Supporting Information for

Formation of octapod MnO nanoparticles with enhanced magnetic properties through kinetically-controlled thermal decomposition of polynuclear manganese complexes

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Experimental Details

Materials and Instrumentation

Oleic acid (OA) 90%, oleyl amine (OAm) 70%, 1-Octadecene (ODE) 70% were purchased from Sigma- Aldrich. All chemicals were used as received without further purification.

Transmission electron microscopy (TEM) and electron diffraction (ED) were performed on a FEI Tecnai TF20 instrument fitted with a field emission gun, operated at 200 keV. TEM samples were prepared by dispersing the sample in hexane and dropping the solution onto an amorphous carbon coated grid. TEM data were obtained and processed using either Digital Micrograph or IMAGEJ 1.41 software.

X-ray diffraction (XRD) patterns were collected on a Philips X'PERT diffractometer using Cu-Kα radiation.

Thermogravimetric (TGA) data were collected on a TGA Q500, with a 10 °C per minute ramp rate under a N_2 atmosphere.

Magnetic data were collected on a Quantum Design MPMS-XL7. Samples were restricted in eicosane and the data corrected for diamagnetism.

Synthesis of manganese oxide nanoparticles

A) With hold step

Mn Precursor (0.5 mmol w.r.t. Mn) was mixed with 10 mL ODE, 1 mL OA and 1 mL OAm in a 3 necked flask.

The mixture was heated to 110 °C under a nitrogen flow for 30 minutes. The nitrogen flow was then reduced and the mixture was slowly heated to the required hold temperature and then held for 2 hours. The reaction was then slowly heated to 300 °C for 1 hour. The heat source was removed and the solution allowed to cool naturally to room temperature.

The particles were precipitated by adding excess acetone and collected via centrifugation at 4,000 r.p.m. for 10 minutes. The waxy brown precipitate obtained was dispersed in 10 mL hexane and re-precipitated with excess acetone. This procedure was repeated at least 3 times.

Hexane dispersions of the particles showed excellent colloidal stability, with no precipitation visible even after weeks of storage at room temperature.

B) With no hold step

Reagent quantities were used as above. After the 110 °C step the reaction mixture was heated slowly to 300 °C for 1 hour, without being held at an intermediate temperature. The heat source was removed and the particles were allowed to cool to room temperature before being collected by centrifugation as described above.

Experimental Details

Biological Studies

To test the potential of the MnO particles for biological applications, the hydrophobic oleate surfactant of the as-synthesized particles was replaced with a water-dispersible PEG derivative ligand. The synthesis of the PEG derivative and subsequent particle functionalisation were performed according to a previously reported method **[1].**

Cell Culture Protocol

Infinity™ Telomerase Immortalised primary human fibroblasts (h-TERT BJ1, Clonetech Laboratories Inc., CA, USA) were seeded onto ethanol sterilised 13 mm diameter coverslips at a density of $1x10^4$ cells per disc in 1 ml of medium and permitted 24 h for cell attachment prior to incubation with particles, cultured at 37ºC, 5% CO_2. The medium used was 71% Dulbeccos Modified Eagles Medium (Sigma Aldrich, MO, USA), 18% medium 199, 10% fetal calf serum (Invitrogen, UK), 0.9% 100 mM sodium pyruvate (Sigma Aldrich). After 24 h cells were incubated with MnO particles for 60 min at 37ºC, 5% CO_2. For the MTT assay, cells were incubated with particles at 3 different concentrations; 0.1, 0.01 and 0.001 mg/mL for 24, 48 and 72 hours. For SEM, cells were incubated with particles at 0.1 mg/mL for 1 hour. Control cells were cultured in the absence of particles.

MTT Assay Protocol

To assess the toxicity of the particles, particle suspensions at the required concentrations were incubated with cells in a 96 well plate for 1 hour at 37ºC. The particle suspension was then removed and 5 L of MTT dye (5 mg/mL in phosphate buffer pH 7.4, Sigma-Aldrich) was added to each well. After 1.5 h of incubation at 37ºC, the medium was removed and any formazan crystals produced were dissolved in 100 µL of DMSO. The absorbance of each well was read on a microplate reader (Dynatech MR7000 instruments) at 550 nm, calibrated to zero absorbance using culture medium without cells.

SEM Imaging Protocol

The cells were cultured and incubated with particles as described in the cell culture protocol section. The cells were fixed with 1.5% glutaraldehyde (Sigma, UK) buffered in 0.1 M sodium cacodylate (Agar, UK) for 1 h at 4ºC. The cells were then postfixed in 1% osmium tetroxide for 1 h (Agar, UK) and 1% tannic acid (Agar, UK) was used as a mordant. Samples were stained in 0.5% uranyl acetate, then dehydrated using a series of increasing methanol concentrations (30, 50, 70 and 90%), followed by a final step using 100% ethanol. The final dehydratation was in hexamethyl-disilazane (Sigma, UK), followed by air-drying. Once dry, the samples were sputter- coated with gold before examination with a JEOL JSM6400 Digital SEM at an accelerating voltage of 6 kV.

[1] J. Xie, C. Xu, N. Kohler, Y. Hou and S. Sun, Adv. Mater., 2007, **19**, 3163–3166.

Experimental Details

MRI Measurements

MRI (magnetic resonance imaging) measurements were performed on a Bruker Biospec 7-T/30-cm system with a gradient coil insert (internal diameter 121 mm, 400 mT/m) and a 72 mm transmit-receive birdcage resonator. The nanoparticle solutions were placed in shortened NMR tubes (Wilmad, 300MHz). The tubes were immersed in a liquid perfluorocarbon (Fombin, Sigma Adrich) to reduce magnetic susceptibility artifacts.

Transverse relaxation times (spin-spin relaxation) T2 were measured using a Multi-Echo Spin-Echo imaging method with the imaging plane perpendicular to the sample tubes. Echo time TE= 9.2ms; Repetition time TR = 10s; Number of echoes 64; Averages = 2; In–plane resolution 312µm x 312µm; slice thickness 2mm; Bandwidth 70KHz; scan time 43minutes.

