Supporting Information

A Markedly Improved Synthetic Approach for the Preparation of Multifunctional Au-DNA Nanoparticle Conjugates Modified with Optical and MR Imaging Probes

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References:

Chemical Synthesis and Characterization Solvents and reagents were purchased from Sigma-Aldrich unless otherwise noted. Synthetic procedures were carried out under ambient conditions unless described otherwise. Chemical characterization was performed using a Varian 500 MHz NMR and a Bruker AutoFlex III MALDI spectrometer. Gd(III) complex purification was performed using a Varian Prostar 500 HPLC using a Waters C8 Sunfire 19 × 150 mm 5 μm column and mobile phases of Millipore water, and HPLC grade acetonitrile. Nanoparticle characterization was performed on JOEL 1230 and Hitachi HD7700 TEMs. UV/Vis/NIR spectra of colloidal solutions were collected on an Agilent UV/Vis spectrophotometer. DLS and zeta-potential measurements were obtained using a Brookhaven ZetaPals zeta potential and particle size analyzer.

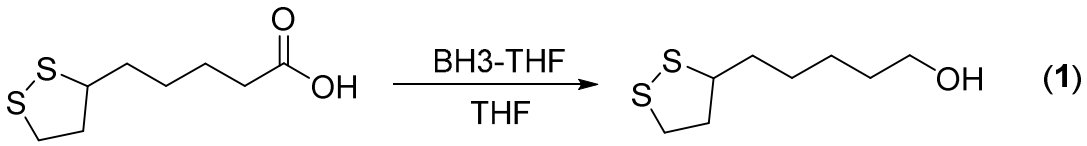
Oligonucleotides were synthesized using solid phase, controlled pore glass beads (CPGs) by standard techniques on a MerMade automated synthesizer. Reagents, protected 3' thiol modifier CPGs, and C6 amino modifier dT modified bases were purchased from Glen Research (Sterling, Va). Oligonucelotides were deprotected from the solid phase using AMA conditions [(1:1 methylamine:ammonium hydroxide (*sat.*)] for sixty minutes. Oligonucleotide purification was performed by HPLC using a mobile phase consisting of 30mM triethyl ammonium acetate buffer pH 7 (TEAA) and acetonitrile (ACN). A method containing a gradient of 75% Acetonitrile over 45minutes was applied, as monitored by backbone and Cy3 wavelengths at 254 and 546 nm, respectively. Post-purification, oligonucleotides were lyophilized and stored at -20 °C. Inorganic Gd(III) complex **4** was synthesized as described below.

ICP-MS was performed on either a computer-controlled (Plasmalab software) Thermo (Thermo Fisher Scientific, Waltham, MA) PQ ExCell ICP-MS equipped with a CETAC 500 autosampler or a computer-controlled (Plasmalab software) Thermo X series II ICP-MS equipped with an ESI (Omaha, NE, USA) SC-2 autosampler.

Chemical Synthesis of 2,2',2''(10(3-(((1-(5-(1,2-dithiolan-3-yl)pentyl)-1,2,3-triazol-4-yl)methyl)amino)-3-oxopropyl)-1,4,7,10-tetraazacyclododecyl-gadolinium(III) complex (4):

The synthesis of dtGd(III) was adapted from Koufaki et. al.,[1]

5-(1,2-dithiolan-3-yl)pentan-1-ol **(1)**



Scheme S1: Chemical reduction of (±) lipoic acid to corresponding alcohol

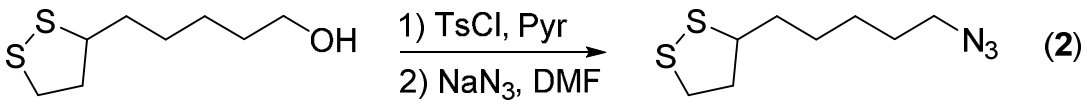
To a two-necked 250 mL round bottom flask was added a stir bar and (±) lipoic acid (1.00 g, 4.8 mmol). To this was added 50 mL of dry THF under nitrogen atmosphere, with stirring until complete dissolution of lipoic acid. The mixture was cooled to 0 °C, at which time was added 1.0 M Borane-THF complex (7.3 mL, 7.3 mmol)) dropwise over ten minutes. The yellow solution was observed to bubble during addition of Borane-THF and was left to warm to room temperature with stirring over 3 hours. The reaction was again cooled to 0 °C and 5 mL of methanol was added dropwise to quench the remaining Borane-THF. After no further bubbling was observed, it was left open to air under positive flow of nitrogen until the majority of solvent had evaporated. The crude mixture was dissolved into 20 mL diethyl ether and extracted with saturated sodium bicarbonate (3 x 20 mL), brine (1 x 20 mL) and the diethyl

