COPPER-CONTAINING PROTEINS

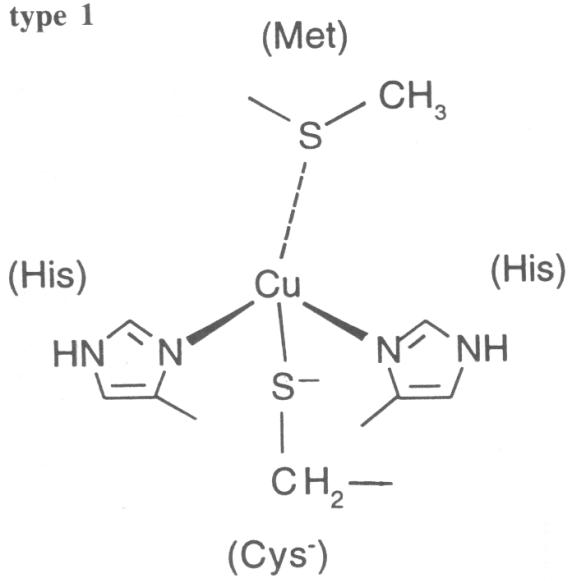
Correspondence of iron and copper proteins.

Function	Fe protein	Cu protein
O_2 transport	hemoglobin (*h*)	hemocyanin
	hemerythrin (*nh*)	
oxygenation	cytochrome P-450 (*h*)	tyrosinase
	methane monooxygenase (*nh*)	quercetinase (dioxygenase)
	catechol dioxygenase (*nh*)	
oxidase activity	peroxidases (*h*)	amine oxidases
	peroxidases (*nh*)	laccase
electron transfer	cytochromes (*h*)	blue Cu proteins
antioxidative function	peroxidases (*h*)	superoxide dismutase
	bacterial superoxide dismutases (*nh*)	(Cu, Zn) from erythrocytes
NO_2^- reduction	heme-containing nitrite reductase (*h*)	Cu-containing nitrite reductase

h, heme system; *nh*, non-heme system.

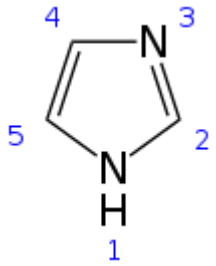
Types of copper centers in proteins

type 1



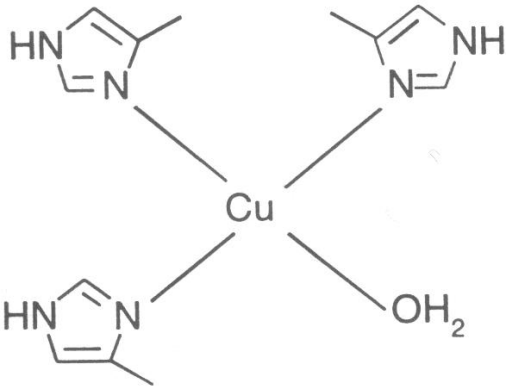
Function:
reversible electron transfer

structure: strongly distorted, (3 + 1) coordination



imidazole

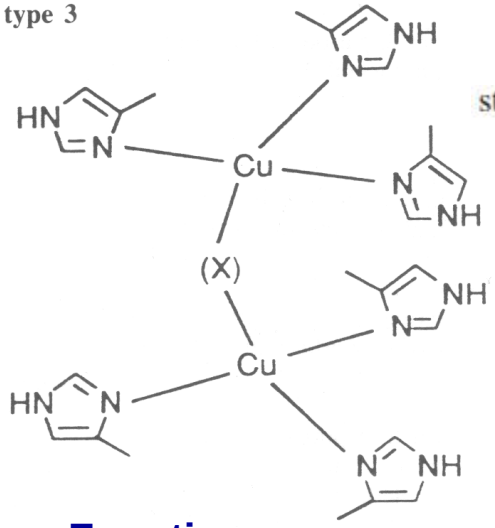
type 2



Function:
oxygen activation

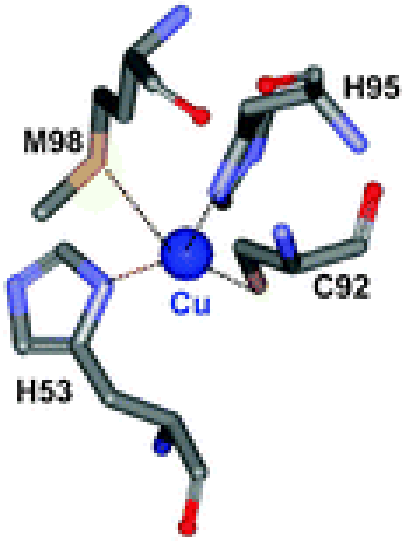
structure: essentially planar with weak additional coordination

type 3

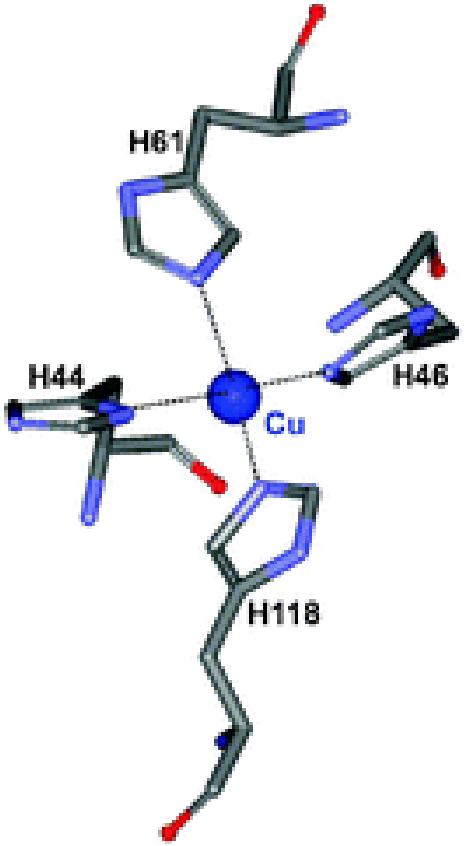


Function:
oxygen uptake

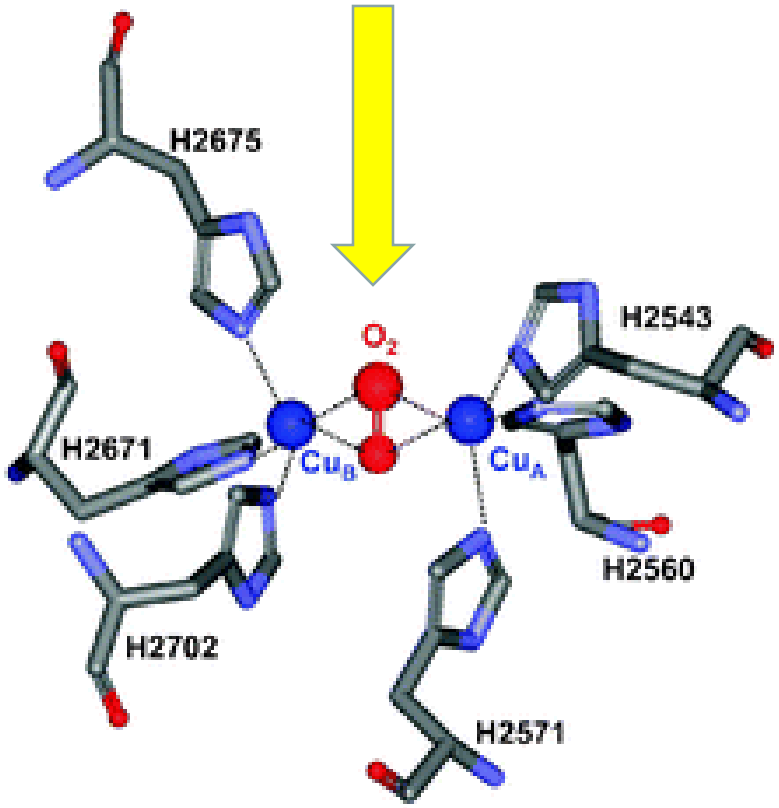
structure: (bridged) dimer



Type 1 copper site
(amicyanin)