Longitudinal relaxation times (spin-Lattice relaxation) T1 were measured using an Inversion Recovery Spin-Echo Echo-Planar-Imaging methods (IR-SE-EPI).
Echo time TE =16.2ms; Repetition time TR =15s ; 64 inversion times at 100ms increments; 4 shot EPI acquisition; In-plane resolution 417µm x 417µm; slice thickness 2mm; Scan time 1hour 4minutes.

The T1 weighted image was acquired using a gradient-echo sequence (FLASH) with: echo-time Te = 2.7ms, repetition time Tr = 30ms, 64 averages, flip-angle 90º, Field of View 4.0 x 4.0cm, matrix 256 x 256, resolution 156µm x 156µm, slice thickness 1mm, scan time 8min 11sec.

Additional Data - TGA

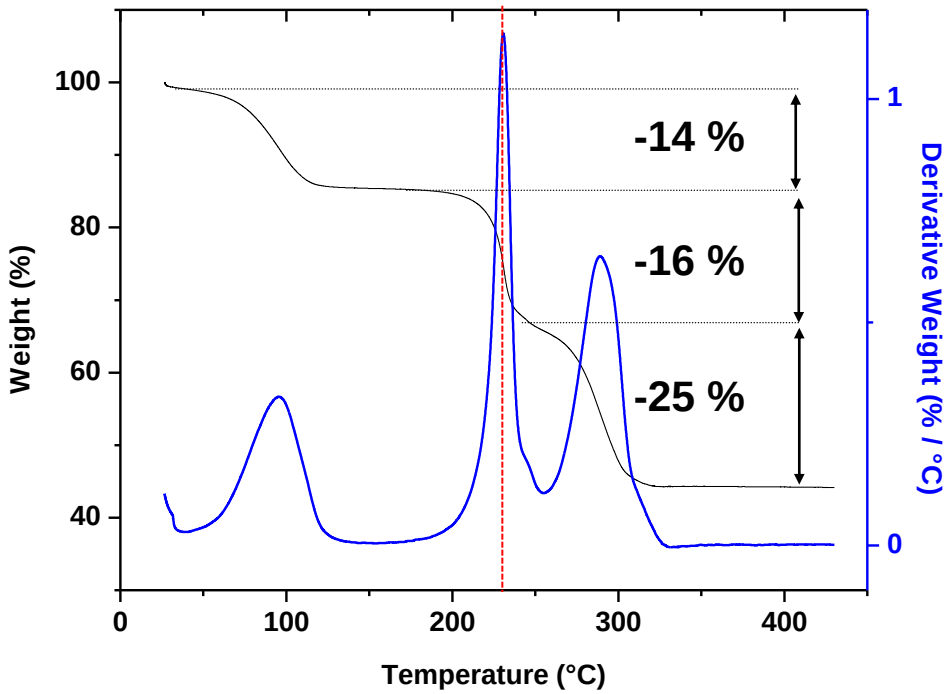


Figure S1: TGA plot (black, left abscissa) and corresponding derivative weight-loss plot (blue, right abscissa) for Mn_{12}-acetate. Numbers indicate percentage weight losses from the original weight over the indicated regions. The dotted red line at 230°C indicates maximum in the derivative weight-loss plot, the temperature subsequently used as the hold temperature during NP synthesis.

Figure S1 shows distinct steps in the decomposition of Mn_{12}-acetate. The first of these (14%) occurs at ~100 °C. This initial weight loss corresponds to the loss of molecules of crystallisation from the parent complex. The 14% loss is higher than would be expected assuming the loss of the two acetic acid and four water molecules from the original cluster (which would give ~9%) and probably arises from the presence of residual water or acetic acid not removed during the washing stage of precursor synthesis.

The second (16%) and third (25%) weight losses correspond to the subsequent decomposition of the cluster core.**[2]** All organic moieties are removed by 300 °C, and the complex undergoes no further decomposition. The remaining 45% of the initial weight agrees with the expected weight corresponding to the formation of 4 formula units of hausmannite (Mn_3O_4) per Mn_{12}-acetate molecule (44%).

[2] B. Folch, J. Larionova, Y. Guari, C. Guerin and C. Reibel, J. Solid State Chem., 2005, **178**, 2368 –2375.

