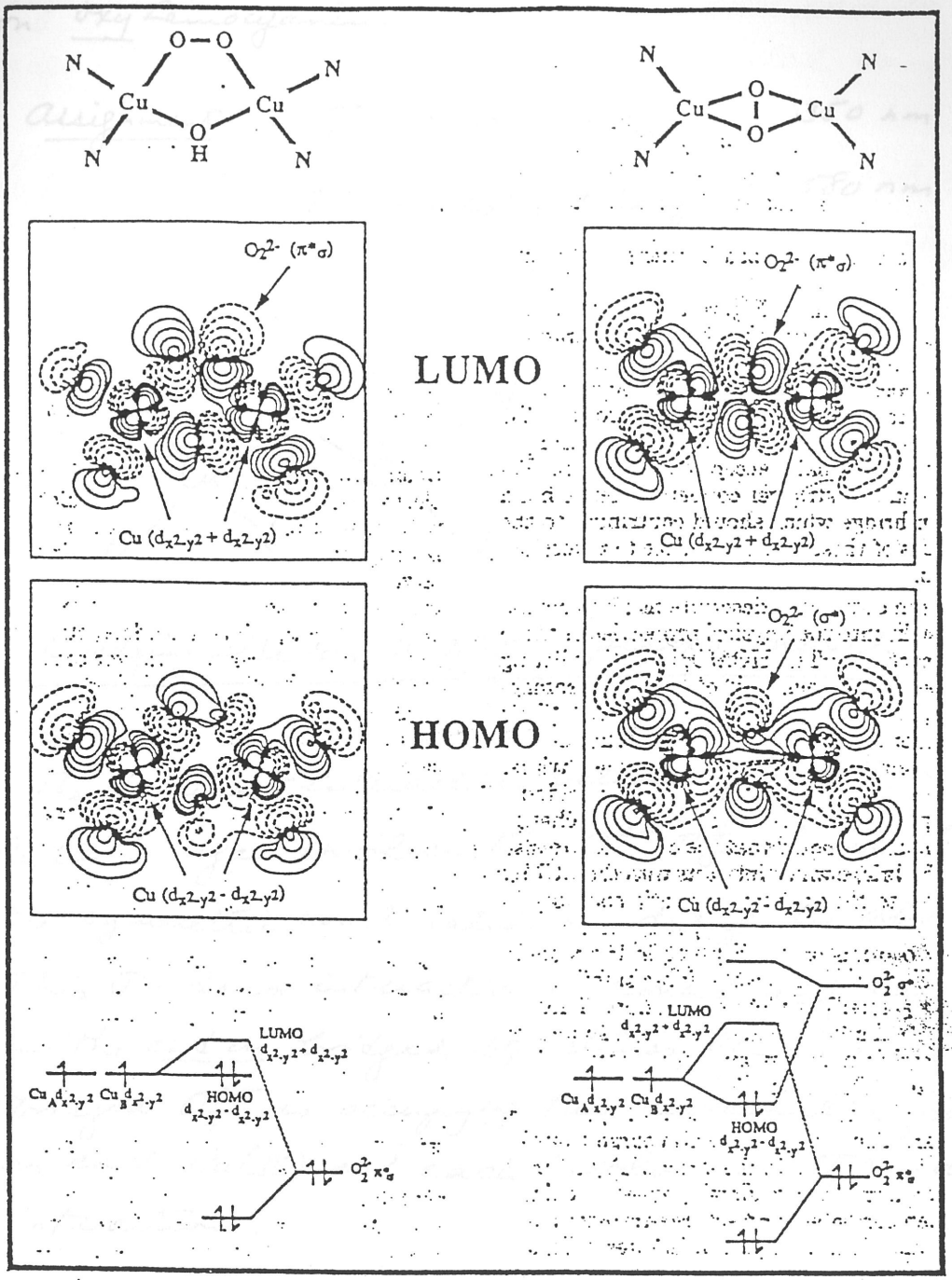


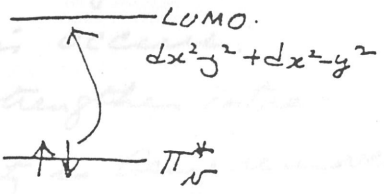
. Geometric and electronic structures for end-on cis-μ-1,2 (C_{2v}) and side-on μ-η^2:η^2 (D_{2h}) models of the oxyhemocyanin active site: top, geometric structures; center, contours of the HOMO and LUMO orbitals of each from broken-symmetry SCF-Xα-SW calculations; bottom, energy-level diagrams showing dominant orbital contributions. (Cu_A and Cu_B refer to each of the two coppers in the dimer.)

cis-μ-1,2 model

CT transitions: a) O_2^{2-} π^*_σ $\longrightarrow$ $d_{x^2-y^2} + d_{x^2-y^2}$ LUMO

b) O_2^{2-} π^*_ν $\longrightarrow$ $d_{x^2-y^2} + d_{x^2-y^2}$ LUMO

(a)



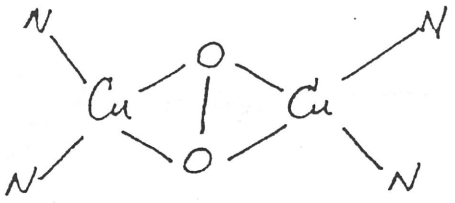
(b)

weaker & to lower energy

Cu-μ-1,2 model seems consistent with uv-visible data on oxyhemocyanin.

