Supplementary Information to

High Magnetic Relaxivity in a Fluorescent CdSe/CdS/ZnS quantum dot functionalized with MRI contrast molecules

S. G. McAdams,[a] D. J. Lewis,[a b] P. D. McNaughter,[a] E. A. Lewis,[b] S. J. Haigh,[b] P. O'Brien,[a b c]* and F. Tuna.[a c]*

[a]School Of Chemistry, University of Manchester, Oxford Road, M13 9P, UK
[b]School of Materials, University of Manchester, Oxford Road, M13 9PL, UK
[c.]Photon Science Institute, University of Manchester, Oxford Road, M13 9PL, UK

Contents

1. Materials

All materials and chemicals were purchased from Sigma-Aldrich, except cadmium oxide (Alfa Aesar); zinc oxide (BDH Reagents); and octadecylamine (ACROS organics). All chemicals were used as received.

2. Instrumentation

High angle annular dark field scanning transmission electron microscope (HAADF STEM) imaging, and energy dispersive X-ray (EDX) spectrum imaging were performed using a probe side aberration corrected FEI Titan G2 80-200 S/TEM "ChemiSTEM™" instrument with an X-FEG electron source operated at 200 kV, with a probe current of ~800 pA and a convergence angle of 21 mrad. EDX spectra and spectrum images were acquired and analysed using Bruker Esprit software. STEM images were acquired using FEI TIA software and analysed using Gatan Digital Micrograph and Image J software. Samples were prepared by dropping a dilute nanoparticle solution onto a holey carbon coated copper grid. Nanoparticle size distribution analysis was performed using Trainable Weka Segmentation [1] software that is part of the open-source Image J / Fiji package.[2] EDX quantification was performed in Bruker Esprit Software using the Cliff-Lorimer approach and with no absorption correction. Nanoparticle surface groups were characterised using a Nicolet iS5 Fourier Transform Infrared Spectrometer with an iD5 ATR accessory on dried powder samples. Elemental analyses were performed at the University of Manchester Micro Analytical Laboratory. ICP-MS measurements were performed at the University of Manchester School of Earth, Atmospheric and Environmental Sciences. Magnetisation data were collected with a Quantum Design MPMS-XL SQUID magnetometer equipped with a 7 T magnet. Samples were measured in gelatine capsules, as restrained by eicosane, to avoid orientation in the magnetic field. Field dependant magnetisation curves were recorded at temperatures of 2 K and 4 K by increasing the magnetic field from 0 to 7 T. Experimental data were corrected for the diamagnetic contribution of the gelatine capsule and eicosane used to mount the samples. Electron paramagnetic resonance (EPR) spectra were recorded in continuous-wave mode with a Bruker EMX 300 EPR spectrometer operating at microwave frequency of ca. 34 GHz (Q-band). UV–VIS absorption spectra were collected using a double beam *UV-1800 Shimadzu Uv-vis spectrophotometer* between $200 - 900$ nm. Samples were measured in quartz cuvettes with path length of 1 cm, and pure solvent was used as a blank. PL emission spectra were collected using a GILDEN Photonics fluoroSENS spectrofluorometer at a 400 nm excitation wavelength, with a 1 nm resolution. Quantum yields were measured using an integration sphere (Edinburgh Instruments). Relaxivity measurements at 1 T (42.5 MHz) were made on a Magritek Spinsolve spectrometer. T_1 weighted fast spin echo MR images (repetition time = 720 ms; echo time = 11 ms; echo train length = 2) were obtained using a 3T MRS 3000 (MR Solutions, Guildford, UK). DLS and Zeta potential measurements were performed at 25°C in water using a Malvern ZetaSizer (Malvern Instruments Ltd, Uk). A dilute suspension of QD@GdL was passed through 0.45 μm PTFE membrane filters (Millex®) prior to measurements. For DLS, data was collected in automatic analysis mode; and the hydrodynamic diameter calculated based on a number weighted distribution.

3. Synthesis

3.1 H₃L and GdL Synthesis

N,*N''*- Bis[*p*-thiophenyl (aminocarbonyl)] diethylenetriamine-*N*,*N'*,*N''*-triacetic acid (H₃L) was synthesised as previously described.[3] ^{1}H NMR (400 MHz, MeOH-*d*₄), δ ppm: 7.24 (4H, d, ^3J = 8.75 Hz); 6.985 (4H, d, ^3J = 8.75 Hz, H$_g$); 4.23 (2H, s); 3.52 (4H, s, H$_d$); 3.49 (4H, s, H$_e$); 3.41 (4H, t, ^3J = 5.43 Hz); 3.22 (4H, t, ^3J = 5.34 Hz) (Fig. S1). Anal. Calcd. For $C_{26}H_{33}N_5O_8S_2(N_2H_6Cl_2)_{0.6}(H_2O)_2$: C 44.2, H 5.8, N12.3. Found: C 44.13, H 5.62, N 12.94

