

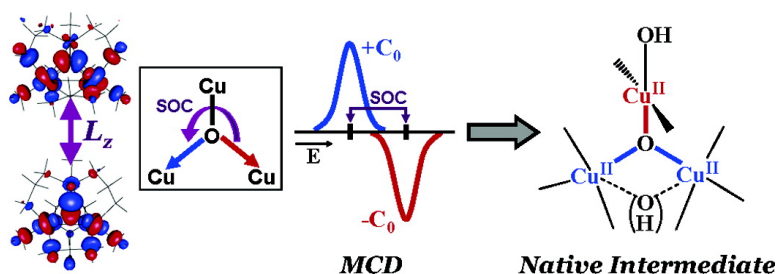
Article

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Variable-Temperature, Variable-Field Magnetic Circular Dichroism Studies of Tris-Hydroxy- and μ_3 -Oxo-Bridged Trinuclear Cu(II) Complexes: Evaluation of Proposed Structures of the Native Intermediate of the Multicopper Oxidases

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Abstract: Multicopper oxidases catalyze the $4e^-$ reduction of O_2 to H_2O . Reaction of the fully reduced enzyme with O_2 produces the native intermediate (NI) that consists of four oxidized Cu centers, three of which form a trinuclear cluster site, all bridged by the product of full O_2 reduction. The most characteristic feature of NI is the intense magnetic circular dichroism pseudo-A feature (a pair of temperature-dependent C-terms with opposite signs) associated with $O \rightarrow Cu(II)$ ligand-to-metal charge transfer (LMCT) that derives from the strong Cu–O bonds in the trinuclear site. In this study, the two most plausible Cu–O structures of the trinuclear site, the tris- μ_2 -hydroxy-bridged and the μ_3 -oxo-bridged structures, are evaluated through spectroscopic and electronic structure studies on relevant model complexes, TrisOH and μ_3O . It is found that the two components of a pseudo-A-term for TrisOH are associated with LMCT to the same Cu that are coupled by a metal-centered excited-state spin–orbit coupling (SOC), whereas for μ_3O they are associated with LMCT to different Cu centers that are coupled by oxo-centered excited state SOC. Based on this analysis of the two candidate models, only the μ_3 -oxo-bridged structure is consistent with the spectroscopic properties of NI. The Cu–O σ -bonds in the μ_3 -oxo-bridged structure would provide the thermodynamic driving force for the $4e^-$ reduction of O_2 and would allow the facile electron transfer to all Cu centers in the trinuclear cluster that is consistent with its involvement in the catalytic cycle.

Introduction

Multicopper oxidases (MCO), which include laccase, Fet3p, and ceruloplasmin, catalyze the $4e^-$ reduction of O_2 to H_2O with concomitant $1e^-$ oxidations of substrates.¹ The electrons are taken up at the type 1 (T1) “blue” Cu site and transferred $\sim 13 \text{ \AA}$ to the trinuclear Cu site comprised of a type 2 (T2) “normal” Cu center and a type 3 (T3) “coupled binuclear” Cu site where O_2 reduction occurs.^{1,2} Reaction of the fully reduced enzyme with O_2 produces the native intermediate (NI). NI, trapped using a rapid freeze–quench technique, has been spectroscopically determined to have all four Cu’s oxidized, with the three Cu^{II} centers in the trinuclear site mutually bridged by the product of full O_2 reduction with at least one Cu–Cu distance of 3.3 \AA .³ The bridged structure promotes strong antiferromagnetic coupling among the three Cu^{II} centers, yielding a spin-frustrated doublet ground state with a low-lying excited state at 150 cm^{-1} .³ Consequently, the T2 signal in the electron paramagnetic resonance (EPR) spectrum is greatly changed from that of the resting oxidized enzyme.³

The most characteristic feature of NI is found in the magnetic circular dichroism (MCD) spectrum, in which a pair of intense C-terms with opposite signs (called a pseudo-A-term) is observed at 316 nm (–) and 364 nm (+), associated with the $O \rightarrow Cu^{II}$ charge-transfer (CT) transitions at the trinuclear site;^{3,4} this is definitive evidence that an oxyl-based radical species, to which NI was initially assigned,^{5,6} is not relevant, as such a radical would exhibit very different MCD features.⁷ However, the physical origin of the pseudo-A-term has been elusive. This requires a detailed understanding of the underlying spin–orbit coupling (SOC) mechanism between CT excited states. As SOC involves essentially a single-center operator, elucidation of the nature of the characteristic MCD pseudo-A feature would reveal a great deal of structural information on the Cu–O chromophore in NI.

Two limiting structures for the trinuclear Cu^{II} site in NI are consistent with its reactivity and ground-state spectral features. One has three μ_2 -OH-ligands, each bridging a Cu^{II} pair, where

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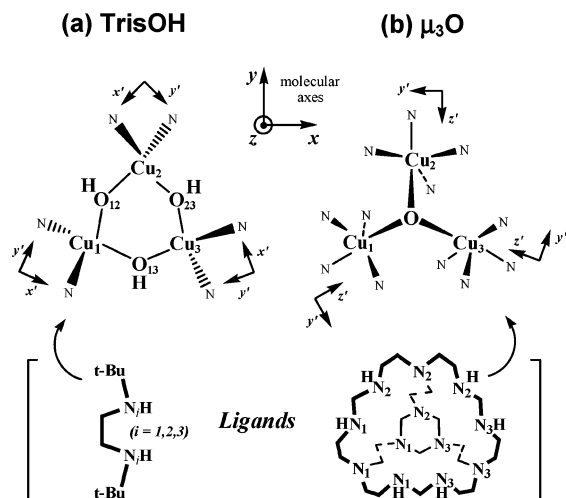
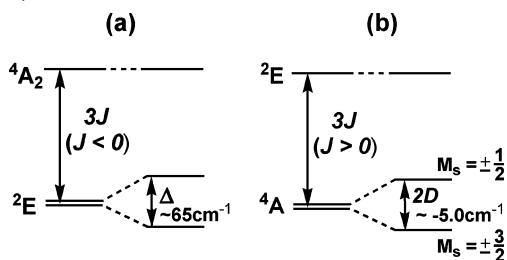


Figure 1. Structures of (a) TrisOH and (b) $\mu_3\text{O}$. Molecular coordinate axes (x, y, z) and local, metal-centered coordinate axes (x', y', z') are indicated.

Scheme 1. Ground-State Energy Diagrams of (a) Antiferromagnetically Coupled TrisOH and (b) Ferromagnetically Coupled $\mu_3\text{O}$



two OH^- -ligands derive from O_2 and the third from an ambient H_2O .³ The other structure has a single μ_3 -oxo ligand bridging all three Cu's at the center of the cluster, with the second oxygen atom from O_2 either remaining bound or dissociated from the trinuclear site. Recently, two model complexes have become available that represent these structures: TrisOH^{8,9} and $\mu_3\text{O}$.¹⁰ TrisOH is D_3 symmetric with three μ_2 -OH-ligands in the Cu_3 plane (Figure 1a). All Cu^{II} pairs are antiferromagnetically coupled, yielding a ${}^2\text{E}$ ground state ($J = -105 \text{ cm}^{-1}$) in which the spin-frustration is lifted by antisymmetric exchange, via ground-to-excited state superexchange, and symmetry lowering effects with a resultant zero-field splitting (ZFS) of 65 cm^{-1} (Scheme 1a).⁸ $\mu_3\text{O}$, on the other hand, is C_3 symmetric with the central μ_3 -oxo ligand $\sim 0.5 \text{ \AA}$ above the Cu_3 plane (Figure 1b). $\mu_3\text{O}$ has a ferromagnetic ${}^4\text{A}$ ground state ($J = +54.5 \text{ cm}^{-1}$),¹⁰ which is also confirmed by EPR.¹¹ The ${}^4\text{A}$ ground state shows a ZFS ($= 2D$) of -5.0 cm^{-1} (Scheme 1b)¹¹ that originates from anisotropic exchange that also involves a ground-to-excited state superexchange, similar to antisymmetric exchange in TrisOH.^{8,11} While the quartet ground state is not consistent with that of NI, the ferromagnetic interaction is caused by the protrusion of the oxo ligand out of the Cu_3 plane, most likely due to the tight macrocyclic ligand structure. If the oxo ligand is closer to the Cu_3 plane, the antiferromagnetic contribution dominates, resulting in a ${}^2\text{E}$ ground state as in TrisOH.¹¹

In this study, we present a systematic evaluation of the electronic transitions observed in the absorption and MCD spectra of TrisOH and $\mu_3\text{O}$. These models are ideal for elucidating the Cu–O bonding interactions in the respective trinuclear motif, as the μ_2 -hydroxo (in TrisOH) and μ_3 -oxo are the only O-ligands present and both complexes have three-fold symmetry. The results obtained here permit insight into the coupling between CT transitions of two ligands to one metal center or of one bridging ligand to two or more metal centers. Most importantly, this comparison allows a specific assignment of the geometric and electronic structures of the oxygen-bound trinuclear Cu^{II} cluster of NI, providing new insight into the O–O bond cleavage reaction in the catalytic cycle of the MCOs.

Experimental Section

Detailed descriptions of the syntheses, crystal structures, and magnetic susceptibilities of TrisOH and $\mu_3\text{O}/\mu_3\text{OH}$ ($\text{p}K_{\text{a}} \approx 4.6$ in aqueous solution) are as reported.^{9,10}

Low-temperature (LT) absorption spectra were collected with a Cary 500 double-beam spectrophotometer (Varian) using a liquid He cryostat (Janis Research Super Vari-Temp). LT MCD spectra were collected with a Jasco J-810-150S spectropolarimeter operating with an S-20 photomultiplier and an Oxford SM4000-8T magnet. MCD spectra were measured at temperatures from 1.78 to 150 K and fields from 0 to $\pm 7 \text{ T}$, and are corrected for baseline effects. The powder samples of TrisOH/ $\mu_3\text{O}/\mu_3\text{OH}$ were prepared anaerobically by finely grinding single crystals under a N_2 atmosphere in a glovebox. The mull samples were prepared by dispersing the powdered material in poly(dimethylsiloxane) (Aldrich) that was uniformly spread between quartz disks (Heraeus-Amersil), loaded into a copper MCD cell, and promptly frozen. The solution samples of $\mu_3\text{O}/\mu_3\text{OH}$ were prepared by dissolving the powder samples in D_2O . Diluted HClO_4 or NaOH was added to the solution to ensure only appreciable amounts of either $\mu_3\text{O}$ ($\text{pD} = 6.1$) or $\mu_3\text{OH}$ ($\text{pD} = 1.6$). Glass samples were prepared by adding 50% (v/v) $\text{D}_2\text{O}/\text{glycerol-}d_3$.

Spin-unrestricted DFT calculations were performed using Gaussian 98¹² and Amsterdam Density Functional¹³ programs and were analyzed using AOMix.¹⁴ All geometry optimizations were performed at the uB3LYP¹⁵/LanL2dz level. TrisOH was optimized in the broken-symmetry¹⁶ state with $\langle S^2 \rangle \approx 1.75$, with the *tert*-butyl groups on the ligands truncated with H-atoms and the dihedral angles of the N-atoms relative to the Cu_3 plane fixed. $\mu_3\text{O}$, on the other hand, was optimized in the high-spin state with $\langle S^2 \rangle \approx 3.75$, with all the ethylene groups linking N-atoms in the macrocyclic ligand truncated with H-atoms.¹¹ C_3 symmetry was imposed on both optimizations to reflect the crystal structures. The resulting geometries were then used for single-point calculations (of both the broken-symmetry and high-spin state calculations) with a triple- ζ Slater-type orbital basis set (ADF basis set TZP) with a single polarization function at the local density approximation of Vosko, Wilk, and Nusair¹⁷ with nonlocal gradient corrections of Becke and Perdew.¹⁸ The total energies of the high-spin quartet and the broken-symmetry states were used to estimate the isotropic exchange constant J of each trimer ($-2J = E_{\text{HS}} - E_{\text{BS}}$; the quartet-doublet energy difference is $|3J|$, as shown in Scheme 1).^{11,16b}

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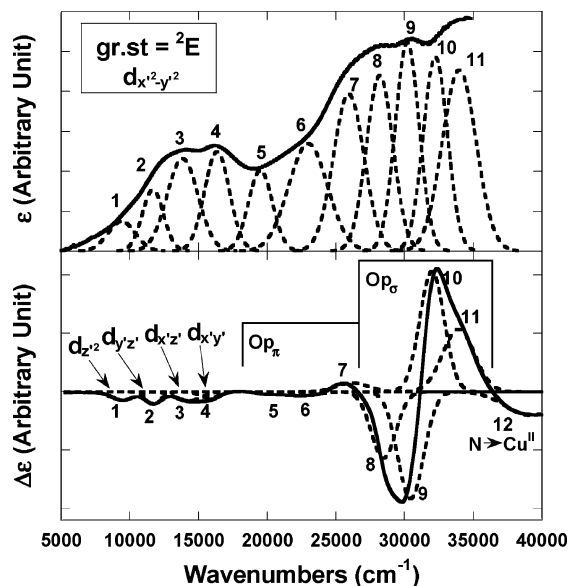


Figure 2. TrisOH mull absorption (top, 10 K) and MCD (bottom, 5.0 K/7.0 T) spectra.

