

SUPPORTING INFORMATION

**O₂-evolving Chlorite Dismutase as a Tool to
Study O₂-Utilizing Enzymes[†]**

*Laura M. K. Dassama,^a Timothy H. Yosca,^b Denise A. Conner,^b Michael H. Lee,^a Béatrice
Blanc,^c Bennett R. Streit,^c Michael T. Green,^b Jennifer L. DuBois,^{c,*} Carsten Krebs,^{a,b,*} J.
Martin Bollinger, Jr.^{a,b,*}*

Departments of ^aBiochemistry and Molecular Biology and ^bChemistry, The Pennsylvania
State University, University Park, Pennsylvania 16802 and ^cDepartment of Chemistry and
Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556

Table S1: EPR spin quantification obtained from a range of packing factors

Packing factor	Total spin (mM)	Mono Mn ^{II} (mM)	X (mM)	Mn ^{IV} /Fe ^{IV} (mM)
0.50	2.18	0.60	0.13	1.45
0.52	2.09	0.62	0.13	1.34
0.54	2.02	0.66	0.12	1.24
0.56	1.95	0.67	0.12	1.16
0.58	1.88	0.69	0.11	1.07
0.60	1.82	0.72	0.11	0.99

Table S2: Spin-Hamiltonian Parameters of the Mn^{IV}/Fe^{IV} intermediate in *Ct* RNR-β₂

Parameter	Fe ^{IV} site	Mn ^{IV} site
A (MHz)	-54.5, -59.5, -43.5 (-55.9, -59.3, -40.5)	247, 216, 243
δ (mm/s)	0.19 (0.17)	
ΔE_Q (mm/s)	-0.62 (-0.75)	
η	-9 (-10)	

FIGURES, CAPTIONS, AND LEGENDS

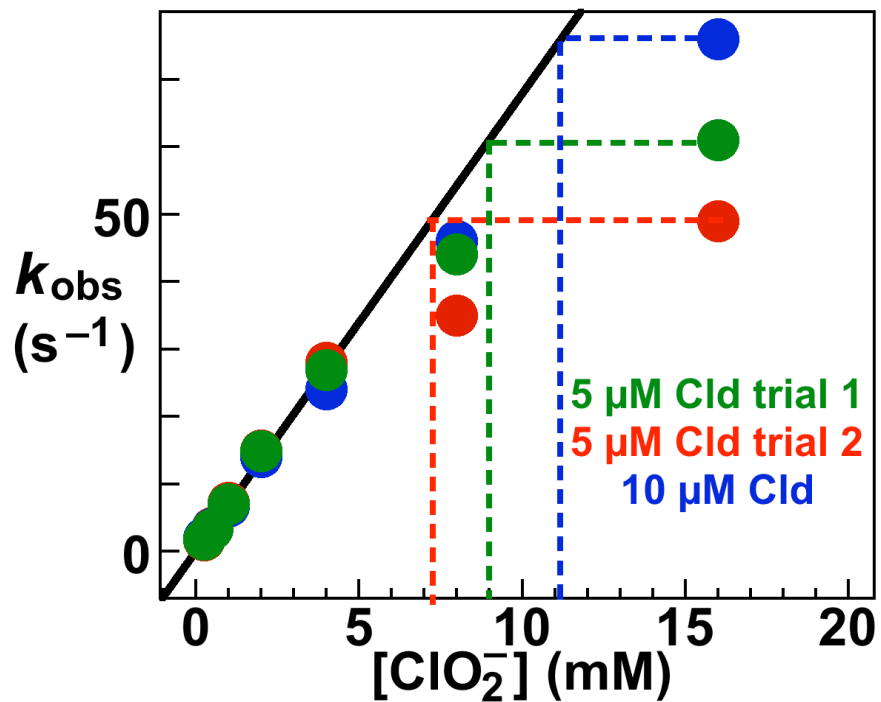


Figure S1. Observed first-order rate-constant for $\text{Mn}^{\text{IV}}/\text{Fe}^{\text{IV}}$ intermediate formation as a function of ClO_2^- from three independent experiments employing two different concentrations of Cld. The onset of the deviation from the first-order dependence on $[\text{ClO}_2^-]$ occurs at a greater value of $[\text{ClO}_2^-]$ with the greater $[\text{Cld}]$ (10 μM), suggesting that the deviation is associated with inefficiency or inactivation of the Cld catalyst at high concentrations of ClO_2^- .

