

# Supporting Information

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## SI Text

**S1. With Burst Conditions of  $O_2^-$  Where  $[Mn^{2+}] \gg [O_2^-]$ .**  $r_2$  is the slow step and  $k_{-3}$  neglected.

$r_3 \gg r_5$ ; because  $r_5$  step happens much later after  $r_3$ ; i.e.,  $r_5$  happens after all  $[MnO_2^+]$  has formed.

In fact, because  $O_2^-$  is limiting reagent,

$$[O_2^-]^i \approx [MnO_2^+]^i. \quad [1]$$

Thus, the rate of  $r_5$  and the overall rate is

$$r = r_5 = [MnO_2^+]^2 k_5 \approx [O_2^-]^2 k_5, \quad [2]$$

which is very similar to the rate law for the self-disproportionation of  $O_2^-$ , except here the rate is based on  $k_5$  and, most importantly, the species present that goes through the slow dismutation is not  $O_2^-$  but  $MnO_2^+$ —that is, there is very little free  $O_2^-$ .

Taking into account the effect  $K_4$  equilibrium

$$K_4 = \frac{[MnO_2^+ - Anion^{n-}]}{[MnO_2^+][Anion^{n-}]}. \quad [3]$$

Using Eq. 1 and letting the equilibrium form,  $x$  is equal to the  $MnO_2^+ - Anion^{n-}$  formed in the reaction. Assume change in  $[Anion^{n-}]$  concentration is negligible:

$$K_4 = \frac{x}{([O_2^-]^i - x)[Anion^{n-}]}. \quad [4]$$

Solve for  $x$

$$x = \frac{K_4 [Anion^{n-}] [O_2^-]^i}{K_4 [Anion^{n-}] + 1}, \quad [5]$$

and after equilibrium forms is equal to

$$[MnO_2^+] = [MnO_2^+]^i - [MnO_2^+ - Anion^{n-}]. \quad [6]$$

Substituting in Eqs. 1 and 5,

$$\begin{aligned} [MnO_2^+] &= [O_2^-]^i - \frac{K_4 [Anion^{n-}] [O_2^-]^i}{K_4 [Anion^{n-}] + 1} \\ &= [O_2^-]^i \left( 1 - \frac{K_4 [Anion^{n-}]}{K_4 [Anion^{n-}] + 1} \right) \end{aligned} \quad [7]$$

Finally, substituting back into Eq. 2,

$$r = \left( [O_2^-]^i \left( 1 - \frac{K_4 [Anion^{n-}]}{K_4 [Anion^{n-}] + 1} \right) \right)^2 k_5 = \frac{k_5 [O_2^-]^2}{(K_4 [Anion^{n-}] + 1)^2} \quad [8]$$

**S2. With Steady-State Conditions of  $O_2^-$  Where  $[Mn^{2+}] \gg [O_2^-]$  ( $k_{-3}$  Neglected).** The rate of formation of  $O_2^-$  is equal to  $X$ , steady-state equations can be setup such that

$$X = r_3 = r_5 \quad [9]$$

$$r_3 = [Mn^{2+}][O_2^-]k_3 \quad [10]$$

$$r_5 = [MnO_2^+]^2 k_5 \quad [11]$$

$$K_4 = \frac{[MnO_2^+ - Anion^{n-}]}{[MnO_2^+][Anion^{n-}]}, \quad [12]$$

then solving for the steady-state concentration of  $[MnO_2^+]$  using Eqs. 9 and 11

$$[MnO_2^+] = \frac{\sqrt{X}}{\sqrt{k_5}}, \quad [13]$$

then using Eq. 12

$$[MnO_2^+ - Anion^{n-}] = K_4 [MnO_2^+][Anion^{n-}], \quad [14]$$

substituting Eq. 13 into 14

$$[MnO_2^+ - Anion^{n-}] = \frac{\sqrt{X}[Anion^{n-}]}{\sqrt{k_5}} K_4, \quad [15]$$

and then using that the steady-state concentration of  $[MnO_2]$  and  $[MnO_2^+ - Anion^{n-}] + [Mn]$  will equal the initial concentration of Mn ( $[Mn]^i$ )

$$[MnO_2] + [MnO_2^+ - Anion^{n-}] + [Mn^{2+}] = [Mn^{2+}]^i \quad [16]$$

substituting Eqs. 13 and 15 into 16 and solving for  $[Mn^{2+}]$

$$[Mn^{2+}] = [Mn^{2+}]^i - \frac{\sqrt{X}}{\sqrt{k_5}} \left( 1 + [Anion^{n-}] K_4 \right). \quad [17]$$

