

Supporting Information

Gadolinium-doped magnetite nanoparticles from a single-source precursor

F. J. Douglas,^a D. A. MacLaren,^{*b} N. Maclean,^a I. Andreu,^c F. J. Kettles,^a F. Tuna,^d
C. C. Berry,^e M. Castro^c and M. Murrie ^{*a}

a. WestCHEM, School of Chemistry, University of Glasgow, Glasgow G12 8QQ, UK,

E-mail: Mark.Murrie@glasgow.ac.uk; Tel: +44 141 330 4486.

b. SUPA, School of Physics and Astronomy, The University of Glasgow, Glasgow G12 8QQ, UK,

E-mail: dmaclaren@physics.org; Tel: +44 141 330 5886.

*c. Instituto de Ciencia de Materiales de Aragón (ICMA), CSIC – Universidad de Zaragoza, Campus Río Ebro,
María de Luna, 3, 50018 Zaragoza, Spain.*

d. National EPR Centre, University of Manchester, Oxford Road, Manchester, M13 9PL, UK.

e. Centre for Cell Engineering, CMVLS, University of Glasgow, Glasgow G12 8QQ, UK.

Characterisation of the iron/gadolinium precursor complex

The air-dried precipitate was analysed to be $[\text{Fe}_{1.7}\text{Gd}_{1.3}\text{O}(\text{O}_2\text{CPh})_7(\text{H}_2\text{O})_3]$, analysis (%) calculated (found): C, 48.35 (48.51); H, 3.40 (3.20); Fe, 7.80 (8.15); Gd, 16.79 (16.75); N, 0 (0). Selected IR data: $\nu = 1618, 1599, 1561, 1401, 1325, 1301, 1176, 1024, 720, 674, \text{cm}^{-1}$. The oxo-centred trimer structure is commonly found for iron carboxylate complexes. Due to difficulty in growing single-crystals of precursor, attempts to confirm the trimeric structure were made using IR spectroscopy (Fig S1). The data for the Fe/Gd precursor complex show strong similarity to the iron-only benzoate complex $[\text{Fe}_3\text{O}(\text{O}_2\text{CPh})_6(\text{H}_2\text{O})_2(\text{MeCN})]$, prepared by an analogous Fe-only route, strongly suggesting a similar trimeric core. The IR data show strong COO frequency absorption bands typical for oxo-centred trimers with 1,3-bridging benzoate ligands and in addition, two new weaker bands at 1618 and 1325 cm^{-1} are consistent with a monodentate benzoate ligand.[2] Furthermore, there are two new bands in the 500-800 cm^{-1} region (indicated) which is the region where the $\nu_{\text{as}}(\text{M}_3\text{O})$ band would be expected to be split into two components (we assume $\nu_{\text{as}}\text{Fe}_2\text{GdO A}_1$ and B_2).[3] Hence, a possible trimeric structure with an average powder composition of $\sim 2:1$ Fe:Gd based on the analytical and IR data is presented in Figure S2.

