Supporting Information

O$_2$ Reduction to Water by High Potential Multicopper Oxidases: Contributions of the T1 Copper Site Potential and the Local Environment of the Trinuclear Copper Cluster

Alina Sekretaryova, Stephen M. Jones, Edward I. Solomon*

Department of Chemistry, Stanford University, 333 Campus Drive, Stanford, California 94305-5080, United States
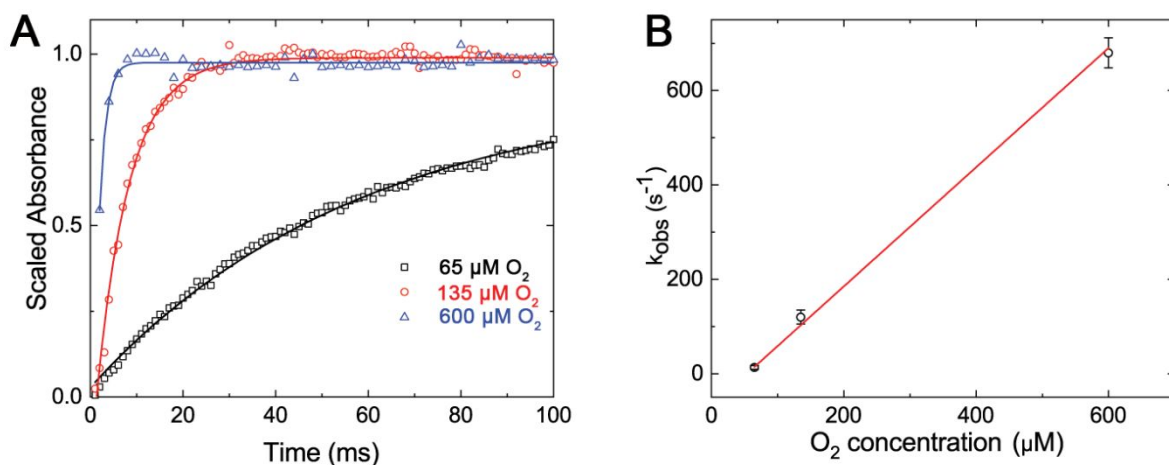
Figure S1. Reaction of the reduced *TvL* with O_2 at various concentrations monitored by a rapid scan stopped-flow instrument at pH 6.0 and 4 °C; [Lc] = 50 μM. (A) Absorption traces of the T1 (610 nm) charge transfer band, various O_2 concentrations are shown by different colors: $[O_2]$ = 65 μM in black, 135 μM in red, 600 μM in blue; open dots are experimental data, solid lines are kinetic fits to the pseudo-first order rate