Supporting Information

Cellular Fates of Manganese(II) Pentaazamacrocyclic Superoxide Dismutase (SOD) Mimetics – Fluorescently Labeled MnSOD Mimetics, X-ray Absorbance Spectroscopy and X-ray Fluorescence Microscopy Studies

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UV/Vis and fluorescence spectra characteristics of newly synthesized complexes

Table S1. Absorption maxima (λ_{max}) and extinction coefficients (ε) determined for all MnPAMs.

complex	$\boldsymbol{\lambda_{max}}$ **in nm**	$\boldsymbol{\varepsilon_{\lambda max}}$ **in l mol^{-1} cm^{-1}**		**[MnPAM]$_0$ in mM**
manganese(II) pyane	266	4427.3	± 36.1	0.2
dimanganese(II) bipyane	240	20426.8	± 862.7	0.2
manganese(II) pyane-rhodB	564	110977.9	± 4527.8	0.042
manganese(II) pyane-pyrene	278	30530.4	± 1679.1	0.2
manganese(II) pydiene	300	4070.8	± 92.6	0.2
M40404	264	4257.8	± 79.7	0.1
manganese(II) pyane-CH_2NH_2	266	2729.2	±110.7	0.2
M40403	264	3777.3	± 124.6	0.2

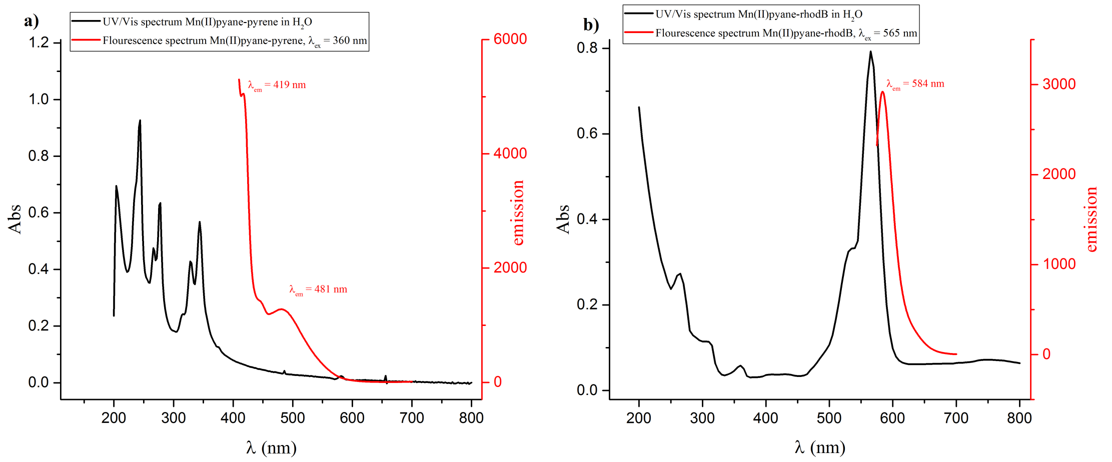

Figure S1. Absorption (black) and fluorescence (red) spectra of (a) manganese(II) pyane-pyrene and (b) manganese(II) pyane-rhodB. Spectra were obtained in aqueous solution, manganese(II) pyane-pyrene was excited at 360 nm, manganese(II) pyane-rhodB at 565 nm.

SOD activity

Buffer composition for phosphate dependence measurements

The buffer was maintained by phosphate and HEPES with its capacity held at 60 mM (by variation of the NaP_i/HEPES ratio; [NaP_i] + [HEPES] = 60 mM). For each HEPES and NaP_i concentration the ionic strength (μ) was calculated and the total ionic strength of the buffer solution was set to 100 mM by the addition of the respective amount of sodium chloride (see Table S2). The pH was adjusted to 7.4. For the calculation of the ionic strength the Henderson-Hasselbalch equation was employed and the following p*K*a values were used:
NaP_i:

$H_2PO_4^- \rightarrow HPO_4^{2-} + H^+$ p*K*a = 7.21
(Partanen, J. I.; Covington, A. K. *Journal of Chemical & Engineering Data* **2005**, *50*, 1502-1509.)

HEPES:

$R\text{-}SO_3H \rightarrow R\text{-}SO_3^- + H^+$ p*K*a = 7.5

(Long, R. D.; Hilliard, N. P.; Chhatre, S. A.; Timofeeva, T. V.; Yakovenko, A. A.; Dei, D. K.; Mensah, E. A. Beilstein *J Org Chem* **2010**, *6*, 31.)

Table S2. Composition and ionic strength (μ) of the buffers used.

[NaPi] mM / μ mM	[HEPES] mM / μ mM	[NaCl] mM
0	60 / 26.0	74
1 / 2.2	59 / 25.6	72.2
2.5 / 5.5	57.5 / 24.9	69.6
5 / 11	55 / 23.8	65.2
10 / 22.4	50 / 21.7	55.9
20 / 44.8	40 / 17.3	37.9
30 / 67.2	30 / 13.0	19.8
40 / 89.6	20 / 8.7	1.7
50 /112	0/ 0	0

Table S3. k_{obs} values determined for the reaction of manganese(II) pyane-rhodB, manganese(II) pyane-pyrene and manganese(II) pyane-CH_2NH_2 with superoxide at different pH conditions and buffers.