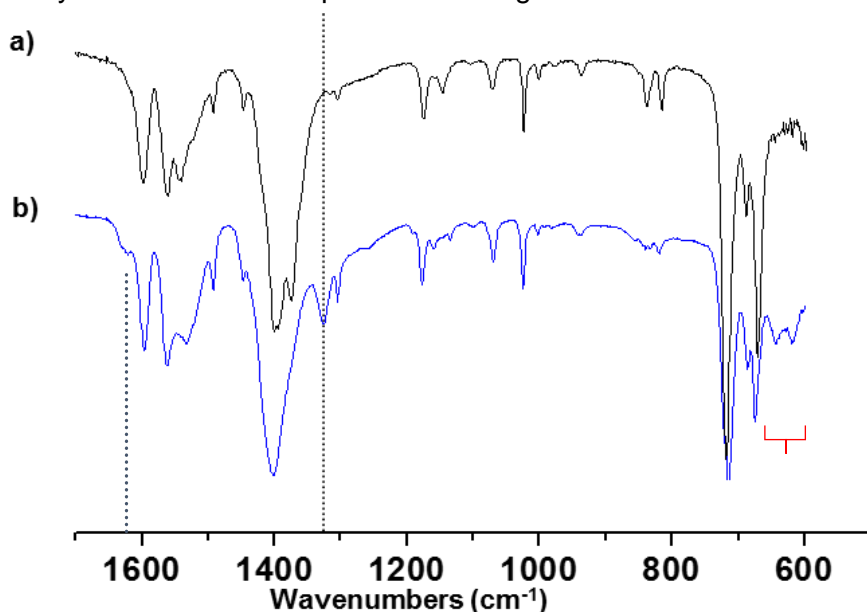


Figure S1: IR spectra of a) $[\text{Fe}_3\text{O}(\text{O}_2\text{CPh})_6(\text{H}_2\text{O})_2(\text{MeCN})]$ and b) $[\text{Fe}_{1.7}\text{Gd}_{1.3}\text{O}(\text{O}_2\text{CPh})_7(\text{H}_2\text{O})_3]$. New bands at 1618 and 1325 cm^{-1} and in the 500 – 800 cm^{-1} region are indicated.

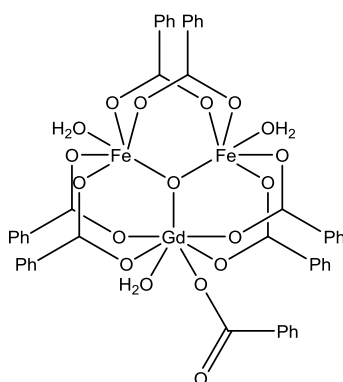


Figure S2: Possible structure of the Fe/Gd precursor.

- [1] A. Ferguson, J. McGregor, E. K. Brechin, L. H. Thomas and M. Murrie, *Dalton Trans.*, 2009, 9395-9397.
[2] K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds* (4th Ed, Wiley)
[2] M. Yazdanbakhsh, N. Lotfian, H. Tavakkoli and R. Boese, *Cent. Eur. J. Chem*, 2011, 9, 585-589.

Gd:Fe₃O₄ and Fe₃O₄ nanoparticles - additional details

Gd:Fe₃O₄ nanoparticles

Following dispersion of the nanoparticles in hexane (see main text), the remaining solution was centrifuged for a further 15 minutes but no more solid was collected. This step was performed to see if any smaller particles were formed, as we found previously that gadolinium-containing homometallic carboxylate precursors routinely form small Gd₂O₃ NPs under reflux conditions.[4] As no small Gd₂O₃ particles were observed by electron microscopy, we assume that any gadolinium not incorporated into the magnetite particles remains dissolved in the reaction mixture.

Control batch of Fe₃O₄ nanoparticles

The iron-only precursor [Fe₃O(O₂CPh)₆(H₂O)₂(MeCN)] was prepared by an analogous route to the iron/gadolinium precursor [5] and subsequently used to prepare a control batch of similarly sized undoped Fe₃O₄ nanoparticles (see Fig S3) using the method described in the main text for the Gd:Fe₃O₄ nanoparticles.

Aqueous phase transfer

The Gd:Fe₃O₄ nanoparticles and the control batch of Fe₃O₄ nanoparticles were then prepared for biological studies by rendering them water soluble by ligand exchange with a polyethylene glycol based ligand.[6] PEG diacid 600 (200 mg) was mixed with N-hydroxysuccinimide (NHS, 4 mg), N,N'-dicyclohexylcarbodiimide (DCC, 6 mg) and dopamine hydrochloride (DPA, 3 mg) in a solution containing CHCl₃ (4 mL), DMF (1 mL), and anhydrous Na₂CO₃ (20 mg). This mixture was then stirred for two hours at room temperature to ensure complete formation of the PEG-DPA ligand. 15 mg of oleate-coated particles was then added and the resultant mixture was left to stir for 24 hours under N₂ protection. The modified particles were precipitated by adding hexane and collected via centrifugation for 5 minutes at 4,000 r.p.m before being dried under a gentle N₂ flow. The particles can then be dispersed in water, giving a pale-brown dispersion (see Fig. S3). The aqueous particle dispersion was purified by dialysis against DI water using SnakeSkinTM dialysis tubing (Thermo-Scientific, MWCO = 7,000) for 24 hours, changing the water 3 times during the 24 hour period. Finally the particles were filtered through a 200 nm syringe filter to remove any large aggregates or biological contaminants.

