Supporting Material:

Spectroscopic and Kinetic Studies of Perturbed Trinuclear Copper Clusters: The Role of Protons in Reductive Cleavage of the O-O Bond in the Multicopper Oxidase Fet3p.

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Figure S1. Room temperature absorption spectra (A) and X-band EPR spectra at 77 (B) of holo WT Fet3p and E487D, E487A, and D94E mutants

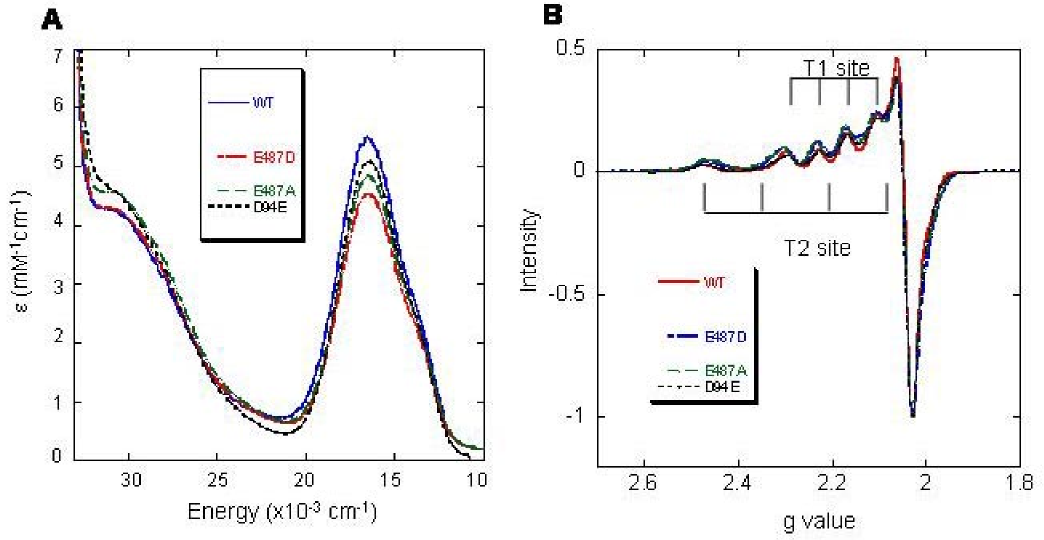

Figure S2. Gaussian Fits of absorption and CD spectra

	T1D		T1DE487D		T1DE487A		T1DD94E	
Band no.	energy[a]	$\Delta\varepsilon/\varepsilon$[b]	energy[a]	$\Delta\varepsilon/\varepsilon$[b]	energy[a]	$\Delta\varepsilon/\varepsilon$[b]	energy[a]	$\Delta\varepsilon/\varepsilon$[b]
1 d-d	10200	N/D	10200	N/D	10200	N/D	10200	N/D
2 d-d	12500	-8.41	12500	-6.3	12500	-4.61	12430	-18.4
3 d-d	14080	1.39	14080	4.19	14080	1.56	15490	3.46
4 d-d	15120	-9.47	15120	-5.95	15120	-2.18	13190	-0.99
5 d-d	17300	18.9	17300	18.5	17300	19.0	17200	23.1
6 d-d	19630	31.2	19630	13.1	19630	12.0	19100	29.7
7 His π	24770	-0.68	24770	-0.68	24750	-0.19	23900	-1.11
8 μ-OH	28000	0.38	28000	0.33	28000	0.48	28250	0.22
9 μ-OH	30400	-0.18	30650	-0.2	30800	-0.10	30800	-0.17

[a] Energies are in cm-1 and obtained through a simultaneous fit of the CD and absorption spectra. [b] The Khun anisotropy factors are a ratio of the CD intensity ($\Delta\varepsilon$ $M^{-1}cm^{-1}$) to the absorption intensity (ε $mM^{-1}cm^{-1}$).

Figure S3. EPR Spin Hamiltonian Parameters

	T1D	T1DE487D	T1DE487A	T1DD94E
g_x	2.041	2.041	2.041	2.038
g_y	2.055	2.055	2.055	2.055
g_z	2.243	2.243	2.243	2.243
Ax^a	17	17	17	17
Ay^a	15	15	15	15
Az^a	190	190	190	188

