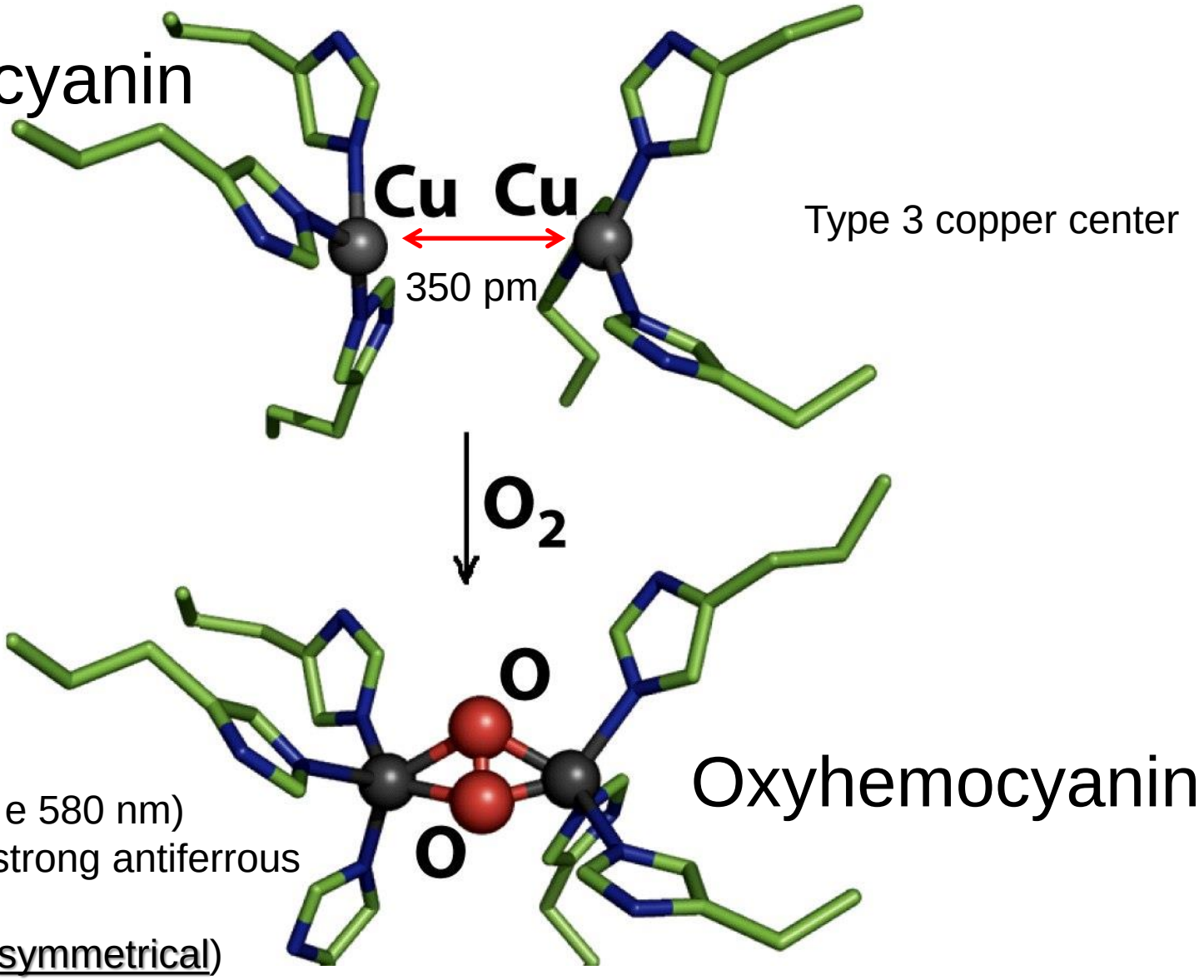


O_2 transport mediated by Cu-proteins: Hemocyanin

Hemocyanin

Up to 1500 kDa,
Each unit 75 kDa

Colorless
2 Cu(I) (S = 0)



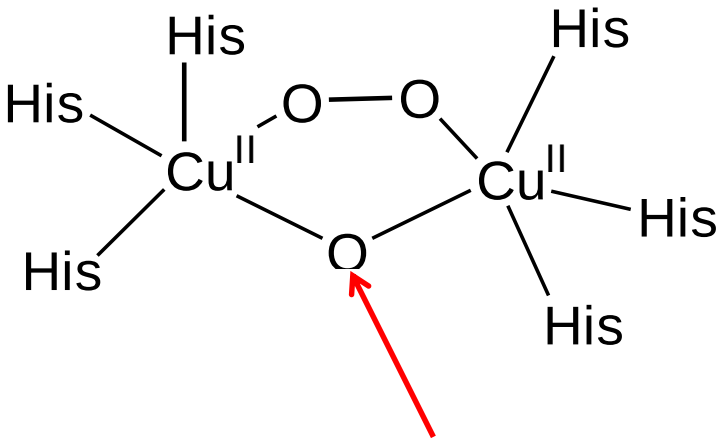
Type 3 copper center

Oxyhemocyanin

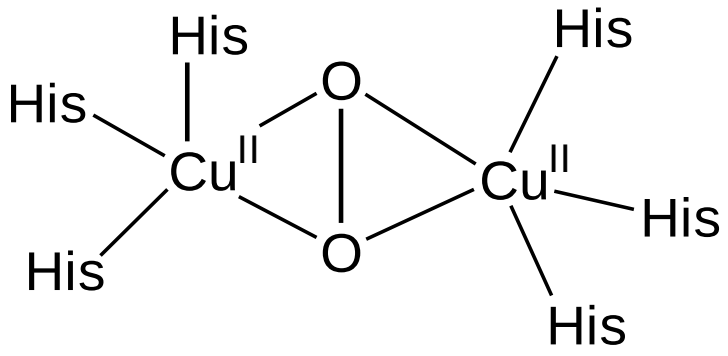
Purple (LMCT 350 e 580 nm)
2 Cu(II) (diamag., strong antiferrous coupling)
IR: 755 cm^{-1} (O_2^{2-}, symmetrical)

O_2 transport in molluscs (snails, squids) e arthropods (crabs, lobsters, shrimps, scorpions)

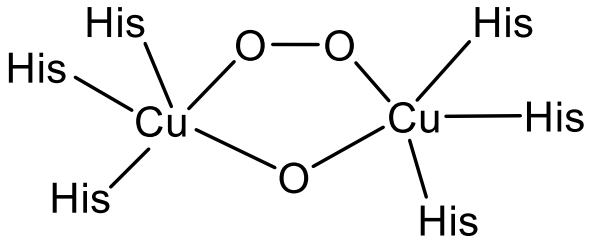
Possible symmetrical cooordination modes of peroxide ion



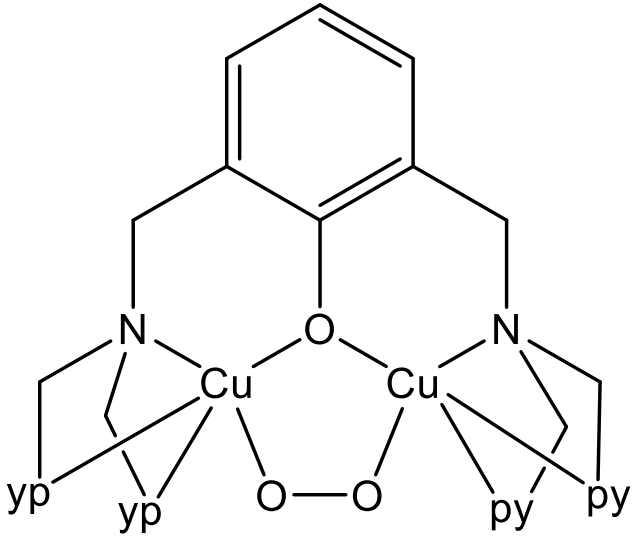
tyrosinate?

