Supporting Information for

Gd-Functionalised Au Nanoparticles as Targeted Contrast Agents in MRI: Relaxivity Enhancement by Polyelectrolyte Coating

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1. Materials and Methods

All chemicals i.e. $GdCl_3.6H_2O$, DTPA, $HAuCl_4.3H_2O$, Biotin, avidin-agarose, polyethyleneimine (PEI), t-butyl bromoacetate, ethanol amine, dithiobutyric acid, sephadex (G 100) were purchased from Aldrich except H-lysine-t-butyl ester hydrochloride that was purchased from Novabiochem. All chemicals were used without further purification. Transmission electron microscopy images of gold nanoparticles were acquired on FEI I2 electron microscope. Thermogravimetric analysis of gold nanoparticles was done on STA 625 thermal analyzer. All ^{1}H and ^{13}C spectra were run on JEOL ECX 400 and ECX 270 spectrometers. UV/Vis. spectra were acquired on a Hitachi U-3000 spectrophotometer. ICP characterisation of Gd-DTPA@AuNPs was done at MEDAC ltd Surrey, UK.

T_1 weighted images were acquired on a spin echo based RARE sequence on Bruker Avance II 600 MHZ (14 T) bruker NMR spectrometer with the GREAT 40 gradient system. Imaging parameters were: time of repetition (TR) 400.0 ms, time of echo (TE) 15.0 ms, the thickness of the slice 2.0 mm, MTX = 128 × 128, FOV = 2.5cm × 2.5cm. Spin lattice relaxation (T_1) of Gd-DTPA, Gd-loaded-AuNPs, $GdCl_3$ and PEI-protected-Gd-loaded-AuNPs was measured on 300 MHz (7.0 T) Bruker NMR spectrometer. The inversion recovery method with T_1-delay list ranging from 0.0001 seconds to 4.0 seconds was used to determine the spin lattice relaxation time (T_1). The concentration of [Gd] in Gd-DTPA, Gd-loaded-AuNPs, $GdCl_3$ and PEI-protected-Gd-loaded-AuNPs was kept from 0.00 mM to 1.0 mM.

2. **Synthesis of diethylenetriaminepentaacetic acid (DTPA) based ligand**.

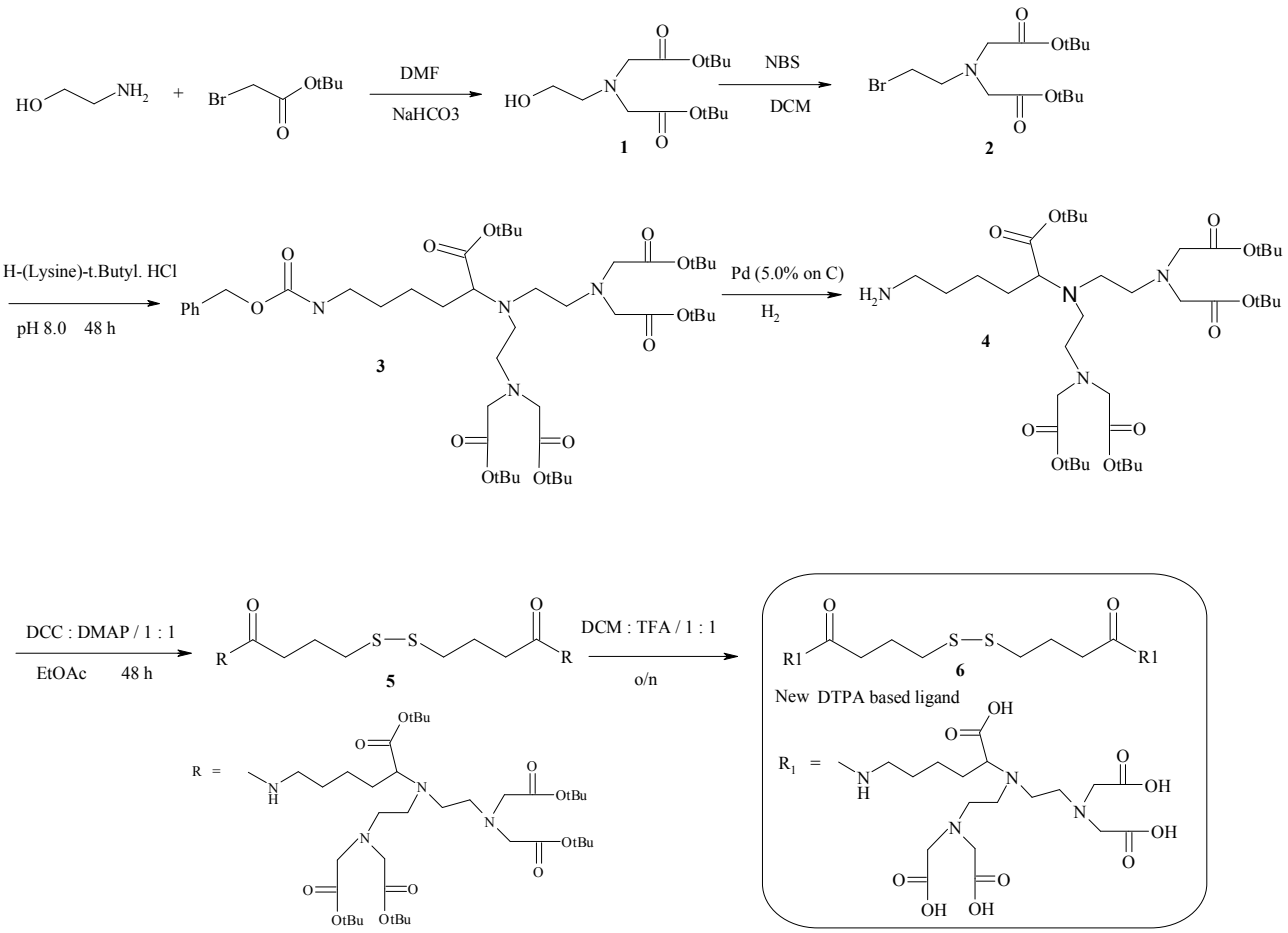
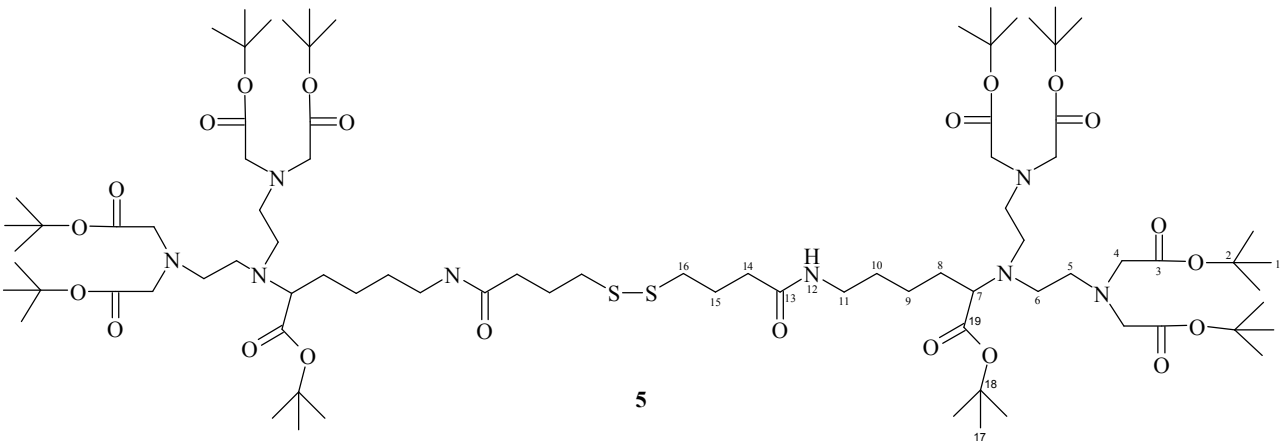


Figure S1: Scheme for synthesis of DTPA based ligand

Compounds **1-4** were synthesized and characterized by following the literature procedure [1, 2] without any changes in the recipe. Compound **5** is a new compound that was synthesized by coupling of compound **4** with dithiobutyric acid. For coupling reactions the standard coupling reagents (DCC (dicyclohexylcarbodimide) and DMAP (dimethylaminopyridine) were used.[3] The detailed procedure for synthesis of compound **5** is shown below.

