Supplementary Information

Effect of Antioxidants on Enzyme-catalysed Biodegradation of Carbon Nanotubes

Gregg P. Kotchey,[a] James A. Gaugler,[a] Alexander A. Kapralov,[b] Valerian E. Kagan[a,b] and Alexander Star*[a]

[a]Department of Chemistry and [b]Department of Environmental and Occupational Health
University of Pittsburgh, Pittsburgh, Pennsylvania, USA

astar@pitt.edu

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Table S1. Initial Experimental Conditions

Sample	o-SWCNTs (1 mg/mL)	H_2O_2 (18.75 mM)	AR MPO (2.0 µM)	BE MPO	NaCl (5 M)	AA (250 mM)	GSH (250 mM)	PB/DTPA (0.1 M/ 300 µM)
o-SWCNT	7 µL	0	0	0	0	0	0	243 µL
o-SWCNT + MPO	7 µL	0	0	4.8 µL	0	0	0	238.2 µL
o-SWCNT + H_2O_2	7 µL	1 µL	0	0	0	0	0	242 µL
o-SWCNT + MPO + H_2O_2 − Cl^-	7 µL	1 µL	0	4.8 µL	0	0	0	237.2 µL
o-SWCNT + MPO + H_2O_2 + Cl^-	7 µL	1 µL	4 µL	0	7 µL	0	0	231 µL
o-SWCNT + AA	7 µL	0	0	0	0	1 µL	0	242 µL
o-SWCNT + MPO + AA	7 µL	0	0	4.8 µL	0	1 µL	0	237.2 µL
o-SWCNT + MPO + H_2O_2 − Cl^- + AA	7 µL	1 µL	0	4.8 µL	0	1 µL	0	236.2 µL
o-SWCNT + MPO + H_2O_2 + Cl^- + AA	7 µL	1 µL	4 µL	0	7 µL	1 µL	0	230 µL
o-SWCNT + GSH	7 µL	0	0	0	0	0	1 µL	242 µL
o-SWCNT + MPO + GSH	7 µL	0	0	4.8 µL	0	0	1 µL	237.2 µL
o-SWCNT + MPO + H_2O_2 − Cl^- + GSH	7 µL	1 µL	0	4.8 µL	0	0	1 µL	236.2 µL
o-SWCNT + MPO + H_2O_2 + Cl^- + GSH	7 µL	1 µL	4 µL	0	7 µL	0	1 µL	230 µL

