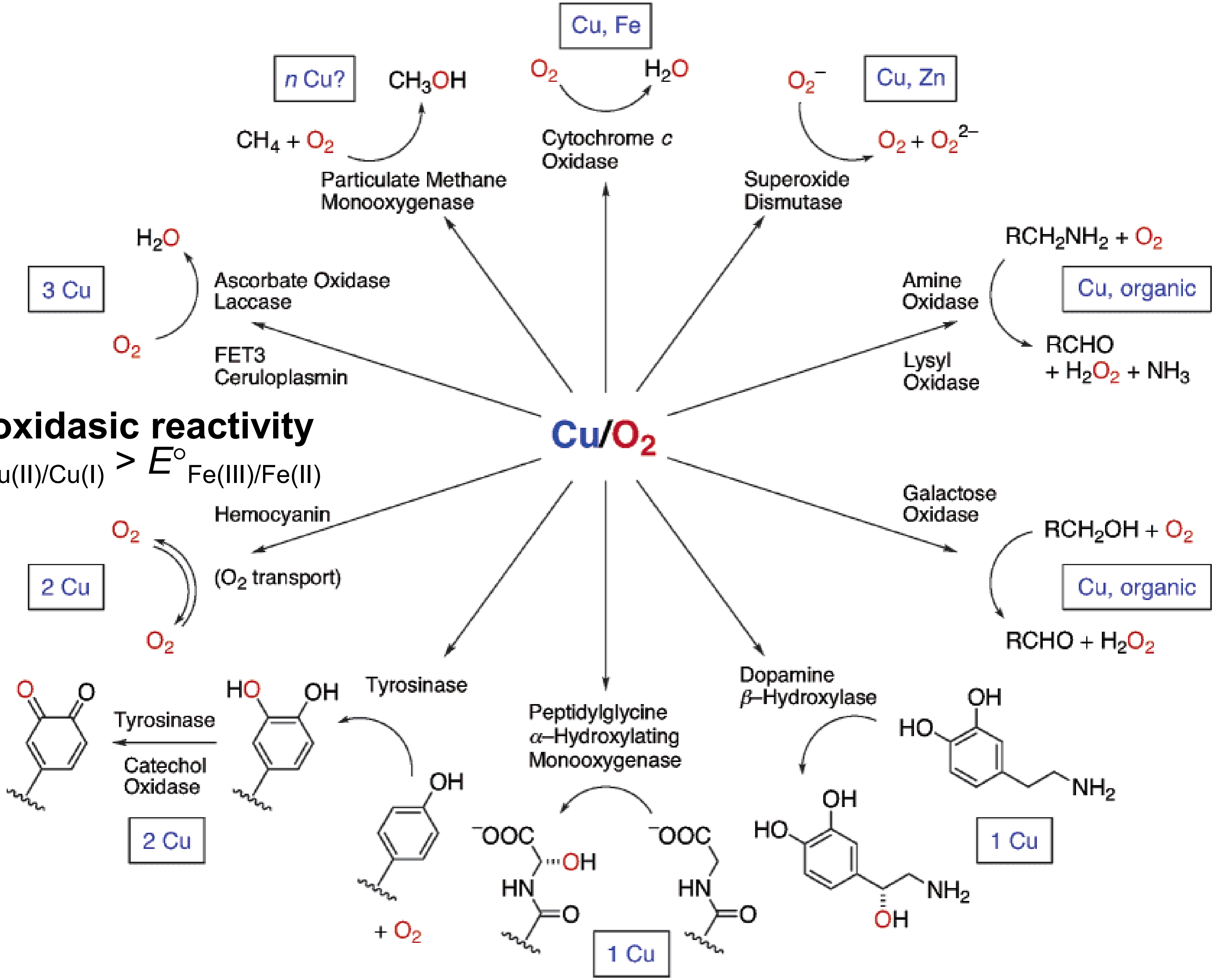
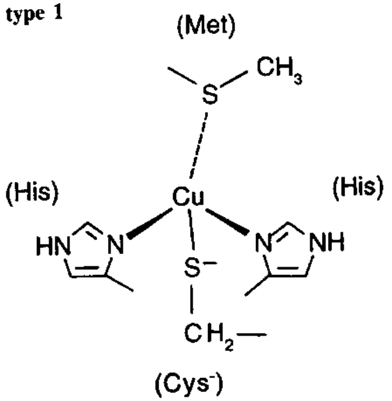
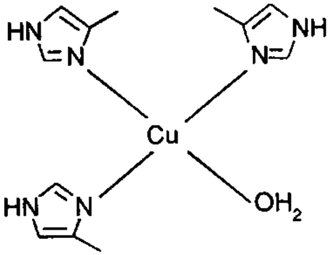
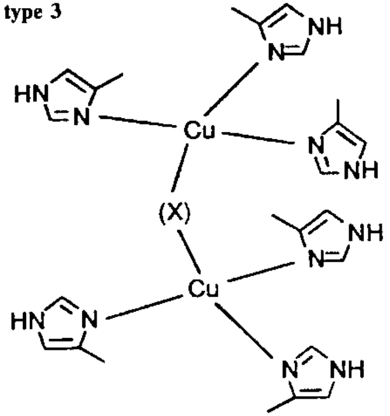
Copper proteins



ferroxidasic reactivity

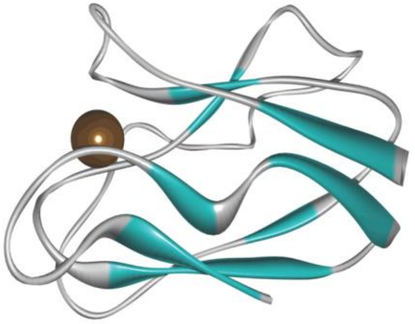
$E^\circ_{\mathrm{Cu(II)/Cu(I)}} > E^\circ_{\mathrm{Fe(III)/Fe(II)}}$

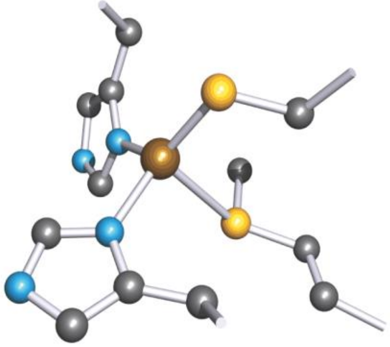
Table 10.2 Characteristics of 'classical' copper centers in protein

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

Plastocyanin
(from spinach)

10.5 kDa,
ca. 100 a.a.



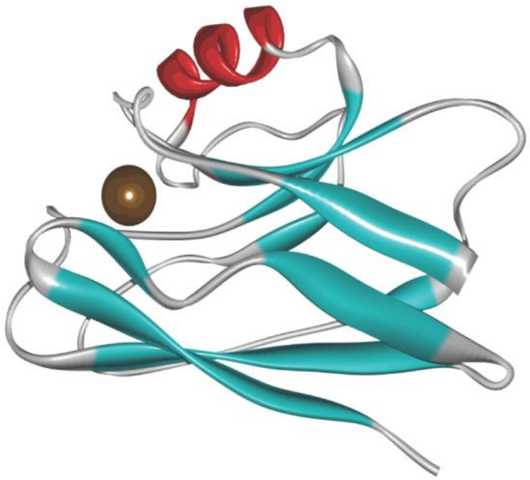


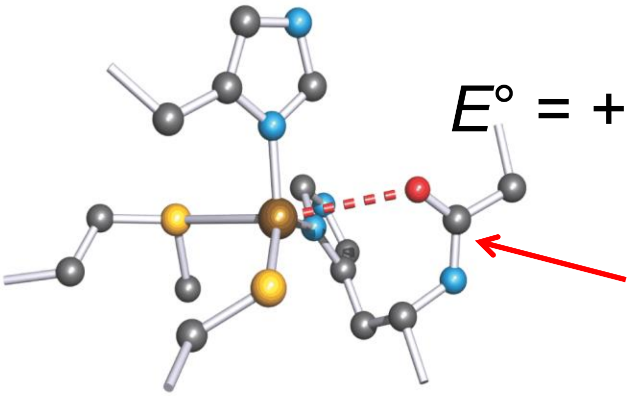
$E° = +370$ mV

3 + 1 coordination

Azurin
(from bacteria)

14.5 kDa,
ca. 130 a.a.





