

**Acid-base and Electrochemical Properties of Manganese *meso*(*ortho*- and *meta*-*N*-ethylpyridyl)porphyrins: Voltammetric and Chronocoulometric Study of Protolytic and Redox Equilibria<sup>†</sup>**

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**Supplementary Material**

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<sup>†</sup> Dedicated to the memory of Ivan Kos, our dear friend and colleague.

<sup>‡</sup> Taken in part from Tin Weitner's Ph.D. thesis submitted to the University of Zagreb.

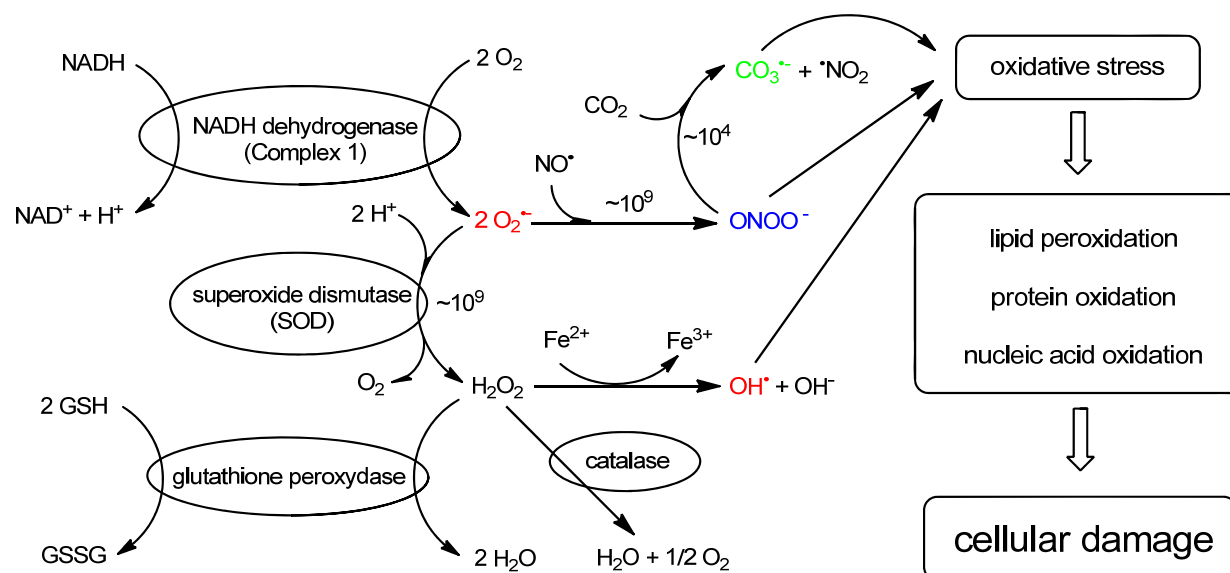


Figure S1. A schematic representation of cellular oxidative stress. The mitochondrial electron transport system is listed here as a source of  $O_2^{\bullet-}$ .

