Supporting information for:

High Relaxivity Gd(III)-DNA Gold Nanostars: Investigation of Shape Effects on Proton Relaxation

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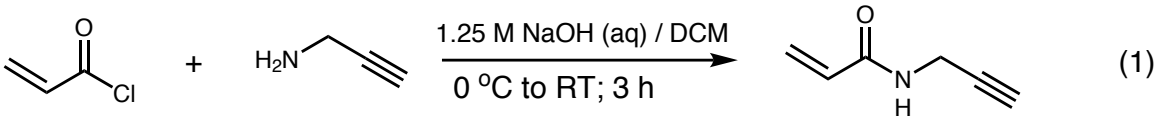
Synthesis of Inorganic Complexes:

Chemical Synthesis of complex 5:

2-bromo-N-(prop-2-yn-1-yl)acetamide **(1)**



(1)

Scheme S1: Synthetic scheme of monopropionate arm

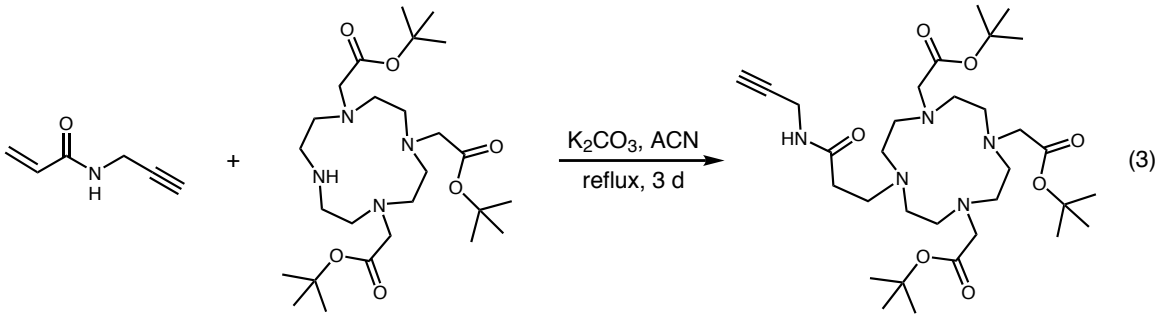
Synthesis of the primary alkyne arm proceeds via modification of a literature procedure.[1, 2]

A 500 mL round bottom flask equipped with a magnetic stir bar is charged with propargylamine (1.25 g, 22.7 mmol) in 125 mL dicholromethane and cooled to 0° C. To the stirring mixture is added 50 mL of 1.25 M aqueous NaOH, resulting in a biphasic solution which is further stirred 5 min for equilibration to 0° C. Acryloyl Chloride (6.16g, 68.1 mmol) is added dropwise by syringe to the denser dichloromethane layer over 5 minutes resulting in a yellow orange solution containing a visible precipitate. The stirring solution is allowed to warm to room temperature over 2.5 hours at which time it is poured into a separatory funnel containing 50mL each of water and dichloromethane. The aqueous layer is extracted twice, and the dichloromethane is combined and further extracted three times with sodium bicarbonate (taking care to add the dichloromethane fraction to sodium bicarbonate due to the vigorous evolution of carbon dioxide). The resultant organic fraction is dried over sodium sulfate and concentrated by rotary evaporation yielding a brown oil. Purification is achieved by way of silica gel flash chromatography in 1:1 ethyl acetate: hexanes (R_f = 0.5 by UV irradiation), resulting in a tan solid (1.78g, 72%). The final compound is characterized by NMR.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.31 (dd, *J* = 17.0, 1.4 Hz, 1H), 6.13 (dd, *J* = 17.0, 10.3 Hz, 1H), 5.68 (dd, *J* = 10.3, 1.3 Hz, 1H), 4.12 (dd, *J* = 5.3, 2.6 Hz, 2H), 2.24 (t, *J* = 2.6 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 165.57, 130.39, 127.67, 79.60, 72.04, 29.58.

***tri-tert-butyl 2,2',2''-(10-(3-oxo-3-(prop-2-yn-1-ylamino)propyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate* (3)**

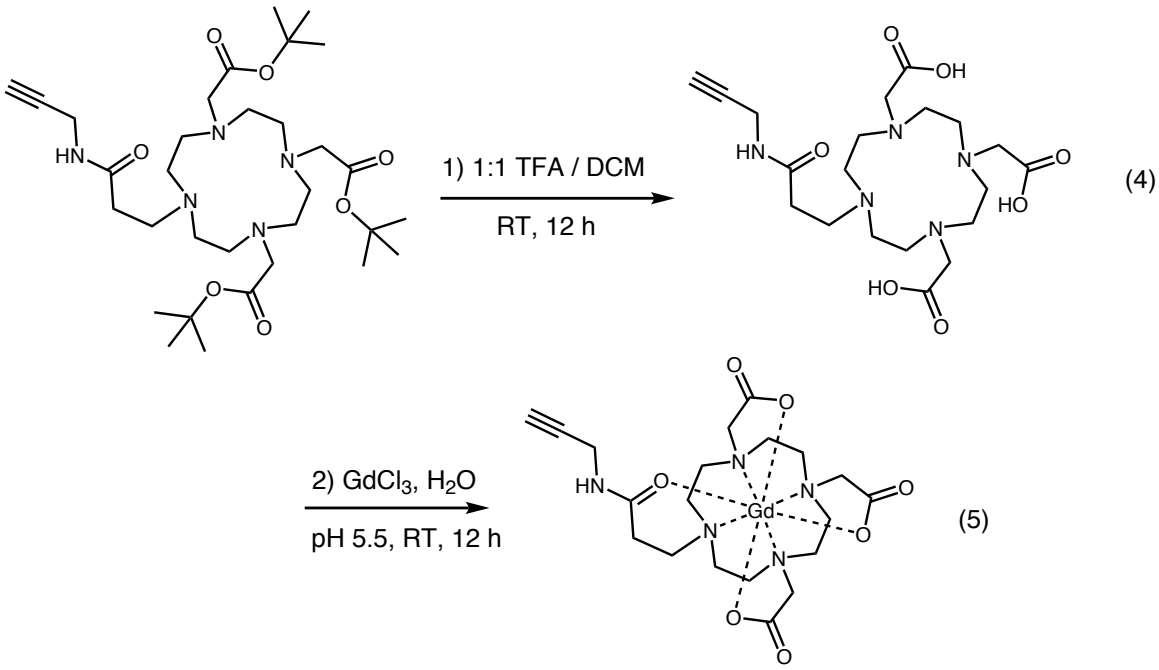


Scheme S2: Synthetic scheme of 1,4 conjugate addition of monopropionate arm to DO3A macrocycle

To a 500 mL two necked round bottom flask containing a magnetic stir bar is added previously prepared[3] DO3A-HBr (1.95g, 3.26 mmol) and K_2CO_3 (2.25g, 16.3 mmol) under N_2. To these solids is added 230 mL of dry acetonitrile, resulting in a clear solution containing a white suspension. The solution is stirred five minutes at room temperature at which time 2-bromo-N-(prop-2-yn-1-yl)acetamide (1.6 g, 14.9 mmol) is added . The mixture is brought to reflux with stirring for 3 days. After reaction, the mixture is filtered over Celite and dried by rotary evaporation resulting in a brown oil, which is taken on to the next step without further purification. The modified ligand is characterized by ESI-MS.

(*m/*z) observed: 624.9, calculated: 624.4 $[M + H]^+$.

***1-(N-(prop-2-yn-1-yl)2-oxopropyl)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecyl-gadolinium(III) complex* (5)**



Scheme S2: Synthetic scheme of *tert*-butyl deprotection and Gd(III) metalation

To a 250 mL round bottom flask containing a magnetic stir bar and crude tri-tert-butyl 2,2',2''-(10-(3-oxo-3-(prop-2-yn-1-ylamino)propyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate is added 25 mL of 1:1 dichloromethane : trifluoroacetic acid. The mixture is stirred at room temperature for 12 hours to complete deprotection of the acetate arms. Complete formation of **(4)** is assessed by the disappearance of **(3)** by ESI-MS (624.9 $[M + H]^+$) and appearance of an *m/z* of 454.2 $[M - H]^-$.