[a] All hyperfine coupling constants, A values, are shown in X x 10^{-4} cm^{-1}

Figure S4. Gaussian fit of MCD spectra

	T1D	T1DE487D	T1DE487A	T1DD94E
Band no.	energy/cm^{-1}	energy/cm^{-1}	energy/cm^{-1}	energy/cm^{-1}
1 *xy*	(+) 9800	(+) 9800	(+) 9800	(+) 10000
2 z^2	(-) 10540	(-) 10540	(-) 10540	(-) 11080
3 *xz*	(+) 11700	(+) 11700	(+) 11700	(+) 11540
4 *yz*	(-) 13940	(-) 13940	(-) 13940	(-) 14380
5 His π	(-) 16700	(+) 18930	(+) 18720	(-) 16800
6 OH π	(-) 20140	(+) 21980	(+) 22330	(-) 20400
7 His π	(-) 24800	(+) 26590	(+) 25510	(-) 25280
8 His π	(+) 28270	(+) 27930	(+) 27500	(+) 28300
9 His π	(+) 30800	(+) 30940	(+) 30960	(+) 30900

Figure S5. Room temperature CD spectrum of T1D PI from 5000 – 33,000 cm^{-1}

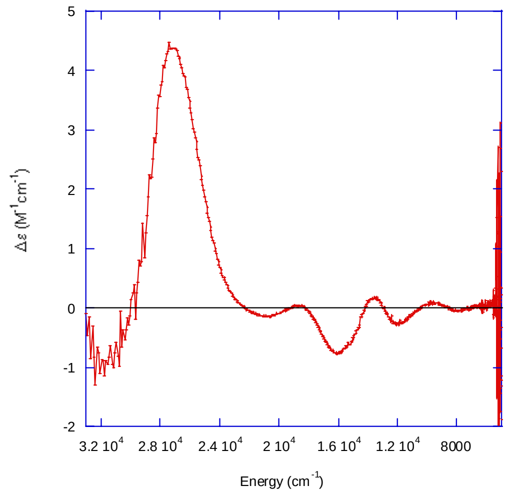

Figure S6. Gaussian Fits of absorption and CD spectra

	T1D PI		T1DE487D PI		T1DE487A PI		T1DD94E PI	
Band no.	energy[a]	Δε/ε[b]	energy[a]	Δε/ε[b]	energy[a]	Δε/ε[b]	energy[a]	Δε/ε[b]
2	11690	-0.72	11600	-0.63	12000	-0.78	12430	-0.62
3	14230	0.54	14270	13.4	14310	0.74	13920	1.44
4	15280	-1.61	15100	-5.95	15150	-1.76	14680	-4.39
5	16540	-0.98	16510	-0.64	16470	-0.58	16060	-0.83
6	20850	-0.15	20650	-0.10	21130	-0.02	20920	-0.06
7	24800	0.26	25020	0.77	25040	0.21	24550	0.30
8	27580	0.94	27730	1.12	27580	1.20	27210	0.76
9	30625	-0.21	31340	-0.40	30820	-0.15	30660	-0.19

[a] Energies are in cm-1 and obtained through a simultaneous fit of the CD and absorption spectra. [b] The Khun anisotropy factors are a ratio of the CD intensity (Δε $M^{-1}cm^{-1}$) to the absorption intensity (ε $mM^{-1}cm^{-1}$).

S7. For analysis of kinetic isotope effects using the Westheimer model, a linear transition state is assumed of type A------B------C where in this case A is the starting point, a carboxylate and C is the ending point, the μ2-OH in the native intermediate, and B is the proton of interest.

The kinetic isotope effect (KIE) is given by the following formula:

KIE = (zero-point energy) x (mass and moments of inertia) x (excited vibrational states)

In the Westheimer model the KIE is only dependent upon the zero-point energy (ZPE) as the other components are approximated to be 1, which is a fair approximation under the conditions of the experiments performed herein.

The KIE was then calculated for 3 possible transition states (TS): a product-like TS, a reactant-like TS, and a symmetric TS where the proton (B) is located near the midpoint between A and C. The results are summarized in the following table:

Position of TS	KIE
Reactant	1.00
Symmetric	6.90
Product	0.70

In all cases, the initial A-B bond frequency (that for a carboxylate O-H stretch) was taken as 3000 cm^{-1} and the final product B-C bond frequency (that for the μ2-OH stretch in the native intermediate) was taken as 3600 cm^{-1}. And the ZPE was calculated as:

$\mathrm{ZPE} = \exp[1/2(U_{\mathrm{Hreactant}} - U_{\mathrm{Dreactant}}) - (U_{\mathrm{HTS}} - U_{\mathrm{HTS}})]$ and $U_i = h\nu_i/k_BT$

Where h is Planck's constant (6.626×10^{-34} J s), k_B is Boltzmann's constant (1.38066×10^{-23} J K^{-1}), and T the absolute temperature (T = 293 K for the calculations in this paper).