Finally, using Eqs. 9, 10, and 14,

$$X = r_3 = [Mn^{2+}][O_2^-]k_3 \quad [18]$$

$$X = \left( [Mn^{2+}]^i - \frac{\sqrt{X}}{\sqrt{k_5}} \left( 1 + [Anion^{n-}] K_4 \right) \right) [O_2^-] k_3 \quad [19]$$

$$[O_2^-] = \frac{x}{\left( [Mn^{2+}]^i - \frac{\sqrt{X}}{\sqrt{k_5}} \left( 1 + [Anion^{n-}] K_4 \right) \right) k_3} \quad [20]$$

Because of the steady state, the forward rate is just  $X$ , the formation of  $O_2^-$ . However, once the equilibrium is formed,  $Mn^{2+}$ ,  $O_2^-$ , and  $MnO_2$  will form steady-state concentrations that depend on the rates  $k_3$  and  $k_5$ , on the initial concentration of Mn, and on the rate of  $O_2^-$  formation. This steady state will be lost if too much  $O_2^-$  forms and the  $Mn^{2+}$  is overwhelmed; that occurs (from Eqs. 17 and 20) when

$$\frac{\sqrt{X}}{\sqrt{k_5}} \left(1 + [\text{Anion}^{n-}]K_4\right) > [\text{Mn}^{2+}]^i. \quad [21]$$

### S3. A More General Solution with $k_{-3}$ Neglected

			Stoichiometry
3	$\text{Mn}^{2+} + \text{O}_2^- \rightarrow$	$\text{MnO}_2$	2
5	$2 \text{MnO}_2 + 2\text{H}^+ \rightarrow$	$\text{H}_2\text{O}_2 + \text{O}_2$	1
4	$\text{MnO}_2^+ + \text{Anion}^{n-} \rightarrow$	$\text{MnO}_2^+ - \text{Anion}^{n-}$	0
Net reaction	$2 \text{O}_2^- + 2 \text{H}^+ \rightarrow$	$\text{H}_2\text{O}_2 + \text{O}_2$	

the sum of steady-state concentrations of  $\text{Mn}^{2+}$ ,  $\text{MnO}_2^+$ , and  $[\text{MnO}_2^+ - \text{Anion}^{n-}]$  will be constant:

$$[\text{Mn}^{2+}]^i = [\text{Mn}^{2+}] + [\text{MnO}_2^+] + [\text{MnO}_2^+ - \text{Anion}^{n-}]. \quad [28]$$

Substituting with Eqs. 24 and 26,

$$\frac{2r}{[\text{O}_2^-]k_3} + \frac{\sqrt{r}}{\sqrt{k_5}} + K_4 \frac{[\text{Anion}^{n-}]\sqrt{r}}{\sqrt{k_5}} - [\text{Mn}^{2+}]^i = 0, \quad [29]$$

and solve for  $r$  (negative root):

$$r = \frac{(k_3 + k_4k_3[\text{Anion}^{n-}])^2}{8k_5} [\text{O}_2^-]^2 + \left[ \frac{[\text{Mn}^{2+}]^i k_3}{2} - \frac{(k_3 + k_4k_3[\text{Anion}^{n-}]) \sqrt{\frac{(k_4^2[\text{Anion}^{n-}]^2 + 2K_4[\text{Anion}^{n-}] + 1)k_3^2[\text{O}_2^-]^2 + 8[\text{Mn}^{2+}]^i k_3 k_5 [\text{O}_2^-]}}{k_5}}}{8\sqrt{k_5}} \right] [\text{O}_2^-]. \quad [30]$$