$E° = +308$ mV

glycine

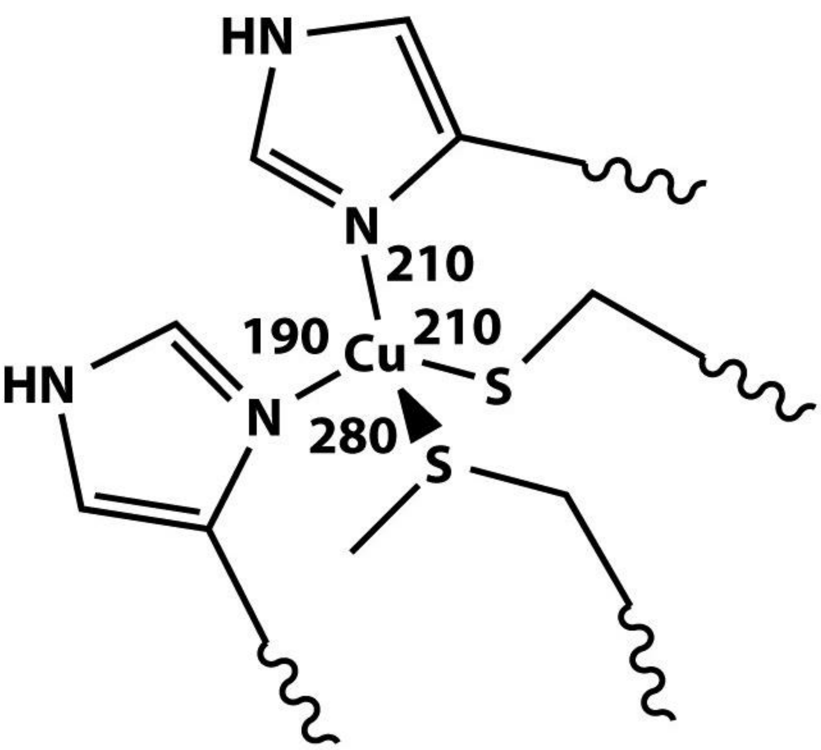
3 + 1 + 1 coordination

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

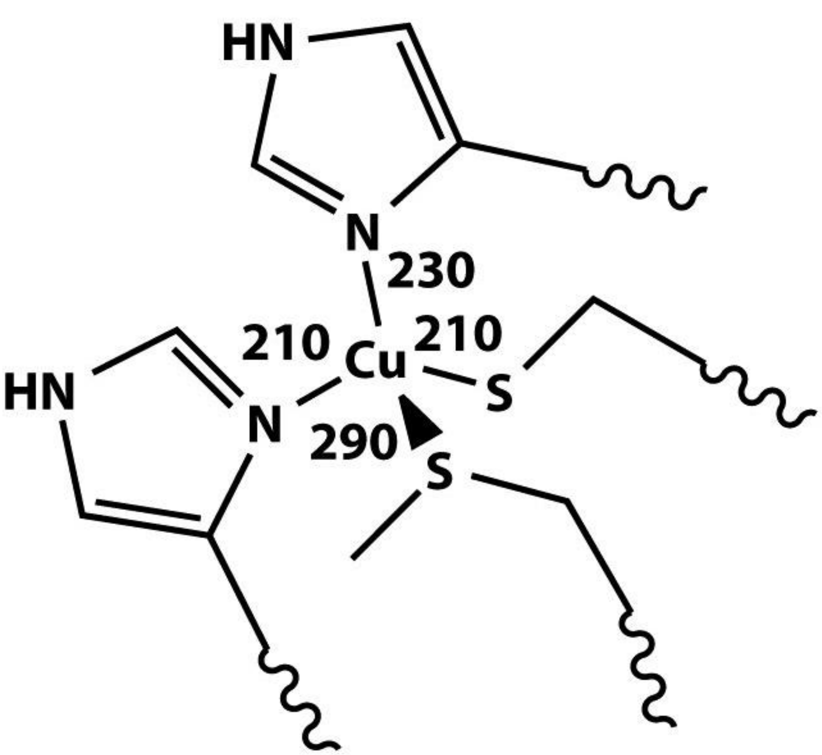
Blue copper proteins

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

Example of entatic state



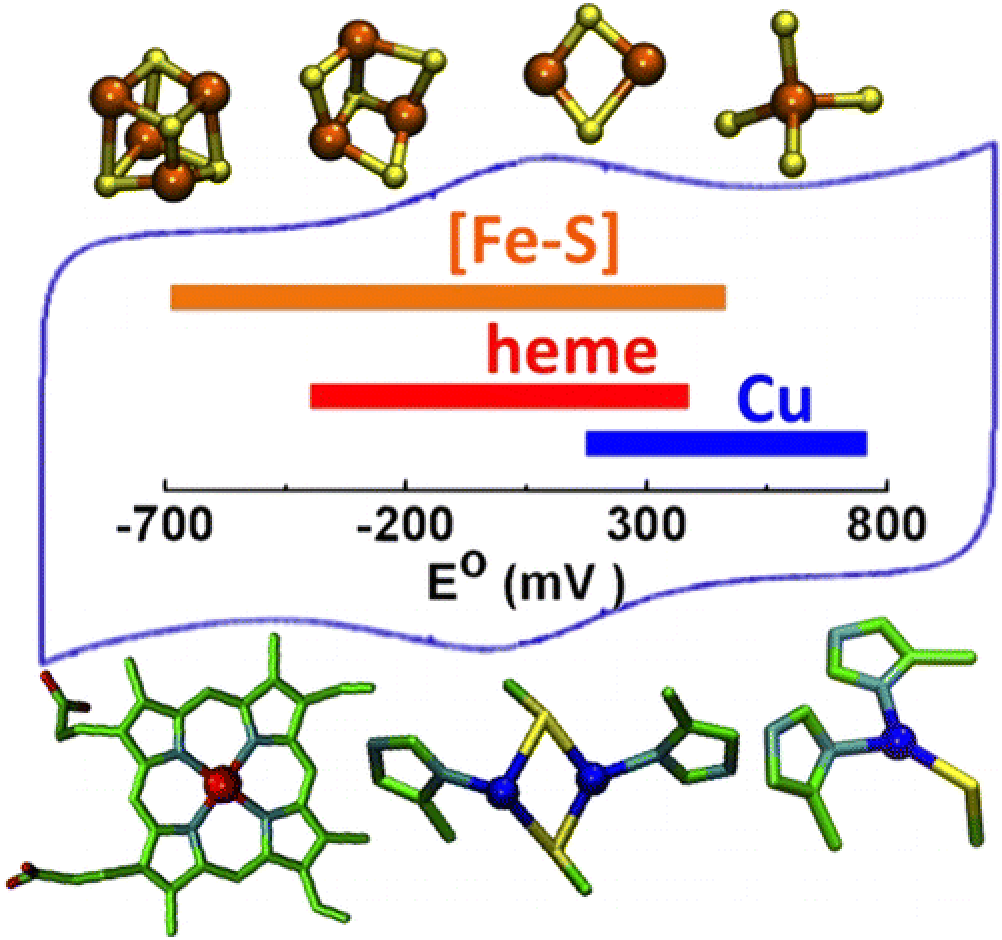
Oxidized plastocyanin



Reduced plastocyanin

*Blue-copper proteins are **very efficient and fast 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



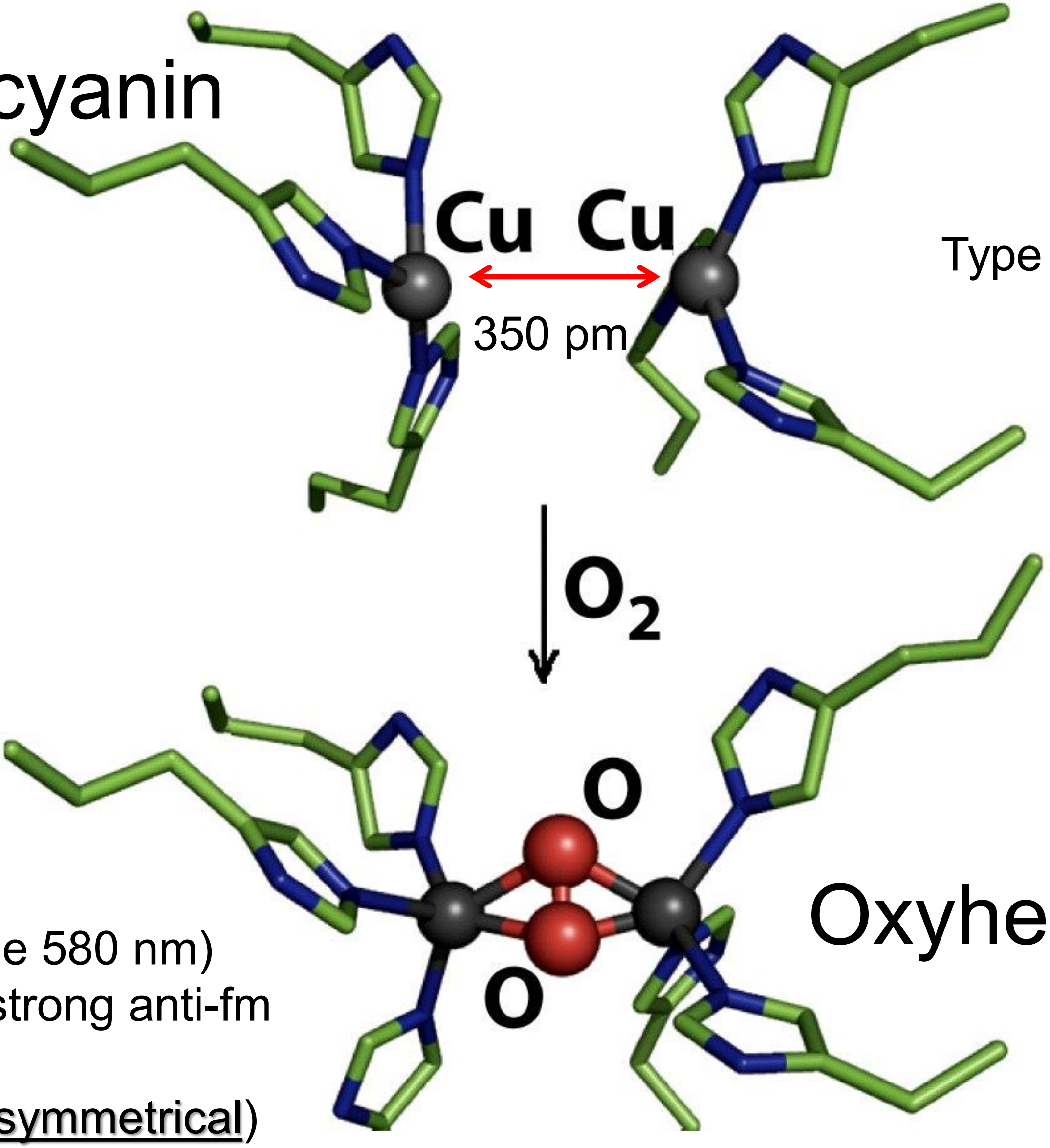
O_2 transport mediated by Cu-proteins: Hemocyanin

Hemocyanin

Up to 1500 kDa,
Each unit 75 kDa

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

Type 3 copper center

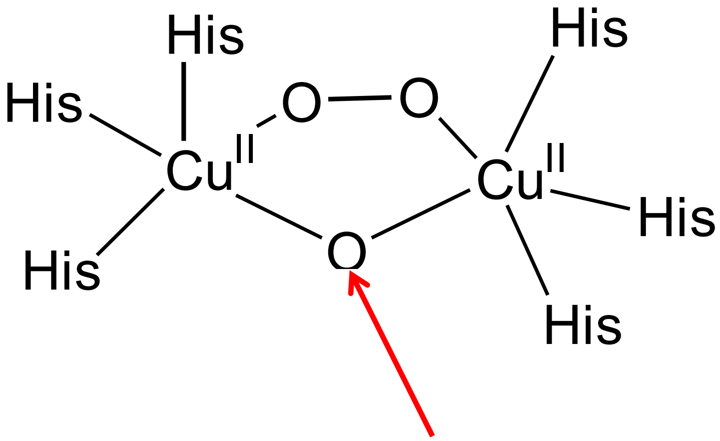


Oxyhemocyanin

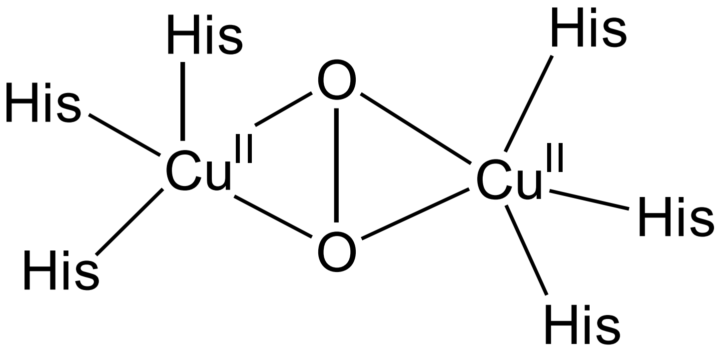
Purple (LMCT 350 e 580 nm)
2 Cu(II) (diamag., strong anti-fm coupling)
IR: 755 cm^{-1} (O_2^{2-}, <u>symmetrical</u>)

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

Possible symmetrical cooordination modes of peroxide ion

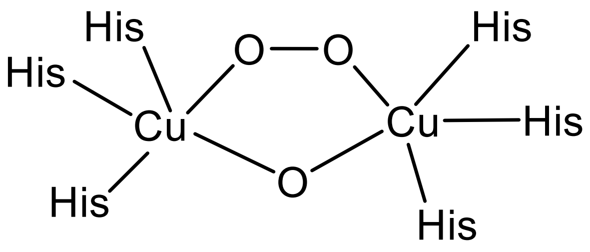


tyrosinate? OH?

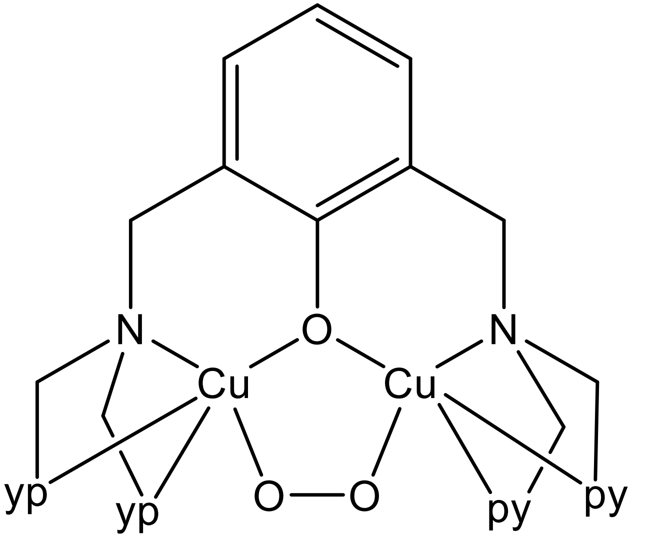


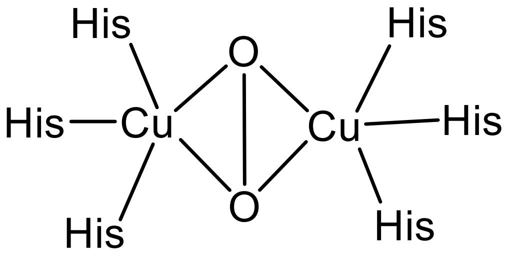
In the hypothesis of end-on coordination for the peroxide ion, the presence of a bridging oxygen atom was hypothesized to account for the strong anti-ferromagnetic coupling between the two Cu(II) centers