Table 1. Gaussian-Resolved Peak Parameters of the Absorption and MCD Spectra of TrisOH

band	assignment	ν_{\max} (cm^{-1})	MCD intensity ^a	abs. intensity ^a	fwhm	polarization ^b
1	Cu d_{z^2}	9 470	-72	0.14	2080	x,y
2	Cu $d_{y^2-z^2}$	11 730	-103	0.31	1330	x,y
3	Cu $d_{x^2-z^2}$	14 270	-79	0.46	2230	z
4	Cu d_{xy}	15 830	-55	0.50	1660	z
5	O p_{π}	19 980	-19	0.40	1690	x,y
6	O p_{π}	22 440	-29	0.54	3350	x,y
7	O p_{π}	26 250	74	0.79	2730	x,y
8	O p_{σ}	28 470	-571	1.04	2100	x,y
9	O p_{σ}	30 450	-916	1.04	2210	x,y
10	O p_{σ}	32 000	1027	0.97	2090	x,y
11	O p_{σ}	33 940	533	0.91	2720	x,y
12	N	39 210	-203			z

^a Presented in arbitrary units. ^b From VTVH MCD (see Results, section 2(a)).

Results

1. Absorption and MCD. (a) TrisOH. The absorption (10 K) and MCD (5.0 K/7.0 T) spectra of the mull TrisOH sample are given in Figure 2. The absorption intensities above 25 000 cm^{-1} vary and are considerably reduced due to scattered light effect.¹⁹ However, the band positions are reproducible. Between 5000 and 40 000 cm^{-1} , 12 bands are resolved as indicated by the Gaussian curves produced from the simultaneous fits to both spectra (Table 1). All bands show field- and temperature-dependent MCD C -term behaviors, and no change in the spectral shape is observed between 1.8 and 150 K.

Three regions can be distinguished in these spectra. The first region consists of bands 1–4 (9400–15900 cm^{-1}) with weak absorption and MCD intensities. The band energies are similar to the $d-d$ energies of mononuclear Cu^{II} complexes with geometries similar to those of the individual metal sites in TrisOH (i.e., distorted square planar).²⁰ These bands all have

(19) When a THF solution of TrisOH was diluted in 2-methyl-THF (to form a glassing solution), a different MCD spectrum than the solid-state mull spectrum was obtained. To directly correlate with the crystal structure, only the mull spectra are presented in this article.

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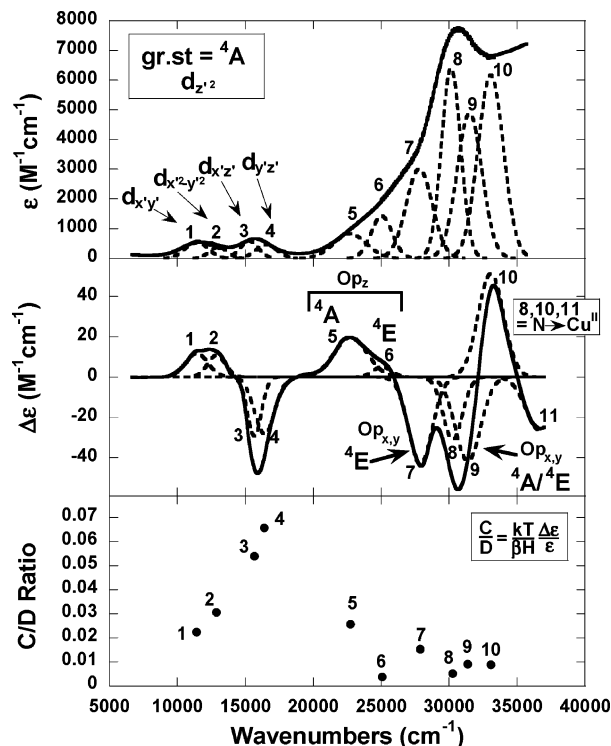


Figure 3. $\mu_3\text{O}$ solution absorption (top, 10 K) and MCD (middle, 5.0 K/7.0 T) spectra and C/D ratios between the MCD and absorption intensities (bottom).

(-) C -terms, deviating from the MCD sum rule.²¹ It indicates that their MCD intensity is governed by SOC between the ground and low-lying excited states (i.e., SOC between the ZFS components of the 2E) rather than SOC between excited states (see below). The second region consists of bands 5–7 (19 900–26 300 cm^{-1}) with medium absorption and weak MCD intensities, and the third region consists of bands 8–12 (28 400–40 000 cm^{-1}) with high absorption and MCD intensities. Their high energies indicate ligand-to-metal CT (LMCT) transitions. In both regions, pseudo- A -terms with negative bands at lower energies are observed. The MCD sum rule is obeyed that indicates SOC between the CT excited states. From the structure of TrisOH, the $\text{OH}^- \rightarrow \text{Cu}^{\text{II}}$ CT transitions are expected to be purely x,y -polarized, while those for the amine $\text{N} \rightarrow \text{Cu}^{\text{II}}$ CT are expected to be z -polarized. Therefore, the assignments of these CT bands can be made using the polarizations of the bands obtained from variable-temperature, variable-field (VTVH) MCD (see below): bands 5–7 are the x,y -polarized $\text{OH}^- \pi \rightarrow \text{Cu}^{\text{II}}$ LMCT with relatively low intensities at low energies, bands 8–11 are the x,y -polarized $\text{OH}^- \sigma \rightarrow \text{Cu}^{\text{II}}$ LMCT with high intensities, and band 12 is the z -polarized $\text{N} \rightarrow \text{Cu}^{\text{II}}$ LMCT (Table 1).

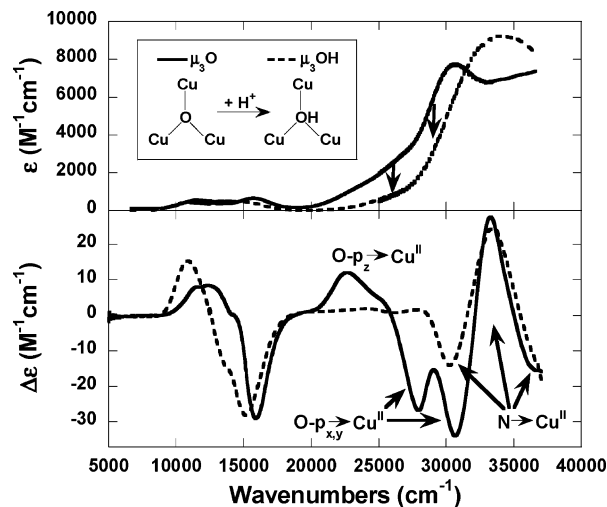
(b) $\mu_3\text{O}$. The absorption (10 K) and MCD (5.0 K/7.0 T) spectra of the $\mu_3\text{O}$ solution are presented in Figure 3. The frozen-glass sample allowed for consistent absorption/MCD intensities to be observed and the molar extinction coefficients ϵ , $\Delta\epsilon$ and the C/D ratio evaluated. These spectra are similar to those obtained from mull samples in the band positions and the overall spectral features (Figure S1), which suggests that the molecular structure in solution is similar to that of the crystal structure. Between 5000 and 37 000 cm^{-1} , 11 bands are resolved as

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Table 2. Gaussian-Resolved Peak Parameters of the Absorption and MCD Spectra of $\mu_3\text{O}$

band	assignment ^a	ν_{max} (cm^{-1})	$\Delta\epsilon_{\text{max}}$ ($\text{M}^{-1}\text{cm}^{-1}$)	ϵ_{max} ($\text{M}^{-1}\text{cm}^{-1}$)	fwhm	C/D ratio ^a	polarization ^b
1	Cu d_{xy}	11 420	11.8	559	1930	0.022	
2	Cu $d_{x^2-y^2}$	12 880	10.8	375	1540	0.031	
3	Cu d_{yz}	15 680	-29.3	579	1190	0.054	
4	Cu d_{yz}	16 420	-28.3	457	1740	0.066	
5	O p_z	22 750	19.8	822	2840	0.026	x,y ($z = 3\%$)
6	O p_z	25 080	5.1	1425	1580	0.004	x,y
7	O $p_{x,y}$	27 880	-43.3	3014	2010	0.015	x,y
8	N	30 270	-31.1	6411	1660	0.005	z
9	O $p_{x,y}$	31 380	-41.8	4876	2170	0.009	x,y
10	N	33 070	51.4	6173	2200	0.009	z
11	N	36 670	-26.4				z

^a $C/D = (kT/\beta H)(\Delta\epsilon/\epsilon) = 1.064(\Delta\epsilon/\epsilon)$ when $H = 7.0$ T and $T = 5.0$ K. ^b From VTVH MCD (see Results, section 2(b)).

**Figure 4.** Comparison of the $\mu_3\text{O}$ (solid) and $\mu_3\text{OH}$ (dotted) solution absorption (top, 10 K) and MCD (bottom, 5.0 K/7.0 T) spectra.

indicated by the Gaussian curves obtained from the simultaneous fits to both spectra (Table 2). All bands show MCD C -term behavior, and no change in the MCD spectral shape is observed between 1.8 and 150 K.

Three spectral regions can be identified. The first region consists of bands 1–4 (11 400–16 500 cm^{-1}) with weak absorption and strong MCD intensities. The relatively large C/D ratios are indicative of d – d transitions, involving large metal-based contributions to the orbitals in the transitions. The band energies are also similar to the d – d energies of mononuclear Cu^{II} complexes, with geometries similar to those of the metal sites in $\mu_3\text{O}$ (i.e., distorted trigonal bipyramidal).²⁰ MCD pseudo- A -terms are observed, indicating that the intensities are governed by SOC between the associated excited states. Thus, in contrast to TrisOH, SO mixing into the ground state is limited, as expected for a non-degenerate 4A ground state. The second region consists of bands 5–7 and 9 (22 700–31 400 cm^{-1}) that are absent in the spectra of $\mu_3\text{OH}$ (Figure 4). These bands are the oxo $\rightarrow \text{Cu}^{\text{II}}$ CT transitions as protonation shifts them much higher in energy. The lower intensities of bands 5/6 as compared to bands 7/9 suggest the former can be assigned as the weak out-of-plane O p_z (π /pseudo- σ) $\rightarrow \text{Cu}^{\text{II}}$ CT transitions and the latter as the strong in-plane O $p_{x,y}$ (σ) $\rightarrow \text{Cu}^{\text{II}}$ CT transitions. Finally, the third region of $\mu_3\text{O}$ consists of bands 8, 10, and 11 (30 200–36 700 cm^{-1}) that are also present in the $\mu_3\text{OH}$ spectrum with similar energies and intensities (Figure 4). These bands are assigned as the N $\rightarrow \text{Cu}^{\text{II}}$ CT transitions. The

assignments presented are consistent with those indicated by VTVH MCD (see below).

2. VTVH MCD. (a) TrisOH. VTVH MCD saturation curves have been obtained for all 12 bands in the MCD spectrum of TrisOH. Bands 1, 2, and 5–11 show a non-nesting behavior (Figures 5b and S2), whereas bands 3, 4 (Figures 5a and S2c), and 12 (Figure 5c) show nesting behavior, which is unique in an $S_{\text{tot}} = 1/2$ system.

The polarization of an electronic transition in a randomly oriented sample can be determined from the shape and nesting of its magnetic saturation curves. A detailed VTVH MCD analysis of an x,y -polarized O $\rightarrow \text{Cu}^{\text{II}}$ CT transition (band 9) has been previously reported.⁸ TrisOH has a ZFS 2E ground state (Scheme 1a), and an x,y -polarized transition does not show nesting behavior as long as the higher energy ZFS component of the 2E ground state is not populated. Therefore, the non-nested bands 1, 2, and 5–11 are purely x,y -polarized. This is consistent with the assignment of bands 5–11 as the $\text{OH}^- \rightarrow \text{Cu}^{\text{II}}$ CT transitions since the three OH^- -ligands lie in the Cu_3 plane. Alternatively, the nesting behavior of bands 3, 4, and 12 originates from the nonlinear behavior of the ground-state energy levels in the magnetic field when the molecular z -axis is not aligned with the field. This supports the assignment of band 12 as a z -polarized N $\rightarrow \text{Cu}^{\text{II}}$ CT transition since the N-ligands lie off the Cu_3 plane. Bands 3 and 4 exhibit nesting behaviors as these d – d transitions gain intensities from higher energy CT transitions, such as the z -polarized band 12.