2(a) Synthesis of compound 5



5

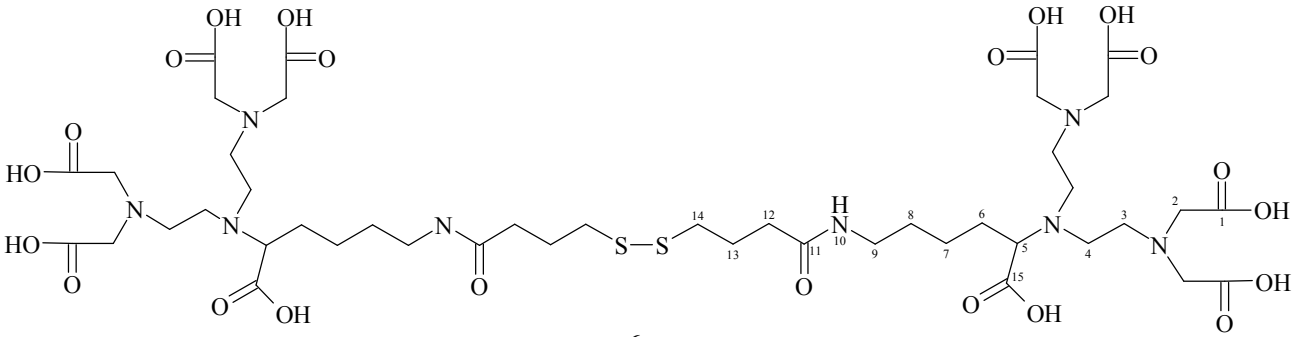
Compound **4** (0.4530 g, 0.6088 mmol) was taken in a 50 mL round bottom flask containing EtOAc (10 mL). Dithiobutyric acid (0.0658 g, 0.2767 mmol) was added and was solution cooled to 0 ^{o}C. Dicyclohexylcarbodiimide (DCC) (0.0570 g, 0.2767 mmol) and dimethylaminopyridine (DMAP) (0.0337 g, 0.2767 mmol) were added as a mixture of solids and solution was stirred for 48 h at rt. After stirring, the solid residues were removed by filtration and filtrate was evaporated to get the highly viscous yellow coloured crude product. The crude compound was purified by column chromatography using silica gel as stationary phase and ethyl acetate : n-hexane (2/1) as mobile phase. R_F 0.65. Yield 53% (0.545 g). Compound **5** was characterized by NMR and HRMS with the help of similar compounds reported in literature.[4]

^{1}H NMR ($CDCl_3$): δ (ppm) 1.40 (s, 90H, H^{1}), 1.64-1.17 (br, 12H, H^{8}**,** H^{9} **&** H^{10}), 1.98 (m, 4H, H^{15}), 2.26 (t, 2H, H^{14}), 2.80-2.60 (m, 20H, H^{5}**,** H^{6} **&** H^{16}), 3.23-3.16 (m, 6H, H^{7} & H^{11}), 3.40 (s, 16H, H^{4}).

^{13}C NMR ($CDCl_3$): δ (ppm) 19.81 (C^{9}), 21.07 (C^{15}), 24.40 (C^{1}), 24.52 (C^{17}), 25.26 (C^{8}), 25.68 (C^{10}), 30.89 (C^{14}), 34.28 (C^{16}), 35.50 (C^{11}), 46.39 (C^{5}), 49.81 (C^{4}), 52.16 (C^{6}), 60.14 (C^{7}), 76.93(C^{18}), 77.06 (C^{2}), 166.89 (C^{3}), 168.24 (C^{19}), 168.95 (C^{13}).

HR ESI-MS: m/z $[M + H]^{2+}$, calcd. 846.5382, observed: 846.5363

2(b) Synthesis of compound 6



6

Compound **6** was obtained by deprotection of t-butyl ester groups of compound **5** by modification in literature procedure.[4a] Compound **5** (117 mg, 0.069 mmol) was taken in 25 mL round bottom flask. Trifluoroacetic acid (TFA) (1.0 mL) was added to the solution of compound **5** in dichloromethane (DCM) (1.5 mL). The reaction mixture was stirred overnight at rt. After overnight stirring, DCM and TFA were evaporated using rotaty evaporator at rt. The crude product (compound **6**) was dissolved in water (10.0 mL), filtered and dialyzed using 500 MWCO (Float-A-Lyzer) membrane against demineralised water (2.0 L) for 48 hr. After dialysis, water was evaporated below 40 ^{o}C to get white product. Yield 77.73% (60.0 mg).

^{1}H NMR (D_2O): δ (ppm) 1.34 (m, 4H, **7**), 1.45 (m, 4H, H^{8}), 1.77-1.56 (br, 4H, H^{6}), 1.92 (t, 4H, H^{13}), 2.28 (t, 4H, H^{12}), 2.66 (t, 4H, H^{12}), 3.22-2.96 (m, 16H, H^{3+4}), 3.53-3.52 (br, 6H, H^{5+9}), 3.94 (s, 16H, H^{2})

^{13}C NMR (D_2O): δ (ppm) 23.42 (C^{7}), 24.81 (C^{13}), 27.82 (C^{6}), 28.19 (C^{8}), 34.35 (C^{12}), 37.14 (C^{14}), 38.92 (C^{9}), 46.29 (C^{4}), 52.89 (C^{3}), 55.92 (C^{2}), 63.26 (C^{5}), 169.65 (C^{1}), 175.09 (C^{15}), 175.70 (C^{11}).

HR ESI-MS: m/z $[M-H]^{1-}$ Calcd. 1129.4286, Observed: 1129.4306

3. Synthesis and Characterization of gold nanoparticles (AuNPs)

The gold nanoparticles (AuNPs) stabilized by DTPA based ligand **6** were prepared by modification of literature procedure.[5] A 1.0 % w/w solution of hydrogen tetrachloroaurate trihydrate

($HAuCl_4 \cdot 3H_2O$) (4.12 mL, 0.1 mmol) was taken in a 25 mL round bottom flask. To this flask, DTPA-based ligand **6** (60 mg, 0.1 mmol) dissolved in 10.0 mL deionised water was added. A 10.0 mL freshly prepared aqueous solution of sodium borohydride ($NaBH_4$) (40 mg, 1.0 mmol) was added immediately after the addition of ligand **6** into flask. The colour of the solution turned dark brown after the addition of sodium borohydride solution, which confirmed the formation of gold nanoparticles. After stirring for 10 min, water was evaporated below 40 ^oC using rotary evaporator. The excess of free ligand was removed by gel separation chromatograpy using Sephadex (G-100) gel column as stationary phase and 0.1M NaCl as mobile phase. The purified AuNPs were characterized by NMR, UV/Vis., TEM and TGA.

Yield: 64% (35.0 mg).

3(a) NMR of Gold Nanoparticles

In ^1H NMR spectrum of gold nanoparticles, two major changes were observed as compared to ^1H NMR spectrum of free ligand. The first change is the complete disappearance of ^1H peak of CH_2S group as reported in literature.[6] Secondly, all peaks became broader. These two evidences confirmed the successful adsorption of ligand on AuNPs surface. The NMR spectrum of AuNPs attached ligand along with free ligand is shown in following figure S1.