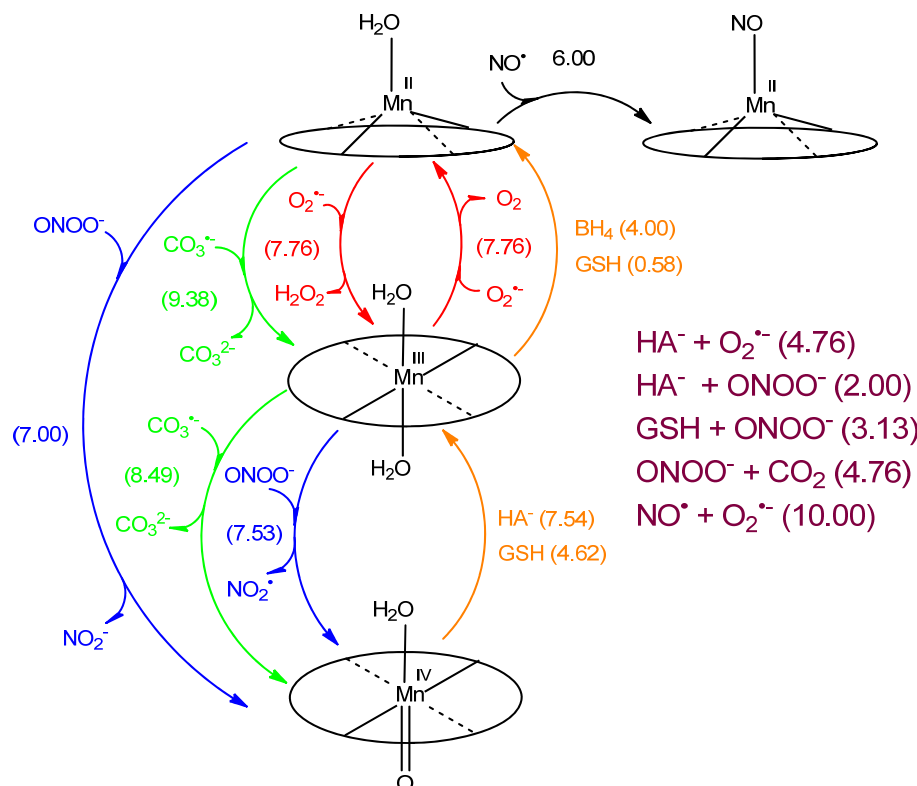


Figure S2. Reactivity scheme of manganese porphyrins in the presence of cellular reductants in aqueous solutions:  $HA^- \equiv$  monodeprotonated ascorbate,  $BH_4 \equiv$  tetrahydrobiopterin,  $GSH \equiv$  glutathione. The numbers in parentheses indicate logarithms of individual reaction rate constants,  $\log k$ , given in  $M^{-1} s^{-1}$  (refs. 15, 23-29). Recently, a substantial amount of data indicated that MnPs react with protein thiols in the same fashion they react with GSH (refs. 30-31). Such reactions appear to be key ones in MnPs' anticancer mechanism of action. It should also be noted that the water exchange kinetics of MnPs does not limit the rate of redox kinetics under *in vivo* conditions (ref. 32).

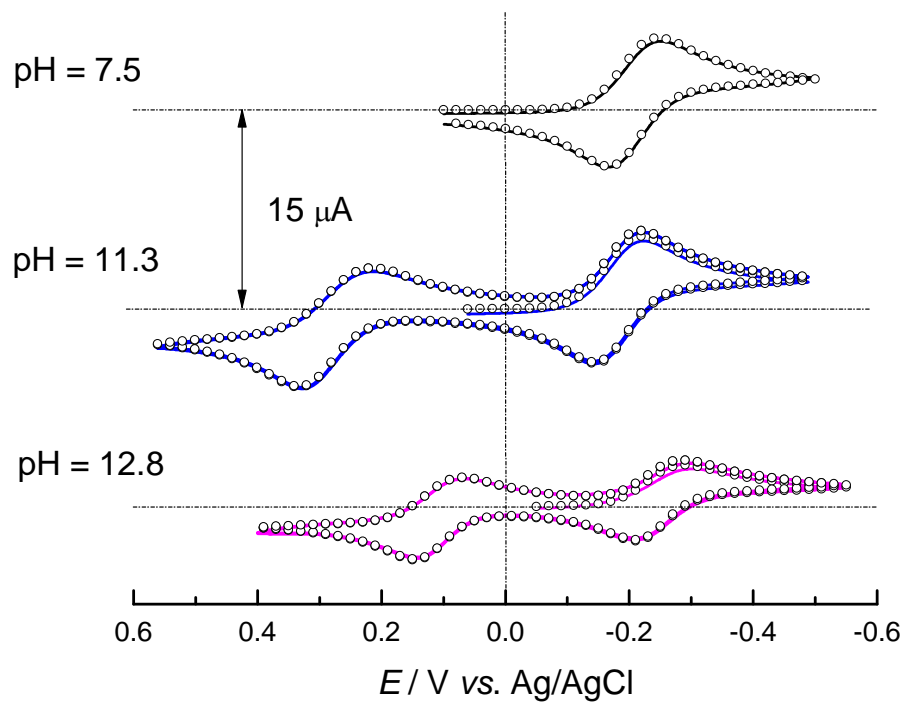


Figure S3. Dependence of cyclic voltammograms of aqueous solutions of MnTE-3-PyP on pH,  $\nu = 0.1 \text{ V s}^{-1}$ ,  $[\text{NaCl}] = 0.1 \text{ M}$ ,  $\theta = 25 \text{ }^\circ\text{C}$ , ( $\circ$ ) simulation in DigiElch.