Assignment		
$\pi_\sigma^* \longrightarrow d_{x^2-y^2} + d_{x^2-y^2}$	350 nm
$\pi_\nu^* \longrightarrow d_{x^2-y^2} + d_{x^2-y^2}$	580 nm

(3)



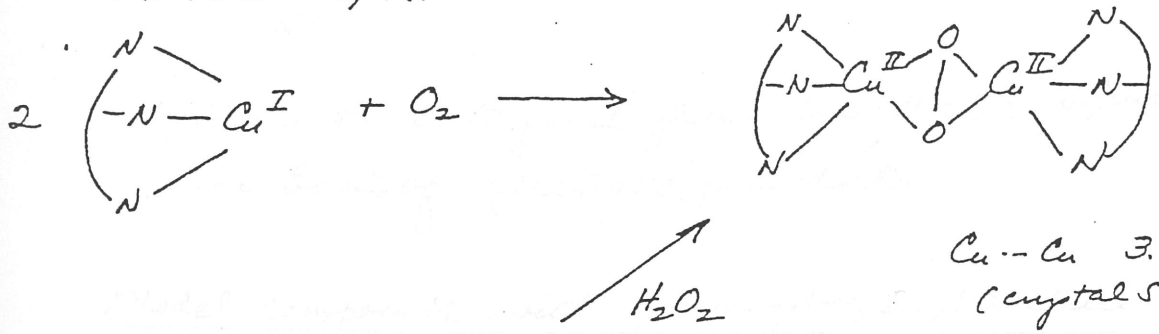
Bridging side-on μ-η^2:η^2 peroxo model

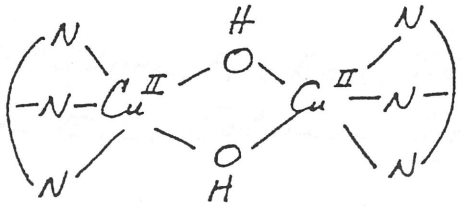
(a) The dominant interaction between peroxide and Cu(II)'s again involves the O_2^{2-} π_σ^*-level destabilizing the symmetric combination of $d_{x^2-y^2}$ Cu orbitals. This σ-donor interaction is considerably larger than in the end on bridged structures, because the side on bridged O_2^{2-} is occupying two coordination positions on each Cu(II) and each involves a π_σ^*-donor interaction.

(b) In this case, the HOMO is also stabilized through a bonding interaction with the high-energy σ^* orbital of the peroxide, which is empty. Thus, the peroxide is acting as a π-acceptor ligand. This interaction is probably important toward weakening the O-O bond for bond cleavage, should this occur. In contrast, π_σ^* - Cu($d_{x^2-y^2}$) interactions strengthen intra-peroxide O-O bond, since electron density is being removed from π_σ^* (antibonding) orbital.

Kitajima has structurally defined the first side-on peroxide bridge (μ-η^2:η^2) in a transition metal complex.

N. Kitajima, K. Fujisawa, Y. Moro-oka and K. Toriumi JACS (1989) 111, 8975-8976; N. Kitajima, K. Fujisawa, C. Fujimoto, Y. Moro-oka, S. Hashimoto, T. Kitagawa, K. Toriumi, K. Tatsumi, A. Nakamura. JACS (1992) 114, 1277-12





Cu--Cu 3.560 Å (crystal structure)

Absorption spectrum

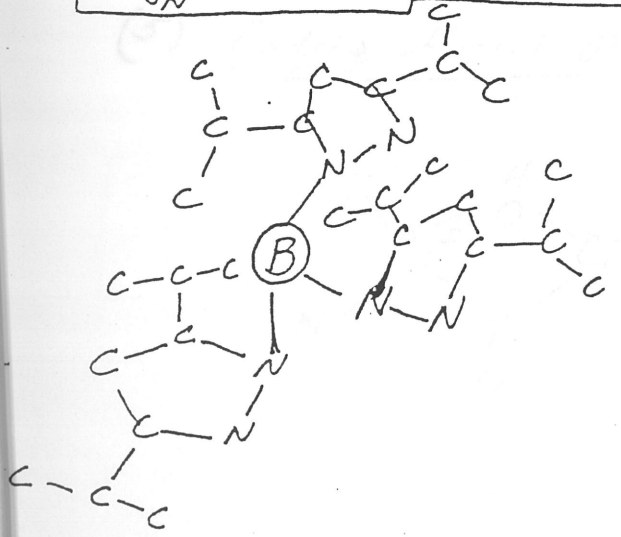
λ_{max} 349 nm (ϵ = 2100
551 nm (800)

Resonance Raman

$^{16}O-^{16}O$ 741 cm^{-1}

(JACS (1992) 114, 1277-1291)

$\left(\text{N}\right)_3 \equiv HB(3,5\text{-}iPr_2pz)_3$



Oxyhemocyanin

λ_{max} 350 nm (ϵ ~ 20,000
580 nm (ϵ ~ 1000)