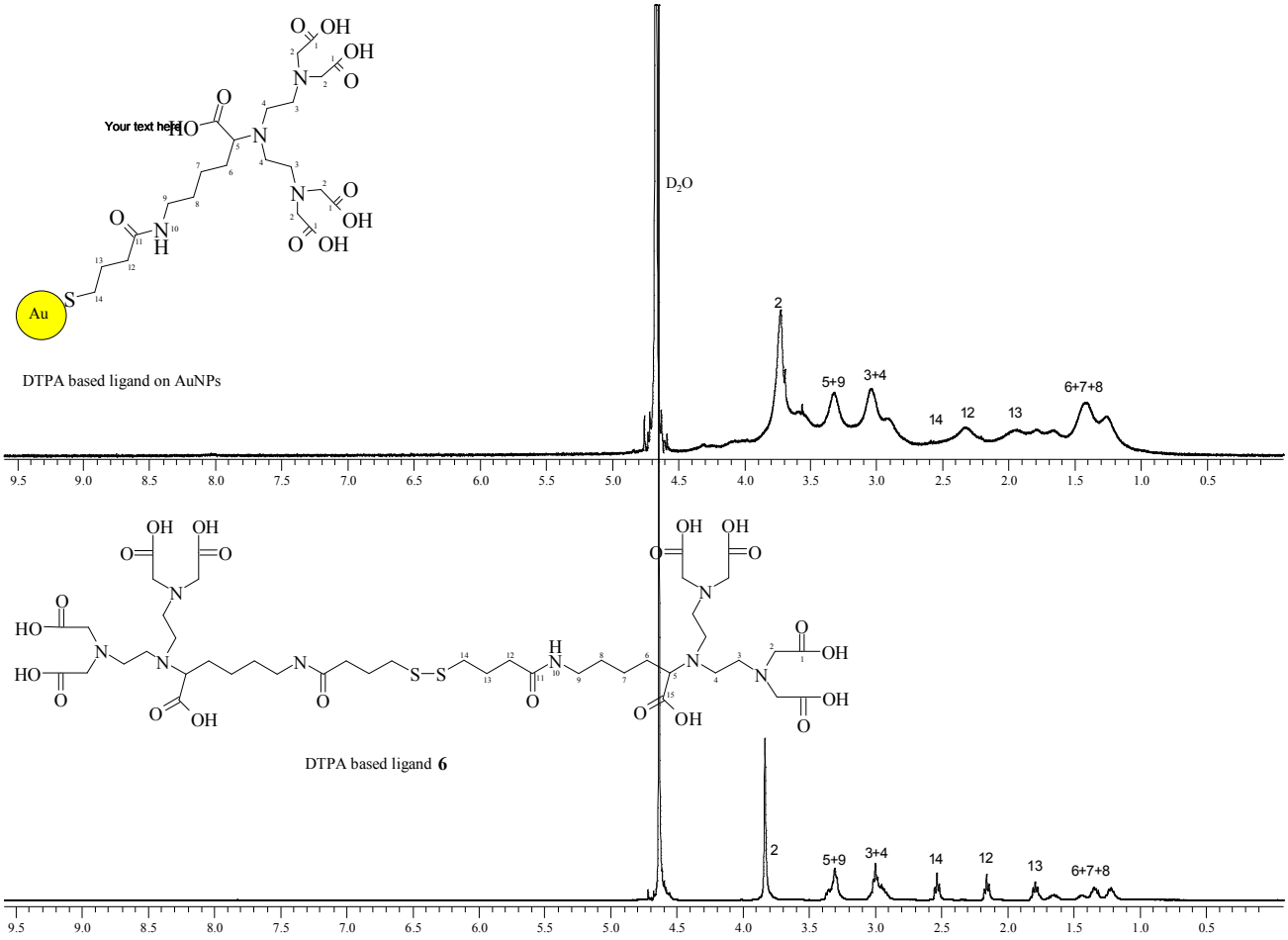


Figure S1: ^{1}H NMR Spectra of free ligand (compound 6) and of adsorbed on gold nanoparticles surface.

3(b) UV-Vis. Spectrum of Gold Nanoparticles

UV-Vis. spectrum of gold nanoparticles (shown in figure S2) showed surface plasmon band (SPB) at 520-525 nm, which is consistent with the formation of small Au nanoparticles.[7]

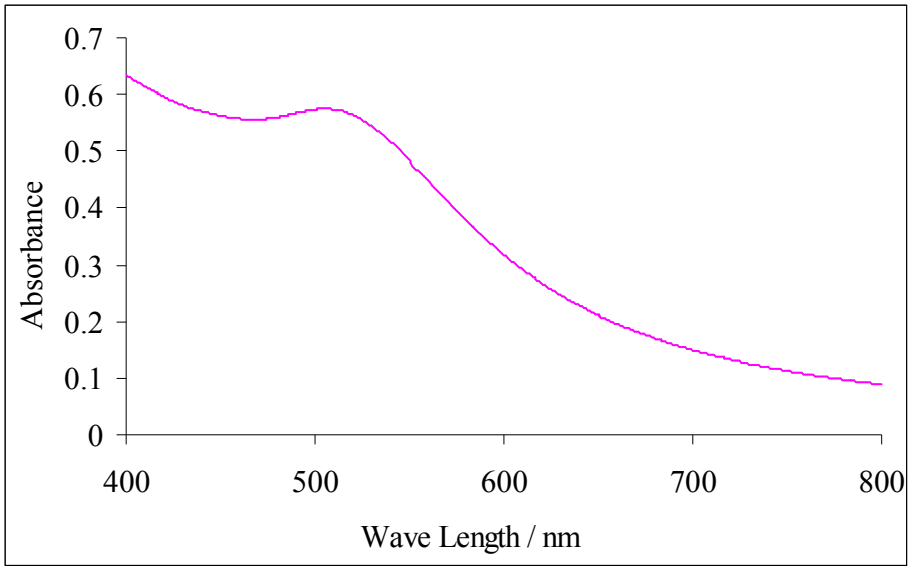


Figure S2: UV/Vis. spectrum of gold nanoparticles

3(c) TEM of Gold Nanoparticles

Average diameter of gold nanoparticles was calculated from TEM picture acquired by electron microscope and is found 1.75 nm ± 0.42. The TEM picture and histogram of diameter of AuNPs are shown in figure S3.