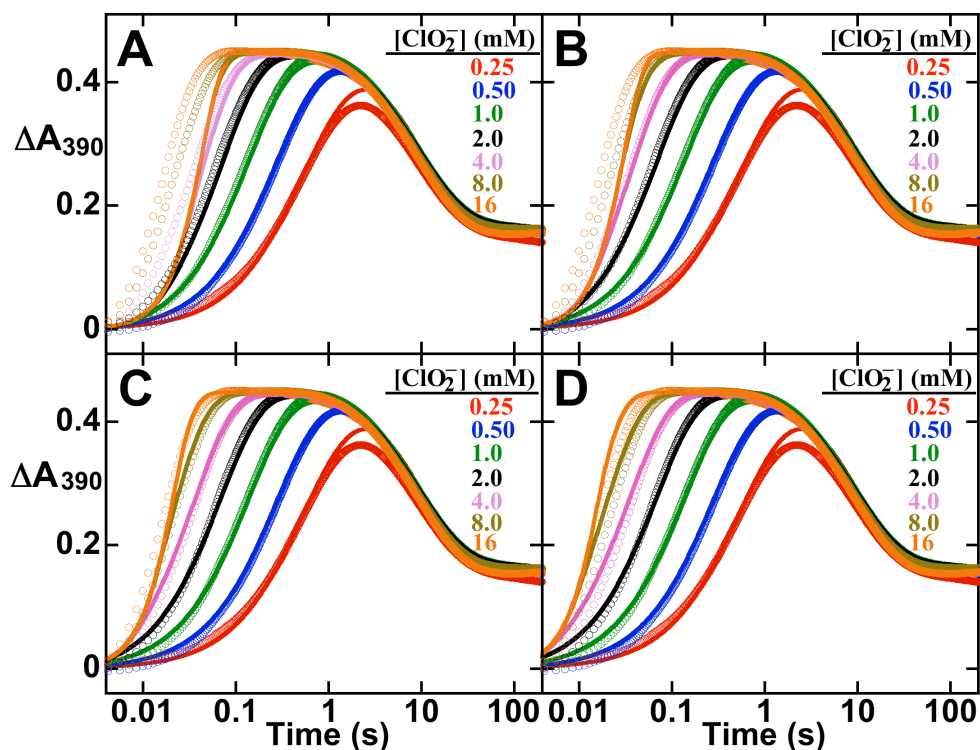


Figure S2. Kinetic simulations to assess the k_{cat} of ClD under the reaction conditions described in Fig. 1B. The simplest two-step kinetic model for ClD, in which ClO_2^- binds non-covalently with a K_D of 215 μM [the reported K_M of the reaction (1)] and is converted in a single step to free O_2 and Cl^- with a phenomenological rate constant of k_{cat} , was assumed, and k_{cat} was varied. Simulated traces with the same range of $[\text{ClO}_2^-]$ used in Fig. 1B and with k_{cat} values $\leq 60,000 \text{ s}^{-1}$ (A and B) give noticeably poor agreement with the data for $[\text{ClO}_2^-] > 4 \text{ mM}$. An assumed k_{cat} of $120,000 \text{ s}^{-1}$ (C) gives much better agreement between the simulated traces and the data, while a value of $200,000 \text{ s}^{-1}$ (D) is obviously too high. This analysis suggests that the true k_{cat} under these conditions is $\sim 120,000 \text{ s}^{-1}$, in agreement with published results (2).

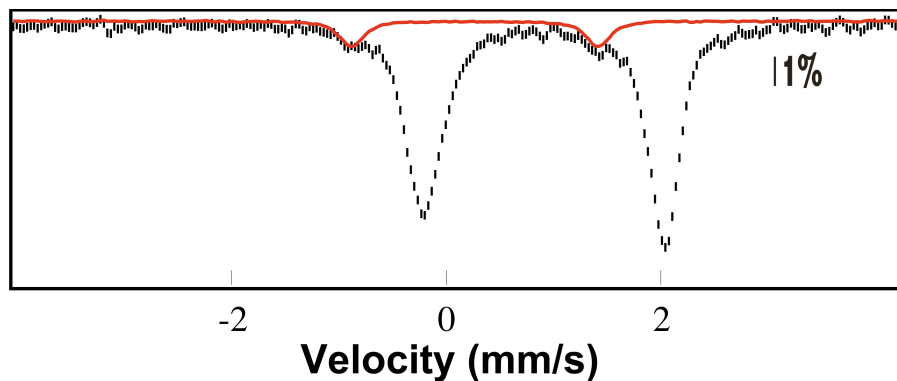


Figure S3. Mössbauer spectrum (4.2 K/53 mT parallel field) of a freeze-quench sample prepared by mixing a solution of 10 mM Fe^{II}-Mb (2.5 mM ⁵⁷Fe-Mb and 7.5 mM ⁵⁶Fe-Mb) with 0.25 equivalent volumes of a solution of 100 mM ClO₂⁻ and freeze-quenching quenched after 15 ms. The spectrum has a $7 \pm 3\%$ contribution from oxy-Mb (shown in red).

REFERENCES

1. Streit, B. R., and DuBois, J. L. (2008) Chemical and steady-state kinetic analyses of a heterologously expressed heme dependent chlorite dismutase, *Biochemistry* 47, 5271-5280.
2. Streit, B. R., Blanc, B., Lukat-Rodgers, G. S., Rodgers, K. R., and DuBois, J. L. (2010) How Active-Site Protonation State Influences the Reactivity and Ligation of the Heme in Chlorite Dismutase, *J. Am. Chem. Soc.* 132, 5711-5724.