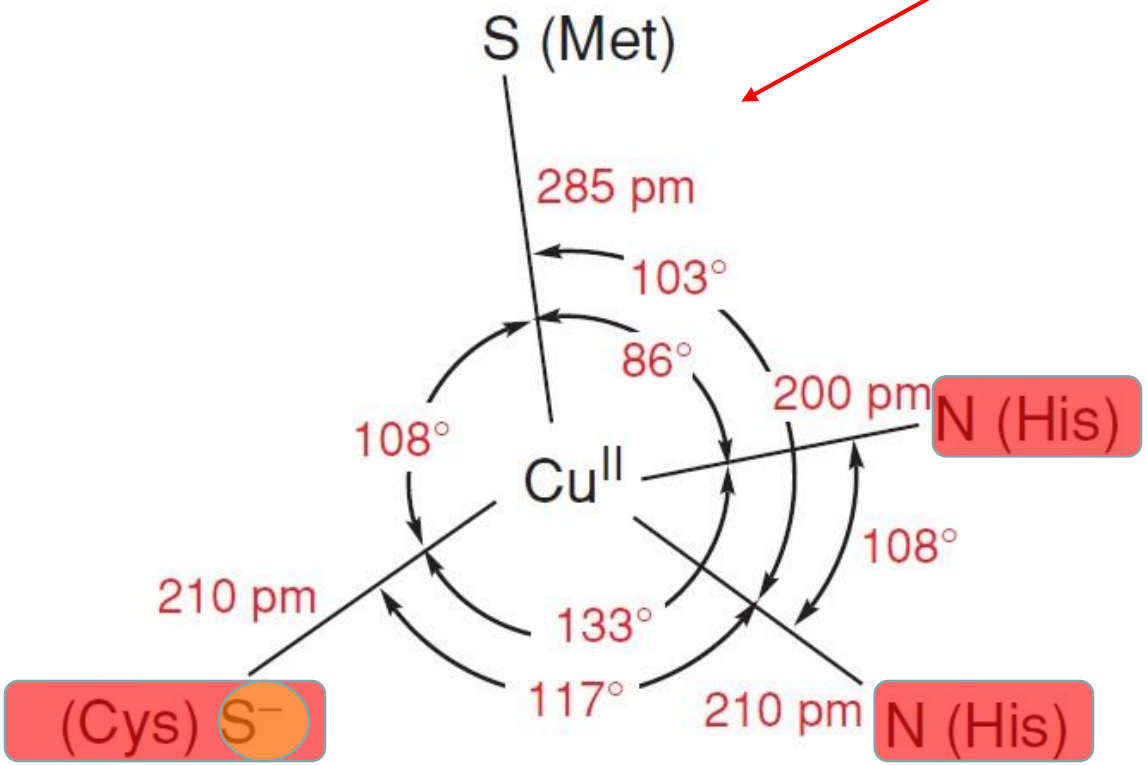
Type 2 copper site
(superoxide dismutase)



Type 3 copper site
(hemocyanin)

Type 1

Pseudo tetrahedron



The blue-copper proteins

Plastocyanin

Azurin

Electron transfer $Cu^{+} \rightleftharpoons Cu^{++} + e$

Ascorbate oxidase

Laccase

"Blue" oxidase $O_2 \longrightarrow H_2O$

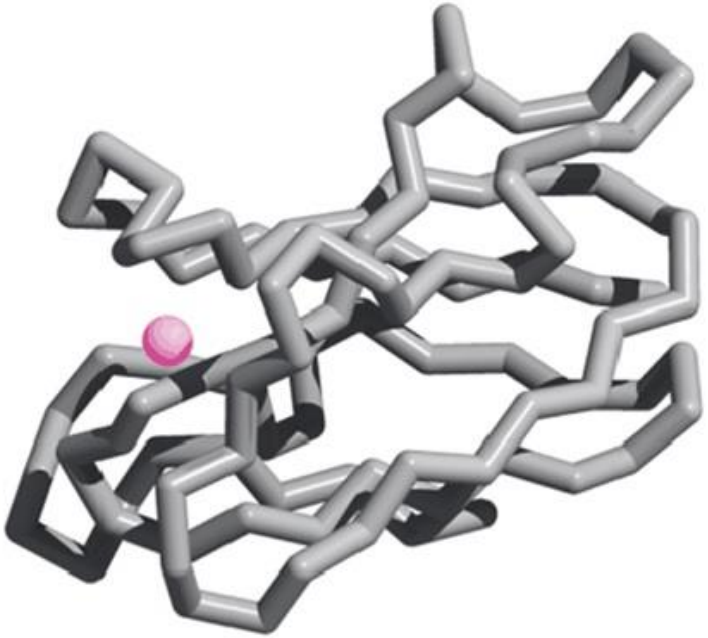
Blue Copper Proteins

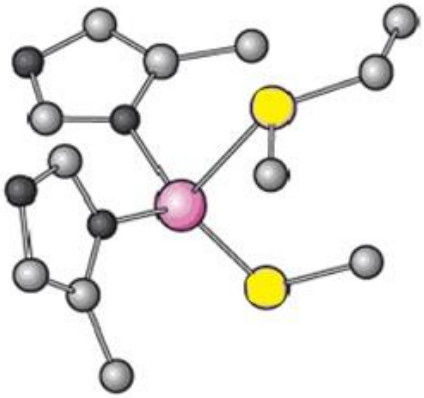
- ALL blue copper proteins contain at least one Type 1 centre. For examples, *plastocyanin* and *azurin*.

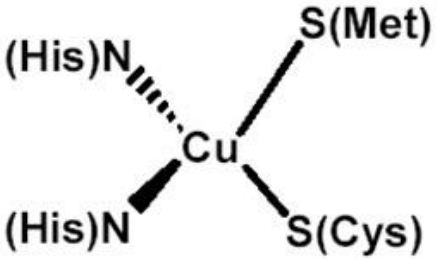
- *Plastocyanin* – present in blue-green algae and used to transport electrons between Photosystem I and II in these photosynthetic pathways.

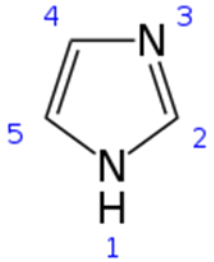
- *Azurin* – occur in some bacteria and are involved in electron transport in the conversion of $[NO_3]^-$ to N_2. Typically the protein chain is ~ 128-129 amino acid residues M ~ 14,600.

The Blue Copper Protein – Plastocyanin (spinach)