(b) $\mu_3\text{O}$. VTVH MCD saturation curves at all 11 bands in the MCD spectrum of $\mu_3\text{O}$ have been obtained (Figure S3). In contrast to TrisOH, it is not feasible to distinguish the polarization solely on the basis of the presence of nesting behavior since most of the saturation curves are nested due to the low ZFS ($2D = -5.0$ cm^{-1}).¹¹ Therefore, simulations were performed using eq 1:²²

$$\frac{\Delta\epsilon}{E} = -\frac{\gamma}{4\pi} \int_0^\pi \int_0^{2\pi} \tanh\left(\frac{g_e\beta H}{2kT}\right) \frac{\sin\theta}{g} (l_x^2 g_x M_{yz}^{\text{eff}} + l_y^2 g_y M_{xz}^{\text{eff}} + l_z^2 g_z M_{xy}^{\text{eff}}) d\theta d\phi \quad (1)$$

where $g_e = 2.0023$, β is the Bohr magneton, H is the magnetic field, l_i is the angular momentum operator along axis i , g_i is the g -value along axis i , M_{ij}^{eff} is the effective MCD transition dipole moment, and integration of this expression over θ and ϕ accounts for orientational averaging in a frozen solution sample.

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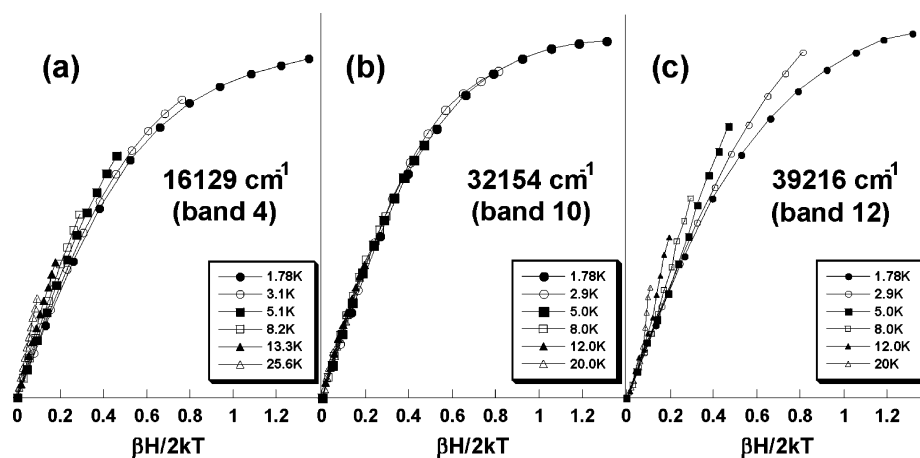


Figure 5. Representative VTVH MCD plots of TrisOH mull sample at temperatures below 30 K.

Individual transition polarizations are projected from the calculated M_{xy} , M_{xz} , and M_{yz} values.

The resulting polarizations are given in Table 2. Bands 5–7 and 9, assigned above as the oxo \rightarrow Cu^{II} CT transitions, are dominantly x,y -polarized. Note that a small amount of z -polarization character ($\sim 3\%$) in band 5 is unequivocally obtained, as a clear distinction can be made between the saturation behaviors of band 5 and the purely x,y -polarized band 6 (Figure S3). Alternatively, bands 8, 10, and 11 are dominantly z -polarized, which is consistent with their assignment as the N \rightarrow Cu^{II} CT transitions (see above). Bands 1–4 also exhibit significant z -polarization as these $d-d$ transitions gain intensities from higher energy CT transitions.

3. Electronic Structure Calculations. (a) TrisOH. In the optimized geometry, the OH[−]-ligands remain in the Cu₃ plane and the D_3 symmetry is maintained. The bond lengths and angles are in excellent agreement with experiment (in parentheses):⁹ $r(\text{Cu}-\text{Cu}) = 3.69 \text{ \AA}$ (3.64 \AA), $r(\text{Cu}-\text{O}) = 1.96 \text{ \AA}$ (1.91 \AA), $r(\text{Cu}-\text{N}) = 2.07 \text{ \AA}$ (2.02 \AA), and $\angle(\text{Cu}-\text{O}-\text{Cu}) = 140.5^\circ$ (144.2 $^\circ$). J is estimated to be -361 cm^{-1} , which is consistent with the antiferromagnetism observed in experiment ($J = -105 \text{ cm}^{-1}$).^{8,9} The relatively large J -value most likely comes from the tendency of pure DFT functionals to give overly covalent descriptions of the ground states in metal complex systems.²³

The MO energy diagram relevant to the Cu–O bonding interactions is presented in Figure 6 for the high-spin $|\alpha_1\alpha_2\alpha_3\rangle$ and the broken-symmetry $|\alpha_1\beta_2\alpha_3\rangle$ states (subscripts 1–3 refer to the Cu centers). The MOs are labeled “ x^2-y^2 ” for the HOMO/LUMOs (nos. 106–108) having mostly Cu $d_{x^2-y^2}$ characters, “O p_π ” for MOs (nos. 91–93) having mostly out-of-plane OH[−] p_z characters ($z \parallel C_3$ -axis) that form weak π -bonds with the Cu centers, and “O p_σ ” for MOs (nos. 79–81) having mostly in-plane OH[−] $p_{x,y}$ characters (along the Cu–Cu directions) that form strong σ -bonds with the Cu centers. The contours of the unoccupied MOs (α_{108} , β_{107} , and β_{108}) and the occupied O p_σ MOs (β_{79} – β_{81}) in the broken-symmetry state are presented in Figure 7,²⁴ and their compositions are listed in Table 3.²⁵

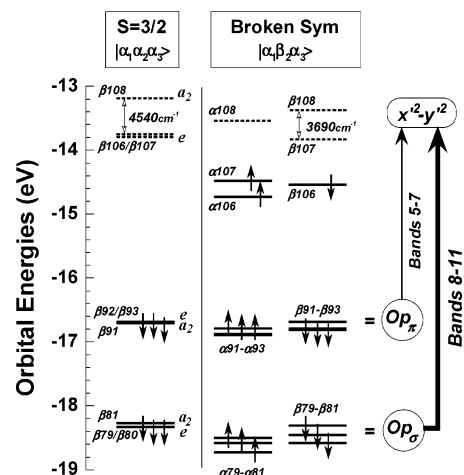


Figure 6. Energy level diagram of TrisOH in the high-spin $|\alpha_1\alpha_2\alpha_3\rangle$ and the broken-symmetry $|\alpha_1\beta_2\alpha_3\rangle$ states (1–3 refer to the Cu centers). O p_π refers to the out-of-plane O p_z -based donor MOs associated with the weak LMCT bands 5–7, and O p_σ refers to the in-plane O $p_{x,y}$ -based donor MOs associated with the strong LMCT bands 8–11. The high-spin state MOs are labeled with the symmetries of the D_3 group.

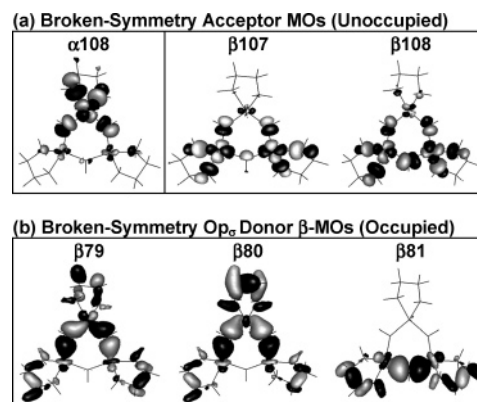


Figure 7. Contours of the broken-symmetry wave functions of TrisOH: (a) unoccupied, metal-based acceptor MOs α_{108} , β_{107} , and β_{108} and (b) occupied, ligand O p_σ -based donor MOs β_{79} – β_{81} .

The high-spin quartet ($S_{\text{tot}} = 3/2$) state which corresponds to the 4A_2 state in Scheme 1a is well described by the single determinant $|\alpha_1\alpha_2\alpha_3\rangle$ shown in Figure 6. The MOs have a_2 and

(23) Szilagy, R. K.; Metz, M.; Solomon, E. I. *J. Phys. Chem. A* **2002**, *106*, 2994–3007.

(24) Additional contours of the unoccupied β -MOs (β_{106} – β_{108}) in the high-spin state and the occupied O p_π (β_{91} – β_{93}) and O p_H (β_{67} – β_{69}); based on the in-plane OH[−] $p_{x,y}$ -orbitals along the O–H bonds) MOs in the broken-symmetry state are given in Figures S4 and S5.

(25) Complete MO energies and compositions of the acceptor and donor MOs in the high-spin and broken-symmetry states are given in Tables S2–S4 for TrisOH and in ref 11 for $\mu_3\text{O}/\mu_3\text{OH}$.

Table 3. Energies (eV) and Compositions (%)^a of the Acceptor and the O p_σ Donor MOs of the Broken-Symmetry State Calculation on the TrisOH Model²⁵

MO no.	E (eV)	Cu ₁ /Cu ₃	Cu ₂	O ₁₂ /O ₂₃		O ₁₃	
				2p _{xy}	2p _z	2p _{xy}	2p _z
unoccupied							
<i>x</i> ² − <i>y</i> ²							
α108	−13.52	2	52	6	2	1	0
β108	−13.36	26	2	3	1	9	3
β107	−13.82	29	2 ^b	3	1	1	0
occupied							
<i>x</i> ² − <i>y</i> ²							
α107	−14.48	20	5	1	0	13	5
α106	−14.73	17	16 ^b	8	0	1	0
β106	−14.52	6	35	6	2	3	1
O p _σ							
β81	−18.29	13	0	0	0	48	0
β80	−18.45	5	10 ^b	21	0	0	0
β79	−18.59	5	19	20	0	0	0

^a Results from ADF calculation using TZP basis set are presented. MO compositions were obtained from Mulliken population analysis. ^b d_{x²−y²} character, not d_{x²−y²}.

e symmetries in the D₃ group (contours in Figure S4). The a₂ and *e* MOs are split in energy due to different orbital and exchange interactions. These splittings are more pronounced within the metal-based β-MOs (β106–β108 = 4540 cm^{−1}) than within the ligand-based MOs (β91–β93; β79–β81).

Alternatively, the low-spin doublet (S_{tot} = 1/2) state, which corresponds to the ²E ground state in Scheme 1a, has a multi-determinant wave function that requires a linear combination of three broken-symmetry spin configurations, |α₁β₂α₃⟩, |α₁α₂β₃⟩, and |β₁α₂α₃⟩.²⁶ The three-fold symmetry of TrisOH permits the orbital and exchange interactions of the three spin configurations to be effectively described by one. In the broken-symmetry |α₁β₂α₃⟩ state calculated here, the MOs result from mixing of the a₂ and *e* MOs of the high-spin state due to symmetry lowering. Moreover, inequivalent exchange interactions exist among the three centers (cf. α₁–α₃ (↑↑) vs α₁–β₂ or β₂–α₃ (↑↓)). Consequently, the spin-polarized energy levels become non-degenerate despite the D₃ symmetry of the molecule, particularly with the two unoccupied β-MOs being split by 3690 cm^{−1}.

The MO compositions (Table 3) and relative energies of the broken-symmetry calculation (i.e., ²E ground state) can be used to explain the orbital origin of the experimentally observed OH[−] → Cu^{II} CT transitions. The relative intensities can be predicted from the ligand character in the acceptor MOs: the d_{x²−y²}-orbitals are involved in strong σ-bonds with OH[−] p_{xy} (1–9%) and weak π-bonds with OH[−] p_z (0–3%). Also, the relative transition energies can be predicted from the MO energies of the donor MOs. Thus, the high-energy/intensity bands 8–11 originate from the O p_σ → d_{x²−y²} CT transitions and the low-energy/intensity bands 5–7 from the O p_π → d_{x²−y²} CT transitions, as indicated in Figure 6. Note that group theory predicts 2²A₁ + 2²A₂ + 4²E CT states associated with either O p_σ or O p_π → d_{x²−y²} CT transitions.²⁷ These multi-determinant CT states are different linear combinations of the single determinants obtained by exciting electrons from donor (O p_σ or O p_π) to acceptor MOs (the LUMOs, α108, β107, and β108) for all possible M_S = +1/2 spin configurations. These CT states

(26) Griffith, J. S. *Struct. Bond.* **1972**, *10*, 87–126.

(27) ²A₁ + 2²A₂ + 4²E (and ⁴A₁ + ⁴A₂ + 2⁴E) states are obtained in the D₃ group by transferring each of the six available O p_σ or O p_π electrons at the three OH[−]-ligands to the hole on either of the two adjacent Cu-atoms, resulting in 48 microstates. Transitions to remote Cu are not considered as these will not have significant CT intensity.