	$\boldsymbol{k_{obs}}$, $\mathbf{s^{-1}}$		
[MnL] (×10^{-7} M)	60 mM HEPES 7.4	60 mM HEPES 8.1	50 mM NaPi 7.4
manganese(II) pyane-rhodB			
4.5	32.4184	19.73122	36.43202
9.0	35.97444	21.12627	38.9253
23	48.15904	29.23956	41.0477
45	82.74797	49.33548	51.02608
manganese(II) pyane-pyrene			
4.5	32.02504	20.00518	34.82748
9.0	34.66691	21.45475	36.34141
23	46.3734	26.84046	38.81046
45	70.08606	40.78573	46.72258
manganese(II) pyane-$\mathbf{CH_2NH_2}$			
4.5	39.538	18.75798	31.96485
9.0	48.81368	20.34012	32.68781
23	72.0655	29.5024	35.09463
45	109.2532	48.75351	39.62045

Binding constants of hydrogen phosphate

Table S4. k_{obs} and k_{cat} values determined for the reaction of manganese(II) pyane with superoxide at different phosphate concentrations.

	k_{obs}, s^{-1}									
[MnL] ($\times 10^{-7}$ M)	0 mM P_i	1 mM P_i	2.5 mM P_i	5 mM P_i	10 mM P_i	20 mM P_i	30 mM P_i	40 mM P_i^a	MnL] (M)	50 mM P_i^a
4.5	35.4	37.0	38.0	36.7	35.7	33.7	36.2	37.4	0.90E-06	27.2
9.0	50.3	46.9	51.0	46.6	42.8	40.4	40.7	43.5	2.3E-06	36.0
23	86.2	83.0	83.0	75.5	67.8	61.6	55.1	55.1	4.5E-06	54.3
									9.0E-06	95.6
k_{cat} ($\times 10^7$ $M^{-1}s^{-1}$)	2.78	2.58	2.47	2.15	1.80	1.55	1.08	0.955		0.840

[a]Friedel, F. C.; Lieb, D.; Ivanovic-Burmazovic, I. *J. Inorg. Biochem.* **2012**, *109*, 26-32.

Table S5. k_{obs} and k_{cat} values determined for the reaction of M40403 with superoxide at different phosphate concentrations.

	k_{obs}, s^{-1}									
[MnL] ($\times 10^{-7}$ M)	0 mM P_i	1 mM P_i	2.5 mM P_i	5 mM P_i	10 mM P_i	20 mM P_i	30 mM P_i	40 mM P_i^a	[MnL] (M)	50 mM P_i^a
2.3	36.0	50.6	53.5	58.5	29.9	40.1	36.0	35.0	0.90E-06	31.4
4.5	44.9	56.3	59.5	62.1	32.0	43.1	37.8	37.0	1.8E-06	33.7
9.0	55.6	64.4	61.9	67.0	35.1	45.9	38.4	39.0	4.5E-06	38.1
23	80.8	79.2	71.5	73.8	43.0	49.6	43.4	40.9	9.0E-06	47.4
k_{cat} ($\times 10^7$ $M^{-1}s^{-1}$)	2.12	1.35	0.804	0.706	0.631	0.422	0.347	0.259		0.19

[a]Friedel, F. C.; Lieb, D.; Ivanovic-Burmazovic, I. *J. Inorg. Biochem.* **2012**, *109*, 26-32.

Table S6. k_{obs} and k_{cat} values determined for the reaction of dimanganese(II) bipyane with superoxide at different phosphate concentrations.

	k_{obs}, s^{-1}								
[MnL] ($\times 10^{-7}$ M)	0 mM P_i	1 mM P_i	2.5 mM P_i	5 mM P_i	10 mM P_i	20 mM P_i	30 mM P_i	40 mM P_i	50 mM P_i
2.3	35.9	37.0	31.9	32.2	35.7	34.4	34.0	34.1	27.0
4.5	42.5	41.0	34.8	34.4	38.0	36.7	35.5	36.3	27.2
9.0	51.7	47.5	42.7	41.2	39.4	41.7	40.1	39.3	31.1
23	73.0	62.9	56.7	55.1	57.8	56.0	55.1	54.9	46.7
k_{cat} ($\times 10^7$ $M^{-1}s^{-1}$)	1.77	1.25	1.22	1.14	1.10	1.07	1.06	1.03	1.02

Table S7. k_{obs} and k_{cat} values determined for the reaction of manganese(II) pyane-rhodB with superoxide at different phosphate concentrations.

	k_{obs}, s^{-1}								
[MnL] ($\times 10^{-7}$ M)	0 mM P_i	1 mM P_i	2.5 mM P_i	5 mM P_i	10 mM P_i	20 mM P_i	30 mM P_i	40 mM P_i	50 mM P_i
4.3	31.4	33.4	32.5	33.4	33.9	33.8	38.5	37.7	27.0
8.7	33.7	36.0	36.0	34.7	35.9	36.9	41.4	38.4	27.2
22	44.3	45.9	44.2	41.2	42.0	38.2	45.4	43.9	31.1
443	73.5	67.2	62.4	56.0	56.0	54.8	56.4	51.9	46.7
k_{cat} ($\times 10^7$ $M^{-1}s^{-1}$)	1.09	0.876	0.763	0.588	0.568	0.520	0.445	0.376	0.35