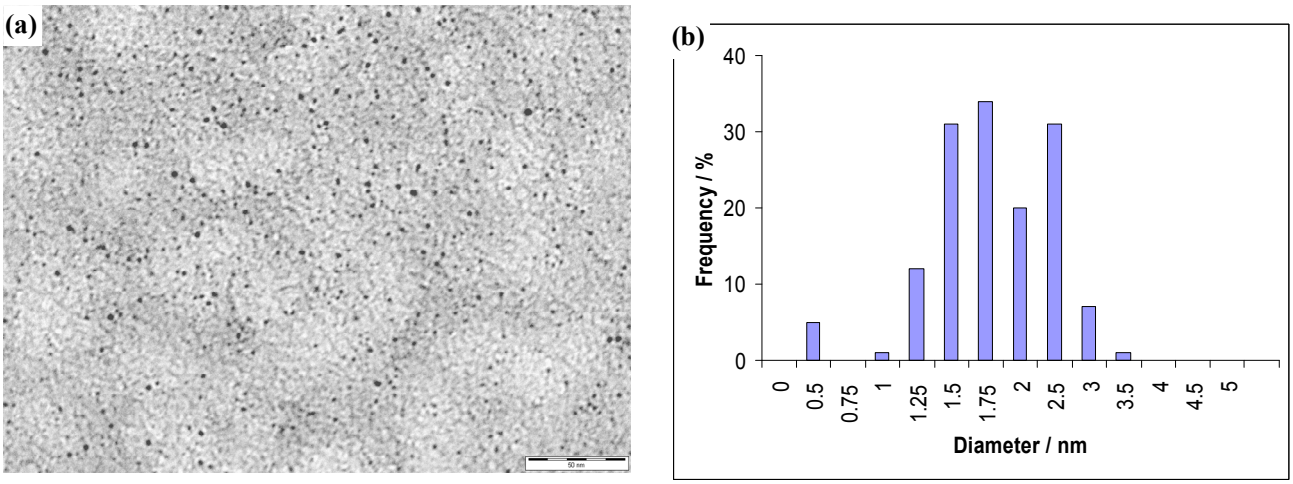


Figure S3: TEM picture (a) and histogram (b) of gold nanoparticles

3(d) Thermogravimetric analysis (TGA) of Gold Nanoparticles

TGA of gold nanoparticles showed about 42 % weight loss (show in figure S4) that is consistent with UV/Vis. data.

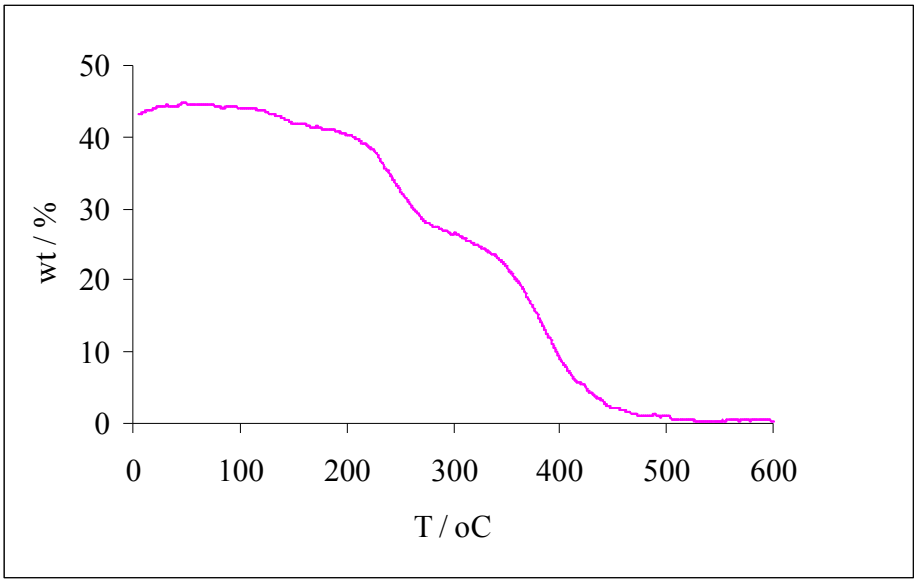


Figure S4: TGA graph of gold nanoparticles

4. Determination of Spin-Lattice Relaxation Time (T1) by Inversion Recovery Method

The longitudinal relaxation constant for the water ^1H signal, T_1 was measured using an inversion recovery method[8]. The T_1 was calculated using following equation S1

$$M_z = M_0(1 - 2\exp(-\tau / T_1)) \qquad \text{Eq. S1}$$

with measured magnetisation M_z, a function of the equilibrium magnetisation M_o and the variable time delay " τ ". In practice T_1 was measured from regression of $\ln(I_z) = \ln(I_\infty) - \tau/T_1$ for the measured integral of the signal (I_τ) after a time delay (τ) with respect to integral after full relaxation (I_∞). Spin-lattice relaxivity (r_1) was calculated from slope of plot $1/T_1$ vs [Gd] using a simple equation S2:

$$\frac{1}{T1} = r_1[Gd] \qquad \text{Eq. S2}$$

The inversion recovery curves for Gd@AuNPs are shown in figure S8.

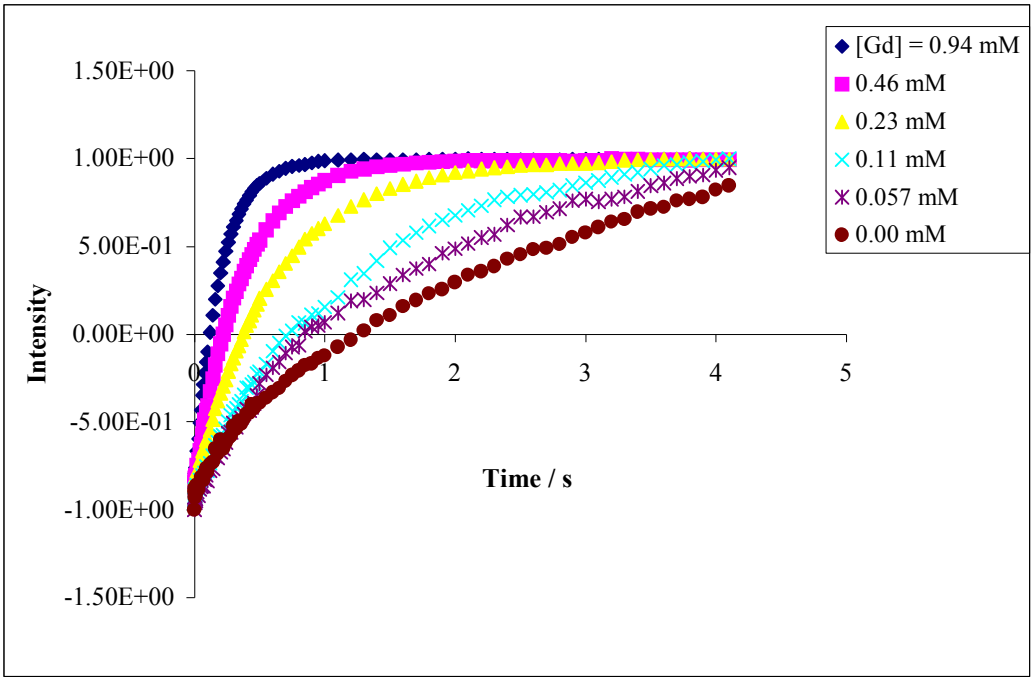
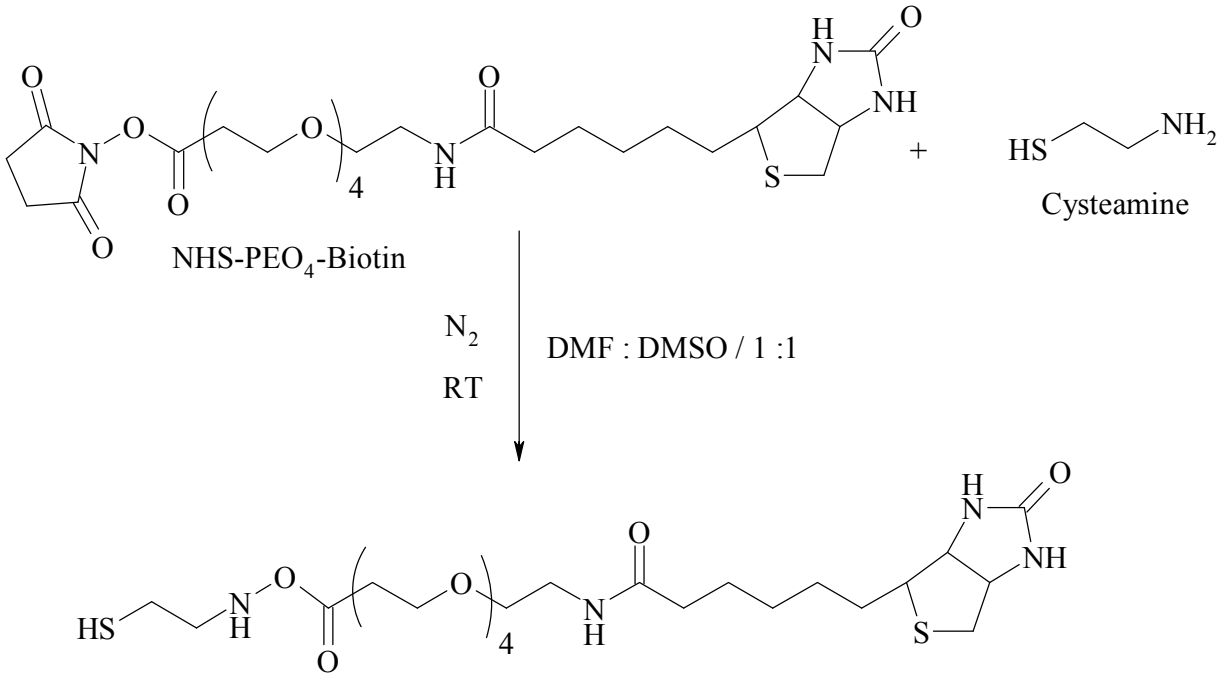


