

Supporting Information for

Oxidation Reactions Performed by Soluble Methane

Monooxygenase Hydroxylase Intermediates H_{peroxo} and Q

Proceed by Distinct Mechanisms

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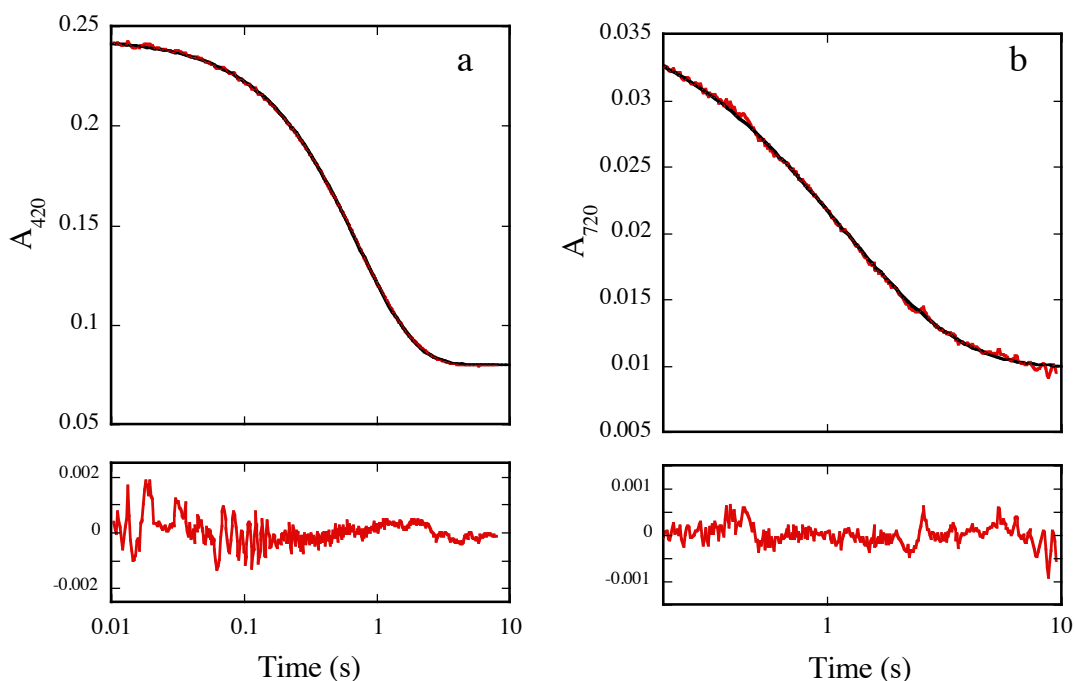


Fig. S1. Representative fits (black lines) of data (red lines) probing the reactions of Q (a) and H_{peroxo} (b) with 96 mM CH_3NO_2 at 4 °C and pH 7.0. 200 μM MMOH_{red} and 400 μM MMOB were mixed rapidly with excess O_2 , the reaction mixture was aged for 12 s (a) or 2 s (b), and then buffer containing 192 mM CH_3NO_2 was introduced. Data probing the reaction with Q fit nicely to one exponential decay phase (a). Data monitoring the reaction with H_{peroxo} fit nicely to two exponential decay phases, where the rate constant for the second process was fixed at the value monitored in experiments probing the reaction with Q (b). Fit residuals are shown in the bottom plots. These results and fitting procedures are typical for all substrates employed unless otherwise noted.