Table S8. k_{obs} and k_{cat} values determined for the reaction of manganese(II) pyane-pyrene with superoxide at different phosphate concentrations.

	k_{obs}, s^{-1}						
[MnL] ($\times 10^{-7}$ M)	0 mM P_i	1 mM P_i	2.5 mM P_i	5 mM P_i	10 mM P_i	20 mM P_i	30 mM P_i
4.50	30.1	28.5	27.1	28.8	29.33	30.2	29.9
9.00	36.2	29.7	28.9	29.4	30.2	30.6	31.8
23	40.8	35.6	37.3	33.8	36.0	34.3	35.7
45	62.6	50.7	46.6	46.0	45.5	45.0	44.4
k_{cat} ($\times 10^7$ M^{-1}s^{-1})	0.770	0.560	0.490	0.430	0.410	0.370	0.350

Table S9. k_{obs} and k_{cat} values determined for the reaction of manganese(II) pyane-CH_2NH_2 with superoxide at different phosphate concentrations.

	k_{obs}, s^{-1}					
[MnL] ($\times 10^{-7}$ M)	0 mM P_i	1 mM P_i	2.5 mM P_i	5 mM P_i	20 mM P_i	40 mM P_i
4.5	38.7			37.7		
9.0	44.7	44.6	38.4	39.2	42.0	40.3
23	49.4	53.5	48.2	44.4	47.3	47.4
45	92.9	77.9	67.3	64.7	60.5	55.2
k_{cat} ($\times 10^7$ M^{-1}s^{-1})	1.09	0.876	0.763	0.588	0.568	0.520

For the binding constant of phosphate, the obtained catalytic rate constants (k_{cat}) were plotted vs. the respective hydrogen phosphate (HPO_4^{2-}) concentration and fitted along equation 1.

$$\boldsymbol{k_{cat}} = \boldsymbol{k_o} + [(\boldsymbol{k_\infty} - \boldsymbol{k_o}) \times \frac{\boldsymbol{K} \times [\boldsymbol{HPO_4^{2-}}]}{\boldsymbol{1+K} \times [\boldsymbol{HPO_4^{2-}}]}] \qquad \text{Eq 1}$$

k_{cat} : catalytic rate constant

k_0 : catalytic rate constant at 0 mM phosphate concentration

k_∞: catalytic rate constant at infinite phosphate concentration

K: binding constant for HPO_4^{2-}

[HPO_4^{2-}]: concentration of HPO_4^{2-}

The binding constants for dimanganese(II) bipyane were obtained by fitting the data along equation 2.

$$\boldsymbol{k_{cat}} = \left[\boldsymbol{k_0} + \boldsymbol{k_1} \boldsymbol{K_1} \left[\boldsymbol{HPO_4^{2-}}\right]\right] + \frac{\boldsymbol{k_2 K_1 K_2} \left[\boldsymbol{HPO_4^{2-}}\right]^{\boldsymbol{2}}}{\boldsymbol{1+K_1} \left[\boldsymbol{HPO_4^{2-}}\right]} \qquad \text{Eq 2}$$

k_{cat} : catalytic rate constant

k_0 : catalytic rate constant at 0 mM phosphate concentration

k_1: catalytic rate constant of *mono*-HPO_4^{2-} adduct

k_2: catalytic rate constant of *bis*-HPO_4^{2-} adduct

K_1: binding constant for first HPO_4^{2-}

K_2: binding constant for second HPO_4^{2-}

[HPO_4^{2-}]: concentration of HPO_4^{2-}

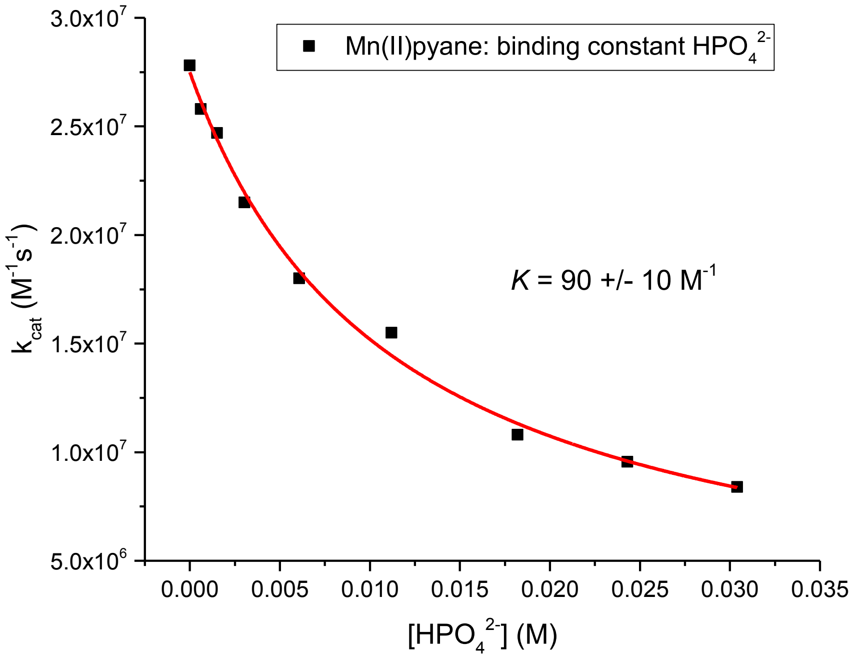

Figure S2. SOD catalytic rate constants k_{cat} as a function of the hydrogen phosphate concentration of manganese(II) pyane, fitted in the equation Eq. 1 (pH = 7.4; 60 mM total HEPES/phosphate buffer concentration).