Additional Data - Diffraction

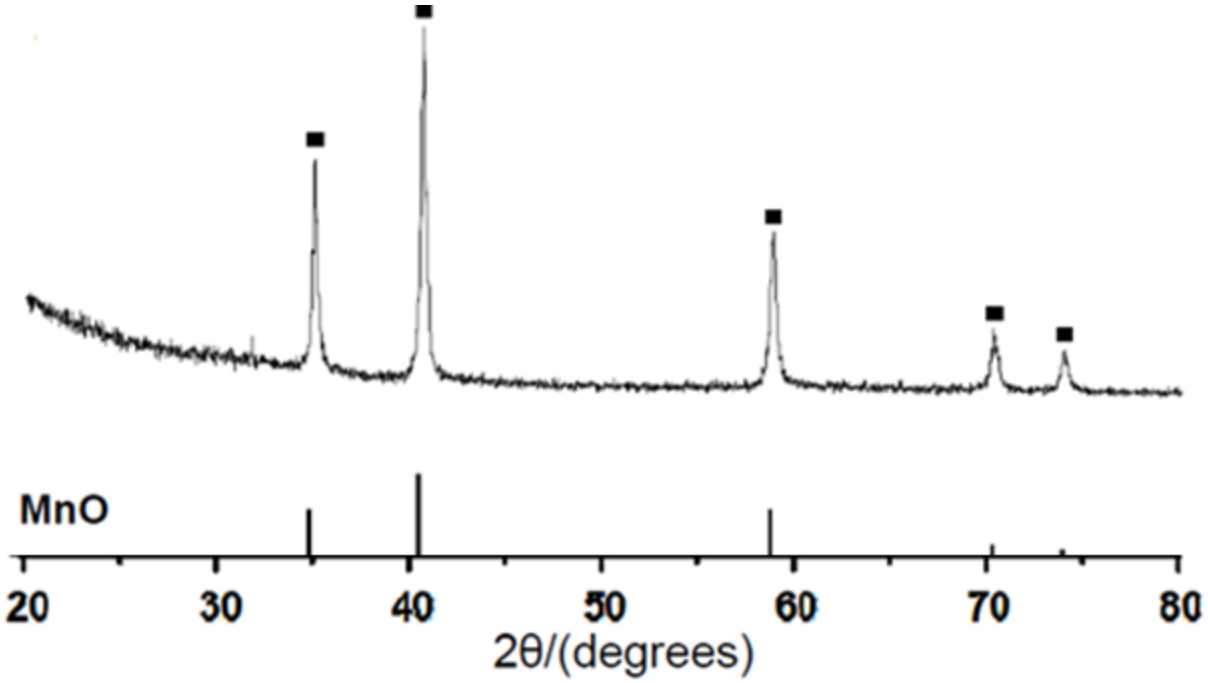
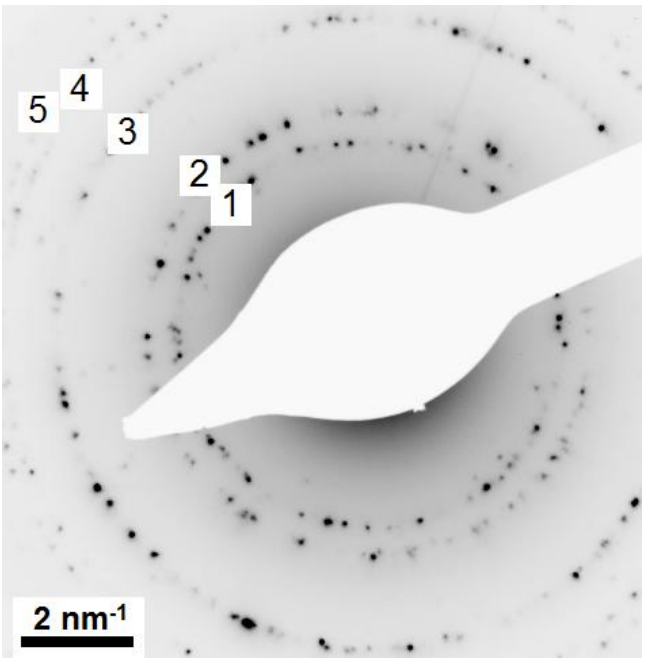


Figure S2: X-Ray Diffraction Pattern for as-obtained spherical MnO nanoparticles, showing agreement with cubic MnO reference pattern (JCPDS:07-0230).



Ring	d(nm)	Assignment
1	0.256	MnO (111)
2	0.220	MnO (200)
3	0.155	MnO (220)
4	0.135	MnO (311)
5	0.128	MnO (222)

Figure S3: Selected area electron diffraction pattern from MnO octapod nanoparticles.

Additional Data - TEM

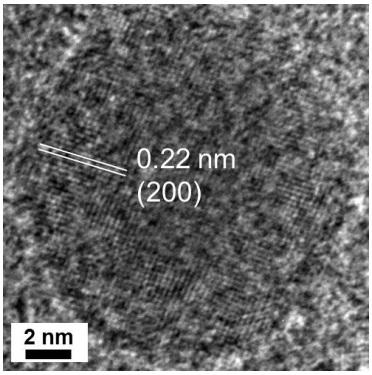


Figure S4: HRTEM image of 17 nm spherical particle. The lattice fringes are visible and have been indexed to MnO (200) reflections.

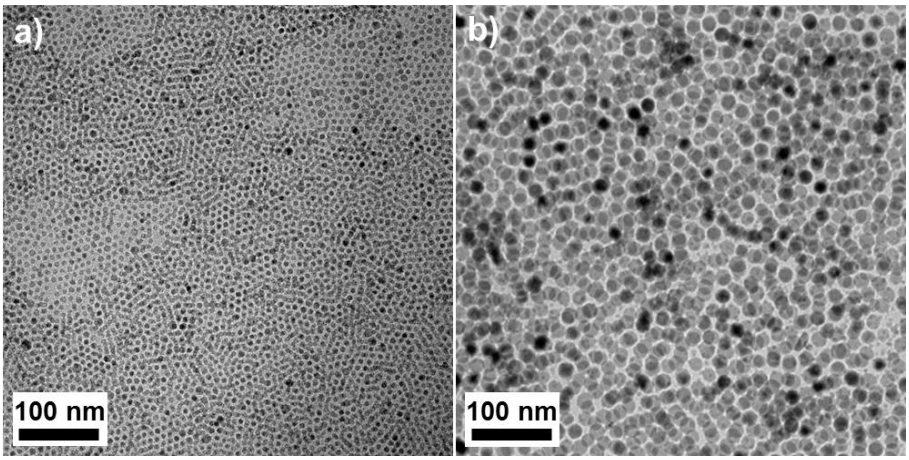


Figure S5: TEM images of superlattices formed by a) 7 nm and b) 17 nm spherical particles.