ether layer was dried over sodium sulfate and evaporated by rotary evaporation. The crude mixture was purified by silica gel flash chromatography using conditions of 1:1 ethyl acetate : hexanes, visualized by CAM stain (R_f = 0.45). Product is a viscous yellow oil. Yield: 0.879g, 95.3%

^{1}H NMR (500 MHz, Chloroform-*d*) δ 3.64 (t, *J* = 6.5 Hz, 1H), 3.57 (dq, *J* = 8.6, 6.4 Hz, 1H), 3.24 – 3.06 (m, 1H), 2.52 – 2.41 (m, 1H), 1.97 – 1.86 (m, 0H), 1.75 – 1.35 (m, 4H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 62.95, 56.75, 40.41, 38.59, 35.02, 32.68, 29.24, 25.71.

3-(5-azidopentyl)-1,2-dithiolane (2)



Scheme S2: Tosylation and azide substitution of 5-(1,2-dithiolan-3-yl)pentan-1-ol

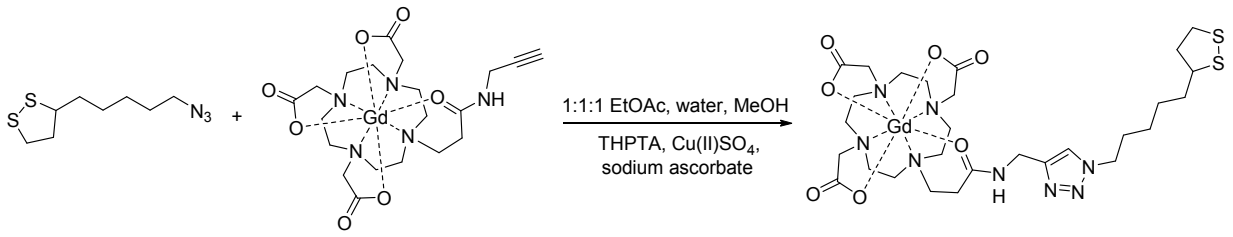
To a 100 mL round bottom flask with a magnetic stir bar was added the lipoic alcohol from above (0.879 g, 4.5 mmol) and 30 mL of pyridine with cooling to 0 °C. To the stirring mixture was added tosyl chloride (1.91 g, 10.0 mmol), and was left to stir overnight. Complete conversion to product was observed by thin layer chromatography under conditions of 1:1 ethyl acetate : hexanes and visualized by CAM (R_f = 0.65). The crude mixture was dissolved into 50 mL of diethyl ether and was extracted with 1M HCl (*aq.*) (2 x 50 mL), saturated sodium bicarbonate (1 x 50 mL), and brine (1 x 50 mL). The organic layer was dried over sodium sulfate and was positively identified by crude NMR and ESI-MS: (*m/*z) observed: 369.2, calculated: 369.5 $[M + Na]^+$. The crude product was taken on to azide substitution without further purification. Into 20 mL of DMF was dissolved the tosylated lipoic alcohol and sodium azide (0.468 g, 7.2 mmol). To the stirring mixture was added a spatula tip of potassium iodide and the reaction was left to stir at 60 °C for 3 days. Thin layer chromatography using 1:1 ethyl acetate : hexanes appeared to show only starting material, but crude ESI-MS indicated complete conversion to product, (*m/*z) observed: 240.8, calculated: 240.3 $[M + Na]^+$. After rotary evaporation of DMF, crude product is extracted with diethyl ether and each of water, saturated aqueous sodium bicarbonate and brine (50 mL each). The extracted diethyl ether is dried over sodium sulfate and evaporated under vacuum yielding one spot by TLC, CAM (R_f = 0.65), and a clean NMR of the 3-azidopentyl substituted 1,2 dithiolane . Product is a viscous yellow oil. Yield: 0.721 g, 69.2 % overall yield.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 3.57 (dq, *J* = 8.6, 6.4 Hz, 1H), 3.27 (t, *J* = 6.9 Hz, 2H), 3.23 – 3.08 (m, 2H), 2.47 (m, *J* = 13.0, 6.6, 5.4 Hz, 1H), 1.92 (dq, *J* = 12.7, 7.0 Hz, 1H), 1.78 – 1.38 (m, 9H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 62.95, 56.75, 40.41, 38.59, 35.02, 32.68, 29.24, 25.71.