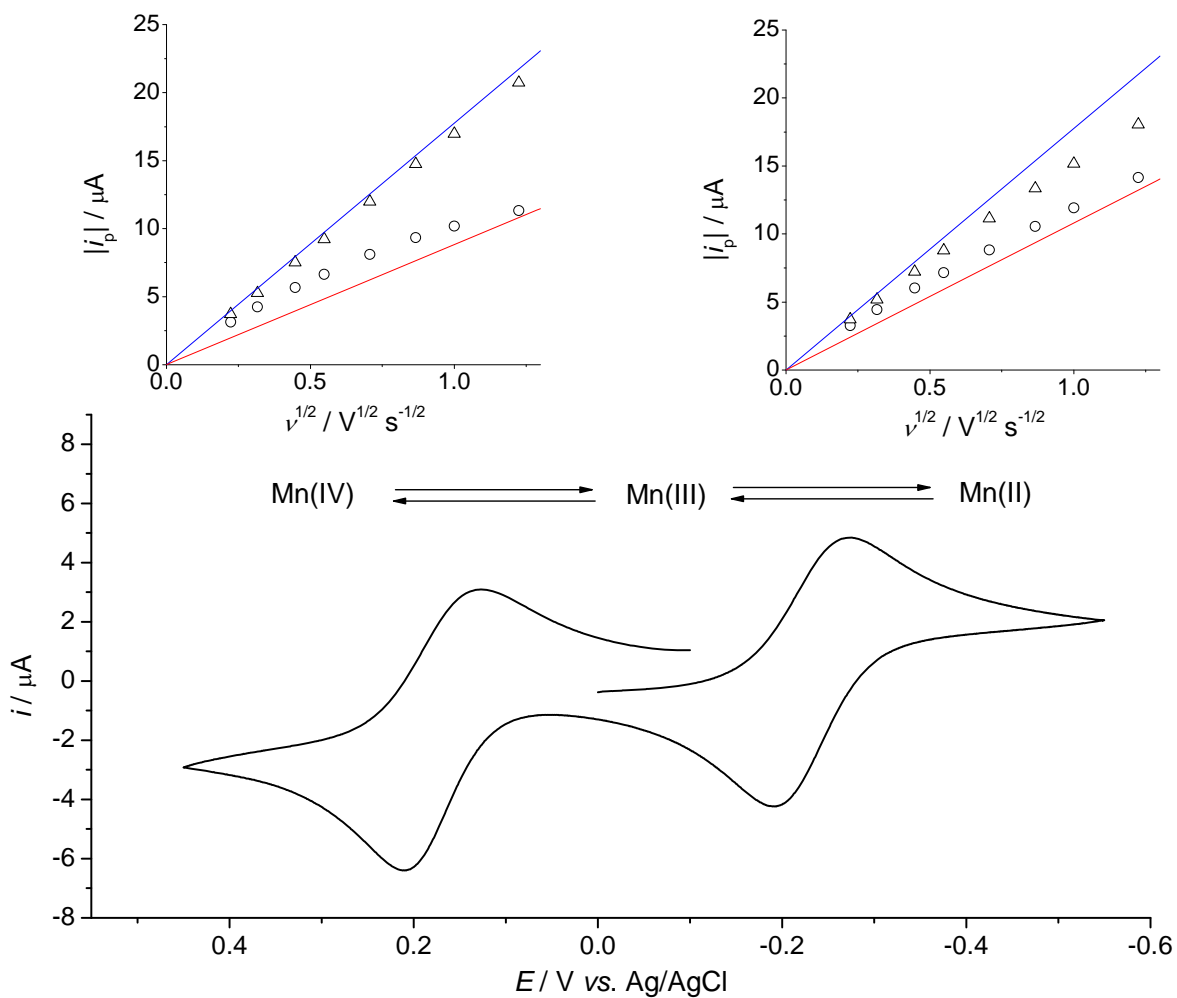


Figure S4. Cyclic voltammogram of 0.52 mM aqueous solution of MnTE-3-PyP, pH = 12.3,  $[\text{Na}_2\text{HPO}_4] = 50 \text{ mM}$ ,  $[\text{NaCl}] = 0.1 \text{ M}$ ,  $\nu = 0.1 \text{ V s}^{-1}$ ,  $\theta = 25 \text{ }^\circ\text{C}$ . Inset left: Dependence of  $|i_p|$  on  $\nu^{1/2}$  for the  $\text{Mn}^{\text{IV}}\text{P}/\text{Mn}^{\text{III}}\text{P}$  redox transition:  $i_{\text{pc}}$  ( $\circ$ ),  $i_{\text{pa}}$  ( $\Delta$ ), Randles-Ševčík equation for the reversible electron transfer (—), peak-current equation for the irreversible electron transfer,  $\alpha = 0.2$  (—). Inset right: Dependence of  $|i_p|$  on  $\nu^{1/2}$  for the  $\text{Mn}^{\text{III}}\text{P}/\text{Mn}^{\text{II}}\text{P}$  redox transition:  $i_{\text{pc}}$  ( $\circ$ ),  $i_{\text{pa}}$  ( $\Delta$ ), Randles-Ševčík equation for the reversible electron transfer (—), peak-current equation for the irreversible electron transfer,  $\alpha = 0.3$  (—). The value of  $D_{\text{app}}$  obtained by chronocoulometry at the same pH (Table 2) was used for calculation.