$^{16}O-^{16}O$ stretch ~ 750 cm^{-1} (v. low)

stretch (intraperoxide stretch)

a) monomer 808 cm^{-1} (2.9 mdynes/Å

b) trans end-on dimer 830 cm^{-1} (3.1 mdynes/Å

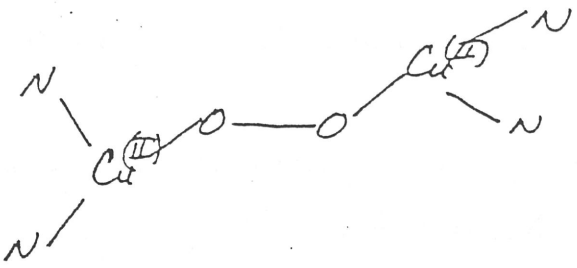
c) side-on bridged dimer 750 cm^{-1} (2.5 mdynes/

monomer: J.E. Pate, R. Cruse, K.D. Karlin, & E.I. Solomon JACS 109, 2624-2630 (1987)

trans-μ-1,2 Dimer, M.J. Baldwin et al. JACS (1991) 113, 8671-8679.

Other possibilities (but less likely)

(4) <u>bridging trans-μ-1,2 peroxy model</u>



difficult to distinguish from cis-μ-1,2 structure on the basis of spectroscopic data

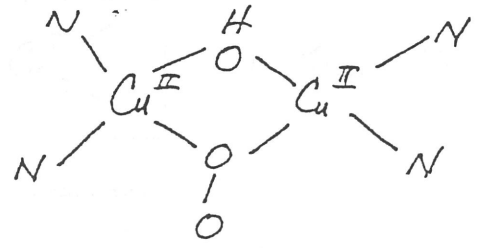
<u>Model compounds with trans-μ-1,2 structure</u>

M.J. Baldwin, P.K. Ross, J.E. Pate, Z. Tyeklár, K.D. Karlin and E.I. Solomon (1991) <u>113</u>, 8671-8679

$[\{Cu(TMPA)\}_2 O_2]^{2+}$ TMPA = tris(2-methylpyridyl)-amine

ν ($^{16}O-^{16}O$ stretch) 832 cm^{-1}

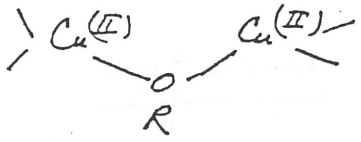
(5) <u>bridging μ-1,1 peroxy model</u>



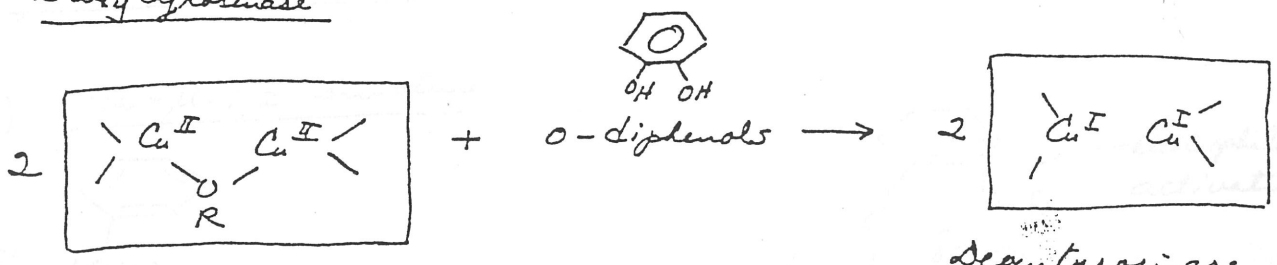
Tyrosinase

As in hemocyanin, tyrosinase (Neurospora crassa) contains a type 3 Cu center (i.e., binuclear copper cluster).

Met tyrosinase is the resting form of the enzyme. In this form of the enzyme, the two copper(II) ions are antiferromagnetically coupled, implicating a bridging ligand.