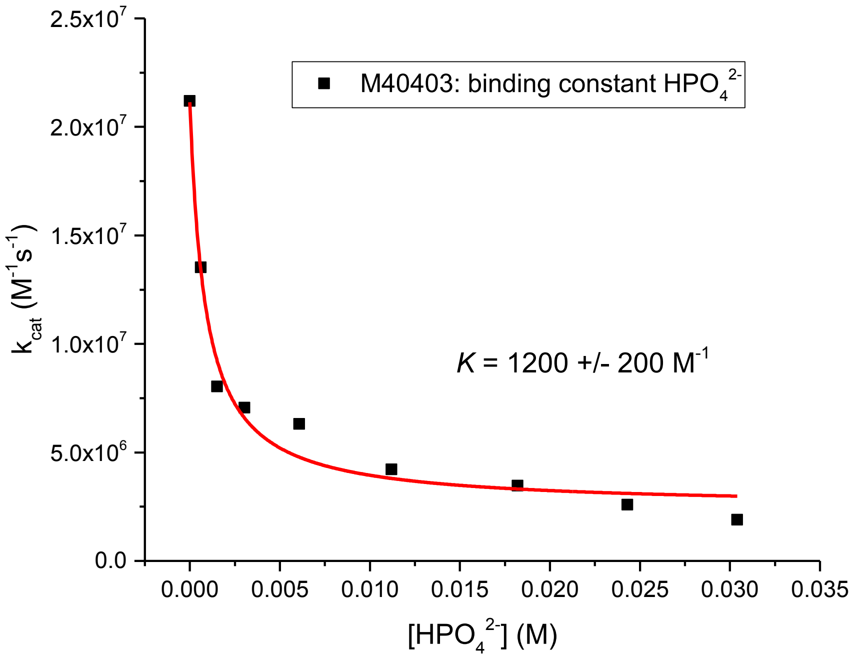

Figure S3. SOD catalytic rate constants k_{cat} as a function of the hydrogen phosphate concentration of M40403, fitted in Eq. 1 (pH = 7.4; 60 mM total HEPES/phosphate buffer concentration).

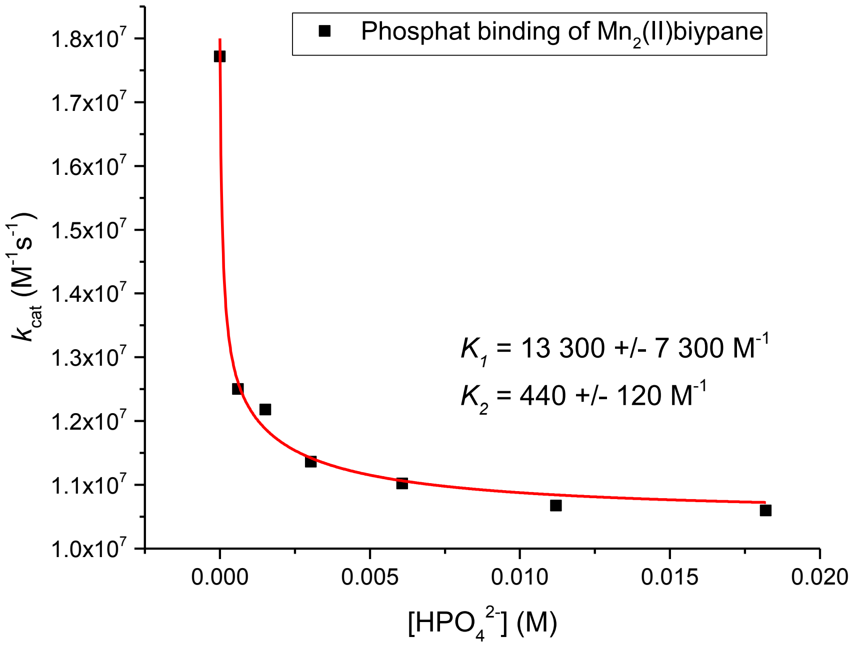

Figure S4. SOD catalytic rate constants k_{cat} as a function of the hydrogen phosphate concentration of dimanganese(II) bipyane, fitted in Eq. 2 (pH = 7.4; 60 mM total HEPES/phosphate buffer concentration).