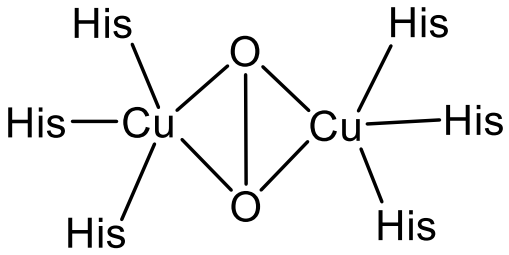


Models for O_2 binding to hemocyanin

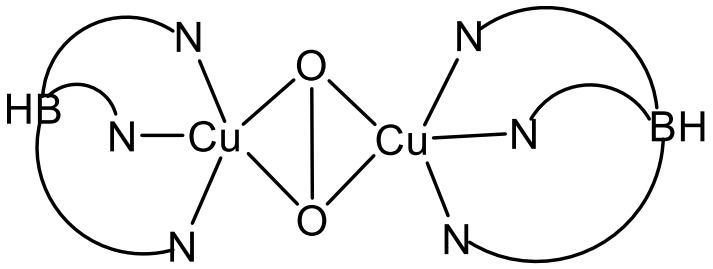


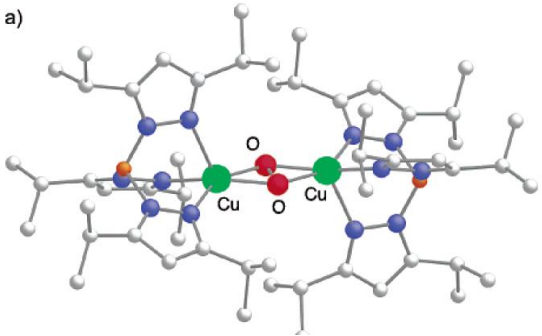
Wrong hypothesis



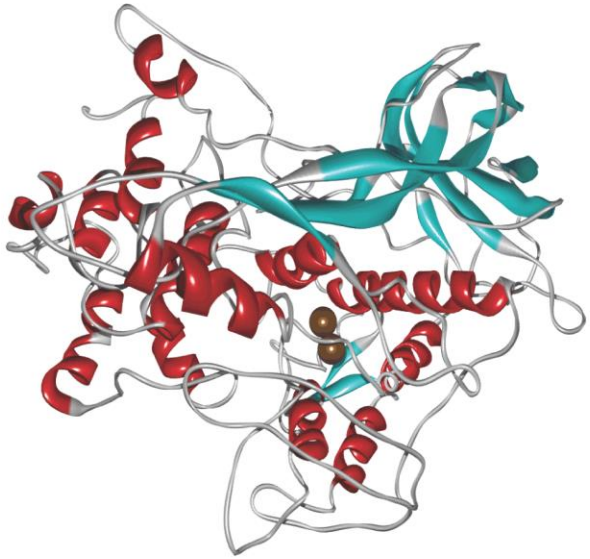


Correct hypothesis

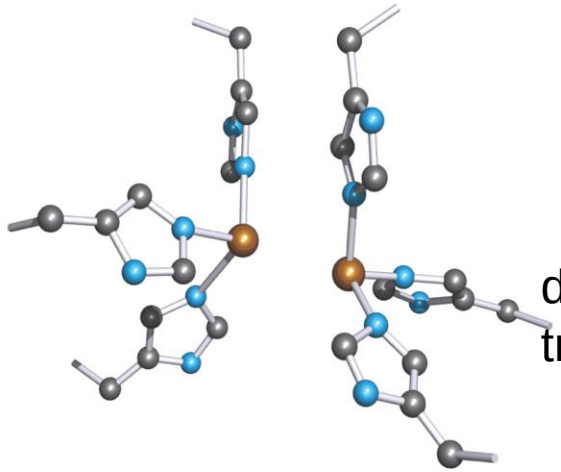




Hemocyanin

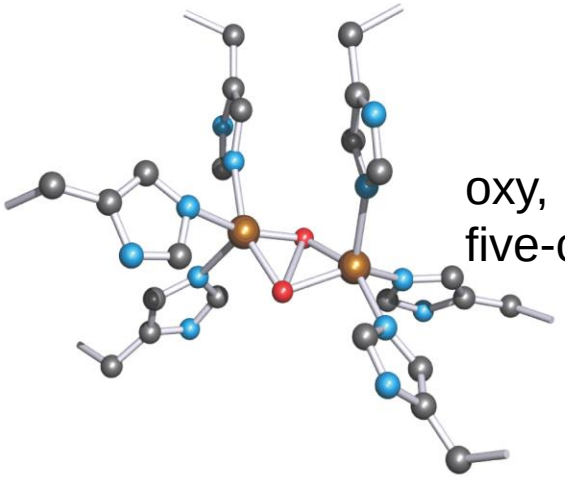


(a)



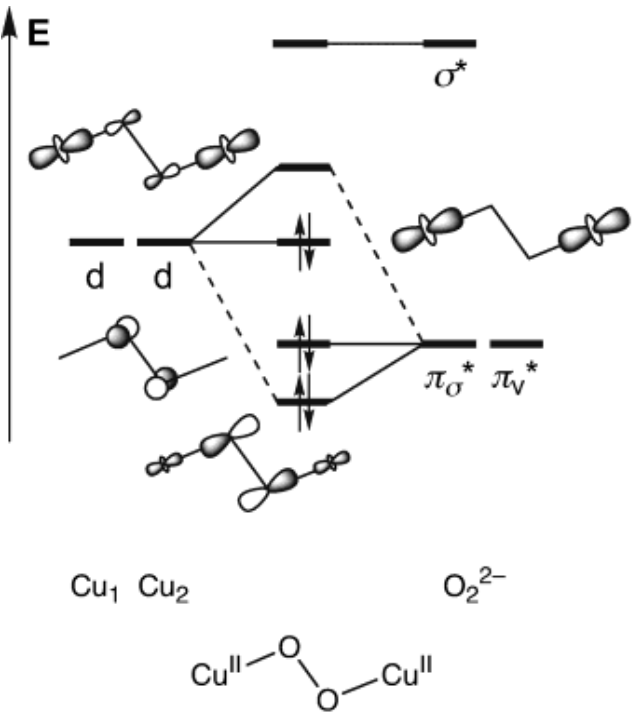
(b)

deoxy, Cu(I)
tri-coordinate



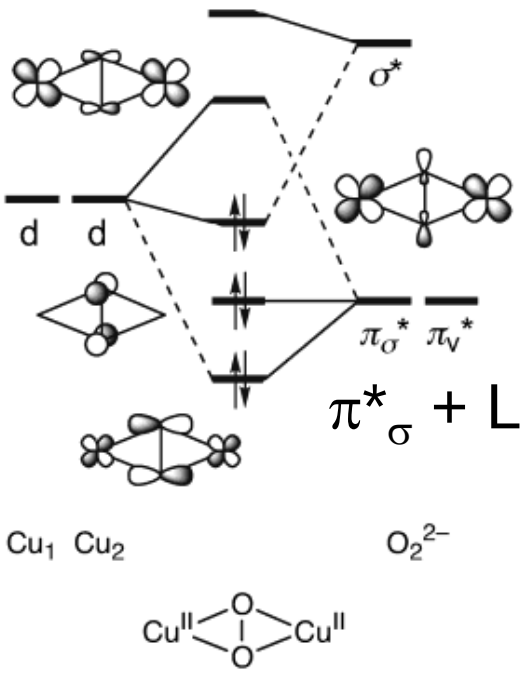
(c)

oxy, Cu(II)
five-coordinate



σ^* + HOMO Cu ($d_{x2-y2} - d_{x2-y2}$)

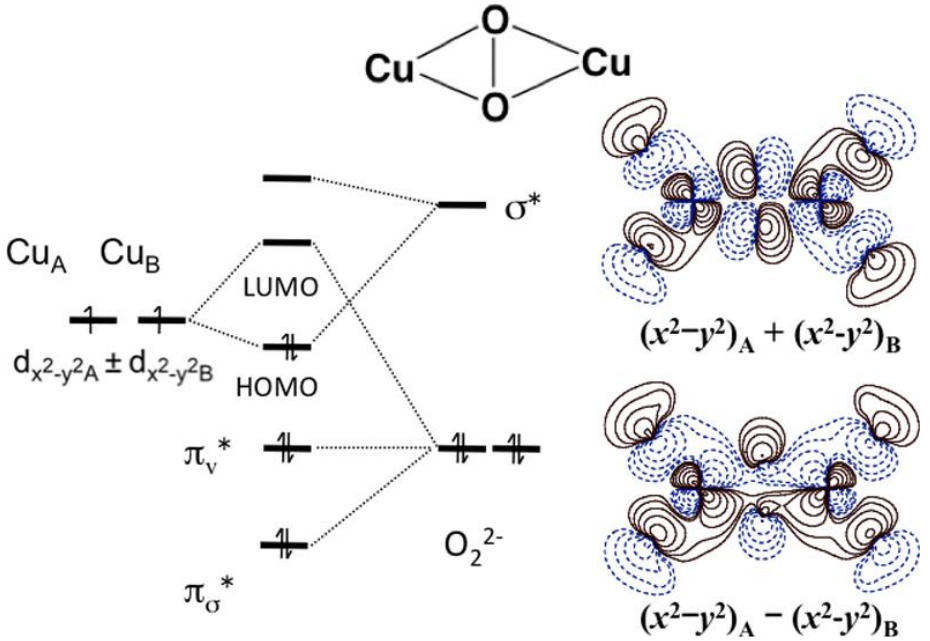
$2Cu(II) \rightarrow O_2^{2-}$



π^*_σ + LUMO Cu ($d_{x2-y2} + d_{x2-y2}$)

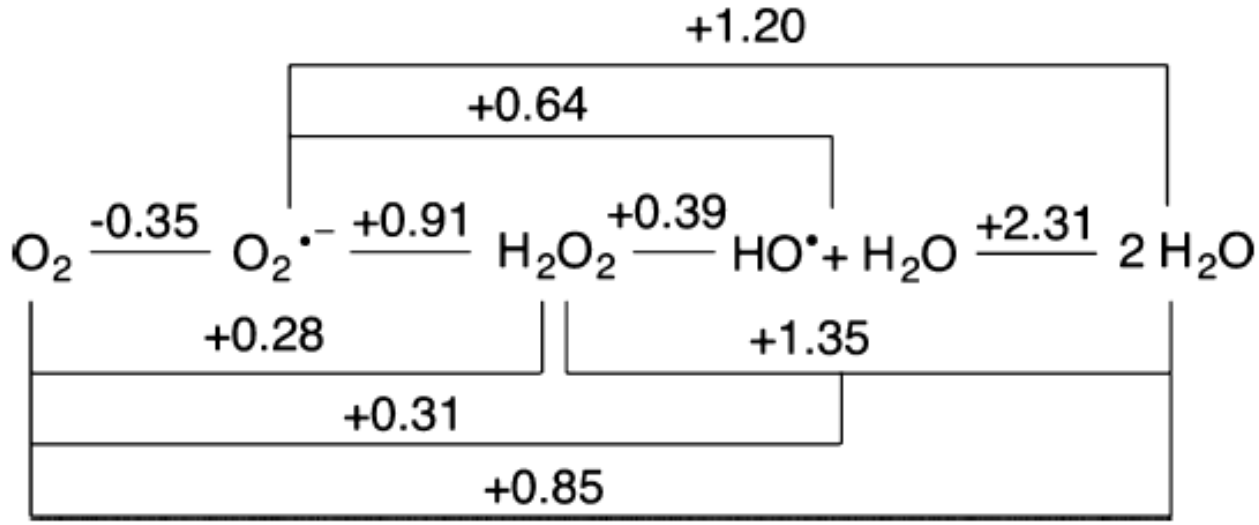
$O_2^{2-} \rightarrow 2Cu(II)$

IR: 755 cm^{-1} (O_2^{2-}, <u>symmetrical</u>)