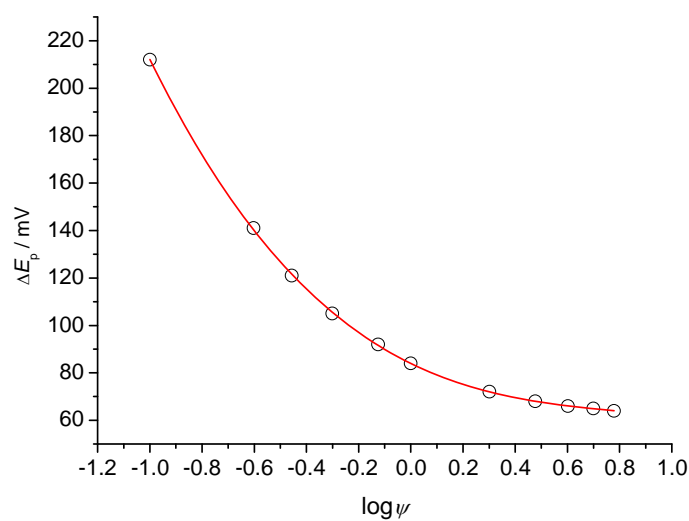


Figure S5. Dependence of  $\Delta E_p$  on  $\log \psi$  ( $\circ$ ) and the polynomial model ( $\text{---}$ ) given by the expression:  $\Delta E_p = -21.358 (\log \psi)^3 + 52.932 (\log \psi)^2 - 53.849 \log \psi + 83.97$ ,  $R^2 = 0.9999$ .

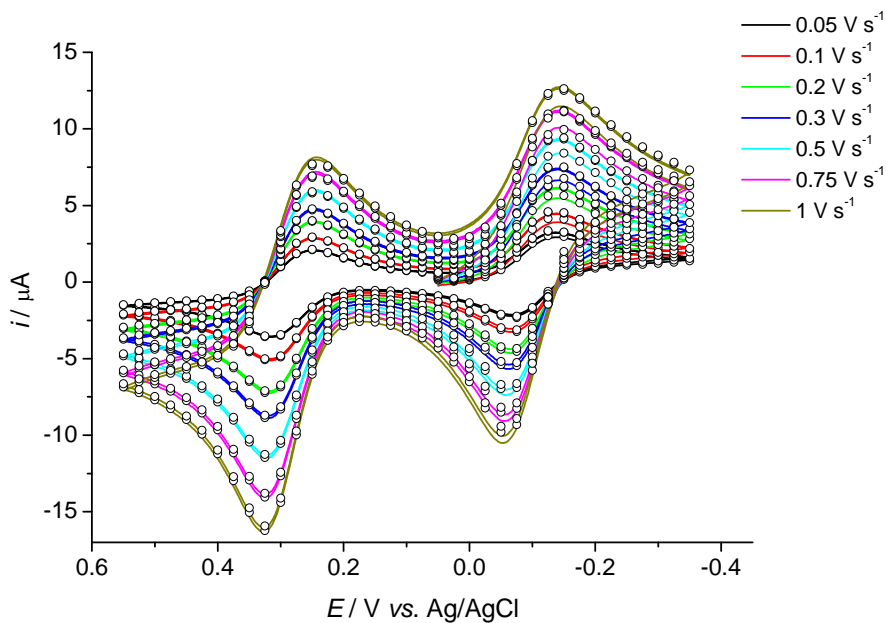


Figure S6. A typical simulation of the recorded cyclic voltammograms in DigiElch software: measured curves ( $\text{---}$ ) and simulation ( $\circ$ ),  $[\text{MnTE-2-PyP}] = 0.52 \text{ mM}$ ,  $\text{pH} = 11.3$ ,  $[\text{CAPS}] = 50 \text{ mM}$ ,  $[\text{NaCl}] = 0.1 \text{ M}$ ,  $\theta = 25 \text{ }^\circ\text{C}$ .

