Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

Supporting Information

RGD-Targeted MnO Nanoparticles as T_1 Contrast Agents for Cancer Imaging – the Effect of PEG Length *In Vivo*

Juan Gallo,[1, 2] *Israt S. Alam,*[1] *Ioannis Lavdas,*[1] *Marzena Wylezinska-Arridge,*[3] *Eric O. Aboagye* [1,*] *and Nicholas J. Long* [1, 2, *]

[1] Comprehensive Cancer Imaging Centre, Department of Surgery & Cancer, Hammersmith Campus, Imperial College London, Du Cane Road, London, W12 0NN, UK

[2] Department of Chemistry, Imperial College London, South Kensington, London, SW7 2AZ, UK

Correspondence to: n.long@imperial.ac.uk; eric.aboagye@imperial.ac.uk

[3] Biological Imaging Centre, Medical Research Council (MRC) Clinical Science Centre, Imperial College London, Du Cane Road, London, W12 0NN, UK

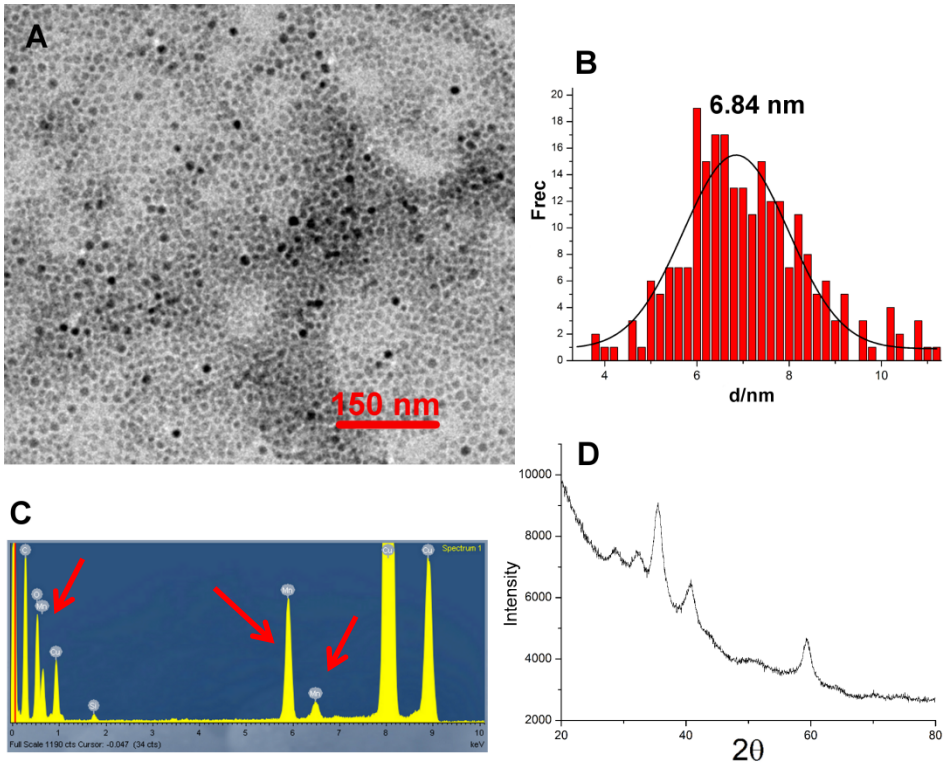

Figure S1. **A**, Overview TEM micrograph of MnO nanoparticles showing a homogeneous population of around 7 nm of diameter (scale bar 150 nm); **B**, Size distribution histogram obtained measuring the particles from TEM images such as A; **C**, EDXS spectra obtained from sample A showing mainly Mn peaks (red arrows). Remaining peaks correspond to Cu and Si coming from the sampler holder. **D**, XRD spectra of a sample of MnO nanoparticles showing characteristic peaks from manganese oxide.

