

Supporting information for:

Peroxidase reactions suggest a heterolytic/nucleophilic O-O joining mechanism in chlorite dismutase †

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Figure S1. Pseudo first order rate constant for Compound I formation from the reaction of ferric *DaCld* WT with PAA, measured as a function of [PAA] in 0.2 M citrate-phosphate buffer at pH 6, 20 °C. The apparent second order rate constant $k = 1.9 \pm 0.003 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$.

Figure S2. Time-resolved RR spectra for the reaction of *DaCld* WT with 3 equivalents of PAA at pH 5.8. The reaction mixture contains 68 μM *DaCld* WT and 200 μM PAA in 0.2 M phosphate buffer pH 5.8. Each spectrum represents a 15 s acquisition using 406.7 nm as the excitation frequency with 12 mW at the sample. The reaction was performed in a spinning NMR tube at 20 °C. Visible spectrum for these reaction times show the 404 nm species described in the text. Inset: The RR spectrum of the ferryl component of the reaction mixture obtained by subtracting the appropriate amount of Fe(III) resting enzyme spectrum (black) (as judged by the loss of the $1494 \text{ cm}^{-1} \nu_3$) from the reaction mixture spectrum obtained in 15 s (red).

Figure S3. The X-band EPR spectrum of *DaCld* reacted with PAA pH 5.8. *DaCld* (22 μM , final concentration) reacted with 9 equivalents of PAA at 22 °C was flash frozen in an acetone/liquid nitrogen bath after 14 s reaction time. Instrument parameters were 200 G sweep width, 4 G modulation amplitude, 2.0 mW power, 100 kHz modulation frequency, and 77K temperature.

Figure S4. Plot of ν_4 frequency as a function of time for the reaction of *DaCld* with PAA at pH 8.0. The reaction mixture contains 58 μM *DaCld* WT and 480 μM PAA in 0.2 M phosphate buffer pH 8.0. Each black point represents the ν_4 from a 15 s spectrum using 406.7 nm excitation. The solid red line is a single exponential fit that indicates a $t_{1/2}$ of $\sim 100\text{s}$. It is possible that other processes that are involved in the ultimate

bleaching of the enzyme are occurring in addition to Compound II conversion to resting enzyme .

Figure S5. Intermediates in the reaction of *DaCld* WT with PAA. Approximately ~15 μM *DaCld* WT (7.5 μM final) was mixed with 100 μM peracetic acid (50 μM final) all in 0.2 M citrate-phosphate buffer at pH 6, 20 °C. The spectrum of the first intermediate, $[\text{Fe}^{\text{IV}}=\text{O}(\text{P}\cdot^+)]$, is shown in red. The spectrum for the second intermediate ($[\text{Fe}^{\text{IV}}=\text{O}(\text{P})(\text{AA}\cdot^+)]$ or $[\text{Fe}^{\text{IV}}=\text{O}(\text{P})+(\text{AA}\cdot^+)]$ or $[\text{Fe}^{\text{IV}}=\text{O}(\text{P})]$ or a mixture of any of those species) formed after 8.7 seconds, is in blue. The final spectrum after 300 second is shown in green, intervening spectra are omitted for clarity.

Figure S6. Pseudo first order rate constants for the reaction of *DaCld* WT Compound I with ascorbate (top) and for the conversion of $[\text{Fe}^{\text{IV}}=\text{O}(\text{P})+(\text{AA}\cdot^+)]$ and Compound II to the ferric species (bottom) as a function of [ascorbate] in 0.2 M citrate-phosphate buffer at pH 6, 20 °C. The slope of the line for the top plot yields the second order rate constant of $k = 2.5 \pm 0.3 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$. The bottom plot was fit to a square hyperbola to yield a maximum rate of $9.3 \pm 0.5 \text{ s}^{-1}$ and apparent $K_d = 3.5 \pm 1.2 \mu\text{M}$.

Figure S7. Pseudo first order rate constants for ferric *DaCld* WT formation from the intermediate in the reaction of ferric *DaCld* with peracetic acid in 0.2 M citrate-phosphate buffer at pH 8, 20 °C., measured as a function of [PAA]. The second order rate constant is $k = 1.3 \pm 0.01 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$.