Cu enzymes that activate or reduce O_2

pH = 7



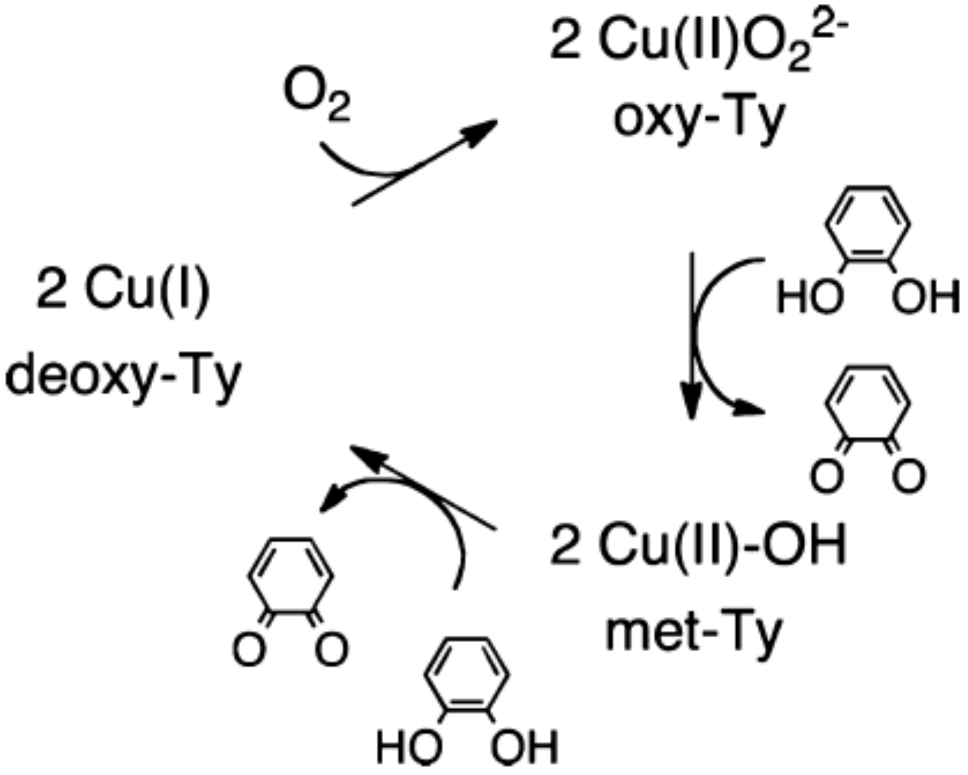
...the mono-electron reduction of O_2 to superoxide is thermodynamically disfavored ($E° = –0.35$ V)

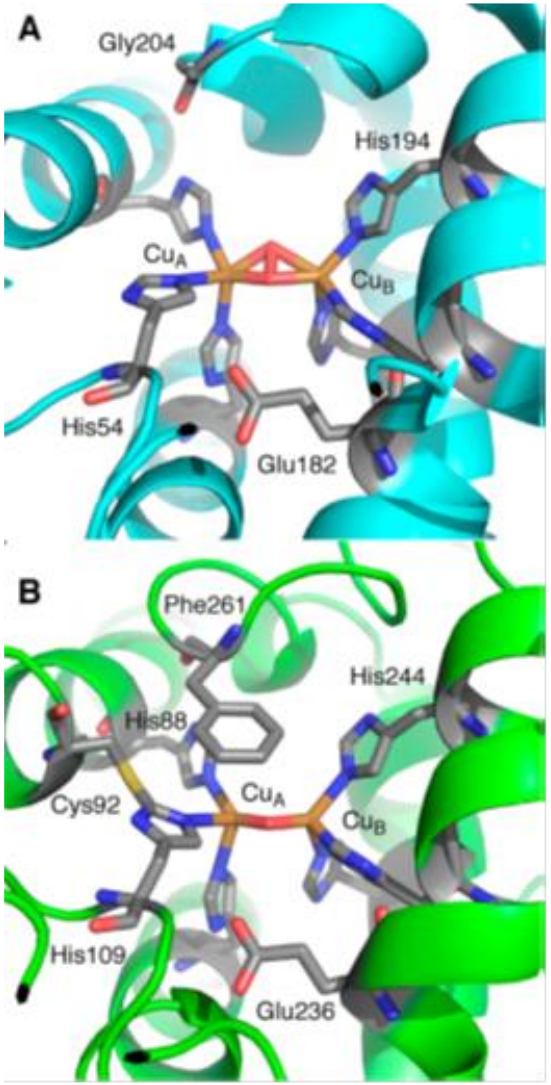
providing two electrons requires either the presence of at least two Cu ions or a Cu ion and a redox-active organic cofactor

Polyphenol oxidases

Tyrosinase, Catechol-oxidase

$$2\ o\text{-diphenol} + O_2 \rightarrow 2\ o\text{-quinone} + 2H_2O$$





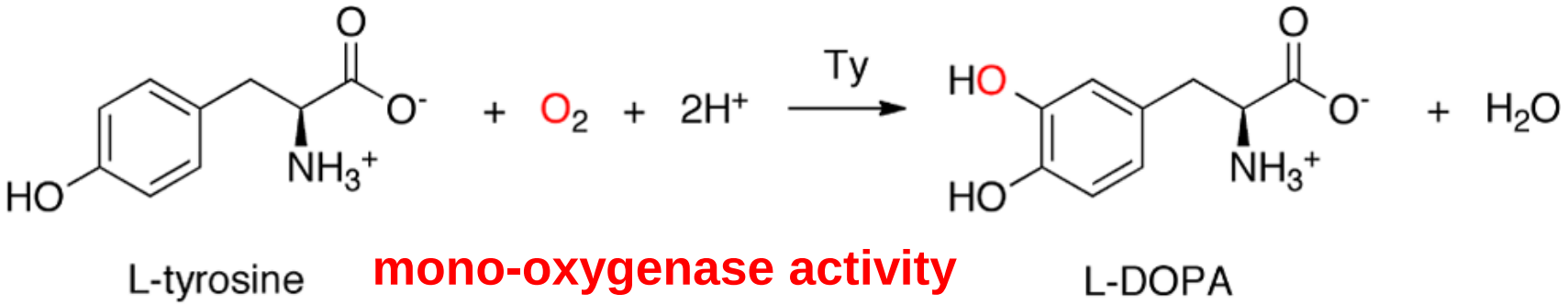
oxy-Ty

met-Ty

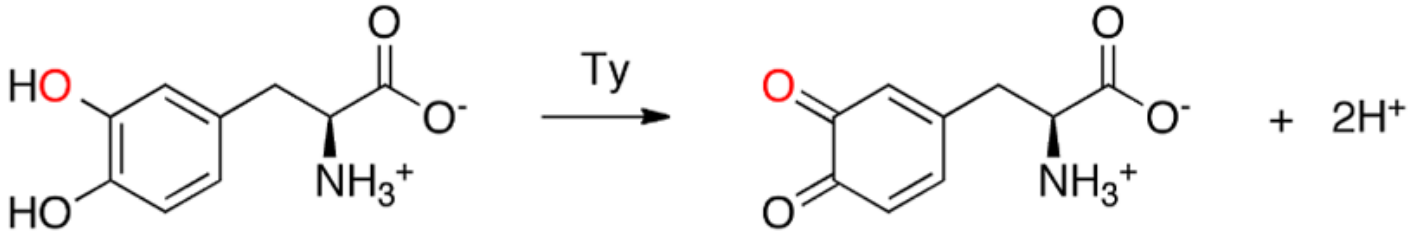
In the cycle 4 electrons get transfered to the two O atoms of O_2 from two diphenol molecules

Tyrosinase as a mono-oxygenase

oxy-Ty is capable to convert both phenols to o-diphenols (oxygenase activity) and o-diphenols to o-quinones, met-T is capable to oxidize only o-diphenols to o-quinones