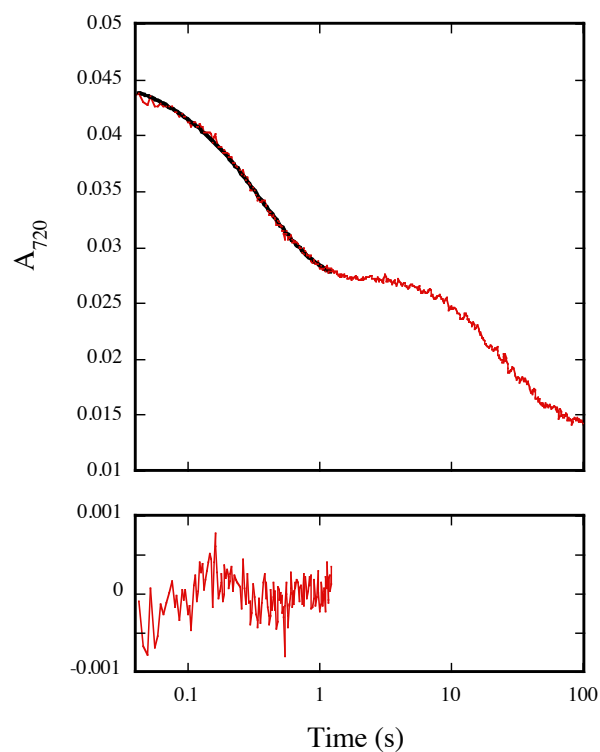


Fig. S2. Representative fit (black lines) of data (red lines) probing the reaction of H_{peroxo} with 272 mM CD_3NO_2 at 4 °C and pH 7.0. 200 μM $MMOH_{\text{red}}$ and 400 μM $MMOB$ were mixed rapidly with excess O_2 , the reaction mixture was aged for 2 s, and then buffer containing 544 mM CD_3NO_2 was introduced. Because the exponential phases corresponding to H_{peroxo} and Q decay are well separated at $[CD_3NO_2] > 200$ mM, the data were fit well by truncating and analyzing each phase separately using independent single exponential processes. Fit (top) and fit residuals (bottom) are shown here for the first, H_{peroxo} , reaction phase.