Figure S8. Pseudo first order rate constants for the reaction of intermediate formed at pH 8 in the reaction of *DaCld* WT with PAA and ascorbate as a function of [ascorbate] in 0.2 M citrate-phosphate buffer at pH 8, 20 °C. . The plot was fit to a square hyperbola to yield a maximum rate of $k = 0.6 \pm 0.01 \text{ s}^{-1}$ and an apparent $K_d = 3.6 \pm 0.3 \mu\text{M}$.

Figure S9. Pseudo first order rate constants for Compound 0 formation from the reaction of the ferric *DaCld* WT with H_2O_2 in 0.2 M citrate-phosphate buffer at pH 6, 20 °C as a function of $[\text{H}_2\text{O}_2]$. The measured $k = 9.6 \pm 0.4 \times 10^1 \text{ M}^{-1}\text{s}^{-1}$.

Figure S10. Pseudo first order rate constants for Compound 0 formation from the reaction of ferric *DaCld* WT and H_2O_2 (top) and conversion to Compound II (bottom) as a function of $[\text{H}_2\text{O}_2]$ 0.2 M citrate-phosphate buffer at pH 8, 20 °C.. The slope of the line for the top plot yields the second order rate constant $k = 1.7 \pm 0.04 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$. The bottom plot is fit to a square hyperbola yielding a maximum rate of $k = 7.9 \pm 0.3 \text{ s}^{-1}$ and apparent $K_d = 1.5 \pm 0.2 \text{ mM}$.

Figure S11. Comparison of *DaCld* potentials to those for HRP as a function of pH.

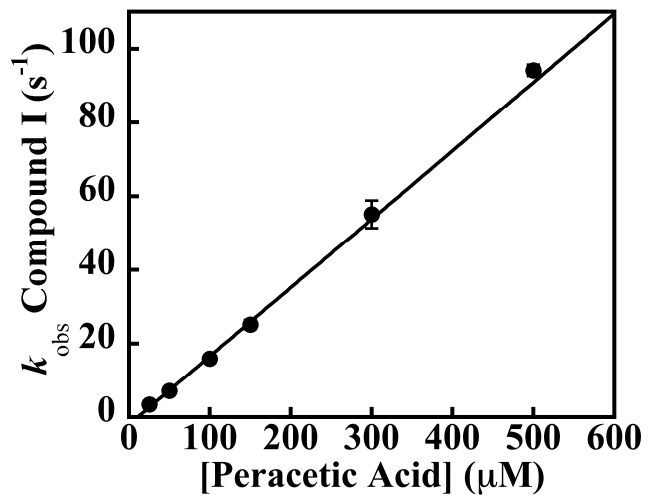


Figure S1.

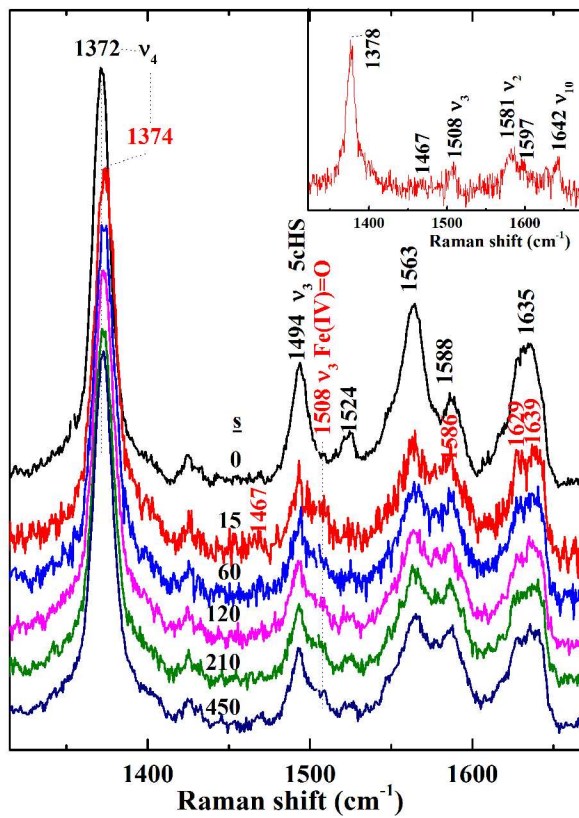


Figure S2.