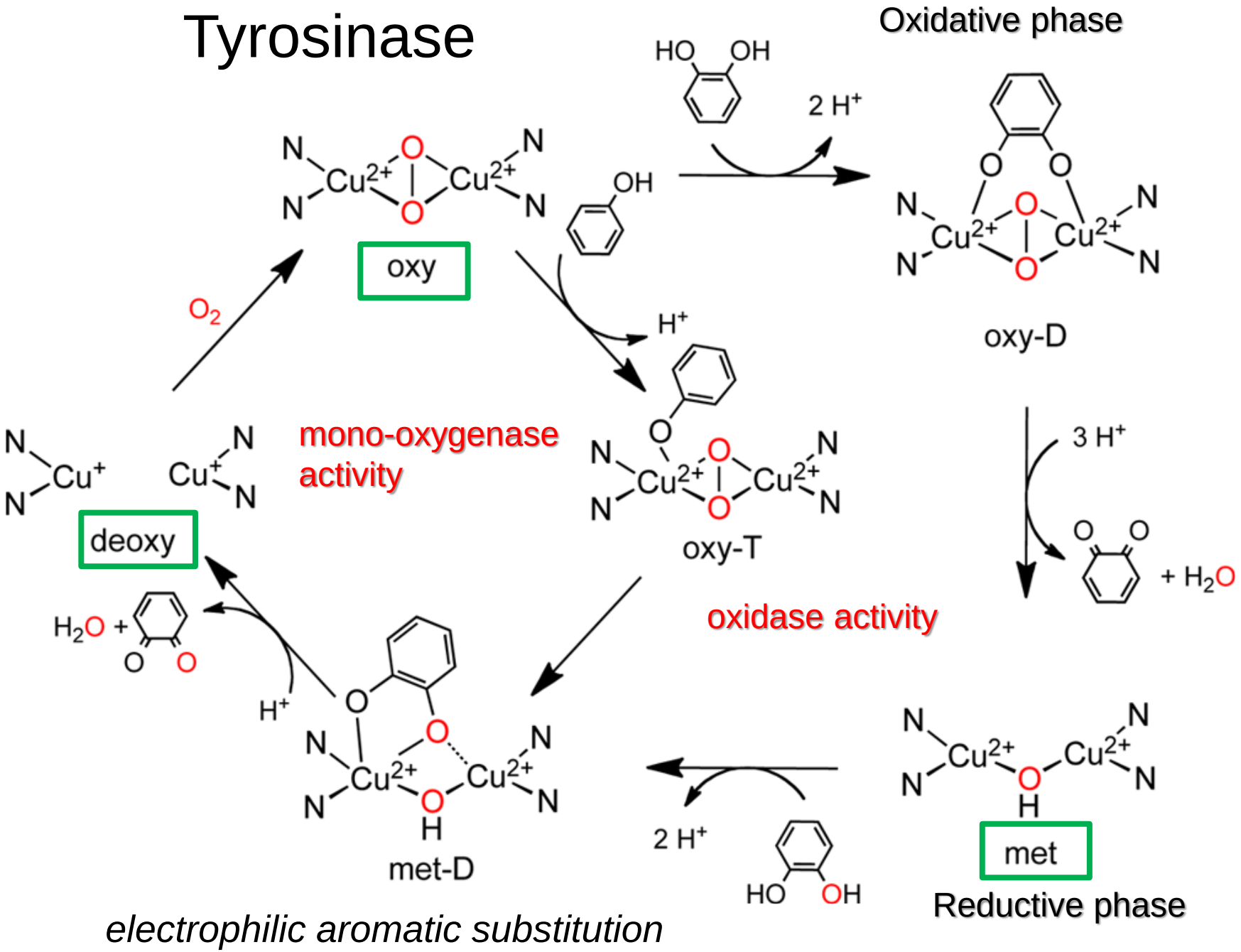
L-tyrosine **mono-oxygenase activity** L-DOPA



L-DOPA *o*-L-DOPAquinone

oxidase activity

Tyrosinase



electrophilic aromatic substitution

Multicopper oxidases, MCOs (blue oxidases)

At least 4 Cu

Organic substrates

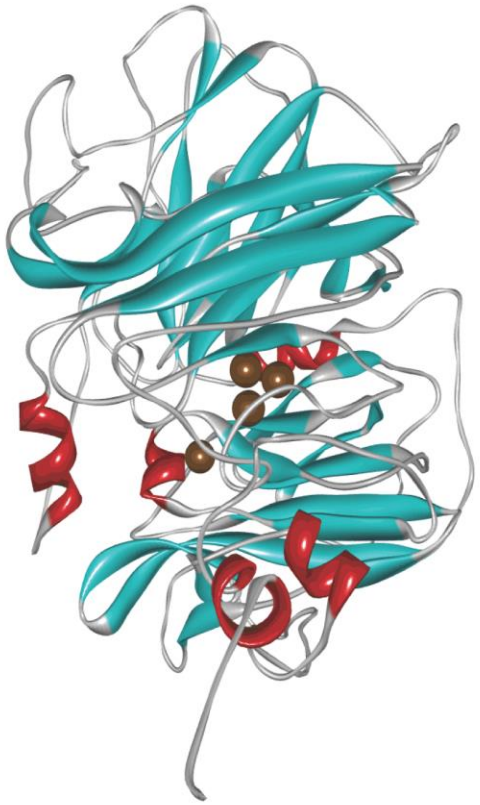
«Metallic» substrates

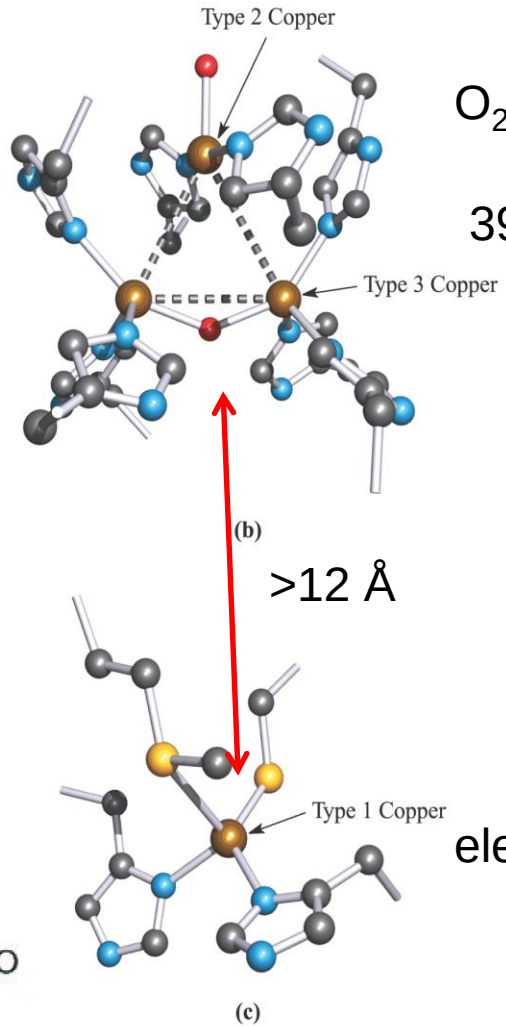
Ascorbate oxidase
Laccase
+500 – +800 mV

Ceruloplasmin
Epestin
Fet3p
Copper-oxidase (CueO)

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

(RH = phenol)





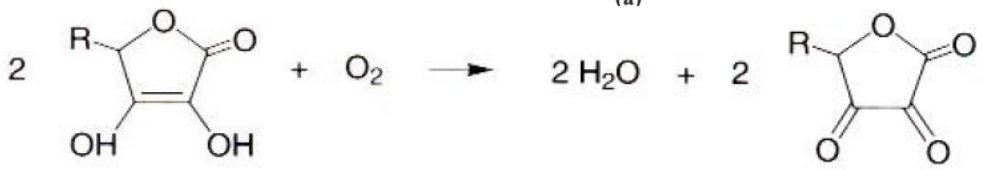
O_2 reduction ($4e^-$)

390 pm

340 pm

R = $CH(OH)CH_2OH$

electron acceptor

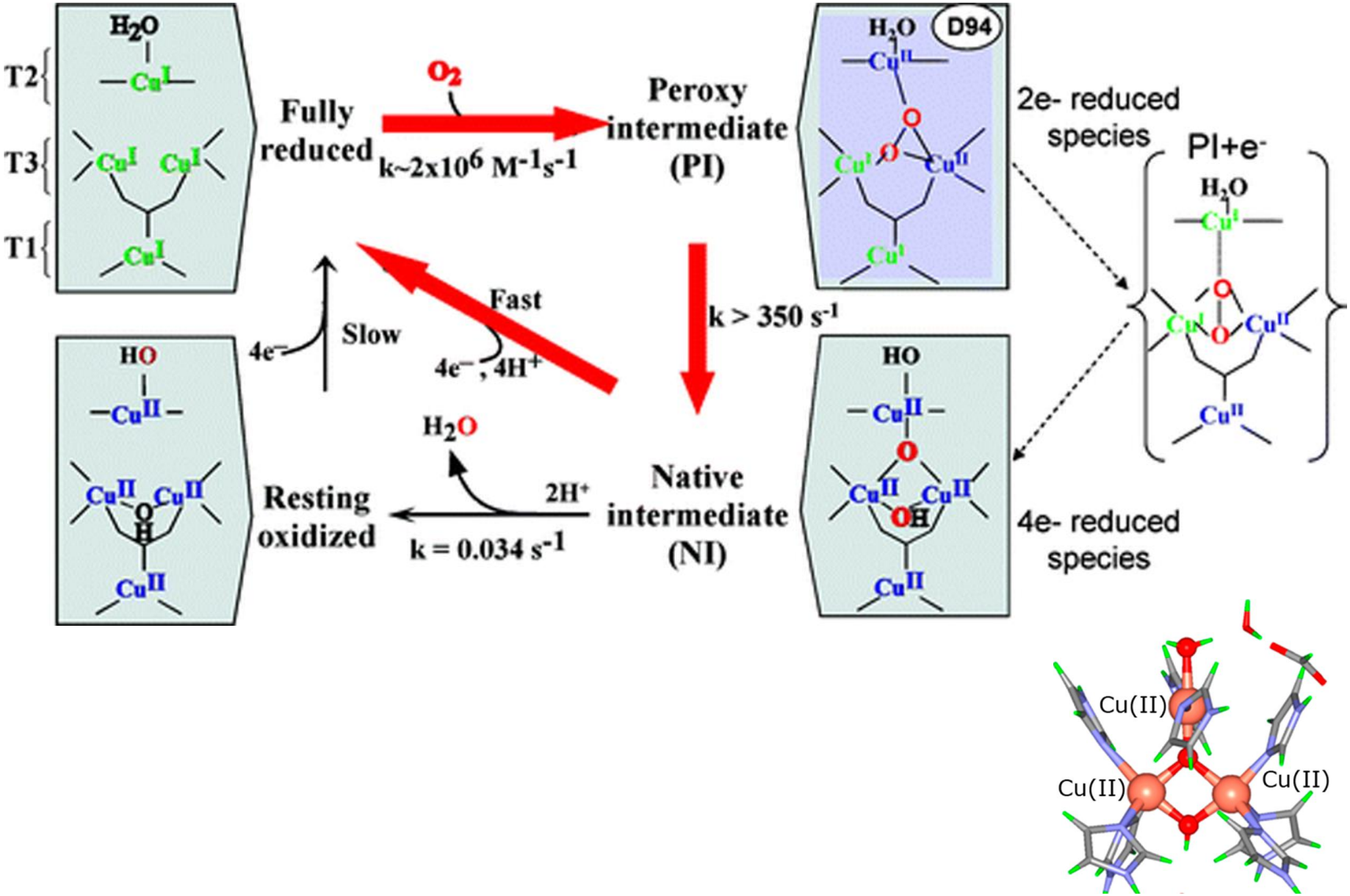


Ascorbate oxidase (from squash)

