Supplementary Material (ESI) for Nanoscale

Oxidative biodegradation of single- and multi-walled carbon nanotubes

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Electronic Supporting Information

Materials and methods

Carbon Nanotubes

SWCNTs were purchased from Unidym (HiPco® Single Walled Carbon Nanotubes, Lot# R1912), MWCNTs from Nanostructured & Amorphous Materials Inc. (NanoAmor, Regular MWCNTs 95% pure, stock No. 1240XH. Outer average diameter was between 20 and 30 nm, and length between 0.5 and 2 μm) and Nanocyl (Thin MWCNT 95+% C purity, Nanocyl 3100®, batch no. 071119, average diameter and length: 9.5 nm and 1.5 μm, respectively). All reagents and solvents were purchased from different commercial suppliers and used as received. Oxidized carbon nanotubes *ox*-SWCNTs **1** and *ox*-MWCNTs **2** and **3** were prepared as already reported.[1-3]

Instruments

TEM was performed on a Hitachi 600 microscope with an accelerating voltage of 75 kV. Raman spectra were recorded using a Renishaw inVia microRaman equipped with a He-Ne Laser (633 nm) and a Leica microscope. When stated, suspensions of CNTs were sonicated in a water bath (Transsonics Digitals Elma, 20 W, 40 kHz). Dynamic light scattering was performed on a Zetasizer Nano S (Malvern Instruments) spectrometer operating under 633 nm laser irradiation.

Biodegradation assays

To study the biodegradation of oxidized CNTs *in vitro*, we evaluated the effects of two different conditions on their morphological features. First, *ox*-SWCNTs **1** and *ox*-MWCNTs **2** and **3** have been subjected to the degrading action of an enzyme free reaction buffer, the phagolysosomal stimulant fluid (PSF). This buffer has been shown to mimic the chemical environment of phagolysosomes where nanomaterials are located after phagocytosis.[4-6] To fully simulate the physiological oxidizing environment of phagolysosomes, H_2O_2 was added

weekly. Briefly, 2 mg of solid CNTs were dispersed in 10 ml of PSF (Table 1) containing a physiological concentration of H_2O_2 (1 mM).[5] After 1 hour of bath sonication, the samples were placed in the dark and stirred during the whole experiment. H_2O_2 (100 µl of a 100 mM solution) was added weekly to maintain the initial concentration of 1 mM.

A second experimental protocol has been carried out by incubating *ox*-SWCNTs **1** and *ox*-MWCNTs **2** and **3** with Horseradish Peroxidase (HRP Type VI, Sigma Aldrich). Briefly, 1 mg of solid CNTs was suspended in phosphate buffered saline (PBS) by 1 min of bath sonication. Following the dispersion of the tubes, 4 ml of HRP solution (0.385 mg/ml) were added and the enzymatic catalysis started, 24 hours later, by mixing 8 ml of H_2O_2 (800 µM) to the dispersion of CNTs in HRP.[7,8] To avoid the possible loss of activity of the HRP, the enzyme was refilled after 30 days by adding 1 ml of a fourfold concentrated HRP solution (1.54 mg/ml). All the solutions were kept in the dark and stirred during the entire experiment and H_2O_2 (250 µl of the 800 µM solution) was added daily.

In both conditions, samples (500 µl) were drawn at time 0, 1, 7, 15, 30 and 60 days and stored at 4°C in the dark until characterization.

Table 1. Composition of phagolysosomal simulant fluid (PSF)[a]

Constituents of PSF	**mg/L**	**Concentration**
Sodium phosphate dibasic anhydrous	142	1 mM
Sodium chloride	6650	113.8 mM
Sodium sulfate (anhydrous)	71	0.5 mM
Calcium chloride dihydrate	29	197 mM
Glycine	450	6 mM
Potassium hydrogen phthalate	4084.6	0.02 M
Alkylbenzyldimethylammonium chloride	50	50 ppm

[a] The pH of the solution is 4.5

Characterization of the degrading CNTs

Initially all samples of CNTs were washed by a methanol/ether mixture (1:3) three times and precipitated (3250g for 7 min at 4°C) to remove the salts from the reaction buffers. CNTs were then resuspended in distilled water (500 µl) by sonication for the different analyses.