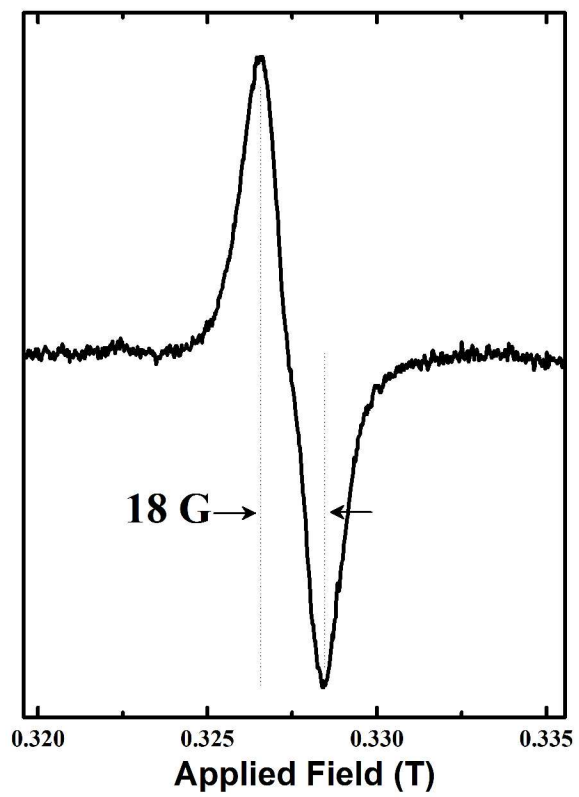


Figure S3.

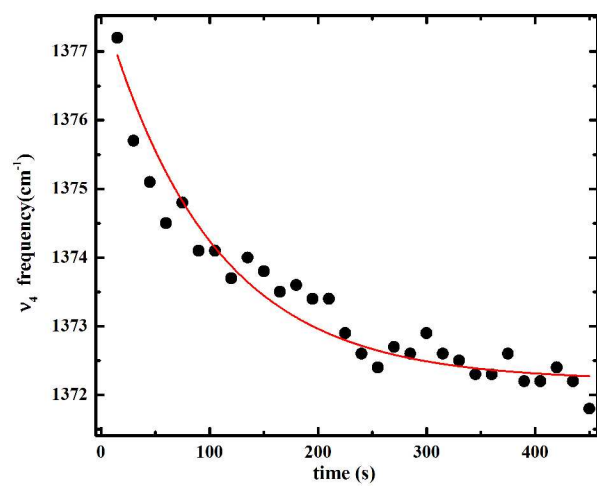


Figure S4.

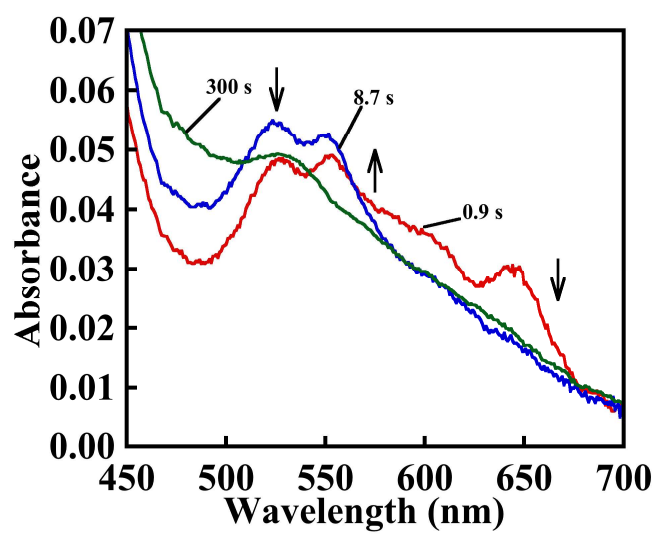
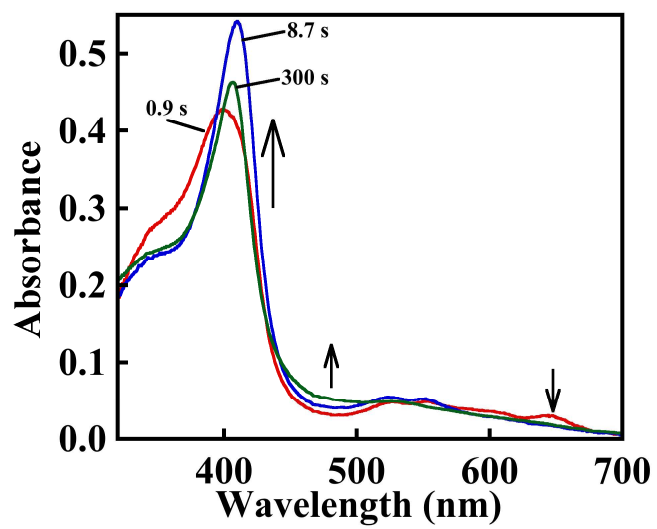


Figure S5.