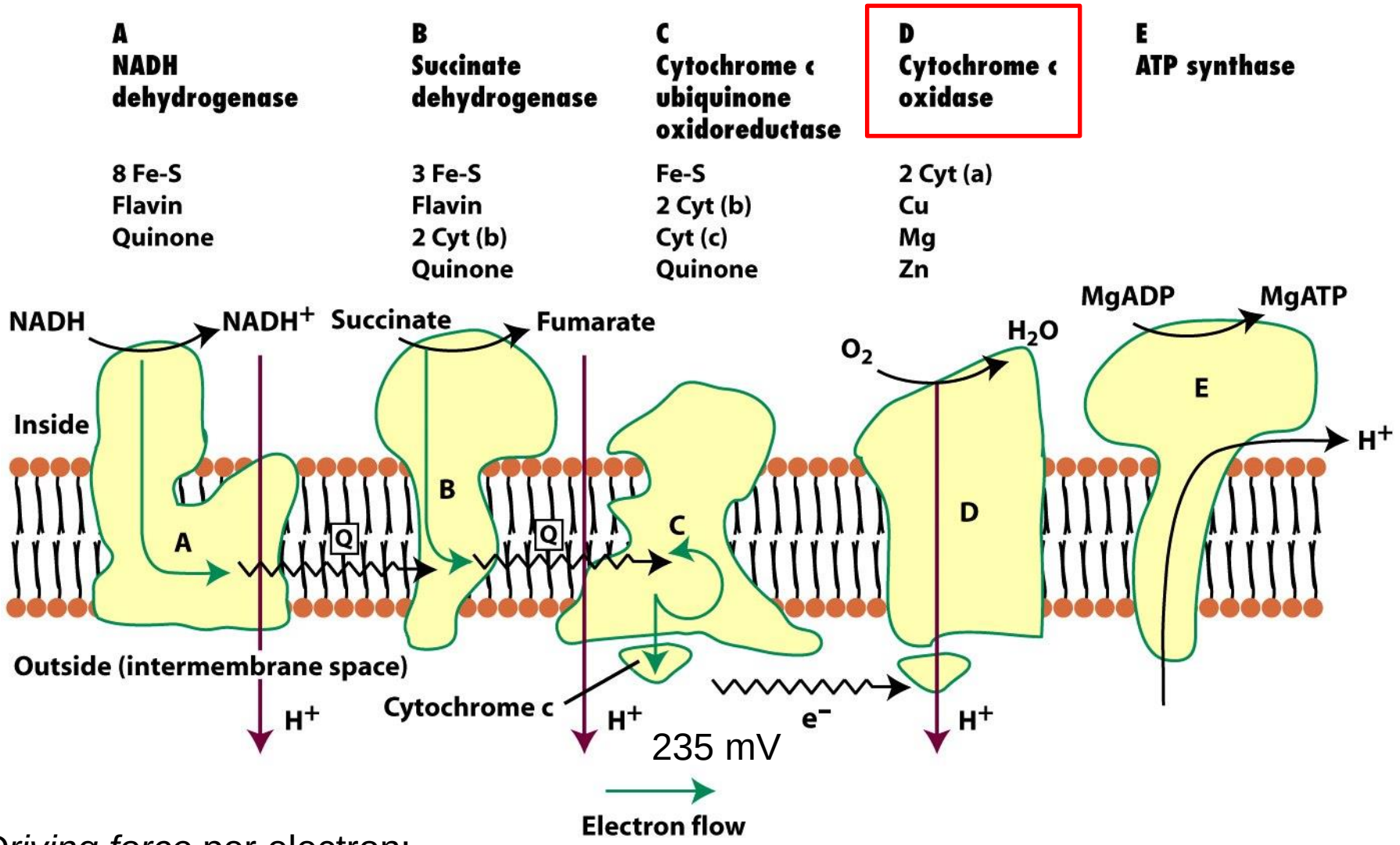
$$4RH + O_2 \rightarrow 4R\cdot + 2H_2O$$

(RH = phenol)

Mechanism of action for multicopper oxidases



Respiratory chain (oxidative phosphorylation)

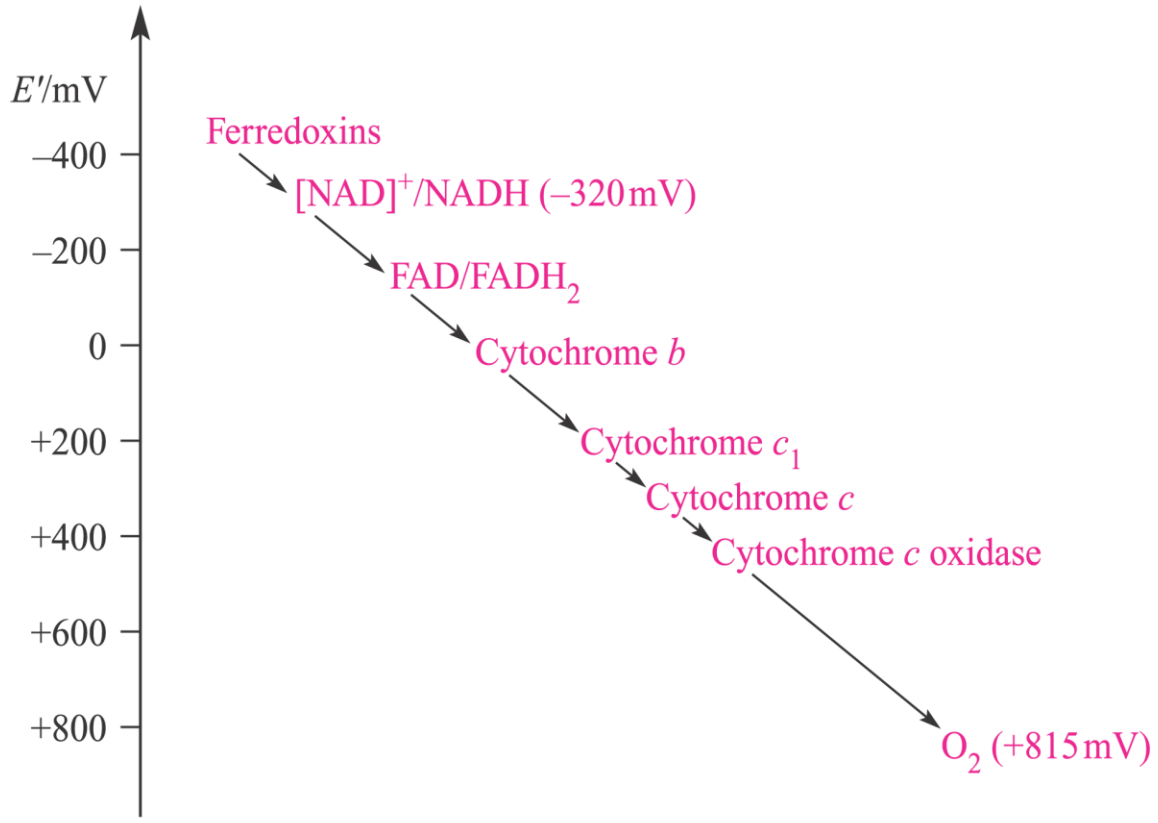


Driving force per electron:
815 – 235 = 580 mV (ca. 13 kcal/mol)