Models for O_2 binding to hemocyanin

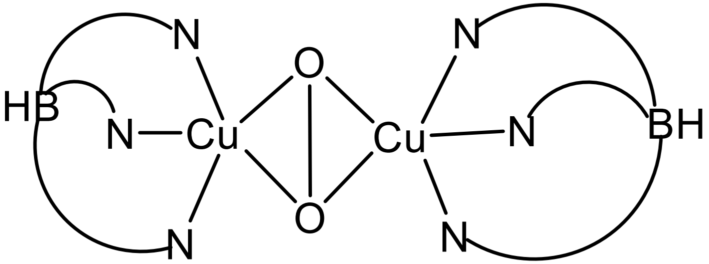


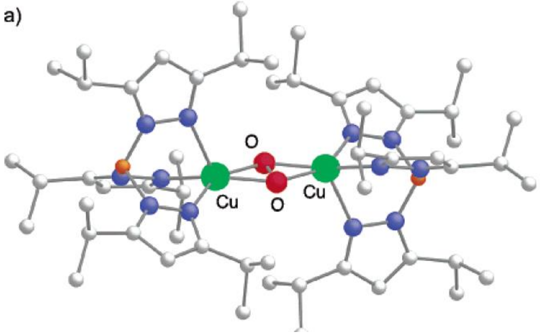
Wrong hypothesis



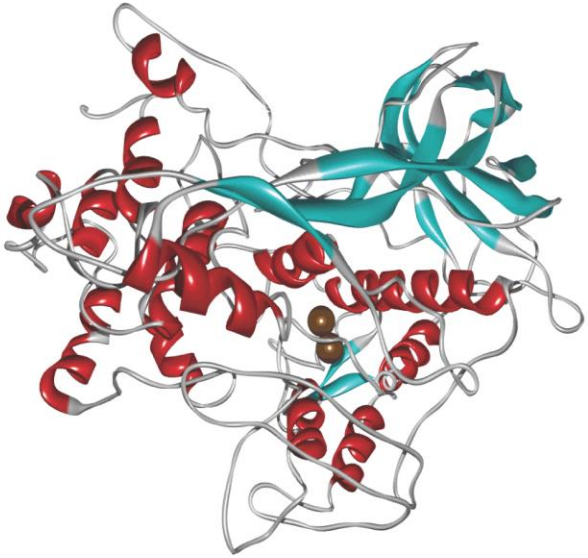


Correct hypothesis

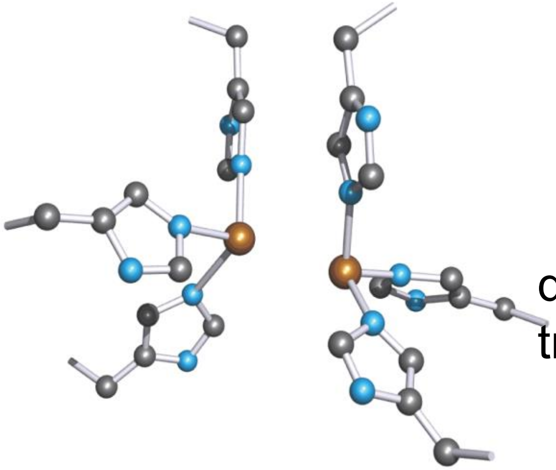




Hemocyanin

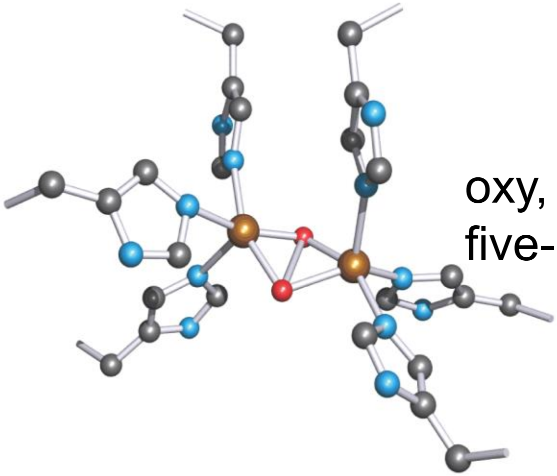


(a)



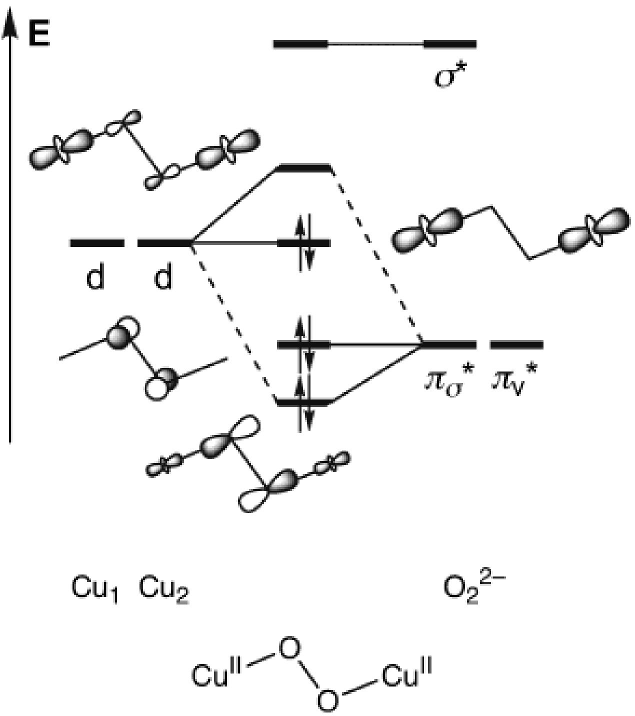
(b)

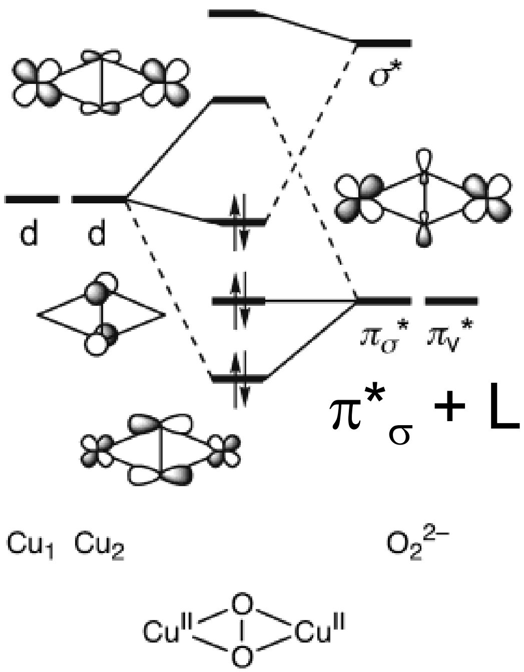
deoxy, Cu(I)
tri-coordinate



(c)

oxy, Cu(II)
five-coordinate





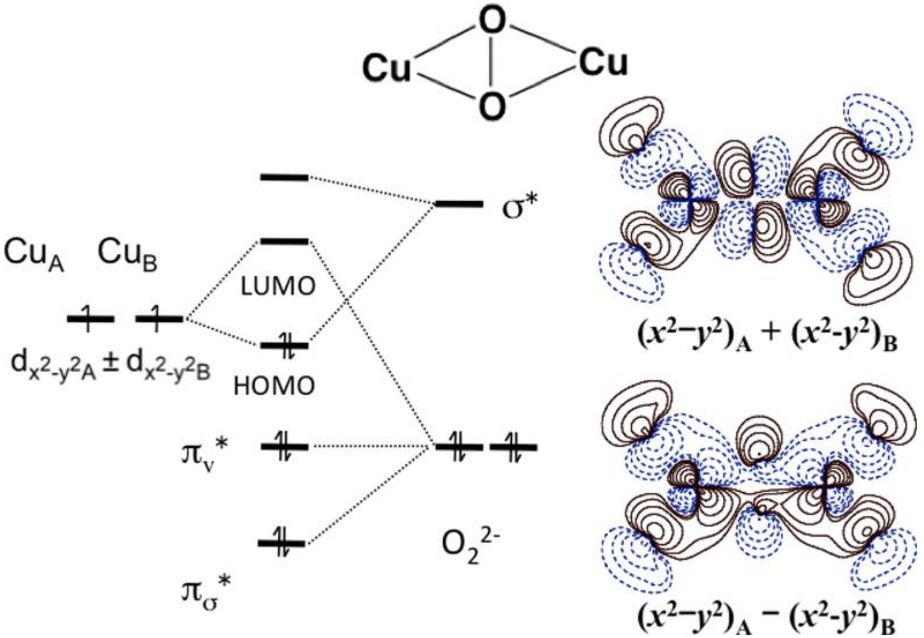
σ^* + HOMO Cu ($d_{x2\text{-}y2} - d_{x2\text{-}y2}$)

2Cu(II) → O_2^{2-}

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

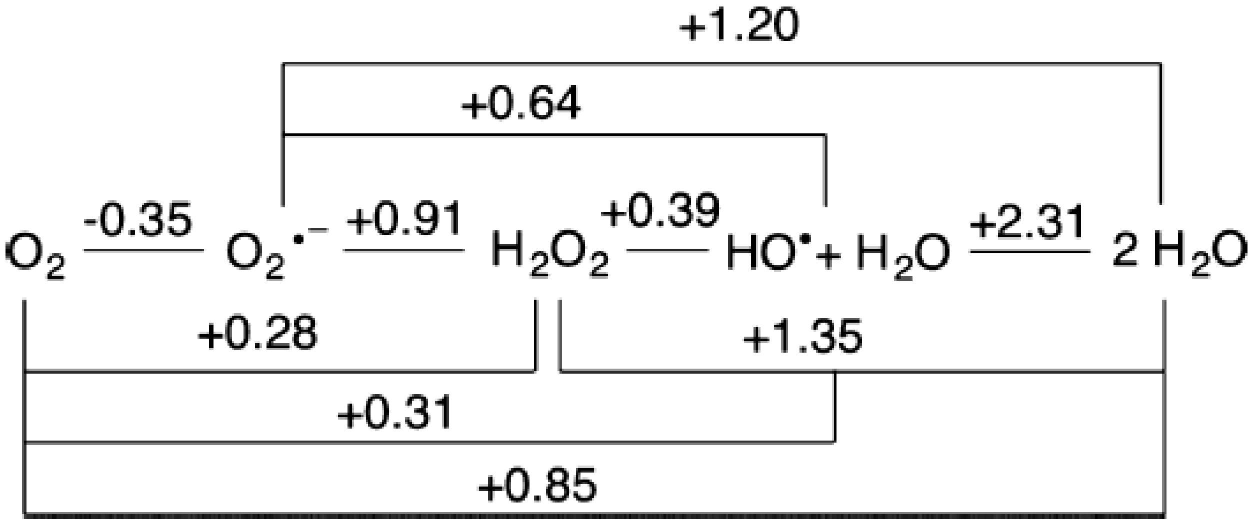
O_2^{2-} → 2Cu(II)

IR: 755 cm^{-1} (O_2^{2-}, symmetrical)



Cu enzymes that activate or reduce O_2

pH = 7



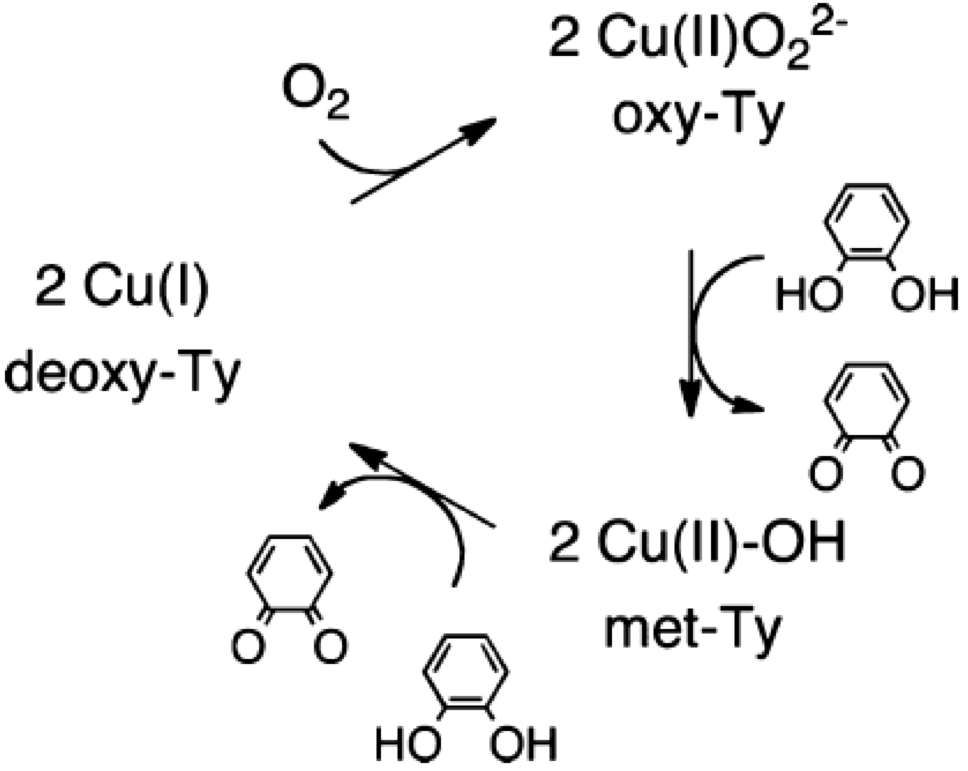
the mono-electron reduction of O_2 to superoxide is thermodynamically disfavored (E° = –0.35 V)

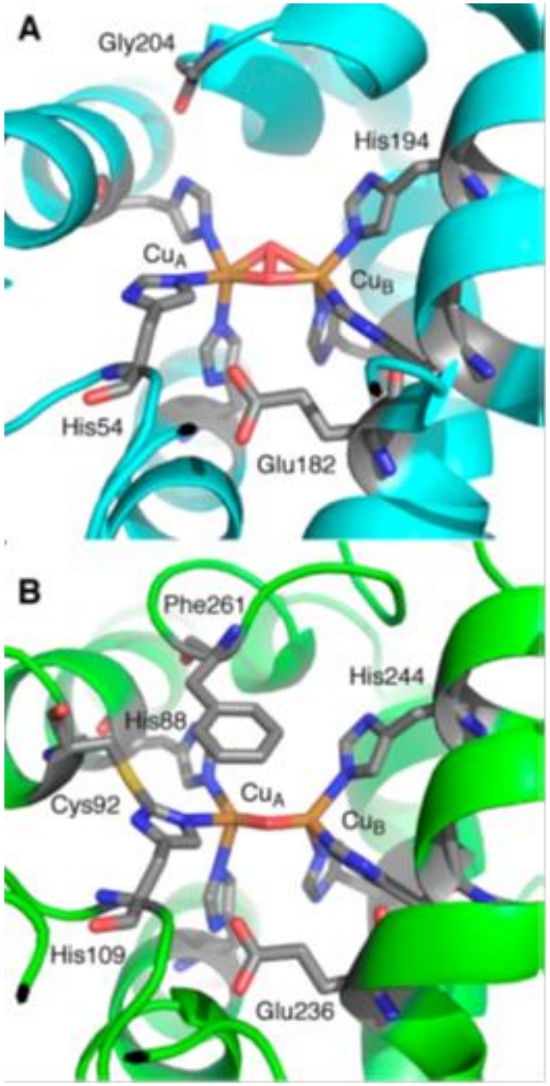
providing two electrons requires either the presence of at least two Cu ions or a Cu ion and a redox-active organic cofactor