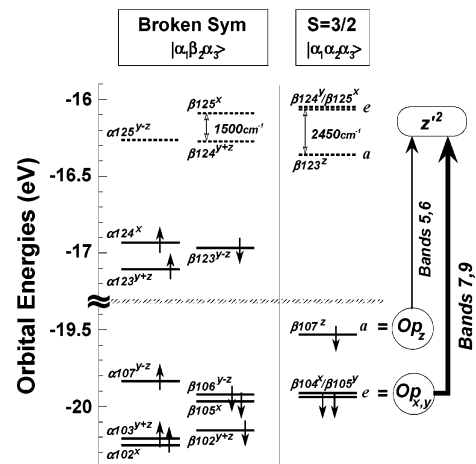


Figure 8. Energy level diagram of μ₃O in the broken-symmetry |α₁β₂α₃⟩ and the high-spin |α₁α₂α₃⟩ states (1–3 refer to the Cu centers). O p_z refers to the out-of-plane O p_z-based donor MOs associated with weak LMCT bands 5 and 6, while O p_{xy} refers to the in-plane O p_{xy}-based donor MOs associated with the strong LMCT bands 7 and 9. The high-spin state MOs are labeled with the symmetries of the C₃ group.

would split in energy dominantly by different bonding and exchange interactions that are described by the broken-symmetry wave function.

Finally, the αLUMO in the broken-symmetry state (Figures 6 and 7a) is localized on one metal, and it is useful for describing the ligand field around each Cu site. The four-coordinated Cu^{II} sites in TrisOH are best described as D_{2d} distorted square-planar, from the relative d-orbital energies (α-MO energies): d_{x²−y²} (−13.5 eV) > d_{z²} (−14.8 eV) > d_{xy} (−15.5 eV) > d_{xz} (−15.9 eV) > d_{xy} (−16.4 eV). These assignments are given in Figure 2 and Table 1.

(b) μ₃O. The optimized geometry is in good agreement with the crystal structure (in parentheses): r(Cu–Cu) = 3.30 Å (3.105/3.125 Å), r(Cu–O) = 1.97 Å (1.869/1.883 Å), ∠(Cu–O–Cu) = 113.6° (112.3/112.2°), and r(O–Cu₃ plane) = 0.51 Å (0.529/0.539 Å). Some increases in the Cu–Cu/O distances can be ascribed to the relaxed ligand structure from the truncation of the ethylene links. *J* is estimated to be +246 cm^{−1},¹¹ consistent with the ferromagnetism observed in experiment (+54.5 cm^{−1}).¹⁰ Again, the relatively large *J*-value originates from the overly covalent description of the ground state by pure DFT functionals.²³

The MO energy diagram relevant to the Cu–O bonding interactions is presented in Figure 8 for the broken-symmetry |α₁β₂α₃⟩ and the high-spin |α₁α₂α₃⟩ states. The MOs are labeled “z²” for HOMO/LUMOs (nos. 123–125) having mostly Cu d_{z²} characters and “O p_z” and “O p_{xy}” for occupied MOs having mostly out-of-plane o_{xo} p_z (z || C₃-axis) and in-plane o_{xo} p_{xy} characters, respectively. The MOs are labeled with x, y, or z to indicate the constituent o_{xo} p-orbital(s). The contours of the unoccupied MOs of the high-spin state (β123–β125) and those of the broken-symmetry state (α125, β124, and β125) are presented in Figure 9, and the compositions of the high-spin state MOs are listed in Table 4.²⁵

As with TrisOH, the high-spin quartet (S_{tot} = 3/2) state, which corresponds to the ⁴A ground state in Scheme 1b, is well described by the single determinant |α₁α₂α₃⟩ presented in Figure 8. The MOs have *a* and *e* symmetries in the C₃ group, in which *a*-MOs compose the out-of-plane o_{xo} p_z- and *e*-MOs compose

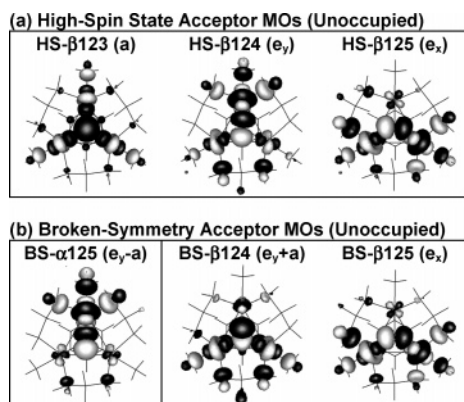


Figure 9. Contours of the unoccupied acceptor MOs of the $\mu_3\text{O}$ wave functions: (a) the high-spin state MOs β_{123} – β_{125} and (b) the broken-symmetry state MOs α_{125} , β_{124} , and β_{125} .

Table 4. Energies (eV) and Compositions (%)^a of the Acceptor and Donor MOs of the High-Spin State Calculation on the $\mu_3\text{O}$ Model²⁵

MO no.	E (eV)	Cu ₁ /Cu ₃	Cu ₂	O		
				2p _x	2p _y	2p _z
unoccupied						
<i>z</i> ² β_{125}	−16.06	25	3 ^b	20	0	0
β_{124}	−16.06	10	33	0	20	0
β_{123}	−16.37	16	16	0	0	20
occupied						
O p _z β_{107}	−19.52	15	15	0	0	18
O p _x β_{105}	−19.92	7	10 ^b	29	0	0
O p _y β_{104}	−19.92	9	6	0	29	0

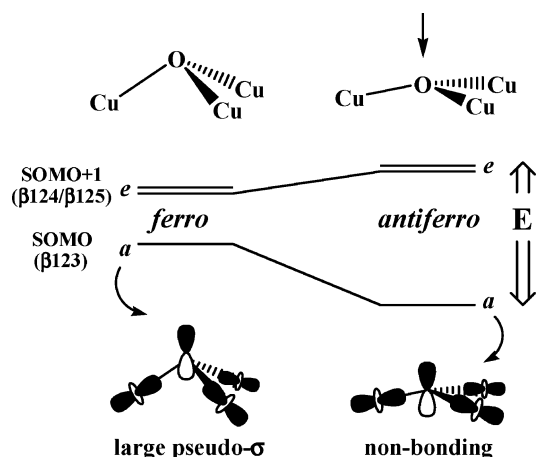
^a Results from ADF calculation using TZP basis set are presented. MO compositions were obtained from Mulliken population analysis. ^b d_{z^2} character, not d_{z^2} .

the in-plane oxo $p_{x,y}$ -orbitals. Alternatively, the bonding description of the low-spin doublet ($S_{\text{tot}} = 1/2$) state, which corresponds to the ${}^2\text{E}$ state, is described by the broken-symmetry $|\alpha_1\beta_2\alpha_3\rangle$ state. As indicated above with TrisOH, $|\alpha_1\beta_2\alpha_3\rangle$ is just one of three broken-symmetry states that constitute the multi-determinant ${}^2\text{E}$ state wave function. Nevertheless, the three-fold symmetry of $\mu_3\text{O}$ permits the overall orbital and exchange interactions to be adequately described by the $|\alpha_1\beta_2\alpha_3\rangle$ determinant. The MO energy levels are split by these bonding and exchange effects, particularly with the two unoccupied β -MOs being split by 1500 cm^{-1} .

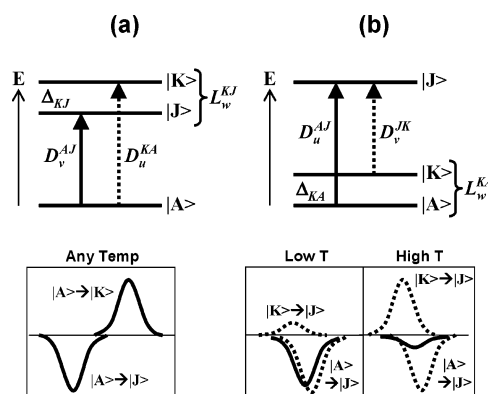
The MO compositions (Table 4) and relative energies of the high-spin state calculation can be used to explain the orbital origin of the experimentally observed oxo \rightarrow Cu^{II} CT transitions. The d_{z^2} -orbitals are involved in strong σ -bonds with the oxo p_y/p_x (each 20% in the acceptor MOs β_{124}/β_{125}) and weaker pseudo- σ -bonds with the oxo p_z (22% in β_{123}). As a result, the e -MOs are 2450 cm^{-1} more destabilized than the a -MO. Likewise, the O $p_z \rightarrow d_{z^2}$ CT transitions are expected to have low energies/intensities as compared to the O $p_{x,y} \rightarrow d_{z^2}$ CT transitions with higher energies/intensities. Group theory predicts $3^4\text{A} + 3^4\text{E}$ oxo \rightarrow Cu^{II} CT states in C_3 symmetry.²⁸ In contrast to the low-spin doublet CT states, these high-spin CT states can be represented by single determinants. Thus, individual MO configuration can be assigned to each CT state (see below).

(28) $4\text{A} + 3^4\text{E}$ and $6^2\text{A} + 6^2\text{E}$ states in the C_3 group are obtained by transferring one of the six available oxo p_x , p_y , and p_z electrons to each hole available on the three Cu-atoms. Note that of the doublet CT states, $4^2\text{A} + 4^2\text{E}$ are associated with the in-plane oxo $p_{x,y} \rightarrow \text{Cu}^{\text{II}}$ CT transitions, whereas $2^2\text{A} + 2^2\text{E}$ are associated with the out-of-plane oxo $p_z \rightarrow \text{Cu}^{\text{II}}$ CT transitions.

Scheme 2. Effect of the Oxo Ligand Position on the Ground State of $\mu_3\text{O}$



Scheme 3. Two Possible MCD C-Term Mechanisms



Note that the d_{z^2} –O p_z bonding interactions are substantial ($p_z = 22\%$ in β_{123}) due to the oxo ligand being $\sim 0.5\text{ \AA}$ above the Cu_3 plane. The resulting pseudo- σ -bonds cause destabilization of the singly occupied MO (SOMO = unoccupied βLUMO (β_{123}) of a -symmetry) and narrow its energy gap with the SOMO + 1 (unoccupied $\beta\text{LUMO} + 1$ (β_{124}/β_{125}) of e -symmetry).²⁹ Consequently, the ferromagnetic contribution in the exchange interaction dominates over the antiferromagnetic contribution, resulting in the ${}^4\text{A}$ ground state. However, if the oxo ligand is shifted into the Cu_3 plane, the Cu–O pseudo- σ -bonds are lost and the SOMO energy is stabilized, increasing the SOMO/SOMO + 1 energy gap. As a result, the antiferromagnetic contribution, being proportional to the square of the SOMO/SOMO + 1 energy gap, dominates over the ferromagnetic contribution (\sim constant), yielding an antiferromagnetic ${}^2\text{E}$ ground state (Scheme 2).¹¹ Thus, the ferromagnetism is not intrinsic to the $\mu_3\text{-oxo}$ structure and this is still a valid model of NI.

Alternatively, when $\mu_3\text{O}$ is protonated (i.e., $\mu_3\text{OH}$), the Cu–O bonding interactions are considerably reduced (O $p_z = 1.0\%$ in β_{123} and O $p_x/p_y = 10.6\%$ in β_{124}/β_{125}).¹¹ The OH-based donor MOs of $\mu_3\text{OH}$ are significantly stabilized, leading to the absence of the $\text{OH}^- \rightarrow \text{Cu}^{\text{II}}$ CT bands in the absorption/MCD spectra (Figure 4). Note that the $\text{OH}^- \rightarrow \text{Cu}^{\text{II}}$ CT transitions are still observed in TrisOH due to the low coordination number

(29) Hay, P. J.; Thibault, J. C.; Hoffmann, R. *J. Am. Chem. Soc.* **1975**, *97*, 4884–4899.

of the Cu sites (4 vs 5 in $\mu_3\text{O}$) that stabilizes the d-manifold by $\sim 5000\text{ cm}^{-1}$ and, thus, red-shifts the CT bands.³⁰

Finally, the αLUMO of the broken-symmetry state (Figures 8 and 9b) is localized on one metal, and each of the five-coordinated Cu^{II} sites in $\mu_3\text{O}$ is best described as having a distorted trigonal bipyramidal ligand field from the relative d-orbital energies ($\alpha\text{-MO}$ energies) obtained from the broken-symmetry calculation: d_{z^2} (-16.3 eV) $>$ $d_{x^2-y^2}$ (-17.6 eV) $>$ $d_{x^2-y^2}$ (-18.0 eV) $>$ d_{xz} (-18.7 eV) $>$ d_{yz} (-19.0 eV). These assignments are given in Figure 3 and Table 2.

MCD Analysis

1. LF Region: Ground- vs Excited-State SOC. One of the features that distinguish TrisOH and $\mu_3\text{O}$ is the shape of the MCD bands in the d–d region, where only (–) C -terms are observed for TrisOH, whereas both (+) and (–) C -terms are observed for $\mu_3\text{O}$. To explain this difference, the mechanisms governing the MCD transition intensity need to be evaluated.