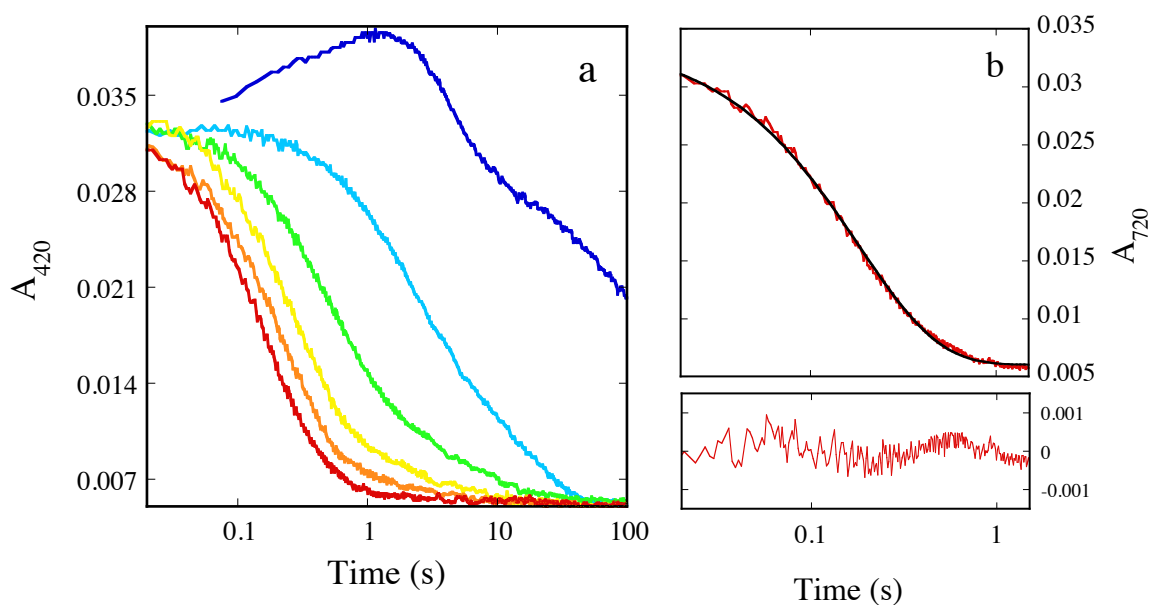


Figure S3. (a) Representative absorbance profile for the reaction of $50 \mu\text{M}$ MMOH_{red} with a mixture of excess O_2 and $\text{CH}_3\text{CH}_2\text{CHO}$ in the presence of 2 equiv MMOB at 4°C and 720 nm . $[\text{CH}_3\text{CH}_2\text{CHO}] = 0 \text{ mM}$ (blue), 5.8 mM (cyan), 24.9 mM (green), 69.7 mM (yellow), 122.3 mM (orange), and 214.9 mM (red). Data collected on separate occasions with different batches of protein yielded similar results. (b) Representative fit of data (red line) depicted in (a) collected in the presence of 214.9 mM $\text{CH}_3\text{CH}_2\text{CHO}$ to a single exponential decay process (black line). Fit residuals are depicted below the plot.

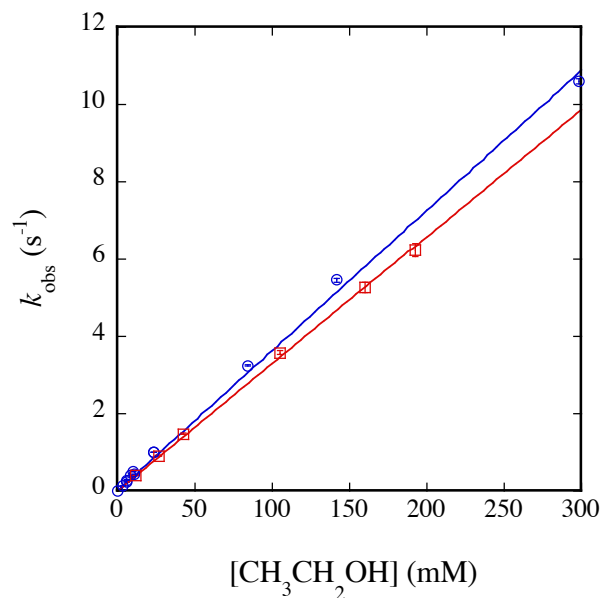


Fig. S4. Plot of k_{obs} versus $[\text{CH}_3\text{CH}_2\text{OH}]$ (circles, blue line) or $[\text{CD}_3\text{CD}_2\text{OH}]$ (squares, red line) for reaction with Q at 4 °C and pH 7.0. 200 μM MMOH_{red} and 400 μM MMOB were mixed rapidly with excess O_2 , the reaction mixture was aged for 12 s, and then buffer containing the appropriate concentration of ethanol was introduced. Data were analyzed as noted in the text. Error bars represent one standard deviation at the 95% confidence level.

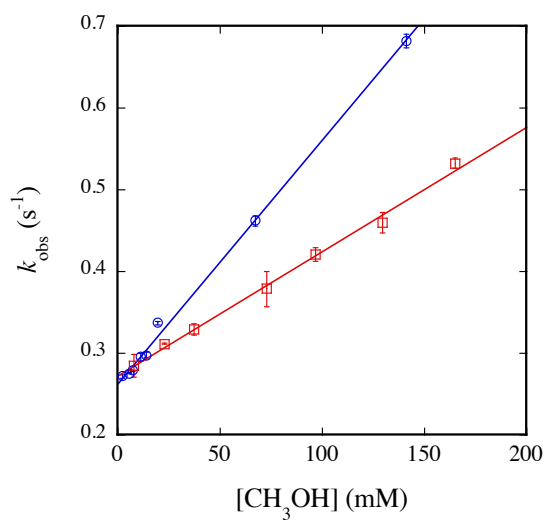


Fig. S5. Plot of k_{obs} versus $[\text{CH}_3\text{OH}]$ (circles, blue line) or $[\text{CD}_3\text{OH}]$ (squares, red line) for reaction with H_{peroxo} at 4 °C and pH 7.0. 200 μM MMOH_{red} and 400 μM MMOB were mixed rapidly with excess O_2 , the reaction mixture was aged for 2 s, and then buffer containing the appropriate concentration of methanol was introduced. Data were analyzed as noted in the text. Error bars represent one standard deviation at the 95% confidence level.