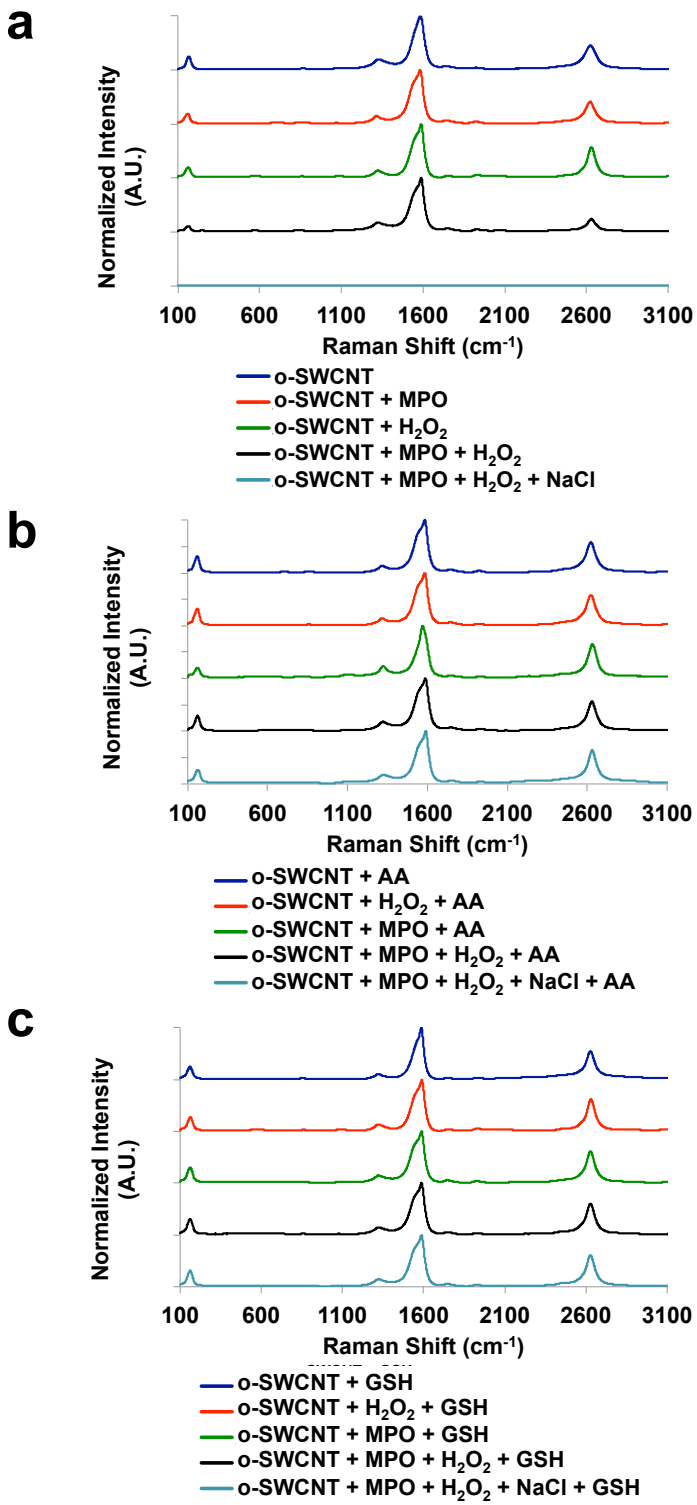

Fig. S1 Raman spectroscopy performed on (a) o-SWCNTs samples, (b) o-SWCNTs samples treated with AA, and o-SWCNTs samples treated with GSH.

