Supporting Information

High Signal Contrast Gating with Biomodified Gd Doped Mesoporous Nanoparticles

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Experimental Section

1. Native Gd-DOTA MSNs

Gd-DOTA doped mesoporous silica nanoparticles were synthesized by a surfactant templated and base catalyst procedure using a delayed co-condensation technique providing preferential internal functionalisation[1]. Initially, a mixture of 6.9mmol triethanolamine (TEA), 1.77mmol cetyltrimethylammonium bromide (CTAB), 0.89mol H_2O, and 0.04mol ethanol was heated to 80˚C. 6.49mmol tetraethylorthosilicate (TEOS) was then added dropwise under vigorous stirring. 60min later, equimolar amounts of TEOS and (3-aminopropyl)triethoxysilane (APTES) mixture (15µmol) were added. After a further 60min of stirring, the reaction was stopped with centrifugation (13200rpm, 20min) and several washes with ethanol. The as-synthesised MSNs were sonicated in acidic ethanol for 30min to remove the surfactant. The resulting aminated MSNs were subsequently reacted with 10µmol 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid mono-N-hydroxysuccinimide ester (DOTA-NHS ester) at room temperature for 24h. Chelation was achieved by the addition of two molar equivalents of Gd^{3+} ($GdCl_3$) at room temperature for 24h. The obtained Gd-DOTA MSNs were collected by a series of washing and centrifugation. The final Gd concentration of Gd-DOTA MSNs was examined by ICP-MS to be 8070±250µg of Gd per g of MSNs.

2. Protein conjugated Gd-DOTA MSNs were synthesis with the following various linkages:

Note: the surface functionalisation was maintained at 87.5pmol/mg of MSNs and consistent across all samples investigated**.**

2.1 Protein directly conjugated MSNs:

40mg of Gd-DOTA MSNs was mixed with 0.03mmol of APTES in 1: 2 H_2O ethanol mixture (4ml: 8ml) and reacted at room temperature for 24h. After amination,

nanoparticles were washed in ethanol twice and redispersed in 20ml anhydrous DMF with 0.06mmol of succinic anhydride to convert the terminal amine groups to carboxyls overnight at room temperature followed by washing in ethanol (twice). The obtained carboxyl modified Gd-DOTA MSNs were coupled with 3.5nmol of biotinylated BSA by reacting with *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) and *N*-Hydroxysuccinimide (NHS) overnight, washing in ice-cold water and collecting with centrifugation. Microarray spotting was used to examine the presence of protein after conjugation reaction (see below).

2.2 PEG linked protein conjugated MSNs:

40mg of Gd-DOTA MSNs was mixed with 3.5nmol of silanized PEG5000 or APTES (for the subsequent PEG2000 modification) in 1: 2 H_2O: ethanol mixture at room temperature for 24h and followed by reacting with 7nmol of succinic anhydride at room temperature overnight. 3.5nmol of Bis-amino-PEG2000 was then reacted with the aforementioned APTES linked particles via EDC/NHS coupling. After pegylation, the particles were washed in ethanol twice and redispersed in 20ml anhydrous DMF with 7nmol of succinic anhydride at room temperature overnight. The obtained modified Gd-DOTA MSNs were coupled with 3.5nmol of biotin-BSA as with the native nanoparticles, washed in ice-cold water and collected by centrifugation. Microarray spotting was subsequently used to confirm the presence of "functional" protein (see below).

2.3 Biotin linked protein conjugated MSNs:

40mg of Gd-DOTA MSNs was mixed with 70nmol or 350nmol of APTES in 1: 2 H_2O ethanol mixture and reacted at room temperature for 24h. After amination, the modified MSNs were coupled with 70nmol or 350nmol of biotin by reacting with *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) and *N*-Hydroxysuccinimide (NHS). The results shown in figure S2 (b) indicated particles with 70nmol of biotin (0.04wt%, mass equivalent with respect to MSN mass) minimized potential STV driven aggregation; the subsequent contrast gating was, thus, carried out with this particle. The as-synthesized biotinylated Gd-DOTA MSNs was then redispersed in 1.75μM of streptavidin aqueous solution to obtain streptavidin conjugated Gd-DOTA MSNs. In control experiments, the as-synthesized biotinylated Gd-DOTA MSNs were redispersed in 1.75μM of molecular biotin, biotin quenched streptavidin

or BSA aqueous solution. For competitive displacement with biotinylated BSA, the streptavidin capped particles were redispersed in 7μM of biotin-BSA solution with gentle shacking overnight.