[4] F. J. Douglas, PhD Thesis, University of Glasgow, 2012.

[5] A. W. Ferguson, PhD Thesis, University of Glasgow, 2007.

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

Biological Studies – Experimental details

Cell Culture Protocol

Infinity™ Telomerase Immortalised primary human fibroblasts (h-TERT BJ1, Clontech Laboratories Inc., CA, USA) were seeded onto ethanol sterilised, 13 mm diameter coverslips at a density of 1×10^4 cells per coverslip in 1 ml of medium and permitted 24 h for cell attachment prior to incubation with PEG600-DPA functionalised particles, cultured at 37 °C, 5% CO₂. 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 particles for 60 min at 37 C, 5% CO₂. 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 electron microscopy, cells were incubated with particles at 0.1 mg/mL for 1 hour. Control cells were cultured in the absence of particles.

SEM, TEM – Cell characterisation

The cells were cultured and incubated with particles as described previously. The cells were fixed with 1.5% glutaraldehyde (Sigma, UK) buffered in 0.1 M sodium cacodylate (Agar, UK) for 1 h at 4 °C. 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 dehydration 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 operating at an accelerating voltage of 6 kV.

For TEM, the cells were cultured and incubated with particles as described previously and fixed and stained as for SEM. The thermanox coverslips were then treated with (1:1) dried absolute alcohol:resin overnight to evaporate the alcohol. The cells were then embedded in Spur's resin and ultra thin sections were cut. Images were taken on a Leo 912AB TEM operated at 120 kV.

Immunofluorescence Microscopy

Cells were incubated with 0.1 mg/mL NP dispersions for 1 hour at 37°C in a 24 well plate as previously described. The medium was then removed and 4% formaldehyde/PBS was added to fix the cells (15 mins, 37°C). The fixative was removed and permeabilising buffer was added for 5 mins at 4°C. The buffer was removed and 1% bovine serum albumin (BSA) in PBS was added (5 mins, 37°C). A solution containing rhodamine phalloidin and the primary antibody anti-β-tubulin in a 1:50 ratio with PBS/BSA was prepared. The BSA was removed from the wells and 200 μL of the phalloidin/anti-β-tubulin solution was added to each well (1 hour, 37°C). The stains were removed and the cells were washed gently with a PBS/0.5% Tween (detergent) solution three times. The Tween solution was removed and 200 μL of relevant biotinylated secondary antibody in a 1:50 PBS/BSA solution was added (1 hour, 37°C). The stain was removed and the cells were gently washed again with PBS/0.5% Tween. Finally, the tertiary FITC-streptavidin component was added (30 minutes, 4°C). The coverslips were then removed from the wells and a drop of fluorescent mountant that contains DAPI was added to the cell area. The coverslip was then stored until required for imaging. A control sample was prepared in analogous way though in the absence of particles.