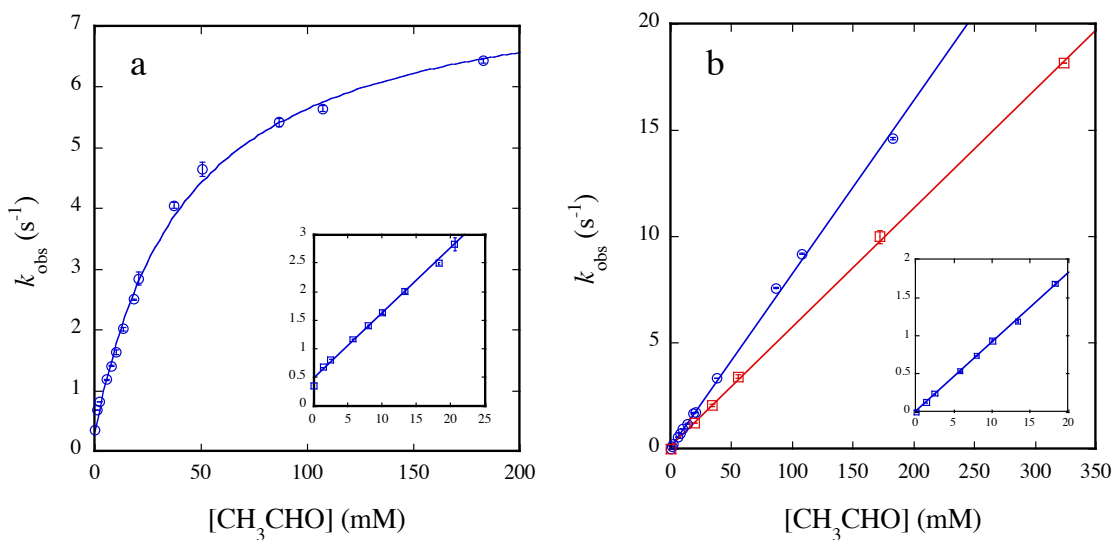


Fig. S6. Plot of k_{obs} versus $[\text{CH}_3\text{CHO}]$ (circles, blue line) or $[\text{CD}_3\text{CDO}]$ (squares, red line; inset) for reactions with H_{peroxo} (a) and Q (b) at 4 °C and pH 7.0. 200 μM MMOH_{red} and 400 μM MMOB were mixed rapidly with excess O_2 , the reaction mixture was aged for 2 s (H_{peroxo}) or 12 s (Q), and then buffer containing the appropriate concentration of acetaldehyde was introduced. Data were analyzed as noted in the text. Data collected at low $[\text{CH}_3\text{CHO}]$ (k_{init}) is depicted in the insets. Error bars represent one standard deviation at the 95% confidence level.