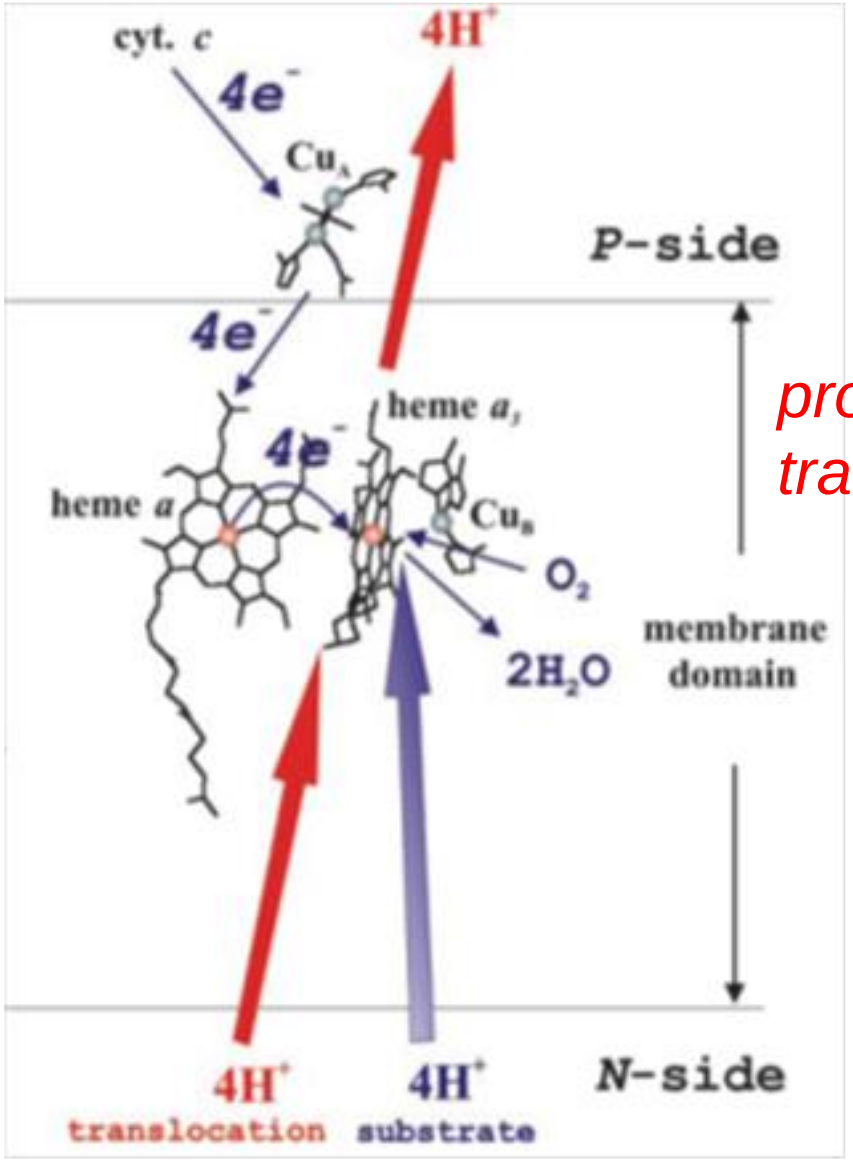
Oxidation of Fe^{2+} in Cyt c

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

The sequence of electron transfers in mitochondria



Cytochrome *c* oxidase is also a proton pump

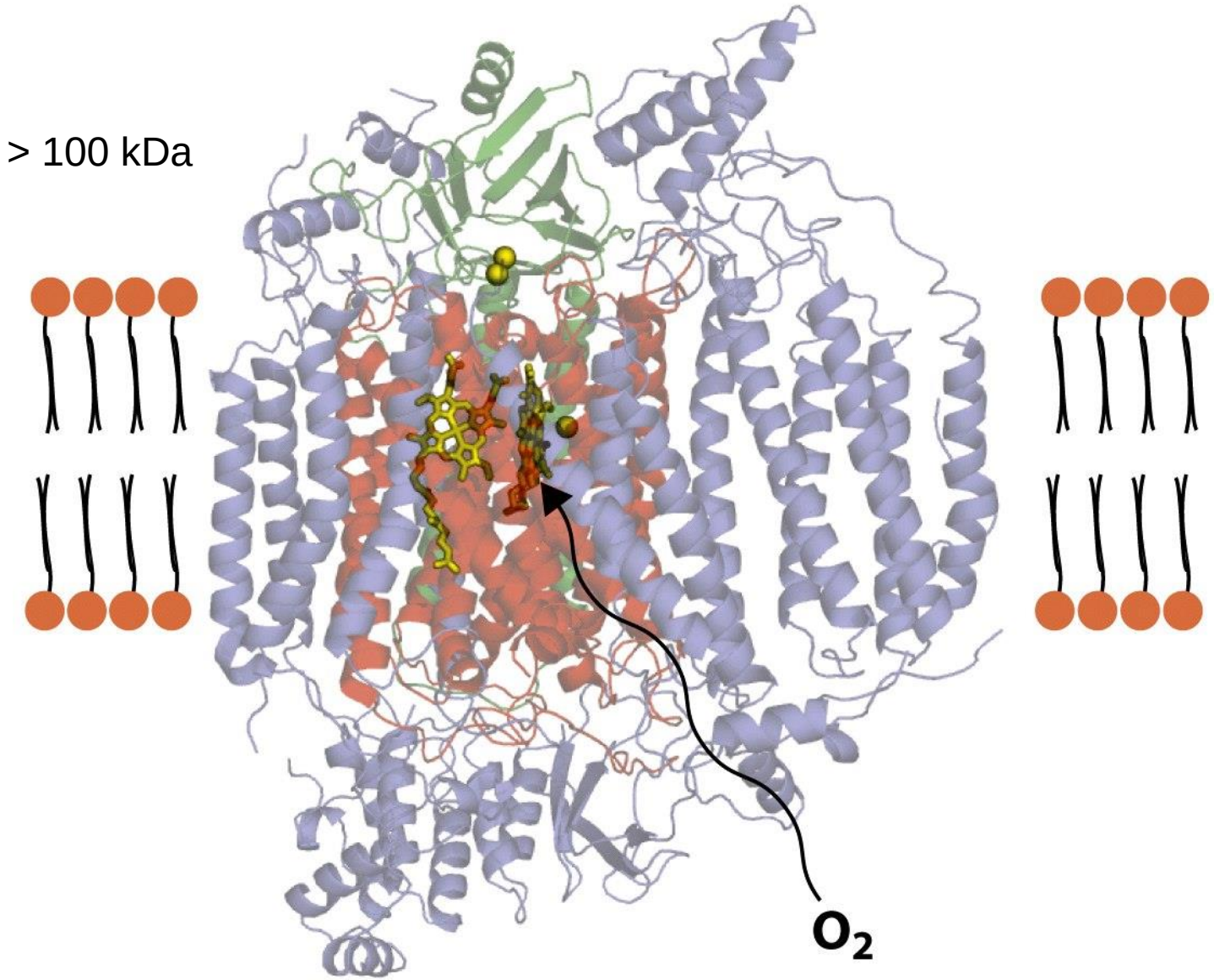


proton coupled electron transfer, PCET

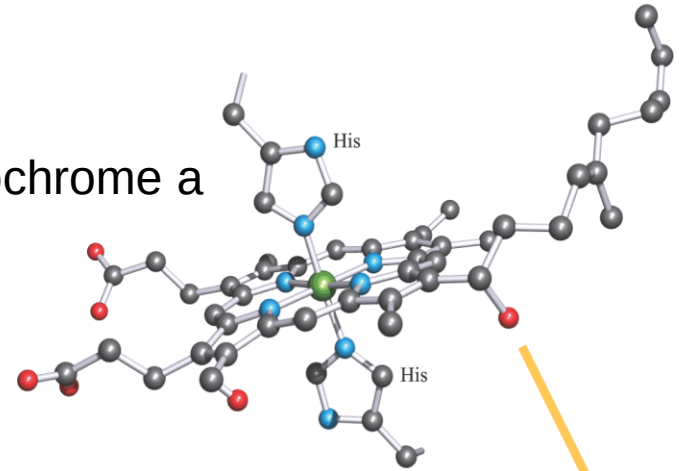
$O_2(g) + 4e^- + 8H^+(inside) \rightarrow 2H_2O(l) + 4H^+(outside)$