$$r = \frac{r_3 r_4 r_5}{v_3 r_4 r_5 + r_{-3} v_2 r_5 + r_{-3} r_{-4} v_5} = \frac{r_3}{v_1} \quad [22]$$

$$= \frac{k_3 [\text{O}_2^-] [\text{Mn}^{2+}]}{2}$$

(Eq. 22 adapted from ref. 1),

and then substituting in values and solving for  $[\text{Mn}^{2+}]$ ,

$$[\text{Mn}^{2+}] = \frac{2r}{[\text{O}_2^-]k_3}, \quad [23]$$

rearranging the order of reactions,

$$r = \frac{r_5 r_3 r_4}{v_5 r_3 r_4 + r_{-5} v_3 r_4 + r_{-5} r_{-3} v_4} = \frac{r_5}{v_5} = [\text{MnO}_2^+]^2 k_5, \quad [24]$$

and then substituting in values and solving for  $[\text{MnO}_2^+]$ ,

$$[\text{MnO}_2^+] = \frac{\sqrt{r}}{\sqrt{k_5}}, \quad [25]$$

rearranging the order of reactions again,

$$r = \frac{r_4 r_5 r_3}{v_4 r_5 r_3 + r_{-4} v_5 r_3 + r_{-4} r_{-5} v_3} = \frac{r_4 r_5 r_3}{r_{-4} v_5 r_3}$$

$$= \frac{k_4 [\text{Anion}^{n-}] [\text{MnO}_2^+] k_5 [\text{MnO}_2^+]^2}{k_{-4} [\text{MnO}_2^+ - \text{Anion}^{n-}]}, \quad [26]$$

and then substituting in values and solving for  $[\text{MnO}_2^+ - \text{Anion}^{n-}]$  and substituting  $\text{MnO}_2^+$  with Eq. 26,

$$[\text{MnO}_2^+ - \text{Anion}^{n-}] = \frac{k_4 [\text{Anion}^{n-}] \sqrt{r}}{\sqrt{k_5 k_{-4}}} = K_4 \frac{[\text{Anion}^{n-}] \sqrt{r}}{\sqrt{k_5}}, \quad [27]$$

**S4. Alternate Mechanism Where First and Second Intermediates React, Instead of Proposed Where Two First Intermediates React.** Using  $\text{A} = \text{PO}_4^{2-}$ ,  $\text{B}$  = first intermediate,  $\text{C}$  = second intermediate, and  $\text{D}$  = any product (dioxygen or hydrogen peroxide):



and



Equilibrium is

$$\frac{[\text{C}]}{[\text{A}][\text{B}]} = K. \quad [33]$$

Rate law is

$$[\text{B}][\text{C}]k = \text{rate}. \quad [34]$$

For fastest rate,  $[\text{B}][\text{C}]$  should be at a maximum.

Also,  $[\text{B}] + [\text{C}] = \text{initial } [\text{B}]$ , concentration of  $\text{B}$  before  $\text{B}$  and  $\text{C}$  come to equilibrium. Label  $[\text{B}]_0$ , which is a constant,

$$[\text{B}] + [\text{C}] = [\text{B}]_0, \quad [35]$$

and

$$[\text{C}] = [\text{B}]_0 - [\text{B}]. \quad [36]$$

Solving for a maximum of  $[\text{B}][\text{C}]$  by taking the differential and solving for zero, which should be the maximum if  $[\text{B}]$  and  $[\text{C}]$  are positive real numbers, and substituting the above equation,

$$[B][C]d[B] = 0 \quad [37]$$
$$[B]([B]_o - [B])d[B] = 0 \quad [38]$$
$$([B][B]_o - [B]^2)d[B] = 0 \quad [39]$$
$$[B]_o - 2[B] = 0 \quad [40]$$
$$\frac{[B]_o}{2} = [B] \quad [41]$$

Maximum rate is when

$$\frac{[B]_o}{2} = [B] = [C]. \quad [42]$$

Inserting back in equilibrium to find  $[A]$  when  $[B] = [C]$ :

$$\frac{[C]}{[A][B]} = \frac{[B]}{[A][B]} = \frac{1}{[A]} = K. \quad [43]$$

Therefore, to make the fastest rate where  $[B] = [C]$ , the concentration of  $[A]$  should equal  $1/K$ , as long as  $[A] \gg [B]_0$ .