Polyphenol oxidases

Tyrosinase, Catechol-oxidase

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





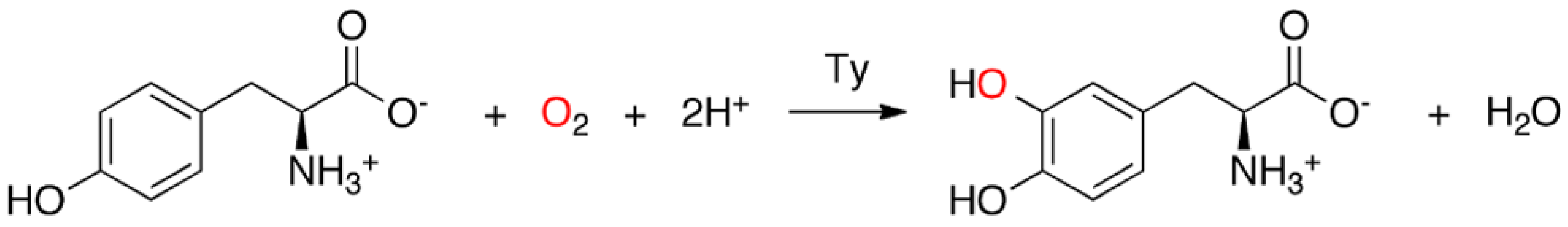
oxy-Ty

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

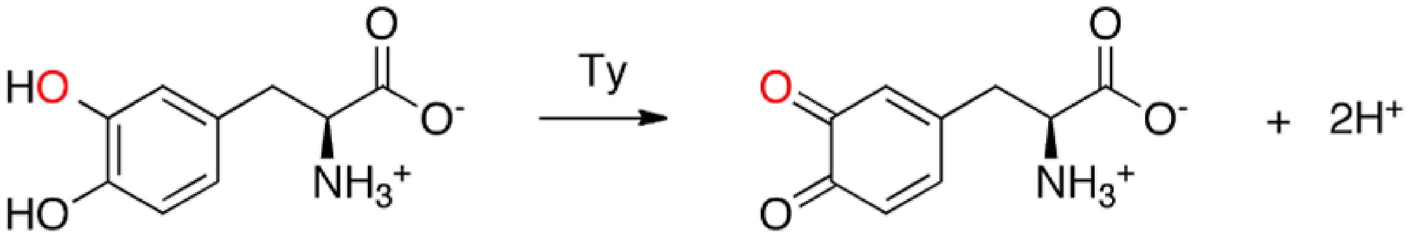
oxy-Ty is capable to convert **both** phenols to o-diphenols (oxygenase activity) **and** o-diphenols to o-quinones, met-T is capable to oxidize only o-diphenols to o-quinones



L-tyrosine

mono-oxygenase activity

L-DOPA

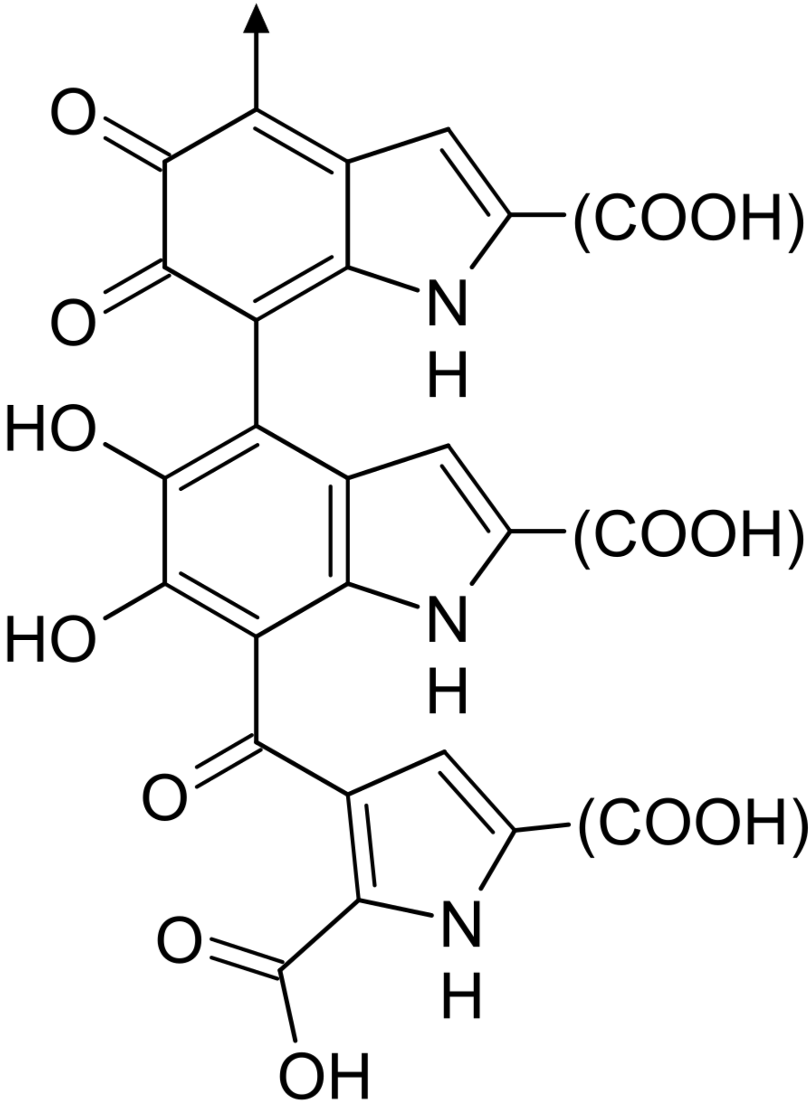


L-DOPA

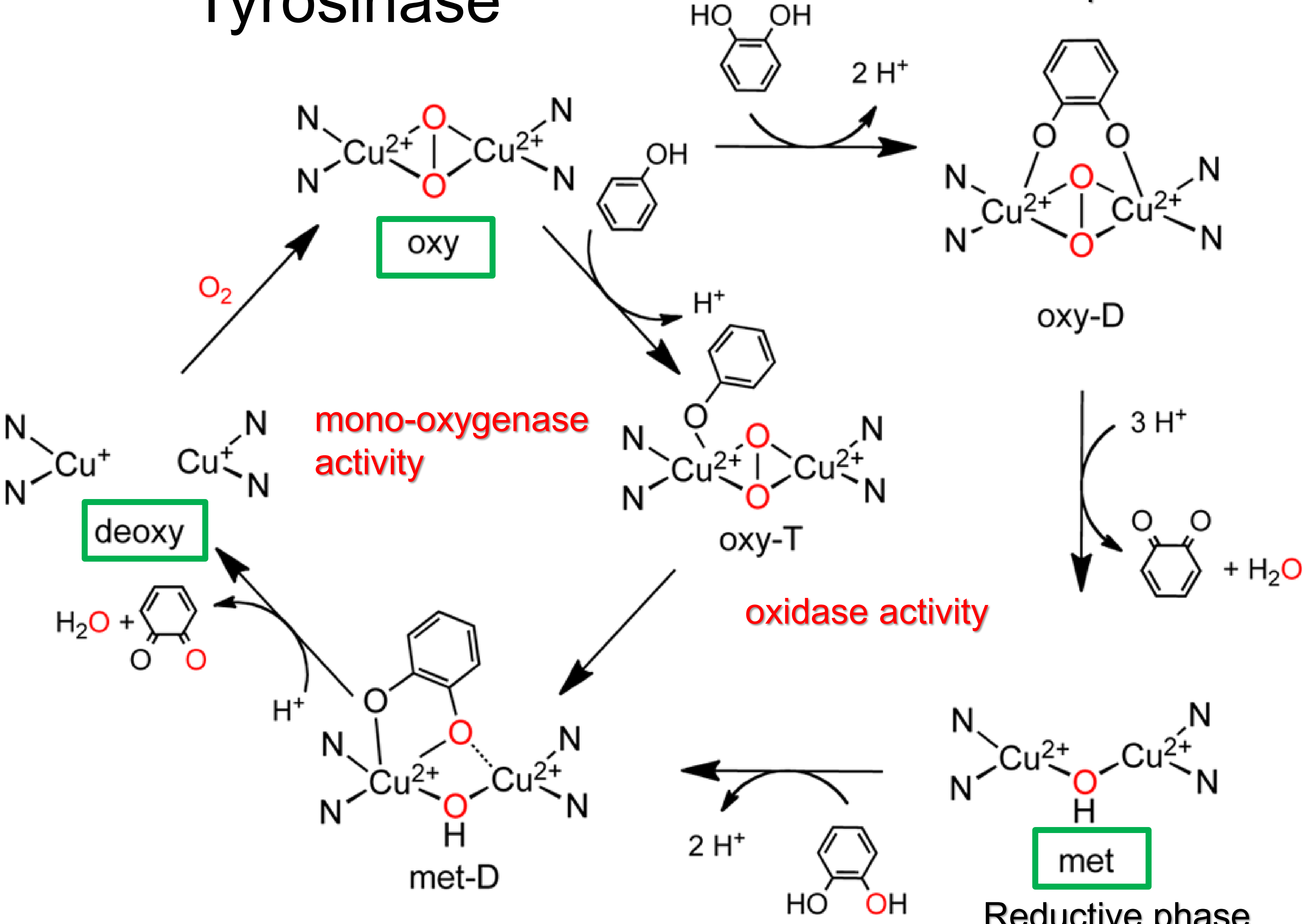
o-L-DOPAquinone

oxidase activity

The oxygenation tyrosine, followed by oxidation to an o-quinone, eventually leads to the production of **melanin**



Tyrosinase



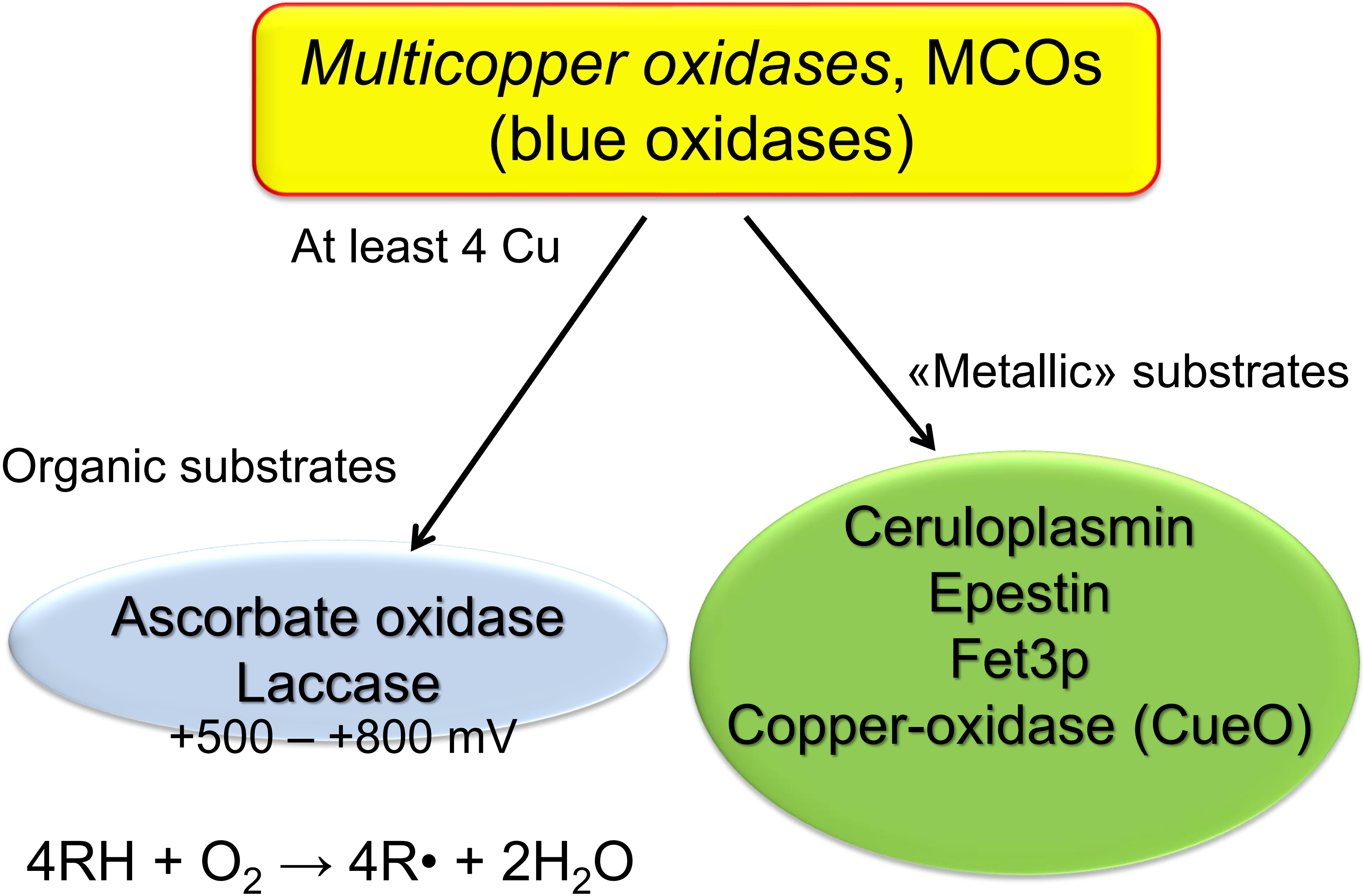
Oxidative phase

mono-oxygenase activity

oxidase activity

Reductive phase

electrophilic aromatic substitution



Multicopper oxidases, MCOs (blue oxidases)