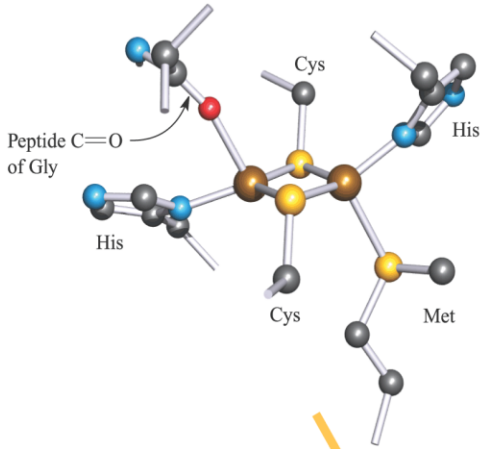
Reacts with cytochrome C

> 100 kDa



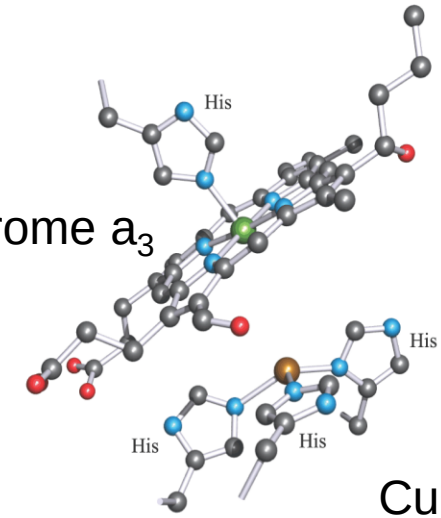
cytochrome a

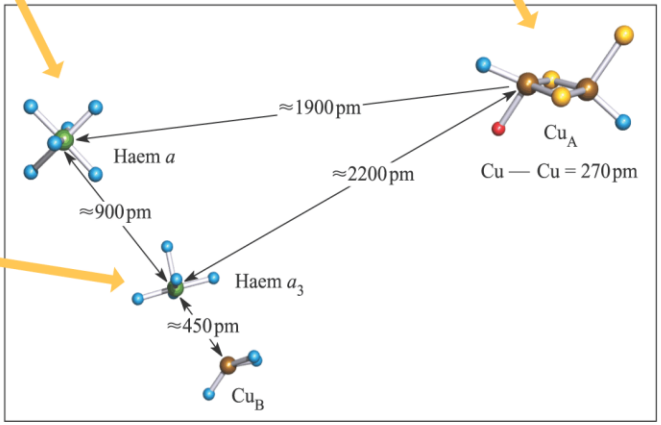




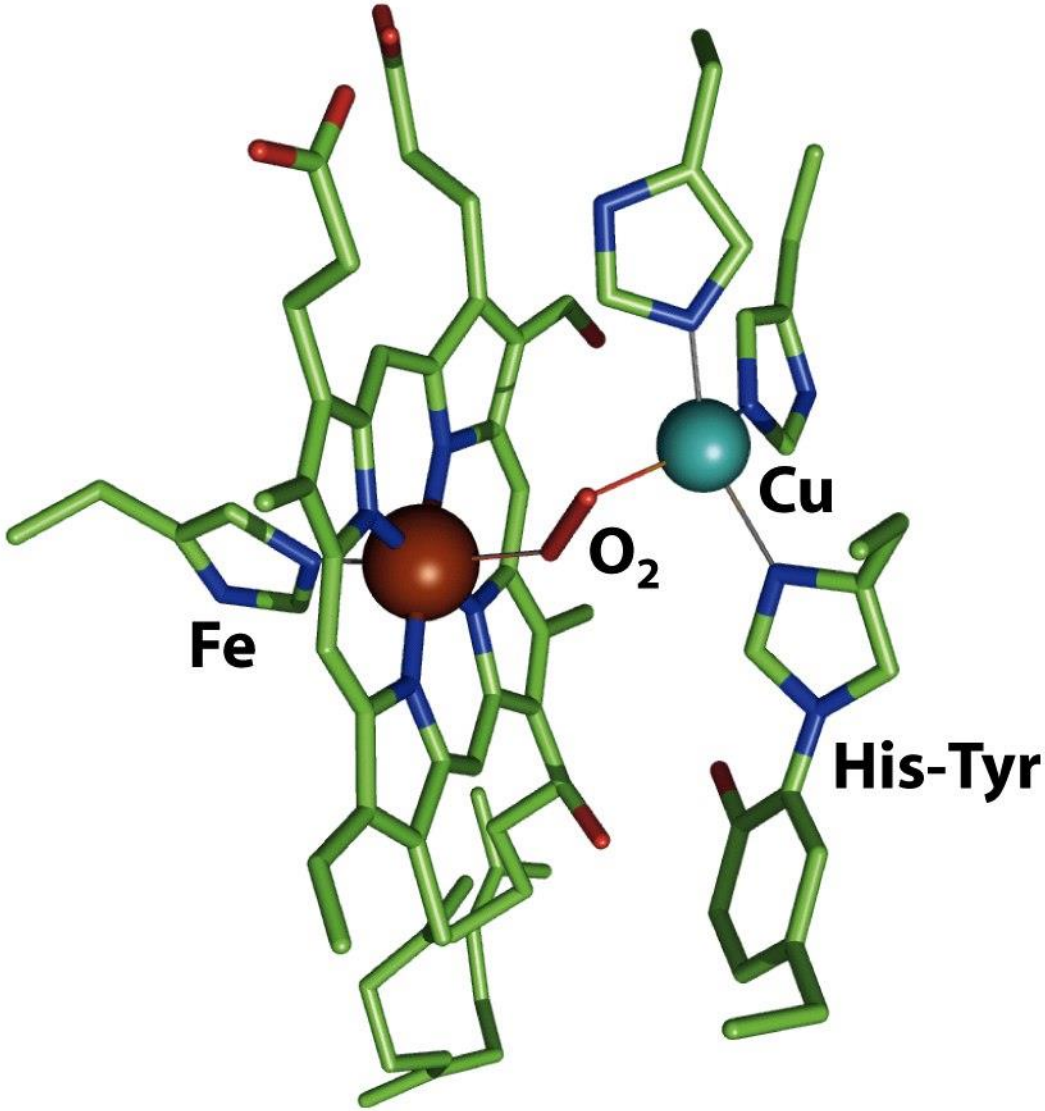
Cu_A

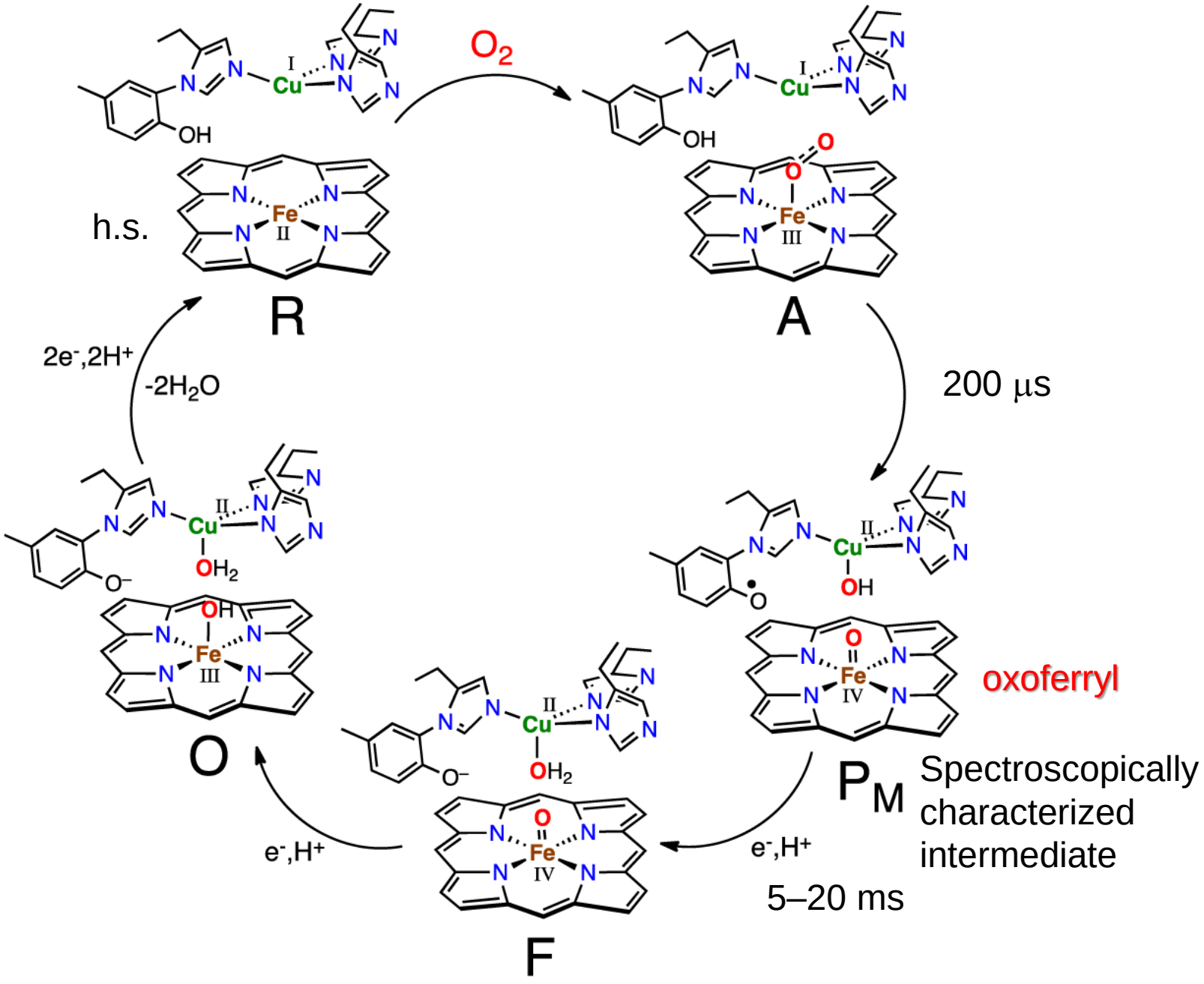
cytochrome a_3



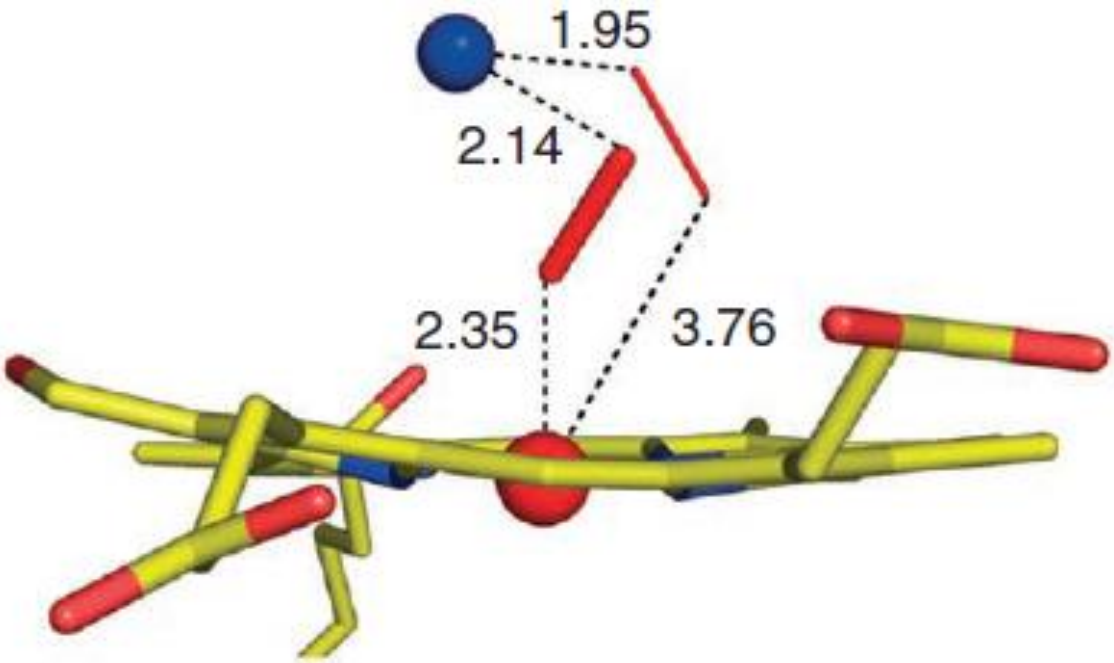


Cu_B (type 2 copper)

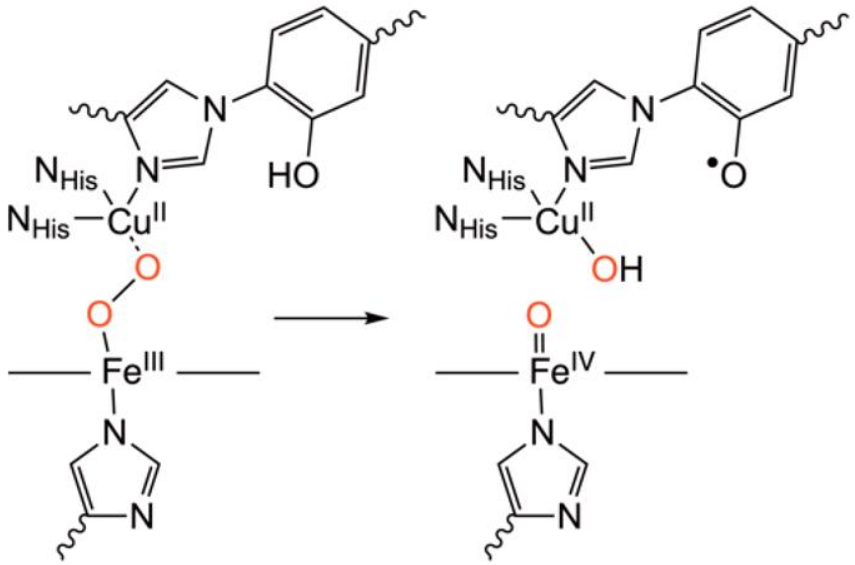

Fe
O_2
Cu
His-Tyr


O2
h.s.
R
A
200 μs
oxoferryl
PM
Spectroscopically characterized intermediate
5–20 ms
e-,H+
F
e-,H+
O
2e-,2H+
-2H2O