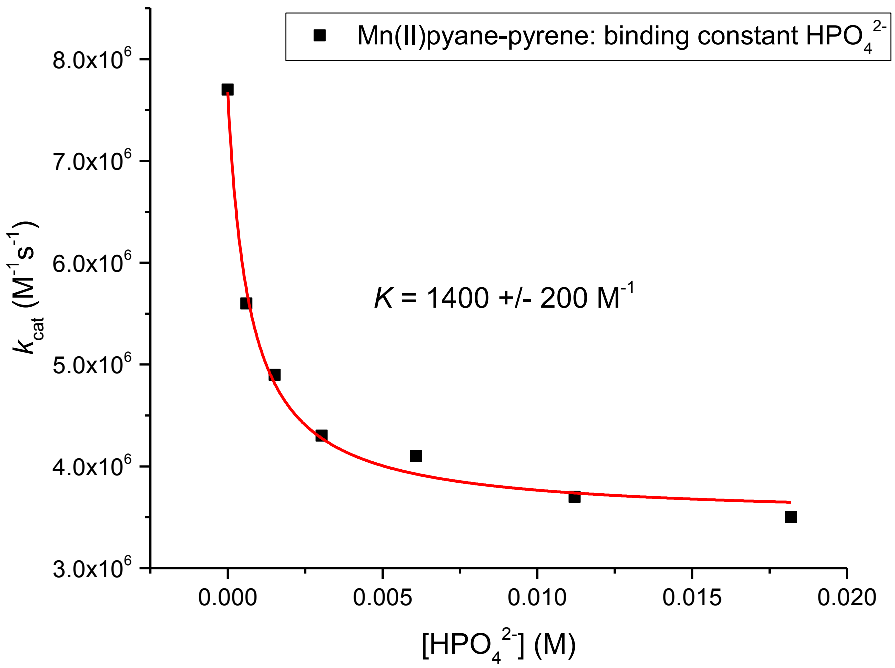

Figure S5 SOD catalytic rate constant k_{cat} as a function of the hydrogen phosphate concentration of manganese(II) pyane-pyrene, fitted in Eq. 1 (pH = 7.4; 60 mM total HEPES/phosphate buffer concentration).

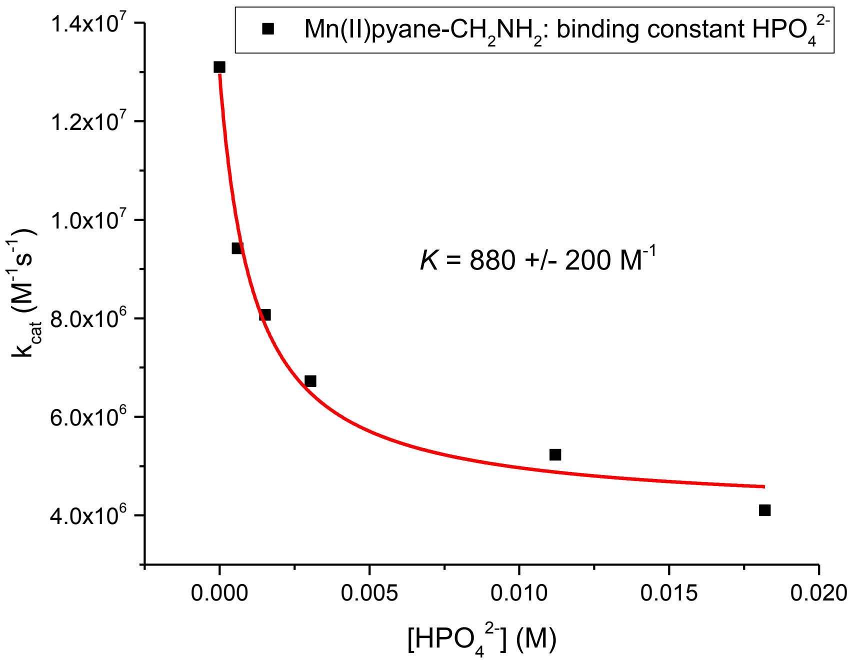

Figure S6. SOD catalytic rate constant k_{cat} as a function of the hydrogen phosphate concentration of manganese(II) pyane-CH_2NH_2, fitted in Eq. 1 (pH = 7.4; 60 mM total HEPES/phosphate buffer concentration).