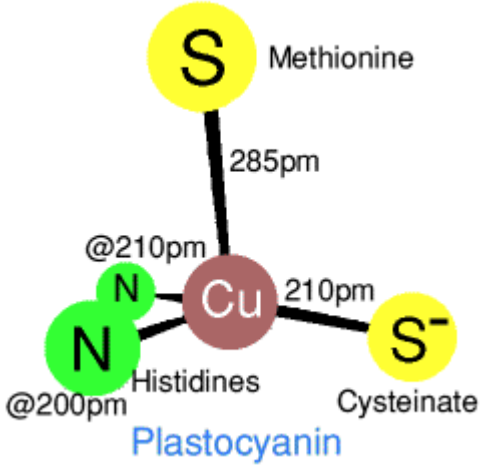




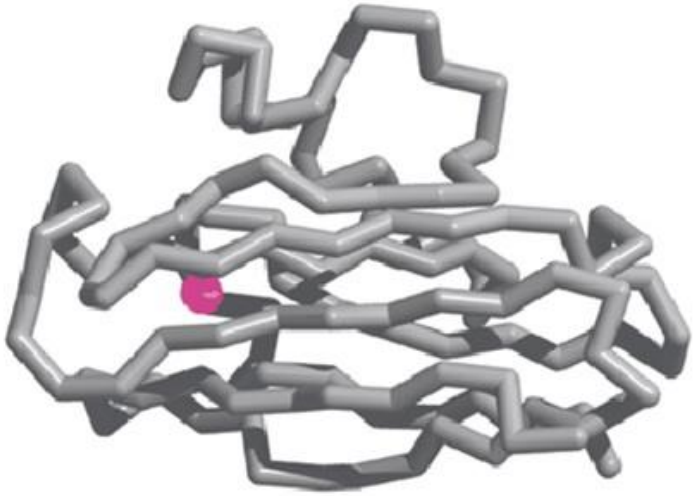


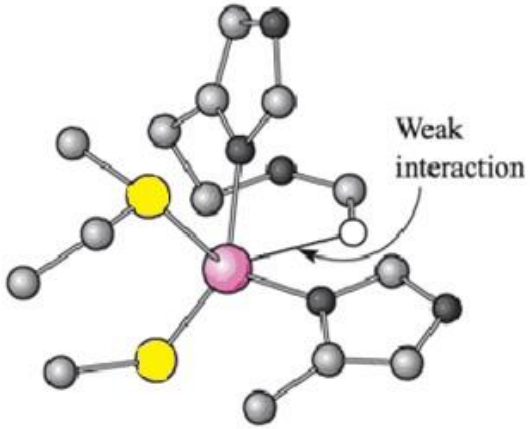


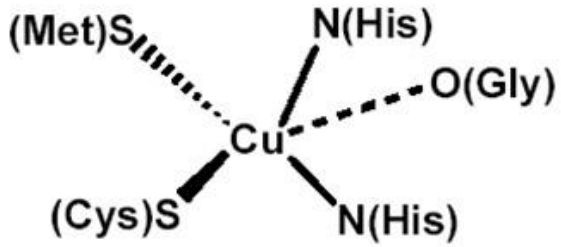
imidazole

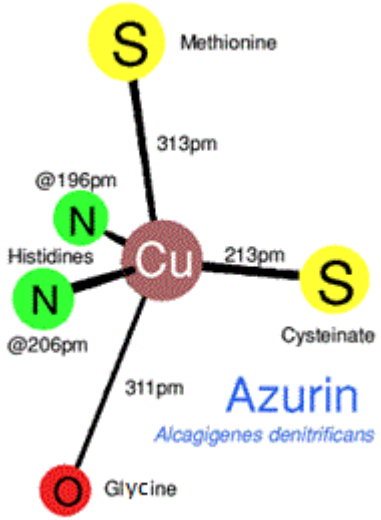


The Blue Copper Protein – Azurin (bacterium)

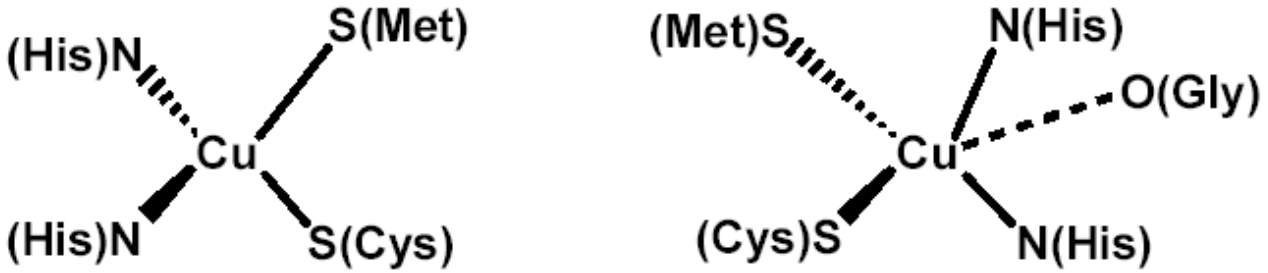








The Blue Copper Proteins – Plastocyanin & Azurin



- Tetrahedral Cu(II) centres with Cu-S(Met) > Cu-S(Cys)
- Cu-L distances lengthen ~ .05 - .10 Å when reduced to Cu(I) BUT coordination geometry remains the same!!!
- Thus coordination sphere is suitable for Cu(I) and Cu(II).
- This facilitates VERY rapid electron transfer as no significant atomic rearrangements are necessary.
- High reduction potentials of 370 (plastocyanin) and 308 mV (azurin) indicates probably more favourable for Cu(I).

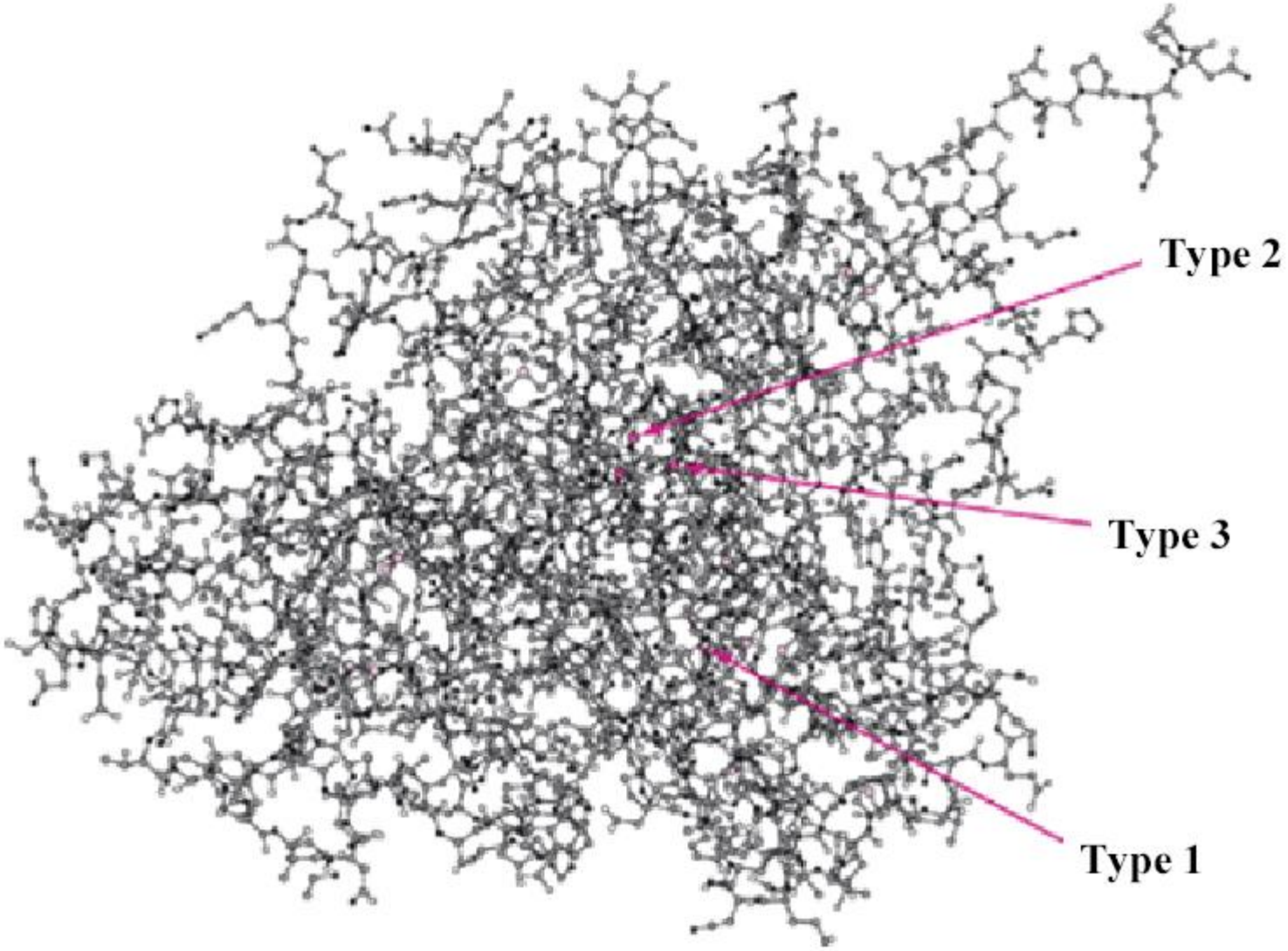
Blue Copper Proteins

- ☆ Multicopper blue copper proteins include *ascorbate oxidase* and *laccase*.