At least 4 Cu

«Metallic» substrates

Organic substrates

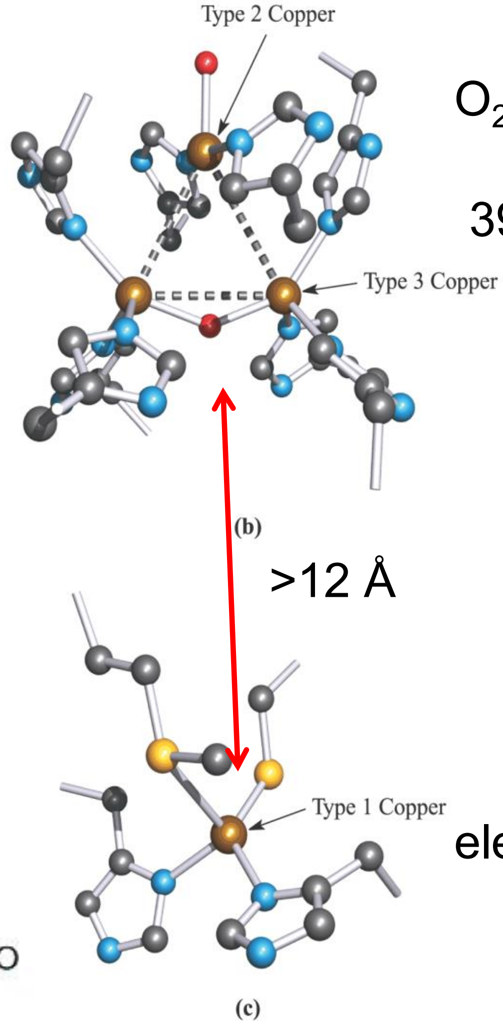
Ascorbate oxidase
Laccase
+500 – +800 mV

Ceruloplasmin
Epestin
Fet3p
Copper-oxidase (CueO)

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

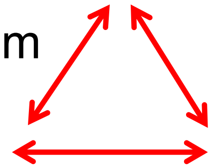
(RH = phenol)


(a)



>12 Å

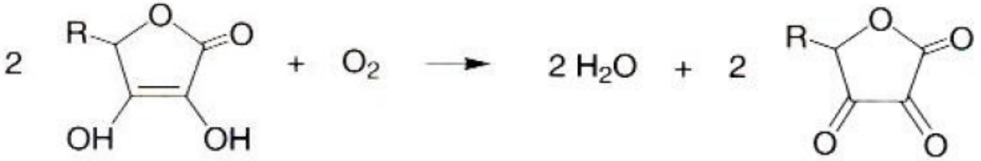
O_2 reduction ($4e^-$)


390 pm

340 pm

electron acceptor

R = $CH(OH)CH_2OH$

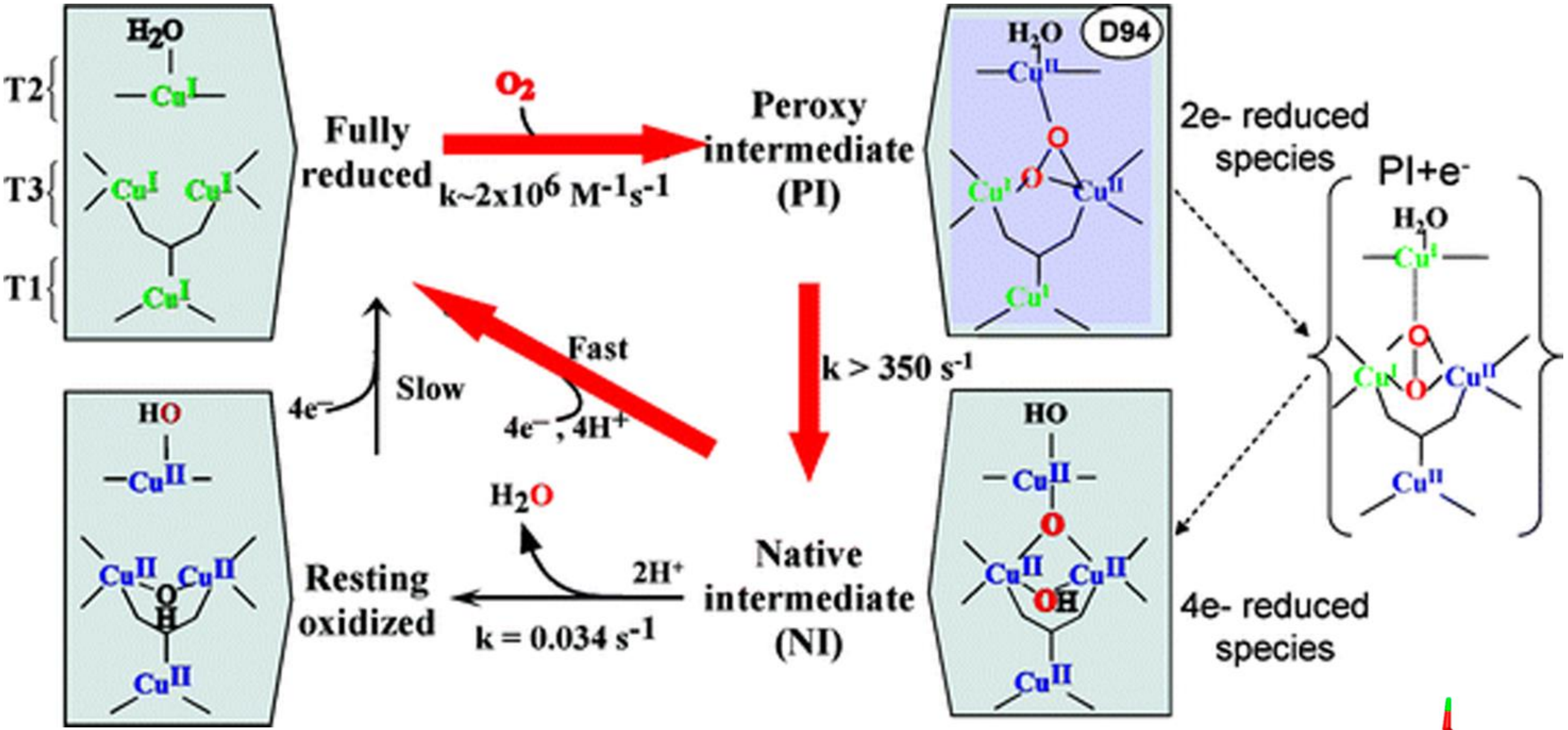


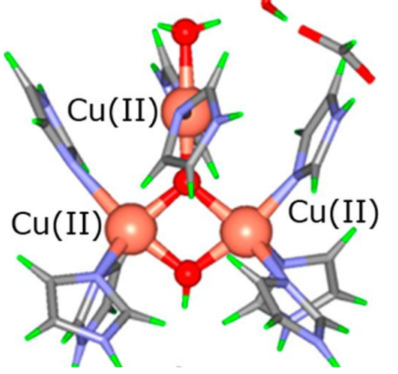
Ascorbate oxidase (from squash)

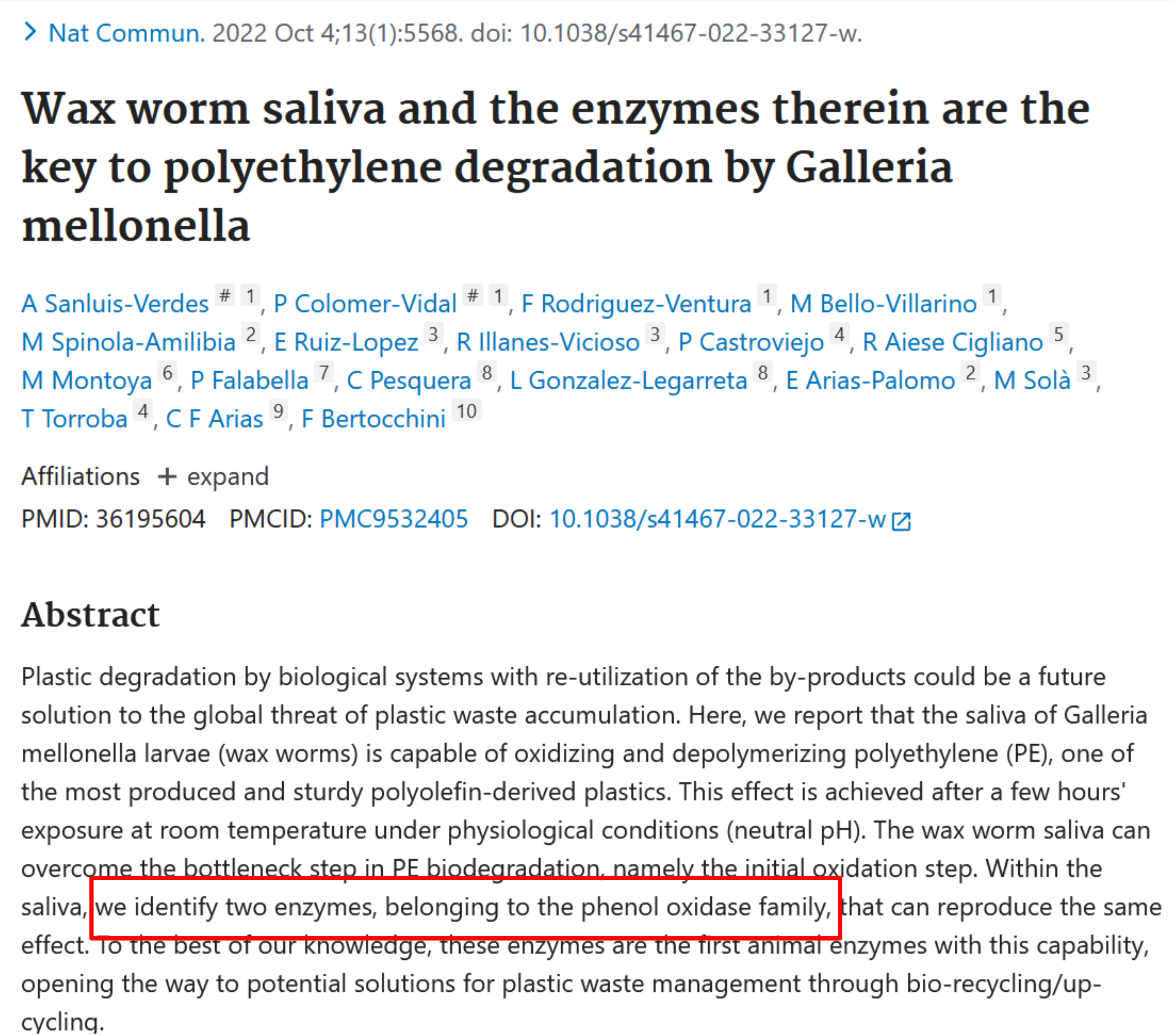
$$4RH + O_2 \rightarrow 4R\cdot + 2H_2O$$

(RH = phenol)

Mechanism of action for multicopper oxidases







Nat Commun. 2022 Oct 4;13(1):5568. doi: 10.1038/s41467-022-33127-w.