Table S7. Electrochemical parameters obtained from the recorded cyclic voltammograms of MnTE-2-PyP and MnTE-3-PyP, [NaCl] = 0.1 M,  $\theta = 25$  °C. All potentials are given vs. the Ag/AgCl electrode.

redox couple	MnP	pH	$E_{1/2} \pm \sigma /$ V vs. Ag/AgCl	$k_{app}^0 \pm \sigma /$ $10^{-2} \text{ cm s}^{-1}$	$\alpha_{app} \pm \sigma$
$\text{Mn}^{\text{III}}\text{P}/\text{Mn}^{\text{II}}\text{P}$	MnTE-2-PyP	7.5	$-0.061 \pm 0.001$	$0.44 \pm 0.04$	$0.433 \pm 0.007$
		8.4	$-0.061 \pm 0.001$	$0.48 \pm 0.05$	$0.435 \pm 0.008$
		9.0	$-0.062 \pm 0.001$	$0.51 \pm 0.06$	$0.457 \pm 0.005$
		9.5	$-0.061 \pm 0.002$	$0.54 \pm 0.07$	$0.453 \pm 0.008$
		10.1	$-0.060 \pm 0.001$	$0.53 \pm 0.04$	$0.453 \pm 0.006$
		10.5	$-0.065 \pm 0.001$	$0.55 \pm 0.04$	$0.491 \pm 0.007$
		11.0	$-0.090 \pm 0.003$	$0.55 \pm 0.03$	$0.438 \pm 0.006$
		11.5	$-0.121 \pm 0.002$	$0.46 \pm 0.04$	$0.338 \pm 0.003$
		11.9	$-0.137 \pm 0.001$	$0.34 \pm 0.03$	$0.385 \pm 0.003$
		12.5	$-0.187 \pm 0.003$	$0.30 \pm 0.06$	$0.335 \pm 0.004$
		13.0	$-0.238 \pm 0.002$	$0.25 \pm 0.03$	$0.336 \pm 0.008$
		13.5	$-0.270 \pm 0.005$	$0.20 \pm 0.02$	$0.280 \pm 0.006$
	MnTE-3-PyP	7.5	$-0.230 \pm 0.002$	$0.22 \pm 0.02$	$0.322 \pm 0.003$
		8.7	$-0.229 \pm 0.001$	$0.27 \pm 0.03$	$0.317 \pm 0.004$
		9.7	$-0.223 \pm 0.001$	$0.35 \pm 0.01$	$0.324 \pm 0.005$
		10.2	$-0.226 \pm 0.002$	$0.38 \pm 0.03$	$0.334 \pm 0.002$
		10.8	$-0.221 \pm 0.002$	$0.40 \pm 0.03$	$0.313 \pm 0.003$
		11.3	$-0.223 \pm 0.002$	$0.51 \pm 0.09$	$0.202 \pm 0.009$
		12.0	$-0.236 \pm 0.003$	$0.51 \pm 0.07$	$0.204 \pm 0.010$
		12.3	$-0.244 \pm 0.004$	$0.48 \pm 0.03$	$0.164 \pm 0.009$
$\text{Mn}^{\text{IV}}\text{P}/\text{Mn}^{\text{III}}\text{P}$	MnTE-2-PyP	10.1	$0.394 \pm 0.003$	$0.14 \pm 0.02$	$0.172 \pm 0.002$
		10.5	$0.354 \pm 0.005$	$0.51 \pm 0.02$	$0.314 \pm 0.004$
		11.0	$0.296 \pm 0.004$	$0.47 \pm 0.05$	$0.364 \pm 0.005$
		11.5	$0.267 \pm 0.004$	$0.38 \pm 0.03$	$0.360 \pm 0.004$
		11.9	$0.245 \pm 0.005$	$0.38 \pm 0.04$	$0.262 \pm 0.003$
		12.5	$0.206 \pm 0.003$	$0.52 \pm 0.05$	$0.243 \pm 0.003$
		13.0	$0.164 \pm 0.003$	$0.45 \pm 0.10$	$0.255 \pm 0.011$
		13.5	$0.130 \pm 0.002$	$0.31 \pm 0.08$	$0.191 \pm 0.009$
	MnTE-3-PyP	10.2	$0.459 \pm 0.014$	$0.03 \pm 0.01$	$0.107 \pm 0.002$
		10.8	$0.376 \pm 0.016$	$0.08 \pm 0.01$	$0.135 \pm 0.002$
		11.3	$0.301 \pm 0.007$	$0.31 \pm 0.10$	$0.281 \pm 0.006$
		12.0	$0.213 \pm 0.005$	$0.47 \pm 0.05$	$0.468 \pm 0.008$
		12.3	$0.178 \pm 0.004$	$0.50 \pm 0.04$	$0.484 \pm 0.008$
		12.8	$0.129 \pm 0.003$	$0.48 \pm 0.04$	$0.487 \pm 0.008$
13.2	$0.088 \pm 0.003$	$0.58 \pm 0.10$	$0.510 \pm 0.006$		