COMMUNICATION
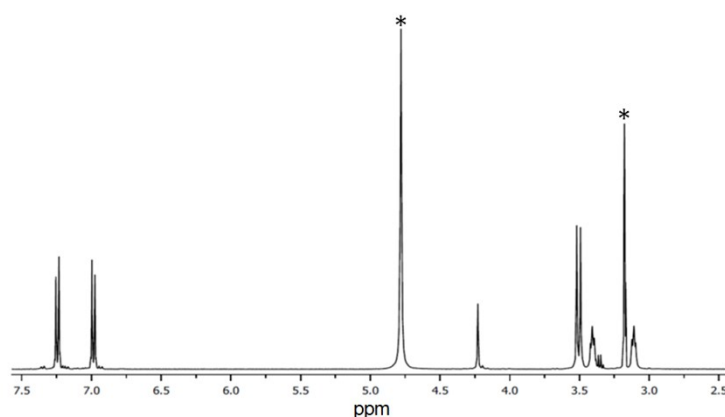
Fig. S1. *NMR of N,N''-Bis[p-thiophenyl(aminocarbonyl)]diethylenetriamine-N,N',N''-triacetic Acid.* * denotes solvent peaks

GdL was synthesised as previously described.[3] H_3L (0.25 g, 0.40 mmol) and $GdCl_3 \cdot 6H_2O$ (0.40 mmol) were dissolved in degassed methanol (3 mL) with mild sonication. GdL precipitated as a white powder with the addition of acetonitrile (30 mL), and collected via suction filtration under N_2. The complex was washed with acetonitrile (30 mL) and diethyl ether (30 mL), dried under vacuum, and finally stored under nitrogen at - 20 °C. MS (ES-TOF[+]) *m/z*: 763 {M+H}[+].

3.2 CdSe Quantum Dot Synthesis

3.4 nm CdSe QD cores were synthesised following a previously reported protocol.[4] Once the growth period was complete, the heat source was removed and the reaction cooled. When the temperature reached 60°C, hexane (30 mL) was injected into the reaction solution to prevent solidification. The QDs were isolated with the addition of acetone and centrifugation (10,000 rpm for 15 minutes). The supernatant was discarded and the pellet resuspended in chloroform. This washing/centrifugation procedure was repeated another twice. The final pellet was resuspended in chloroform and stored under N_2 at room temperature in a glass vial. CdSe QD concentration was calculated based upon UV-vis absorption spectra.[5]

3.3 CdSe/CdS/ZnS Quantum Dot Synthesis

CdSe/CdS/ZnS QDs were synthesised using the SILAR method following a previously reported protocol.[6] The injection volume of each precursor was calculated based on equations described by Reiss *et al.*[7] to synthesise a CdSe core with five monolayers of CdS and two monolayers of ZnS. The first Cd precursor injection volume is halved as the CdSe QD surface is expected to already consist of 50% Cd atoms.

To synthesise CdSe/CdS/ZnS QDs, octadecene (6 mL) and octadecylamine (2 g) were stirred in a 100 mL three-necked flask, and heated to 100 °C under vacuum, and held for 1 hour before cooling to room temperature. Next, the CdSe QD stock solution (2.86 mL ; 0.366 μmol) was injected into the reaction flask using standard air-free techniques. The reaction was heated to 100 °C under vacuum and held for 30 minutes, and then heated to 245 °C under N_2. The precursors were alternately injected at 10 minute intervals, and after the final injection the temperature was raised to 260 °C and held for 30 minutes. Once complete, the reaction was allowed to cool to 60 °C and hexane (30 mL) was injected into the reaction solution to prevent solidification. The CdSe/CdS/ZnS QDs were precipitated using acetone, and isolated using centrifugation (10 000 rpm; 10 minutes). The pellet was then resuspended in chloroform, and this washing/centrifugation procedure was repeated another twice. The final pellet was suspended in chloroform and stored under N_2.

3. 4 Ligand exchange: QD@GdL

In previous work the 'footprint' of a EuL complex was calculated as 0.398 nm^2. It follows that a maximum of 557 GdL complexes can be loaded onto the surface of a CdSe/CdS/ZnS QD 8.4 nm in diameter (see section 9.3). To synthesise QD@GdL, GdL at a 2 fold excess was stirred with CdSe/CdS/CdSe QDs in a 1:1 methanol/chloroform solution. After 24 hours stirring under N_2, the solution was centrifuged (4000 rpm; 15 minutes) to isolate the hybrid, followed by washing with methanol through five rounds of centrifugation (4000 rpm; 15 minutes). Finally, the pelleted QD@GdL was resuspended in methanol for optical and TEM studies; and PBS buffer for relaxivity measurements.