Biological Studies – Effect of the particles on cells

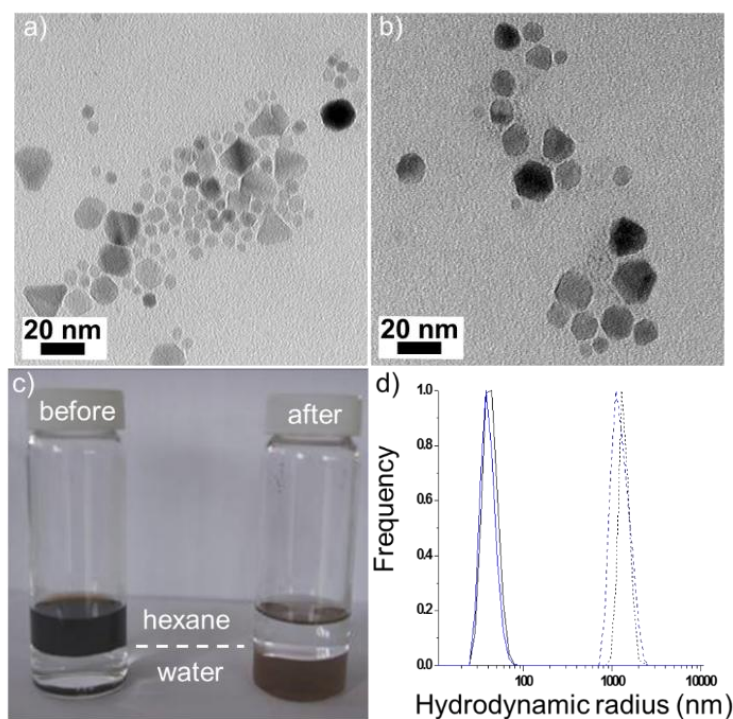


Figure S3. TEM images of a) Fe₃O₄ and b) Gd:Fe₃O₄ PEG functionalised NPs deposited from their aqueous dispersions. c) Photograph showing dispersion of doped particles in hexane and water before and after ligand exchange respectively. d) Hydrodynamic radii of undoped (black) and doped (blue) particles before and after functionalisation (solid and dotted lines respectively).

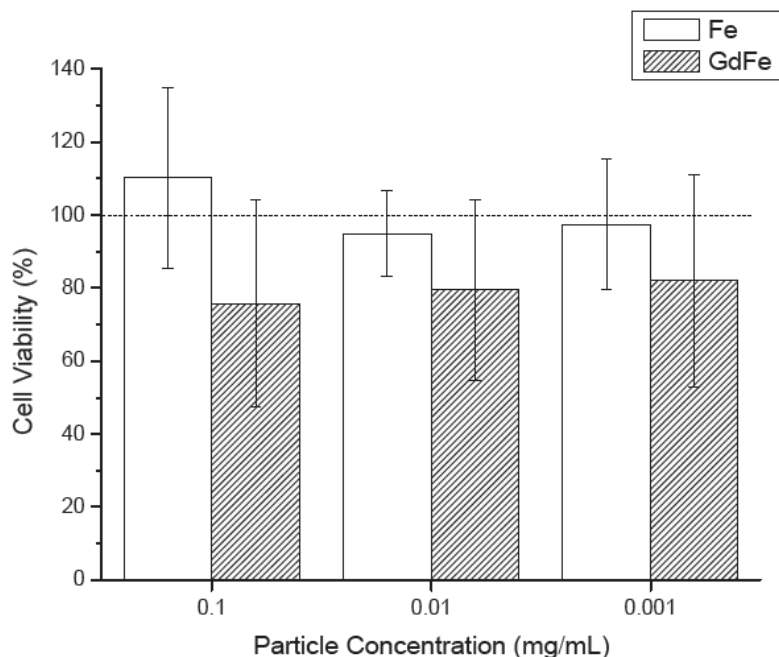


Figure S4. MTT toxicity assays of Fe₃O₄ and Gd:Fe₃O₄ PEG functionalised NPs, showing good cell viability for the undoped Fe₃O₄ NPs and a moderate cell viability for the Gd:Fe₃O₄ NPs.