3. Gd-DOTA doped nonporous silica nanoparticles (Gd-DOTA SNPs)

Nonporous silica nanoparticles were synthesized by traditional Stöber methods. A mixture of 1ml NH_4OH, 20ml ethanol and 1ml TEOS was stirred at room temperature for 24h. 116mg of as-synthesised SNPs were mixed with 0.04ml APTES in 10ml toluene under stirring at 70˚C for 20min. 90mg of the resulting aminated nanoparticles were mixed with 160μl triethylamine and 1.5mg DOTA-NHS ester in 3ml DMF at room temperature for 24h. Chelation was achieved by the addition of two molar equivalents of Gd^{3+} ($GdCl_3$) at room temperature for 24h.The final Gd-DOTA doped silica nanoparticles were collected by an extensive series of washings in ethanol and centrifugation.

4. Particle characterization

All characterization techniques and measurements were carried out on two different batches of nanoparticles of each sample type. The above nanoparticles were characterized with Zetasizer Nano (Malvern Instruments) dynamic light scattering (DLS)/zeta potential for particle distribution and surface charge and JEOL JEM-2100 LaB6 Transmission Electron Microscope (TEM) for particle size and morphology. A 7T horizontal bore magnet interfaced to a Varian Inova console (Varian Inc., Palo Alto, CA) and a 100mm internal diameter birdcage transmit and receive coil were used to acquire magnetic resonance imaging (MRI). Relaxivity experiments were repeated three times for at least three different sample concentrations. Analysis of Gadolinium concentration was carried out using ICP-MS and samples were measured by $LiBO_2$ fusion by Viridian Partnership, Surrey.

5. TEM sample preparation

TEM images were obtained on a Jeol JEM-2100, 200 kV, LaB6 instrument, operated at 120 kV with a beam current of about 65 mA. Samples for TEM were prepared by deposition and drying of a drop of the powder dispersed in water onto a formvar-coated 300-mesh copper grid. Diameters were measured using the ImageJ version

1.40 software; average values were calculated by counting a minimum of 100 particles.

6. Microarray spotting

Streptavidin arrays were printed in 3 x 3 features, across 9 repetitions, with print buffer on amine modified glass slides (supplied by Genetix) by a BioOdyssey Calligrapher MiniArrayer (humidity controlled at 50%, temperature 4°C). (Figure S5a) All proteins and protein modified MSNs were labelled using standard protocols supplied by the dye manufacturer, ATTO-TEC Gmbh. Spotted volumes were allowed to incubate on the slide for 60min prior to a blocking step in 1% BSA in PBST for 60min. Atto655 NHS ester labelled proteins (1:1 labelling ratio) or protein modified MSNs were then incubated at a concentration of 7nM in 1% BSA in PBST for 40min. Slides were washed in PBST twice for 10 min then twice in deionised water for 2min. The slides were dried under a stream of nitrogen and scanned at a pixel resolution of 5μm with 633nm lasers under the Cy5 detection protocol (ScanArray Express, Perkin Elmer).