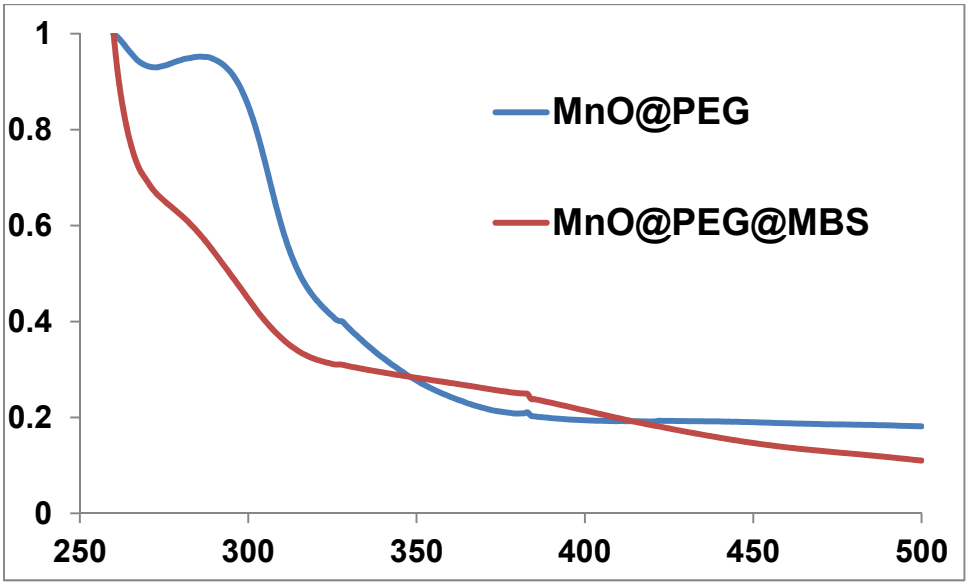

Figure S2. Normalised UV-Vis spectra of MnO@PEG_{5000} nanoparticles (red) and MnO@PEG_{5000}@MBS (blue). A shoulder coming from the maleimide ring can be observed at around 290 nm in the MBS sample. Both spectra were recorded in water solutions.

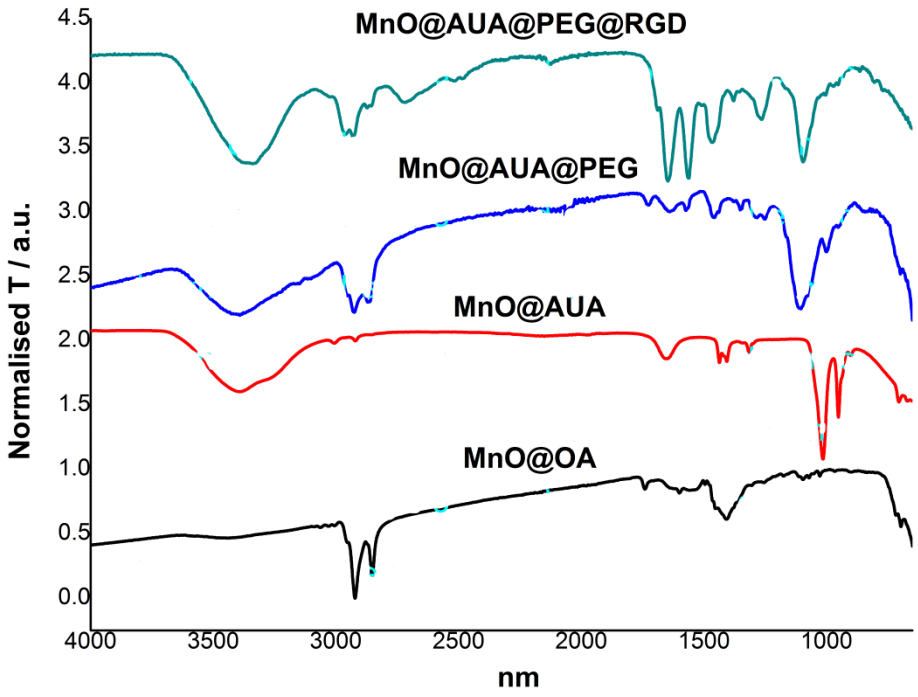

Figure S3. Evolution of the FT-IR spectra along the functionalization of MnO nanoparticles. **Black**, original oleic acid-coated nanoparticles. **Red**, MnO nanoparticles after the ligand exchange with 11-aminoundecanoic acid (AUA). The peak from the carboxylic group (around 1650 nm) shifted to lower wavenumber. **Blue**, MnO nanoparticles after the coupling of PEG. A broad band coming from the C-O-C bonds in PEG can be observed at around 1200 nm. **Green**, final RGD functionalosed MnO nanoparticles. Two clear peaks around 1650 and 1600 nm can be observed coming from the peptidic bonds.