Biological Studies – Effect of particles on cell cytoskeleton and morphology

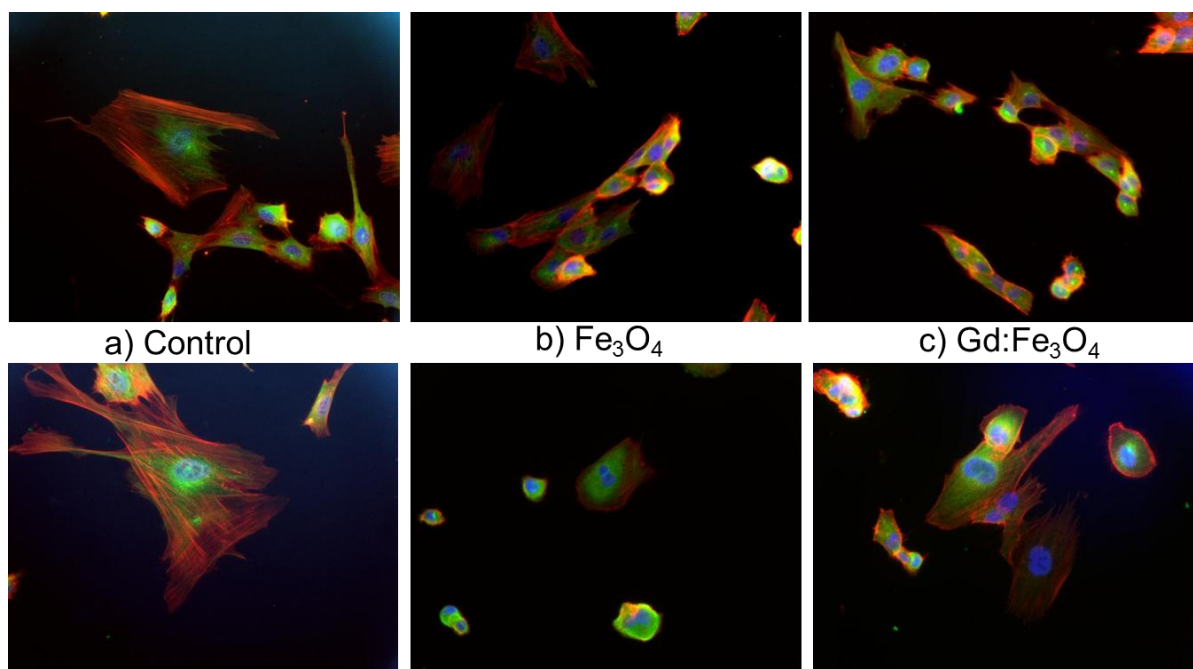


Figure S5: Optical fluorescence microscopy (20x magnification) of the three stained cell samples a) control, b) incubated with Fe₃O₄ NPs and c) incubated with Gd:Fe₃O₄ NPs.

For the control cells (Fig. S5a) the fluorescence microscopy pictures show the elongated and well-spread cells (upper and lower pictures) that would be expected for a typical healthy cell sample on a flat surface (like a coverslip). The nucleus of each cell is visible in blue (DAPI staining) and each nucleus appears spherical, indicating no abnormalities. The F-actin (stained red) is filamentous and provides the main structural support to the cell cytoskeleton. In a control cell this typically appears along the cell periphery and throughout a cell spread on a flat surface in the form of stress fibres across the cell. The β -tubulin, visible in green is an additional part of the cell cytoskeleton and is involved in cell trafficking (*i.e.* movement of endocytosed material through the cell to storage by the nucleus). In a healthy cell, β -tubulin should be evenly dispersed throughout the cell, so Fig. S5a) indicates that normal β -tubulin distribution is observed in the control cells.

The micrographs of the cells which have been exposed to particles (Fig. S5b,c) for the undoped and doped particles respectively) show that there is a difference in cell morphology when compared to the control cells. The elongated cells seen in the control sample were not as prevalent in the particle samples, and instead the cells appear to have adopted a more rounded shape — although elongated cells were still visible in some areas of the coverslip. The long strands of actin (stained red) that were visible in the control cells are not as apparent in the cells that have been incubated with particles, and only small amounts of actin are visible at the cell edge. This implies that the introduction of particles has altered the cell cytoskeleton, perhaps as the cells change to uptake particles. Interestingly, the DAPI staining of the Fe₃O₄ and Gd:Fe₃O₄ samples reveals that some of the nuclei of the rounded up cells are in fact dividing, and the cells were in the process of cell division when they were fixed. It is not currently clear why the cells incubated with particles would undergo the cell division process, though cell division would explain the rounded-up shape of the cells, which is normal for a dividing cell.

Biological Studies – Effect of particles on cell; interaction with and uptake into cell body.