Figure S5: Inversion recovery curves for Gd-loaded-AuNPs

5. Synthesis of biotin terminated thiol (BTT)

Biotin terminated thiol (BTT) was synthesized by following the literature procedure.[9] NHS- (PEO) $_4$-biotin (1.17mg, 1.98×10^{-3} mmole) was taken in 5.0 ml round bottom flask. To this flask, 19.44mM (0.1 mL) solution of cysteamine (0.15 mg, 1.98×10^{-3} mmol) in DMSO was added. The flask was put under vacuum in a Schlenk line, to avoid any contact with air or moisture. Anhydrous DMSO (0.9 mL) and anhydrous DMF (1.0 mL) were then added carefully with pre-dried syringes. The reaction mixture was stirred over night under the blanket of nitrogen at room temperature. The unreacted biotin terminated thiol could be removed by dialysing against deionised water using 500 MWCO dialysing membrane

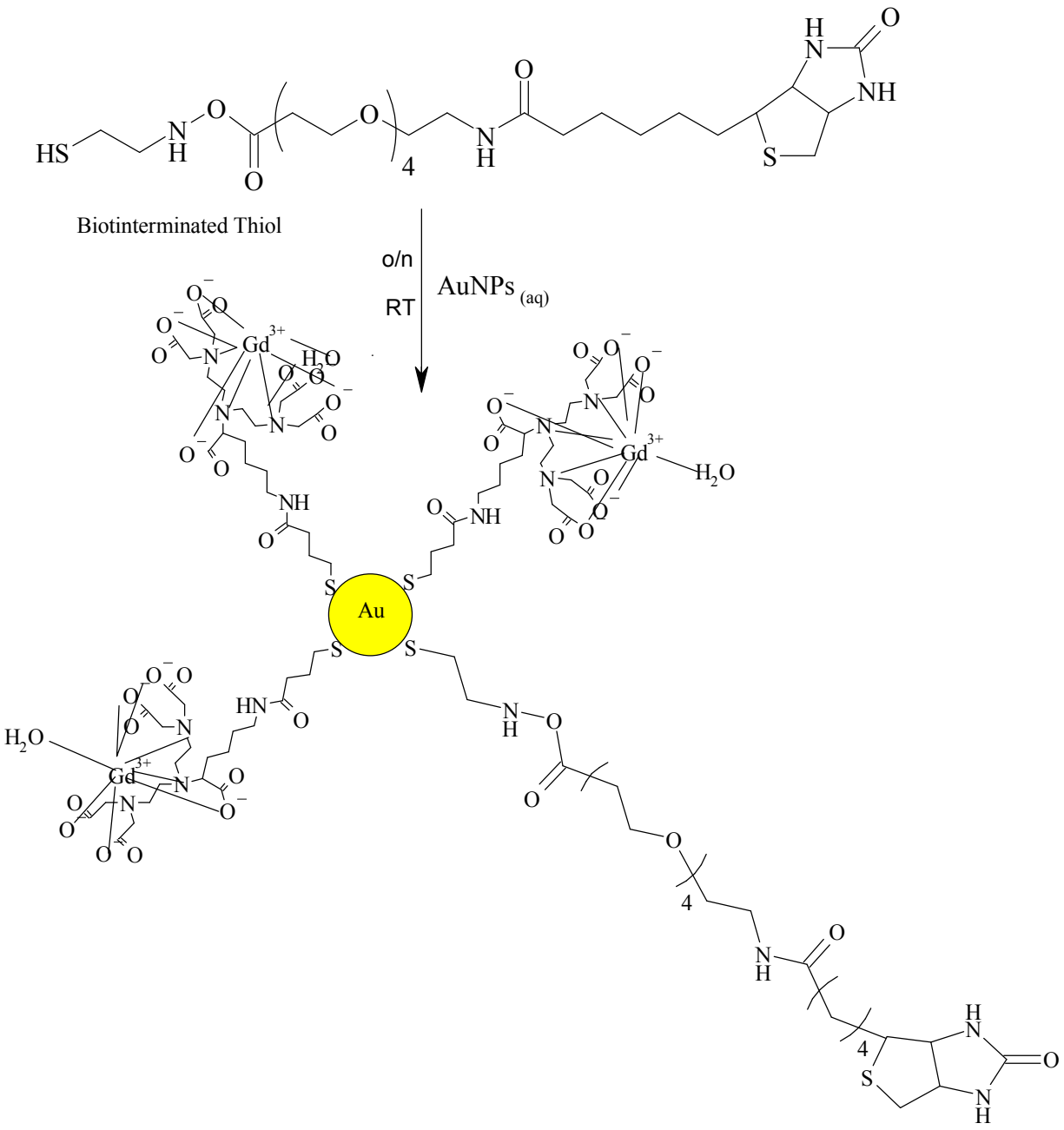
HR ESI-MS: m/z $[M-H]^{1+}$ calcd. 567.2557, observed: 567.2517



Biotinterminated Thiol

6. Attachment of Biotin terminated thiol on AuNPs surface

Biotin terminated thiol (BTT) was attached on gold nanoparticles (AuNPs) surface by modifying the literature procedure.[10] AuNPs (1.0 mg, 1.6875×10^{-5} mmol) were taken in 5.0 mL round bottom flask. To this flask, 1.5759 mM solution of BTT (20.0 mg, 3.15×10^{-5} mmol) in DMSO/DMF mixture were added. The reaction mixture was stirred overnight at rt. The unreacted BTT was removed by dialysis against deionised water for over night using 12-14kDa MWCO membrane.


Biotinterminated Thiol
o/n
RT
AuNPs (aq)
Au

7. Formation of Poly(ethylene imine) (PEI) and Poly (amino amide) (PAMAM) layers around Gd@AuNPs

Poly(ethylene imine) with the average molecular weight 423, 1800 and 60000 Da (PEI **1**, PEI **2** and PEI **3,** respectively) and two poly(amino amide) PAMAM dendrimers (**G0** and **G 4**) were used to coat the surface of negatively charged Gd-DTPA@AuNPs. Gd-DTPA@AuNPs (5.5 mg, 3.36×10^{-3} mmol) were dissolved in deionised water (5.0 mL). To this solution, appropriate amount of diamine (0.013 mmol, 4:1 amine:Gd ratio) was added. The solution was stirred for 15 minutes at room temperature. Polyelectrolyte protected Gd-DTPA@AuNPs were characterised by UV/Vis, TEM and relaxivity measurements. A sample of Gd-DTPA@AuNPs nanoparticles coated with PEI **2** was dialysed using a dialysis membrane with 12 kDa molecular weight cut-off over a period of 24 h. No reduction in relaxivity was observed which suggests that poly(ethylene imine) does not leach from the nanocomposite.