***2,2',2''(10(3-(((1-(5-(1,2-dithiolan-3-yl)pentyl)-1,2,3-triazol-4-yl)methyl)amino)-3-oxopropyl)-1,4,7,10-tetraazacyclododecyl-gadolinium(III) complex* (4)**

The synthesis utilized previously synthesized ***1-(N-(prop-2-yn-1-yl)2-oxopropyl)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecyl-gadolinium(III) complex* (3)** as described previously.[2] Design parameters for the copper(I) catalyzed azide-alkyne cycloaddition reaction conditions described below were derived from discussion of work by Finn and co-workers.[3]



Scheme S3: Chemical synthesis of dtGd(III) complex **4**

To a 10 mL round bottom flask containing a stir bar, nitrogen atmosphere, and water (2 mL) was added copper(II) sulfate pentahydrate (0.007 g, 0.03 mmol) and *tris*-hydroxypropyltriazolylamine (0.026 g, 0.06 mmol). To this mixture was further added complex **3** (0.1 g, 0.16 mmol) with stirring. After complete dissolution was observed, sodium ascorbate (0.06 g, 0.3 mmol) was added. To a separate vial was dissolved compound **2** (0.52 g, 0.24 mmol) and ethyl acetate (2 mL). The dissolved **2** in ethyl acetate was then combined with the stirring aqueous solution of complex **3** and copper, THPTA and sodium ascorbate making a biphasic mixture. To the stirring mixture was then added methanol (2 mL) to coalesce the phases, and the reaction was left to stir overnight at room temperature. ESI-MS was used to indicate complete reaction of starting materials and positively identify complex **4**. Purification of complex 4 was achieved using high performance liquid chromatography (HPLC) using a Waters C8 Sunfire (semipreparative column on a Varian ProStar 500 system with freshly purified water and HPLC grade acetonitrile as the mobile phase. Specifically, purification was achieved at a flowrate of 15 mL/min using a method which held 5% acetonitrile from 0-5 minutes, followed by a linear ramp to 40% between 5-20 minutes and to 100% between 20-25 minutes. Complex **4** elutes between 13.4 and 13.8 minutes as monitored by UV absorbance at 200/210 nm. Final compound is an off white solid. Yield 0.561 g, 41.5 %. (Final characterization was confirmed by high resolution ESI-TOF MS: (*m*/z) observed: 850.1980, calculated: 851.08 $[M + Na]^+$.