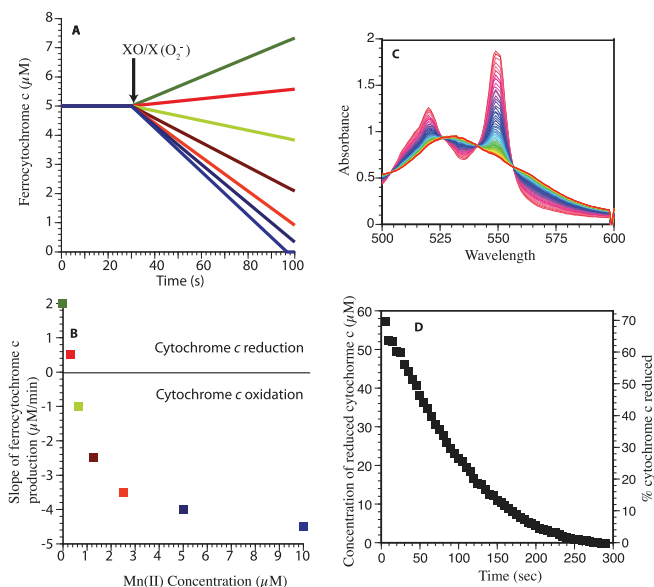
For phosphate,

$$\frac{1}{K} \approx 50 \text{ mM}. \quad [44]$$

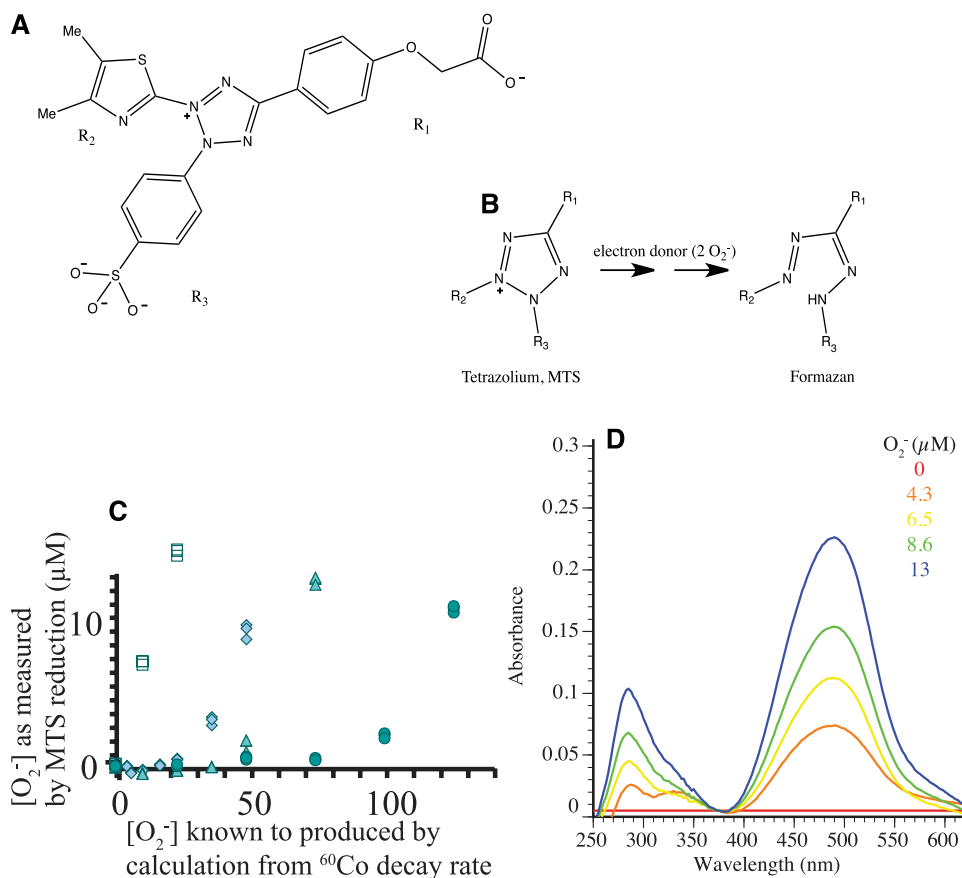
**55. Calculations of SOD and Superoxide Concentrations in Vivo.** We calculated that 1  $\mu\text{M}$  of CuZnSOD is necessary for normal growth, as follows. It has been shown that on average there are 519,000 molecules of CuZnSOD per yeast cell (2), and that a yeast cell volume is 75 fL (3). Therefore, the average concentration of CuZnSOD is  $\sim 10 \mu\text{M}$ . Further, research has shown that  $<10\%$  of normal CuZnSOD is necessary for normal growth (4).