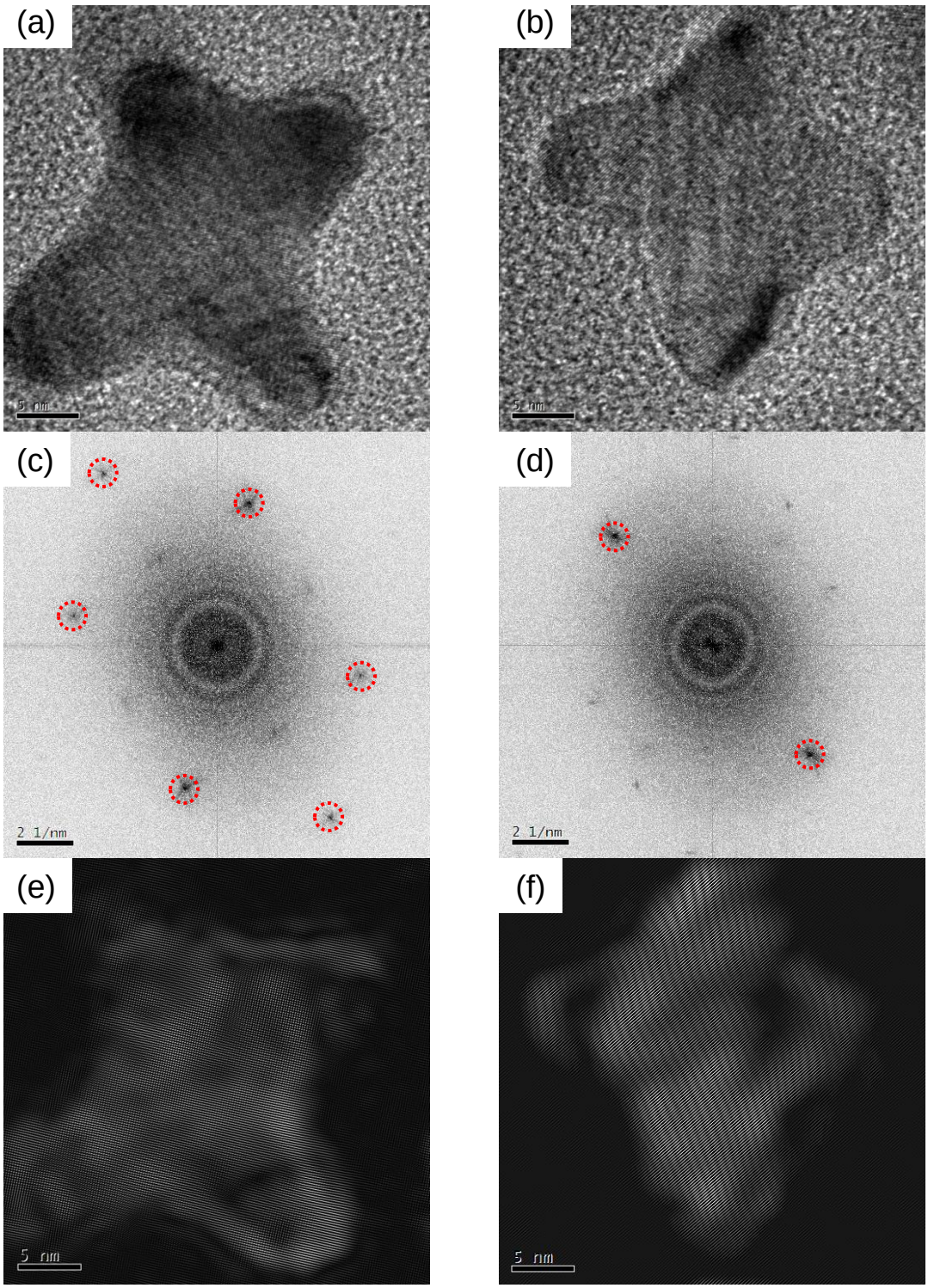


Figure S6: (a,b) High resolution TEM images of typical octapods (at different orientations with respect the electron beam) with (c,d) Fourier transforms showing clear lattice spots that are consistent with single crystal structure throughout the particles. Those peaks encircled in red in the Fourier transforms were used to generate the filtered images (e,f), which clearly show continuity of the crystal lattice throughout the nanoparticle. High resolution imaging was hindered by the presence of residual organic solvent that cracked under the electron beam; removal of the organic led to nanoparticle oxidation.

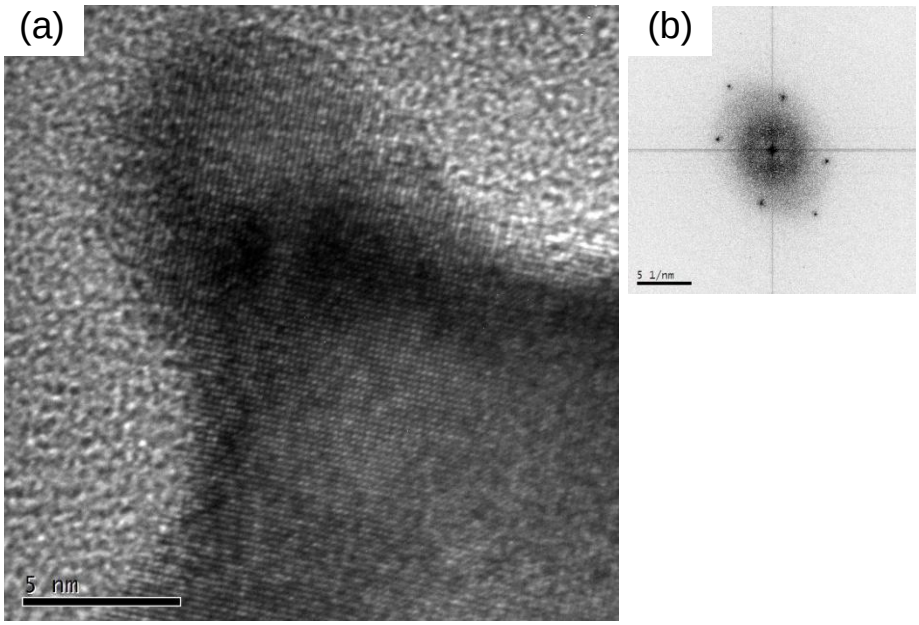


Figure S7: (a) A higher magnification TEM image of the corner of a typical octapod, clearly showing its single-crystal nature. The lattice fringe orientation is consistent with an underlying cubic structure with elongated [111] corners. (b) The associated Fourier transform.