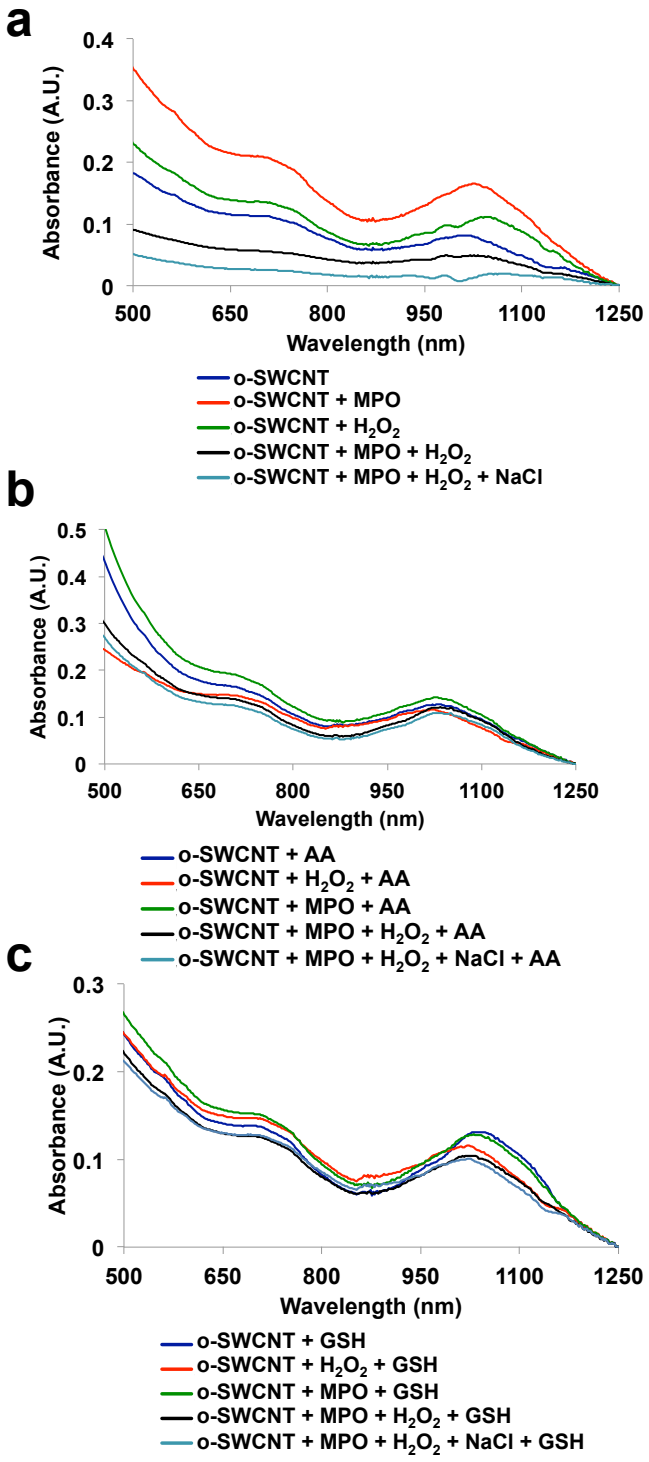

Fig. S2 vis−NIR absorption spectra for (a) o-SWCNTs samples, (b) o-SWCNTs samples treated with AA, and o-SWCNTs samples treated with GSH.

Table S2. Area of S_{22} Peaks

Sample	Area of S_{22} Peak
o-SWCNT	8.135
o-SWCNT + MPO + H_2O_2	2.980
o-SWCNT + MPO + H_2O_2 + NaCl	1.962
o-SWCNT + MPO + H_2O_2 + GSH	7.425
o-SWCNT + MPO + H_2O_2 + NaCl + GSH	7.981
o-SWCNT + MPO + H_2O_2 + AA	9.698
o-SWCNT + MPO + H_2O_2 + NaCl + AA	10.627