Sample	Hydrodynamic size (nm)	Z potential (mV)
MnO@AUA	**31.3 ± 6.2**	**-22.8 ± 1.2**
MnO@AUA@PEG_{600}	**40.2 ± 10.2**	**-18.2 ± 0.3**
MnO@AUA@PEG_{600}@RGD	**42.3 ± 12.1**	**-19.0 ± 0.7**
MnO@AUA@PEG_{5000}	**50.6 ± 9.6**	**-17.1 ± 0.4**
MnO@AUA@PEG_{5000}@RGD	**56.7 ± 13.2**	**-17.3 ± 0.3**

Table S1. Hydrodynamic size and Zeta-potential measurement from the two different families of MnO nanoparticles used for this study. All values represent the average ± standard deviation of three independent measurements. All measurement were performed in 5 µg Mn/mL MnO solution in pH 7.4 10 mM PBS.

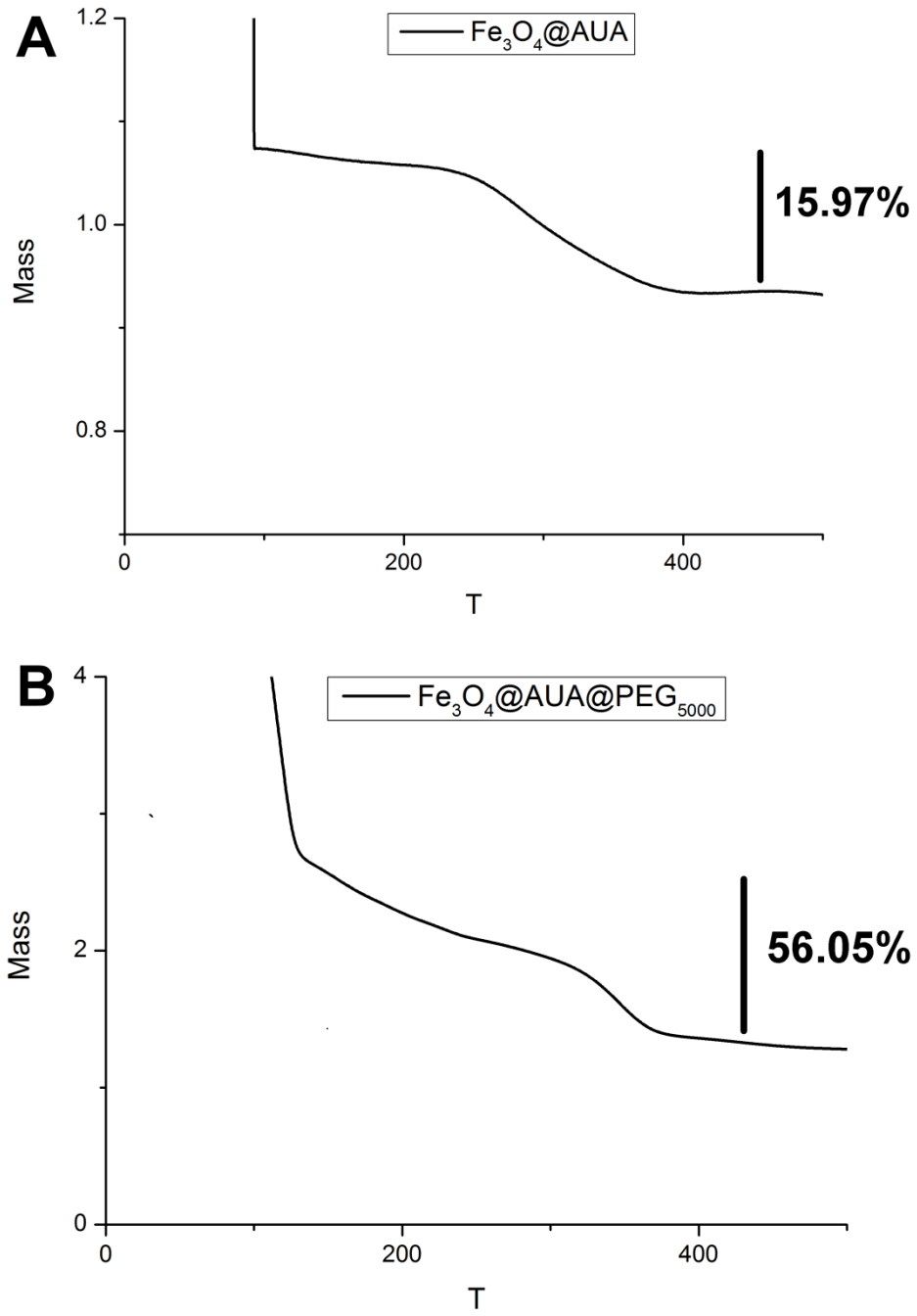

Figure S4. **A**. TGA analyses of Fe_3O_4@AUA under N_2 flow. **B**. TGA analyses of Fe_3O_4@AUA@PEG_{600} under N_2. The samples were heated first to 100 ^oC for 30 mins to remove the water (solvent), and then heated up to 500 ^oC.

Cytotoxicity assessment

The cytotoxicity of the nanoparticles was measured against M21 cells. A sulforhodamine B (SRB) protocol was followed to calculate the IC50 (half maximal inhibitory concentration) of the nanoparticles. [1] M21 melanoma cells were incubated for 72 hours with