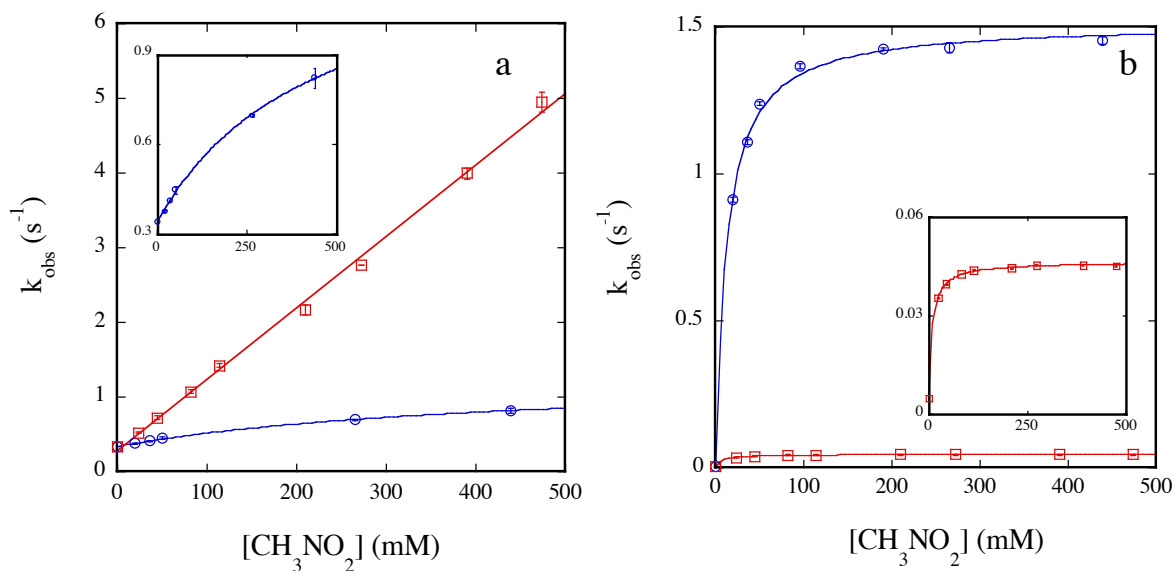


Fig. S7. Plot of k_{obs} versus $[\text{CH}_3\text{NO}_2]$ (circles, blue line) or $[\text{CD}_3\text{NO}_2]$ (squares, red line; inset) for reactions with H_{peroxo} (a) and Q (b) at 4 °C and pH 7.0. 200 μM MMOH_{red} and 400 μM MMOB were mixed rapidly with excess O_2 , the reaction mixture was aged for 2 s (H_{peroxo}) or 12 s (Q), and then buffer containing the appropriate concentration of nitromethane was introduced. Data were analyzed as noted in the text. Insets show clearer plots of data displaying small rate constants. Error bars represent one standard deviation at the 95% confidence level.

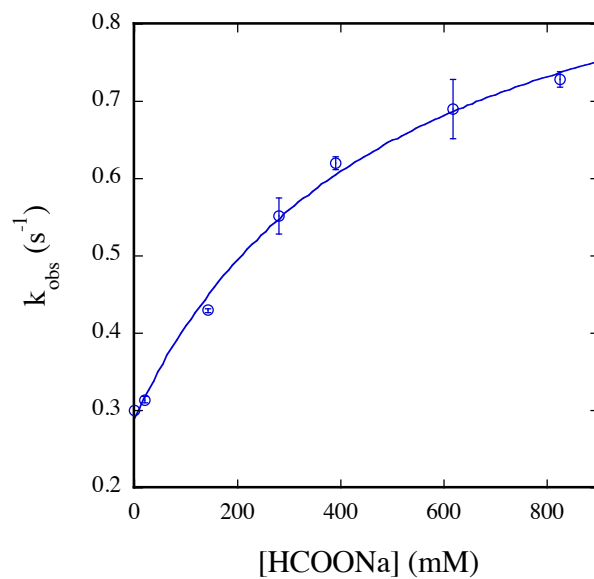


Fig. S8. Plot of k_{obs} versus [HCOONa] for reaction with H_{peroxo} at 4 °C and pH 7.0. 200 μM MMOH_{red} and 400 μM MMOB were mixed rapidly with excess O_2 , the reaction mixture was aged for 2 s, and then buffer containing the appropriate concentration of sodium formate was introduced. Data were analyzed as noted in the text. Error bars represent one standard deviation at the 95% confidence level.