For the purposes of characterization, a portion of (**4**) was removed, evaporated and purified by HPLC. The crude mixture is brought up in water and purified by semipreparative HPLC on a reverse phase column, eluting via the use of the following method, where the mobile phase consists of Millipore water and HPLC grade acetonitrile (ACN): initial conditions of 0% ACN were held constant for 5 min, ramp to 50% ACN between 5–30 min, wash at 100 % ACN for 5 min followed by return to 0% ACN. The product peak elutes between 13.7 and 14.5 minutes, as monitored by UV absorption at 210 nm. The product is collected, and lyophilized to a fluffy white solid.

^{1}H NMR (500 MHz, Deuterium Oxide) δ 4.03 (s, 2H), 3.98 (d, J = 2.5 Hz, 2H), 3.68 – 3.09 (m, 28H), 2.82 (t, J = 6.3 Hz, 1H), 2.62 (t, J = 2.5 Hz, 1H).

^{13}C NMR (126 MHz, D_2O) δ 173.83, 171.44, 169.70, 79.46, 71.85, 55.27, 53.05, 51.29, 50.16, 49.81, 48.53, 48.27, 29.11, 28.78.

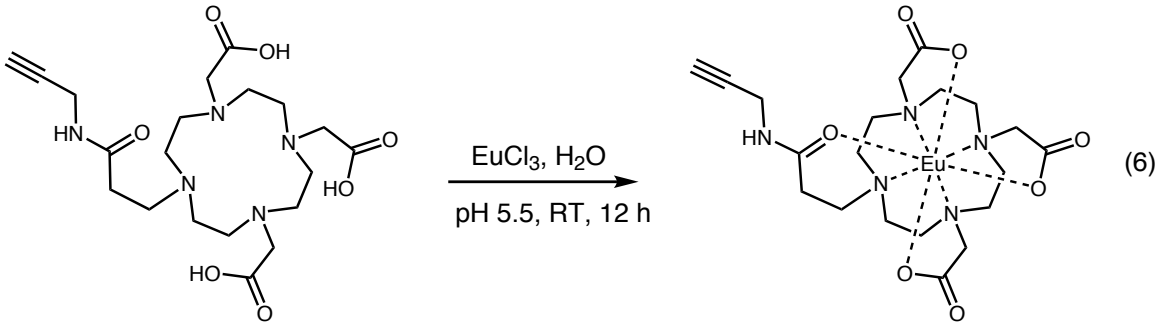
(*m/*z) observed: 456.2455, calculated: 456.24 $[M + H]^+$.

The remaining crude mixture of (**4**) is concentrated by blowing with N_2 , and is subsequently brought up in methanol and dried by rotary evaporation for full removal of trifluoroacetic acid (3 x 25 mL), yielding a pale orange oil. To the unpurified, deprotected chelate is added Millipore water (25 mL) and gadolinium(III) chloride hexahydrate (1.46 g, 3.9 mmol). With addition of $GdCl_3$ is added 1M NaOH dropwise to maintain a pH of 5.5. The mixture is then stirred at room temperature for 24 hours. The crude mixture is purified from water by semipreparative HPLC: initial conditions of 0% ACN were held constant for 5 min, ramp to 100% ACN over 20 min, wash at 100 % ACN for 5 min followed by return to 0% ACN. The desired product elutes from 17-18 mins as monitored by UV-Vis at 201/210 nm and is collected and freeze dried resulting in a fluffy white solid (0.652 g, 1.09 mmol, 65 % over three steps). The final complex is characterized by ESI-MS, and elemental analysis.

ESI-MS (*m/*z) observed: 611.1454, calculated: 611.14 $[M + H]^+$. *Anal.* Calcd. for $C_{20}H_{30}GdN_5O_7 \bullet 3H_2O$: C, 36.19; H, 5.47; N, 10.55. Found: C, 36.24; H, 5.43; N, 10.45.

Chemical Synthesis of complex 6:

***1-(N-(prop-2-yn-1-yl)2-oxopropyl)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecyl-europium(III) complex* (6)**



Scheme S4: Synthetic scheme of Eu(III) metalation

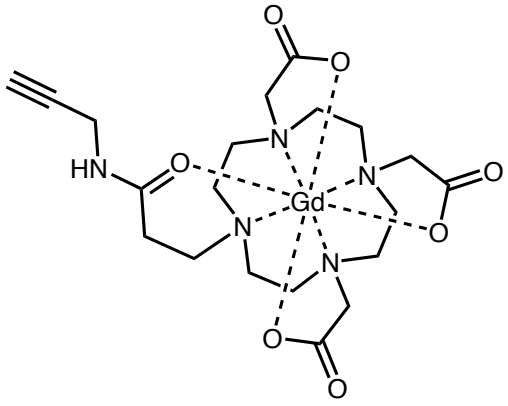
Complex **6** was synthesized by the same procedure as above, until after ligand intermediate (**4**).

To a 100 mL round bottom flask containing a magnetic stir bar and crude **4** (0.04 g, 0.09 mmol) is added Millipore water (25 mL) and europium (III) chloride hexahydrate(0.065 g, 0.18 mmol). With addition of $EuCl_3$ is added 1 M NaOH dropwise to maintain a pH of 5.5. The mixture is then stirred at room temperature for 24 hours. The crude mixture is purified from water by semipreparative HPLC on a reverse phase column, eluting via the use of the following method, where the mobile phase consists of Millipore water and HPLC grade acetonitrile (ACN): initial conditions of 0% ACN were held constant for 5 min, ramp to 100% ACN over 20 min, wash at 100 % ACN for 5 min followed by return to 0% ACN. The desired product elutes from 17-18 mins as monitored by UV-Vis at 201/210 nm and is collected and freeze dried resulting in a fluffy white solid (0.041 g, 0.067 mmol, 74 % yield). The final complex is characterized by ESI-MS.

(*m/*z) observed: 606.2, calculated: 606.14 $[M + H]^+$.

Chemical Synthesis of complex 7:

1-(N-(prop-2-yn-1-yl)2-oxoethyl)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecyl-gadolinium(III) complex **(7)**



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Scheme S5: Structure of previously reported Gd(III) complex used in first generation DNA-Gd^{III}@AuNP

Complex 7 was synthesized by published protocols for use in control water exchange rate studies.[4]

Determination of Water Exchange Rate (τ_m):

Calculation of the inner-sphere water exchange rate of complex **5** was performed by running variable temperature ^{17}O NMR. A coaxial tube insert containing D_2O was placed into a standard NMR tube containing a known concentration of **5** in 10% $H_2{}^{17}O$ in water. The full width half max of the oxygen peak was measured and recorded as a function of temperature on a 400 MHz variable nucleus Varian NMR spectrometer. Data were fit and τ_m calculated as described by Toth and coworkers.[5]

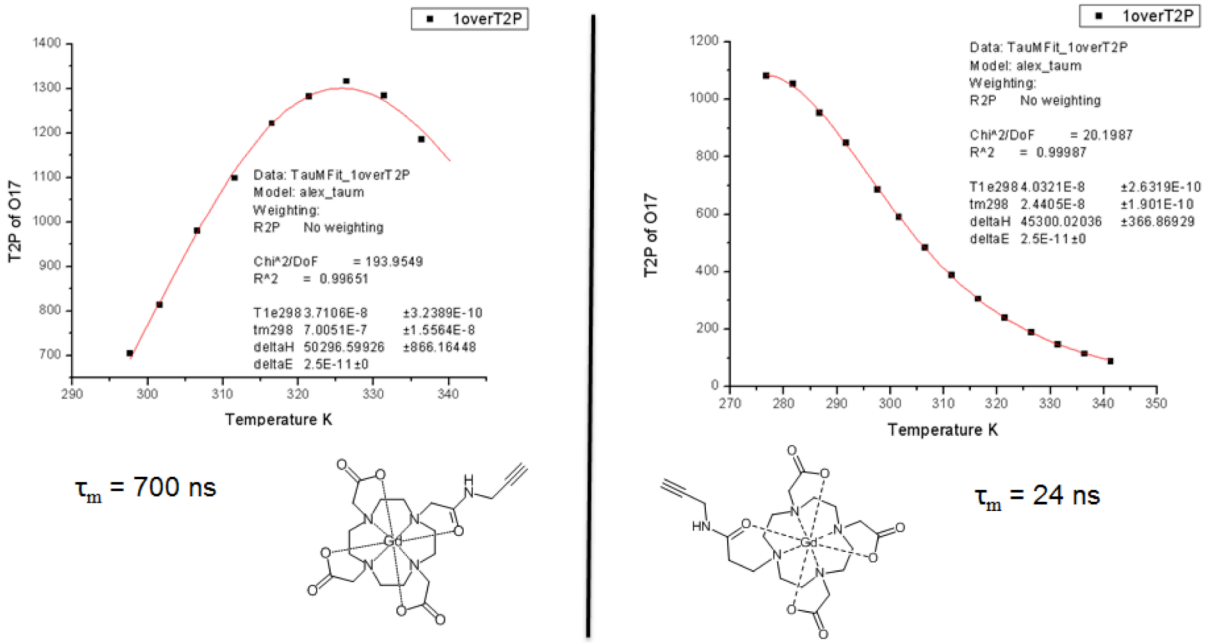


Figure S1: Fitted T_2P of ^{17}O vs. temperature in solution of complex **5** in 10% $H_2{}^{17}O$ at 400 MHz