Transmission electron microscopy. For TEM characterization few microliters of a suspension of CNTs in methanol were deposited on a carbon TEM grid and dried.

For the statistical analysis of the length distribution we have measured:

i) 198 *ox*-MWCNTs **2** (NanoAmor), 393 *ox*-MWCNTs **2** treated with PSF, 413 *ox*-MWCNTs **2** treated with HRP.

ii) 163 *ox*-MWCNTs **3** (Nanocyl), 596 *ox*-MWCNTs **3** treated with PSF, 656 *ox*-MWCNTs **3** treated with HRP.

Raman Spectroscopy. 50 µl of *ox*-SWCNTs **1** and *ox*-MWCNTs **2** and **3** solutions were evaporated on a glass slide and the spectra of agglomerates of about 1 µm or larger have been recorded. The spectra shown in the figures are representative of the recorded spectra.

Dynamic Light Scattering. Samples of *ox*-SWCNTs **1** and *ox*-MWCNTs **2** and **3** (t = 0 and t = 60 days) were dispersed in distilled water to reach a concentration of approximately 20 $\mu g.mL^{-1}$ and sonicated for 1 hour. Measurements were then performed over 12 runs of 10 seconds each.

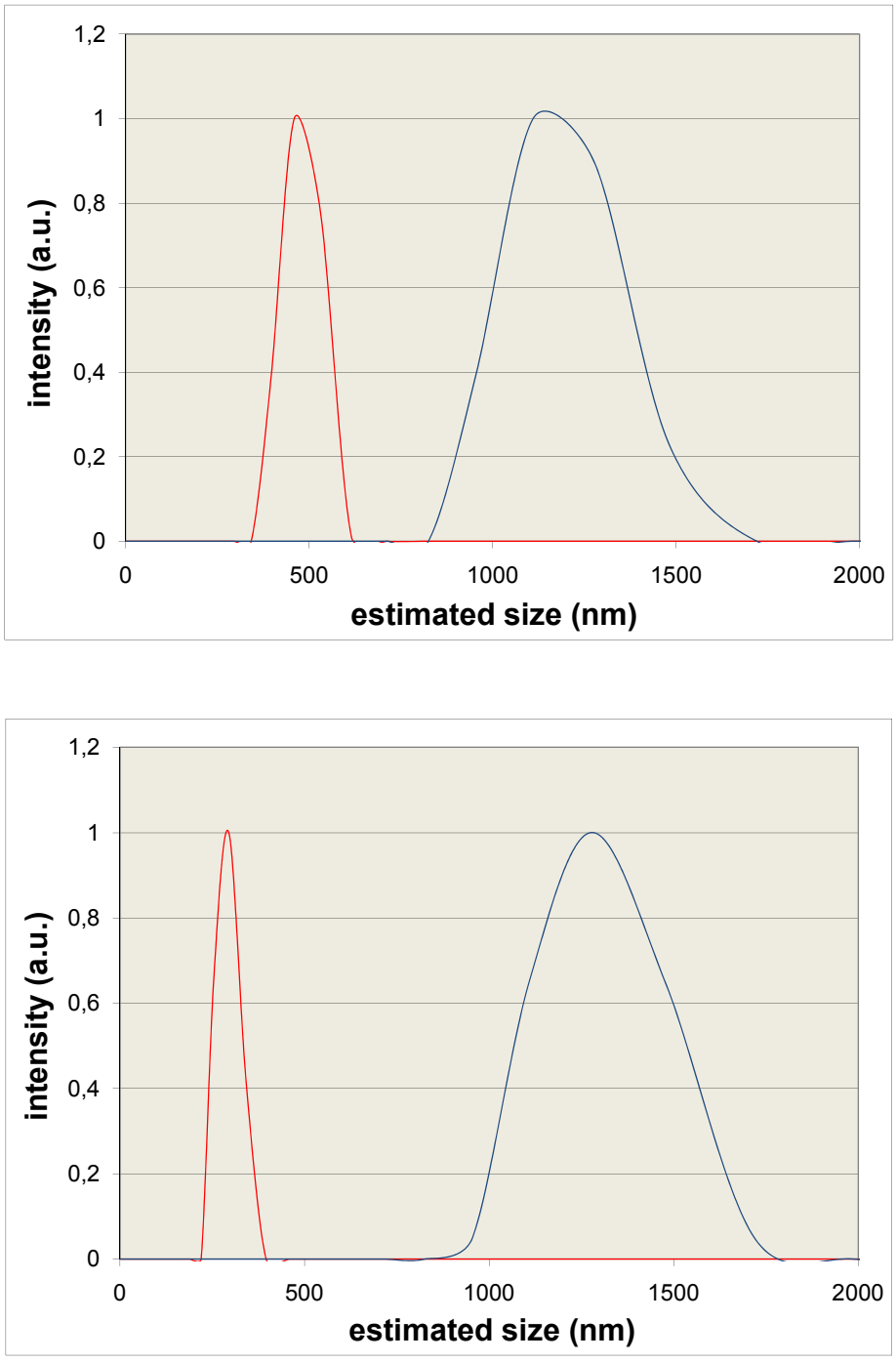

Figure S1. DLS diagrams of *ox*-SWCNTs **1** in PSF (top) and HRP (bottom) at time zero (blue line) and after 60 days (red line).