Additional Data – Spherical Particles Magnetism

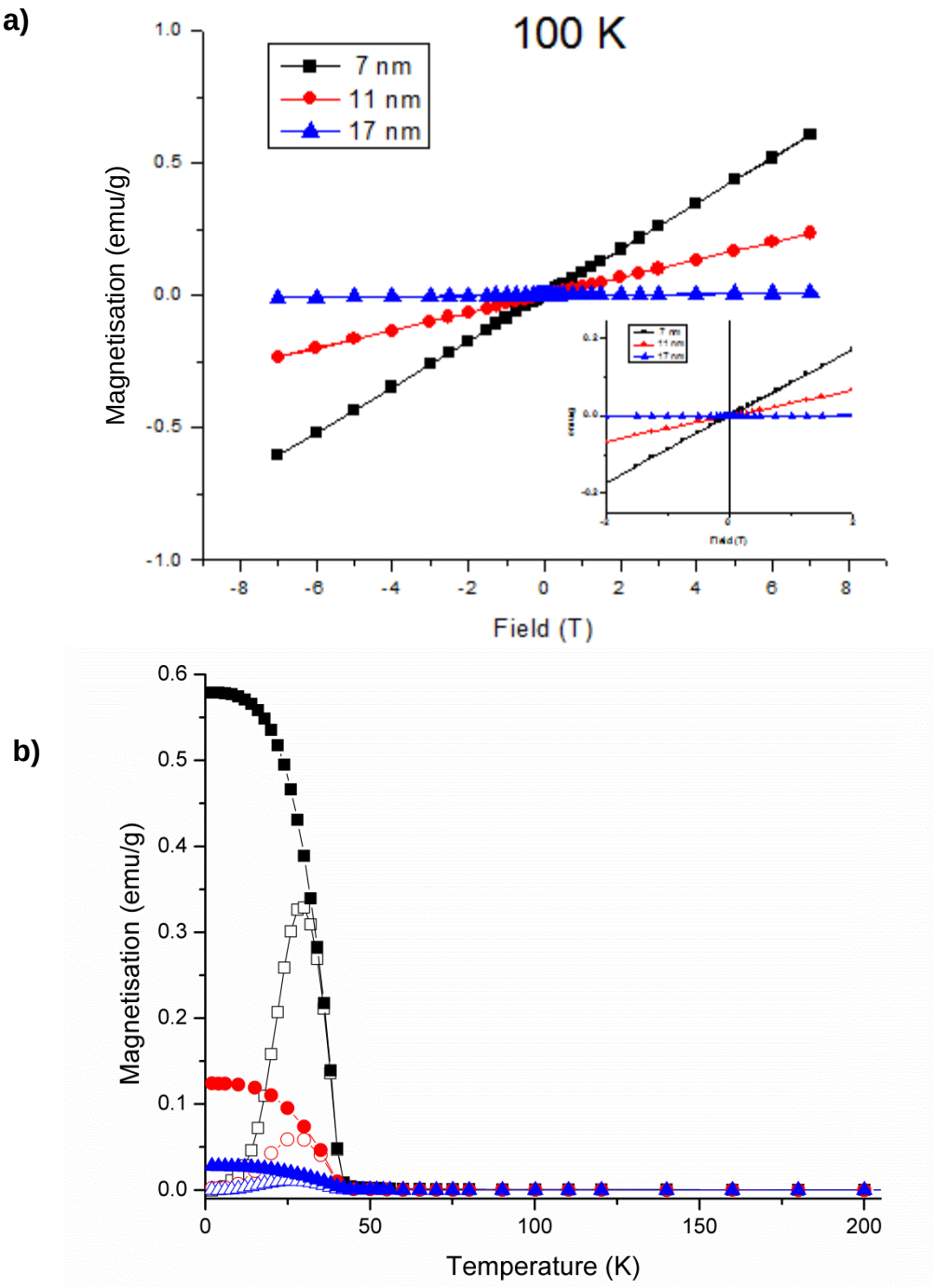
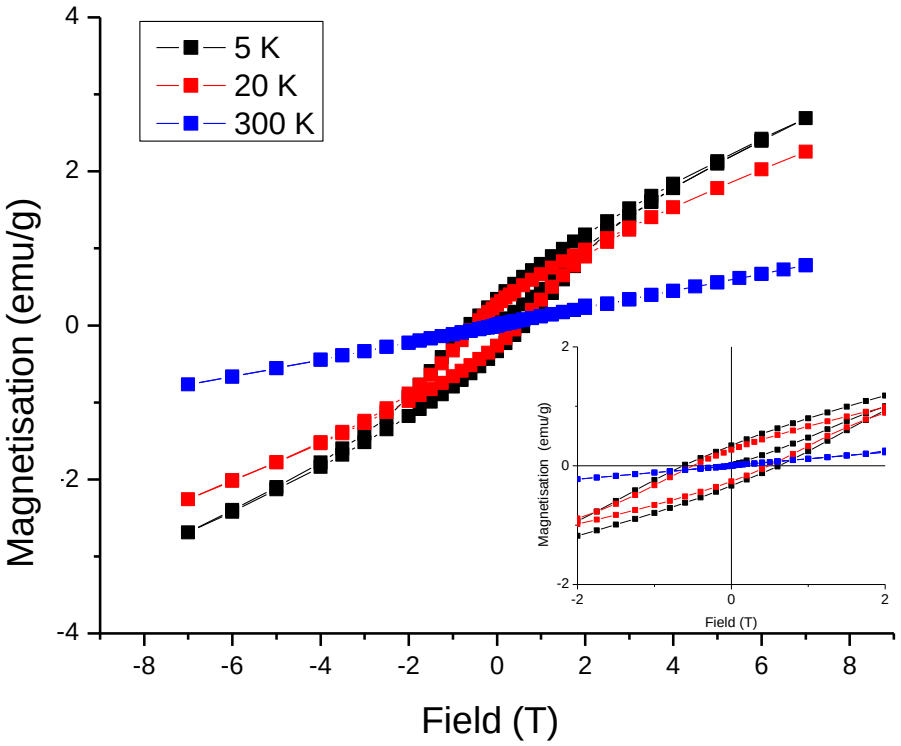


Figure S8:
a) M vs H data for spherical MnO particles recorded at 100 K. Inset is an expansion of the region near the origin.
b) Zero field cooled (open symbols) and field-cooled (closed symbols) data for spherical 7, 11 and 17 nm MnO particles in an applied field of 100 Oe. No clear features around the Néel temperature of bulk MnO are observed for these small particles. [3]

[3] M. A. Morales, R. Skomski, S. Fritz, G. Shelburne, J. E. Shield, Ming Yin, Stephen O'Brien, and D. L. Leslie-Pelecky, Phys. Rev. B, 2007, **75**, 134423.