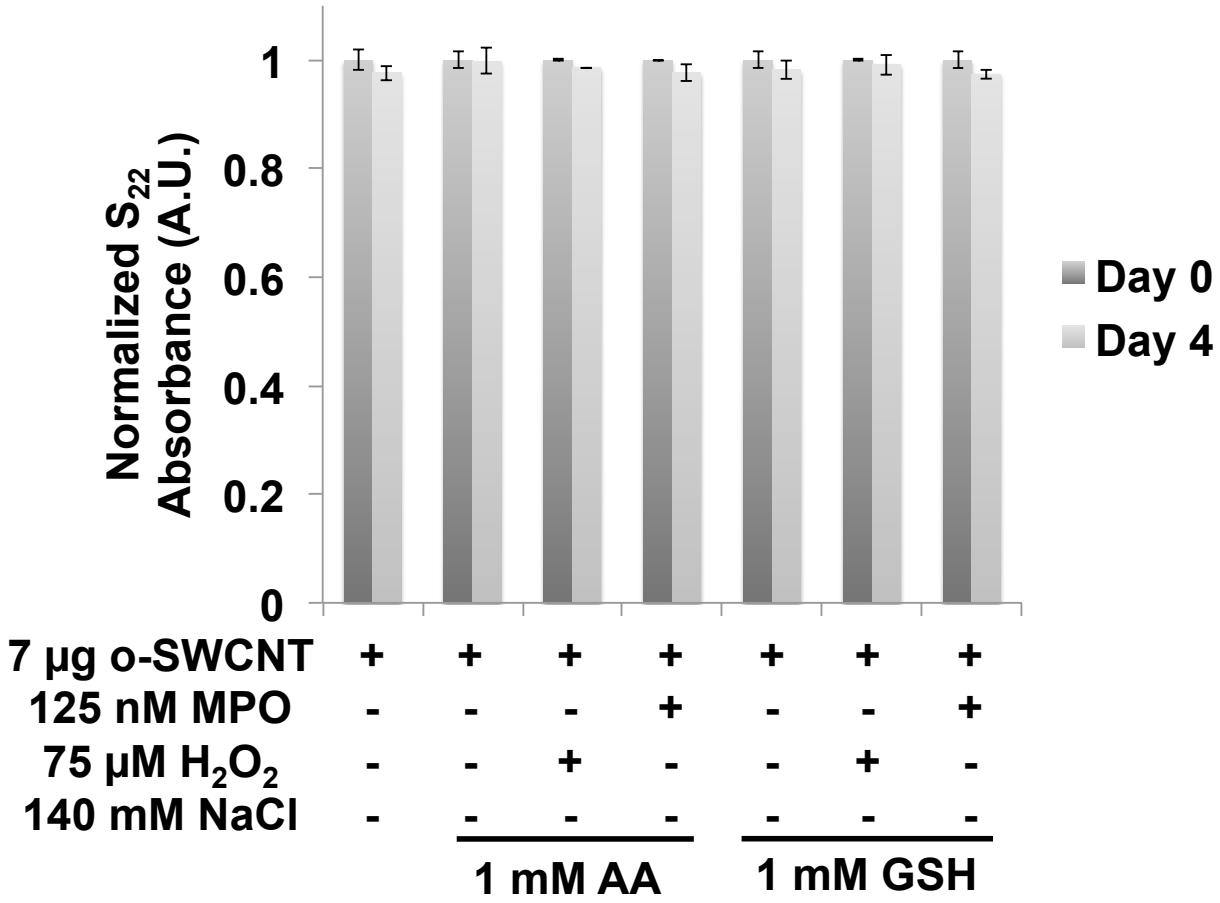

Fig. S3 S_{22} absorbance intensity of o-SWCNTs at day 0 and day 4 for the given control experiments. The error bar represents standard error of the mean with a sample size of three.

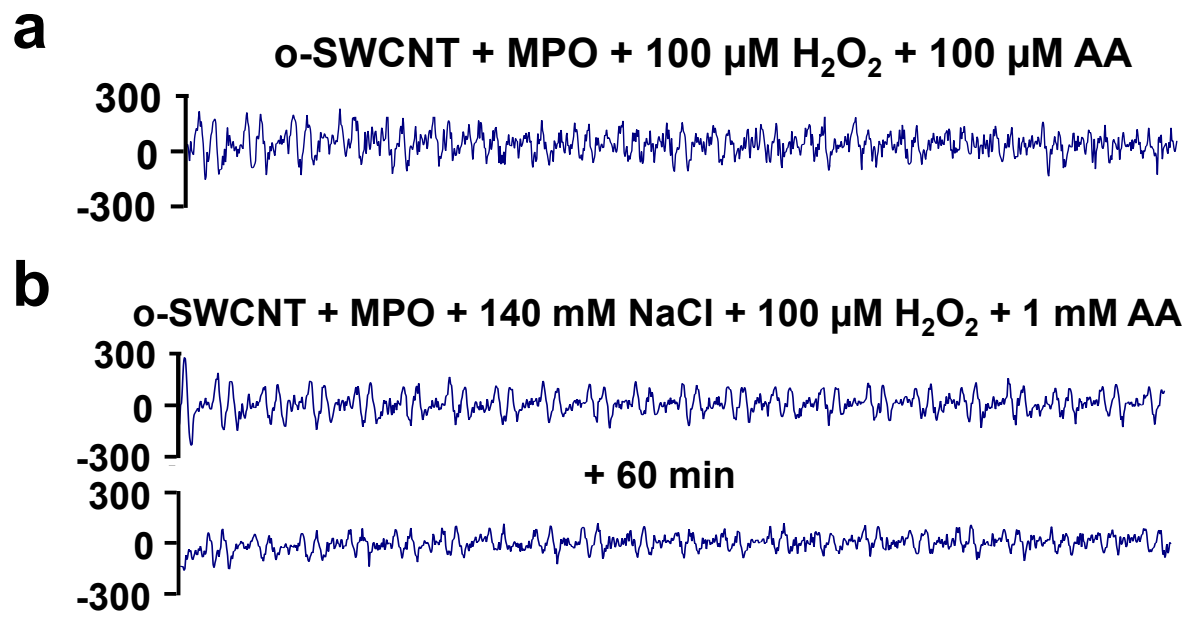

Fig. S4 Results of an electron paramagnetic resonance (EPR) study, which demonstrates the presence of the ascorbate radical (a) under conditions consisting of o-SWCNTs, MPO, and H_2O_2 and (b) after a 60-minute incubation with a system consisting of o-SWCNTs, MPO, NaCl, and H_2O_2.