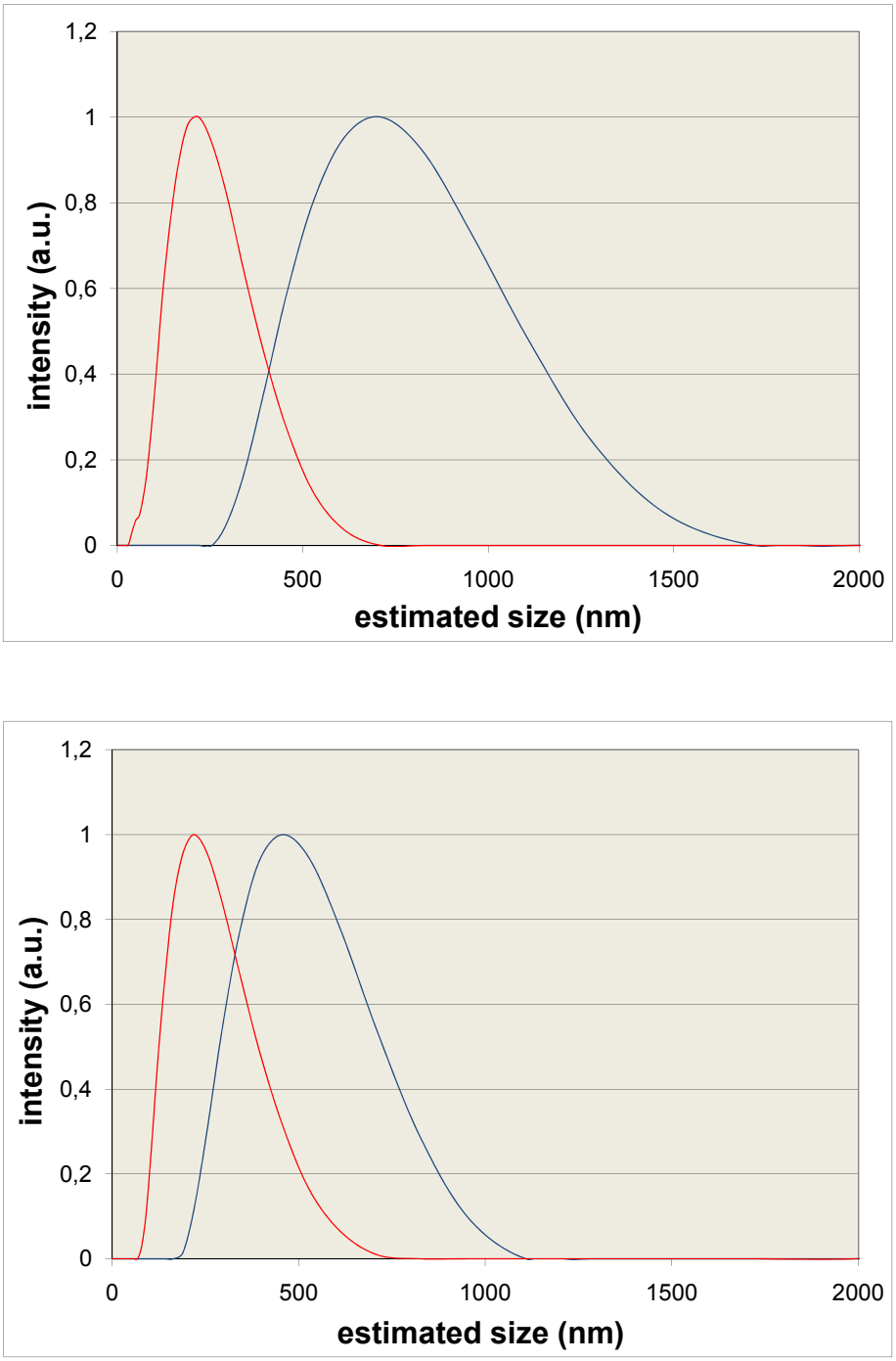

Figure S2. DLS diagrams of *ox*-MWCNTs **2** in PSF (top) and HRP (bottom) at time zero (blue line) and after 60 days (red line).