equation. (B) The dependence of the calculated pseudo-first rate constant on O_2 concentrations, yielding the second-order rate constant of $(1.2\pm0.3)\times10^6$ M^{-1} s^{-1}.
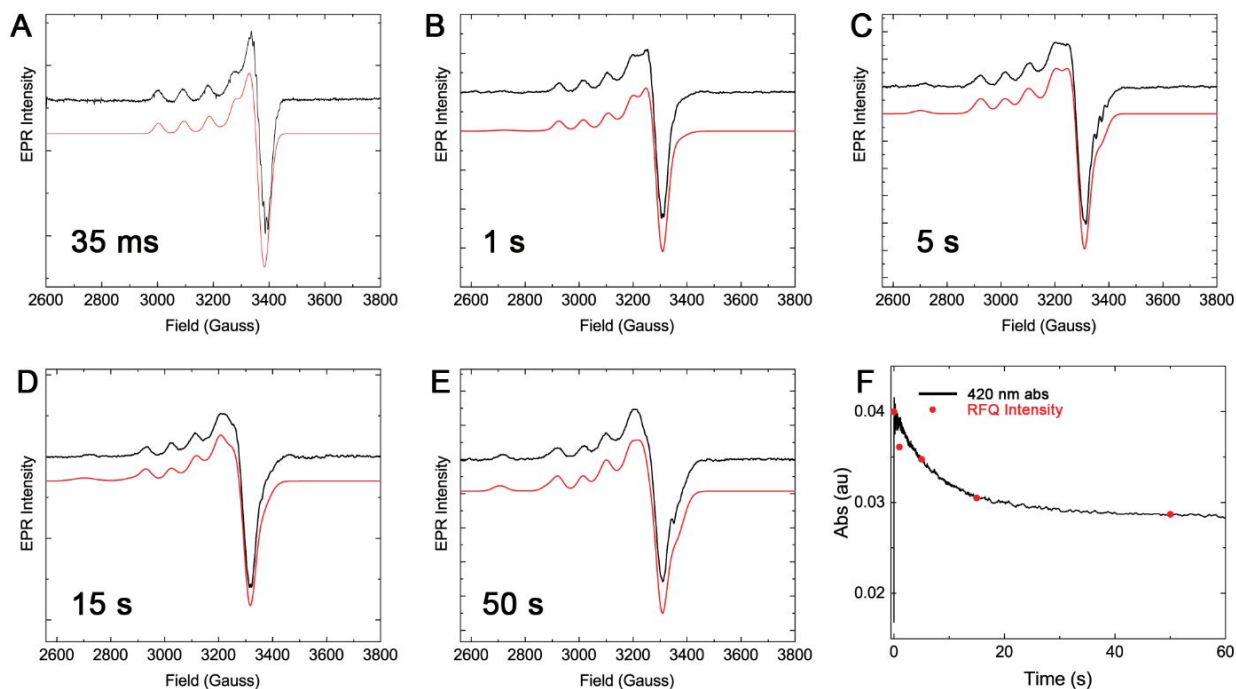
Figure S2. The native intermediate decay kinetics at pH 6.0 monitored by the RFQ-EPR and stopped-flow absorbance. (A)-(E) RFQ-EPR spectra (black) and fits (red) of the native intermediate at 35 ms, 1s, 5s, 15s and 50 s, respectively. (F) Kinetic profiles of UV−vis absorbance at 420 nm (black line) with overlaid relative EPR intensities of the intermediate (red dots).
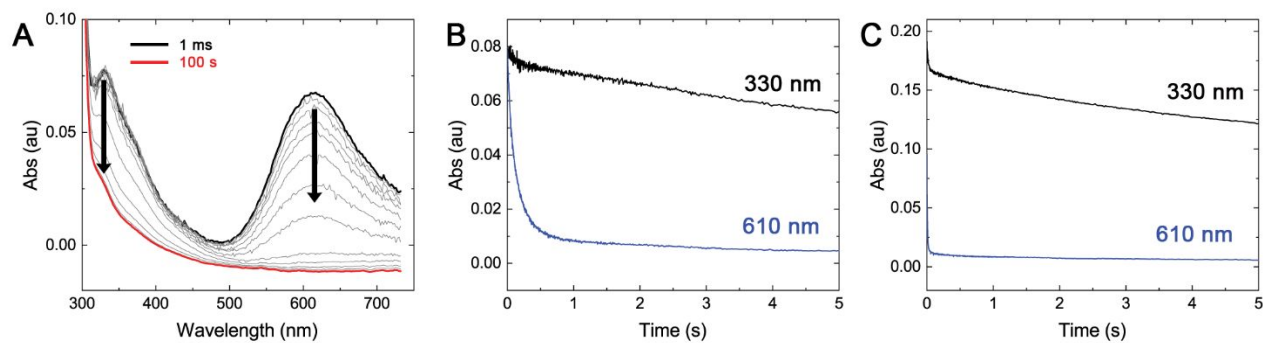
Figure S3. Resting oxidized enzyme reduction by ascorbic acid monitored by a rapid scan stopped-flow instrument at pH 6.0 and 4 °C; [Lc] = [O_2] = 0.05 mM (A) Absorption spectra from 1 ms to 100 s; [Ascorbic