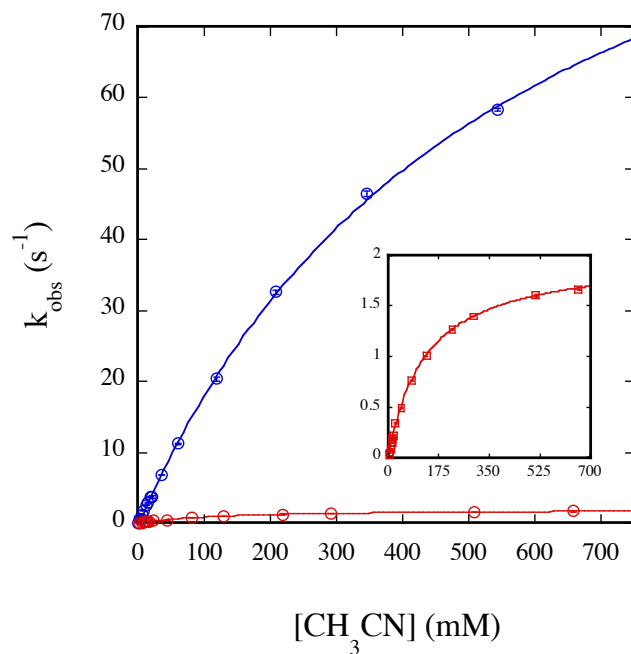


Fig. S9. Plot of k_{obs} versus $[\text{CH}_3\text{CN}]$ (circles, blue line) or $[\text{CD}_3\text{CN}]$ (squares, red line; inset) for reaction with Q at 4 °C and pH 7.0. 200 μM MMOH_{red} and 400 μM MMOB were mixed rapidly with excess O_2 , the reaction mixture was aged for 12 s, and then buffer containing the appropriate concentration of acetonitrile was introduced. Data were analyzed as noted in the text. The inset shows a clearer plots of the $[\text{CD}_3\text{CN}]$ data and fit. Error bars represent one standard deviation at the 95% confidence level.

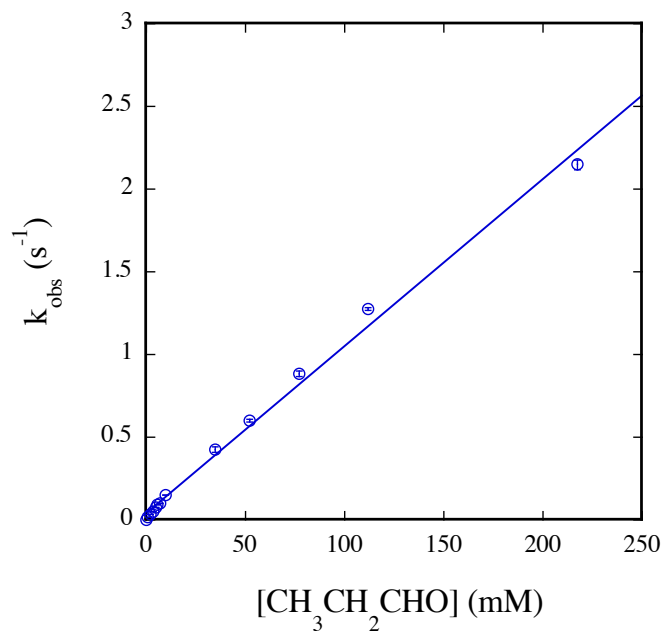


Fig. S10. Plot of k_{obs} versus $[\text{CH}_3\text{CH}_2\text{CHO}]$ for reaction with Q at 4 °C and pH 7.0. 200 μM MMOH_{red} and 400 μM MMOB were mixed rapidly with excess O_2 , the reaction mixture was aged for 12 s, and then buffer containing the appropriate concentration of propionaldehyde was introduced. Data were analyzed as noted in the text. Insets show clearer plots of data displaying small rate constants. Error bars represent one standard deviation at the 95% confidence level.