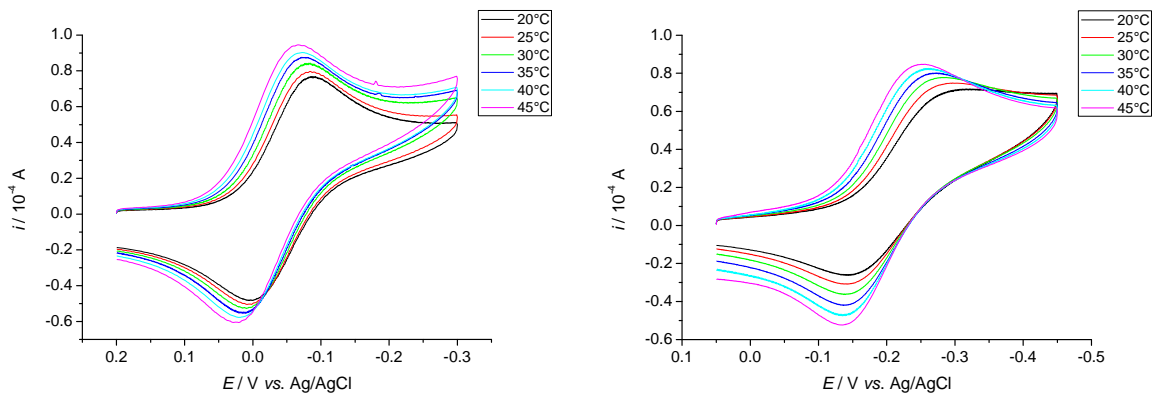


Figure S8. Cyclic voltammograms of MnPs in aqueous solution in the range from 20 °C to 45 °C, pH = 7.5, [NaH<sub>2</sub>PO<sub>4</sub>] = 50 mM, [NaCl] = 0.1 M, left: [MnTE-2-PyP] = 0.65 mM, right: [MnTE-3-PyP] = 0.54 mM.

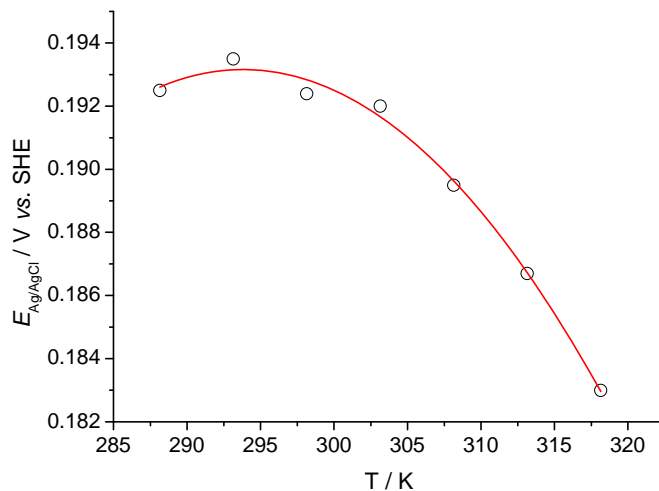


Figure S9. The temperature dependence of the potential of the Ag/AgCl electrode vs. SHE (○) in aqueous solution in the range from 15 °C to 50 °C and the polynomial model (—) given by the expression:  $E_{\text{Ag/AgCl vs. SHE}} = -1.72381 \times 10^{-5} T^2 + 0.01013 T - 1.29508$ ,  $R^2 = 0.9948$ , pH = 7.5, [NaH<sub>2</sub>PO<sub>4</sub>] = 50 mM, [NaCl] = 0.1 M.

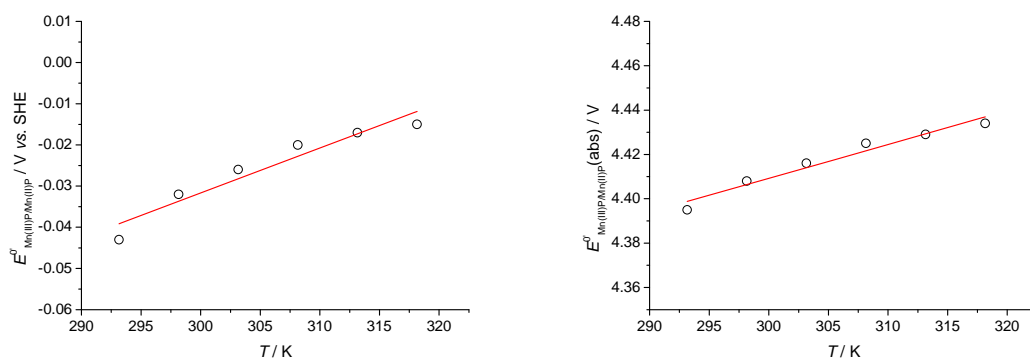


Figure S10. The temperature dependence of the formal reduction potential ( $E^0$  vs. SHE) and the absolute reduction potential ( $E^0(\text{abs})$ ) of  $\text{Mn}^{\text{III}}\text{P}/\text{Mn}^{\text{II}}\text{P}$  redox couple of MnTE-3-PyP:  $[\text{MnTE-3-PyP}] = 0.54 \text{ mM}$ ,  $\text{pH} = 7.5$ ,  $[\text{NaH}_2\text{PO}_4] = 50 \text{ mM}$ ,  $[\text{NaCl}] = 0.1 \text{ M}$ .