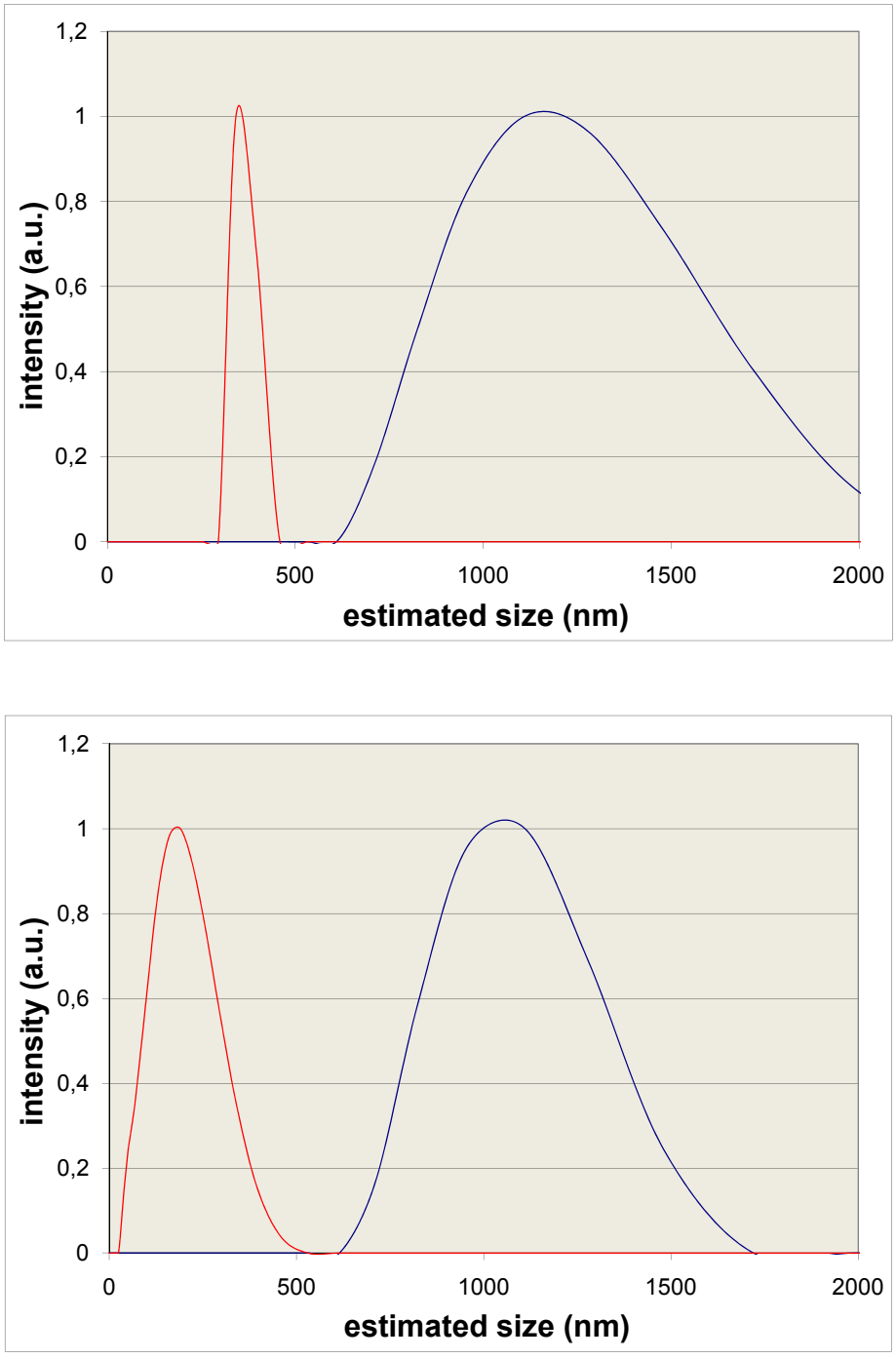

Figure S3. DLS diagrams of *ox*-MWCNTs **3** in PSF (top) and HRP (bottom) at time zero (blue line) and after 60 days (red line).