Additional Data – Octapod Particles Magnetism

a)



b)

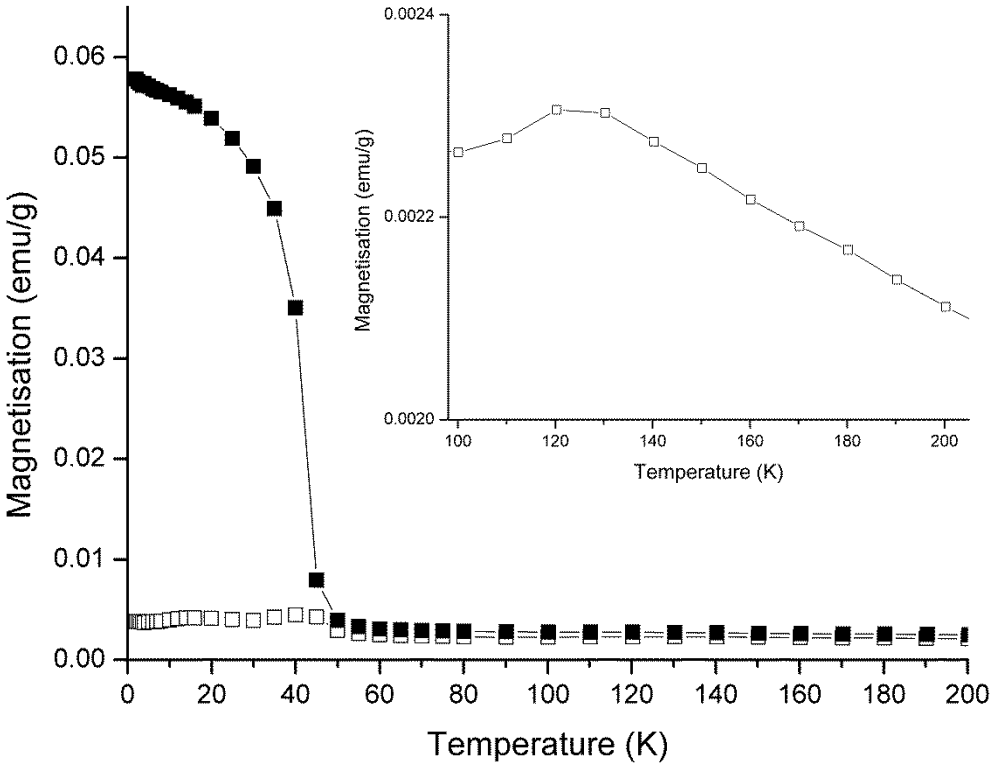


Figure S9:
a M vs H data for the 85 nm octapod MnO particles recorded at 5, 20 and 300 K. Inset is an expansion of the region near the origin.
b) Zero field cooled (open symbols) and field-cooled (closed symbols) data for 85 nm octapod MnO particles in an applied field of 100 Oe. Inset is an expansion of the region from 100 – 200 K showing a broad local maximum around the Néel temperature of bulk MnO.