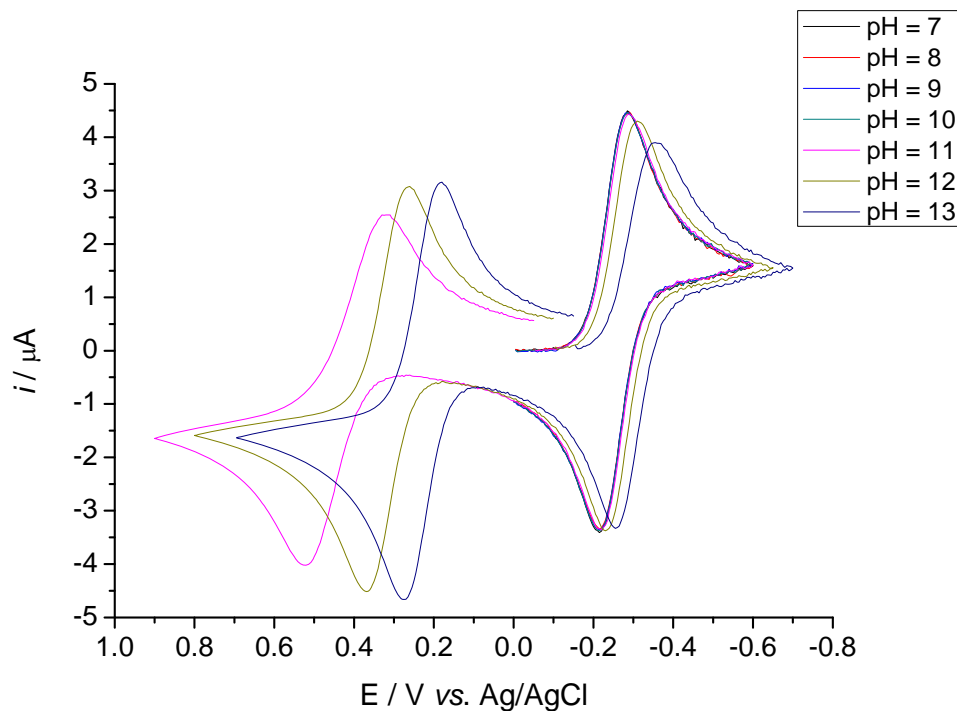


Figure S11. Simulation of the electrochemical behaviour of a 0.5 mM solution of MnTE-3-PyP in the range of pH values from 7 to 13.