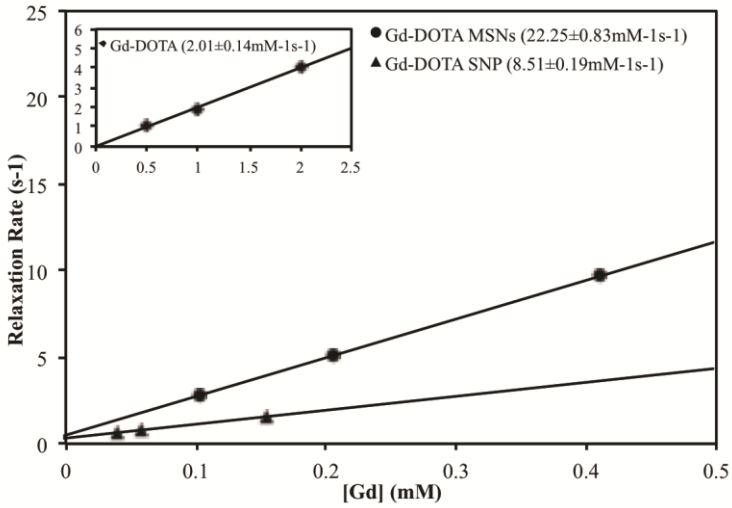
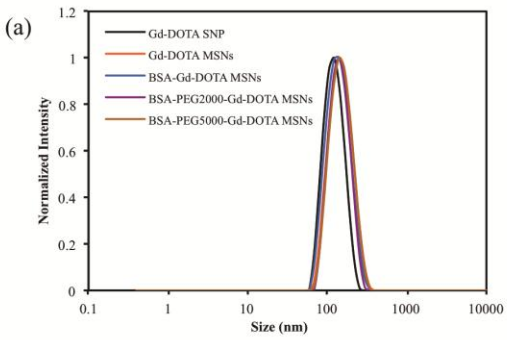
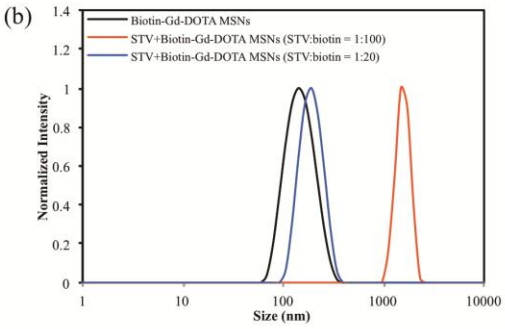
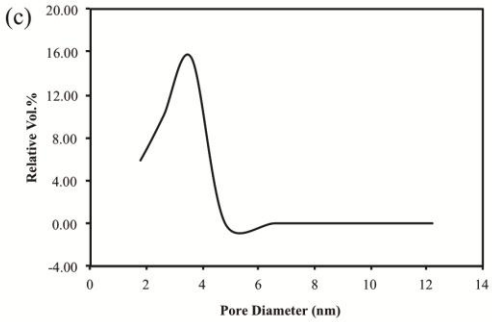


Figure S1 Plots of concentration (mM) versus relaxation rate (1/T1) (s^{-1}) measured at a 7 T magnetic field. The numbers in the brackets are the corresponding relaxivities (20°C).







(d)

Reaction	Z-potential (mV)
Gd-DOTA MSNs	-24.93 (±1.72)
NH_2-Gd-DOTA MSNs	0.89 (±0.27)
COOH-Gd-DOTA MSNs	-21.23 (±1.21)
PEG5000-Gd-DOTA MSNs	-20.47 (±0.25)
PEG2000-Gd-DOTA MSNs	-35.60 (±0.76)

Figure S2 (a) DLS of Gd doped SNPs, Gd doped MSNs and BSA conjugated Gd doped MSNs w/o PEG linker. Five samples demonstrate hydrodynamic size of 115.5nm (PDI:0.08), 138.7nm (PDI:0.09), 128.9nm (PDI:0.09), 135.6nm (PDI:0.07), and 140.9nm (PDI:0.08), respectively and good dispersion. (b) DLS of biotinylated Gd doped MSNs and its streptavidin conjugated derivatives (biotin/STV=100 or 20). Data shown indicates that ratios of 100 and 20 induce significant and negligible levels of aggregation respectively, compared to the parent biotinylated Gd doped nanoparticles. (c) Pore size distribution by BET surface area analysis. Gd-DOTA MSNs have pore sizes of 3.2±1.3nm. (d) Surface charge assessment as a function of particle modification (the silanol acidity responsible for negative charge is quenched on extensive surface amination prior to acidification).

