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Supplementary Information

Synthesis of gadolinium oxide nanodisks and gadolinium doped iron oxide nanoparticles for MR contrast agents

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Experimental Section

1. Materials. Gadolinium (ш) chloride hexahydrate (99%), 1-Octadecene (90%) and α,ω-Bis[2-[(3-carboxy-1-oxypropyl)amino]ethyl]polyethylene glycol (PEG-diacid) (>99%), *N*-Hydroxysuccinimide (98%), *N,N''*- Dicyclohexylcarbodiimide (99%) and Chloroform was purchased from Sigma. Dimethylformamide was purchased from J.T.Baker. Sodium Oleate (>97 %) was purchased from TCI. Oleic acid (90%) and Iron (ш) chloride hexahydrate (98%) was purchased from Alfa Aesar. Ethanol and *n*-hexane were purchased from VWR.

2. Synthesis of Gd_2O_3 nanodisks. Gd_2O_3 nanodisks were prepared by thermal decomposition of gadolinium (ш) oleate in the presence of oleic acid. The procedure for preparing Gd (ш) oleate complex was adapted from previous reported literature of Fe (ш) oleate.[1] Sodium oleate (12.2g, 40.1mmol) and gadolinium (ш) chloride hexahydrate (5.0 g, 13.5 mmol) was dissolved in a mixed solvent containing 30 mL distilled water, 40 mL ethanol and 70 mL hexane. The solution was left to reflux in a water bath (70°C) for 4 hours under a gentle stream of nitrogen. The upper hexane layer was collected and washed three times with 30 mL distilled water using a separatory funnel. Excess solvent was removed *in vacuo* yielding a waxy solid. Gd_2O_3 nanodisks were synthesized by dissolving gadolinium (ш) oleate (1.62 g, 1.78 mmol) in 25 mL 1-Octadecene and then adding a certain amount of oleic acid (0.95 mmol, 1.58 mmol, 1.9 mmol and 2.28 mmol). The solution was then heated with a constant heating rate of 3°C/min to T = 320°C and left to react for 4 hours. After cooling down to room temperature, the NPs were precipitated with a mixture of *n*-hexane, isopropanol and acetone (*v/v/v*= 1:2:2). The precipitate was washed with a mixture of *n*-hexane and acetone (*v /v*= 1:2).

3. Preparation of Gd doped iron oxide (GDIO) NPs. Spherical and cubic GDIO NPs were prepared by thermal decomposition of iron-gadolinium oleate in the presence of oleic acid. The FeGd oleate complex was prepared as described for gadolinium (ш) oleate, except that iron (ш) chloride hexahydrate and gadolinium (ш) chloride hexahydrate was mixed in a molar ratio of 1:0.1 and 1:0.5 before dissolving in a mixed solvent of distilled water, ethanol and hexane. The FeGd-oleate complex (1.62 g) was dissolved in the 25 mL 1-octadecene solvent in the presence of oleic acid (1.58 mmol). The reaction mixture was slowly heated at a constant rate of 3°C/min to T = 320°C. After leaving the reaction for 4 hours, the reaction mixture was cleaned by a procedure as described above.

4. Surface functionalization. The Gd_2O_3, and GDIO NPs were all made water soluble by coating them with PEG-diacid by previously reported protocols.[2] Solutions of *N*-hydroxysuccinimide (NHS, 2mg/mL) and *N, N''*- Dicyclohexylcarbodiimide (DCC, 3mg/mL) was freshly prepared in DMF and chloroform (*v/v*=1:2). PEG (20 mg) and anhydrous Na_2CO_3 (10mg) was dissolved in DMF (1 mL) and chloroform (2 mL) before adding 1 mL of NHS stock solution and 1 mL of DCC. The activation of the carbodiimide-PEG complex was left to react at room temperature for two hours under vigorous stirring. NPs (5 mg) were dissolved in 1mL chloroform and added to the reaction beaker. The reaction was left overnight under a gentle stream of argon. PEG-coated NPs were washed with *n*-hexane (20 mL) three times and acetone (20 mL) before drying and dissolving in MilliQ-water.