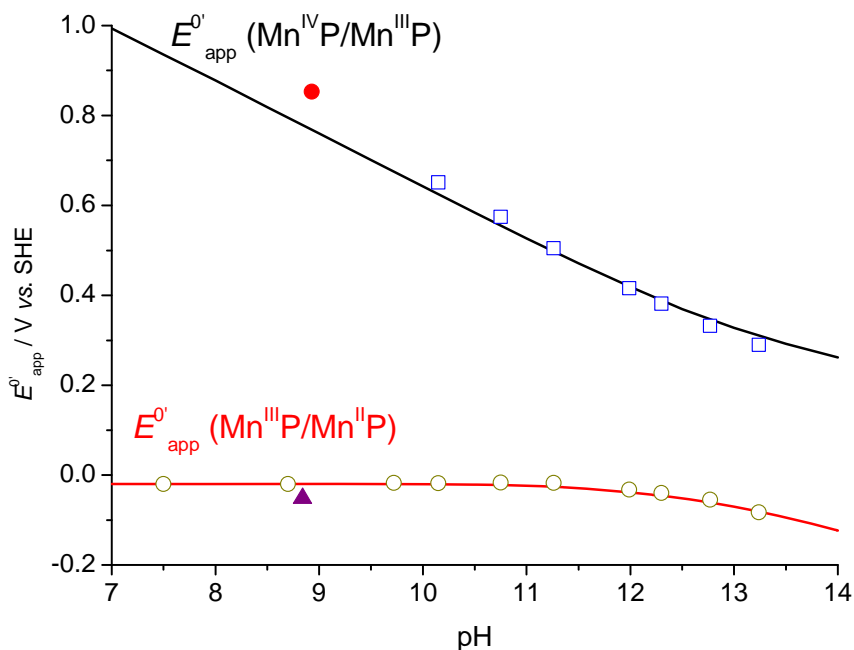


Figure S12. Comparison of the pH-dependent apparent formal reduction potentials,  $E_{app}^{0'}$ , of the  $Mn^{III}P/Mn^{II}P$  and  $Mn^{IV}P/Mn^{III}P$  redox couples of MnTE-3-PyP simulated by eqs. (5) and (8) with various experimental values: (●) oxidation of  $Mn^{III}P$  with octacyanomolybdate (ref. 35), (□) cyclic voltammetry of the  $Mn^{IV}P/Mn^{III}P$  redox couple (Table S7), (▲) reduction of  $Mn^{III}P$  with ascorbate (ref. 35), and (○) cyclic voltammetry of the  $Mn^{III}P/Mn^{II}P$  redox couple (Table S7).

Table S13. Electrochemical parameters for the reduction of the experimentally accessible species of MnTnBu-2-PyP in aqueous solutions,  $[NaCl] = 0.1 M$ ,  $\theta = 25\text{ }^\circ C$ . Values of the deprotonation constants were taken from ref. 50:  $pK_{a1}^I = 10.6$ ,  $pK_{a1}^II = 10.3$ ,  $pK_{a2}^II = 11.2$ ,  $pK_{a3}^II = 10.3$ . As previously in Figures 7, 8, S11 and S12, the experimentally unavailable deprotonation constants were taken to be at least one pH unit beyond the studied pH range, i.e.  $pK_{a2}^I = 14$ ,  $pK_{a3}^I = 14$ ,  $pK_{a4}^I = 15$ ,  $pK_{a1}^{II} = 5$ ,  $pK_{a2}^{II} = 6$ ,  $pK_{a4}^{II} = 14$ .

MnP	couple	index	$E_i^{0'}$ / V vs. SHE
MnTnBu-2-PyP	$Mn^{III}P/Mn^{II}P$	1	0.203
		2	0.185
		3	$\leq 0.020$
	$Mn^{IV}P/Mn^{III}P$	5	$\geq 0.836$
		6	0.528
		7	$\leq 0.309$

Table S14. Thermodynamic parameters for the reactivity of MnTE-2-PyP and MnTE-3-PyP with ONOO<sup>-</sup> in a neutral aqueous medium. All potentials are given in V vs. SHE.

MnP	MnTE-2-PyP	MnTE-3-PyP
$E^0(\text{Mn}^{\text{IV}}\text{P}/\text{Mn}^{\text{III}}\text{P}) / \text{V}$ (eq. 14, pH = 7)	0.980	0.993
$E^0(\text{Mn}^{\text{III}}\text{P}/\text{Mn}^{\text{II}}\text{P}) / \text{V}$ (eq. 11, pH = 7)	0.145	-0.020
$E^0(\text{Mn}^{\text{IV}}\text{P}/\text{Mn}^{\text{II}}\text{P})^* / \text{V}$ (pH = 7)	0.563	0.487
$E^0(\text{ONOO}^-/\text{NO}_2^-) / \text{V}$ (ref. 79)	1.600	1.600
$E^0(\text{ONOO}^-/\text{NO}_2^-) / \text{V}$ (ref. 79)	1.300	1.300
$EMF(1e^-) / \text{V}$	0.620	0.607
$EMF(2e^-)^{**} / \text{V}$	0.738	0.814

\*  $E^0(\text{Mn}^{\text{IV}}\text{P}/\text{Mn}^{\text{II}}\text{P}) = (E^0(\text{Mn}^{\text{III}}\text{P}/\text{Mn}^{\text{II}}\text{P}) + E^0(\text{Mn}^{\text{IV}}\text{P}/\text{Mn}^{\text{III}}\text{P}))/2$ .

\*\*  $EMF = E_c - E_a$  for single-electron and two-electron reduction of ONOO<sup>-</sup>,  $EMF(1e^-)$  and  $EMF(2e^-)$ , where the values of  $E_c$  are the formal potentials  $E^0(\text{ONOO}^-/\text{NO}_2^-)$  and  $E^0(\text{ONOO}^-/\text{NO}_2^-)$ , respectively, whereas the values of  $E_a$  are the formal potentials  $E^0(\text{Mn}^{\text{IV}}\text{P}/\text{Mn}^{\text{III}}\text{P})$  and  $E^0(\text{Mn}^{\text{IV}}\text{P}/\text{Mn}^{\text{II}}\text{P})$ , respectively.