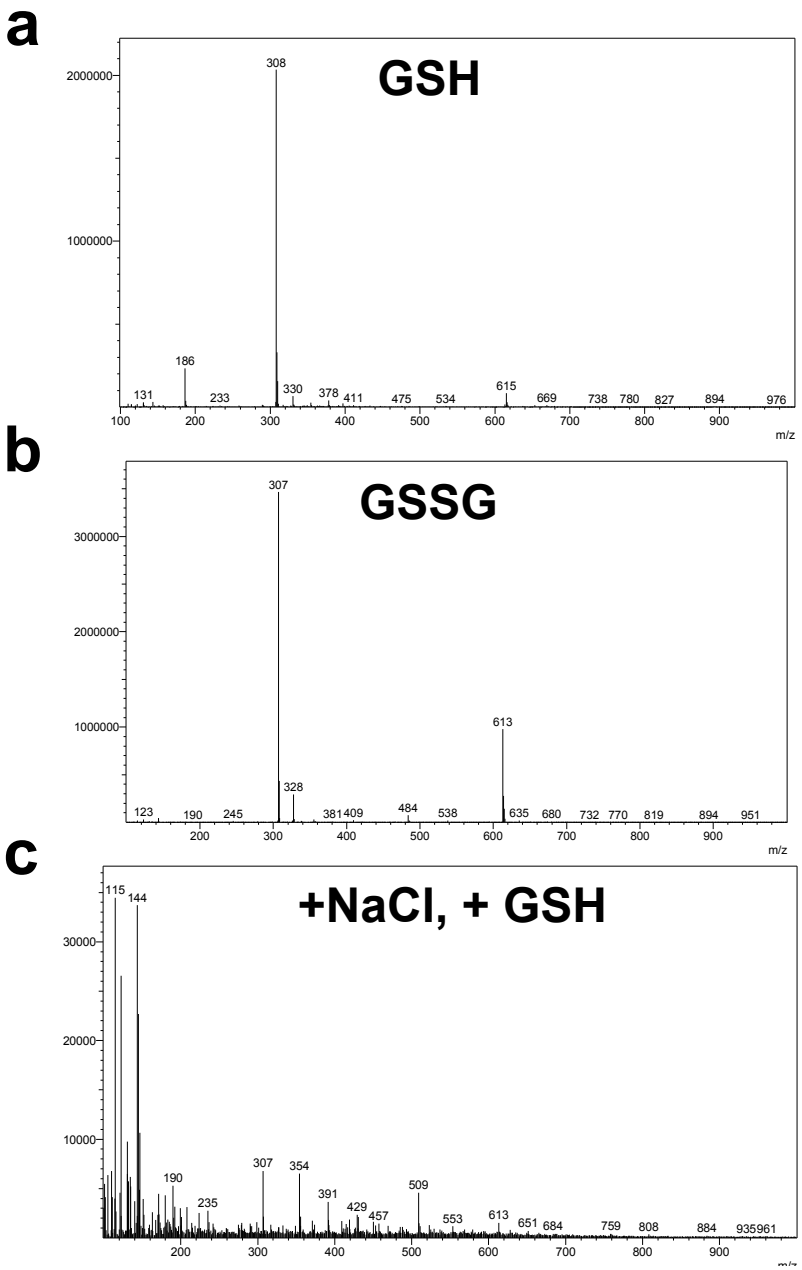

Fig. S5 Electrospray ionization mass spectrometry (ESI-MS) data (positive mode) for (a) GSH, (b) oxidized GSH (GSSG), (c) o-SWCNTs samples treated with both GSH and NaCl.

Table S3. Experimental Conditions for MPO Activity Assay Employing Amplex Red

Sample	Amplex Red (10 mM)	H_2O_2 (10 mM)	AR MPO*	BE MPO*	AA (250 mM)	GSH (250 mM)	PB/DTPA (0.1 M/ 300 µM)
AR MPO + H_2O_2 − Cl^-	3 µL	3 µL	4 µL	0	0	0	590 µL
BE MPO + H_2O_2 − Cl^-	3 µL	3 µL	0	4.8 µL	0	0	589.2 µL
o-SWCNT + AR MPO + H_2O_2 − Cl^- + AA	3 µL	3 µL	4 µL	0	2.4 µL	0	587.6 µL
MPO + H_2O_2 − Cl^- + GSH	3 µL	3 µL	4 µL	0	0	2.4 µL	587.6 µL