Additional Data – Cell Uptake Studies

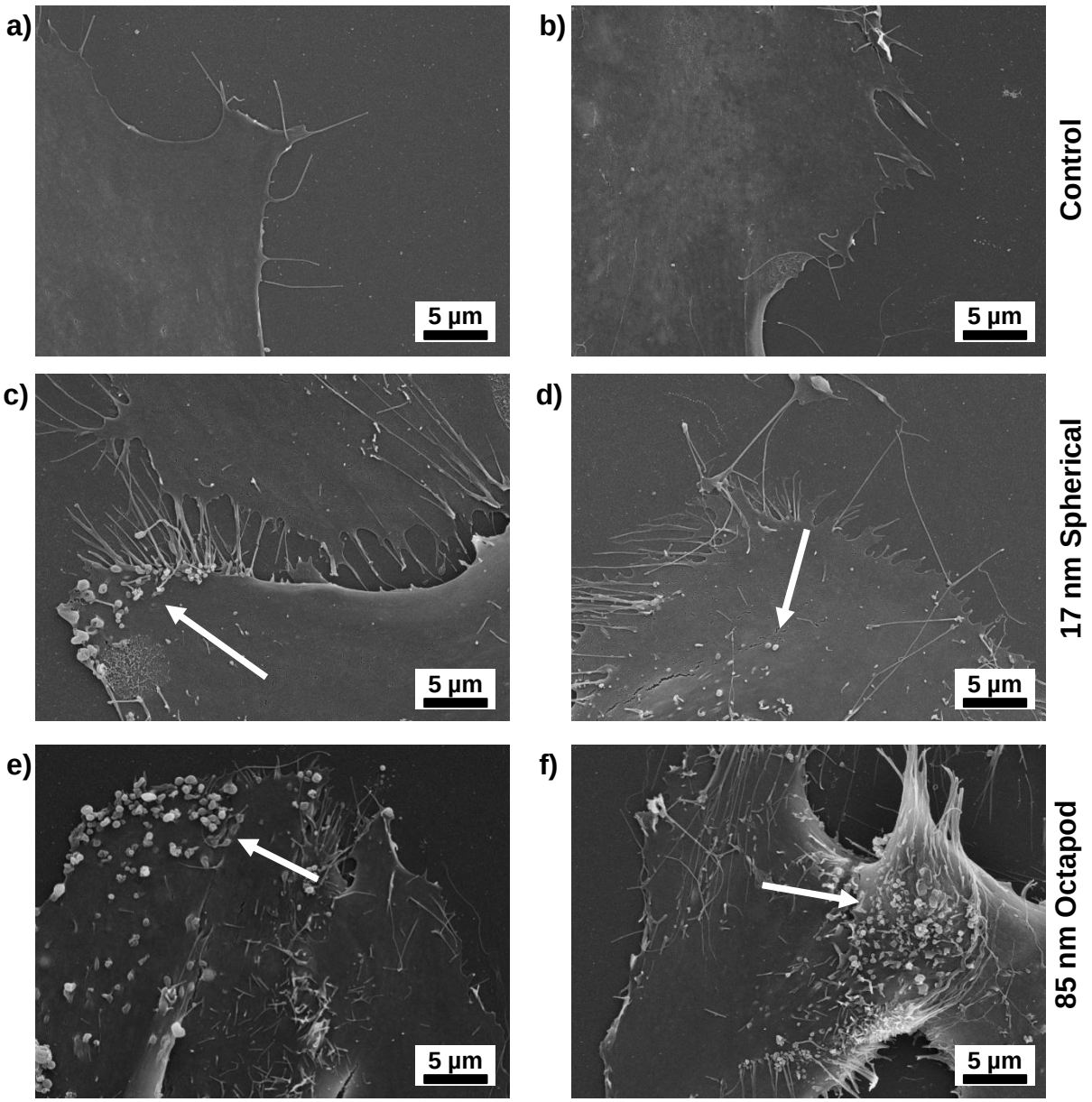


Figure S10:
a, b) SEM images of control cells incubated in the absence of particles. Cells are flat on the surface, indicating that they are healthy. The actin protrusions that are associated with particle uptake are absent, as is expected in the control sample.
c, d) SEM images of cells incubated with 17 nm spherical particles. The cells are flat on the coverslip, which indicates that they are healthy. The actin protrusions that are associated with pinocytosis (the process by which cells uptake material) are visible and marked with white arrows, indicating that the particles are being uptaken.
e, f) SEM images of cells incubated with 85nm octapod particles. Similarly to the cells incubated with spherical particles, the cells are flat on the coverslip, which indicates that they are healthy. The actin protrusions that are associated with particle uptake are present in abundance, indicating the strong interaction between cells and the particles.

Figure S10 (continued):

e, f, continued) The edges of the cells incubated with particles also show more filopodia that the control cells, (the long, spindle-like protrusions). This indicates that the cells are responding to presence of the nanoparticles. Combined with the MTT toxicity data (Figure S8), these results show that the MnO particles, regardless of size and shape and if adequately stabilised can be used in physiological conditions without detrimental effects.

Additional Data – Cell Uptake Studies (MTT)