The MCD intensity is described by A -, B -, and C -terms.^{22,31} The C -term ($= C_0/kT$) dominates the MCD intensity for paramagnetic systems at low temperatures, being several orders of magnitude larger than the other terms. C_0 for a spin-allowed transition from a spatially non-degenerate doublet ground state $|A\rangle$ to an excited state $|J\rangle$ is given by²²

$$C_0(A \rightarrow J) = -\frac{1}{6} \sum_{uvw} \epsilon_{uvw} g_w \sum_{K \neq A, J} (\Delta_{KJ}^{-1} D_u^{KA} D_v^{AJ} L_w^{KJ} + \Delta_{KA}^{-1} D_u^{AJ} D_v^{JK} L_w^{KA}) \quad (2)$$

where $|K\rangle$ is a collection of states that SOC to the $|A\rangle$ ground or $|J\rangle$ excited states, ϵ_{uvw} is the Levi–Civita symbol with $(u, v, w) = (x, y, z)$, g_w is the effective g -value of the doublet in the w -direction, Δ_{KJ} (or Δ_{KA}) is the energy denominator from second-order perturbation theory, $D_v^{AJ} = \langle A S_{\text{tot}} M_S | m_v | J S_{\text{tot}} M_S \rangle$ ($= D_v^{JA}$) is the component of the transition dipole moment between $|A\rangle$ and $|J\rangle$ in the v -direction, and $L_w^{KJ} = \text{Im} \langle K S_{\text{tot}} M_S^K | \sum_{N,i} \xi(r_{iN}) l_{N,w}(i) s_w(i) | J S_{\text{tot}} M_S^J \rangle$ ($= -L_w^{JK}$) is the SOC matrix element between $|K\rangle$ and $|J\rangle$ in the w -direction, where $l_{N,w}(i)$ and $s_w(i)$ are w -components of the orbital and the spin angular momenta of the i th electron relative to nucleus N , and $M_S^K - M_S^J = 0$ or ± 1 . L_w^{KJ} is essentially a one-center function due to the $1/r^3$ dependence of the effective SOC parameter $\xi(r_{iN})$.

Two mechanisms contributing to the C -term intensity can be described on the basis of eq 2.^{22,32} In the first mechanism (Scheme 3a), the MCD intensity is gained by SOC between two nearby excited states $|J\rangle$ and $|K\rangle$ to which orthogonal transitions are made from a single ground state $|A\rangle$. The two transitions result in oppositely signed C -terms with equal intensities (i.e., a pseudo- A -term), abiding by the MCD sum rule that describes the requirement for spectral stability to conserve the total angular momentum of a photon.²¹ A pseudo- A -term is observed in a typical mononuclear Cu system in which the ground state is non-degenerate and has negligible SOC with any excited states due to large energy differences. This

mechanism is applicable to the d–d bands in the $\mu_3\text{O}$ MCD spectrum in which the d–d transitions are considered to be single-site transitions. Also, $\mu_3\text{O}$ has a 4A ground state with no low-lying orbital excited states that can SOC into the ground state. When the d–d excited states of $\mu_3\text{O}$ SOC in the molecular z -direction (L_z^{KJ}), two sets of pseudo- A -terms are obtained: $d_{xy}(+)/d_{yz}(-)$ and $d_{x^2-y^2}(+)/d_{xz}(-)$, where the indicated signs from experiment (Figure 3) agree with the predicted C -term signs of a mononuclear Cu^{II} complex.³³

Alternatively, the second mechanism (Scheme 3b) describes the MCD intensity gained by SOC between the ground state $|A\rangle$ and a low-lying excited state $|K\rangle$ from which two orthogonal transitions are made to a single excited state $|J\rangle$. In the LT region where the low-lying excited state $|K\rangle$ is not populated, only the $|A\rangle \rightarrow |J\rangle$ transition is observed as a single MCD C -term, while the $|K\rangle \rightarrow |J\rangle$ transition serves as a virtual orthogonal transition required for the MCD activity. Therefore, the MCD sum rule is not applicable. This mechanism is appropriate for the all-negative d–d bands in the TrisOH MCD spectrum in which the two SOC pairs $|A\rangle$ and $|K\rangle$ are the two ZFS components of the 2E ground state. These states are split by 65 cm^{-1} (i.e., greater than thermal energy) and SOC via antisymmetric exchange.⁸ The excited-state SOC mechanism may also contribute (e.g., between d_{xz} and d_{yz} via $L_z^{xz/yz}$) but appears to be less effective as no pseudo- A -term is observed. When the low-lying excited state $|K\rangle$ becomes populated at the higher temperatures, the $|K\rangle \rightarrow |J\rangle$ transition gains intensity with opposite C -term sign. If the energy splitting between $|A\rangle \rightarrow |J\rangle$ and $|K\rangle \rightarrow |J\rangle$ transitions is small, the $|K\rangle \rightarrow |J\rangle$ intensity cancels that of the $|A\rangle \rightarrow |J\rangle$ transition. Therefore, atypical of the C -terms, this intensity would deviate from a linear dependence on $1/T$ at sufficiently high temperatures. This deviation is observed at $T > 30\text{ K}$ for the CT band at 331 nm (band 9) in TrisOH.⁸

2. CT Region. (a) TrisOH: Metal-Based SOC between CT Transitions. Between $27\,000$ and $35\,000\text{ cm}^{-1}$ of the TrisOH MCD spectrum (Figure 2), intense and symmetric pseudo- A -terms (bands 8–11) are observed, with the (–) C -term components lower in energy by 2000 – 5000 cm^{-1} . These bands are all x, y -polarized as determined by VTVH MCD, consistent with the positions of the OH^- ligands in the Cu_3 plane. The high intensities observed in both the absorption and MCD spectra imply that $\text{Cu}^{\text{II}}\text{--OH}^-$ σ -bonds are involved in these CT transitions. This is also supported by the MO description from DFT (see above). Thus, bands 8–11 are unambiguously assigned as the $\sigma\text{ OH}^- \rightarrow \text{Cu}^{\text{II}}$ CT transitions.

The presence of pseudo- A -terms requires that there exists SOC between CT excited states. $2^2A_1 + 2^2A_2 + 4^2E$ CT states are associated with the $\sigma\text{ OH}^- \rightarrow \text{Cu}^{\text{II}}$ LMCT,²⁷ and transition to any of these states from the 2E ground state would be x, y -polarized. C_0 associated with any two of these CT transitions can be evaluated by expanding eq 2 with $w = z$ and $(u, v) = (x, y)$:

$$C_0(A \rightarrow J) = -\frac{1}{6} \sum_{K \neq A, J} g_z \Delta_{KJ}^{-1} (D_x^{KA} D_y^{AJ} - D_y^{KA} D_x^{AJ}) L_z^{KJ} \quad (3)$$

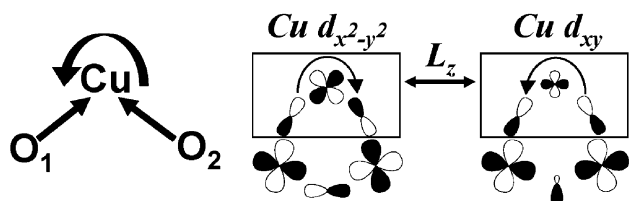
(30) Gamelin, D. R.; Randall, D. W.; Hay, M. T.; Houser, R. P.; Mulder, T. C.; Canters, G. W.; de Vries, S.; Tolman, W. B.; Lu, Y.; Solomon, E. I. *J. Am. Chem. Soc.* **1998**, *120*, 5246–5263.

(31) (a) Stephens, P. J. *Annu. Rev. Phys. Chem.* **1974**, *25*, 201–232. (b) Piepho, S. B.; Schatz, P. N. *Group Theory in Spectroscopy with Applications to Magnetic Circular Dichroism*; John Wiley & Sons: New York, 1983.

(32) Lehnert, N.; Ho, R. Y. N.; Que, L., Jr.; Solomon, E. I. *J. Am. Chem. Soc.* **2001**, *123*, 8271–8290.

(33) Gewirth, A. A.; Solomon, E. I. *J. Am. Chem. Soc.* **1988**, *110*, 3811–3819. Note that the z -axis in this reference corresponds to the y' -axis of the Cu sites in $\mu_3\text{O}$.

(a) TrisOH : Metal-Based SOC



(b) TrisOH : OH-Based SOC (X)

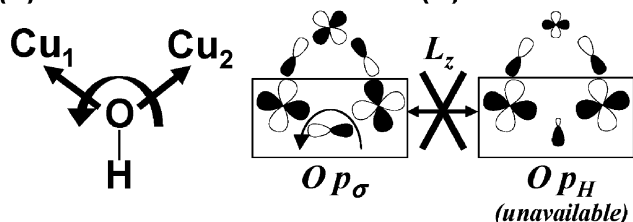
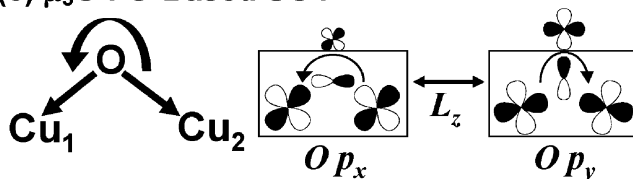
(c) $\mu_3\text{O}$: O-Based SOC

Figure 10. Single-centered (a) metal-based SOC in TrisOH, (b) OH-based SOC in TrisOH, and (c) oxo-based SOC in $\mu_3\text{O}$. The OH-based SOC in TrisOH is disabled as the protonation eliminates one of the in-plane O p-orbitals ($\text{O } p_{\text{H}}$) for the spin-orbit rotation.

Notice that when indices J and K are switched in eq 3, $C_0(A \rightarrow K) = -C_0(A \rightarrow J)$ is obtained, implying that the two orthogonal transitions $|A\rangle \rightarrow |J\rangle$ and $|A\rangle \rightarrow |K\rangle$ must produce a pseudo- A -term upon SOC via L_z .

The SOC matrix element L_z^{KJ} is effectively a localized one-center function, and therefore, contributions that occur between different centers can be neglected. Thus, a single atomic center must be simultaneously involved in both orthogonal CT transitions for MCD activity. We first consider the O-ligand as a possible SOC center since a LMCT would involve the majority of the charge on the donor ligand (Figure 10b). The in-plane OH^- $p_{x,y}$ -orbitals in the donor $\text{O } p_{\sigma}$ MOs, however, cannot rotate into the out-of-plane OH^- p_z -orbitals in the $\text{O } p_{\pi}$ MOs by L_z . Although the OH^- $p_{x,y}$ -orbitals in $\text{O } p_{\sigma}$ can rotate into those in $\text{O } p_{\text{H}}$ (the in-plane OH^- p-orbitals involved in the O–H bond), no significant SOC is expected since the energy differences between the $\text{O } p_{\sigma}$ and $\text{O } p_{\text{H}}$ MOs are large and the $\text{O } p_{\text{H}}$ MOs do not contribute in the Cu–O bonds or in the $\text{OH}^- \rightarrow \text{Cu}^{\text{II}}$ CT transitions. Thus, a pseudo- A -term associated with these transitions in TrisOH cannot be derived from an O-based SOC.

Alternatively, the transitions from two OH^- -ligands to a single Cu site can be considered (Figure 10a). In this case, the Cu site would serve as the SOC center. This metal-based SOC mechanism is illustrated by the two unoccupied acceptor MOs (β_{107}/β_{108} ; Figure 7a) and the two occupied $\text{O } p_{\sigma}$ donor MOs (β_{79}/β_{80} ; Figure 7b) in which both the $d_{x^2-y^2}$ - and d_{xy} -orbitals at the apex Cu_2 center form σ -bonds with the in-plane $\text{O } p_{x,y}$ -orbitals (note that d_{xy} characters at the apex Cu_2 center are found in β_{80} , α_{106} , and β_{107} ; Table 3). Although the donor CT states are mostly OH^- centered, the metal-based SOC would still be operative as the highly covalent Cu–OH σ -bonds allow

a significant amount of metal contribution into the donor $\text{O } p_{\sigma}$ MOs. Thus, L_z can operate between $d_{x^2-y^2}$ and d_{xy} at the same Cu center in two different CT states and provide an efficient SOC mechanism in the TrisOH structure.

According to group theory, L_z induces the out-of-state SOC between 2A_1 – 2A_2 and 2E – 2E and in-state SOC of 2E . However, the in-state SOC in 2E is not expected to be resolved as the splitting would be determined either by the small SOC parameter of the ligand ($\xi_0 \approx 60$ – 70 cm^{-1}) or by a significantly reduced contribution from the metal. On the other hand, the out-of-state CT state splittings are directly related to the MO energy splittings (e.g., $\beta_{107}/\beta_{108} = 3690 \text{ cm}^{-1}$; Figure 6) as the CT states would split in energy by the different bonding and exchange interactions. Therefore, each of the observed pseudo- A -terms in the TrisOH MCD spectrum represents a pair of CT states that interact via out-of-state SOC.