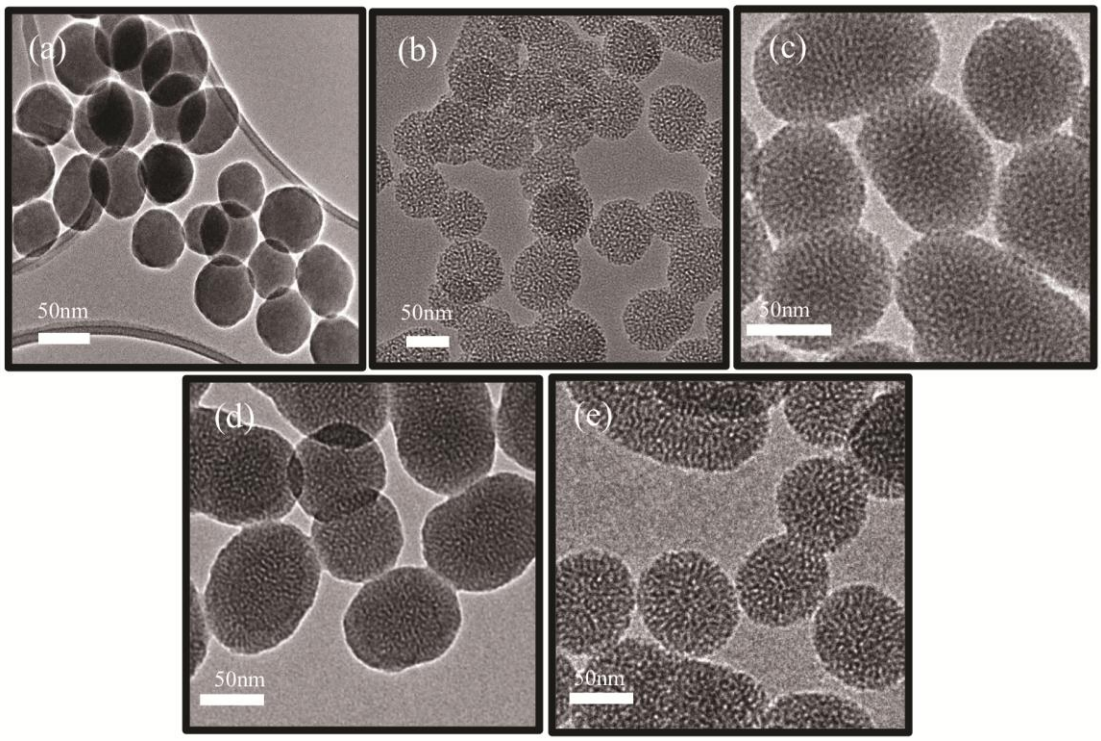


Figure S3 TEM images of (a) Gd-DOTA SNP; (b) Gd-DOTA MSNs; (c) BSA-Gd-DOTA MSNs; (d) BSA-PEG5000-Gd-DOTA MSNs; (e) BSA-PEG2000-Gd-DOTA MSNs, with sizes of 80.9(±7.7)nm, 72.1(±6.3)nm, 75.0(±6.9)nm, 74.4(±6.0)nm, and 75.9(±6.4)nm, respectively.