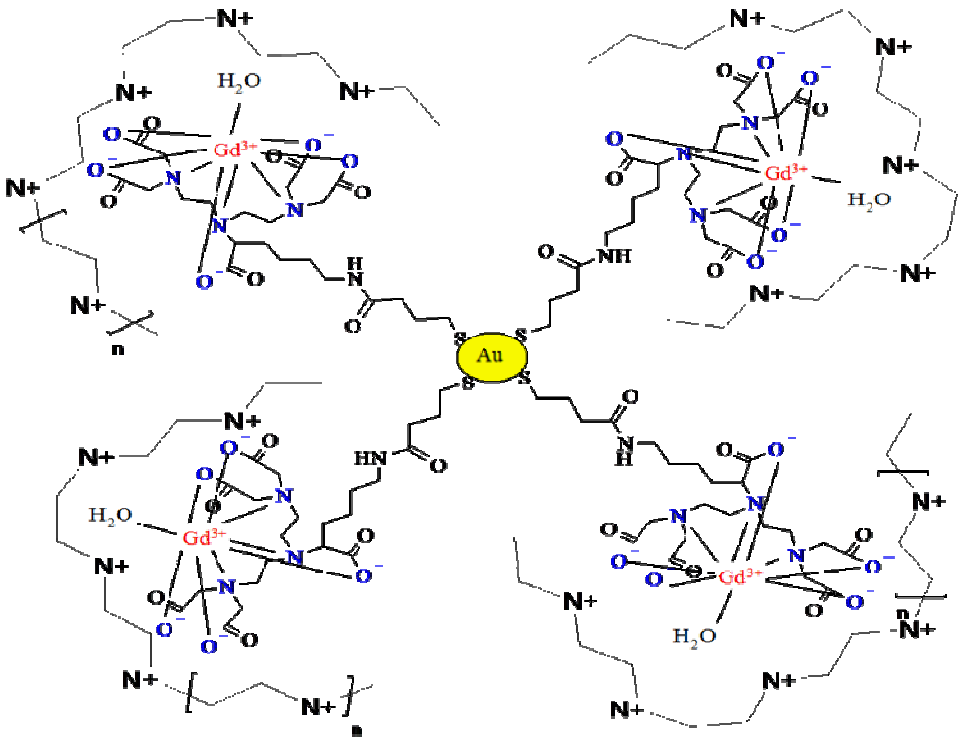


Figure S6: PEI-protected Gd@AuNPs

7a. UV/Vis. of PEI-protected Gd@AuNPs

The UV/Vis. spectrum of PEI-protected Gd@AuNPs were nearly identical to that of Gd@AuNPs (Figure S7).

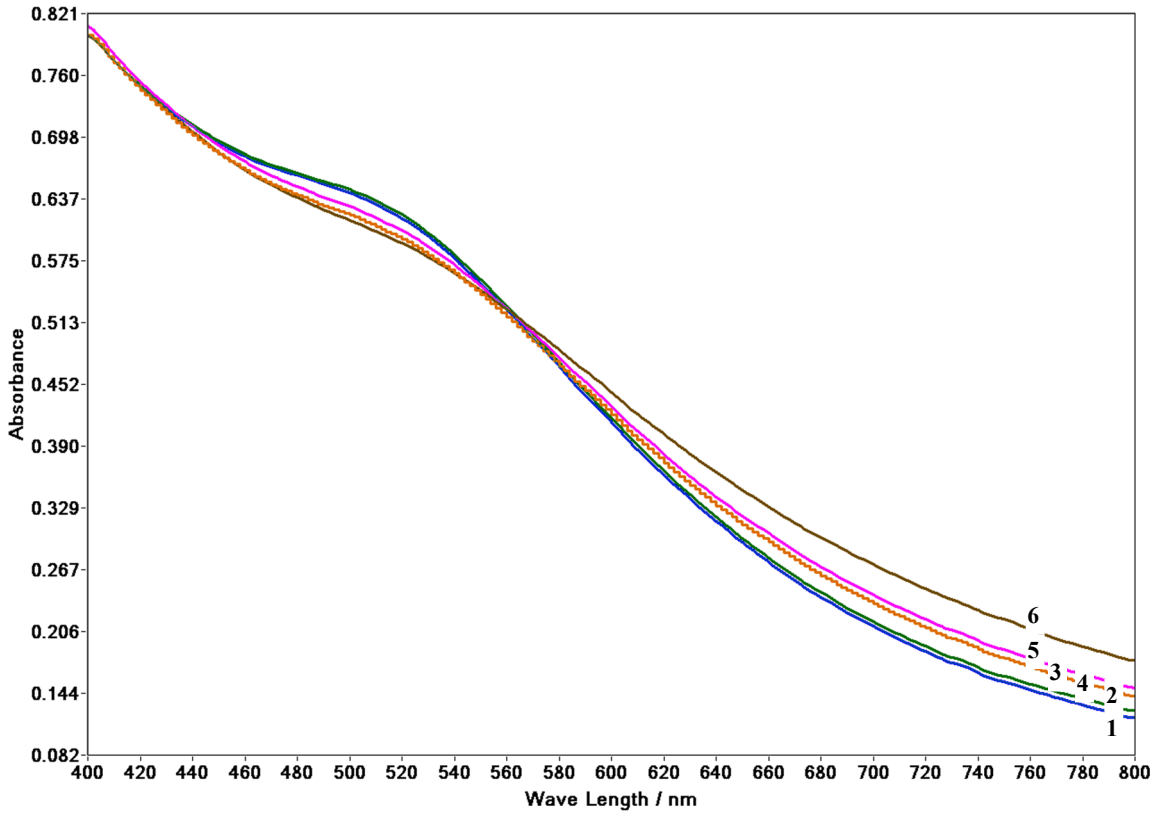


Figure S7: UV/Vis. spectra of Gd@AuNPs (1), PEI1-protected (2), PEI2-protected (3), PEI3-protected (4), PAMAM G0 (5) & PAMAM G4 (6) @ Gd@AuNPs .

7b-I. TEM of Gd-DTPA@AuNPs

The TEM image of gold nanoparticles after Gd loading is shown in Figure S11. TEM image showed no aggregation. Average diameter was (1.77 ± 0.50) nm.