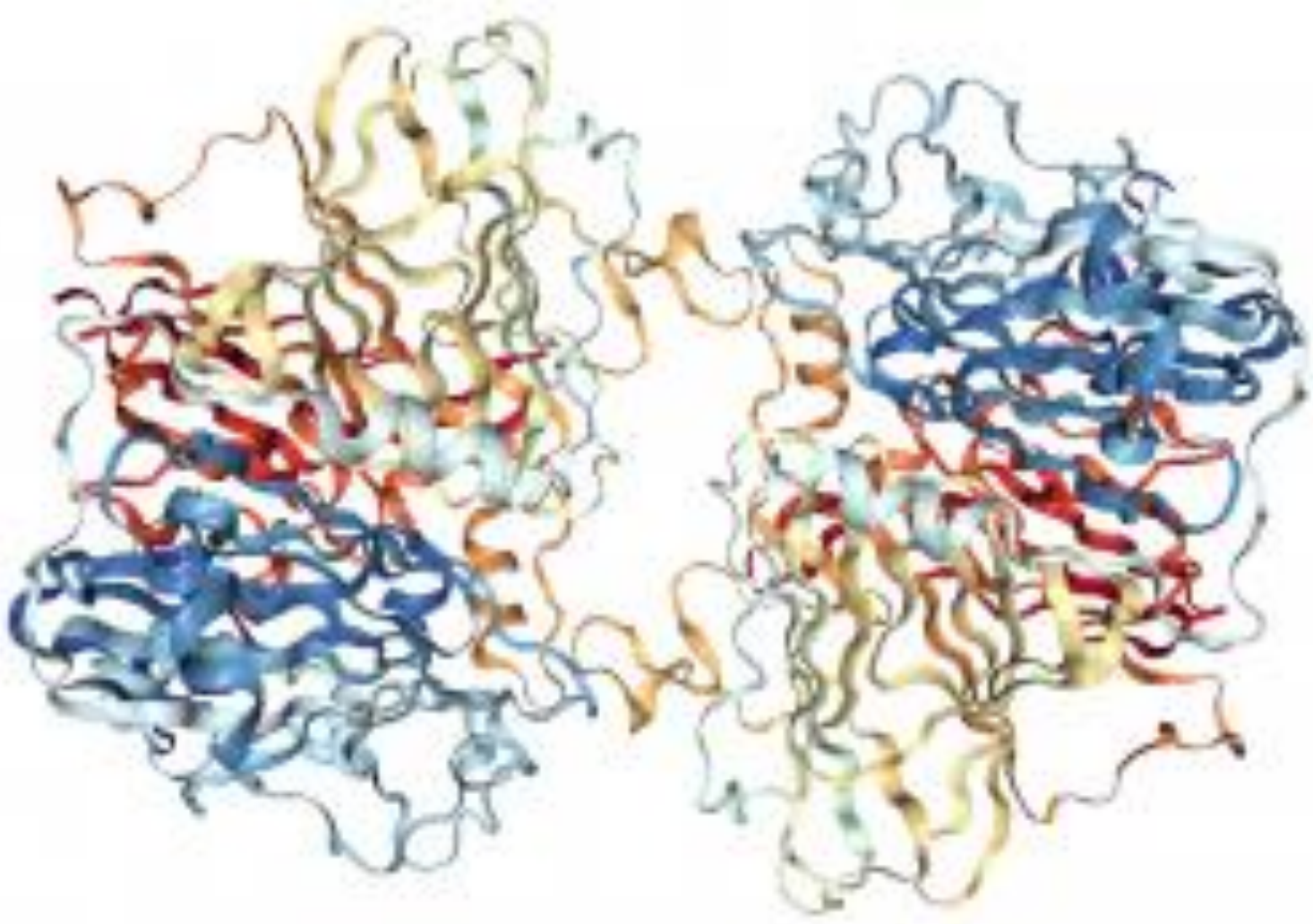
- ☆ These enzymes catalyze the reduction of O_2 to H_2O and the one electron oxidation of a substrate such as phenol.

- ☆ Spectroscopic data suggest the presence of all three types of copper sites.