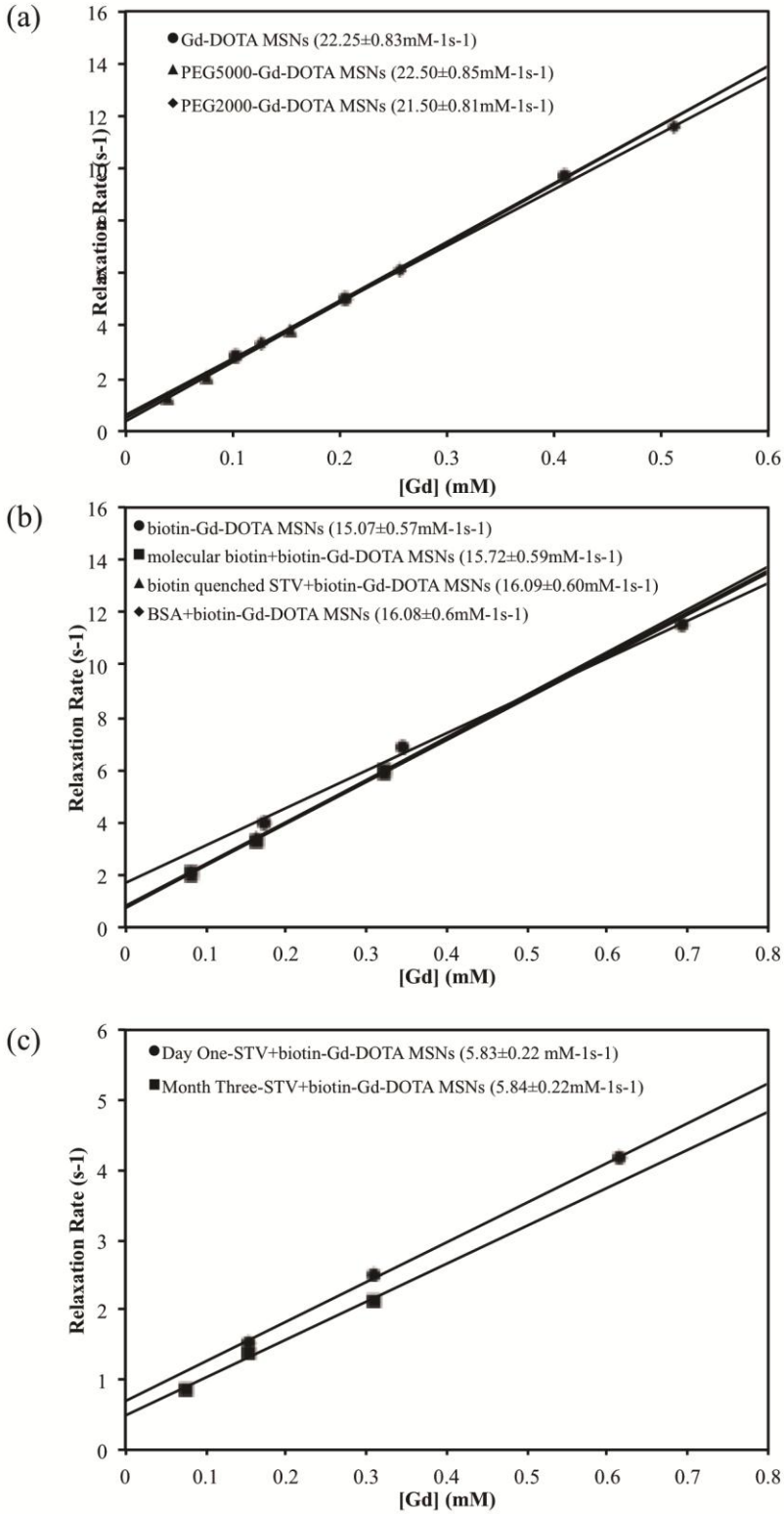


Figure S4 (a) Plots of concentration (mM) versus relaxation rate (1/T1) (s^{-1}) measured at 7 T magnetic field. The numbers in the brackets are the corresponding relaxivity. Data shown indicates PEG alone has negligible effect on relaxivity. (b) Relaxivity assessment of biotin-Gd-DOTA MSNs in the presence of molecular biotin, biotin saturated STV or natively unmodified BSA. Data indicates these three controls have negligible effect on relaxivity, showing high specificity towards STV. (c) Relaxivity assessment of STV capped biotin-Gd-DOTA MSNs. The data indicates the relaxivity characteristic of protein capped particles is highly stable for at least three months (20°C).

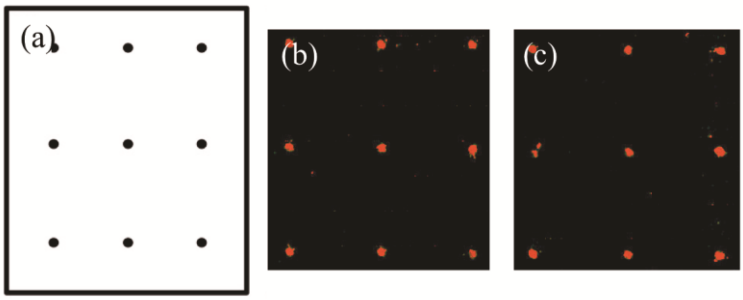


Figure S5 (a) Schematic representation of ordered streptavidin arrays; (b) Microarray images of fluorescent biotin-BSA-Gd-DOTA MSNs targeted spots; (c) Microarray images of biotin-BSA-PEG5000-Gd-DOTA targeted spots. Images demonstrate clean ordered fluorescent array, indicating that vectoring MSNs by an attached protein to another protein had been successfully achieved.

Table S1 Previous reports of responsive paramagnetic contrast

Sensing targets	Contrast agents	Parameter altered
Glucose[2]	Molecular based Gd complex	Rotational correlation time
Beta-galactosidase[3]	Molecular based Gd complex	Hydration number
Ca(II)[4]	Molecular based Gd complex	Hydration number
pH[5]	Poly(amino acid) based Gd complex	Rotational correlation time
Human carboxypeptidase B, thrombin-activatable fibrinolysis inhibitor (TAFI)[6]	Molecular based Gd complex	Rotational correlation time
Human serum albumin[7]	Molecular based Gd complex	Rotational correlation time
Zn(II)[8]	Molecular based Gd complex	Hydration number

Beta-glucuronidase[9]	Molecular based Gd complex	Hydration number
Horseradish peroxidase[10]	Molecular based Gd complex	Rotational correlation time
Porcine liver esterase[11]	Molecular based Gd complex	Hydration number
K(I), Mg(II), Ca(II)[12]	Molecular based Gd complex	Hydration number
Myeloperoxidase[13]	Molecular based Gd complex	Rotational correlation time
Ca(II)[14]	Molecular based Gd complex	Hydration number
Zn(II)[15]	Molecular based Gd complex	Hydration number
Cu(I), Cu(II)[16]	Molecular based Gd complex	Hydration number
pH[17]	Polymer baed Gd complex	Rotational correlation time
This work: Streptavidin, biotinylated BSA	**MSN based contrast agents**	**Water accessibility and motion in the mesopores**

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