Wax worm saliva and the enzymes therein are the key to polyethylene degradation by Galleria mellonella

A Sanluis-Verdes [#, 1], P Colomer-Vidal [#, 1], F Rodriguez-Ventura [1], M Bello-Villarino [1], M Spinola-Amilibia [2], E Ruiz-Lopez [3], R Illanes-Vicioso [3], P Castroviejo [4], R Aiese Cigliano [5], M Montoya [6], P Falabella [7], C Pesquera [8], L Gonzalez-Legarreta [8], E Arias-Palomo [2], M Solà [3], T Torroba [4], C F Arias [9], F Bertocchini [10]

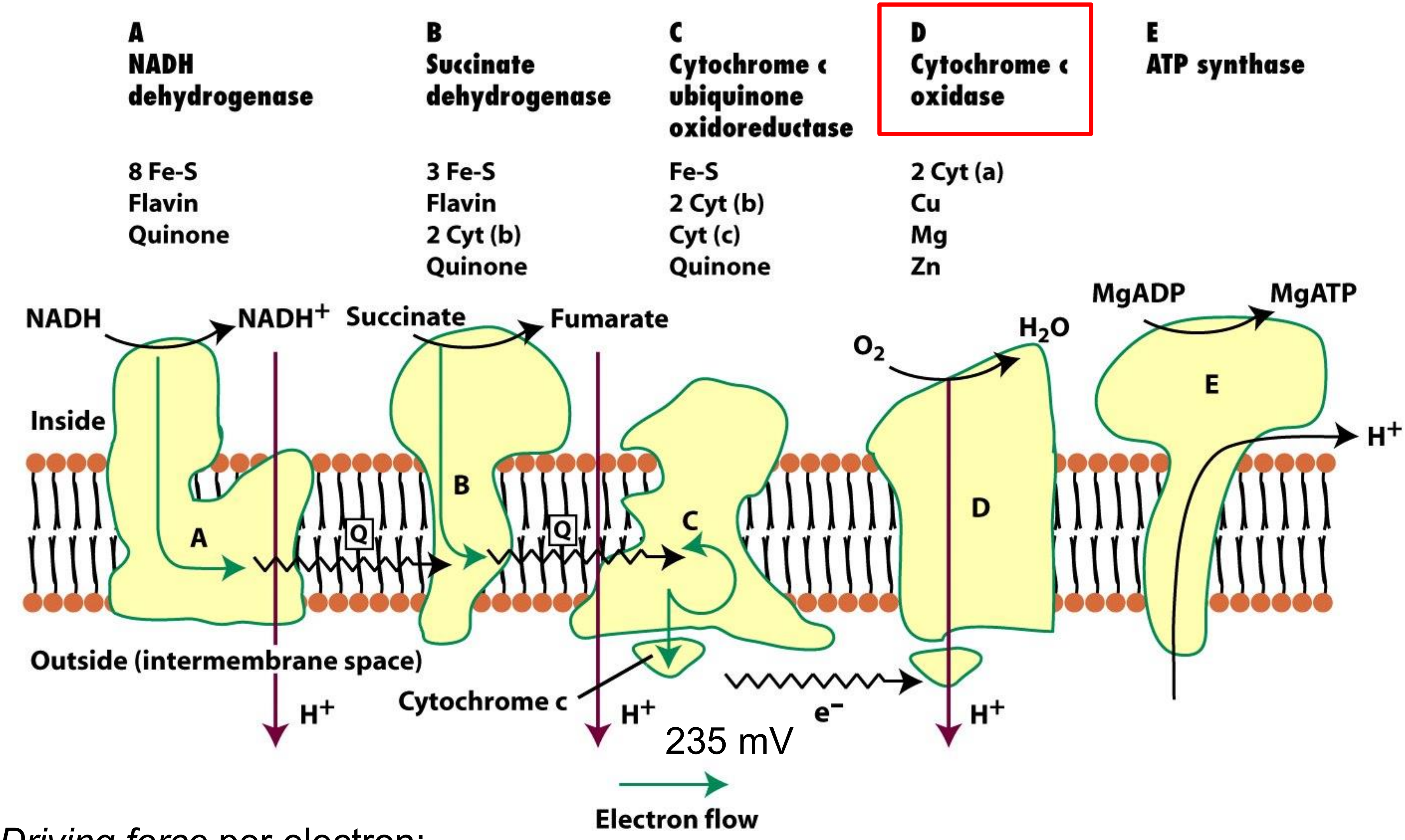
Affiliations + expand

PMID: 36195604 PMCID: PMC9532405 DOI: 10.1038/s41467-022-33127-w

Abstract

Plastic degradation by biological systems with re-utilization of the by-products could be a future solution to the global threat of plastic waste accumulation. Here, we report that the saliva of Galleria mellonella larvae (wax worms) is capable of oxidizing and depolymerizing polyethylene (PE), one of the most produced and sturdy polyolefin-derived plastics. This effect is achieved after a few hours' exposure at room temperature under physiological conditions (neutral pH). The wax worm saliva can overcome the bottleneck step in PE biodegradation, namely the initial oxidation step. Within the saliva, we identify two enzymes, belonging to the phenol oxidase family, that can reproduce the same effect. To the best of our knowledge, these enzymes are the first animal enzymes with this capability, opening the way to potential solutions for plastic waste management through bio-recycling/up-cycling.

Respiratory chain (oxidative phosphorylation)