Peroxide intermediate
X-ray free-electron laser (XFEL)

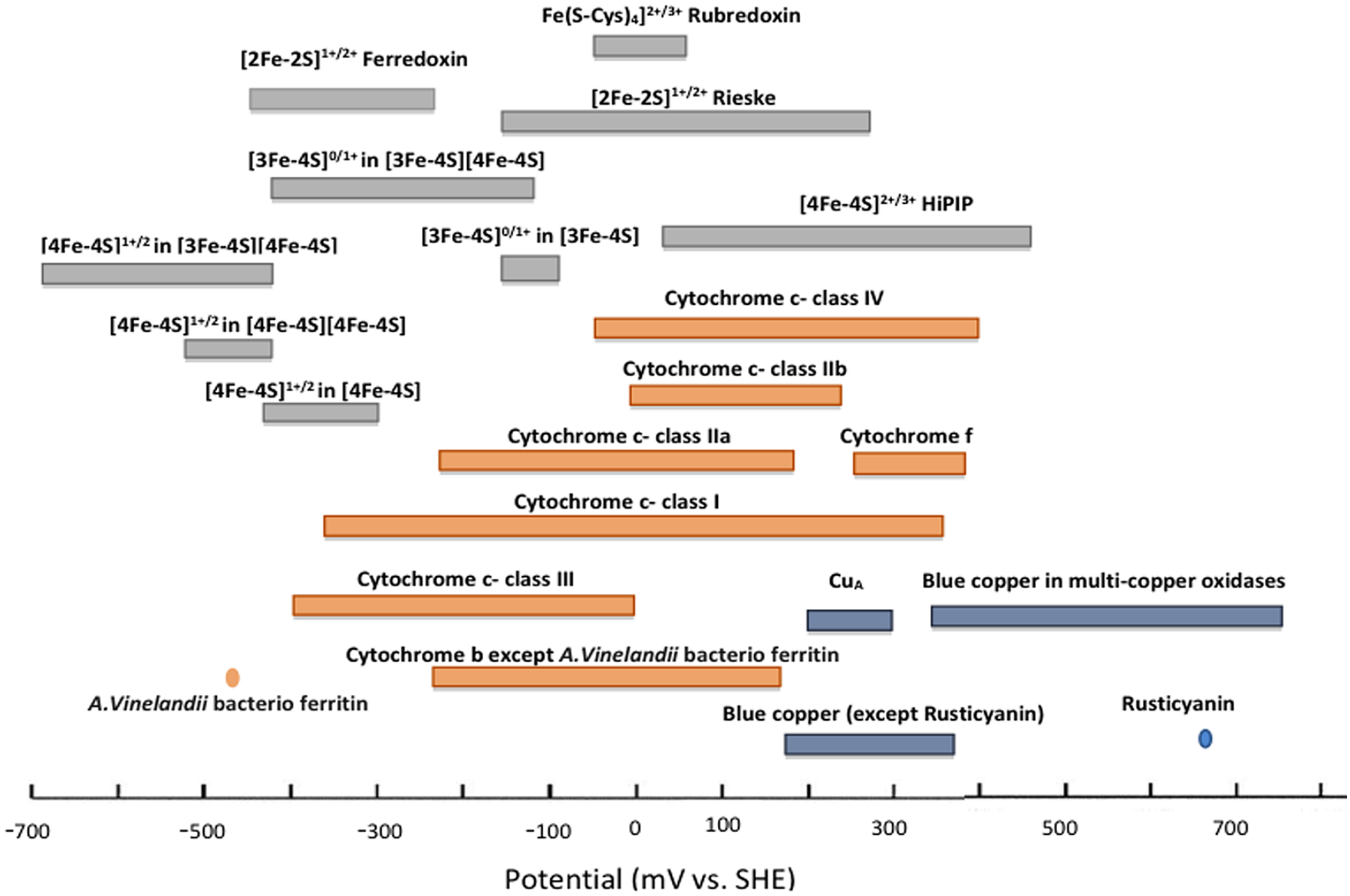


Nature, 2014



(a) Proposed O-O cleavage mechanism in C*c*O

Metallo-proteins for electron transfer



Reactive Oxygen Species (ROS)

Haber-Weiss reaction

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

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

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

Fenton reaction

The net reaction:

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

Superoxide dismutases

Superoxide dismutases are the major ROS detoxifying enzymes of the cell, and catalyze the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen.

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

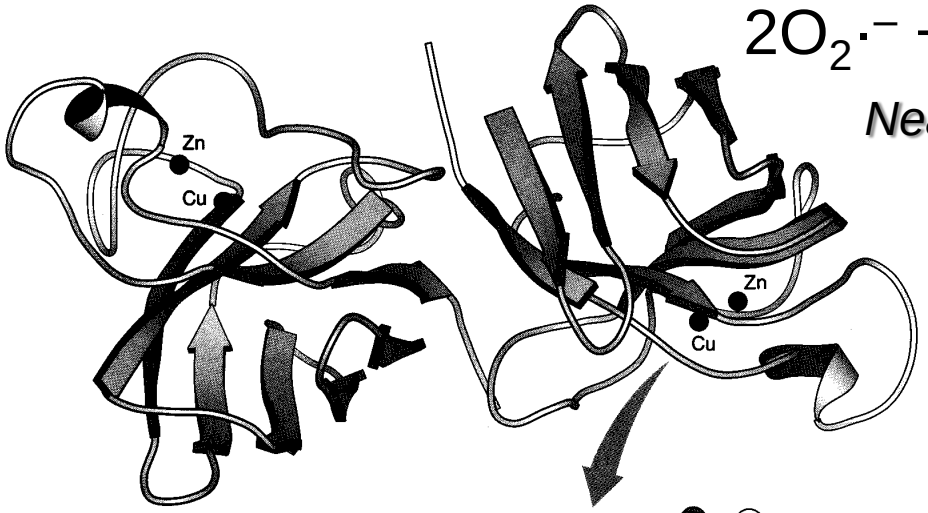
Glutathione peroxidase, peroxiredoxins, and catalase decompose hydrogen peroxide generated by SODs to water.

Three types of SOD are expressed by cells in eukariotes, encoded by separate genes.
Copper- and zinc-containing SOD (CuZnSOD, SOD1) is a homodimer primarily localized to the cytoplasm.
Extracellular SOD (ECSOD) shares significant amino acid homology with CuZnSOD (40–60%), contains both copper and zinc in its active site, but is localized to the extracellular region of the cell.
MnSOD is a homotetramer localized exclusively in the mitochondrial matrix (*see later section on Mn*).

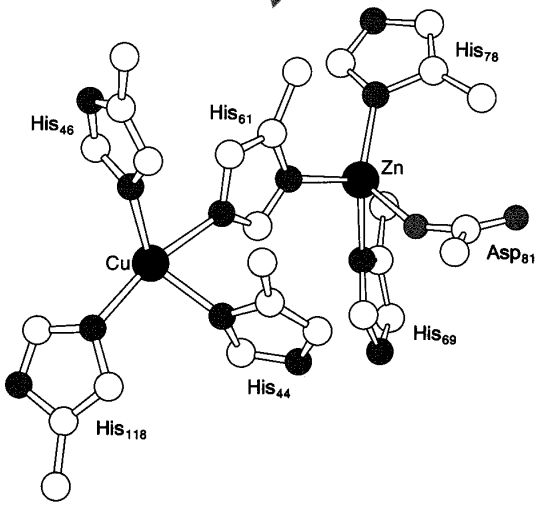
Cu-Zn superoxide dismutase

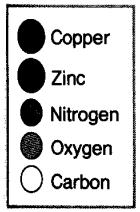
$$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$$

Nearly-diffusive speed

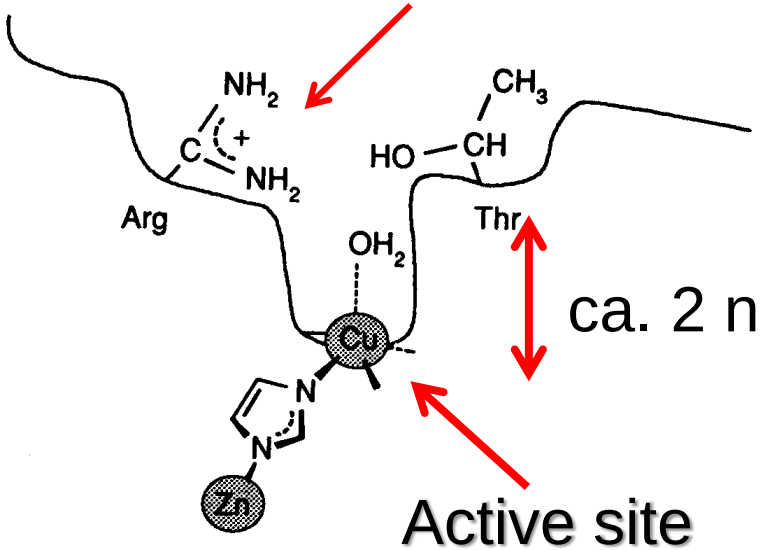


16 kDa





guanidinium group



ca. 2 nm

Active site

Catalytic cycle of Cu-Zn SOD