The C -term signs can be evaluated on the basis of the MO descriptions and eq 3.²² Note that as the doublet CT states are multi-determinant, it is not feasible to make specific assignments to the eight available CT states (${}^2A_1 + {}^2A_2 + 4{}^2E$) that potentially yield four pseudo- A -terms, two of which are clearly resolved as bands 8–11. Nevertheless, it is expected that the dominant contributions in the splitting of these CT states originate from the different orbital and exchange interactions. In particular, the energy splitting of the two unoccupied β -MOs (β_{107} and β_{108}) would most appropriately describe the two energy-split CT states of a pseudo- A -term as these MOs provide the description of the overall bonding interactions and also serve as the two acceptor MOs of electron transitions from a donor MO.³⁵

Thus, we consider the donor $\text{O } p_{\sigma}$ β -MO, β_{79} ,³⁴ and the two acceptor β -MOs, β_{107} and β_{108} (Figure 7), to represent the $|A\rangle \rightarrow |J\rangle$ and $|A\rangle \rightarrow |K\rangle$ transitions, in which the energy splitting between the two CT states $|J\rangle$ and $|K\rangle$ is represented by that of the two acceptor β -MOs (3690 cm^{-1} ; Figure 6).³⁵ The electronic transitions are made from the β_{79} donor MO to the acceptor β_{107} that represents D_y^{AJ} or D_x^{AJ} and to the acceptor β_{108} that represents D_x^{KA} or D_y^{KA} in eq 3. In Figure 11, the graphical descriptions of the signs of the transition dipole moments, the SOC matrix element, and the predicted pseudo- A -term are illustrated. The signs of the transition moments, m_x and m_y , are obtained from the transition densities, which are the products of the donor and acceptor MO densities: the direction of m_x or m_y is taken from the center of the negative overlap (white) to that of the positive overlap (black) in the transition density. The metal-based SOC element L_z^{KJ} (i.e., SOC between $|J\rangle$ and $|K\rangle$) is obtained by the counterclockwise rotation of the SOC-active orbital of the Cu_2 center at the apex position: with $|K\rangle$ higher in energy than $|J\rangle$ ($\Delta_{KJ} > 0$), the counterclockwise rotation is made from the Cu_2 $d_{x^2-y^2}$ -orbital in β_{108} to the d_{xy} orbital in β_{107} , which yields negative overlap of the d-orbitals. Overall, $D_y^{AJ} = 0$, $D_x^{AJ} = -m_x$, $D_x^{KA} = 0$, $D_y^{KA} = -m_y$, $L_z^{KJ} = -L_z$, and $\Delta_{KJ} \approx E(\beta_{108}) - E(\beta_{107}) > 0$ are obtained. Substitution of these parameters into eq 3 yields $(-)$ $C_0(A \rightarrow J)$, and consequently, $(+)$ $C_0(A \rightarrow K)$. This is in agreement with the

(34) β_{80} can be also used as the donor $\text{O } p_{\sigma}$ MO.

(35) α_{125} LUMO of the broken-symmetry wave function (α_{108} for TrisOH and α_{125} for $\mu_3\text{O}$) is also a possible acceptor MO: CT determinants constructed by transferring electrons from the α -spin donor MOs would also constitute CT wave functions. However, the energy splitting of the two β -spin acceptor MOs is more pronounced (for both TrisOH and $\mu_3\text{O}$) and, therefore, would have larger contribution in the splittings of the CT states.

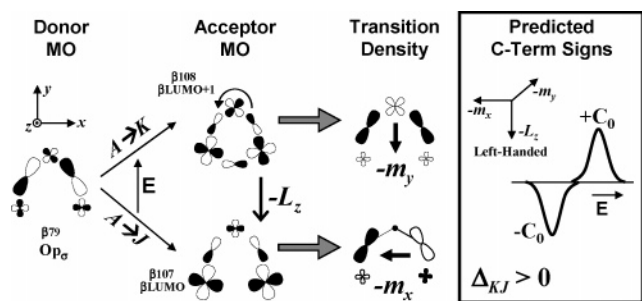


Figure 11. Graphical prediction of the C -term signs of the σ $\text{OH}^- \rightarrow \text{Cu}^{\text{II}}$ CT transitions of TrisOH. The counterclockwise rotation of the SOC-active $d_{x^2-y^2}$ in $\beta 108$ to d_{xy} in $\beta 107$ yields negative overlap ($-L_z$). The directions taken from the center of the negative overlap (white) to that of the positive overlap (black) in the transition densities (the products of the donor and acceptor MO densities) define the signs of the transition moments ($-m_x$ and $-m_y$). The left-handed coordinate system defined by $(-m_x, -m_y, -L_z)$ with positive $\Delta_{KJ} \approx E(\beta 108) - E(\beta 107)$ leads to a pseudo- A -term with a low-energy ($-$) C -term component.

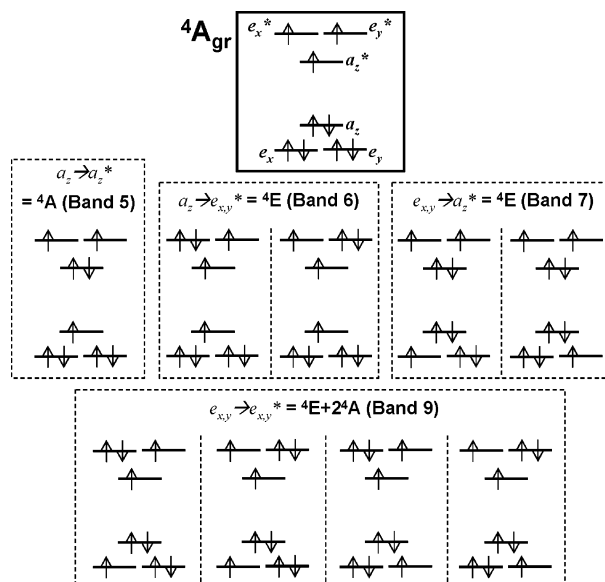
signs of the pseudo- A features observed in the MCD spectrum (Figure 2). The vector components $(-m_x, -m_y, -L_z)$ compose a left-handed coordinate system (with $\Delta_{KJ} > 0$), indicating that right-circularly polarized (RCP) light is absorbed by the $|A\rangle \rightarrow |J\rangle$ transition to produce a ($-$) C -term.²²

(b) $\mu_3\text{O}$ (Ferromagnetic): Oxo-Based SOC between CT Transitions. Asymmetrically shaped pseudo- A -terms are observed between the 20 000 and 35 000 cm^{-1} region in the MCD spectrum of $\mu_3\text{O}$ (Figure 3) that are composed of weak (+) C -terms (bands 5 and 6) and strong ($-$) C -terms (bands 7 and 9), the latter at 3800–9200 cm^{-1} higher in energy. The corresponding absorption intensities are also asymmetric; band 9 is ~ 7 times more intense than band 5. All of these bands are determined to be x,y -polarized from VTVH MCD. However, band 5 has a small but finite amount of z -polarization ($\sim 3\%$), implying that the out-of-plane O p_z -orbital is involved in this transition.

Group theory predicts $3^4A + 3^4E$ CT states for oxo $\rightarrow \text{Cu}^{\text{II}}$ CT transitions.²⁸ These high-spin CT states can be represented by nine single determinants constructed from the high-spin state ground-state MO description given in Figure 8. In Scheme 4, the single determinants of the CT states are described where a_z, e_x, e_y represent the donor MOs and a_z^*, e_x^*, e_y^* represent the acceptor MOs in which the subscripts $x, y,$ and z refer to the oxo p -orbitals in the MOs. Here, the donor–acceptor bonding interactions of the a and a^* MOs (pseudo- σ) are the weakest and those of the e and e^* MOs (σ) are the strongest. Thus, the $^4A(a_z \rightarrow a_z^*)$ CT state would be the lowest in energy, whereas $^4A/^4E(e_{x,y} \rightarrow e_{x,y}^*)$ would be the highest. Also, the $^4E(e_{x,y} \rightarrow a_z^*)$ state would be higher in energy than the $^4E(a_z \rightarrow e_{x,y}^*)$ state as the electron repulsion is expected to be larger in the single-center ligand-based donor MOs, leading to wider energy gap between the donor a_z and $e_{x,y}$ MOs than that of the acceptor a_z^* and $e_{x,y}^*$ MOs. Overall, the relative energy order of the oxo $\rightarrow \text{Cu}^{\text{II}}$ CT states would be $^4A(a_z \rightarrow a_z^*) < ^4E(a_z \rightarrow e_{x,y}^*) < ^4E(e_{x,y} \rightarrow a_z^*) < ^4A/^4E(e_{x,y} \rightarrow e_{x,y}^*)$.

Considering the fact that the positive band 5 has a small z -polarized character ($\sim 3\%$) and that its energy is the lowest of the oxo $\rightarrow \text{Cu}^{\text{II}}$ CT transitions, this band must be associated with the $^4A(a_z \rightarrow a_z^*)$ state. In the zeroth-order, however, while the $^4A \rightarrow ^4E$ transition is x,y -polarized and MCD-active, the $^4A \rightarrow ^4A$ transition is only z -polarized and MCD-inactive. There-

Scheme 4. Single-Determinant Descriptions of the Quartet Ground and CT States of $\mu_3\text{O}$



fore, the presence of the band 5 intensity indicates that there must be SOC between $^4A(a_z \rightarrow a_z^*)$ and an MCD-active 4E state. This intensity borrowing mechanism is described by eq 4:³⁶

$$\langle ^4A_{\text{gr}} M_S | m_{+/-} | ^4A M'_S \rangle = \langle ^4E M_S | H_{\text{SOC}} | ^4A M'_S \rangle \langle ^4A_{\text{gr}} M_S | m_{+/-} | ^4E M_S \rangle / E(^4E - ^4A) \quad (4)$$

where $|^4A_{\text{gr}}\rangle$ is the ground state, H_{SOC} is the SOC operator, $m_{+/-}$ is the electric dipole operator, and $E(^4E - ^4A)$ is the energy separation between the 4E and 4A excited states. This intensity borrowing mechanism is described in Figure 12, in which ZFS states of the 4A and 4E states are labeled with the C_3 double group representations.³⁷ While the SOC splitting of the 4A state is expected to be small due to the lack of orbital angular momentum, the 4E state will exhibit in-state SOC splitting of the eight degenerate states into four equally spaced doubly degenerate states. Before SOC, only the $^4A(\Gamma_6, -3/2) \rightarrow ^4E(\Gamma_5, -3/2)$ and $^4A(\Gamma_6, -3/2) \rightarrow ^4E(\Gamma_4, -3/2)$ transitions absorb LCP and RCP light, respectively ($-3/2$ refers to the M_S value). These transitions will result in two oppositely signed C -terms that may be observed as a pseudo- A -term, depending on the in-state SO splitting of the 4E state; if the splitting is too small, however, the C -terms will not be resolved and the intensities will cancel. As the out-of-state SOC becomes active, the Γ_5 components of the 4A and 4E states are coupled via $L_+S_- + L_-S_+$ ($\Delta M_S = \pm 1$), allowing the MCD-inactive $^4A \rightarrow ^4A$ to gain intensity that involves only left-circularly polarized (LCP) light.