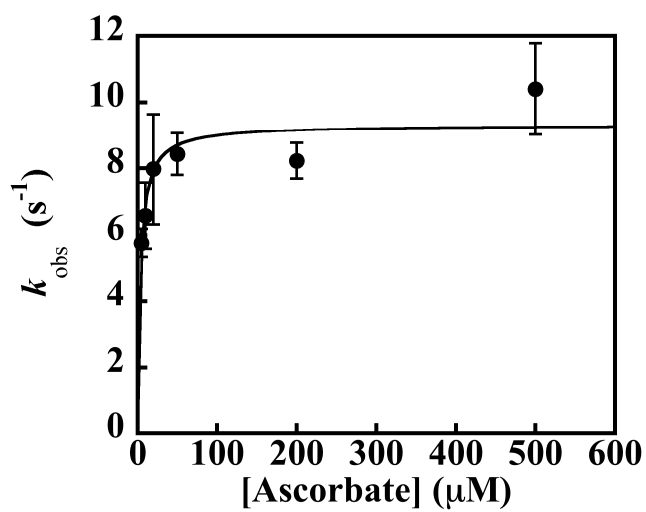
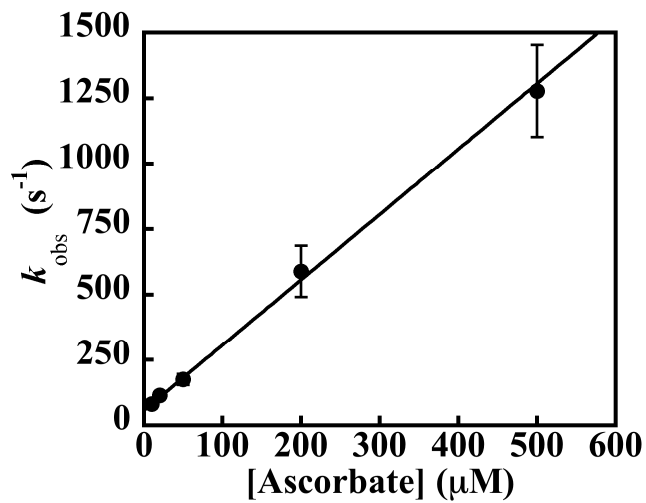


Figure S6.

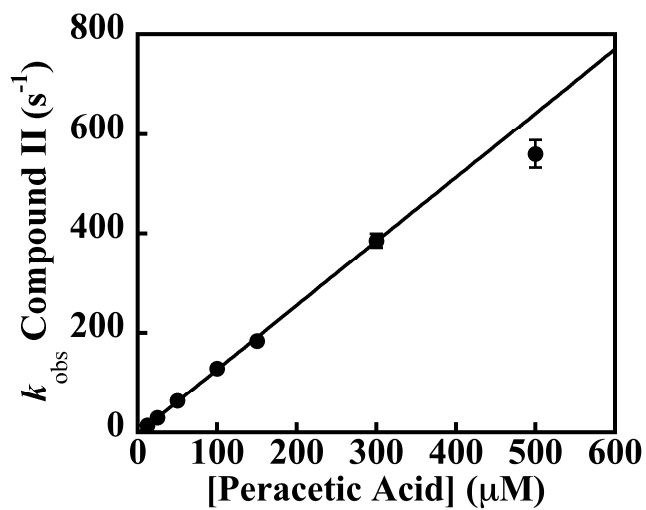


Figure S7.

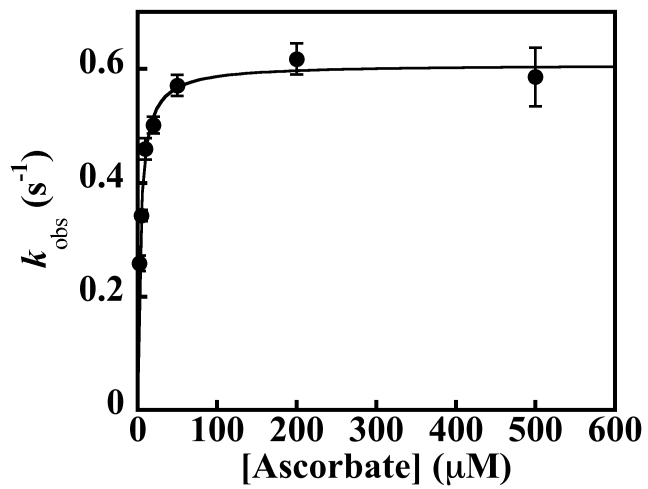


Figure S8.