4. Dynamic Light-scattering: QD@GdL Number Distribution
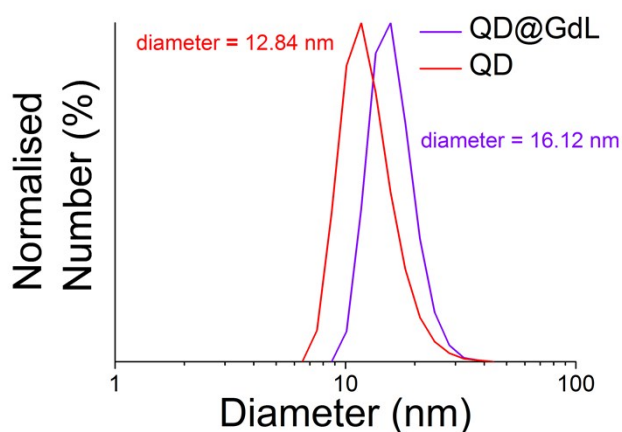

Fig. S2 Number (%) weighted size distribution of QD and QD@GdL obtained using dynamic-light scattering in PBS buffer. PDI reported as 0.21 and 0.32, respectively. Volume weighted size distribution was 16.86 nm (QD) and 19.15 nm (QD@GdL); and intensity weighted size distribution was 69.85 nm (QD) and 104.0 nm (QD @GdL).
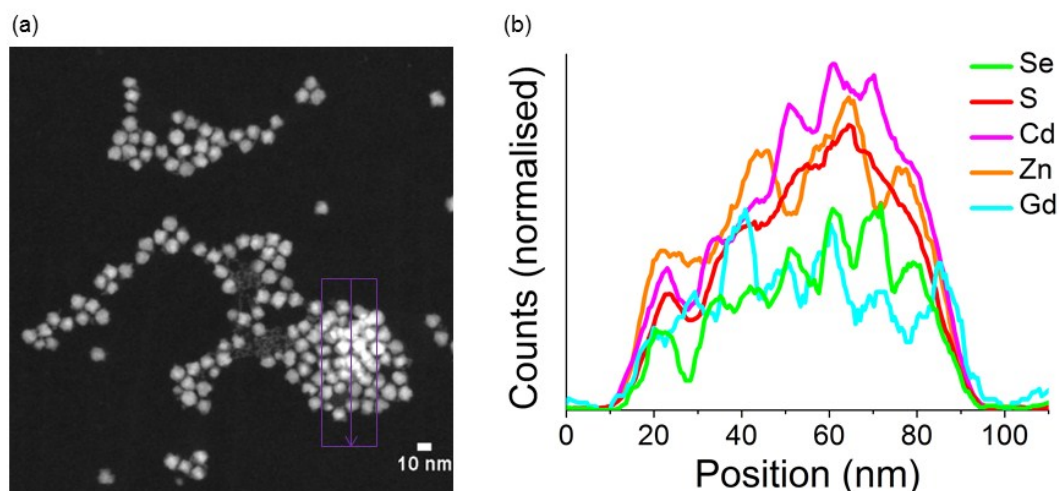
5. Additional QD@GdL HAADF TEM Images, EDX Maps and Spectra, and Size Distribution Histogram
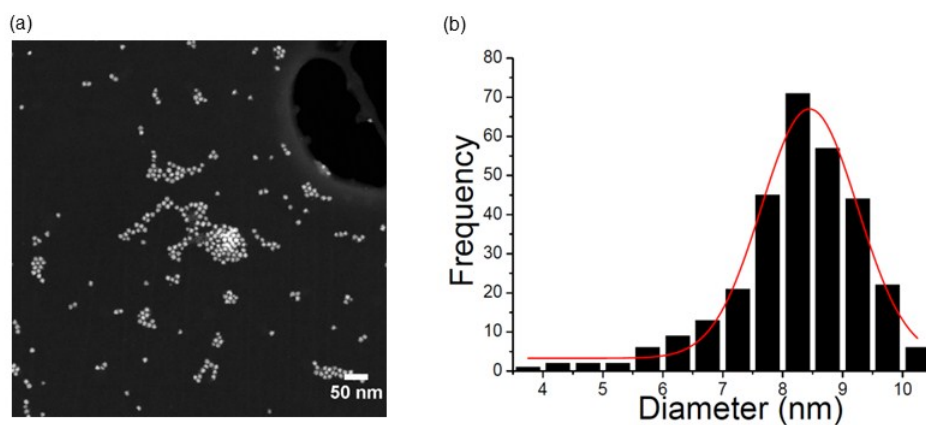