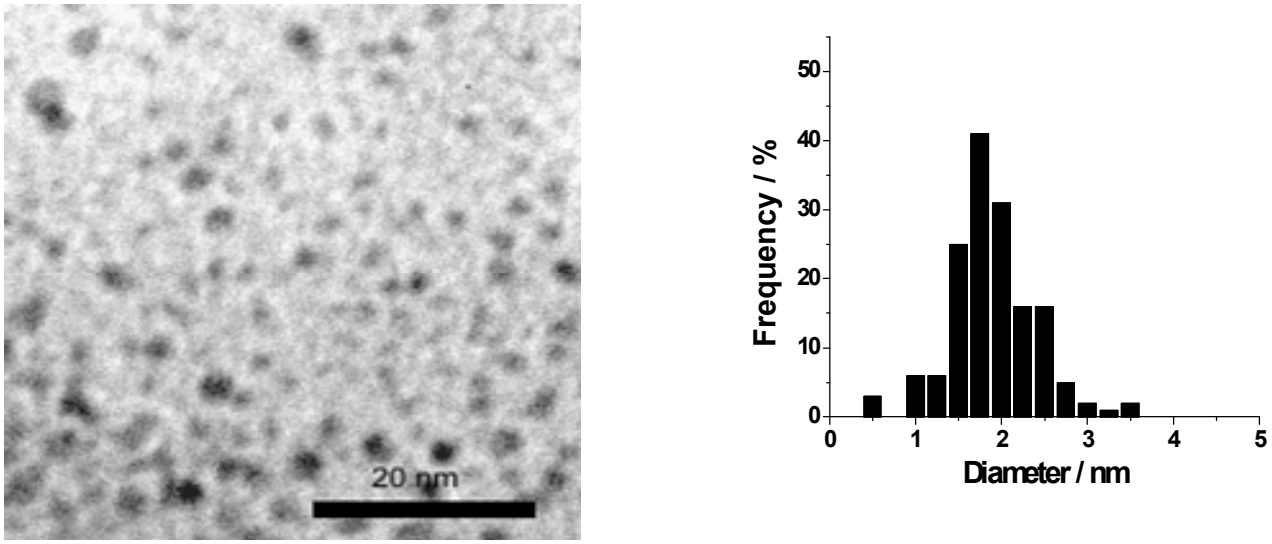


Figure S8: TEM image (a) & Histogram (b) of Gd-loaded AuNPs

7b-II. TEM of PEI 1 Gd AuNPs; average diameter = (1.77 ± 0.43) nm

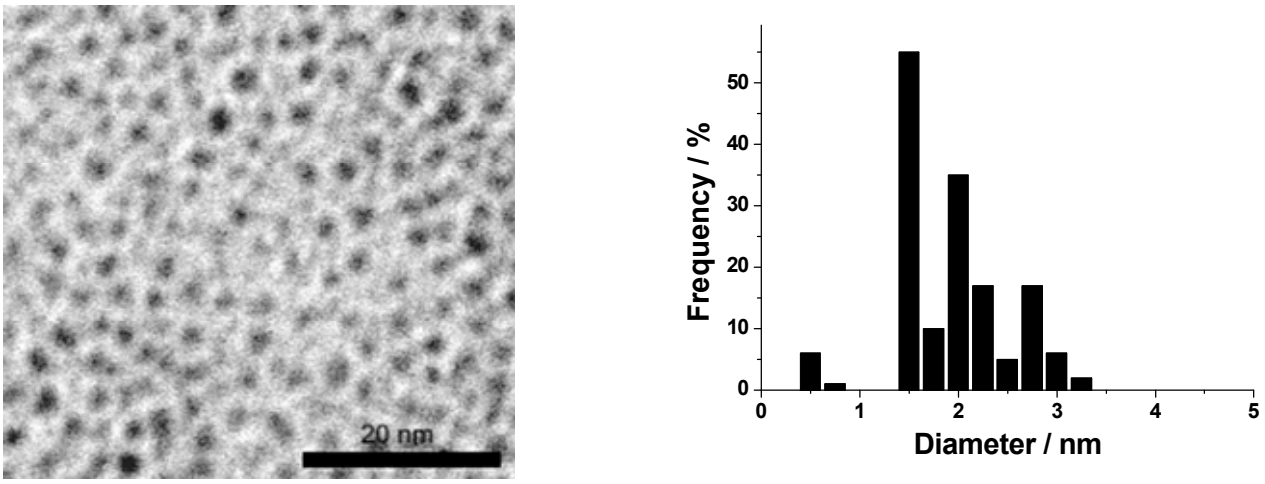


Figure S9: TEM image (a) & Histogram (b) of Gd-loaded AuNPs

7b-III. TEM of PEI 2 Gd AuNPs; average diameter = (1.76 ± 0.40) nm

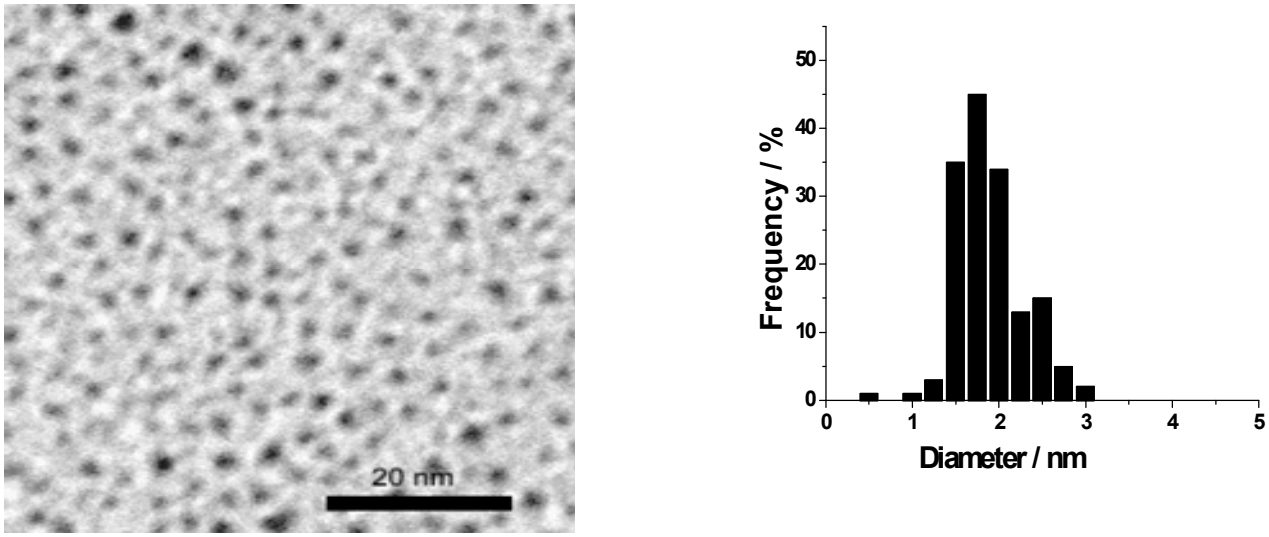


Figure S10: TEM image (a) & Histogram (b) of Gd-loaded AuNPs

7b-IV. TEM of PEI 3 Gd AuNPs; average diameter = (1.76 ± 0.35) nm

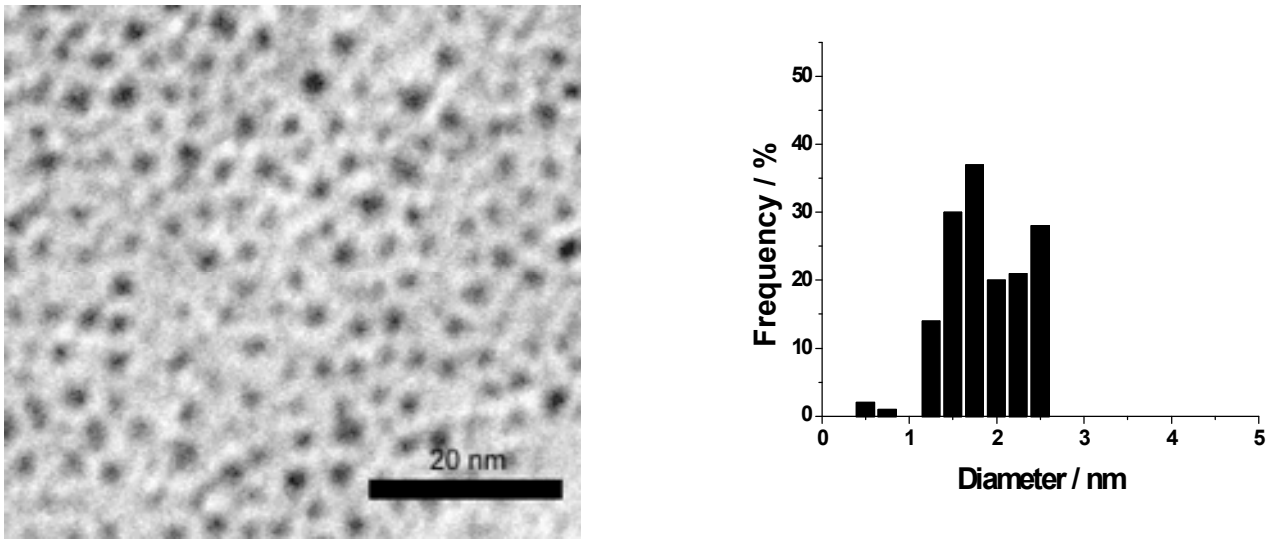


Figure S11: TEM image (a) & Histogram (b) of Gd-loaded AuNPs

7b-V. TEM of PAMAM (G = 0) Gd AuNPs; average diameter = (1.74 ± 0.41) nm

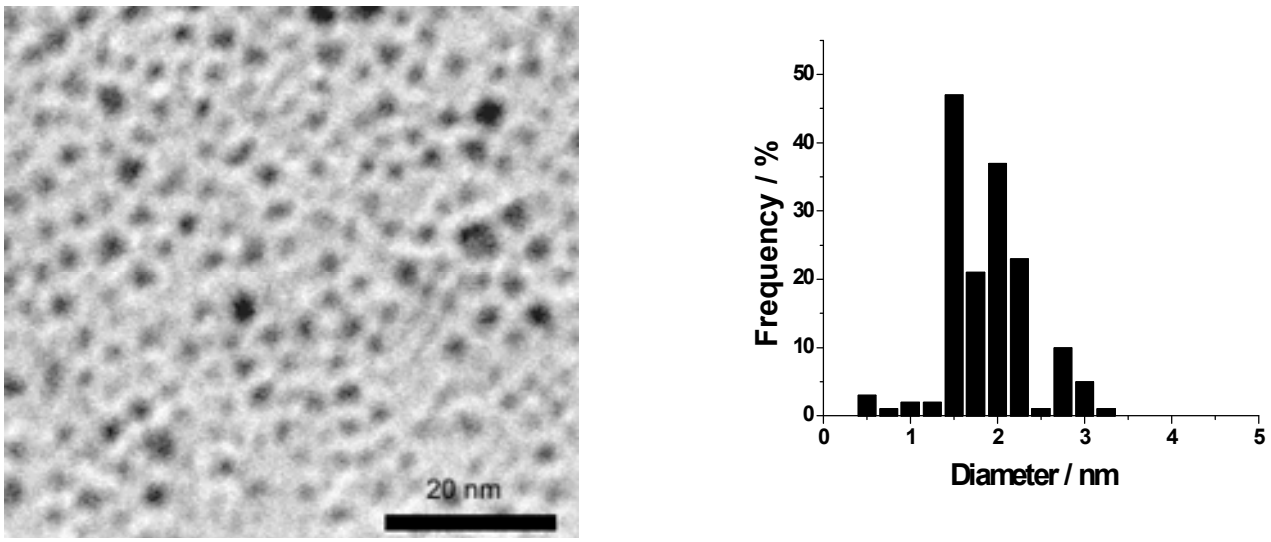


Figure S12: TEM image (a) & Histogram (b) of Gd-loaded AuNPs

7b-VI. TEM of PAMAM (G = 4) Gd AuNPs; average diameter = (1.76 ± 0.51) nm.

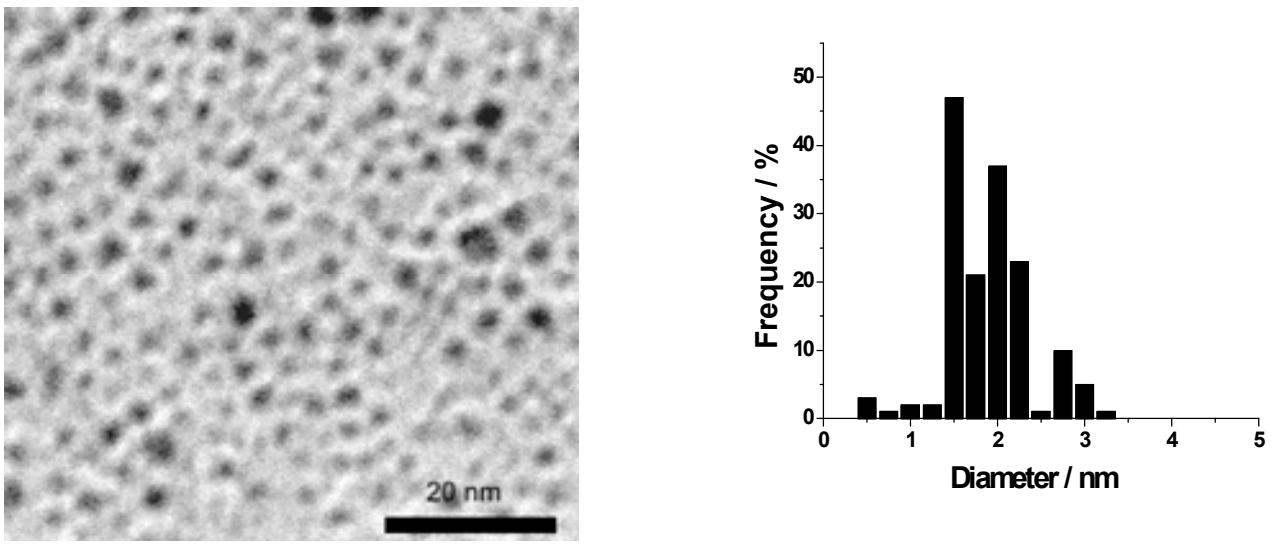


Figure S13: TEM image (a) & Histogram (b) of Gd-loaded AuNPs

8. Relaxivity (r_1) of Gd-DTPA, Gd@AuNPs, PEI-protected and PAMAM-protected Gd@AuNPs.