Under physiological conditions, a slow, relatively constant  $\text{O}_2^-$  generation rate is expected. We estimated this rate at  $6.0 \mu\text{M/s}$ , which was calculated using values of oxygen consumption for yeast of  $0.6 \mu\text{L O}_2 \cdot \text{min}^{-1} \cdot 10^7 \text{ cells}$  (5), yeast volume of  $75 \text{ fL}$  (3), and the assumption that 1% of  $\text{O}_2$  consumed becomes  $\text{O}_2^-$  (6).

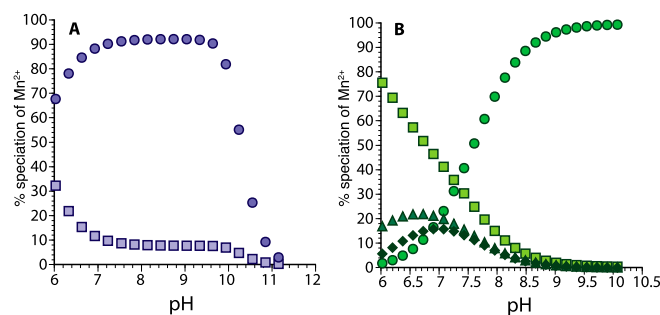
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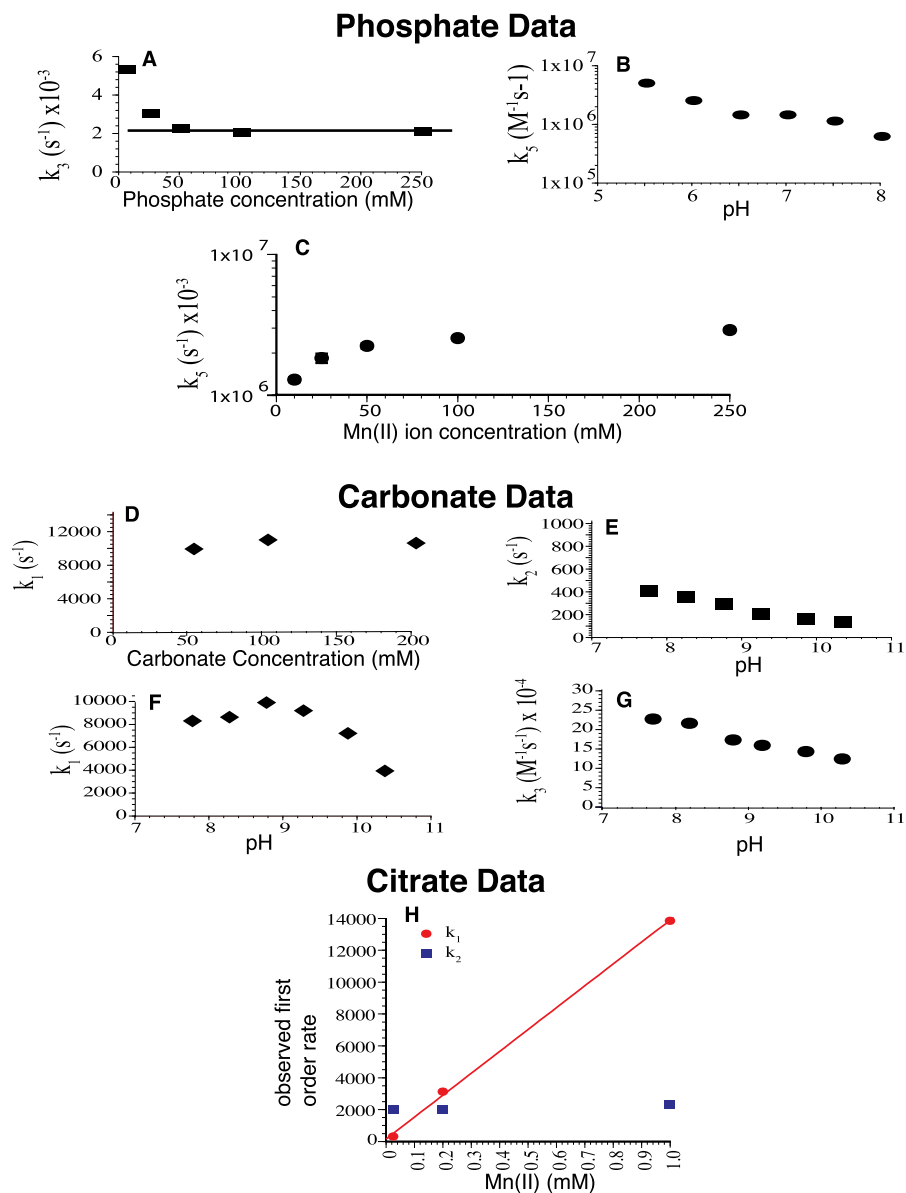
**Fig. S1.** Mn(II) inhibits superoxide reduction of cytochrome c by catalyzing superoxide-mediated oxidation of cytochrome c. (A) Mn(II) inhibits the reduction of cytochrome c by superoxide and leads to cytochrome c oxidation. (B) The slope of the Mn(II)-inhibited reduction of cytochrome c. All solutions were 50 mM phosphate buffer (pH 7.8), 50  $\mu$ M xanthine, and 6 nM xanthine oxidase. The concentration of Mn(II) is the same for A and B for the corresponding color [0, 0.25, 0.5, 1, 2.5, 5, and 10  $\mu$ M Mn(II)]. (C and D) Oxidation of cytochrome c by manganic pyrophosphate. (C) Spectrum for the oxidation of cytochrome c. (D) Calculated concentration of cytochrome c during the oxidation by Mn(III) pyrophosphate from absorbance at 550 nm ( $\epsilon = 21,000 \text{ M}^{-1}\text{cm}^{-1}$ ). For C and D, solutions were pH 7, 100 mM pyrophosphate, 81  $\mu$ M cytochrome c, and 90  $\mu$ M Mn(III) pyrophosphate.