Determination of Inner-Sphere Waters (q):

The number of inner-sphere water molecules coordinated to complex **5** was calculated by the modified Horrock's method.[6] Briefly, the Eu(III) equivalent of complex **5** was synthesized (complex **6** purified and characterized as above) and dissolved to produce equimolar solutions in H_2O and D_2O. The luminescence lifetimes of the solutions were observed for λ_{ex} = 395nm and λ_{em} = 614 nm, analyzed, and compared to indicate that there is 1.1 ± 0.1 inner-sphere waters.

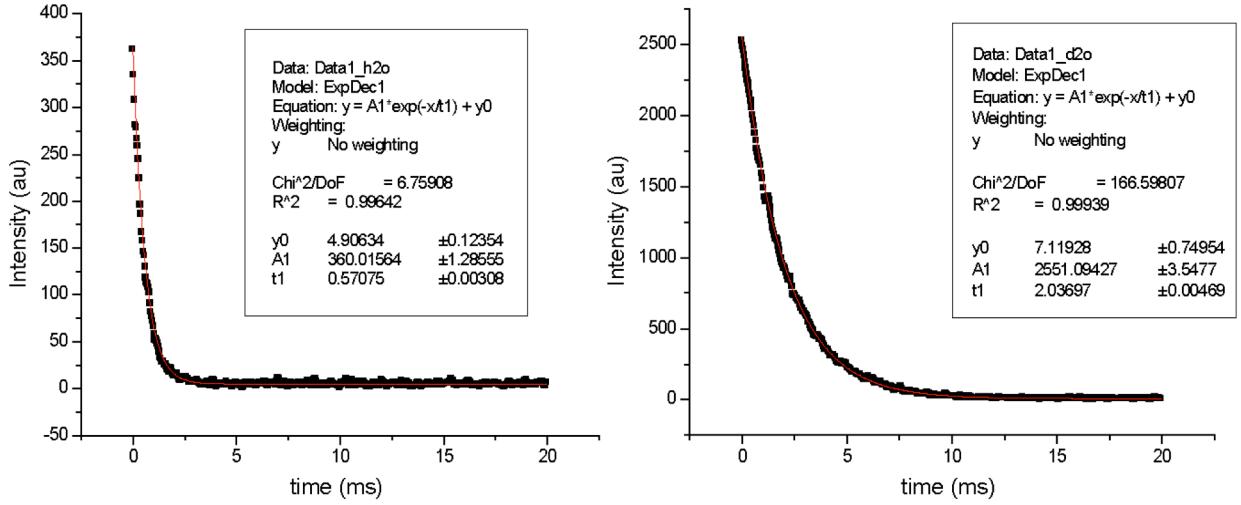


Figure S2: Fitted luminescence lifetimes of **6** in H_2O and D_2O

Nanoparticle Characterization:

Particle size was determined using both transmission electron microscopy (TEM) (Figure S3) and dynamic light scattering (DLS) (Table S1). To approximate the amount of Au atoms per nanostar, the particle volume was estimated from TEM images of over 180 particles. The "fit ellipse" parameter in the Analyze Particles plug-in of ImageJ was used to assign an ellipse of *equal area* to each nanostar's 2D projection. TEM cannot provide measurements of the particle height, the third (out-of-plane) dimension of the nanostar was assumed to be the average of the long and short axis of the fit ellipse. Finally the nanostar volume was calculated as an ellipsoid using the long, short, and out-of-plane axes. For TEM analysis of spherical gold nanoparticles, area was measured using the Analyze Particles plug-in. The volume was then calculated assuming the particles to be perfect spheres. The amount of gold atoms in each type of particle was then calculated using the atomic density of bulk gold (59.1 atoms/nm^3).

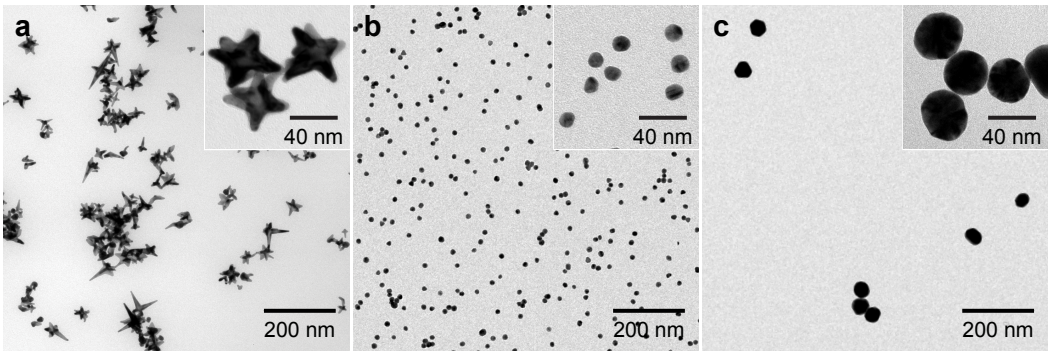


Figure S3: Representative TEM images of gold nanostars (a), 15 nm gold spheres (b), and 40 nm gold spheres (c).

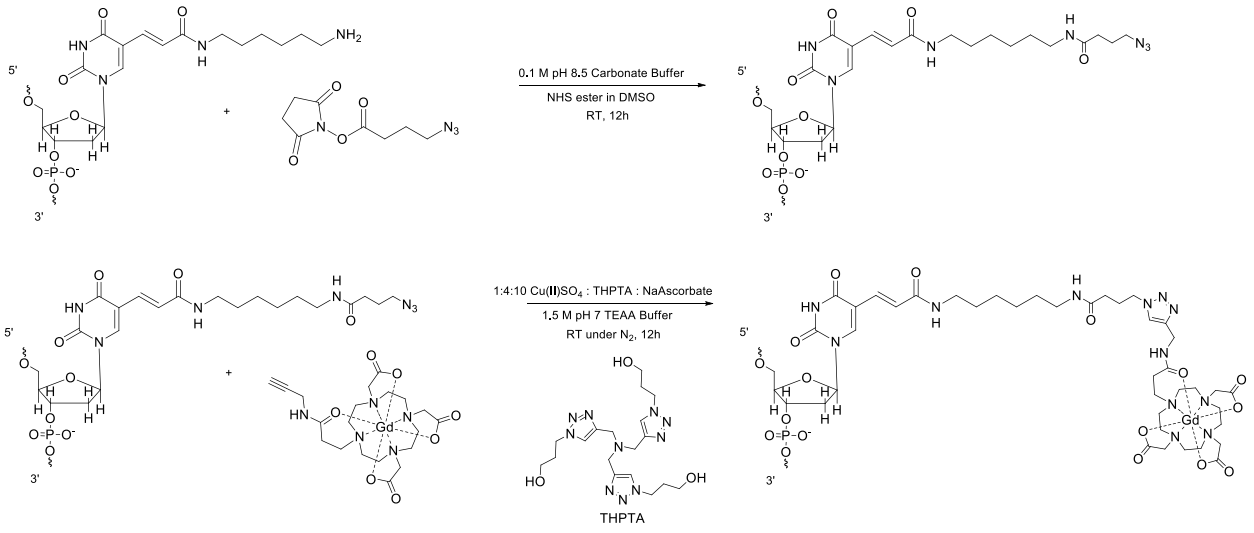
Gd(III)-DNA Synthesis:

Synthesis of the amine modified oligonucleotide was performed on 3' disulfide C3 controlled pore glass beads (Glen Research) using amino modifier C6 dT (Glen Research), indicated as T*, and Cy3 phosphoramidite (Glen Research). The synthesized oligonucleotide consisted of the sequence 3' – S-S-TTT-TTT-TTT-T*TT-T*TT-T*TT-T*TT-T*TT-Cy3 – 5'. Deprotection of the oligonucleotide from the resin was performed using standard 1:1 AMA conditions (ammonium hydroxide:methylamine) at 55 °C for one hour. Strands were filtered away from CPGs and purified by reverse phase HPLC and characterized by MS-MALDI. (*m/*z) observed: 8765, calculated: 8761.71 $[M - H]^-$.