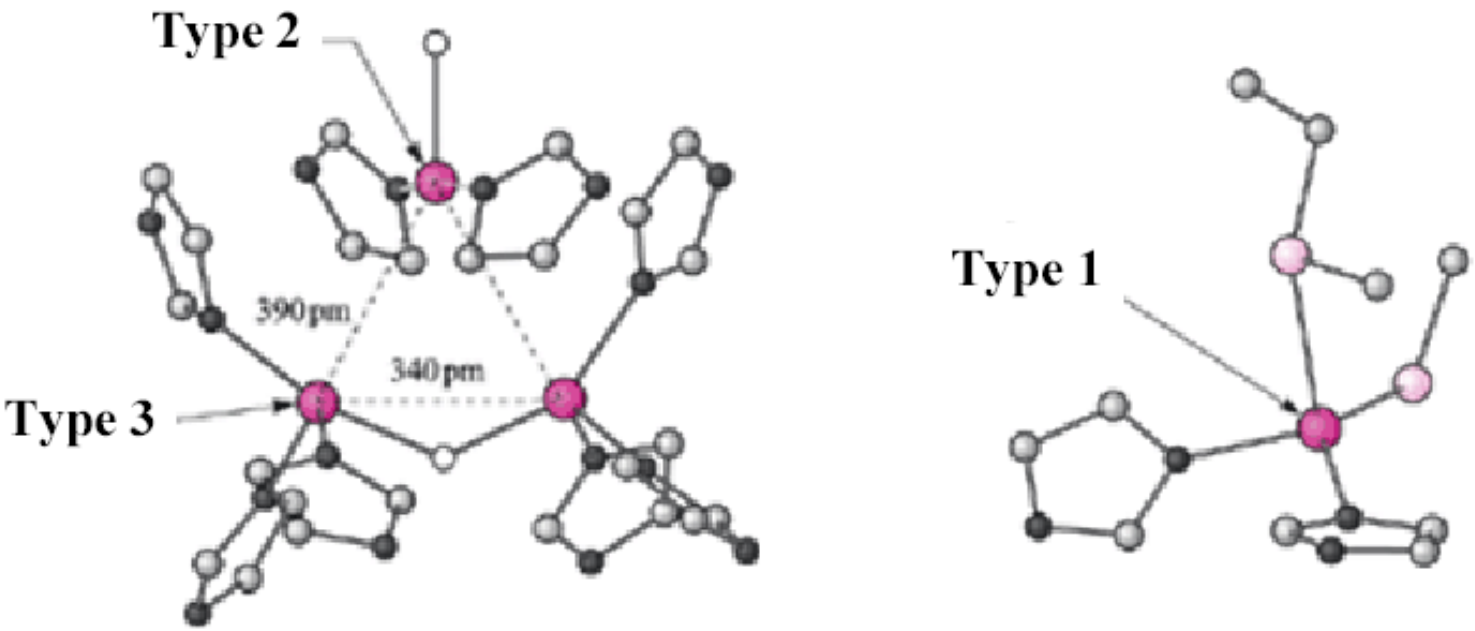
Ascorbate Oxidase



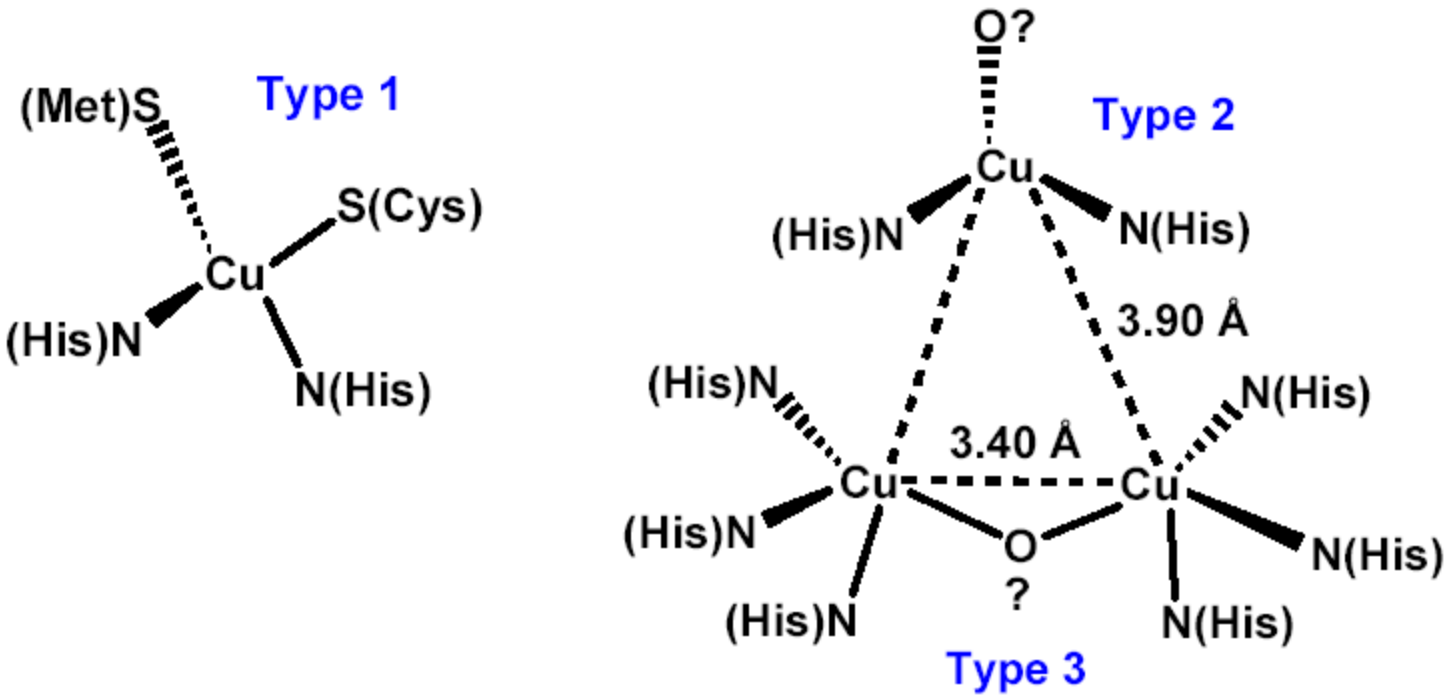
Ascorbate Oxidase



Ascorbate Oxidase



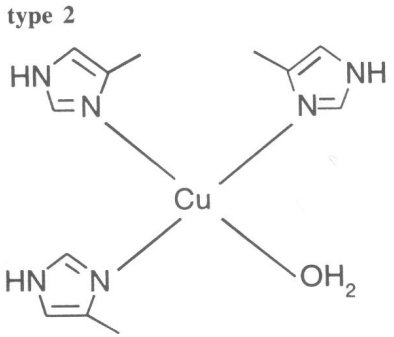
The Blue Copper Protein – Ascorbate Oxidase



- Reduction of O_2 occurs at a Type 2/Type 3 site. The remote (~ 12 Å) Type 1 site acts as the electron acceptor in the oxidation of the organic substrate.

"Non-blue" oxidase* $O_2 \longrightarrow H_2O_2$

Type 1 → O_2



Presence of one type 2 center

$$RR'CHOH + O_2 \xrightarrow{\text{galactose oxidase}} RR'C{=}O + H_2O_2$$

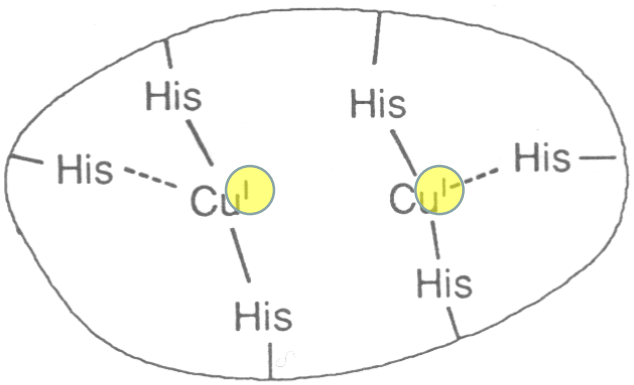
$$RCH_2NH_2 + O_2 + H_2O \xrightarrow{\text{amine oxidase}} RCHO + H_2O_2 + NH_3$$

* without type 1 center

Oxygen transport and oxygenation
“HEMOCYANIN”