**Fig. S2.** (A) Structure of 5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazolyl)-3-(4-sulfophenyl) tetrazolium (MTS). (B) Reduction of MTS to formazan. (C) Magnified version of Fig. 1A near the  $y = 0$  axis. Demonstrates that only after 0, 25, 50, or 100  $\mu\text{M}$  of superoxide was delivered to 0 (empty boxes), 25 (diamonds), 50 (triangles), or 100 (circles)  $\mu\text{M}$  Mn citrate, respectively, did MTS become reduced. Superoxide was generated at  $0.45 \mu\text{M s}^{-1}$  by  $^{60}\text{Co}$  irradiation, and the amount present was determined by measuring MF formation. Solutions contained 0, 25, 50, or 100  $\mu\text{M}$  Mn, 0.5 M ethanol, 150  $\mu\text{M}$  MTS, and 50 mM citrate (pH 7). (D) The spectrum of MF formed by the reduction of MTS by superoxide in the absence of Mn. Irradiating the solutions with  $^{60}\text{Co}$ -generated superoxide caused an increasing absorbance at 490 nm and 290 nm. The solutions were irradiated with 0, 4.3, 6.5, 8.6, and 13  $\mu\text{M}$  superoxide. Solutions contained 50 mM (pH 7) phosphate, 2 U/mL catalase, 50  $\mu\text{M}$  MTS, and 0.5 M ethanol.