Fig. S3 HAADF STEM image of QD@GdL (a); and corresponding histogram of QD@GdL particle size distribution (b).
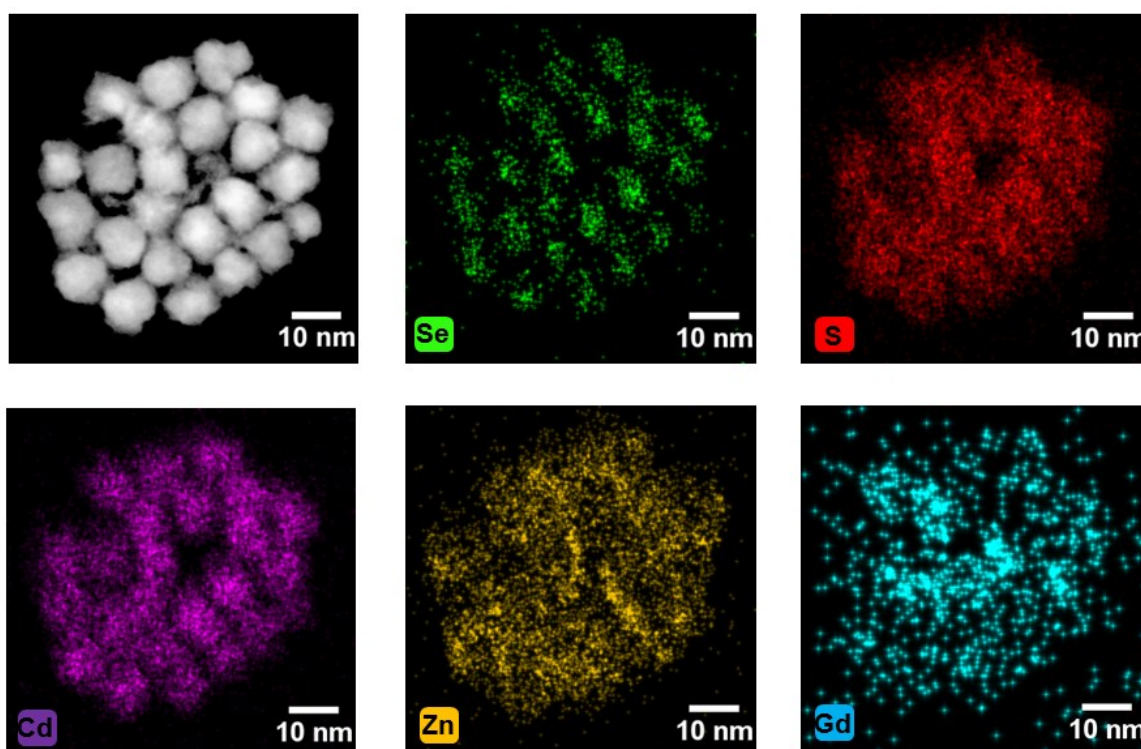

Fig. S4 HAADF STEM image of QD@GdL (a) and corresponding EDX maps of Se (green), S (red), Cd (purple), Zn (orange) and Gd (blue).