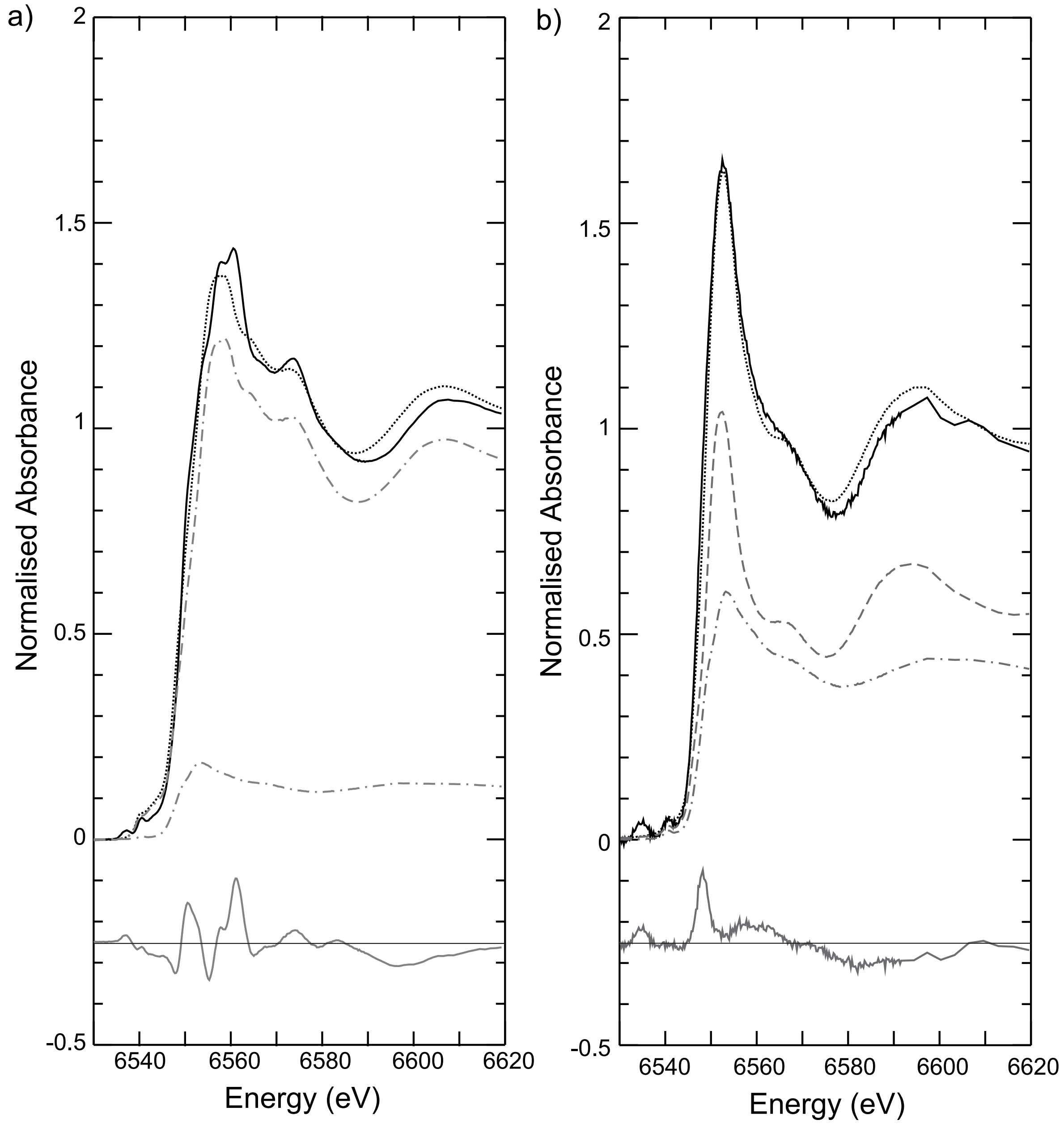

Figure S7. Mn *K*-edge X-ray absorbance spectrum of SH-SY5Y cells treated with 200 μM (a) manganese(II) pydiene or (b) manganese(II) pyane for 24 h (—) fit with a linear combination of $Mn_2(III)O_3$ (· - - - ·), manganese(II) pyane (- - -) and MnPi (· - · - ·). The fit is shown as a dotted line and the residual (solid grey) is offset.