*Diluted 1/200 with PB/DTPA

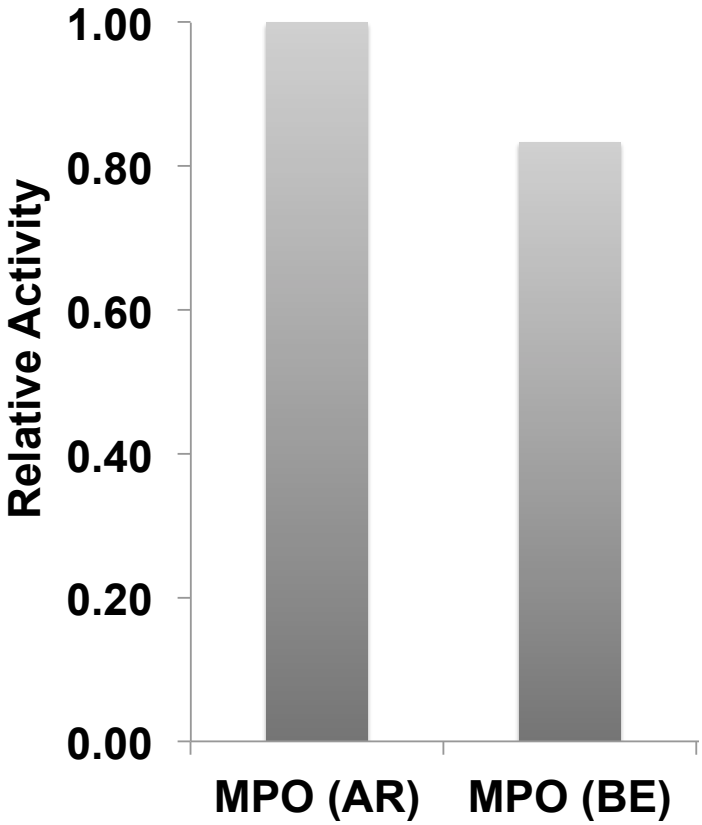

Fig. S6 Comparison of the relative activity of as received (AR) MPO and buffer exchanged (BE) MPO derived from a fluorescence-based kinetic experiment employing Amplex red.

Effect of Changing the Antioxidant Concentration on o-SWCNT Degradation

Next, the effect of changing the antioxidant concentration on o-SWCNT degradation was analysed using a nearly identical procedure as outlined above. Table S3 details the initial experimental conditions, which were performed in triplicate using 96 well plates. The variable AA final concentrations were 50 μM, 100 μM, 250 μM, 500 μM, and 1 mM; the GSH concentrations were 10 nM, 50 nM, 100 nM, 250 nM, 500 nM, 1 μM, 10 μM, 50 μM, 100 μM mM, 250 μM, 500 μM, and 1 mM. Again, the Epoch microplate spectrophotometer was employed to obtain initial (Day 0) absorbance reading at 999 nm. Every hour, 75 μM H_2O_2 (final) and the respective AA (50 μM, 100 μM, 250 μM, 500 μM, and 1 mM) and GSH (10 nM, 50 nM, 100 nM, 250 nM, 500 nM, 1 μM, 10 μM, 50 μM, 100 μM mM, 250 μM, 500 μM, and 1 mM) concentrations (final) were dispensed for a total of 7 additions on day 0 and 8 additions on days 1, 2 and 3. On days 1, 2 and 3, 4 μL of AR MPO and 4.8 μL of BE MPO were added to the samples (according to Table S3, MPO columns). Between additions and overnight, the samples were incubated at 37 °C in an incubator. Finally, on day 4 (96 h), the Epoch microplate spectrophotometer was again used to measure the absorbance at 999 nm. The data is presented in Figure S7 as the relative change compared to when zero antioxidant is utilized.

Table S4. Initial Conditions for Experiments with Different Antioxidant Concentration

Sample	o-SWCNTs (1 mg/mL)	H_2O_2 (18.75 mM)	AR MPO (2.0 μM)	BE MPO	NaCl (5 M)	AA (250 mM)	GSH (250 mM)	PB/DTPA (0.1 M/ 300 μM)
o-SWCNT + MPO + H_2O_2 − Cl^- + AA	7 μL	1 μL	0	4.8 μL	0	1 μL	0	236.2 μL
o-SWCNT + MPO + H_2O_2 + Cl^- + AA	7 μL	1 μL	4 μL	0	7 μL	1 μL	0	230 μL
o-SWCNT + MPO + H_2O_2 − Cl^- + GSH	7 μL	1 μL	0	4.8 μL	0	0	1 μL	236.2 μL
o-SWCNT + MPO + H_2O_2 + Cl^- + GSH	7 μL	1 μL	4 μL	0	7 μL	0	1 μL	230 μL