acid] = 0.25 mM. (B) and (C) Absorption traces of the T1 (610 nm, blue) and TNC (330 nm, black) charge transfer bands in the reduction of RO with 0.25mM and 5 mM ascorbic acid, respectively.
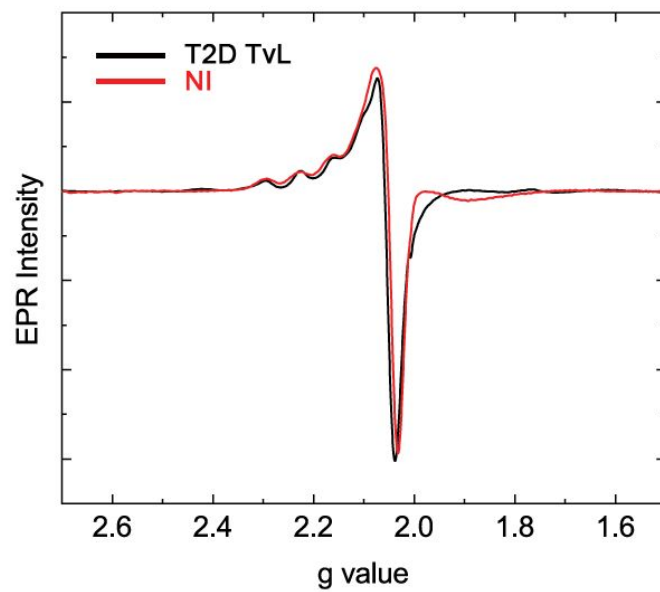
Figure S4. Overlaid EPR spectra of the native intermediate and T2D *TvL* at 20 mW and 5K.
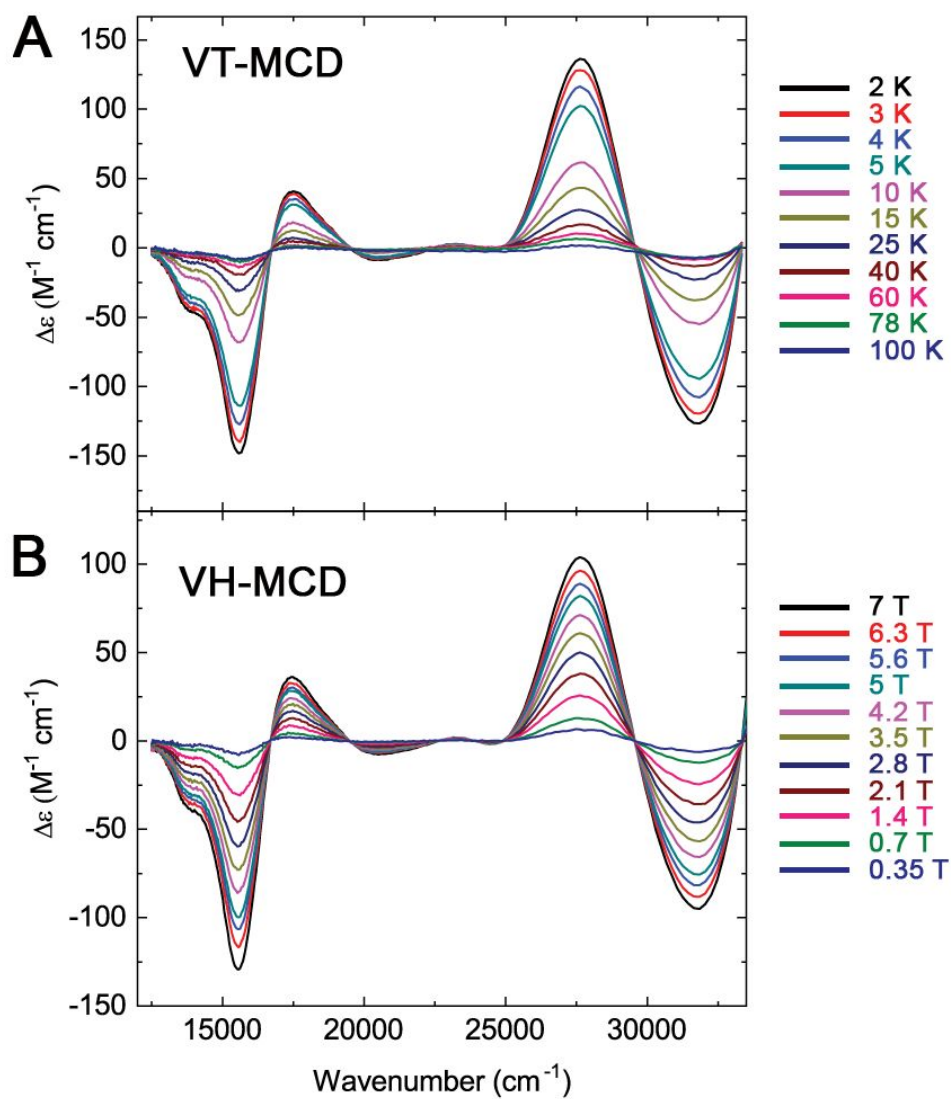
Figure S5. VT and VH-MCD spectra of the native intermediate (A) The native intermediate MCD spectra at 2, 3, 4, 5, 10, 15, 25, 40, 60, 80 and 100 K with a constant field set at 7T. (B) The native intermediate MCD spectra at 0.35, 0.7, 1.4, 2.1, 2.8, 3.5, 4.2, 5.0, 5.6, 6.3, 7T at 5K.
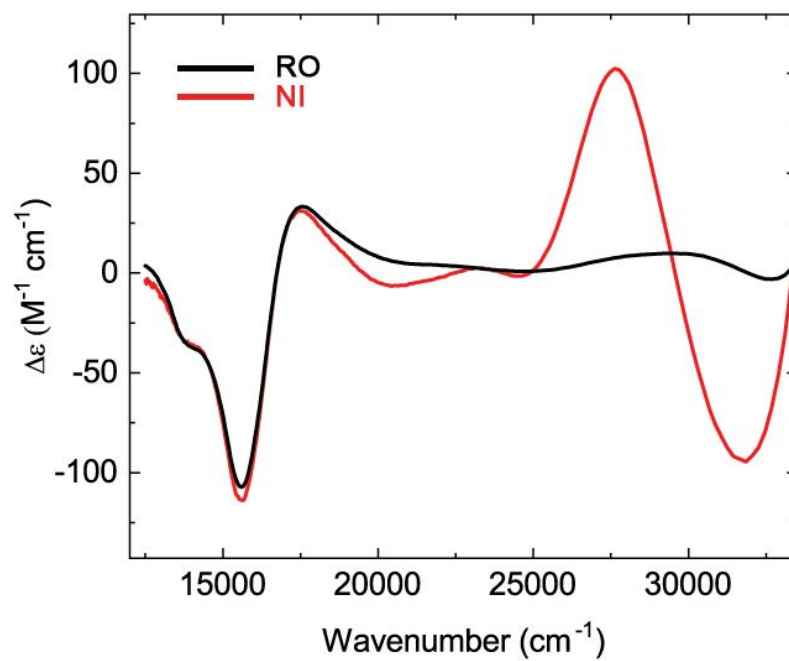
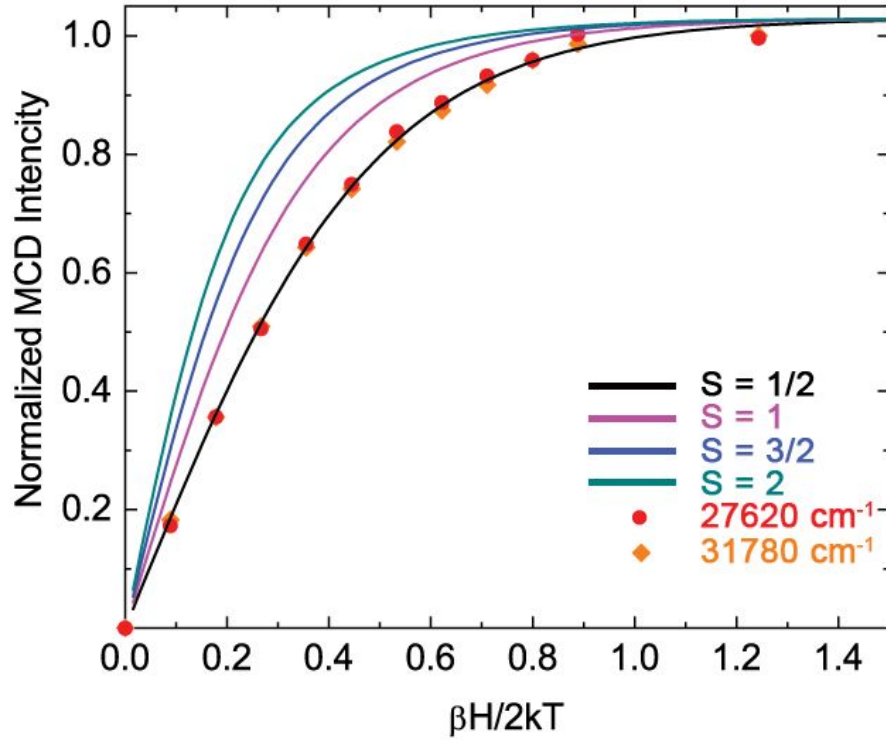**Figure S6**. The native intermediate (red) and resting oxidized (black) MCD spectra at 7T and 5K.