The r_1 values of PEI-protected Gd@AuNPs & PAMAM-protected Gd@AuNPs are shown in table S1.

Table S 1. Comparison of r1 of Gd-DTPA with Gd@AuNPs, PEI-protected-Gd@AuNPs & PAMAM-protected-Gd@AuNPs.

S. No.	Compounds	r_1 / $mM^{-1}s^{-1}$	
		A*	B*
1	Gd-DTPA	3.83	3.83
2	Gd-AuNPs	4.77	4.77
3	PEI-**1**-Gd-AuNPs	6.50	7.45
4	PEI-**2**-Gd-AuNPs	6.74	7.67
5	PEI-**3**-Gd-AuNPs	7.00	8.27
6	PAMAM (**G=0**)-Gd-AuNPs	4.74	5.36
7	PAMAM (**G=4**)-Gd-AuNPs	7.10	8.24
9	$GdCl_3$	12.10	12.10

A*: Amine:Gd molar ratio is 4:1.

B*: Amine:Gd molar ratio is 25:1 (PEI **1**); 500:1 (PEI **2**); 3000:1 (PEI **3**); 5:1 (PAMAM **G0**); 2000:1 (PAMAM **G4**)

9. Characterisation of Gd-loaded AuNPs by ICP

Gd-loaded AuNPs were characterised by ICP. The elemental composition was compared to htat determined by TGA and UV/Vis. titrations using xylenol orange as indicator. The ICP, TGA & UV/Vis. data is summarised in Table S2.

Table S2: ICP and TGA & UV/Vis. data for Gd-DTPA@AuNPs

Technique	Element	Found, %
ICP	Au	45.64
	Gd	11.65
TGA & UV/Vis.	Au	48.13
	Gd	12.25

10. References:

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