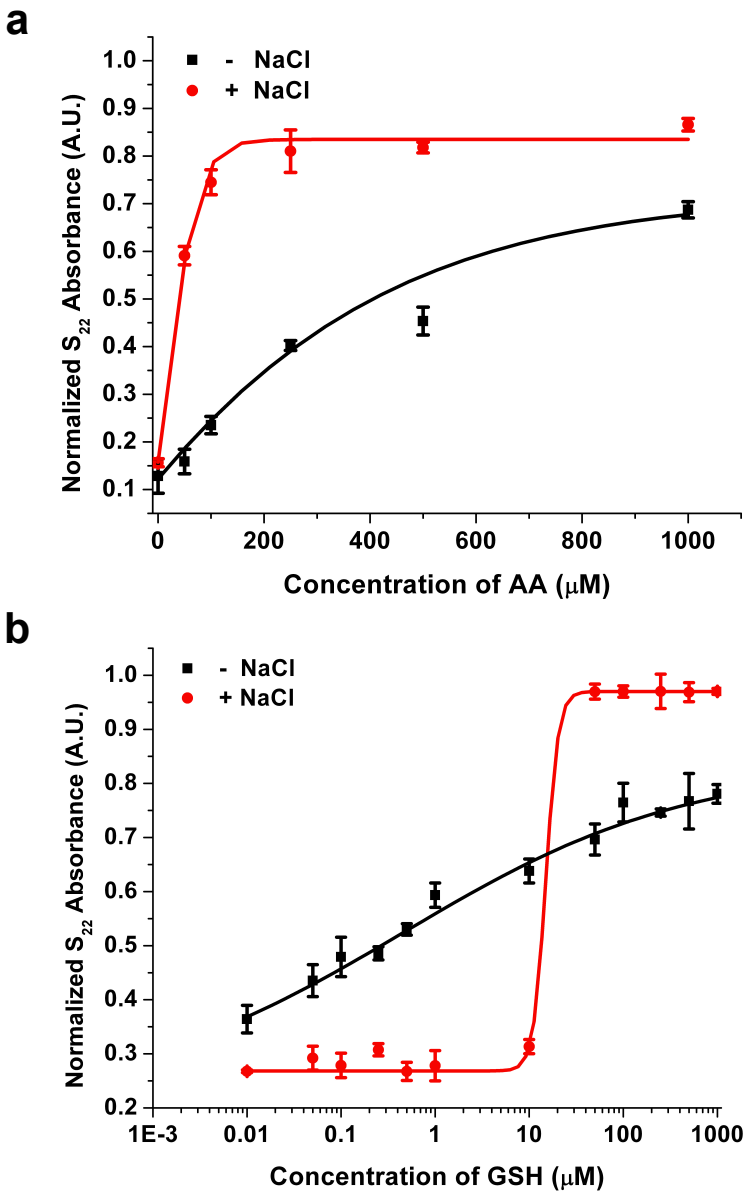

Fig. S7 The effect of changing (a) AA and (b) GSH concentrations on the relative change in S_{22} absorbance for the MPO system with (red) and without (black) NaCl.

Degradation Experiments with Antioxidant Treated o-SWCNTs

To ascertain if the reduction of functional groups belonging to o-SWCNTs by antioxidants hindered enzyme-catalysed degradation, the nanotubes were first treated with the antioxidants and subsequently subjected to enzymatic treatment under ±Cl^- conditions. To this end, 1 mg of P3 o-SWCNTs was added to 10 mL of nanopure water in a glass vial and sonicated for 5 minutes. To this vial, a final concentration of 3.6 mM AA was added for a total of 8 additions per day for 4 days (32 additions total). Between additions, the vial was covered with aluminum foil and incubated at 37 °C with shaking. Note that the concentration of AA utilized was derived from the antioxidant experiments (*i.e.* 1 mM) and proportionally adjusted for the higher concentration of nanotubes (0.1 mg/mL). A second vial was created for GSH following an identical procedure described above. After 96 hours of incubation, the nanotubes were filtered employing TefSep PTFE membrane filters; the nanotubes were washed with 100 mL each of nanopure water, 0.01 M NaOH, 0.01 M HCl, and nanopure water. Finally, the dried antioxidant-treated o-SWCNT samples were weighed and sonicated into 0.1 mM phosphate buffer at a final concentration of 1 mg/mL. Table S3 (ESI†) details the initial experimental conditions, which were performed in triplicate using 96 well plates. Every hour, 75 μM H_2O_2 (final) were dispensed for a total of 7 additions on day 0 and 8 additions on days 1, 2 and 3. On days 1, 2 and 3, 4 μL of AR MPO and 4.8 μL of BE MPO were added to the samples (according to Table S4, MPO columns, ESI†). Between additions, the samples were incubated at 37 °C in an incubator. Finally, on day 4 (96 h), the Epoch microplate spectrophotometer was again used to measure the absorbance at 999 nm.