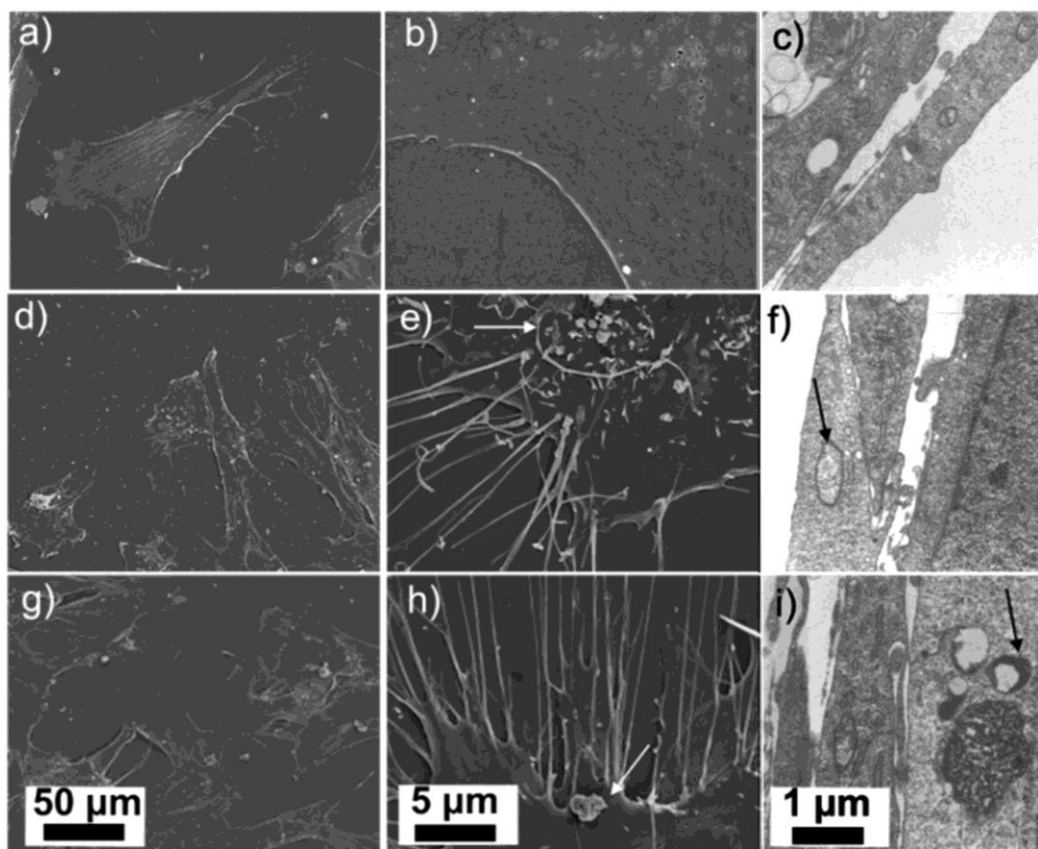


Figure S6. Low magnification SEM (left column), high magnification SEM (middle column) and TEM images (right column) of a--c) Control cells the absence of particles, d--f) Cells incubated with Fe_3O_4 particles and g--i) Cells incubated with $\text{Gd}:\text{Fe}_3\text{O}_4$ particles. White arrows in the SEM images are actin protrusions, which form on the cell surface during incubation in uptake of material. Black arrows in the TEM images show uptake of particles into vesicles.

The EM images of the control cells in the upper panel (which have not been exposed to particles) indicate a typical healthy cell. The SEM images (a,b) show the cell is flat on the coverslip surface and that the cell periphery is relatively smooth. The TEM image c) reveals the vacuoles cells to not contain any discernible high-contrast material. However, the cells obtained from the undoped (d--f) and doped (g--i) particles are quite different in comparison to the control cells. Whilst the SEM images show that after exposure to particles the cells are flat on the surface and appear healthy, the cell surface is highly active, with many actin protrusions (indicated by white arrows); this may be indicative of cellular uptake, for example via endocytosis and pinocytosis. The edges of the cells incubated with particles also show more filopodia than the control cells, which are the long, spindle-like protrusions the cell uses to sense its surroundings and feel its way around a surface. This indicates that the cells are responding to presence of the nanoparticles. TEM images (right column of Fig. S6) show that the vesicles of cells incubated with particles (indicated by black arrows in images f,i) contain more material than the vesicles of the control cells. Given the small particle size and the reduced contrast of the particles against the cell structures (which have been stained with osmium and uranium) it is difficult to get a clear view of the particles within the cell vesicles.