Installation of azide functionality was performed off CPG and began with 1 µmol of 5x amino modifier poly dT DNA in 500 µL of pH 8.5 carbonate buffer (Scheme S4). To the mixture is added 5 mg of azidobutyrate NHS ester (Glen Research) in 100 µL of DMSO. Reaction is allowed to stir overnight and is observed to be complete by full reaction of starting DNA by MALDI-MS (see below). Intermediate 5x azide DNA is purified by reverse phase HPLC. (*m/*z) observed: 9328, calculated: 9327.31 $[M - H]^-$.

Covalent attachment of Gd(III) complex **5** to DNA begins with dissolution of 1 µmol of 5x azide bearing DNA from above, into 500 µL of 1.5 M triethylammonium acetate buffer pH 7. To the mixture (via stock solutions according to Hong, et. al.) [7] is added 100 nmol Cu(II) sulfate, 500 nmol tris-hydroxypropyl triazolyl amine, 10 µmol of **5**, and 1 µmol sodium ascorbate. The reaction is capped and allowed to stir overnight. The resulting 5x Gd(III)-DNA complex is then purified by reverse phase HPLC and characterized by MS-MALDI.

(*m/*z) observed: 12376, calculated: 12372.31 $[M - H]^-$.



Scheme S6: Synthesis of Gd(III) modified poly dT oligonucleotides

Nanoconjugate Synthesis:

Gold nanostars, spherical 15-nm gold nanoparticles, and Cy3-Gd(III)-DNA were synthesized as described in the materials and methods.

DNA was deprotected, and spherical nanoconjugates were functionalized and purified in an analogous procedure as that reported for 13nm gold nanoparticles.[8]

DNA-Gd@stars were functionalized similarly. Specifically, 4.1 OD (260 nm) of DNA (corresponding to ~1500 strands of DNA per nanostar) was dried into a 1.5 mL microcentrifuge tube, to which is added 300 µL of 100 mM dithiothreitol in 180 mM (pH 8.0) phosphate buffer. The solution is left to stir at room temperature for 1 hour. After such time, the DNA is run through a pre-packed G25 sephadex column (NAP-5, GE life sciences) using 180 mM phosphate buffer as the mobile phase, monitoring elution visually by observation of the Cy3 dye on the DNA.

To 10 mL of 2.1 nM nanostars in water is added 11.15 µL of tween 20 (for a total concentration of 0.01% v/v) and deprotected and purified DNA in 0.7 mL 180 mM phosphate buffer. The solution is then sonicated for 30 seconds and left to stir for 30 minutes. Over the subsequent five hours, a solution of NaCl (4.753 M), phosphate buffer (10 mM) and 0.01% tween 20 is added in increments of 178, 181, 184, 187 and 190 µL per hour, with each addition followed by 30 seconds of sonication. During the intervening time, the solution is left to stir at room temperature. The final concentration of NaCl is 450 mM. The solution is left to stir for a further 48 hours.

Purification of DNA-Gd@stars is conducted by three rounds of centrifugation (11 minutes at 15 xg) and resuspension until no further visible dye remains in the supernatant. Particle concentration and loading is determined by ICP-MS by examination of the Gd/Au ratio. When not in use, particles are stored at 4 °C.

Diamagnetic, 24mer poly dT DNA was conjugated to nanostars by the same procedure for use as controls in the NMRD analysis of DNA-Gd@stars. Similarly, the same DNA, and the same functionalization process as above was used for synthesis of 24mer poly dT diamagnetic spherical gold nanoconjugate controls.

After synthesis and characterization, DLS and zeta-potential measurements of all nanoconjugates were conducted (Table S1). Zeta-potential of bare nanostars and spheres was measured in millipore water. After Gd(III)-DNA conjugation, zeta-potential was measured in 1x DPBS (Gibco) to characterize the surface charge under physiological salt conditions.

Table S1: DLS, Zeta-potential, and loading characterization of nanoconjugates

	Hydrodynamic diameter (nm)	**Zeta potential (mV)**	**Gd(III)/particle**	**DNA/particle**
Nanostars	38.8 ± 0.1	-27 ± 5[a]	-	-
DNA-Gd@stars	63.0 ± 0.7	-18 ± 3[b]	1990 ± 450	398 ± 90
Nanospheres (15 nm)	19.5 ± 0.3	-35 ± 3[a]	-	-
DNA-Gd@spheres	53.3 ± 0.4	-17 ± 3[b]	564 ± 15	113 ± 3
Nanospheres (40 nm)	48.5 ± 0.7	NM	-	-
DNA-Gd@spheres$_{40nm}$	68.8 ± 0.7	NM	2055 ± 136	411 ± 27

[a] measured in water, [b] measured in PBS, NM = not measured

The Au composition approximation of each nanoparticle is based on the geometric calculation of the volume multiplied by the density of bulk gold, as described above.

Colloidal Stability of DNA-Gd@stars:

The colloidal stability of the DNA-Gd@stars was examined under a range of pH and salt conditions to ensure their stability for future biological applications. DNA-Gd@stars were incubated in 0 mM, 150 mM, 250 mM, 350 mM and 450 mM NaCl in water; 50 mM HEPES buffer at pH 3, 5, 7, 9, and 11; or fresh cell culture media (DMEM + 10% FBS) and conditioned cell culture media (c. media) for 24 hr. C. media was prepared by incubating adherent PANC-1 cells for 48 hr with DMEM + 10% FBS, then collecting the media and centrifuging (5 min, 1000 x g) to remove dead cells. Photographs, DLS, and UV-VIS spectra were taken after 4 and 24 hr incubations with the different conditions. Figure S4 shows that no visible aggregation occurs under any conditions tested. Under extreme salt (350-450 mM NaCl) and low pH (3) conditions, only slight broadening of the UV-vis spectrum and increase in hydrodynamic is observed. The red-shift in the absorbance peak and increase in diameter in media and c. media is attributed to adsorption of serum proteins.

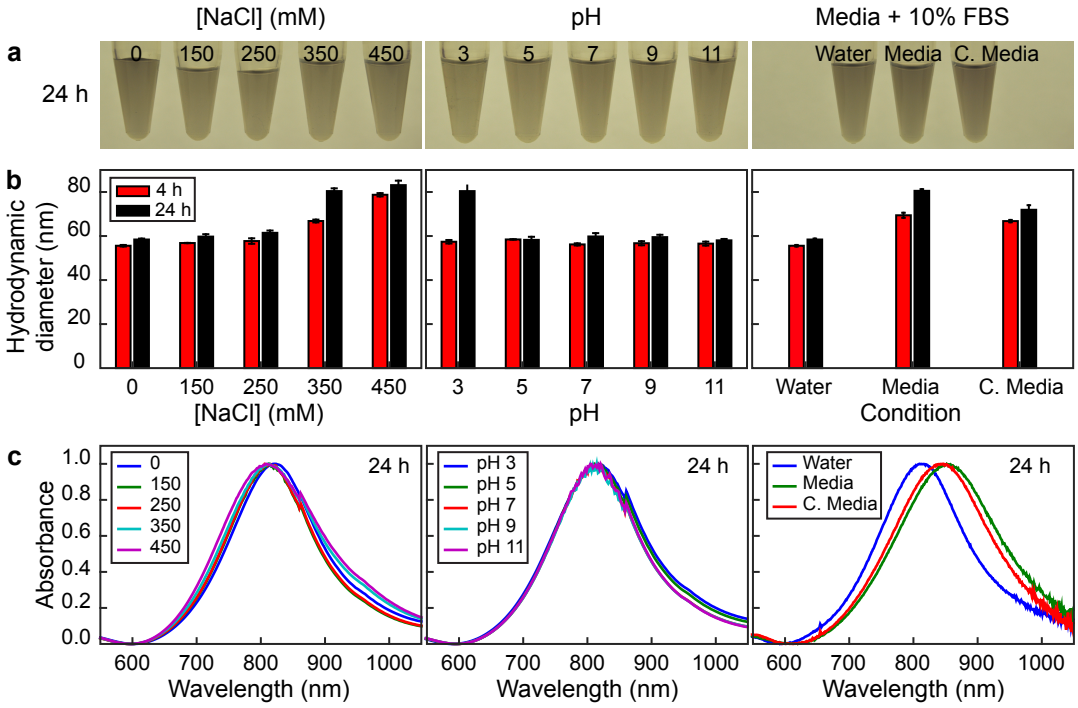


Figure S4. Colloidal stability of DNA-Gd@stars under a range of salt, pH, and cell culture media conditions over 24 hr as characterized by (a) photography, (b) DLS, and (c) UV-Vis.