5. Characterization techniques. Bright field (BF) scanning transmission electron microscopic (STEM) imaging was performed with Hitachi S-5500 operating at 30 keV. High resolution (HR) transmission electron microscopic (TEM) images were collected from JEOL 2010, operating at 200 kV. Samples for TEM investigation were made by evaporating a few µL of NPs solution on a carbon coated copper grids. Bruker DaVinci2 diffractometer with Cu Kα radiation (λ=1.5418 Å) was used for collecting XRD patterns. Samples for XRD were prepared by depositing NPs on a single crystalline silicon holder. Kratos Axis Ultra DLD spectrometer (Kratos Analytical, UK), equipped with a monochromator alumina source ($Al_{k\alpha}$, hν = 1486.6 eV) was used for X-ray photoelectron spectroscopy (XPS) analysis.

6. Relaxometry. T1 and T2 relaxivities at room temperature were determined using a 7T Bruker Biospec Avance 70/20 (Bruker Biospin MRI, Ettlingen, Germany) small animal scanner with Paravision 5.1. A dilution series of the NPs was made and T1 and T2 measurements were performed with a RARE VTR sequence (T1 measurements: TE=8.3 ms, TR=23, 25, 30, 40, 60, 80, 110, 150, 300, 600, 1000, 1600, 3200, 6400, 13000 ms, FOV 6.5x4.8cm^2, MTX=86x64, in plane resolution 750x750 µm^2, slice thicknes: 2mm NEX=1, scan time: 28 min and 24 sec. T2 measurements were performed with identical geometry, but varying TE=8.5-250 ms (with 8.5 ms echo spacing) and TR=13s, NEX=1, scan time: 6 min and 56 sec.). T1 and T2 values for different NP concentrations were obtained by fitting the data with the spin echo MR signal equation. T1 and T2 relaxivities r1 and r2, respectively, were subsequently determined using: $^1/_{T_{1,2}} = \frac{1}{T_{1,2(0)}} + r_{1,2}[CA]$, where $T_{1,2(0)}$ represents T1,2 in the absence of contrast agent (CA). The Gd and Fe content in the NPs were measured by high resolution inductively coupled plasma mass spectroscopy (ICP-MS, Agilent 8800).

7. Cell culture and Imaging

7.1 Glioblastoma culture. Human glioblastoma astrocytoma cells (U-87 MG, ECACC, Salisbury, UK) were cultured in Eagle's Minimal Essential (EMEM) with 1.25% gentamicin (Sigma) and 10% fetal bovine serum (Autogen Bioclear, Wiltshire, UK). The cultures were supplemented with 2mM L-Glutamine, 1% non-essential amino acids (NEAA) (Sigma), and 1mM sodium pyruvate (NaP) (Sigma).

7.2 Cell labeling with PEG modified 11 nm Gd_2O_3 nanodisks and 9 nm spherical GDIO NPs. Human glioblastoma-astrocytoma cells(Sigma) (P>3) were expanded in three 12-well plates (Costar® 3335, Corning CellBIND® surface, Corning Inc., NY, USA) at 37°C, with 5% CO_2, until 50% confluence for LIVE/DEAD® assay. Cells were labelled with Gd_2O_3 and GDIO concentrations of NPs/media volume of 25 µg/mL, 50 µg/mL, 100 µg/mL, and incubated at 37°C for 24 hours. Unlabelled glioblastoma-astrocytoma cultures, at the same stage of confluence, were used as controls.

7.3 LIVE/DEAD® -Assay. After labeling, LIVE/DEAD® cell viability assay (Invitrogen, Life Technologies) was performed on glioblastoma-astrocytoma cultures. LIVE/DEAD® working solution was prepared in 9 mL PBS with 5.4 µL calcein (Invitrogen), and 24 µL ethidium homodimer (Invitrogen). The solution was added to each well in 1:1 (v/v) and left to react for

30 minutes at 37°C. Imaging of LIVE/DEAD® cell viability assay was performed on an Axiovert 200M (Zeiss, Germany) using AxioVision Rel. 4.3 software.