Fig. S5 Additional HAADF STEM image of QD@GdL (a) with an EDX line-scan profile showing elemental distributions (b). Purple box in (a) indicates the line-scan profile area; and arrow the scan direction.
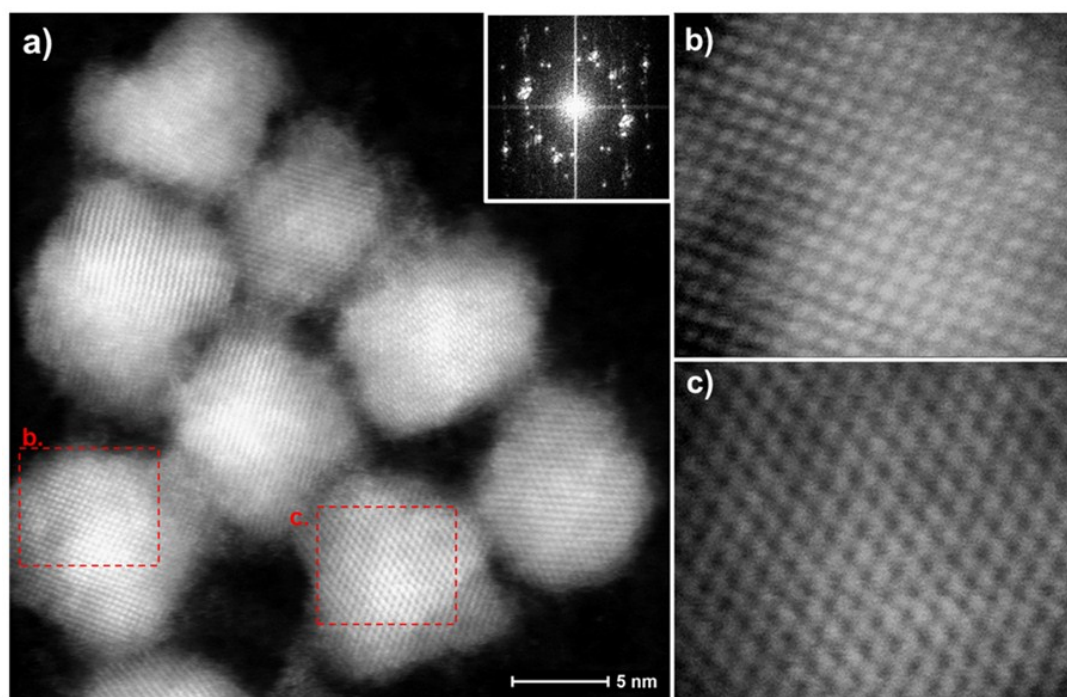
Fig. S6. High resolution HAADF STEM images of a collection of nanocrystals. FFT of (a) is inset in upper right corner of image showing lattice spacings of approximately 3.7, 3.3, 2.6, 2.2, and 1.8 Å. These values agree well with the expected d-spacings of CdSe, corresponding to the (100), (101), (102), (110), and (200) planes respectively. Each quantum dot appears to be single crystalline, enlarge regions indicated by dashed red boxes in (a) are shown in (b) and (c).
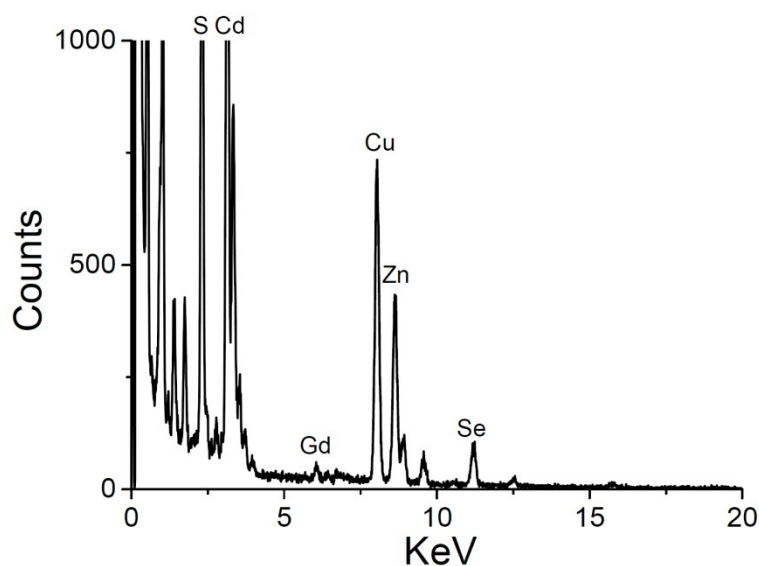
Fig. S7 Summed EDX spectrum corresponding to spectrum image shown in Fig 1. The spectrum confirms the presence of Cd, Zn, S, Se, and Gd. In addition to these elements C, O, and Cu are detected, these elements are associated with the amorphous carbon support and grid bars of the TEM grid used in this work, weak Fe and Zr signals are also detected, these arise from the microscope column and EDX detector collimators. Atomic % of elements calculated as follows: Cd 42%, S 38%, Se 4%, Zn 15%, Gd 1%.

6. FTIR Spectroscopy
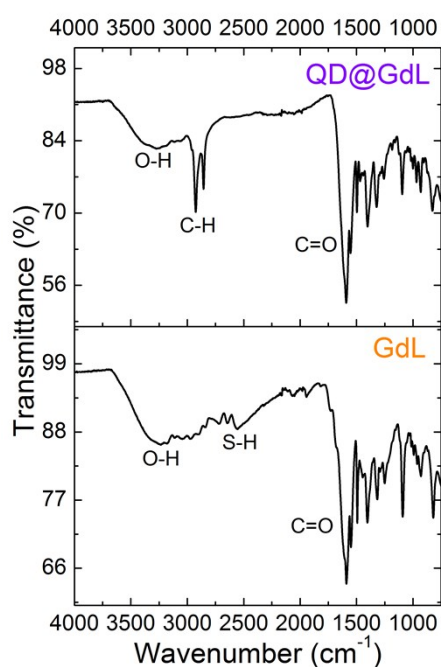
Fig. S8 FT-IR spectra of QD@GdL (top) and GdL (bottom).

7. Magnetic Studies

The number of Bohr magneton (n_B) per formula unit was calculated using the following formula:
n_B = M (emu.g^{-1}) * M_W (g.mol^{-1}) / 5585