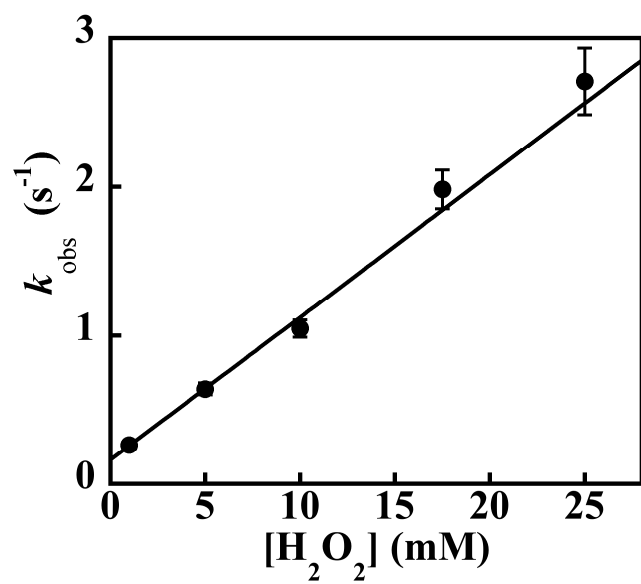


Figure S9.

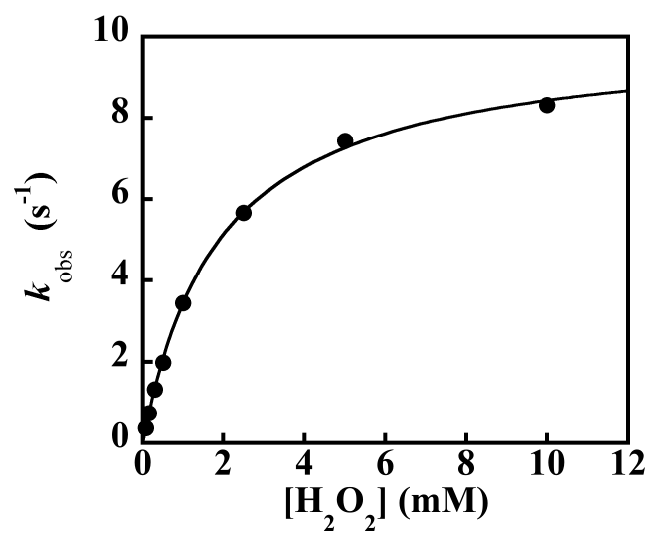
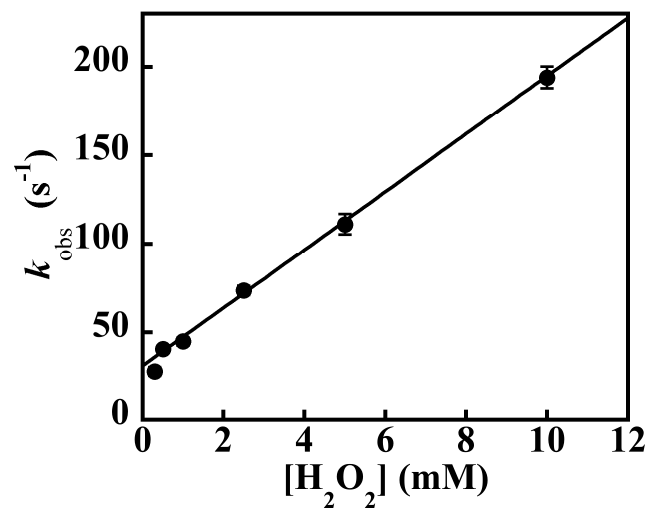


Figure S10.

Estimation of potentials:

Figure S11. The plot illustrates the 0.25 V offset of the *DaCld* potentials relative to the HRP values. The potentials for HRP-C at pH 6.0 are from reference A13. The potential lines for HRP-C are calculated using the Nerst equations whose forms are based on the balanced half cell reactions. The lines for *DaCld* are offset by +0.25V based on the difference in Fe(III)|Fe(II) potentials at pH 7.0.