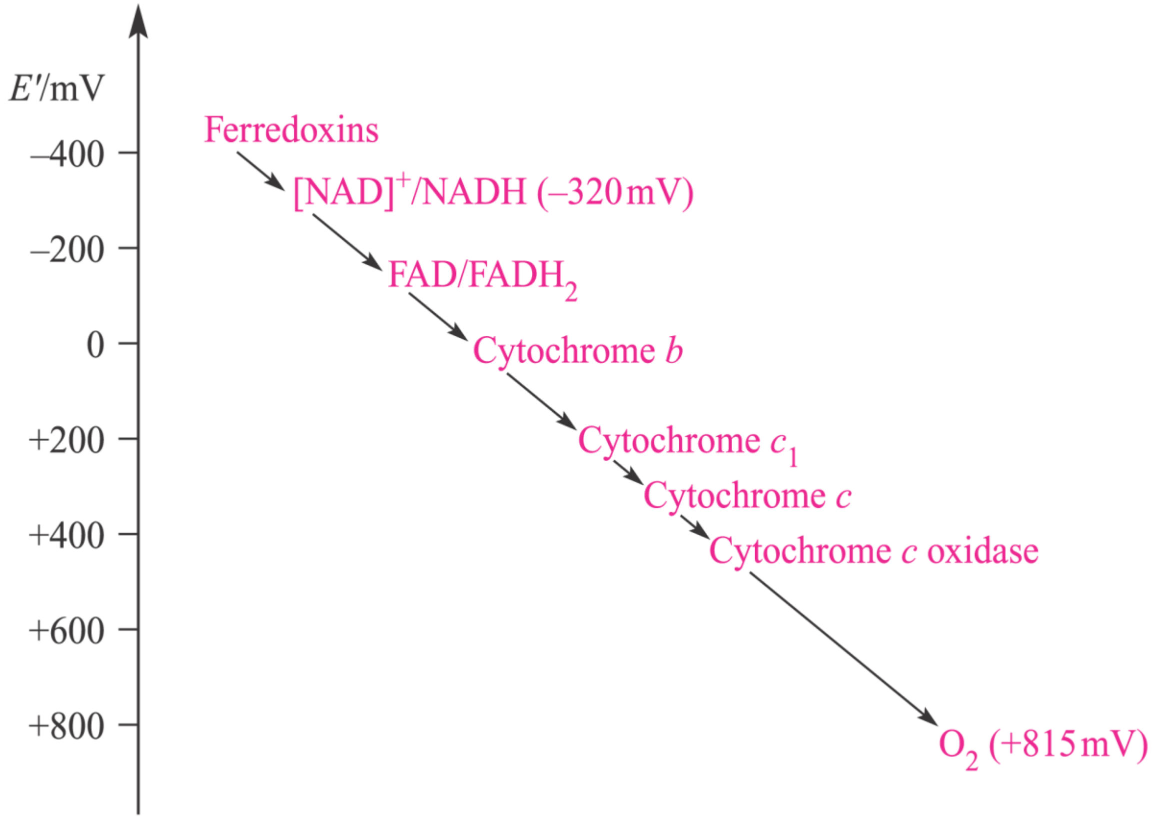
235 mV

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

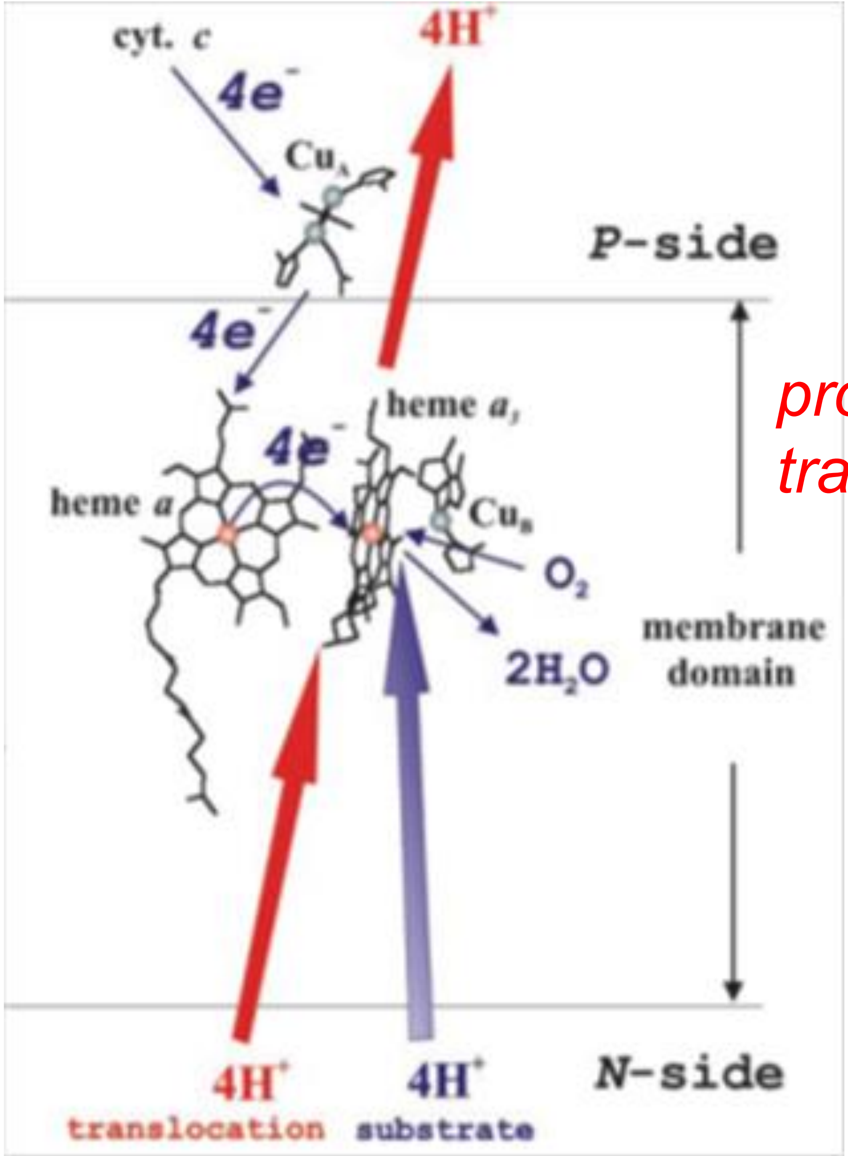
Oxidation of Fe^{2+} in Cyt c

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

The sequence of electron transfers in mitochondria



Cytochrome *c* oxidase is also a proton pump

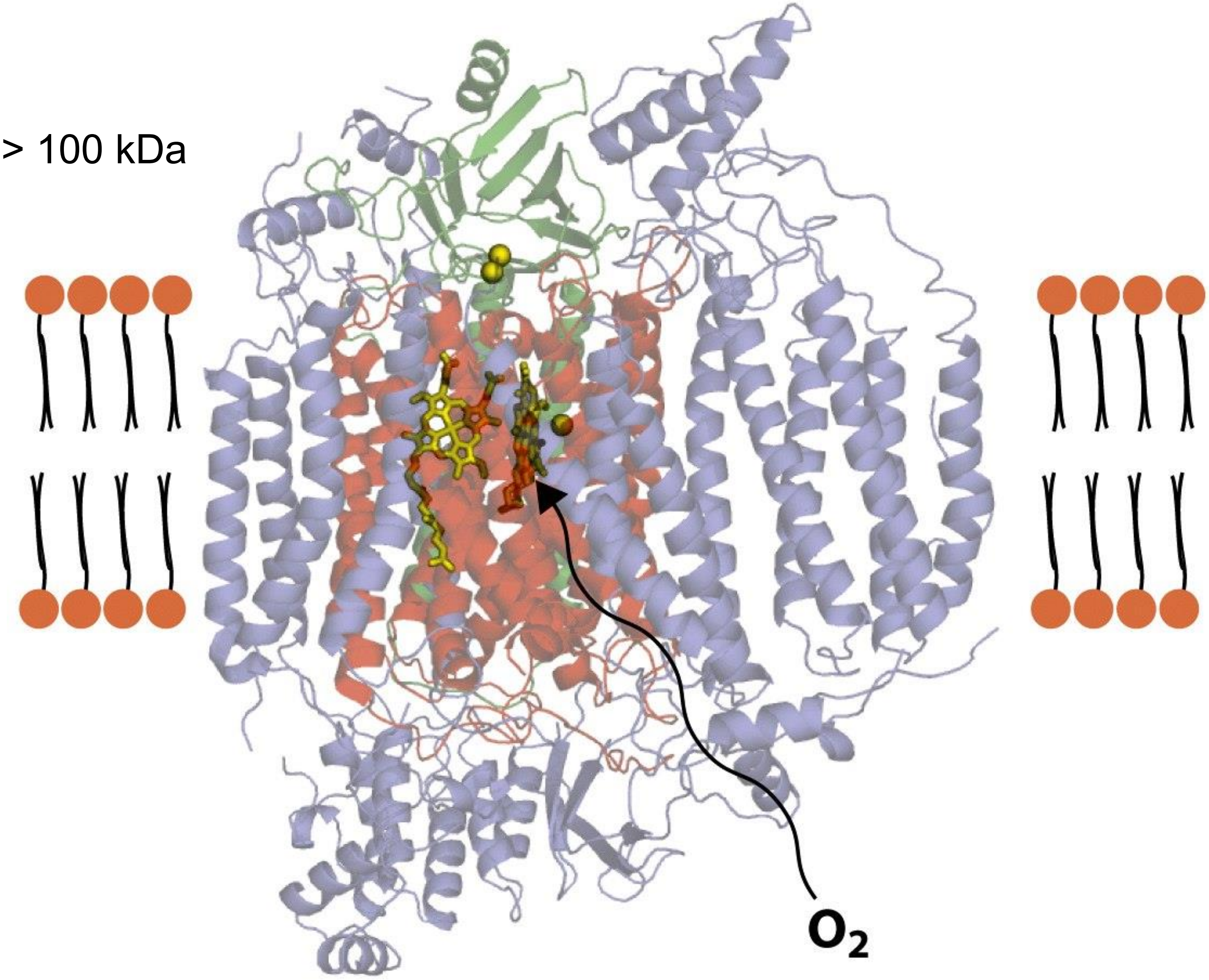


proton coupled electron transfer, PCET

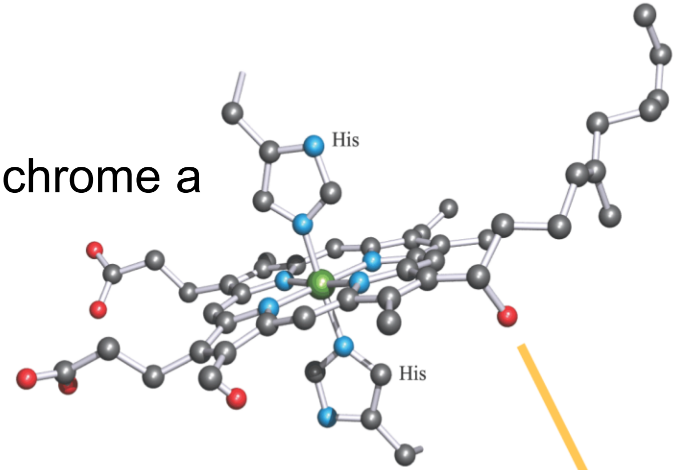
$$O_2(g) + 4e^- + 8H^+(\text{inside}) \rightarrow 2H_2O(l) + 4H^+(\text{outside})$$