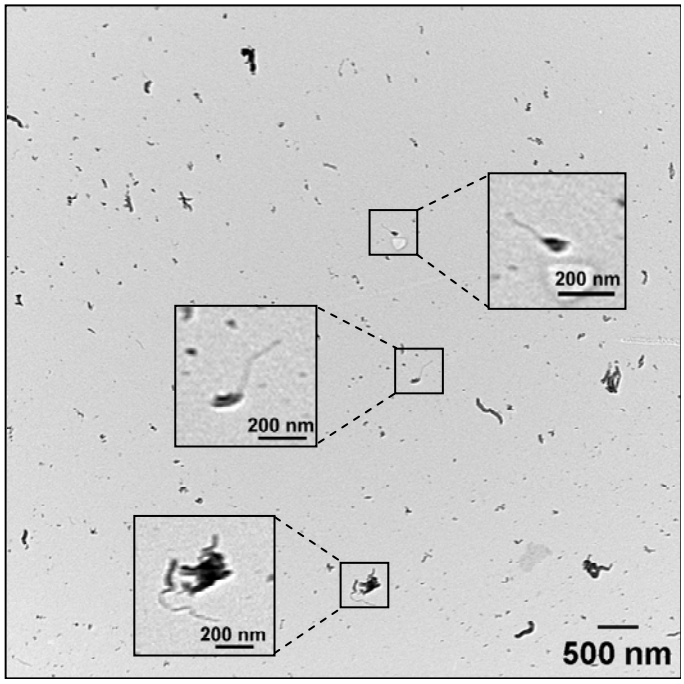

Figure S4. TEM images of *ox*-MWCNTs **2** in HRP after 60 days.

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