**Fig. S3.** The speciation of manganous phosphate and manganous carbonate based on pH. (A) At higher pH the  $\text{MnHPO}_4$  (circles) species dominates over free  $\text{Mn}^{2+}$  (squares) until insoluble hydroxides are formed beyond pH 11. (B) Free  $\text{Mn}^{2+}$  dominates at low pH (squares), and the bicarbonate-bound complexes ( $\text{MnHCO}_3^+$ ) (triangles) and  $\text{Mn}(\text{HCO}_3)_2^{2+}$  (diamonds) also contribute a significant amount, but at higher pH, the  $\text{MnCO}_3$  (circles) species dominates. Calculations were done at 50 mM ligand and 50  $\mu\text{M}$   $\text{Mn}^{2+}$ .



**Fig. S4.** Observed data from pulse radiolysis phosphate data. (A) The dependence of the first steps ( $k_3$ ) upon  $[HPO_4^{2-}]$ . (B and C) The dependence of the second-order rate of decay of  $MnOO^+$  ( $k_5$ ) on pH and Mn(II) ion. Rates fitted on initial  $[O_2^-]$  (1–10  $\mu M$ ). All solutions contained 0.5 M ethanol, and unless noted were pH 7 and contained 50 mM phosphate and 100  $\mu M$   $MnSO_4$ . Carbonate data. (D) The dependence of carbonate on the observed rates of the first reactions. (E–G) The dependence of pH on the observed rates of the first, second, and third reactions. Rates fitted on initial  $[O_2^-]$  (1–10  $\mu M$ ). All solutions contained 0.5 ethanol, and unless noted were pH 8.3 and contained 50 mM carbonate and 100  $\mu M$   $MnSO_4$ . (H) Mn citrate shows a first-order dependence on the reaction with superoxide. Pulse radiolysis was used to determine the pseudo-first-order rate of reaction of manganous citrate with at least three different concentrations of superoxide. Observed first-order rate for  $k_1$  is measured by the formation of the  $MnO_2^+$  at a constant superoxide concentration.  $k_2$  follows the formation of the Mn(III) species from  $MnO_2^+$ . pH was adjusted to 7.0 and citrate concentration was 50 mM.

Table S1. Relevant binding constants for Mn(II) and Mn(III)

			Log K	Ref.
<b>Mn(II)</b>				
Mn(II) + CO <sub>3</sub> <sup>2-</sup>	→	MnCO <sub>3</sub>	4.7	(1)
Mn(II) + HCO <sub>3</sub> <sup>-</sup>	→	MnHCO <sub>3</sub> <sup>+</sup>	1.3	(1)
Mn(II) + Citrate <sup>3-</sup>	→	MnCitrate <sup>-</sup>	3.8	(2)
Mn(II) + P <sub>2</sub> O <sub>7</sub> <sup>4-</sup>	→	MnP <sub>2</sub> O <sub>7</sub> <sup>2-</sup>	7	(2)
Mn(II) + HPO <sub>4</sub> <sup>2-</sup>	→	MnHPO <sub>4</sub>	3.3	(2)
<b>Mn(III)</b>				
Mn(III) + Citrate <sup>3-</sup>	→	MnCitrate	15	(3)
Mn(III) + HPO <sub>4</sub> <sup>2-</sup>	→	MnHPO <sub>4</sub> <sup>+</sup>	11.6	(4)
Mn(III) + H <sub>2</sub> P <sub>2</sub> O <sub>7</sub> <sup>2-</sup>	→	MnH <sub>2</sub> P <sub>2</sub> O <sub>7</sub> <sup>+</sup>	6.5	(5)
MnHPO <sub>4</sub> + e <sup>-</sup>	→	MnHPO <sub>4</sub>	17	(4)
MnP <sub>2</sub> O <sub>7</sub> <sup>-</sup> + e <sup>-</sup>	→	MnP <sub>2</sub> O <sub>7</sub> <sup>2-</sup>	13.3	(5)

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