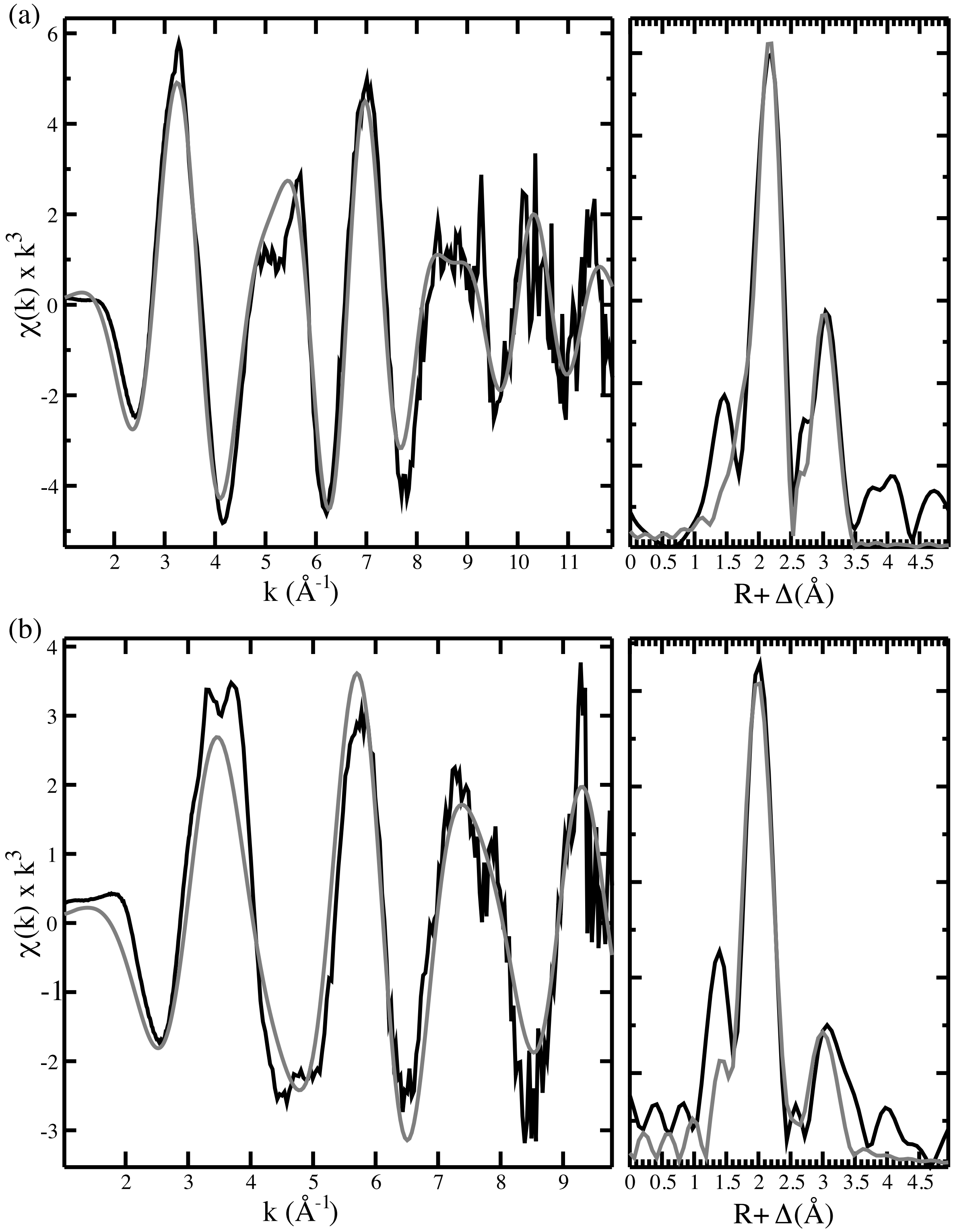

Figure S8. EXAFS (left) and Fourier transforms (right) of (a) M40404 standard solution in PBS and (b) SH-SY5Y cells treated with $MnCl_2$ for 24 hours, shown with their respective fits (grey line). Fit parameters are recorded in Tables 5 and 6.

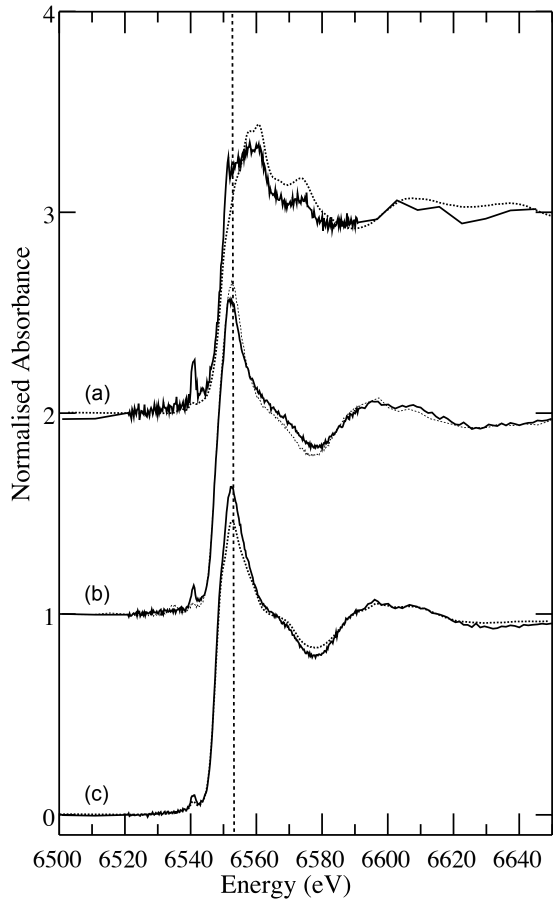

Figure S9. Mn *K*-edge XANES spectra of A549 (solid line) and SH-SY5Y (dashed line) cells treated for 24 h with (a) manganese(II) pydiene, (b) manganese(II) pyane and (c) $MnCl_2$.

Table S10. Elemental areal densities (in μg cm^{-2}) of SH-SY5Y cells treated with MnSOD mimetics, as determined by X-ray fluorescence microscopy. Asterisks indicate the value is significantly different from the control at the $P < 0.05$ significance level (two-tailed t-test assuming unequal variance). Data is presented as mean (standard deviation) of n cells.

	n	Area (μm^2)	P	S	Cl	K	Ca	Mn ($\times 10^{-3}$)	Fe ($\times 10^{-3}$)	Cu ($\times 10^{-3}$)	Zn ($\times 10^{-3}$)
control	5	315(160)	1.2(0.5)	0.6(0.2)	3(1)	0.11(0.06)	0.014(0.003)	0.5(0.2)	8(2)	2.4(0.7)	18(9)
$MnCl_2$	6	300(100)	1.0(0.3)	0.5(0.2)	2.4(0.9)	0.10(0.05)	0.013(0.005)	7(3)*	9(4)	2.3(0.7)	15(5)
Mn(II) pyane	5	260(65)	1.2(0.3)	0.6(0.2)	2.2(0.5)	0.16(0.09)	0.02(0.01)	5(2)*	4(1)	1.6(0.7)	16(3)
Mn(II) pydiene	5	630(110)	0.3(0.1)	0.15(4)	0.15(0.04)	0.02(0.01)	0.02(0.01)	62(6)*	11(5)	3(1)	3.7(0.6)
Mn_2(II) bipyane	5	420(110)	0.3(0.1)	0.17(0.07)	1.2(0.4)	0.04(0.02)	0.011(0.006)	11(2)*	6(3)	2.0(0.6)	9(3)
Mn(II) pyane-pyrene	6	540(150)	0.6(0.3)	0.3(0.1)	7(6)	0.2(0.1)	0.026(0.009)	18(8)*	8(2)	3.7(0.4)	30(10)
Mn(II) pyane[a]	9	250(50)	1.0(0.2)	0.5(0.1)	0.5(0.2)	0.019(0.008)	0.2(0.1)	1.7(0.5)*	6(4)	1.6(0.2)	17(3)
Mn(II) pyane-rhodB[a]	10	270(6)	1.1(0.4)	0.5(0.2)	0.6(0.1)	0.023(0.004)	0.012(0.002)	9(2)*	7.4(0.9)	2.5(0.3)	33(8)
M40404[a]	7	290(130)	0.4(0.2)	0.21(0.09)	0.8(0.1)	0.09(0.05)	0.13(0.06)	50(20)*	32(3)	2.3(0.3)	17(7)

[a]Samples were prepared and imaged separate on a second occasion.