In the $\mu_3\text{O}$ structure, the orbital origin of the SOC would be found at the oxo ligand as this site is the only common center of any two orthogonal oxo $\rightarrow \text{Cu}^{\text{II}}$ CT transitions in this structure. The in-plane O p_x - and O p_y -orbitals are degenerate and can rotate into each other by L_z . Alternatively, the out-of-plane O p_z -orbital can rotate into O p_x and O p_y by L_y and L_x , respectively. As the SOC is oxo-based, the in-state splitting of

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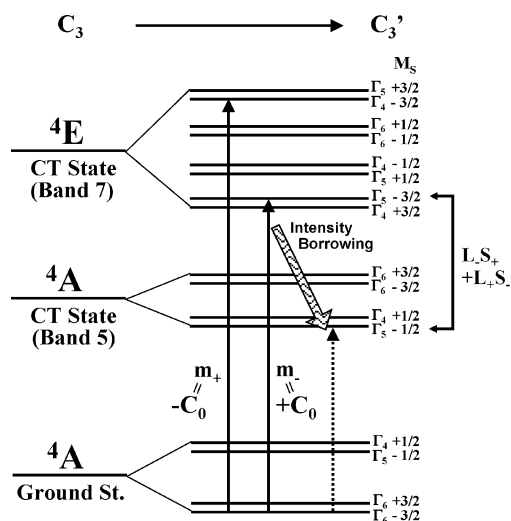


Figure 12. Energy diagram of the ferromagnetic $\mu_3\text{O}$ showing intensity borrowing via SOC ($=L_zS_+ + L_zS_-$). The ZFS states are labeled in the C_3 double group representations.

the ^4E excited state is expected to be small ($\xi_0 \approx 60\text{--}70\text{ cm}^{-1}$) and not resolved in the spectrum. Therefore, the observed pseudo-A-terms with splittings of $3800\text{--}9200\text{ cm}^{-1}$ cannot derive from the in-state SOC splitting of a ^4E excited state. Alternatively, if the positive C-term intensity is shifted into the $^4\text{A}(a_z \rightarrow a_z^*)$ state, the negative C-term component of the in-state pseudo-A-term will no longer be canceled and a pseudo-A-term of the two oppositely signed C-terms would result. From the determinants given in Scheme 4, $^4\text{A}(a_z \rightarrow a_z^*)$ would SOC with $^4\text{E}(e_{x,y} \rightarrow a_z^*)$ as the two determinants differ by a one-electron excitation and the resulting unpaired electrons are in the O p_z - and O p_x/p_y -based donor MOs. This SOC requires rotation of the orbitals in the x - or y -direction ($=L_xS_x + L_yS_y = 1/2(L_+S_- + L_-S_+)$). Therefore, the negative band 7 would correlate to the $^4\text{E}(e_{x,y} \rightarrow a_z^*)$ state. Band 7 is more intense than band 5, which indicates that this transition has additional interactions with the intense $\text{N} \rightarrow \text{Cu}^{\text{II}}$ CT transitions. Finally, the other two x,y -polarized bands 6 and 9 can be correlated to the MCD-allowed ^4E states according to their energy order, with band 6 as $^4\text{A} \rightarrow ^4\text{E}(a_z \rightarrow e_{x,y}^*)$ and band 9 as $^4\text{A} \rightarrow ^4\text{E}(e_{x,y} \rightarrow e_{x,y}^*)$ transitions.³⁸

(c) $\mu_3\text{O}$ with an Antiferromagnetic Ground State. The quartet ground state is not intrinsic to the μ_3 -oxo structure. As shown above, it is possible to have an antiferromagnetically coupled $\mu_3\text{O}$ by shifting the oxo ligand closer to the Cu_3 plane (Scheme 2).¹¹ Therefore, we evaluate the electronic transition properties of an antiferromagnetically coupled $\mu_3\text{O}$, especially for the purpose of extrapolating the quartet $\mu_3\text{O}$ model to the doublet ground state of NI.

If the oxo ligand is in the Cu_3 plane, contributions from the out-of-plane O p_z -orbital in the $\text{Cu}\text{--O}$ bonds can be neglected. For the O $p_{x,y} \rightarrow \text{Cu}^{\text{II}}$ CT transitions, group theory predicts $4^2\text{A} + 4^2\text{E}$ CT states.²⁸ Similar to the TrisOH case, all CT transitions from the ^2E ground state are x,y -polarized (thus, MCD allowed) that can be coupled via L_z , which induces out-of-state SOC between two ^2A or two ^2E states or in-state SOC within the ^2E

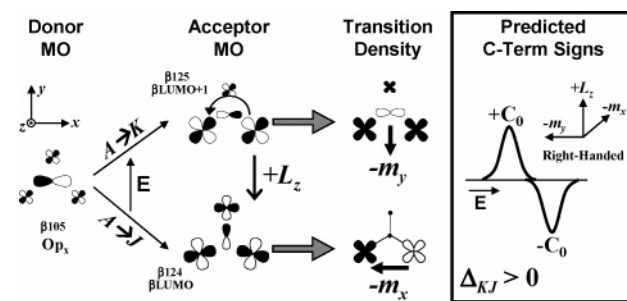


Figure 13. Graphical prediction of the C-term signs of the σ oxo $\rightarrow \text{Cu}^{\text{II}}$ CT transitions of antiferromagnetic $\mu_3\text{O}$. The counterclockwise rotation of the SOC-active O p_x in β_{125} to O p_y in β_{124} yields positive overlap ($+L_z$). The directions taken from the center of the negative overlap (white) to that of the positive overlap (black) in the transition densities (the products of the donor and acceptor MO densities) define the signs of the transition moments ($-m_x$ and $-m_y$). The right-handed coordinate system defined by ($-m_x, -m_y, +L_z$) with positive $\Delta_{KJ} \approx E(\beta_{125}) - E(\beta_{124})$ leads to a pseudo-A-term with a low-energy (+) C-term component.

states. With the oxo-based SOC in the $\mu_3\text{O}$ structure, the in-state splitting of a ^2E CT state would be small and, thus, would not produce a significant MCD feature. On the other hand, the two unoccupied β -MOs (β_{124} and β_{125}) in the broken-symmetry calculations (Figure 8) show an energy difference of 1500 cm^{-1} . As the two unoccupied β -MOs describe the overall bonding and exchange interactions, this energy would be a good estimate of the energy difference of a pair of CT states in a pseudo-A-term.³⁵ Therefore, it is possible to have out-of-state SOC of the eight available CT states to form four pseudo-A-terms.

The signs of the C-terms of a doublet $\mu_3\text{O}$ can be predicted following the same procedure as in the TrisOH case (see above). Here, we implement the donor O p_x β -MO, β_{105} , and the two acceptor β -MOs, β_{124} and β_{125} (Figure 9), to represent the $|A\rangle \rightarrow |J\rangle$ and $|A\rangle \rightarrow |K\rangle$ transitions in which the splitting between $|A\rangle \rightarrow |J\rangle$ and $|A\rangle \rightarrow |K\rangle$ is represented by that of the two acceptor β -MOs (1500 cm^{-1} ; Figure 8).³⁵ In Figure 13, the graphical description of the signs of the transition dipole moment, the SOC matrix element, and the predicted pseudo-A-term is illustrated. The SOC element L_z^{KJ} is obtained from the SOC-active p-orbitals of the oxo center: the counterclockwise rotation from the O p_x in β_{125} to the O p_y in β_{124} yields positive overlap. In addition, the signs of the transition moments, m_x and m_y , are obtained from the center of the negative overlap (white) to that of the positive overlap (black) in the transition densities. Overall, $D_y^{AJ} = 0$, $D_x^{AJ} = -m_x$, $D_x^{KA} = 0$, $D_y^{KA} = -m_y$, $L_z^{KJ} = +L_z$, and $\Delta_{KJ} \approx E(\beta_{125}) - E(\beta_{124}) > 0$ are obtained. Substitution of these parameters into eq 3 yields (+) $C_0(A \rightarrow J)$ and, consequently, (−) $C_0(A \rightarrow K)$. Figure 13 shows that the vector components ($-m_x, -m_y, +L_z$) compose a right-handed coordinate system (with $\Delta_{KJ} > 0$), indicating that LCP light is absorbed by the $|A\rangle \rightarrow |J\rangle$ transition to produce a (+) C-term. Note that the sign of the pseudo-A feature for the antiferromagnetically coupled $\mu_3\text{O}$ is opposite to that of TrisOH. As specific MO descriptions are implemented, the predicted the signs of the pseudo-A-terms would be unique for each Cu_3 structure.

Discussion

1. Structural Assignment of the Trinuclear Cu Site in the Native Intermediate.

Using the combination of absorption, VT/

(38) As the intensity of band 6 is very low and that of band 9 (possibly three bands from $^4\text{E} + 2^4\text{A}$ CT states from $e_{x,y} \rightarrow e_{x,y}^*$ configurations in Scheme 4) is obscured by the intense $\text{N} \rightarrow \text{Cu}^{\text{II}}$ CT transitions, the MCD mechanisms of bands 6 and 9 were not analyzed.

VTVH MCD spectroscopies, and DFT calculations, this study presents a detailed analysis of the electronic spectra of two trinuclear Cu^{II} model complexes that represent two possible oxygen-bridged structures of the trinuclear Cu site in NI. In particular, we have elucidated the different MCD intensity mechanisms involved in the prominent pseudo-*A*-terms observed in the CT region of the MCD spectra of the antiferromagnetic TrisOH and the ferromagnetic $\mu_3\text{O}$. Moreover, shifting the oxo ligand into the Cu_3 plane in $\mu_3\text{O}$ results in antiferromagnetic ground state relevant to NI,¹¹ and intense pseudo-*A*-terms are also expected to be present in the CT region of its MCD spectrum.

The foundation of the difference in the MCD intensity mechanisms of TrisOH and $\mu_3\text{O}$ lies in the distinct excited-state SOC pathways. For TrisOH, a symmetrically shaped pseudo-*A*-term originates from the *x,y*-polarized $\text{OH}^- \rightarrow \text{Cu}^{\text{II}}$ LMCT (Figure 2). In this case, only the metal-centered SOC is possible that requires the same Cu center to be involved in both *C*-term components of the pseudo-*A* feature (Figure 10a). These involve CT from two OH^- -ligands to the same Cu center. The energy splitting of the two *C*-terms in the pseudo-*A* feature derives from the different bonding interaction of the $\text{OH}-\text{Cu}-\text{OH}$ chromophores in the trimeric manifold (Figure 7). Although most of the charge in the donor MOs resides on the OH^- -ligands, an OH-based SOC mechanism is not feasible in TrisOH as the O–H bonding interaction eliminates the in-plane $\text{O } p_{\text{H}}$ -orbital required for SOC via L_z (Figure 10b).

Alternatively, the pseudo-*A* feature originating from the oxo $\rightarrow \text{Cu}^{\text{II}}$ LMCT in the MCD spectrum of the ferromagnetically coupled $\mu_3\text{O}$ (Figure 3) involves intensity borrowing by the *z*-polarized ($^4\text{A} \rightarrow ^4\text{A}$) CT transition from the MCD-allowed *x,y*-polarized ($^4\text{A} \rightarrow ^4\text{E}$) CT transition (Figure 12). This mechanism involves excited-state SOC that requires rotation of an electron from the out-of-plane $\text{O } p_z$ -orbital to one of the in-plane $\text{O } p_x$ - and $\text{O } p_y$ -orbitals. Thus, for $\mu_3\text{O}$, the oxo *p*-orbitals play a dominant role in the SOC of the CT excited states. The same oxo-based SOC mechanism applies to an antiferromagnetically coupled $\mu_3\text{O}$. In this case, the in-plane $\text{O } p_x$ and $\text{O } p_y$ would SOC two σ -type *x,y*-polarized CT transitions, resulting in possibly up to four symmetric and highly intense pseudo-*A*-terms. The energy splitting of the *C*-term components in the pseudo-*A* features would originate from the different bonding interactions of the oxo ligand with the three Cu centers. However, in contrast to TrisOH, this oxo-centered SOC mechanism involves coupling of CT transitions from one oxo ligand to different Cu centers (Figure 10c). In addition, the sign of the pseudo-*A*-term of $\mu_3\text{O}$ would be opposite to that of TrisOH (Figure 13).

These characteristic MCD pseudo-*A* features of the tris-OH-bridged and μ_3 -oxo-bridged structures can now be compared to that of NI. In Figure 14, the exchange coupling coefficients ($-2J$) and the MCD spectrum of tree laccase NI from ref 3 are reproduced. J 's are large and comparable ($-2J = 435\text{--}520 \text{ cm}^{-1}$), but their differences result in the 150 cm^{-1} gap between the ground and the low-lying doublet states as determined by EPR Orbach analysis and VT MCD.³ Consequently, each Cu center in the trinuclear cluster site contributes differently to the ground- and the low-lying excited-state trimer wave functions. As a result, different Cu centers contribute to each *C*-term component of the pseudo-*A* features, as determined from the

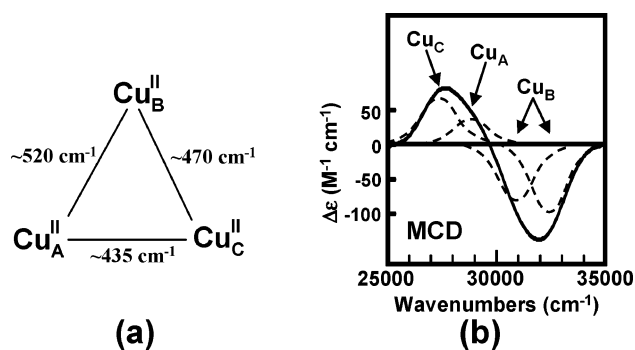


Figure 14. (a) Exchange coupling constants and (b) LT MCD spectrum of NI (data taken from ref 3). The four resolved bands are the $\text{O} \rightarrow \text{Cu}^{\text{II}}$ CT transitions associated with different Cu centers.

temperature dependence of the MCD intensities. The four resolved bands in Figure 14b are labeled by the Cu centers associated with each CT transition; the two bands labeled Cu_B indicate that both CT states have large contributions from the Cu_B center.