Deoxy tyrosinase

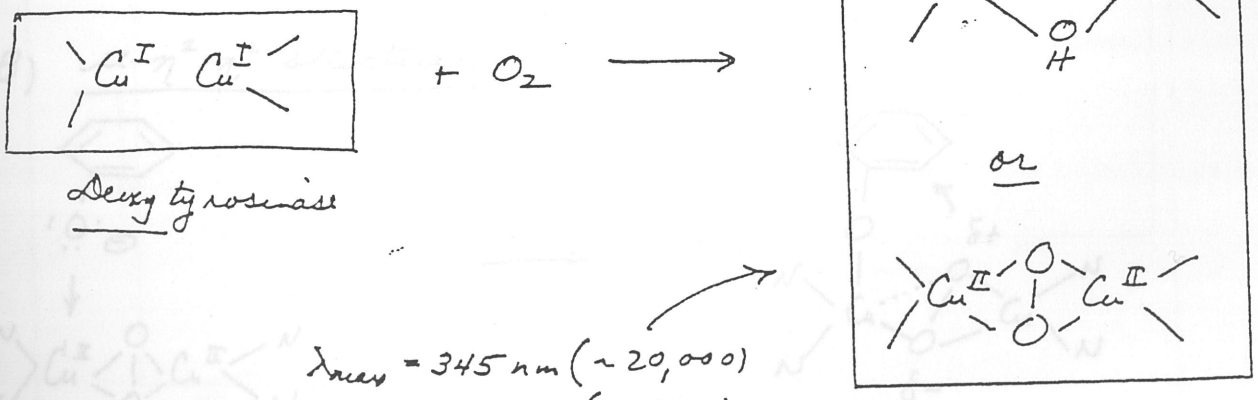


Met tyrosinase

Deoxy tyrosinase

+ o-quinones

Oxy tyrosinase

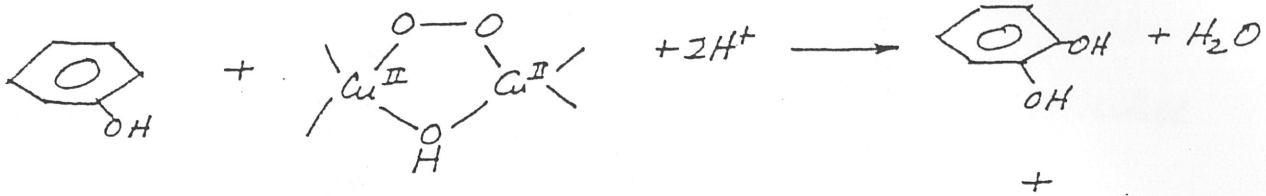


λ_{max} = 345 nm (~20,000)
590 nm (~1,000)
520 (CD band)

Oxy tyrosinase

Oxytyrosinase hydroxylates monophenols to o-diphenols.

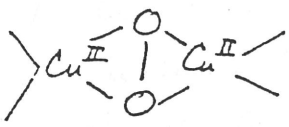
RX



+

Met tyrosinase

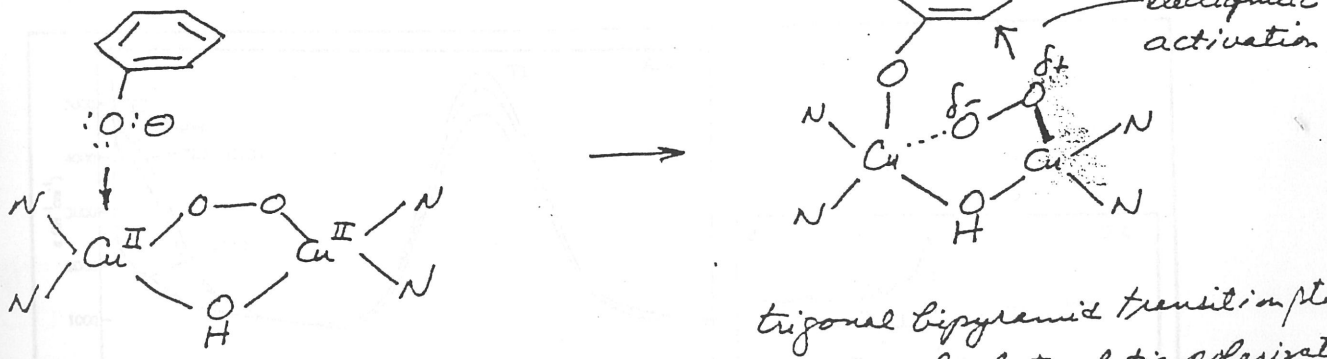
or



Oxytyrosinase

Proposed molecular mechanism

(A) cis-μ-1,2 structure



trigonal bipyramidal transition state followed by heterolytic polarization and cleavage of O—O bond

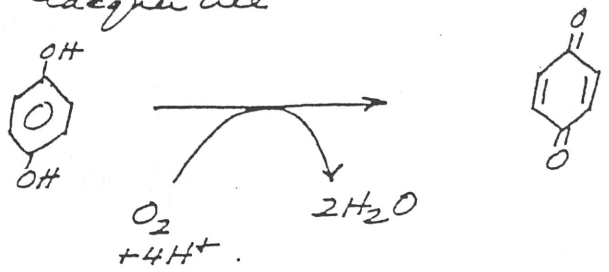
(B) μ-η^2:η^2 structure

Multi copper oxidases

(1) Fungal laccase (*Polyspora versicolor*)

- Tree laccase (*Rhus vernicifera*)
  lacquer tree

(a) RX:

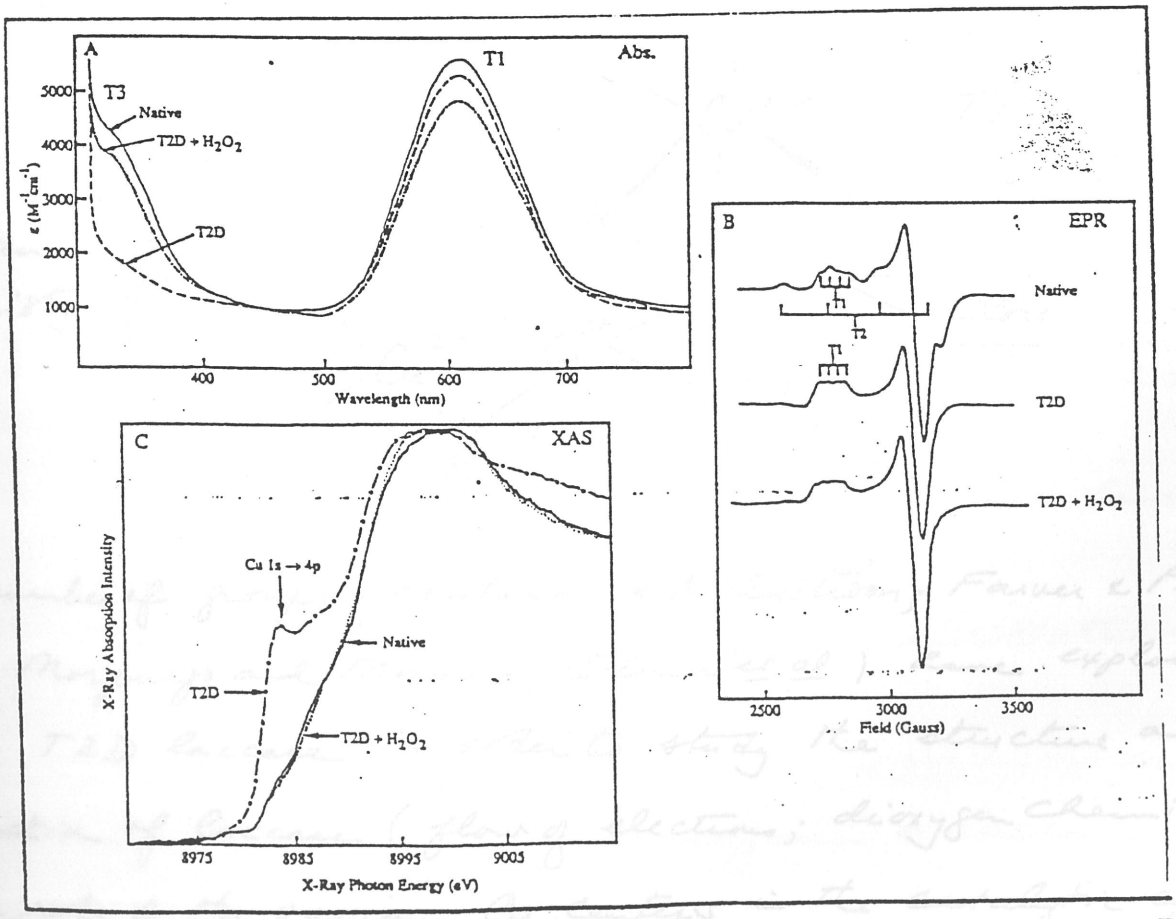


(b) one type 1 Cu center (T1)
one type 2 Cu center (T2)
one type 3 Cu center (T3)
} a total of four copper

type 1 Cu can be substituted by Hg^{2+} ⟹ T1 Hg laccase
(also Co)