Nanoconjugate Relaxivity:

Table S2: Batch to batch characteristics of for DNA-Gd@stars

1.41 T	**r_1 ($mM^{-1} s^{-1}$)**	**r_2 ($mM^{-1} s^{-1}$)**	**Loading Gd/nanostar**	**r_2/r_1 ratio**	**Trials (ICP)**
Batch 1	50.9	101	2170	1.98	3
Batch 2	46.6	88.2	2193	1.89	2
Batch 3	42.3	78.7	1691	1.86	1
Batch 4	67.9	118.6	2177	1.75	1
Batch 5	51.5	93.5	2473	1.82	1
Batch 6	69.1	118.7	1231	1.71	1
Average	54.7	99.8	1989.2	1.84	-
St Dev	11.2	16.3	448.9	0.1	-

Table S3: Measured values of T_1 and corresponding [Gd(III)] measured by ICP-MS for DNA-Gd@stars

sample	[Gd] / mM	T_1 (ms)	T_1 (s)	1/ T_1
1	0.0141	1104.0	1.1	0.90
2	0.0071	1719.0	1.7	0.58
3	0.0038	2275.0	2.3	0.43
4	0.0018	3088.0	3.1	0.32
5	0.0009	3384.0	3.4	0.29

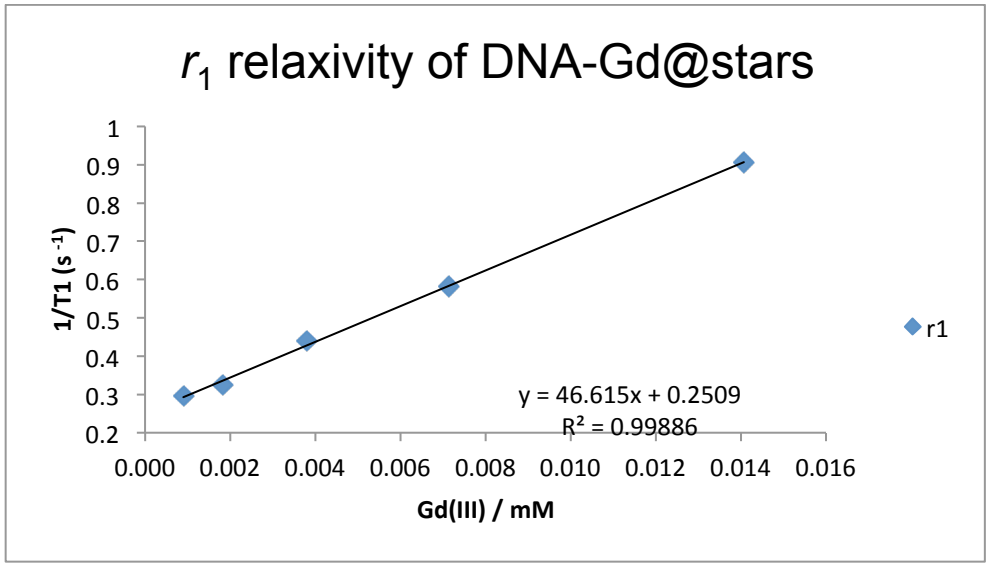


Figure S5: Example of r_1 relaxivity calculation for Batch 2 DNA-Gd@stars

Table S4: Measured values of T_2 and corresponding [Gd(III)] measured by ICP-MS for DNA-Gd@stars

sample	[Gd] / mM	T_2 (ms)	T_2 (s)	1/ T_2
1	0.0141	628.3	0.6	1.59
2	0.0071	949.1	0.9	1.05
3	0.0038	1463.3	1.5	0.68
4	0.0018	1798.7	1.8	0.55
5	0.0009	2379.2	2.4	0.42

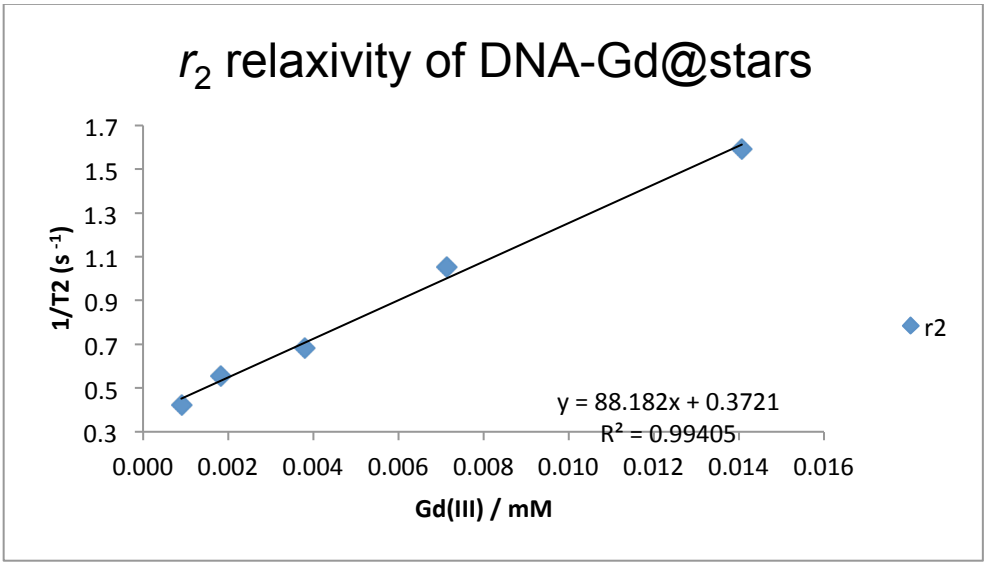


Figure S6: Example of r_2 relaxivity calculation for Batch 2 DNA-Gd@stars

Table S5: Measured values of T_1 and corresponding [Gd(III)] measured by ICP-MS for Gd(III)-DNA

sample	[Gd] / mM	T_1 (ms)	T_1 (s)	1/ T_1
1	0.2333	407	0.41	2.46
2	0.1159	742	0.74	1.35
3	0.0580	1250	1.25	0.80
4	0.0293	1911	1.91	0.52
5	0.0149	2604	2.60	0.38