Type 3 center

Type 2 center

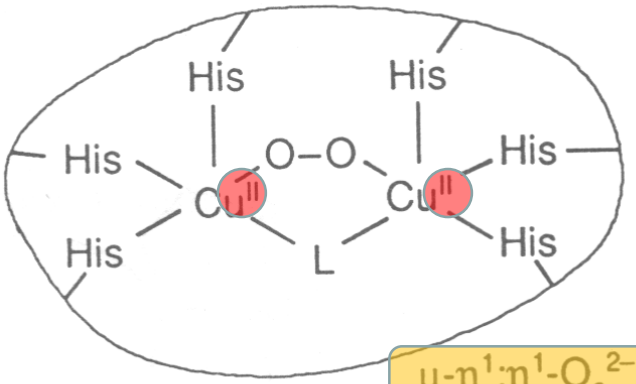


deoxyhemocyanin

$+ {}^3O_2$

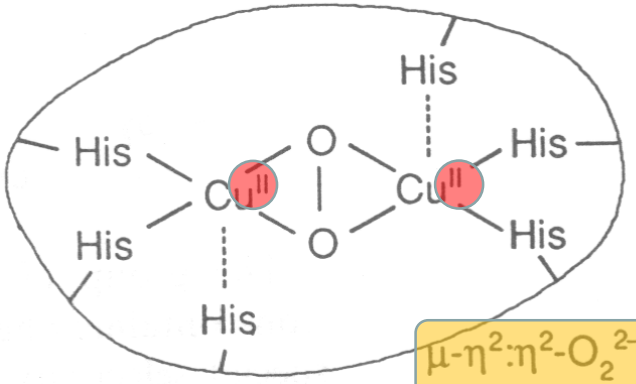
$\rightleftharpoons$

$- {}^3O_2$



$\mu\text{-}\eta^1\text{:}\eta^1\text{-}O_2^{2-}$

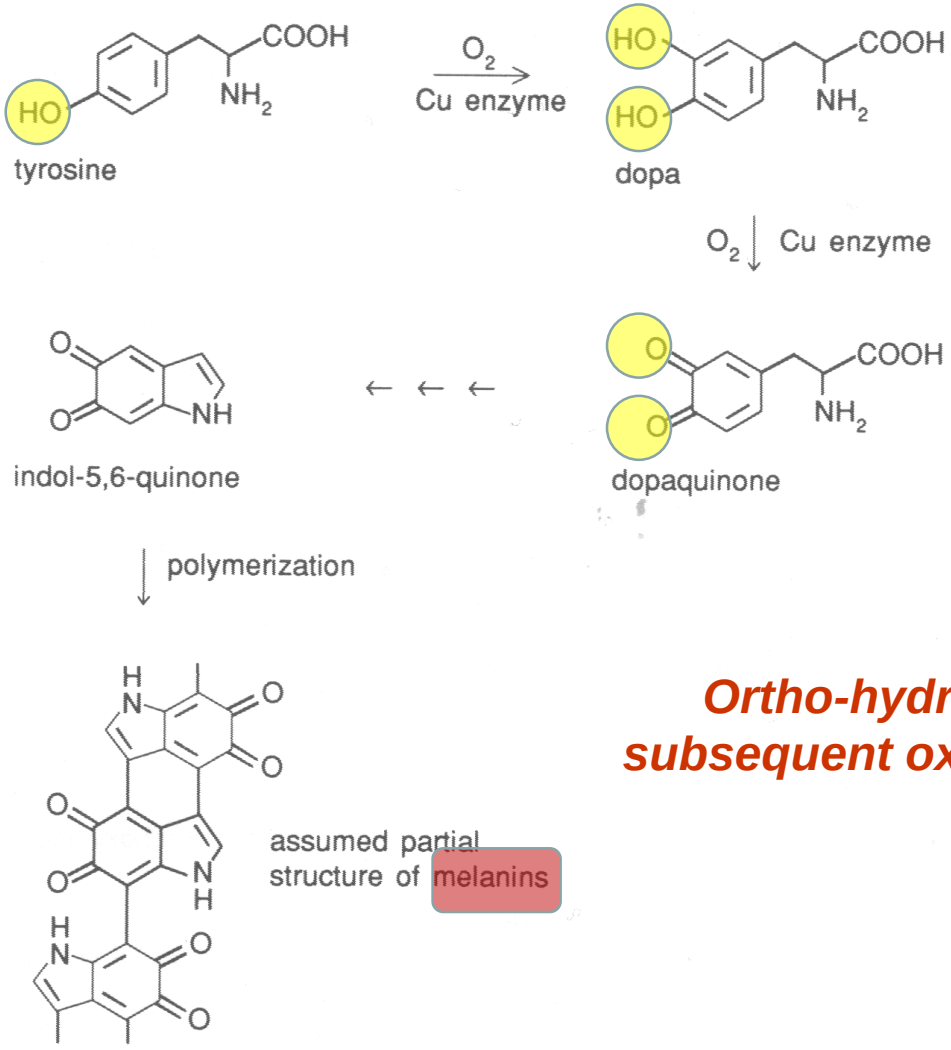
or



$\mu\text{-}\eta^2\text{:}\eta^2\text{-}O_2^{2-}$

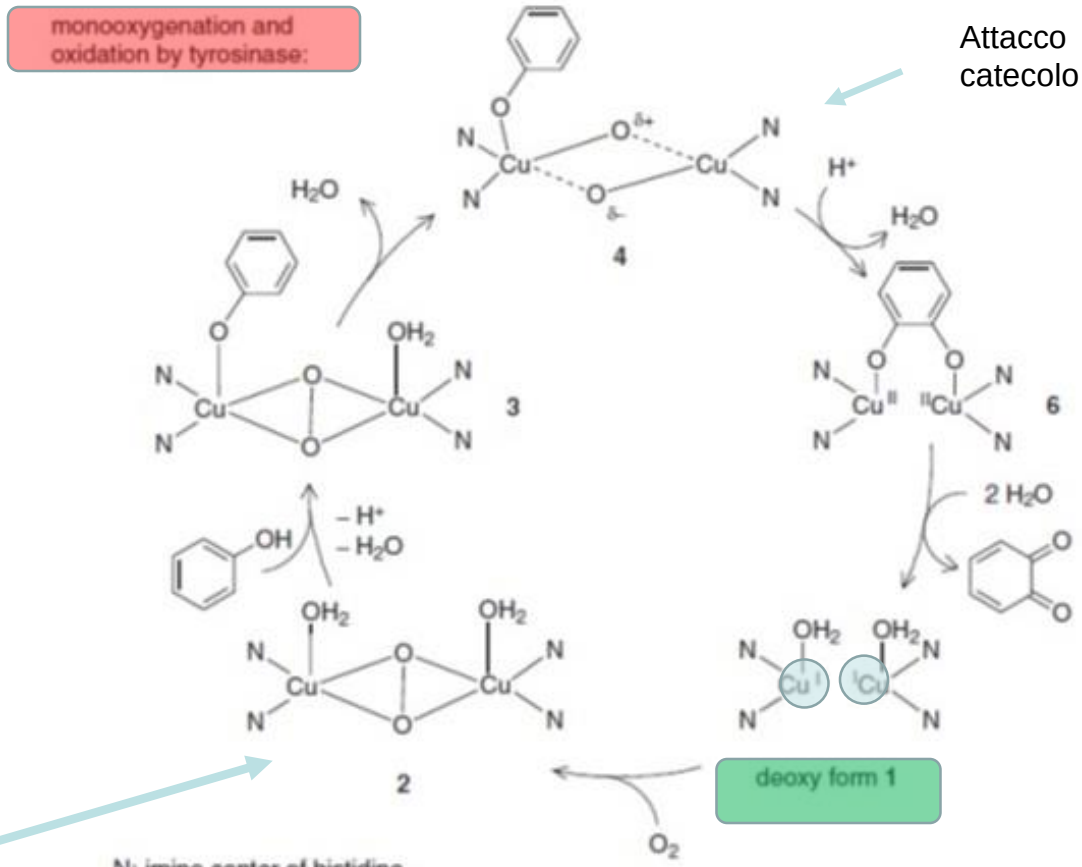
oxyhemocyanin

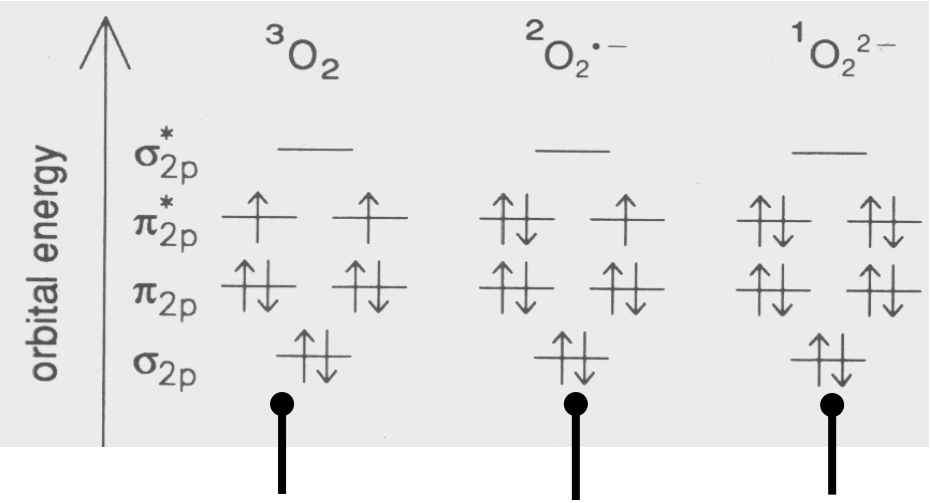
Monooxygenase (introduction of oxygen into substrates)
"TYROSINASE"



Ortho-hydroxylation of phenols and subsequent oxidation to ortho-quinones in skin, fruit etc.

TYROSINASE CYCLE




orbital energy
$^3O_2$
$^2O_2^{\bullet -}$
$^1O_2^{2-}$
σ^*_{2p}
π^*_{2p}
π_{2p}
σ_{2p}
molecular oxygen
superoxide
peroxide

catalase

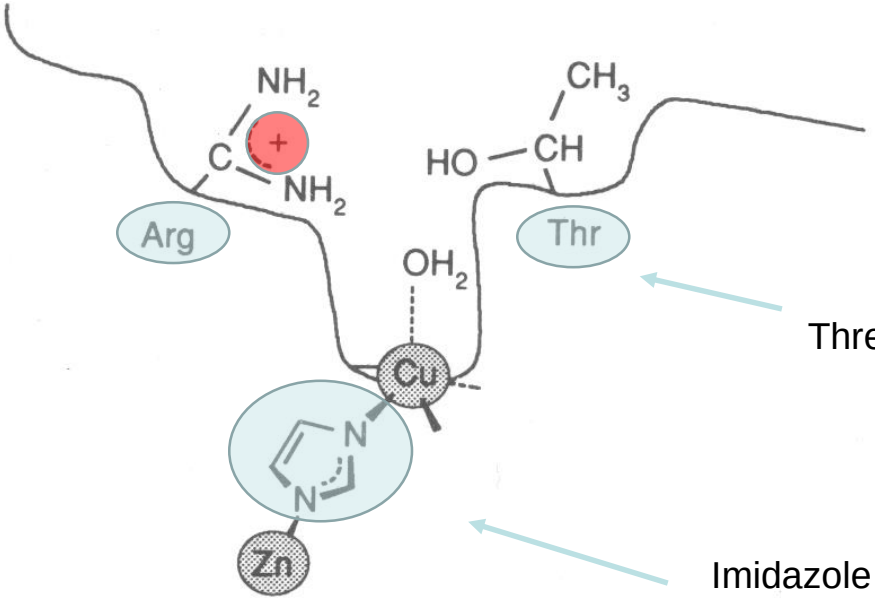
Antioxidant function
"SUPEROXIDE DISMUTASE (SOD)"

overall reaction:

(-0.5) (-I) (0) (oxygen oxidation states)

$2\ O_2^{\bullet -} + 2\ H^+ \xrightarrow{SOD} H_2O_2 + O_2$

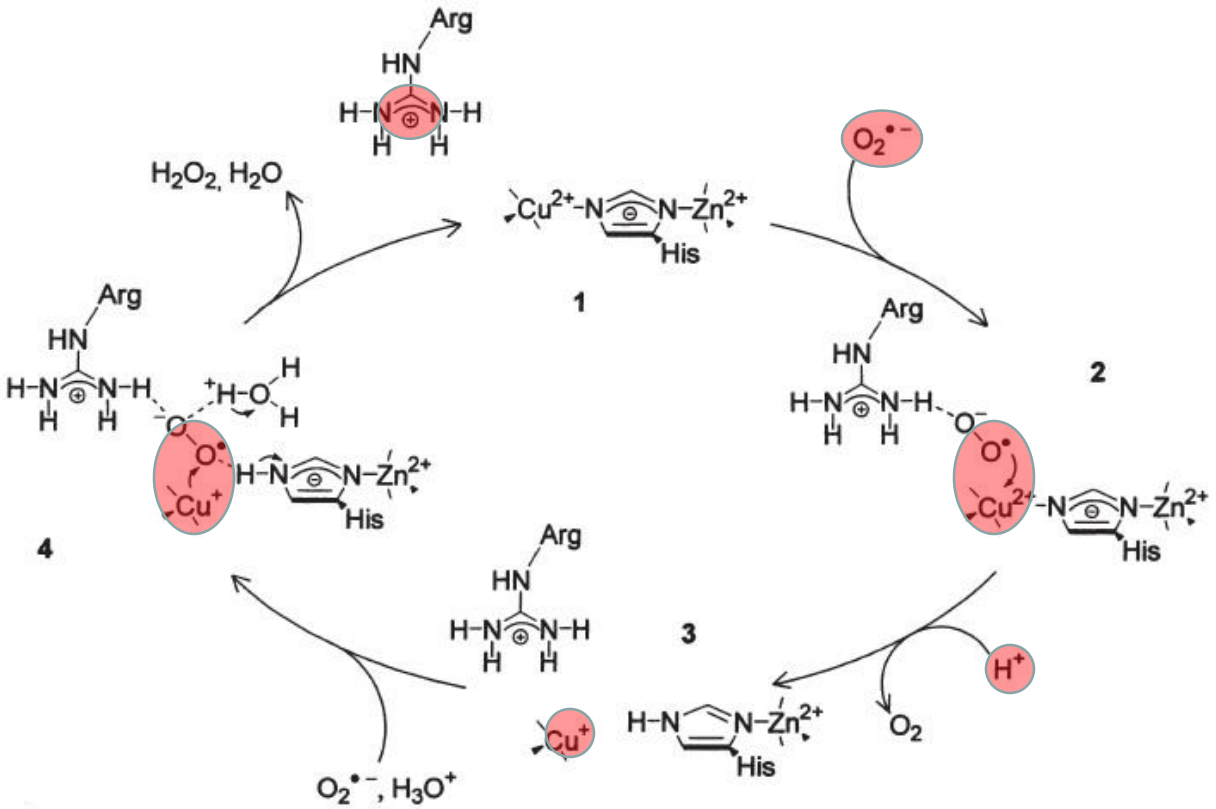
The cavity of $O_2^{\cdot -}$ conversion in SOD

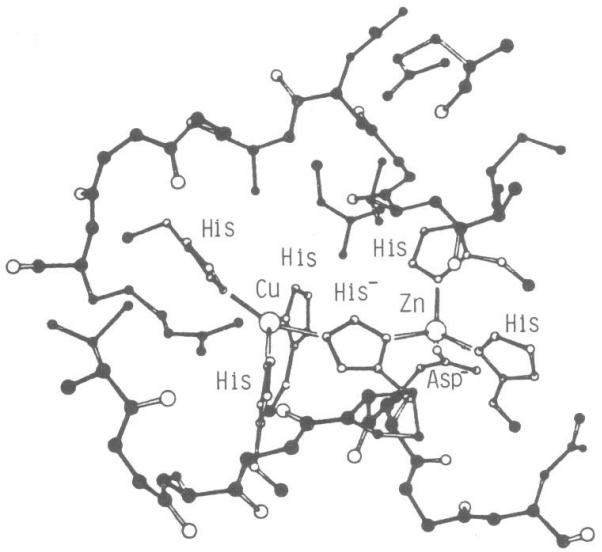


Threonine

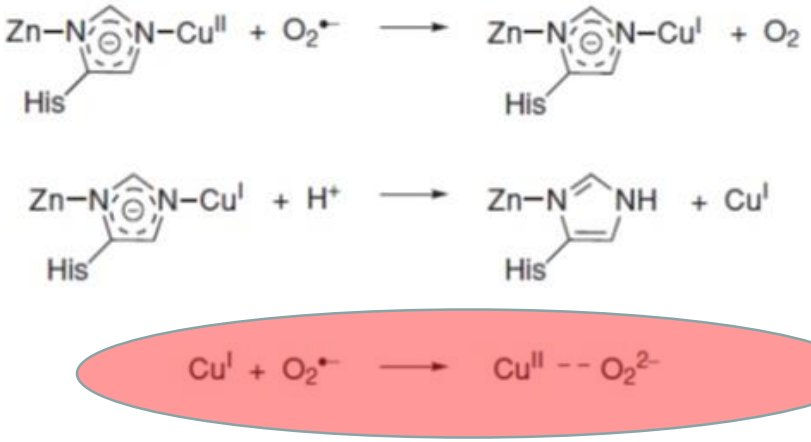
Imidazole → imidazolate

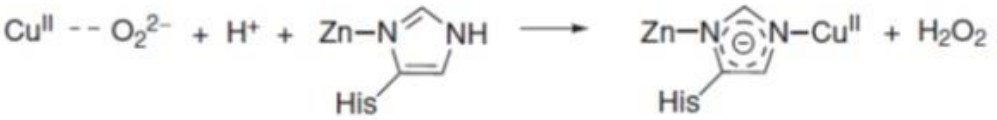
SOD CYCLE


Arg
HN
H–N
N–H
H
H
H_2O_2, H_2O
Cu^{2+}–N
N–Zn^{2+}
His
1
$O_2^{\bullet -}$
2
H–O
H
O
Cu^+
H–N
N–Zn^{2+}
His
4
3
$O_2^{\bullet -}$, H_3O^+
Cu^+
H–N
N–Zn^{2+}
His
H^+
O_2



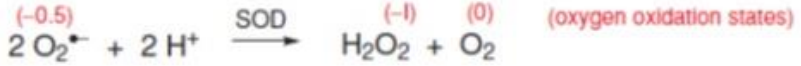
hypothetical mechanism:





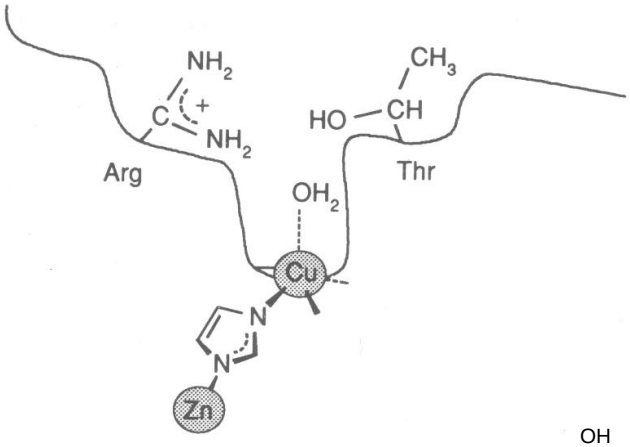
Zn: three-coordinate Zn^{II} center (2 His, 1 Asp^-)
Cu: three-coordinate copper center (3 His)

overall reaction:

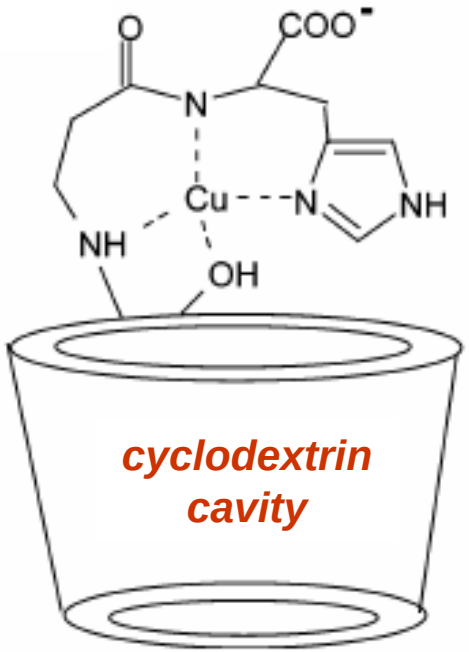


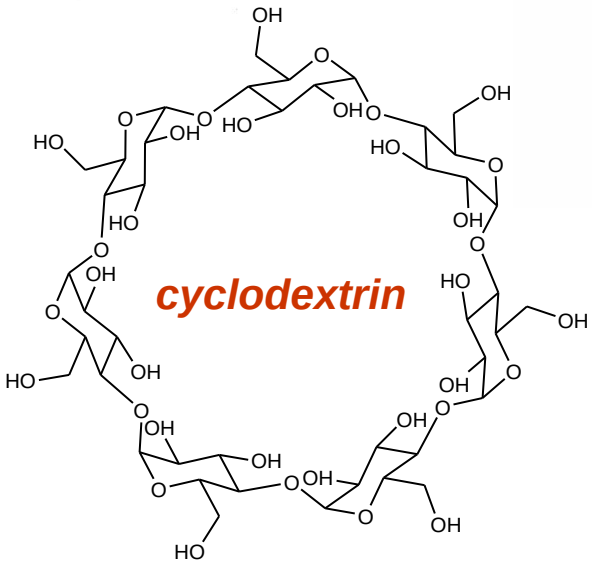
SOD-like model systems

Native Enzime

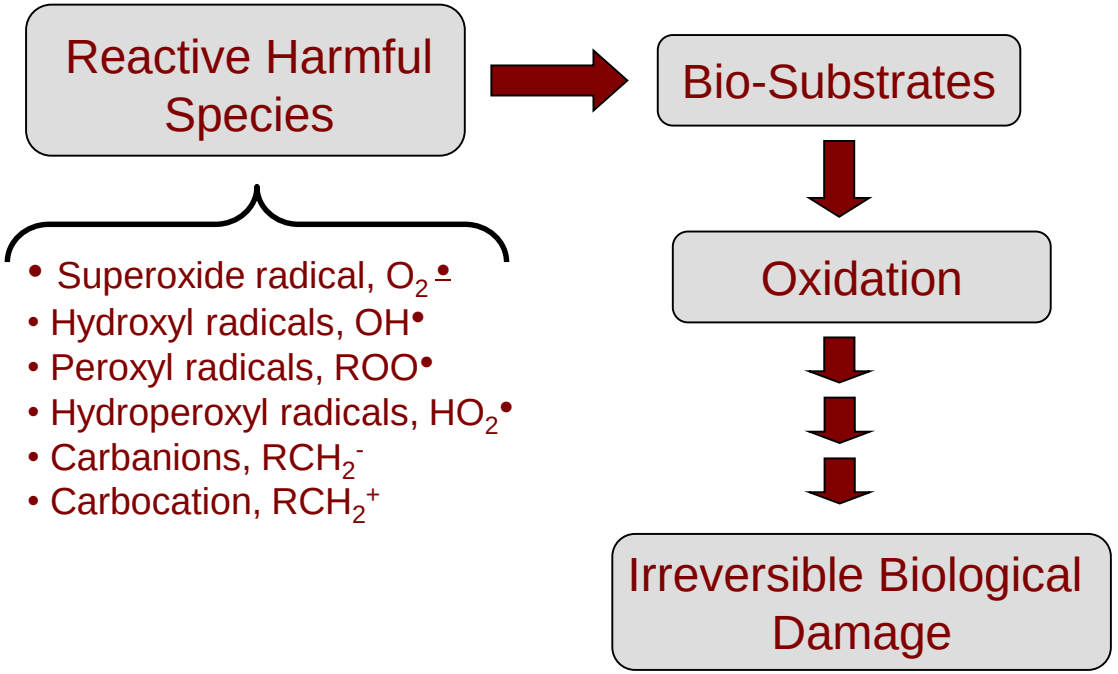


Artificial Enzime





Antioxidant Systems Based on Copper(II)



Copper (II) and its Complexes with Bio-functional Ligands as Antioxidant Systems

- ***bio-compatibility***
- ***suitable redox potentials***

A little bit more on the antioxidant activity of copper

The chain of lipid peroxidation

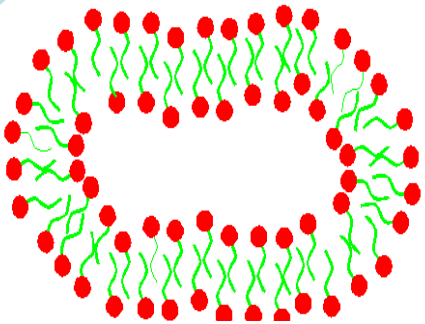
initiation $ROO^{\bullet} + LH \longrightarrow ROOH + L^{\bullet}$

propagation
- $L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$
- $LOO^{\bullet} + LH \longrightarrow LOOH + L^{\bullet}$

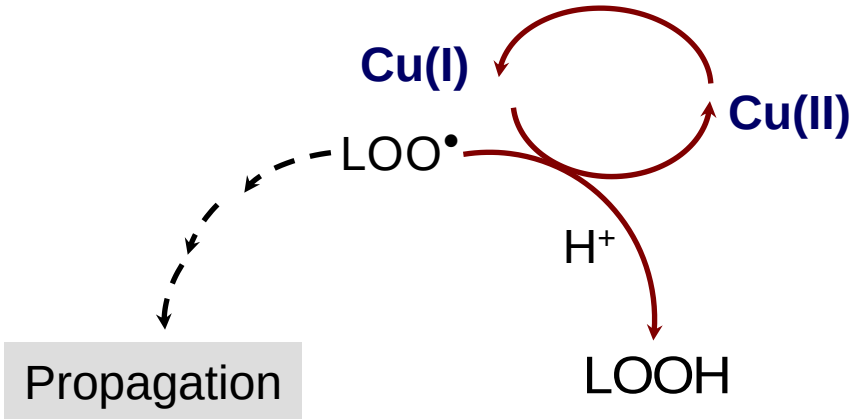
termination $LOO^{\bullet} + LOO^{\bullet} \longrightarrow$ non-radical products

Lipid radical

Lipid peroxyl radical
$ROO^{\bullet}$



Lipids (LH)



New termination route *via* redox quenching of "lipoperoxyl-radicals"