type 2 Cu can be depleted ⟹ T2D laccase

(c) Uv-visible, EPR and X-ray absorption spectra

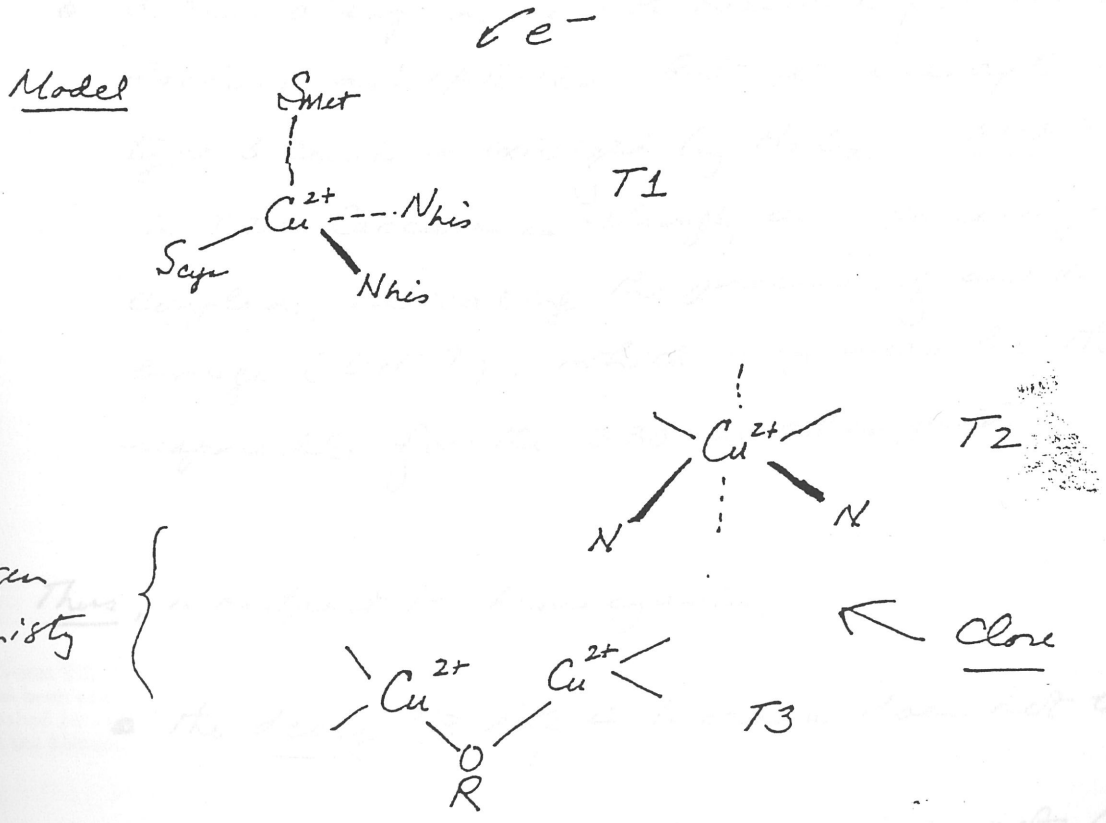


... Spectroscopic comparison of native laccase, T2D laccase, and T2D reacted with excess H_2O_2: (A) absorption, (B) EPR, and (C) X-ray absorption spectra.

- Type 1 center is a fairly typical blue copper site exhibiting an intense cysteine $\pi - Cu^{(II)}$ charge transfer transition at 600 nm ($\epsilon \sim 5000\ M^{-1}cm^{-1}$) and an EPR signal with a small A_{11} value.

- Type 2 center exhibits a normal EPR signal with large A_{11}. Binds ligands (F^-; N_3^-)

- Type 3 center is an antiferromagnetically coupled EPR-silent binuclear copper center (both coppers are d^9!) Site exhibits absorption at 330 nm ($\epsilon \sim 2700\ M^{-1}cm^{-1}$)



A number of groups (Reinhammer & Malmström; Farver & Pecht; L. Morpurgo and Mondovi; Solomon et al) have exploited the T2D laccase in order to study the structure and function of laccase (flow of electrons; dioxygen chemistry and role of the various Cu centers in the catalytic cycle etc)

T2D laccase

- type 3 center is reduced, i.e., in the deoxy $Cu^{(I)}$–$Cu^{(I)}$ state, even in the presence of O_2
- does not react with O_2
- type 3 center is oxidized by H_2O_2 to give met T3 site, but there is no evidence that H_2O_2 binds to the met form to give an oxy T3 derivative
- 330 nm absorption is not observed for the T2D laccase as isolated, but returns after the type 3 center is oxidized by H_2O_2. Met T3 site in T2D laccase is strongly antiferromagnetically coupled, indicating the presence of an endogenous bridge (OH^-?), which may also be the ligand responsible for the 330 nm absorption

Thus, in contrast to hemocyanin,

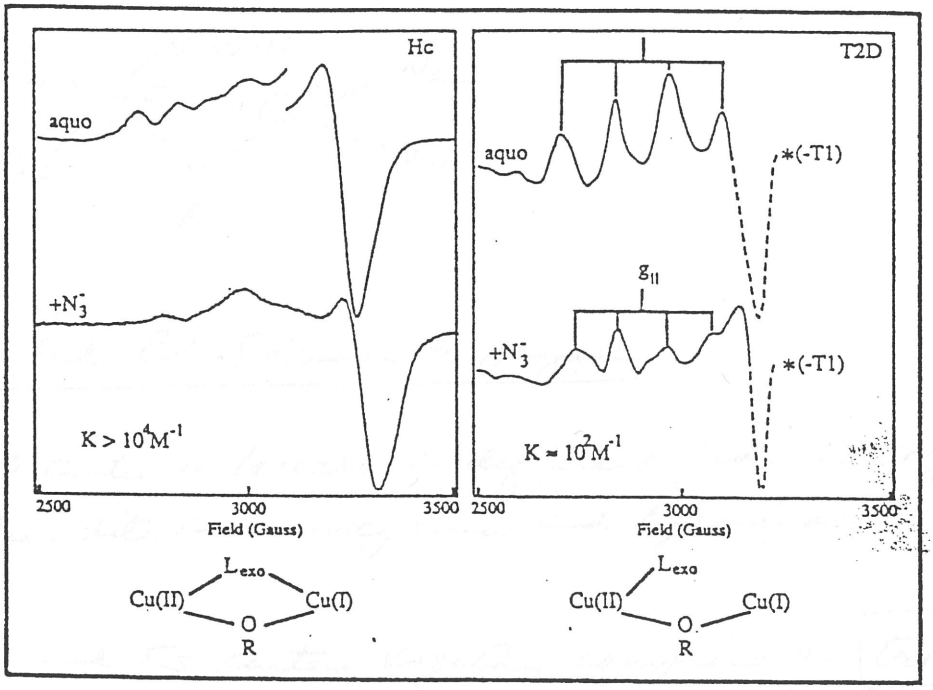
- the deoxy T3 site in laccase does not bind O_2
- the met T3 site in laccase does not bind peroxide
- there is no 330 nm absorption associated with met T3 site.

Conclusion T3 sites of hemocyanin & laccase are different!

Other evidence

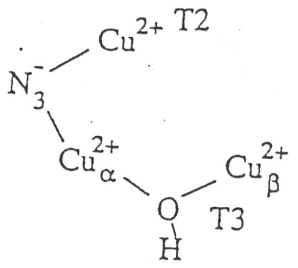
(1) D. Spira-Solomon and E. I. Solomon compared the binding of N_3^- to Half-met hemocyanin and Half-met T2D laccase and noted differences in the binding of this exogenous ligand between the two proteins.