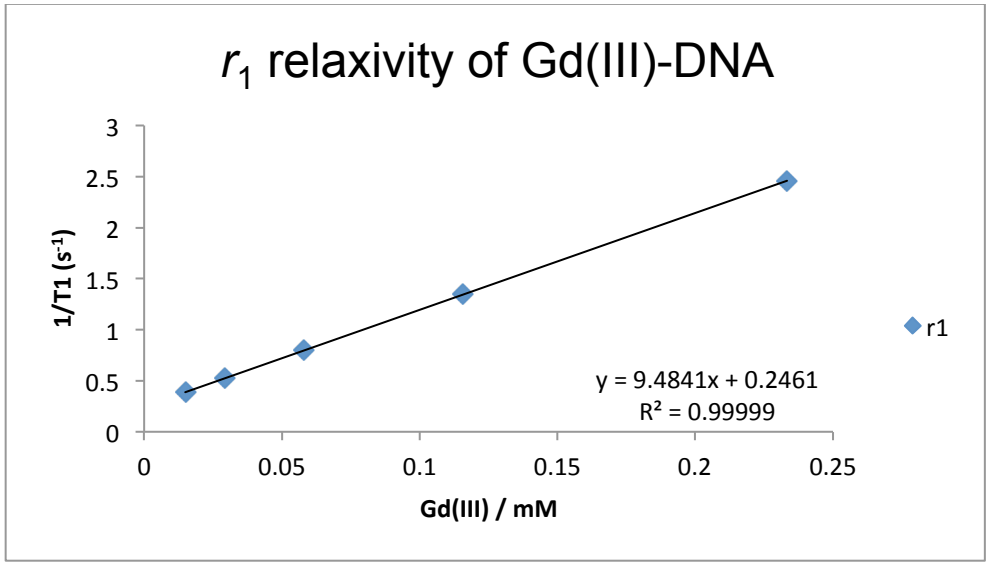


Figure S7: r_1 relaxivity calculation for Gd(III)-DNA

Table S6: Measured values of T_1 and corresponding [Gd(III)] measured by ICP-MS for DNA-Gd@spheres

sample	[Gd] / mM	T_1 (ms)	T_1 (s)	1/ T_1
1	0.1865	327.9	0.33	3.05
2	0.0935	607	0.61	1.65
3	0.0475	1025	1.03	0.98
4	0.0245	1487	1.49	0.67
5	0.0124	1976	1.98	0.51

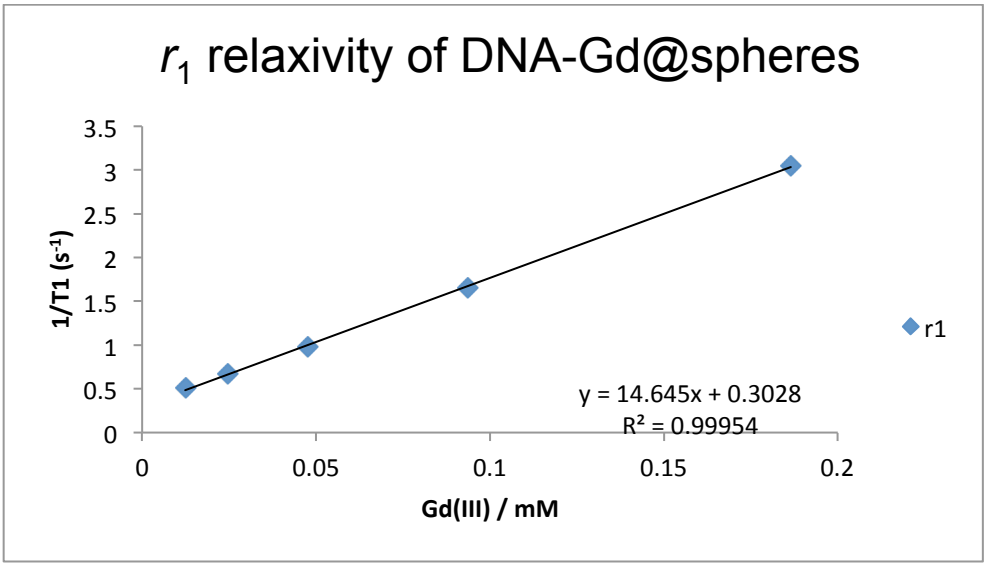


Figure S8: r_1 relaxivity calculation for DNA-Gd@spheres

Table S7: Measured values of T_2 and corresponding [Gd(III)] measured by ICP-MS for DNA-Gd@spheres

sample	[Gd] / mM	T_2 (ms)	T_2 (s)	1/ T_2
1	0.1865	149.4	0.15	6.69
2	0.0935	279.11	0.28	3.58
3	0.0475	495.84	0.50	2.02
4	0.0245	787.4	0.79	1.27
5	0.0124	1156.2	1.16	0.86

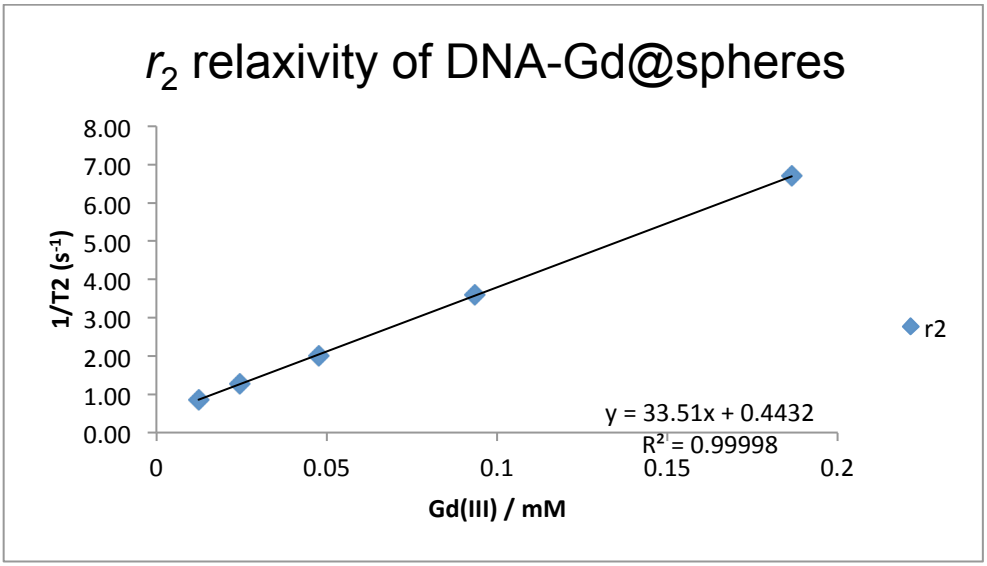


Figure S9: r_2 relaxivity calculation for DNA-Gd@spheres

Table S8: Measured values of T_1 and corresponding [Gd(III)] measured by ICP-MS for 40nm DNA-Gd@spheres

sample	[Gd] / mM	T_1 (ms)	T_1 (s)	1/ T_1
1	0.0148	1970	1.97	0.51
2	0.0074	2612	2.61	0.38
3	0.0037	3098	3.10	0.32
4	0.0019	3450	3.45	0.29
5	0.0009	3659	3.66	0.27

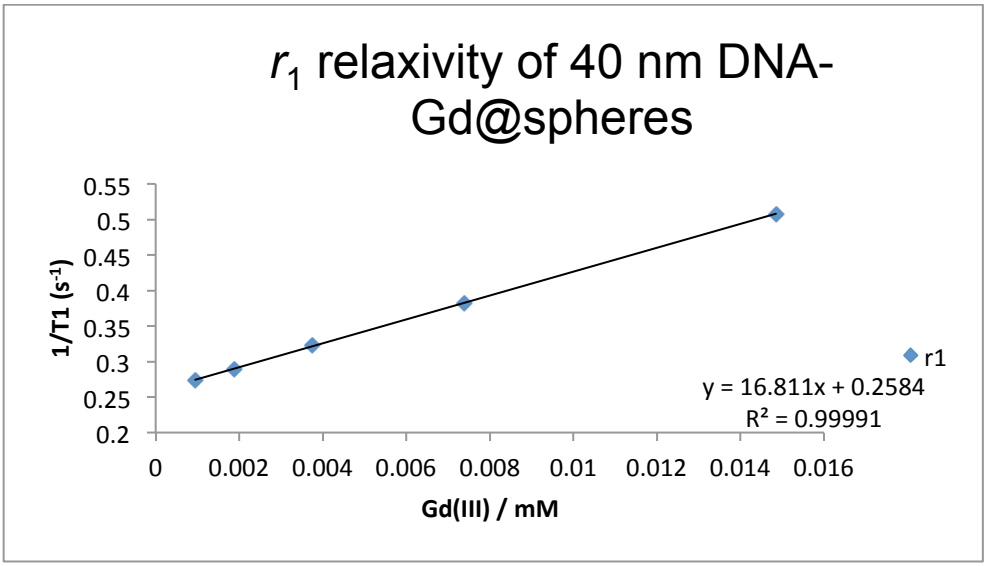


Figure S10: r_1 relaxivity calculation for 40 nm DNA-Gd@spheres

Table S9: Measured values of T_2 and corresponding [Gd(III)] measured by ICP-MS for 40nm DNA-Gd@spheres

sample	[Gd] / mM	T_2 (ms)	T_2 (s)	1/ T_2
1	0.0148	1021.2	1.02	0.98
2	0.0074	1444	1.44	0.69
3	0.0037	1840	1.84	0.54
4	0.0019	2117	2.12	0.47
5	0.0009	2338	2.34	0.43