1. Y. Ridelman, G. Singh, R. Popovitz-Biro, S. G. Wolf, S. Das and R. Klajn, *Small*, 2012, **8**, 654-660.
2. G. Singh, P. A. Kumar, C. Lundgren, A. T. J. van Helvoort, R. Mathieu, E. Wahlstrom and W. R. Glomm, *Part Part Syst Char*, 2014, **31**, 1054-1059.

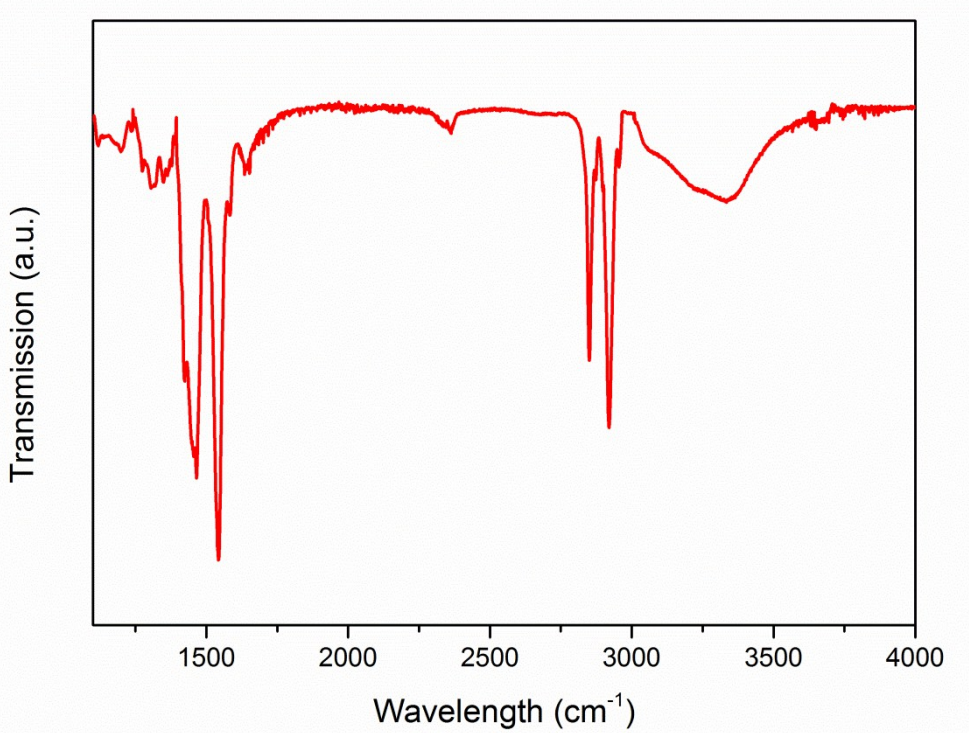

Fig. S1 Infra-red (IR) spectra of Gd-oleate

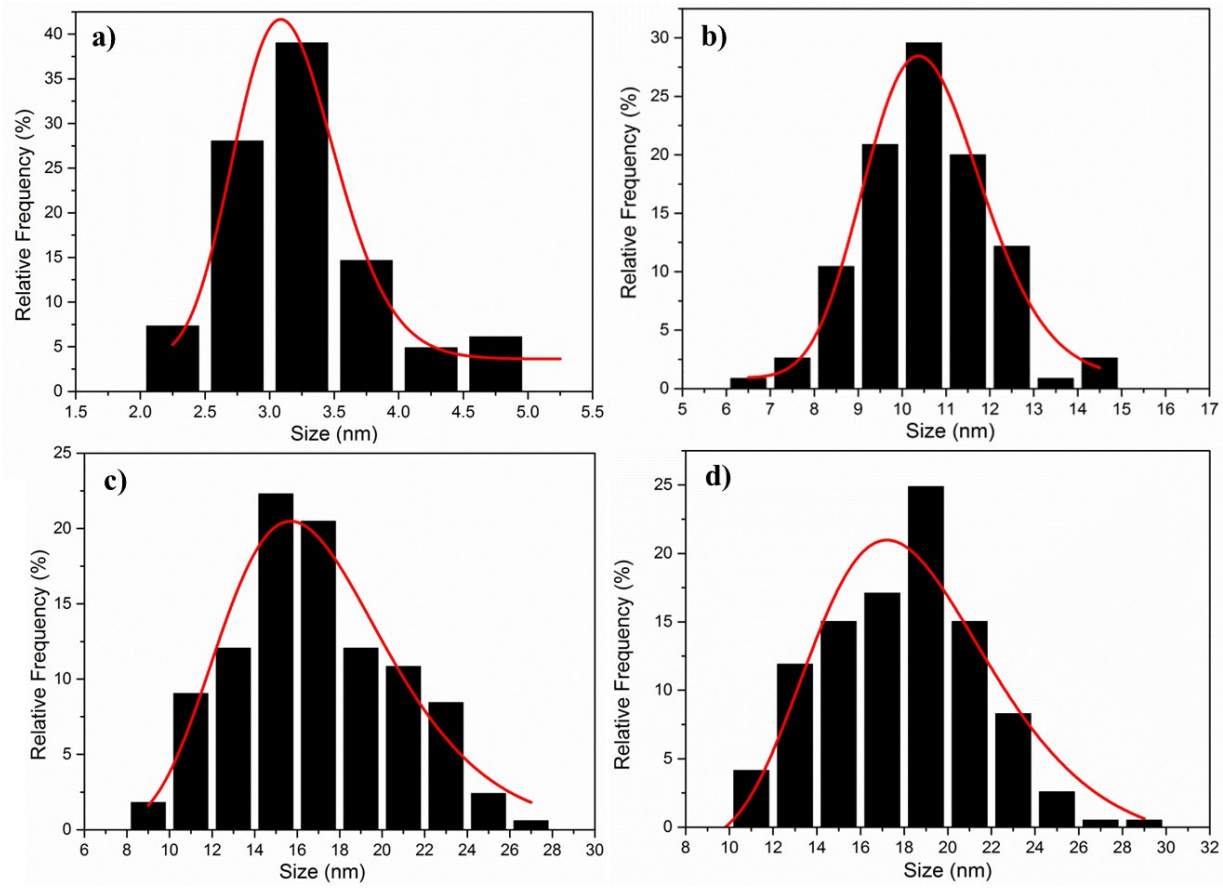

Fig. S2 The histograms show the size distribution of Gd_2O_3 nanodisks by counting more than 200 NPs. a)3±1 nm, b) 11±2 nm, c) 17±3 nm, d) 18±3 nm.

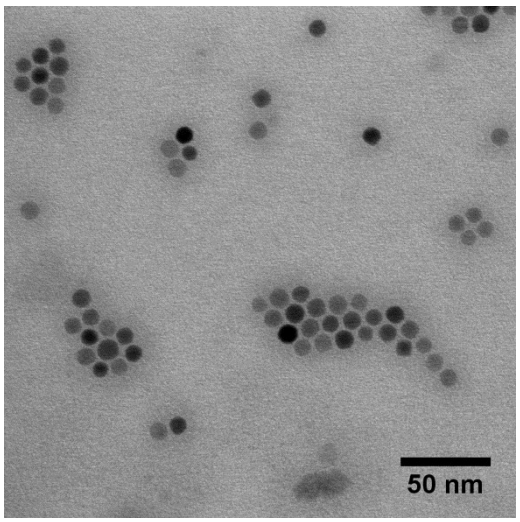

Fig. S3 BFTEM image of 11±2 nm Fe_3O_4 NPs prepared by the thermal decomposition of iron oleate (1.78 mmol) in the presence of 1.58 mmol OA.

Nanoparticle type	Gd (atomic %)	Fe (atomic %)	O (atomic %)	Si (atomic %)	Fe:Gd
GdIO-6 nm (spherical)	0.29	2.93	60.67	36.11	1:0.1
GdIO-4 nm (cubic)	1.45	3.02	70.80	26.09	1:0.5

Table S1 XPS Elemental analysis of GdIO nanoparticles of different shapes prepared from the decomposition of metal precursor containing different ratio of iron and gadolinium salts. XPS measurements were performed on nanoparticles deposited on the silicon (Si) wafer.