Half-met Cu(II) Cu(I) derivative

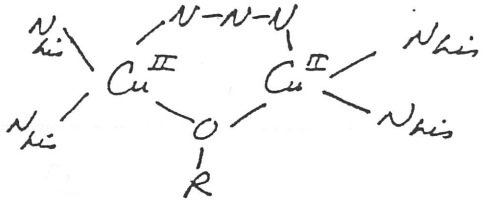


Exogenous ligand binding modes for Hc and T2D laccase. EPR spectra of (left) $^1/_2$-met hemocyanin (Hc) and (right) $^1/_2$-met T2D laccase without (top) and with (bottom) azide bound. The contribution from the type 1 copper of $^1/_2$-met T2D laccase has been eliminated by subtraction of the met T2D spectrum. The $g_\perp$ region is particularly sensitive to this subtraction; thus, the dashed part of the T2D spectrum should be viewed as approximate. Exogenous ligand binding models for each active site are given at the bottom.

(2) Solomon & Students (PNAS, U.S.A. (1985) 82, 3063-3067; JACS (1986) 108, 5318-5328) have also shown on the basis of MCD, Absorption, and EPR experiments that N_3^- binds to native laccase by bridging between T2 and one of the T3 copper.



unlike, in the case of hemocyanin, where it is known that the azide bridges between the two coppers of the binuclear center



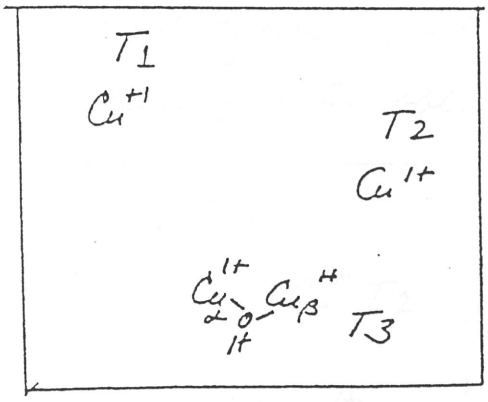
All this has led Ed Solomon to propose

- The T3 center in laccase is different from the coupled binuclear site in hemocyanin and tyrosinase

- The T2 and T3 centers together comprise a **trinuclear** copper cluster, and this trinuclear copper cluster is the minimum structural unit required for O_2 reactivity (oxidase activity)

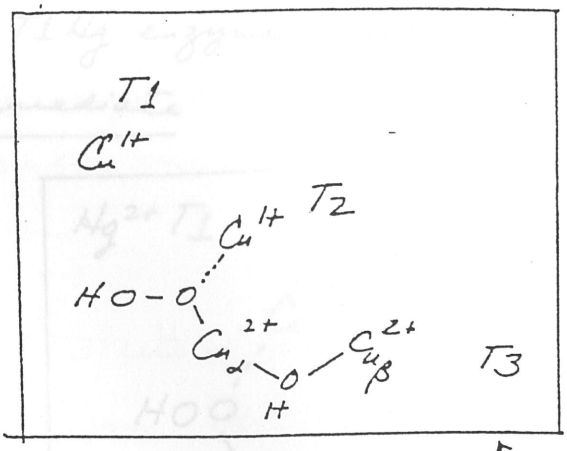
Proposed mechanism of O–O bond cleavage in laccase

Solomon has proposed the following mechanism of O–O bond cleavage in laccase.