Table S11. Nuclear:cytoplasmic ratio of elemental area density (in μg cm^{-2}) of SH-SY5Y cells treated with MnSOD mimetics, as determined by X-ray fluorescence microscopy. Asterisks indicate the value is significantly different from the control at the $P < 0.05$ significance level (two-tailed t-test assuming unequal variance). Data is presented as mean (standard deviation) of n cells.

	n	Area (μm^2)	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn
Control	5	0.3(0.1)	2.3(0.4)	2.3(0.5)	2.1(0.3)	2.1(0.3)	2.1(0.4)	2.0(0.4)	0.9(0.4)	1.0(0.2)	2.1(0.3)
$MnCl_2$	6	0.4(0.2)	2.2(0.4)	2.2(0.5)	2.1(0.4)	2.1(0.4)	2.1(0.4)	2.2(0.4)	2(2)	1.2(0.1)	2.2(0.4)
Mn(II) pyane	5	0.5(0.2)	2.2(0.5)	2.1(0.4)	2.4(0.5)	2.3(0.2)	1.8(0.6)	2.6(0.2)*	3.6(3.5)	1.2(0.2)	2.6(0.9)
Mn (II) pydiene	5	0.3(0.08)	2.0(0.9)	3.9(3.5)	1.1(0.7)	1.8(0.5)	1.6(0.4)	0.3(0.1)*	0.6(0.3)	1.2(0.6)	1.4(0.4)
Mn_2(II) bipyane	5	0.25(8)	2.4(0.5)	2.3(0.5)	1.8(0.2)	2.0(0.3)	2.0(0.3)	1.8(0.4)	2(3)	1.0(0.2)	1.5(0.2)
Mn(II) pyane-pyrene	6	0.4(0.2)	2.9(0.8)	2.2(0.5)	2.1(0.3)	2.3(0.4)	2.1(0.5)	1.3(0.4)*	2.1(2.6)	1.1(0.2)	1.6(0.4)
Mn(II) pyane[a]	9	0.16(0.06)	3.1(0.3)	3.1(0.4)	1.3(0.2)	1.9(0.8)	2.5(0.2)	2.1(0.2)	1.3(0.3)	1.2(0.1)	2.9(0.4)
Mn(II) pyane-rhodB[a]	10	0.19(0.06)	3.4(0.6)	3.6(0.6)	1.6(0.4)	2.0(0.3)	2.7(0.3)	2.4(0.2)	1.4(0.2)	1.7(0.2)	2.8(0.3)
M40404[a]	7	0.34(0.09)	3(1)	2.5(0.6)	1.2(0.2)	1.6(0.2)	2.2(0.8)	1.7(0.5)	1.11(0.07)	1.2(0.1)	3(1)

[a]Samples were prepared and imaged separate on a second occasion.

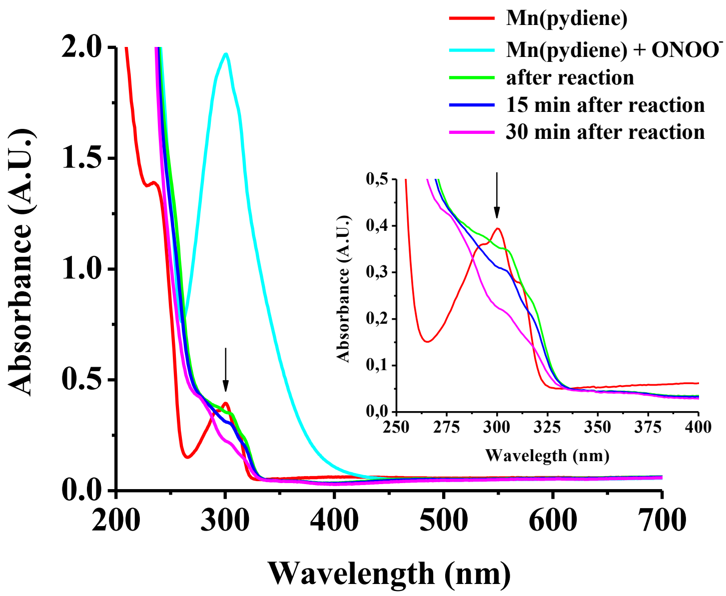

Figure S10. Decomposition of manganese(II) pydiene (50 µM in 100 mM phosphate buffer, pH 7.4) upon treatment with peroxynitrite (1 mM) followed by UV/Vis spectroscopy over a time period of 30 minutes (characteristic absorption band of manganese(II) pydiene is indicated with an arrow).