Reacts with cytochrome C

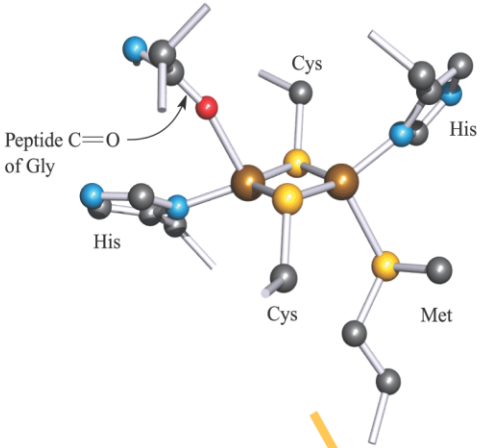
> 100 kDa



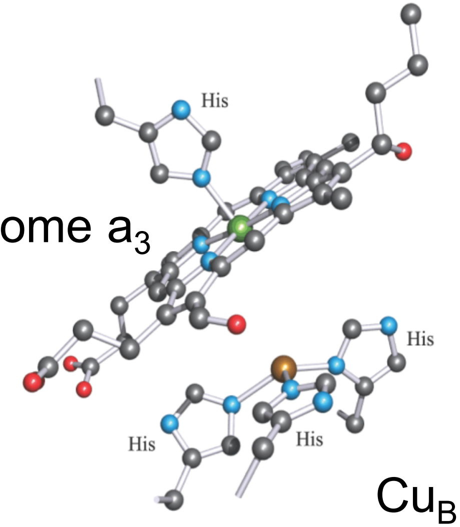
cytochrome a

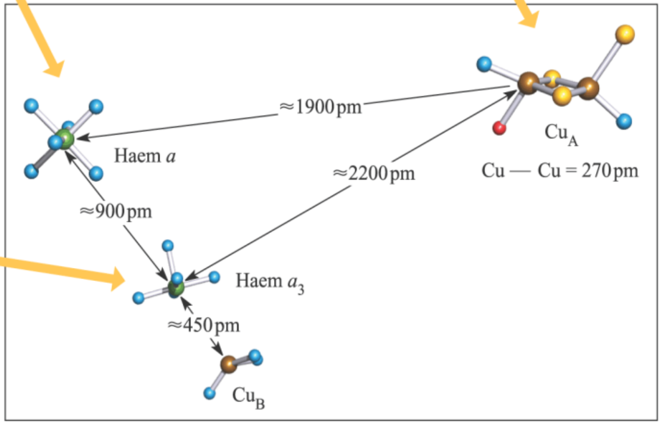


Cu$_A$

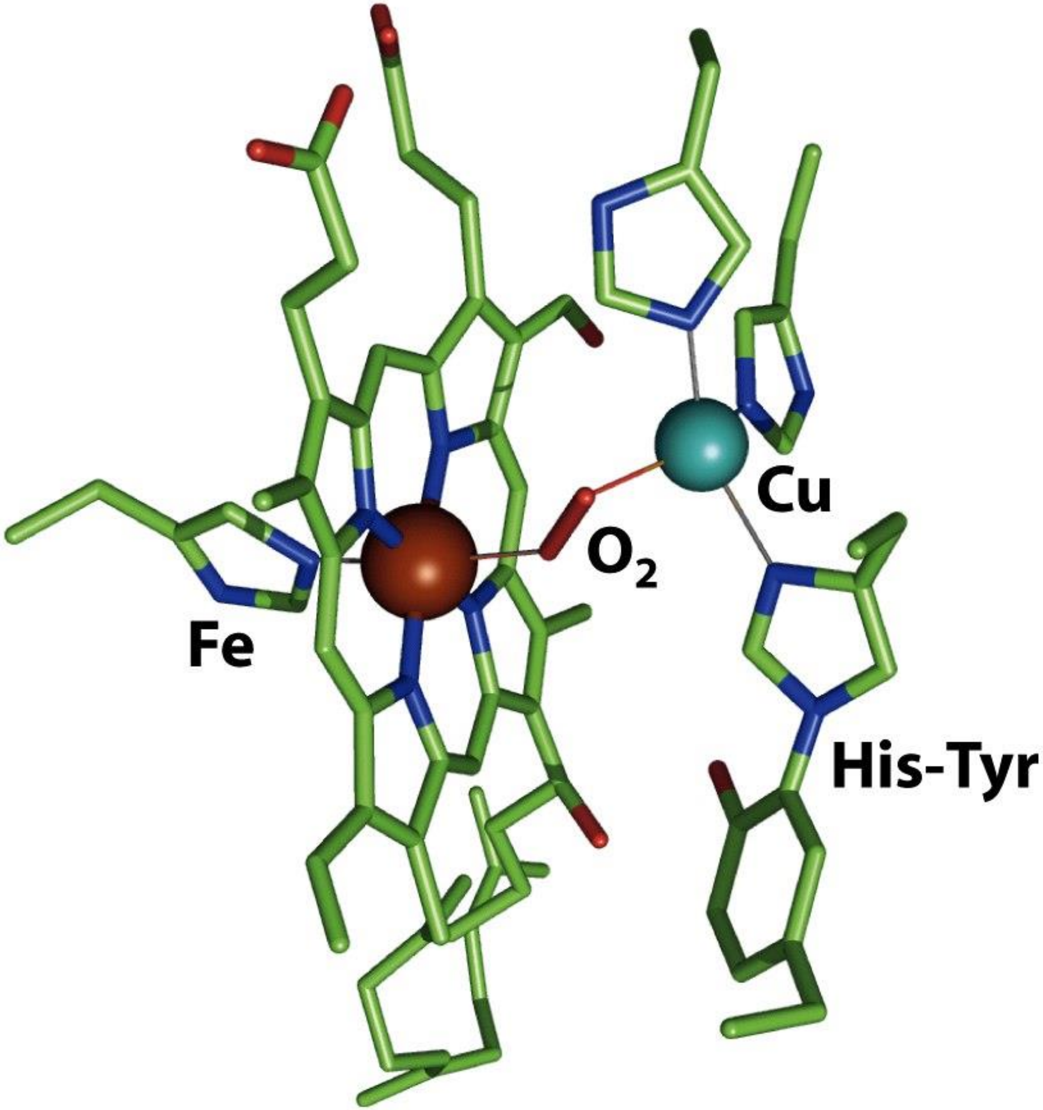


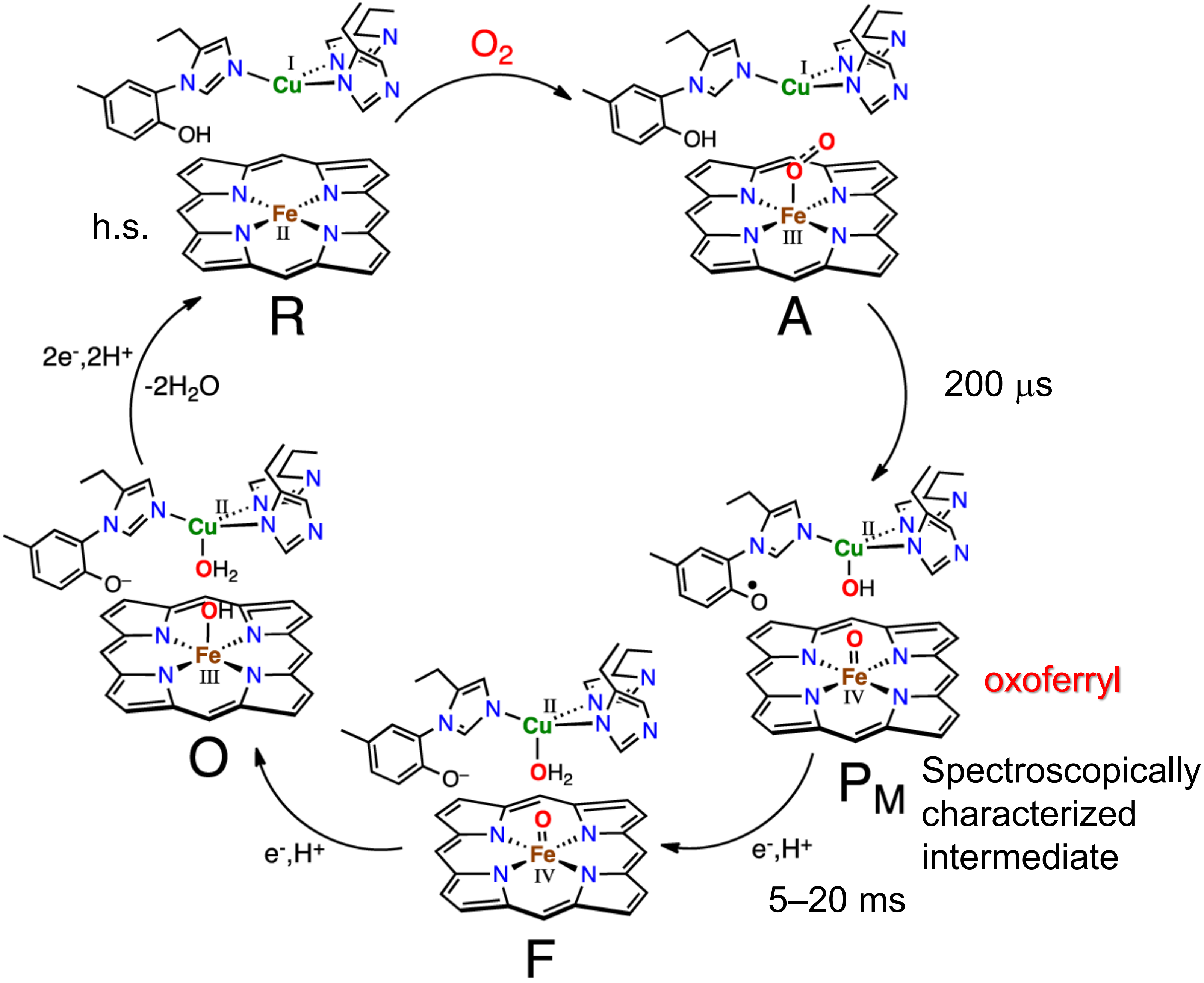
cytochrome a$_3$





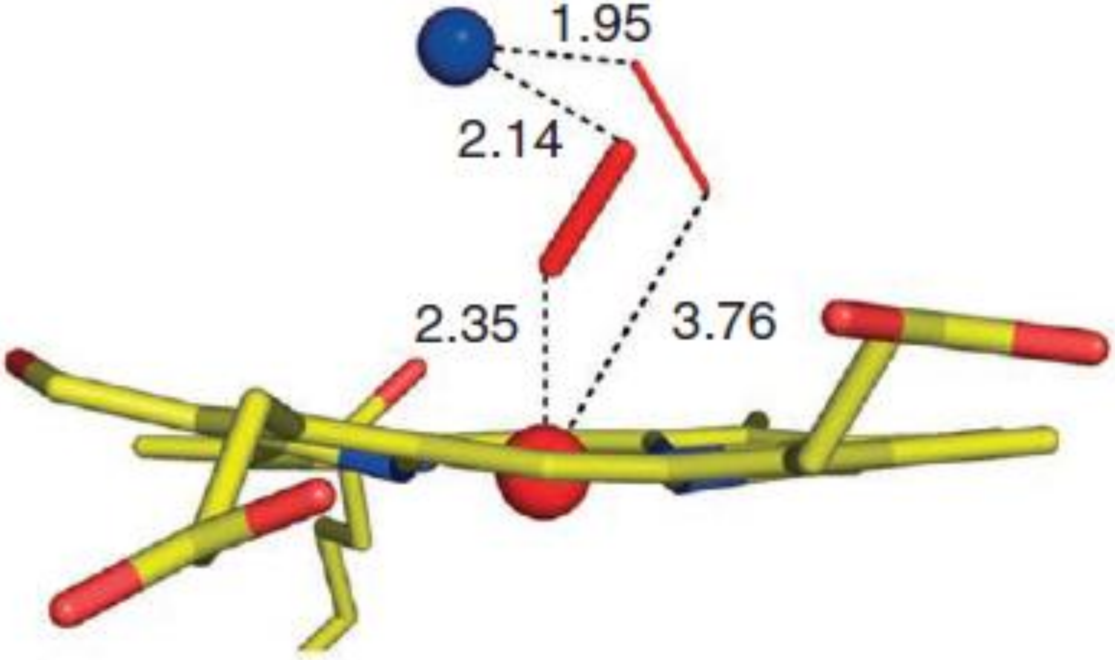
Cu$_B$ (type 2 copper)


Cu
O$_2$
Fe
His-Tyr

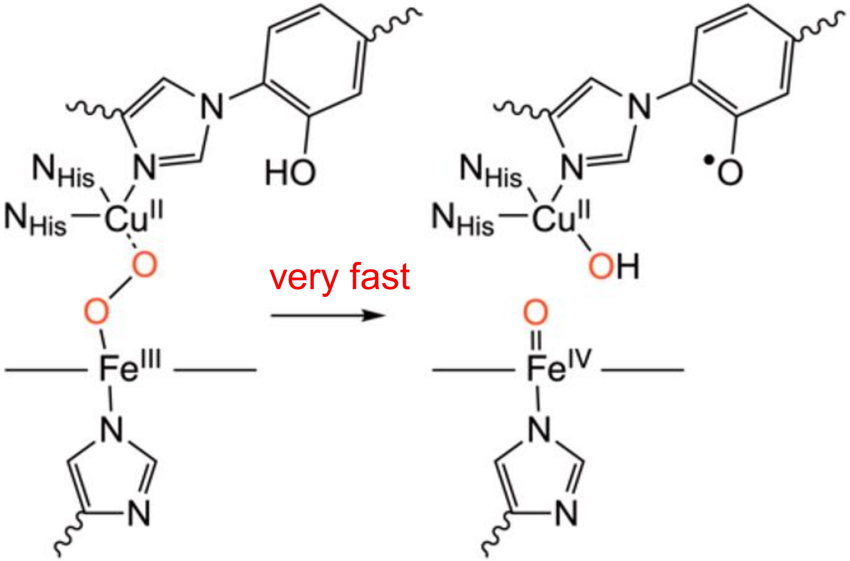


Spectroscopically characterized intermediate

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

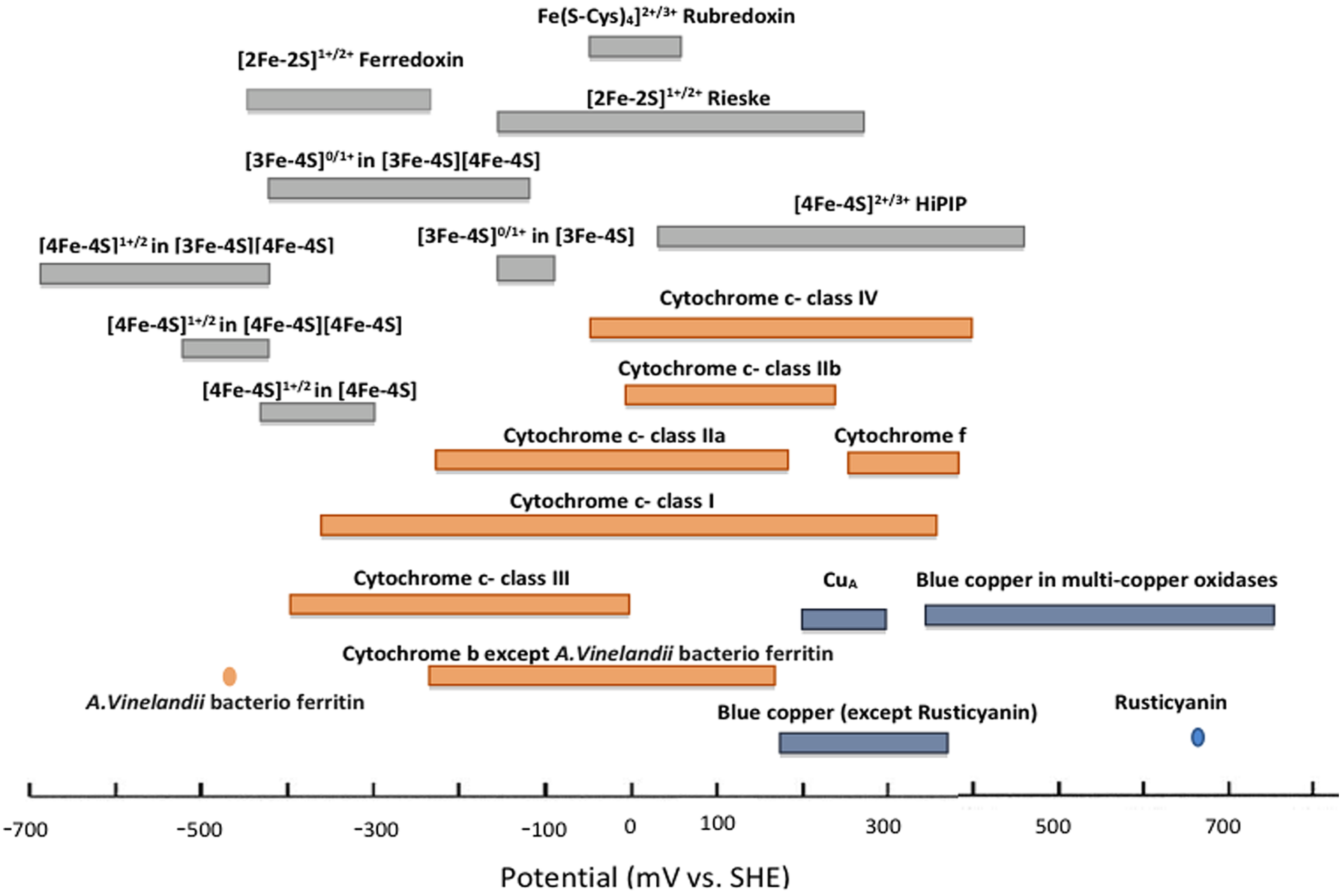


Nature, 2014



(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:

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

The hydroxyl radical •OH, due to its high reactivity, is called HROS

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.

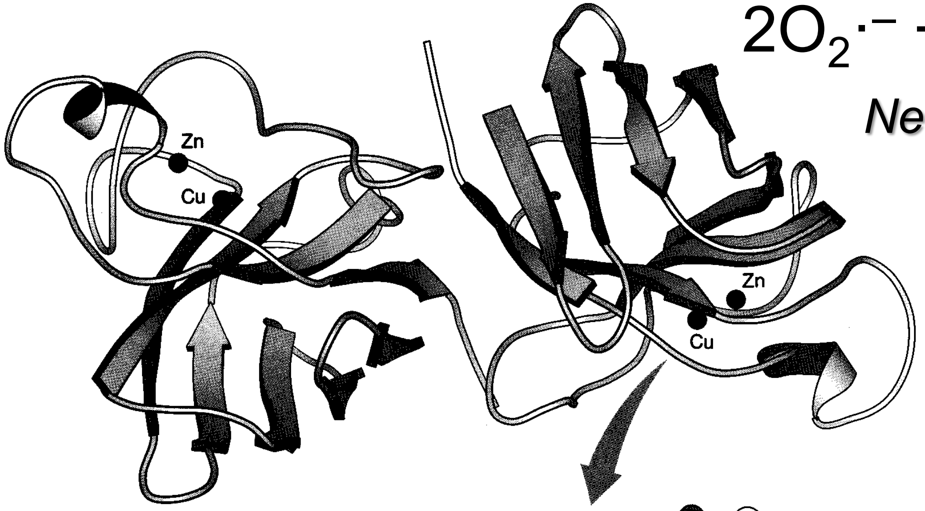
Extracellular SOD (ECSOD) shares significant amino acid homology with CuZnSOD (40–60%), contains both copper and zinc in its active site, but is localized to the extracellular region of the cell.

MnSOD is a homotetramer localized exclusively in the mitochondrial matrix (*see later section on Mn*).

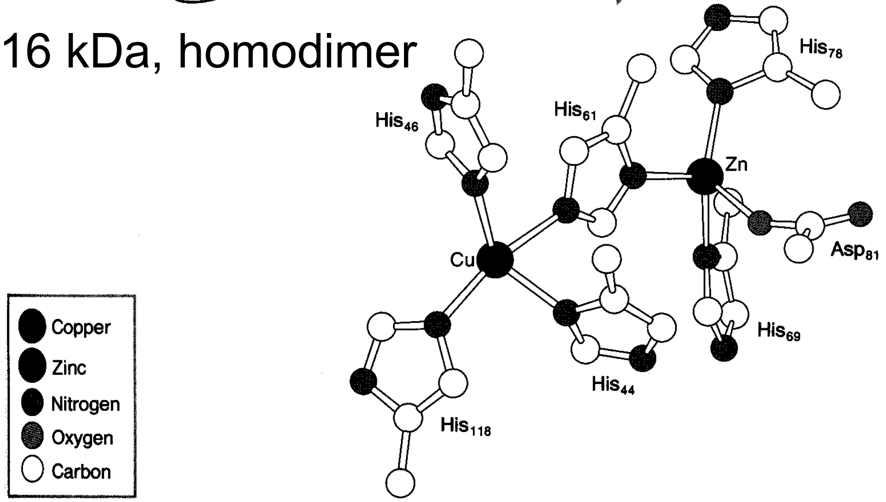
Cu-Zn superoxide dismutase

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

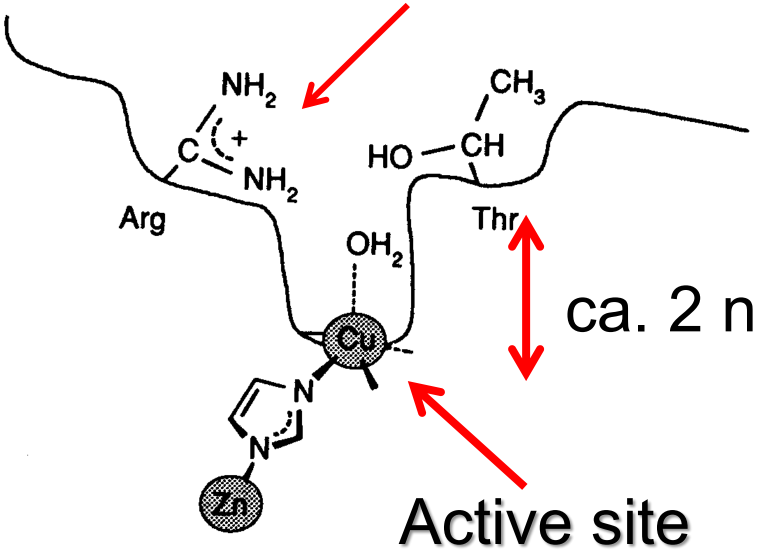
Nearly-diffusive speed



16 kDa, homodimer



guanidinium group



ca. 2 nm

Active site

Catalytic cycle of Cu-Zn SOD