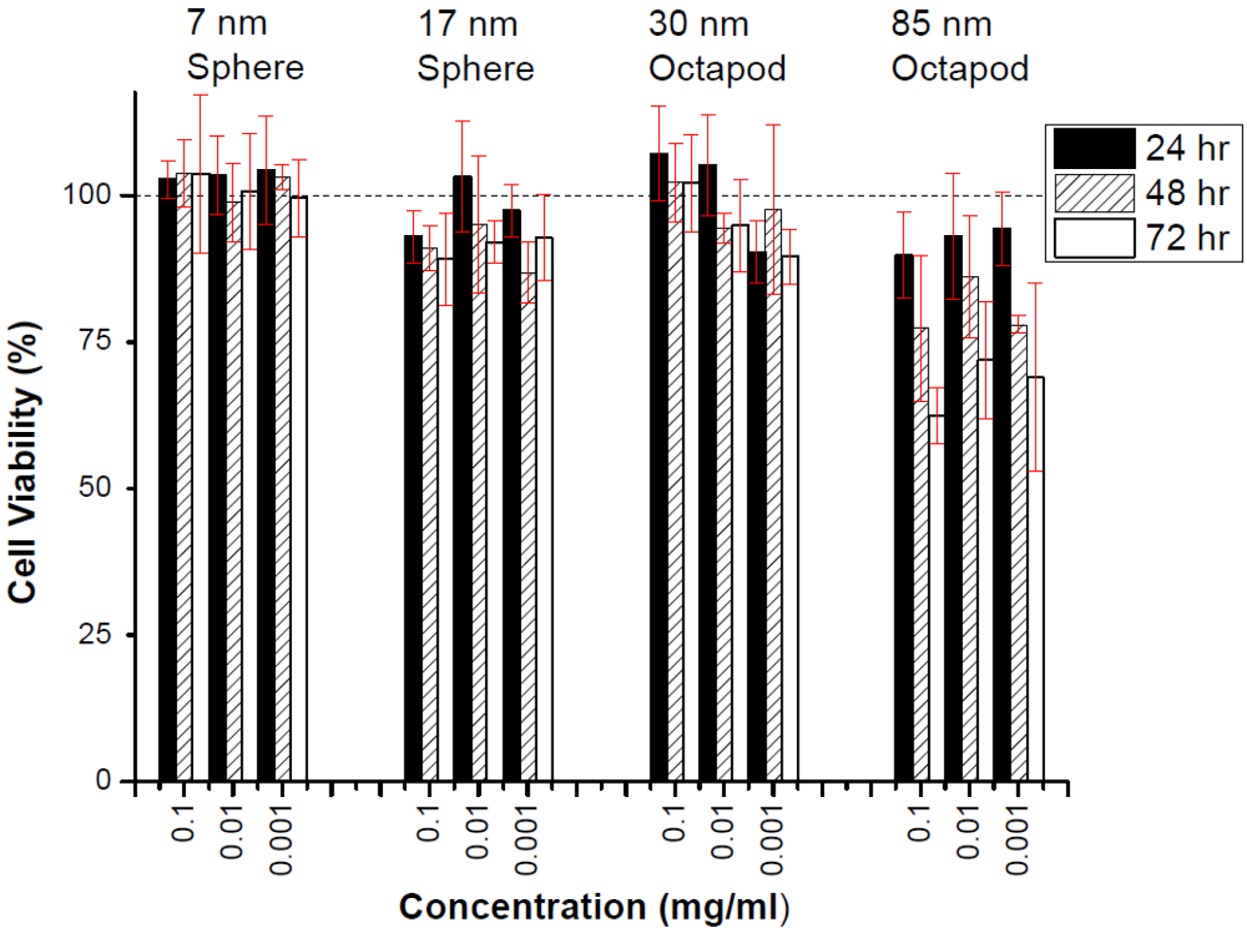


Figure S11:

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) toxicity data for cells incubated with 7, 17 nm spherical particles and 30, 85 nm octapod particles at various concentrations (0.1, 0.01 and 0.001 mg/mL) and different periods of incubation (24, 48 and 72 hr).

An MTT assay allows a quantitative measurement of cell viability upon exposure to particles to be obtained. The above data shows good overall cell viability for both the spherical and octapod particles at each of the concentrations and timescales examined, indicating that the MnO particles studied in this work are viable candidates for future nano-medicinal applications.

MTT toxicity data for the NPs reveal good cell viability over a 48-hour time period , although the 85 nm octapods suggested a slight decrease in viability with increasing time. SEM images of human dermal fibroblast cells incubated with the NPs for one-hour (well within the timeframe for cellular uptake, **Figure S8**) show that the cells respond to the presence of the NPs via actin cell membrane extensions and remain healthy after exposure. The 85 nm octapods show the greatest response, with a lot of cell surface activity (indicating cellular uptake), which may explain the MTT results. Longer-term culture studies are being undertaken to determine the intracellular trafficking routes to verify the particle's biocompatibility alongside signal retention.

Additional Data – MRI relaxation values

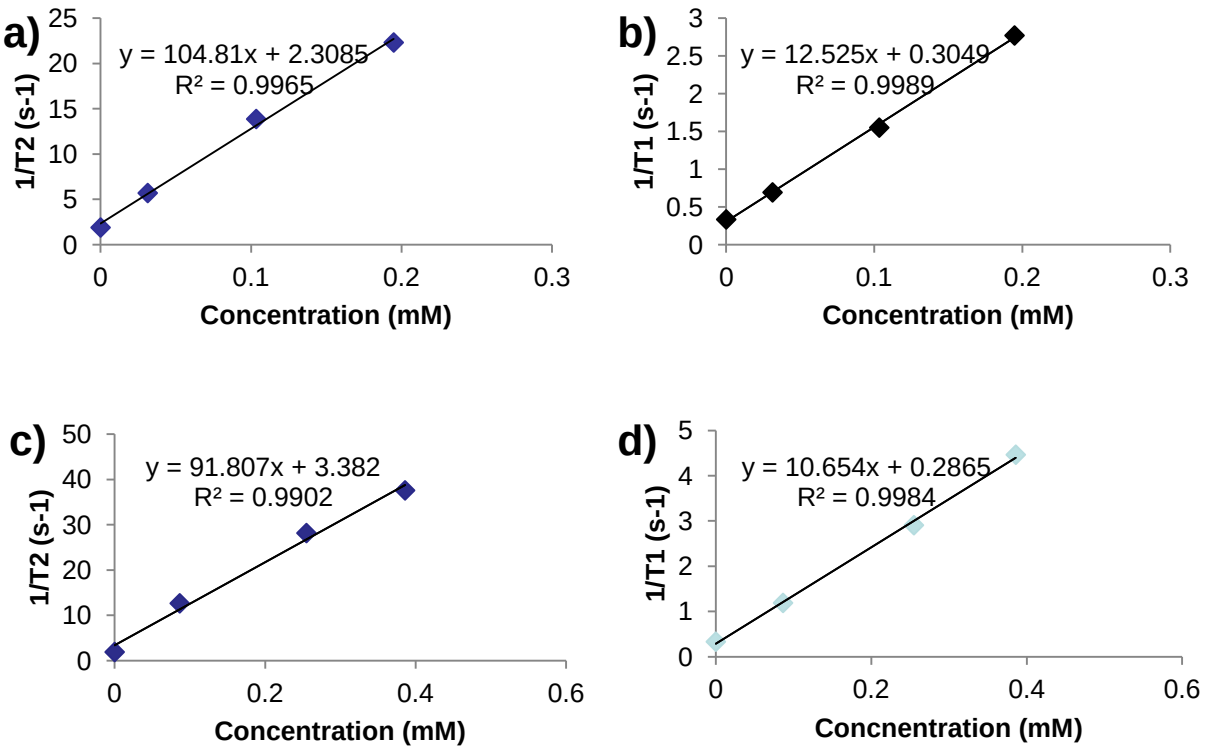


Figure S12:

Calculation of MRI relaxation values from a) Octapod T2, b) Octapod T1, c) Sphere T2 and d) Sphere T1 data. Water-dispersed nanoparticles were prepared as described in previous pages and held in NMR tubes for MRI imaging. T1 and T2 images were collected at 7.0 Tesla as described on page 4 of this document. An initial dispersion of nanoparticles was concentrated by the removal of water and nanoparticle concentrations for all samples were determined using inductively-coupled plasma atomic emission spectrometry (ICP-AES). Note that the different concentrations were used for octapods and spheres. Relaxation values r_1 and r_2 were calculated as shown above using the relationships:

$$\frac{1}{T_1} = \frac{1}{T_{1(water)}} + r_1 C \quad \text{and} \quad \frac{1}{T_2} = \frac{1}{T_{2(water)}} + r_2 C$$