Table S5. Initial Conditions for Experiments with Antioxidant-Treated o-SWCNTs

Sample	AA-o-SWCNTs (1 mg/mL)	GSH-o-SWCNTs (1 mg/mL)	H_2O_2 (18.75 mM)	AR MPO (2.0 µM)	BE MPO	NaCl (5 M)	PB/DTPA (0.1 M/ 300 µM)
AA-o-SWCNT + MPO + H_2O_2 − Cl^-	7 µL	0	1 µL	0	4.8 µL	0	237.2 µL
AA-o-SWCNT + MPO + H_2O_2 + Cl^-	7 µL	0	1 µL	4 µL	0	7 µL	231 µL
GSH-o-SWCNT + MPO + H_2O_2 − Cl^-	0	7 µL	1 µL	0	4.8 µL	0	237.2 µL
GSH-o-SWCNT + MPO + H_2O_2 + Cl^-	0	7 µL	1 µL	4 µL	0	7 µL	231 µL

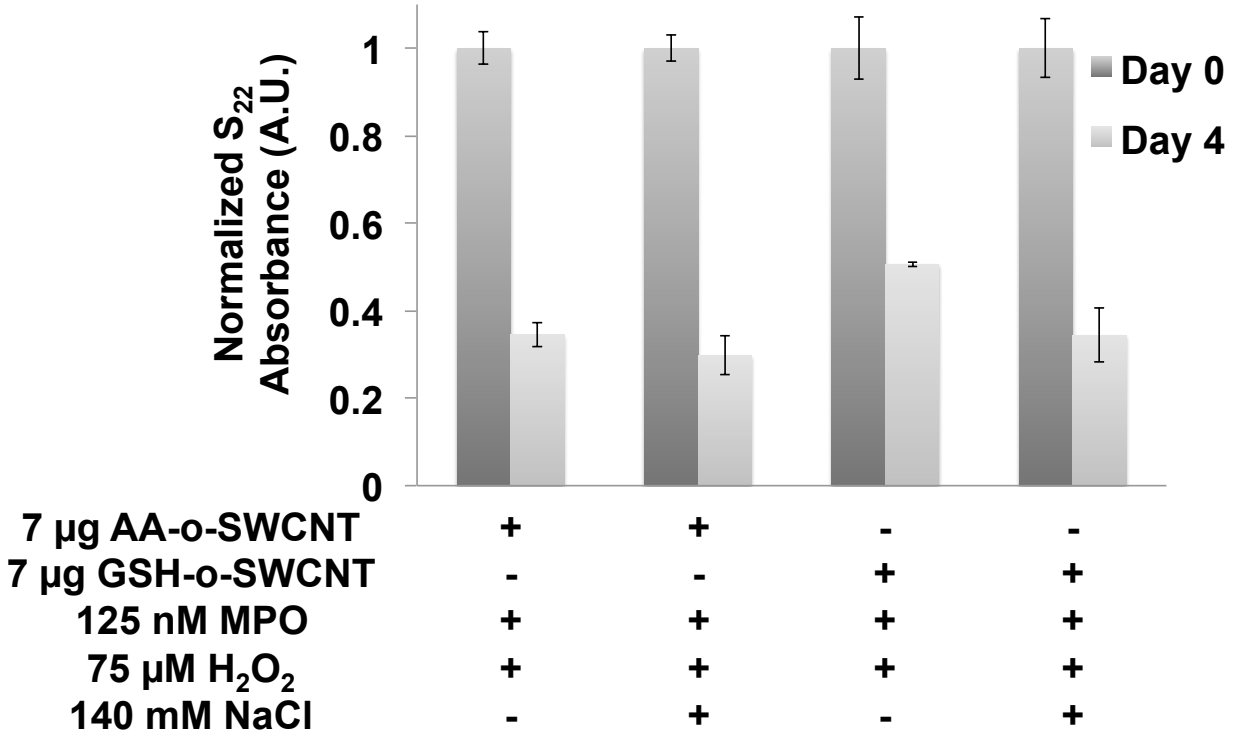

Fig. S8 S_{22} absorbance intensity for antioxidant-treated o-SWCNTs under given experimental conditions at day 0 and day 4. The error bar represents standard error of the mean with a sample size of three.