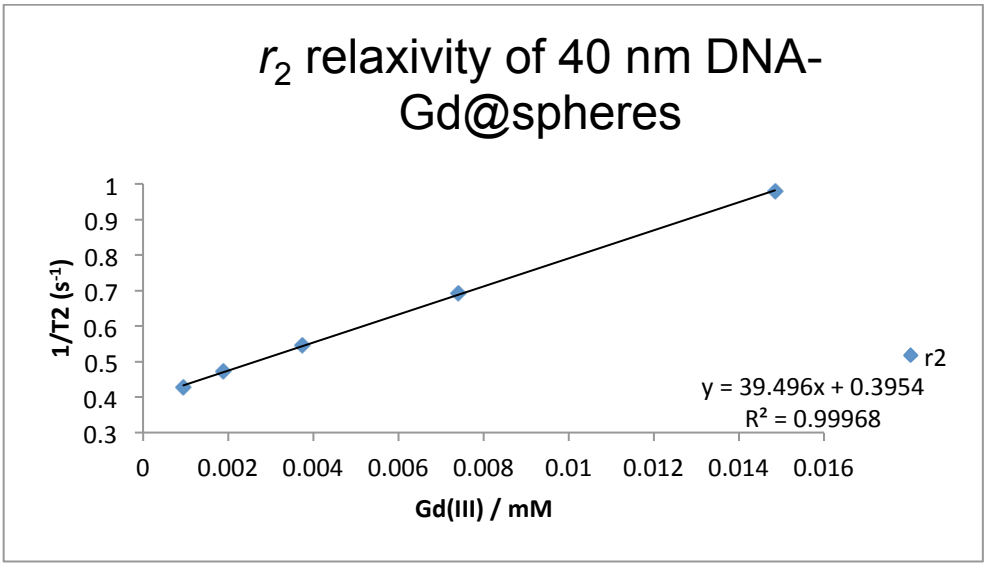


Figure S11: r_2 relaxivity calculation for 40 nm DNA-Gd@spheres

High Field Relaxivity:

MR imaging and T_1/T_2 measurements were performed on a Bruker Pharmscan 7 T imaging spectrometer fitted with shielded gradient coils at 25 °C. Samples were prepared by serial dilution, and confirmation of concentration by ICP-MS for [Gd]. Solutions were imaged in glass capillary tubes of approximate diameter = 1 mm.

Spin−lattice relaxation times (T_1) were measured using a rapid-acquisition rapid-echo (RARE-VTR) T_1-map pulse sequence, with static TE (11 ms) and variable TR (150, 250, 500, 750, 1000, 2000, 4000, 6000, 8000, and 10000 ms) values. Imaging parameters were as follows: field of view (FOV) = 25 × 25 mm^2, matrix size (MTX) = 256 × 256, number of axial slices = 4, slice thickness (SI) = 1.0 mm, and averages (NEX) = 3 (total scan time = 2 h 36 min). T_1 analysis was carried out using the image sequence analysis tool in Paravision 5.0 pl3 software (Bruker, Billerica, MA, USA) with monoexponential curve-fitting of image intensities of selected regions of interest (ROIs) for each axial slice.

Spin−spin relaxation times (T_2) were measured using a multislice multiecho (MSME) T_2-map pulse sequence, with static TR (5000 ms) and 32 fitted echoes in 11 ms intervals (11, 22, through , 352 ms). Imaging parameters were as follows: field of view (FOV) = 25 × 25 mm^2, matrix size (MTX) = 256 × 256, number of axial slices = 4, slice thickness (SI) = 1.0 mm, and averages (NEX) = 3 (total scan time = 48 min). T_2 analysis was carried out using the image sequence analysis tool in Paravision 5.0 pl3 software (Bruker, Billerica, MA, USA) with monoexponential curve-fitting of image intensities of selected regions of interest (ROIs) for each axial slice.

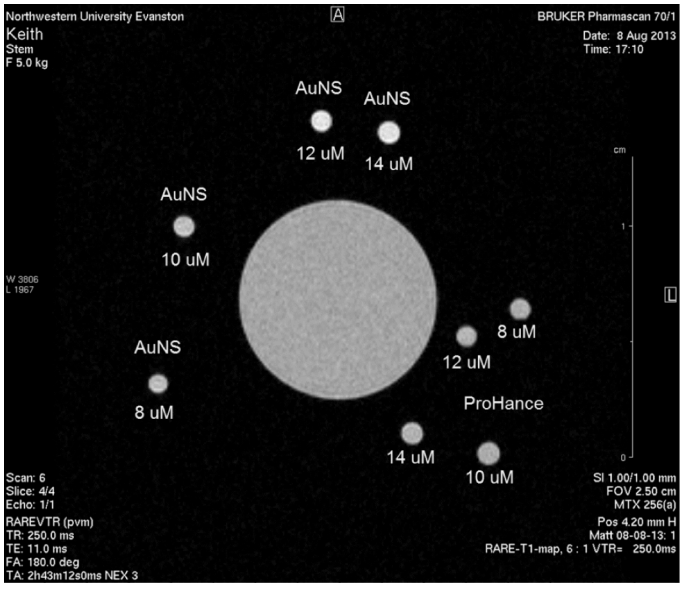


Figure S12: Solution phantoms of DNA-Gd@stars (here AuNS) and [Gd(HP-DO3A)(H_2O)] – ProHance

Table S10: T_1 times and concentrations used for calculation of high field (7T) relaxivity

	ProHance 1		ProHance 2		ProHance 3		ProHance 4	
slice 1		st. dev.		st. dev.		st. dev.		st. dev.
T_1 time [msec]	2720.0	31.0	2788.9	32.2	2854.0	36.1	2896.6	36.9
slice 2								
T_1 time [msec]	2748.1	39.9	2836.4	37.9	2928.3	42.0	3037.5	33.2
slice 3								
T_1 time [msec]	2683.9	25.1	2753.9	33.1	2834.6	28.5	2939.7	21.8
slice 4								
T_1 time [msec]	2660.9	27.3	2719.1	27.2	2736.6	37.3	2725.1	60.6

	DNA-Gd@stars12 uM		DNA-Gd@stars 14 uM		DNA-Gd@stars 10 uM		DNA-Gd@stars 8 uM		H_2O	
slice 1		st. dev.		st. dev.		st. dev.		st. dev.		st. dev.
T_1 time [msec]	2203.6	44.2	2183.4	26.4	2403.2	53.5	2633.2	40.1	3088.1	33.8
slice 2										
T_1 time [msec]	2303.1	41.2	2337.9	23.1	2337.9	67.4	2583.5	42.7	3208.2	46.9
slice 3										
T_1 time [msec]	2304.1	27.4	2188.9	34.2	2537.3	11.7	2465.8	44.8	3131.5	34.4
slice 4										
T_1 time [msec]	2194.1	50.7	2209.2	27.2	2378.9	43.4	2421.5	63.0	3077.8	37.3

Theoretical SBM Calculations:

Simulation describing the largest relaxivity values achievable at 35 MHz due to the presence of one regularly coordinated water molecule (with proton-Gd(III) distance of 3.05 Å), as a function of the proton residence time (with optimized reorientation time and electron relaxation time).

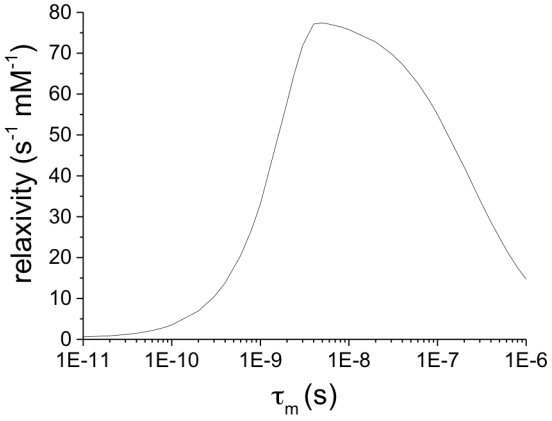


Figure S13: Simulated values of relaxivity achievable by one inner-sphere water molecule versus τ_m

NMRD Analysis:

Table S11: Components of relaxivity at varying field strengths. Here we abbreviate inner-sphere (IS), second-sphere (SS) and outer-sphere (OS) for clarity.