where M is the magnetisation (emu.g^{-1}), M_W the molecular weight (g.mol^{-1}); and 5585 is Avogadro's number multiplied with the Bohr magneton μ_B (6. 022 x10^{23} mol^{-1} * 9.28x10^{-21} erg.G^{-1}).
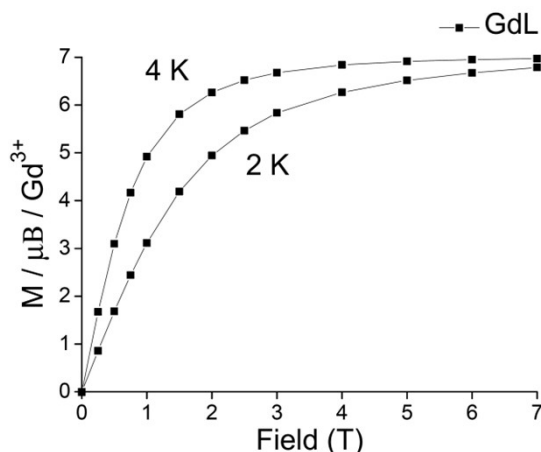

Fig. S9. Plot of M vs H at 2 and 4 K for GdL

To confirm the ICP-MS determined ratio of QDs to GdL, the following calculation was performed:
The M_W of one QD was calculated as 9.0 x 10^5 g.mol^{-1} (Section 9.1), and the ratio of QD to GdL as 1 to 6.2 x 10^2 (Section 9.2).

Therefore the total MW of the hybrid is 9.0 x 10^5 g.mol^{-1} + (6.2 x 10^2 * 763.08 g.mol^{-1}) = 1.4 x 10^6 g.mol^{-1}

Multiplying by a M_{sat} (Figure 3b) of 17.26 emu.g^{-1}gives 2.4 x 10^7 emu.mol^{-1}

Dividing by 5585 gives 4.3 x 10^3 μ_B / 6.2 x 10^2 Gd^{3+}

Dividing by 6.2 x 10^2, i.e. the number of GdL per QD, gives 6.9 μ_B / Gd^{3+} (which approaches the expected M_{sat} value of 6.96 μ_B per Gd^{3+} ion, calculated with g = 1.99 as determined by EPR)

8. Calculations

For all the following calculations, with the exception of QD volume and surface area, all errors were propagated using the equations:[8]

Additions (y = a + b − c)
$$\sigma y = \sqrt{(\sigma a)^2 + (\sigma b)^2 + (\sigma c)^2}$$

Multiplications (y = a * b/c)
$$\sigma y = y\sqrt{(\sigma a/a)^2 + (\sigma b/b)^2 + (\sigma c/c)^2}$$

8.1 CdSe/CdS/ZnS QD Molecular Weight Calculations

ICP-MS determined the ppm (mg/L) of the following elements in a QD@GdL stock solution (Table S1).

Table S1. ICP-MS results of QD@GdL.

Element	Mean ppm (mg.L^{-1}) (n=3)	Standard Deviation
Cd	38.77	0.2017
Zn	10.93	0.05914
Se	4.399	0.09472
Gd	7.475	0.03334

Table S2. Molar concentration of elements in QD@GdL stock solution.

Element	M (mol.L^{-1})
Cd	3.449×10^{-04}
Zn	1.672×10^{-04}
Se	5.571×10^{-05}
Gd	4.754×10^{-05}

The total mass (grams) of each layer constituting the CdSe/CdS/ZnS QDs in one litre were calculated as follows:

ZnS molecular weight (MW) * moles of Zn (Table S2) = 97.45 g.mol^{-1} * 1.672×10^{-04} mol.L^{-1} = 1.629×10^{-02} g.L^{-1}

CdSe MW * moles of Se (Table S2) = 191.37 g.mol^{-1} * 5.571×10^{-05} mol.L^{-1} = 1.066×10^{-02} g.L^{-1}

CdS MW * (moles of Cd – moles of Se) = 144.76 g.mol^{-1} * (3.449×10^{-04} mol.L^{-1} – 5.571×10^{-05} mol.L^{-1})= 4.178×10^{-02} g.L^{-1}

By dividing the mass of each material by the density, the total volume (cm^3) of each material was calculated:

Mass of ZnS / density of ZnS = 1.629×10^{-02} g / 4.09 g.cm^{-3} = 3.983×10^{-03} cm^3

Mass of CdSe / Density of CdSe = 1.066×10^{-02} g / 5.82 g.cm^{-3} = 1.832×10^{-03} cm^3

Mass of CdS / Density of CdS = 4.178×10^{-02} g / 4.82 g.cm^{-3} = 8.668×10^{-03} cm^3

The volume of each of component is calculated as a percentage of the total material volume (1.45×10^{-2} cm^3) (Table S3).