Therefore, we can eliminate TrisOH as a possible model for NI since the MCD intensity mechanism for TrisOH requires the two *C*-term components of a pseudo-*A*-term to be associated with the same Cu center. Alternatively, $\mu_3\text{O}$ uses an oxo-based SOC mechanism that couples CT transitions to two different Cu centers, consistent with NI. Therefore, we propose that $\mu_3\text{O}$ represents the $\text{Cu}-\text{O}$ bonding structure in NI. In addition, the sign of the pseudo-*A*-term of NI is opposite to that experimentally observed for TrisOH (Figure 2), but is in accordance with that predicted for the antiferromagnetic $\mu_3\text{O}$ (Figure 13). Thus, the sign of the pseudo-*A*-term adds further support for the μ_3 -oxo-bridged structure.

The oxo bridge would provide an efficient ground-to-excited superexchange pathway to promote antisymmetric exchange, in addition to the low symmetry of the trinuclear site, to generate a splitting of 150 cm^{-1} between the ground and the low-lying doublet states of NI.¹¹ The antisymmetric exchange also provides a mechanism that leads to the uniquely low *g*-values observed in the LT EPR spectrum of NI ($g = 2.15, 1.86, 1.65$).³ We have demonstrated, using a combination of single-crystal EPR and VT/VTVH MCD, that antisymmetric exchange in an antiferromagnetically coupled trimeric Cu^{II} system intermixes the ZFS doublets of the spin-frustrated ^2E ground state.⁸ As a result, the energy levels behave nonlinearly when the magnetic field is rotated away from the C_3 -axis of the trimer. Thus, the low *g*-values in NI demonstrate the anisotropic SOC provided by the μ_3 -oxo bridge.

Specific assignments of the four resolved bands in the pseudo-*A*-term in the MCD spectrum of NI can be considered on the basis of the ligand environments around the T2 and T3 Cu centers. The T2 Cu would have a four-coordinate square planar site with the μ_3 -oxo ligand, two histidines, and an OH^- -ligand.³⁹ Each of the T3 Cu's would have the μ_3 -oxo ligand and three histidines. In addition, an extra OH^- bridge could exist between the T3 Cu centers as considered in a recent QM/MM study.⁴⁰ This OH^- -ligand would be the second oxygen product of the O–O bond cleavage. It is not clear whether this ligand remains at the trinuclear site as an extra OH^- -ligand (possibly as a

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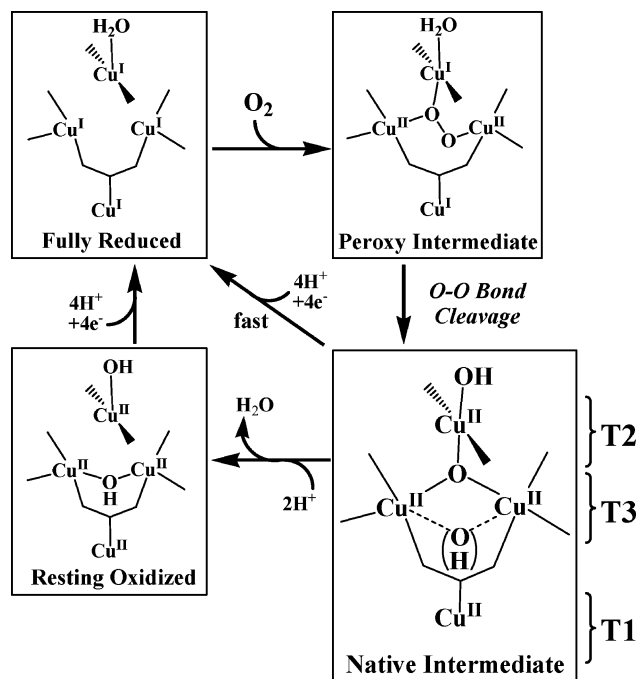


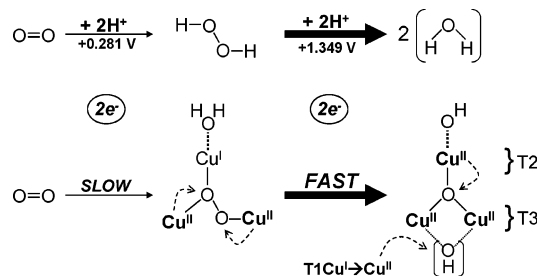
Figure 15. Proposed mechanism for the 4e⁻ reduction of O₂ to H₂O by the multicopper oxidases.

bridging ligand between the T3 Cu's) or whether it dissociates to conserve the total charge of this site.³⁹ Depending on the presence of the OH⁻-ligand, the T3 Cu centers would be either four-coordinated tetrahedral or five-coordinated trigonal bipyramidal/square pyramidal sites.

The splitting of the *C*-terms in the pseudo-*A* features of NI strongly depends on the relative energies of the singly occupied acceptor d-orbitals at the two Cu sites that are involved in the oxo → Cu^{II} CT transitions. The relative energies of these acceptor d-orbitals can be considered in the context of ligand field theory. Two factors exist that will destabilize the acceptor d-orbital of the T2 Cu relative to those of the T3 Cu centers. First, the T2 Cu site has a square planar ligand field that destabilizes the acceptor orbitals by several thousand cm⁻¹ (~4000–5000 cm⁻¹)⁴¹ relative to the ligand field environment of the T3 Cu sites. Second, the strong donor OH⁻-ligand at the T2 site would destabilize the acceptor d-orbital by an additional several thousand cm⁻¹. Overall, it is likely that the acceptor d-orbital of the T2 Cu is higher in energy than those of the T3 Cu's. Therefore, the two higher energy (–) *C*-terms in Figure 14b (labeled Cu_B) would correspond to the oxo → T2 Cu^{II} CT, and the two lower energy (+) *C*-terms (labeled Cu_A and Cu_C) would correspond to the oxo → T3 Cu^{II} CT transitions. This assignment suggests stronger electronic couplings of the T2 to the T3 Cu's relative to that of the two T3 Cu's (520/470 cm⁻¹ vs 435 cm⁻¹; Figure 14a). This difference in exchange coupling is dependent on the nature of the magnetic orbitals on each Cu (a ligand field effect) and their relative interaction with bridging ligand(s), providing an experimental test for possible specific structural models of NI (i.e., whether an additional OH⁻ bridge is present between the T3 Cu's).

2. Mechanistic Implications. The overall molecular mechanism for the 4e⁻ reduction of O₂ to H₂O by the MCO is presented in Figure 15, with the most plausible structures

Scheme 5. Comparison of O₂ Reduction in Aqueous Solutions and in the Multicopper Oxidases^a



^a The reduction potentials given for the aqueous solution reaction are formal potentials at pH 7 for O₂ at unit activity. The dotted arrows depict transfer of electrons from copper to oxygen centers.

available for each state. In the catalytic cycle, the fully reduced trinuclear site reacts with O₂ to form the peroxy intermediate (PI)^{42,43} via a 2e⁻ process ($k \approx 2 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$),⁴⁴ in which two electrons have been transferred from the T3 Cu's to O₂. In a recent QM/MM study, an optimized structure of PI with the peroxide-like O₂ species that internally bridges the T2 and T3 centers has been reported.⁴⁰ This structure is also supported by the crystal structure of a peroxy adduct form of CotA.⁴⁵ PI undergoes a second 2e⁻ process ($k > 350 \text{ s}^{-1}$)⁴³ to form NI with all four Cu centers oxidized; this process involves the reductive cleavage of the O–O bond. Since the first 2e⁻ step is rate determining and the second is fast in the native enzyme, this is effectively a 4e⁻ reduction of O₂. Finally, NI undergoes either a fast re-reduction to the fully reduced enzyme or a slow decay process ($k = 0.034 \text{ s}^{-1}$)⁴⁶ to the resting oxidized form that does not involve a redox reaction.

There are several important implications of the μ_3 -oxo-bridged structure of NI in the overall catalytic process. First, the μ_3 -oxo bridge provides a relatively stable structure that serves as the thermodynamic driving force for the 4e⁻ process of O₂ reduction. Scheme 5 depicts the 2e⁻ steps of O₂ reduction in aqueous solution and in the MCO. In aqueous solutions at pH 7, both 2e⁻ reductions of O₂ (O₂ to H₂O₂ and H₂O₂ to H₂O) are accessible, with the second step having a larger driving force due to the 5-fold larger reduction potential. The Cu centers in the trinuclear cluster would similarly react with O₂, as these serve both as the source of electrons (from reduced Cu^I) and as Lewis acids (the resultant oxidized Cu^{II}) in place of H⁺ for efficient 4e⁻ reduction of O₂. Moreover, the reaction would be driven by the significant decrease of the reduction potentials of the Cu centers by the Cu^{II}–O σ -bonding interactions, in contrast to the redox properties of the coordinatively unsaturated metal centers in the resting enzyme, where $E^\circ(\text{T1/T2/T3}) \approx +400 \text{ mV}$.^{1b} In the first 2e⁻ process, an internal peroxide bridge is formed by oxidizing the T3 Cu's and is also bridged to the reduced T2 Cu, which allows facile ET from the T2 Cu to the peroxide for the second 2e⁻ step (second electron from T1 Cu), leading to cleavage of the O–O bond and the μ_3 -oxo structure of NI. This structure would be advantageous over the tris-OH-

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bridged structure, as the latter requires a larger rearrangement of the internal oxygen and would be entropically less favorable with coordination of an additional H₂O for the third OH⁻ bridging ligand and a proton.

Second, the μ_3 -oxo-bridged structure provides efficient ET pathways from the T1 site to all of the Cu centers in the trinuclear cluster, which allows for fast reduction of the fully oxidized trinuclear site in NI to generate the fully reduced site for further turnover with O₂. The reduction rate of the resting oxidized form is very slow; the electronic isolation of the T2 from the T3 centers is potentially responsible for this behavior.⁴⁷ In NI, the strong in-plane Cu–O σ -bonds of the μ_3 -oxo-bridged structure, as demonstrated by the large superexchange coupling constants and the highly intense pseudo-A-term in MCD (Figure 14b), provide effective exchange coupling for rapid ET from the T1 site to all the Cu centers in the trinuclear cluster. Thus, NI is the catalytically relevant fully oxidized form of the enzyme, with the μ_3 -oxo-bridged structure allowing efficient turnover in the catalytic cycle. Note that the trinuclear cluster site is surrounded by four negatively charged residues to neutralize the highly positive trinuclear cluster site.³⁹ Therefore, the oxo-bridged structure in NI would be deficient of positive charges and, as a result, would be readily protonated, most likely at the oxo ligand to produce H₂O.

Finally, the structural definition of NI provides insight into the slow decay process of NI to the resting oxidized form of the enzyme. ¹⁸O isotope ratio mass spectrometry (IRMS)^{42,48} and ¹⁷O EPR⁶ studies have shown that one oxygen atom of O₂ after turnover is present in the resting oxidized enzyme and is bound terminally to the paramagnetic T2 site. This would be the OH⁻-ligand on the T2 Cu that lies outside the trinuclear cluster. As this oxygen atom likely derives from the μ_3 -oxo ligand in NI, the decay process would require a large structural

rearrangement of this atom from inside to outside of the cluster, resulting in a slow process. Note that this decay process would require protons, one at the μ_3 -oxo and a second at the external OH⁻-ligand. The large translation of the μ_3 -hydroxo ligand to the outside of the cluster would leave a coordinatively unsaturated trinuclear cluster that would be stabilized by the combined effects of the extensive hydrogen-bonding network and the charge compensation by the surrounding carboxylate residues.³⁹

In conclusion, we have provided a plausible structural description of the native intermediate form of the multicopper oxidases based on the characteristic pseudo-A feature observed in the charge-transfer region of its MCD spectrum. By elucidating the SOC origin of the pseudo-A intensity mechanism, we have found that only a μ_3 -oxo-bridged structure is consistent with the spectroscopic properties of this key enzymatic intermediate. In-depth knowledge of the geometric and electronic structures of this intermediate in the enzymatic cycle of the MCOs provides insight into (1) the 4e⁻ reduction of O₂ in the MCOs, (2) the fast re-reduction in the turnover step of the catalytic cycle, and (3) the slow decay of NI to the resting enzyme in which both forms are fully oxidized but only the former contains a catalytically active trinuclear Cu cluster site.

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Supporting Information Available: Complete ref 12, the full MCD spectrum of μ_3 O, VTVH MCD plots of μ_3 O and TrisOH, the MO contours of TrisOH (the high-spin state β_{106} – β_{108} and the broken-symmetry state O p _{π} and O p_H β -MOs), the molecular coordinates of the TrisOH model in the DFT calculations, and the MO energies and compositions of the TrisOH in the high-spin and broken-symmetry states. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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