Figure S7. Saturation magnetization of the NI MCD spectrum at 27620 and 31780 cm-1. Temperature is set at 1.89K and the applied field varied from 0 to 7 T. Solid lines are normalized Brillouin curves using g value of 2.06 and the spin states as shown. The curves were generated using the Brillouin function, Bs (x), for a ground state with various given spin states according to equations:

$$M = NgS\beta B_s(x) \tag{S1}$$

$$B_s(x) = \left[\frac{2S+1}{2S}\coth\left(\frac{2S+1}{2S}x\right) - \frac{1}{2S}coth\left(\frac{1}{2S}x\right)\right] \tag{S2}$$

where x is gSβH/kBT, N is Avogadro's number, S is a given spin state, β is the Bohr magneton (9.274×10-24 J T-1), H is the applied field in tesla.

Table S1. Experimental spectroscopic parameters for the Native Intermediate in *TvL*

Band	Assignment	Energy, cm^{-1}	$\Delta\varepsilon$, M^{-1} cm^{-1}	ε, M^{-1} cm^{-1}	C/D
1	μ_3-oxo $\rightarrow$ Cu^{II}	24700	-8.7	1052	-0.0078
2	μ_3-oxo $\rightarrow$ Cu^{II}	27350	126	1360	0.087
3	μ_3-oxo $\rightarrow$ Cu^{II}	28400	38	2300	0.015
4	μ_3-oxo $\rightarrow$ Cu^{II}	31000	-178	2110	-0.079
5	μ_3-oxo $\rightarrow$ Cu^{II}	32250	-89	1105	-0.075