64MHz	25 °C	Component %	37 °C	Component %
IS	21.2	38.7	25.5	57.6
SS	31.5	57.4	17.2	38.8
OS	2.1	3.9	1.6	3.6
total r_1	54.8	100.0	44.3	100.0

32MHz	25 °C	Component %	37 °C	Component %
IS	66.4	67.5	66.9	78.4
SS	29.6	30.1	16.5	19.3
OS	2.5	2.5	1.9	2.3
total r_1	98.4	100.0	85.3	100.0

Table S12: NMRD best fit parameters for DNA-Gd@stars

NMRD best fit parameters (DNA-Gd@stars)	**25 °C**	**37 °C**
Δ_t (cm^{-1})	0.019	0.019
t_v (ps)	10 ps	12 ps
ZFS (cm^{-1})	0.06	0.06
θ°	67	67
$^*q$	1	1
$^*r_{Gd\text{-H}}$ (Å)	3.05	3.05
$^*\tau_m$ (ns)	39	22
$^*q^{SS}$	4[a]	4[a]
$r^{SS}_{Gd\text{-H}}$ (Å)	3.5	3.5
τ_m^{SS} (ns)	0.58	0.30
$^*d^{OS}_{Gd\text{-H}}$ (Å)	3.6	3.6
$^*D_{diff}$ (m^2/s)	3.3×10^{-9}	2.3×10^{-9}
$^*\tau_r$ (ns)	> 1000	> 1000

*Fixed parameters

Δ_t, transient zero field splitting; τ_v, correlation time for electron relaxation; ZFS, static zero field splitting; θ, angle between the z-axis of the static zero field splitting and the Gd-water molecule vector; q_i, number of water molecules in the ith coordination sphere; r_i, (average) distance between water protons and the gadolinium ion; τ_{Mi}, lifetime of water molecules; d, distance of closest approach; D_{diff}, diffusion coefficient.

Table S13: NMRD best fit parameters for 15 nm DNA-Gd@spheres

NMRD best fit parameters (DNA-Gd@spheres)	**25 °C**	**37 °C**
Δ_t (cm^{-1})	0.017	0.017
t_v (ps)	8.5 ps	6 ps
ZFS (cm^{-1})	0.06	0.06
θ°	67	67
$^*q$	1	1
$^*r_{Gd\text{-H}}$ (Å)	3.05	3.05
$^*\tau_m$ (ns)	30	25
order parameter	0.35	0.35
$^*\tau_{fast}$(ns)	2.3	0.45
$^*d^{OS}_{Gd\text{-H}}$ (Å)	3.6	3.6
$^*D_{diff}$ (m^2/s)	2.3×10^{-9}	3.3×10^{-9}
$^*\tau_r$ (ns)	> 1000	> 1000

*Fixed parameters

Cell Pellet Imaging:

MR imaging and T_1/T_2 measurements were performed on a Bruker Pharmscan 7 T imaging spectrometer fitted with shielded gradient coils at 25 °C. For cell pellet MRI phantoms, ~ 1×10^6 PANC-1 cells were incubated in 25 cm^2 T-flasks with ProHance, DNA-Gd@stars or DNA-Gd@spheres (24 hr incubation, [Gd(III)]=3-6μM). Cells were washed 3x with PBS, and harvested with 500 μL of trypsin. After addition of 500 μL of fresh complete media, cells were transferred to 1.5 mL microcentrifuge tubes and washed twice via centrifuged at 1000 × g at for 5 min. The supernatant was removed; the cell pellets were resuspended in 1 mL of complete media, added to 5 3/4″ flame-sealed Pasteur pipets, and centrifuged at 100 × g at 4.0 °C for 5 min. The bottom sections of the flame-sealed pipets were then scored with a glass scribe, broken into small capillaries, and imaged using a RF RES 300 1H 089/023 quadrature transmit receive 23 mm volume coil (Bruker BioSpin, Billerica, MA, USA).

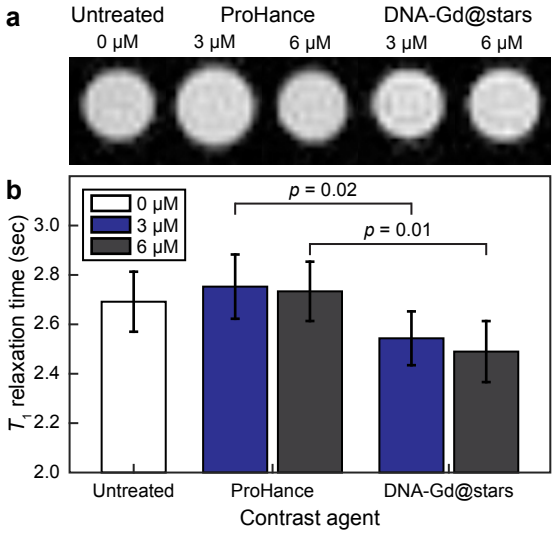


Figure S14: (a) MRI phantoms of PANC-1 cells treated with no contrast agent, ProHance, or DNA-Gd@stars. Tube diameter = 1 mm (b) T_1 relaxation times calculated from the average of 5 slices of each phantom. Error bars represent the standard deviation of the mean. *P*-values are calculated by a two-tailed t-test between equimolar incubation concentrations of ProHance and DNA-Gd@stars.

Table S14: Raw data used for calculation of average cell pellet T_1 relaxation times. Here, NP are DNA-Gd@spheres and NS are DNA-Gd@stars.

ROI name	Water	Control	Prohance 3uM	NS 3 uM	Prohance 6 uM	NS 6 uM
slice 1 (s)	3.246	2.608	2.670	2.498	2.678	2.463
slice 2 (s)	3.378	2.826	2.877	2.647	2.853	2.618
slice 3 (s)	3.345	2.717	2.759	2.584	2.779	2.530
slice 4 (s)	3.370	2.777	2.878	2.613	2.806	2.545
slice 5 (s)	3.205	2.530	2.580	2.375	2.552	2.292
average (s)	3.309	2.692	2.753	2.544	2.734	2.490
st. dev. (s)	0.078	0.121	0.130	0.109	0.120	0.123

Nanoconjugate Biocompatibility:

Viability was measured with Cell-Titer 96 proliferation assay (Promega). 10,000 PANC-1 cells were grown in each well of a 96-well plate and incubated with varying concentrations (0-6 µM [Gd(III)], 5 wells per condition) of DNA-Gd@stars and DNA-Gd@spheres in complete growth medium for 24 hr. After treatment, wells were washed 1x with PBS to remove excess nanoconjugates. After 100 ul of cell culture medium and 20 ul of pre-mixed MTS/PMS reagent was added to each well, the plate was incubated for 3 hr at 37 °C to allow viable cells to convert MTS to soluble formazan. The absorbance at 490 nm was recorded using a Synergy 4 microplate reader. The results indicate that at the concentrations tested, neither nanoconjugate causes a decrease in cell viability (Figure S15). Since the absorbance peak of the 15 nm Au spheres is 524 nm, and the nanostars also have a small absorbance peak near 520 nm, it was necessary to check if the absorbance of the nanoparticles in the cells affected the measured absorbance of formazan. After the initial reading, the same microplate was centrifuged for 15 minutes at 4000 xg to sediment any detached cells or nanoconjugates. 60 ul of the supernatant (now containing only soluble formazan) of each well was transferred to a fresh microplate and the absorbance at 490 was recorded again. The relative absorbance was not affected by the presence of the nanoparticles.

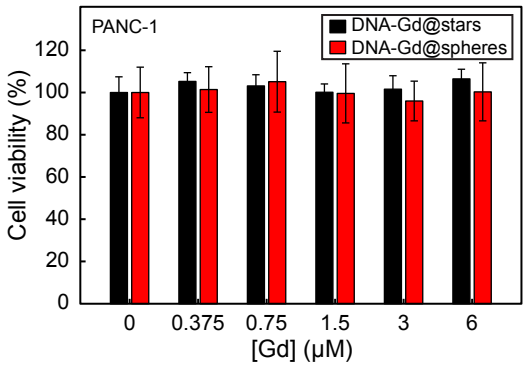


Figure S15: Viability of PANC-1 cells after 24 h incubation with DNA-Gd@stars and DNA-Gd@spheres.

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