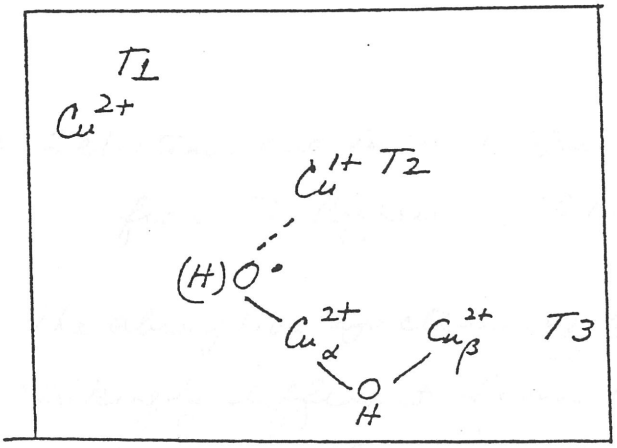
fully reduced laccase

O_2



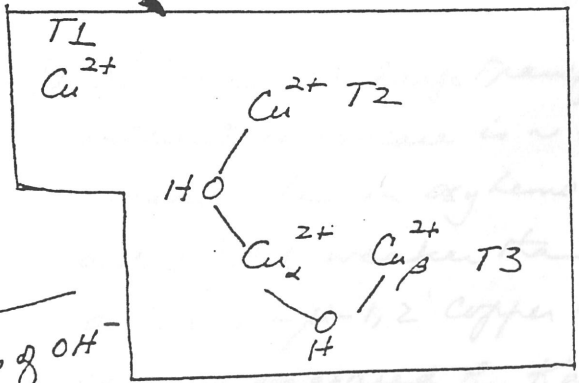
peroxo-intermediate

intramolecular electron transfer



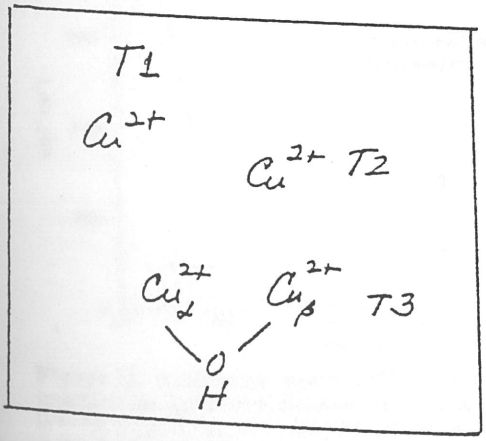
3-electron reduced oxygen radical

intramolecular electron transfer



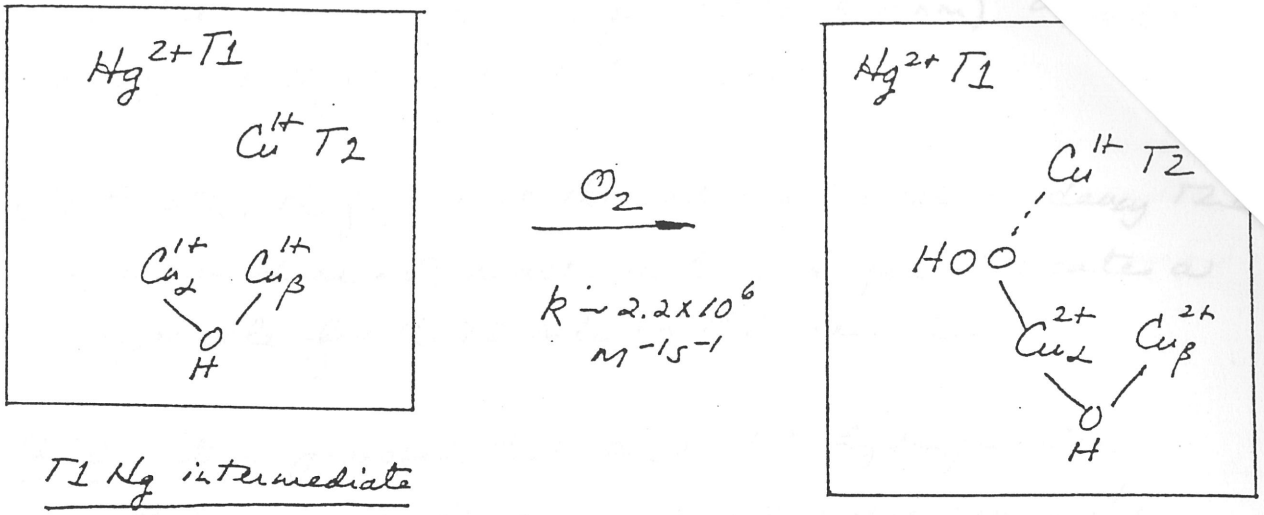
loss of OH^- bridge between T2 & T3 Coppers

fully oxidized enzyme



resting enzyme

To test hypothesis, Solomon has reacted T1 Hg enzym dioxygen to obtain peroxo-intermediate



T1 Hg intermediate

peroxo-intermediate

(a) 2 electrons are initially transferred to dioxygen from T3 Copper (CD/MCD/XAS)

(b) The absorption spectrum of peroxo intermediate is strikingly different from that of oxyhemocyanin, requiring a different mode of binding of peroxide to trinuclear copper cluster

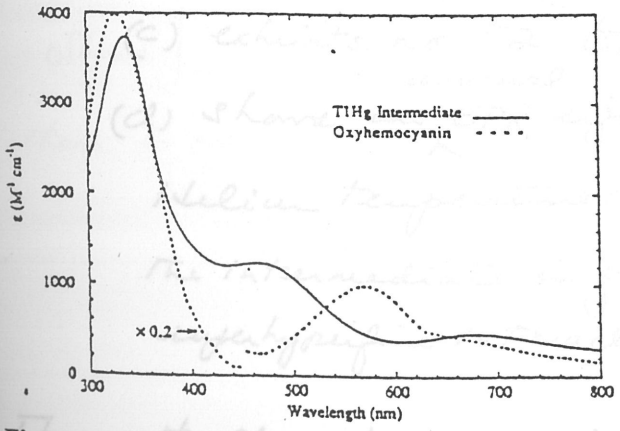


Figure 18. Absorption spectra of T1Hg laccase peroxide level intermediate (solid) and oxyhemocyanin (dashed). Note that the low-wavelength region of the oxyhemocyanin spectrum is scaled down 5-fold.

(i) $O_2^{2-} \rightarrow Cu(II)$ charge transfer intensity in laccase is ~ 5 fold weaker than in oxyhemocyanin and 3-fold weaker than in a trans-μ-1,2 copper model complex prepared by Kaden et al.

(ii) No $O_2^{2-} \rightarrow Cu(II)$ charge transfer band with $\lambda > 500$ nm.

Conclusion Not end on peroxo copper(II)