Table S3. Volume of each material in stock solution calculated as percentage of the total material volume.

Material	Percent of total volume (%)
ZnS	27.50
CdSe	12.65
CdS	59.85

The volume of a 8.4 ± 0.94 nm (mean ± SD) (Figure S2) CdSe/CdS/ZnS quantum dot was calculated using the following equation: $V = (4/3) \pi r^3$

$= (4/3) \pi (4.2 \times 10^{-7} \text{ cm})^3 = 3.1 \times 10^{-19} \text{ cm}^3$

The error in the radius was propagated to the volume using the following equation: [9] $dV = 4 \pi r^2 dr$

$= 4 \pi (4.2 \times 10^{-7} \text{ cm})^2 (4.7 \times 10^{-8}) = 1.0 \times 10^{-19} \text{ cm}^3$

Therefore the volume of a CdSe/CdS/ZnS QD is $3.1 \times 10^{-19} \text{ cm}^3 \pm 32\%$

The volumetric contribution of each material to a single QD was calculated based on previously calculated percentages (see table S3): Percentage contribution per material * QD Volume

ZnS : $27.5 \% * 3.1 \times 10^{-19} \text{ cm}^3 = 8.5 \times 10^{-20} \text{ cm}^3$

CdSe : $12.65 \% * 3.1 \times 10^{-19} \text{ cm}^3 = 3.9 \times 10^{-20} \text{ cm}^3$

CdS: $59.85 \% * 3.1 \times 10^{-19} \text{ cm}^3 = 1.9 \times 10^{-29} \text{ cm}^3$

The number of moles in each QD layer was calculated through the following equation:

Moles per layer = (volume * density) / molecular weight

ZnS : $(8.5 \times 10^{-20} \text{ cm}^3 * 4.09 \text{ g.cm}^{-3}) / 97.45 \text{ g.mol}^{-1} = 3.5 \times 10^{-21} \text{ moles}$

CdSe: $(3.9 \times 10^{-20} \text{ cm}^3 * 5.82 \text{ g.cm}^{-3}) / 191.37 \text{ g.mol}^{-1} = 1.2 \times 10^{-21} \text{ moles}$

CdS: $(1.9 \times 10^{-29} \text{ cm}^3 * 4.82 \text{ g.cm}^{-3}) / 144.76 \text{ g.mol}^{-1} = 6.3 \times 10^{-21} \text{ moles}$

The weight of each element in a QD was calculated using the equation:

(moles of atoms * 6.022×10^{23}) * AMU (Atomic Mass Unit)

Cd: (moles of CdSe + moles of CdS) * 6.022×10^{23}) * $112.411 \text{ g.mol}^{-1}$ = $(1.2 \times 10^{-21} + 6.3 \times 10^{-21}) * 6.022 \times 10^{23}) * 112.411 \text{ g.mol}^{-1}$ = $5.1 \times 10^5 \text{ g.mol}^{-1}$

Se: (moles of CdSe) * 6.022×10^{23}) * 78.96 g.mol^{-1} = $(1.19 \times 10^{-21}) * 6.022 \times 10^{23}) * 78.96 \text{ g.mol}^{-1}$ = $5.7 \times 10^4 \text{ g.mol}^{-1}$

Zn: (moles of ZnS) * 6.022×10^{23}) * 65.38 g.mol^{-1} = $(3.58 \times 10^{-21}) * 6.022 \times 10^{23}) * 65.38 \text{ g.mol}^{-1}$ = $1.4 \times 10^5 \text{ g.mol}^{-1}$

S: (moles of ZnS + moles of CdS) * 6.022×10^{23}) * $32.065 \text{ g.mol}^{-1}$ = $(3.58 \times 10^{-21} + 6.20 \times 10^{-21}) * 6.022 \times 10^{23}) * 32.065 \text{ g.mol}^{-1}$ = $1.9 \times 10^5 \text{ g.mol}^{-1}$

Therefore the molecular weight of a CdSe/CdS/ZnS QD is equal to the addition of the calculated MW of each element

= Cd + Se + Zn + S

$= 5.1 \times 10^5 \text{ g.mol}^{-1} + 5.7 \times 10^4 \text{ g.mol}^{-1} + 1.4 \times 10^5 \text{ g.mol}^{-1} + 1.9 \times 10^5 \text{ g.mol}^{-1}$

$= 9.0 \times 10^5 \text{ g.mol}^{-1} \pm 1.6 \times 10^5$

8.2 ICP-MS: QD to GdL Ratio Calculation

The mass of each QD layer in 1 litre was previously calculated (Section 9.1), therefore the total mass in 1 litre is:

Total QD mass in 1 litre = ZnS + CdSe + CdS

$= 1.629 \times 10^{-02} \text{ grams.L}^{-1} + 1.066 \times 10^{-02} \text{ grams.L}^{-1} + 4.178 \times 10^{-02} \text{ grams.L}^{-1} = 6.873 \times 10^{-02} \text{ grams.L}^{-1}$

The number of moles of QDs equals total weight of QD in 1 litre divided by the MW (section 9.1):

= 6.873 x10^{-02} grams / 9.0 x 10 ^{5} g.mol^{-1} = 7.6 x10^{-8} moles.L^{-1}

Multiplying this value by Avogadro's number gives the number of QDs.L^{-1}: 4.6 x10^{16} QDs.L^{-1}

The molar concentration of Gd was calculated based from ICP-MS data (Table S2).

If 4.754 x10^{-5} moles.L^{-1}Gd = 4.754 x10^{-5} moles.L^{-1} GdL

Multiplying this value by Avogadro's number, the number of GdL.L^{-1}:

= 2.863 x10^{19} GdL.L^{-1}

Therefore the QD:GdL ratio in 1 litre equals:

 = 4.6 x 10^{16} QDs: 2.863 x10^{19} GdL **= 1 QD: 6.2 x 10^2 ± 1.2 x 10^2 GdL**

8.3 Maximum Loading of GdL per QD Calculation

The surface area of a 8.4 ± 0.94 nm (mean ± SD) CdSe/CdS/ZnS QD was calculated using the following equation:

A=4 π r^2 = 4 * π* (4.2)^2 = 2.2 x 1o^2 nm^2

The propagation of error from the diameter to surface area was calculated using the following equation:[9]

dA= 8 π r *dr* = 8 * π * (4.2) * 0.47 = 50 nm^2

Therefore, the surface area of a 8.40 ± 0.94 nm QD is 2.2 x 10^2 ± 50 nm^2

The footprint of GdL was previously determined as 0.398 nm^2.[3]

Dividing 2.2 x 10^2 ± 50 nm^2 by 0.398 nm^2 gives an estimated maximum GdL loading of 5.5 x 10^2 ± 1.3 x 10^2 per QD.

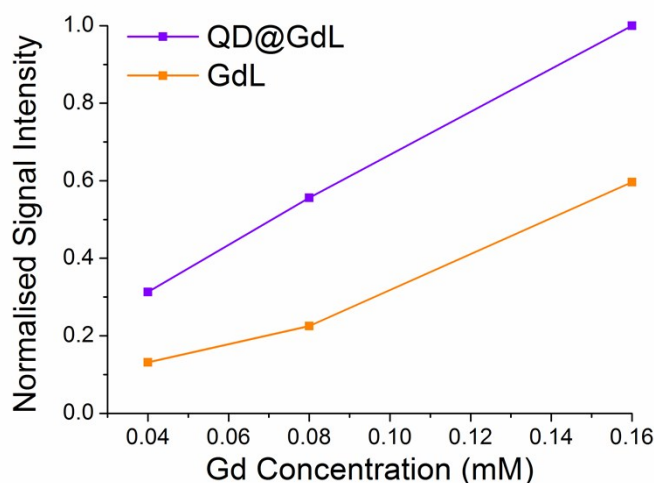## 9. T_1 Weighted MR Images: Signal Intensity

Fig. S10 Normalised signal intensity of T_1 weighted MR images.

10. References

1 M. Hall, E. Frank, G. Holmes, B. Pfahringer, P. Reutemann and I. H. Witten, *SIGKDD Explor Newsl*, 2009, **11**, 10–18.

2 J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J.-Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak and A. Cardona, *Nat. Methods*, 2012, **9**, 676–682.

3 D. J. Lewis, P. B. Glover, M. C. Solomons and Z. Pikramenou, *J. Am. Chem. Soc.*, 2011, **133**, 1033–1043.

4 Q. Sun, Y. A. Wang, L. S. Li, D. Wang, T. Zhu, J. Xu, C. Yang and Y. Li, *Nat. Photonics*, 2007, **1**, 717–722.

5 W. W. Yu, L. Qu, W. Guo and X. Peng, *Chem. Mater.*, 2003, **15**, 2854–2860.

6 R. Xie, U. Kolb, J. Li, T. Basché and A. Mews, *J. Am. Chem. Soc.*, 2005, **127**, 7480–7488.

7 P. Reiss, M. Protière and L. Li, *Small*, 2009, **5**, 154–168.

8 D. Skoog, D. West, F. Holler and S. Crouch, *Fundamentals of Analytical Chemistry*, Cengage Learning, 2013.

9 R. Larson and B. Edwards, *Calculus*, Cengage Learning, 2013.