increasing concentrations of the nanoparticles followed by fixation and protein content determination. Finally the IC50 of the nanoparticles was calculated to be 0.14 mM of Mn. To obtain this value, M21 cells were seeded in 96-well flat-bottom microplates at densities of 1500 cells per well (150 µL), which were then left overnight at 37 ^{o}C to resume exponential growth. After 24 h recovery, 50 µL of various concentrations (final concentrations ranging between 0.005 mM to 3 mM) of MnO nanoparticles made up in media were added to the wells in triplicate. For control wells, the same volume of complete culture medium was included in each experiment. Following 72 h of continuous MnO nanoparticle exposure to the cells under 5% CO_2 atmosphere at 37 ^{o}C, cell survival was assessed by SRB (sulforhodamine B) described elsewhere. [1] The IC_{50} (the concentration of MnO nanoparticles that inhibited 50% of cell growth) was obtained concentration vs. % survival of control curve.

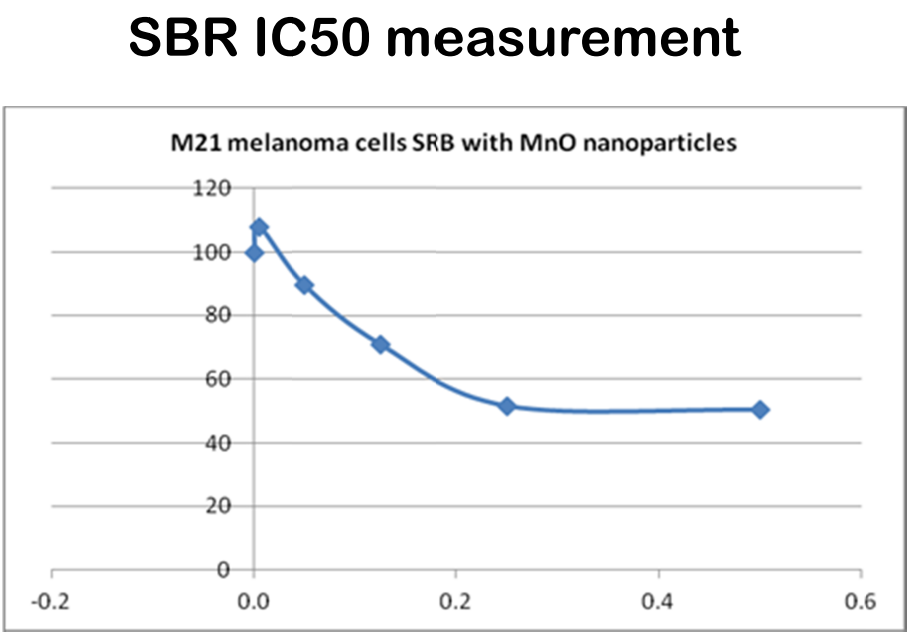

Figure S5. Cytotoxicity measurement of MnO@AUA@PEG.

[1] V. Vichai and K. Kirtikara, *Nature Protocols* **1**, 1112-1116 (2006).