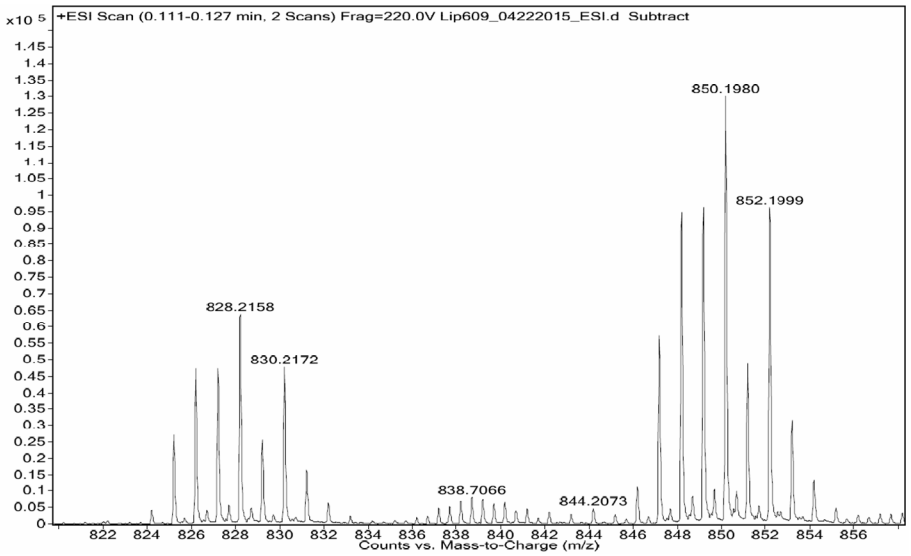


Figure S1. High resolution ESI-TOF spectrum of dtGd(III) complex **4** for $[M + H]^+$ and $[M + Na]^+$

Metals Analysis by ICP-MS: Quantitation of metal concentration was assessed by initial acid digestion of nanoconjugate samples, followed by dilution of acid into water and analysis by ICP-MS. Gadolinium and gold samples were prepared by different dilution factors such that were within the range of the selected standard concentrations. Specifically, Gd analyses were conducted by addition of 5 ul of nanoconjugate sample into 120 ul of 1:1 concentrated nitric acid: concentrated hydrochloric acid (TraceSelect Nitric acid, >69% ; TraceSelect HCl, fuming 37%) for digestion of metal contents. Au analyses were made by addition of 5 uL of nanoconjugate sample to 500 uL of 1:1 HNO_3:HCl as above, and mixed thoroughly. Millipore water and multi-element internal standard (CLISS-1, Spex Certiprep, Metuchen, NJ, USA) containing 6Li, Sc, Y, In, Ho, Bi were added to produce a solution of 2% nitric acid (v/v), 2% HCl (v/v) and 5.0 ng/mL internal standard up to a total sample volume of 3 mL (Gd) and 10 mL (Au) after 20-fold dilution of original aliquot. Individual Au/Gd mixed-metal elemental standards were prepared at 0, 0.78125, 1.5625, 3.125, 6.25, 12.5, 25.0, 50.0, 100, and 200 ng/mL concentrations with 2% nitric acid (v/v), 2% HCl (v/v) and 5.0 ng/mL internal standards up to a total sample volume of 5 mL. Each sample was acquired using 1 survey run (10 sweeps) and 3 main (peak jumping) runs (100 sweeps). The isotopes selected were ^{197}Au, ^{156,157}Gd and ^{115}In, ^{165}Ho, and ^{209}Bi (as internal standards for data interpolation and machine stability).

Relaxivity (r_1): A stock solution of backfilled dt-Gd(III) SNA conjugates was made (700 uL). This stock was serially diluted four times for a total of five solutions. Solutions were heated to 37 °C and two hundred uL of each concentration was placed into a Bruker minispec mq60 NMR spectrometer (60 MHz) for measurement of T_1 relaxation time. Data were collected using an inversion recovery pulse sequence using 4 averages, a 15s repetition time and 10 data points. The remaining volumes of each solution were utilized for ICP analysis of [Gd(III)]. The inverse of the longitudinal relaxation time ($1/T_1$, s^{-1}) was plotted *versus* the Gd(III) concentration (mM). By applying a linear fit to this data, the slope that is generated is defined as the relaxivity of the agent (mM^{-1} s^{-1}). Relaxivities of DNA-Gd@spheres, Gd(III)-DNA and complex 5 were collected by the analogous procedure.

dt-Gd(III) relaxivity

Table S1. Measured values of T_1 and corresponding [Gd(III)] measured by ICP-MS for dt-Gd(III)

sample	[Gd] / mM	T_1 (ms)	T_1 (s)	1/ T_1
1	3.10	64	0.064	15.63
2	1.60	125	0.125	8.01
3	0.79	235.2	0.235	4.25
4	0.41	439	0.439	2.28
5	0.21	787	0.787	1.27

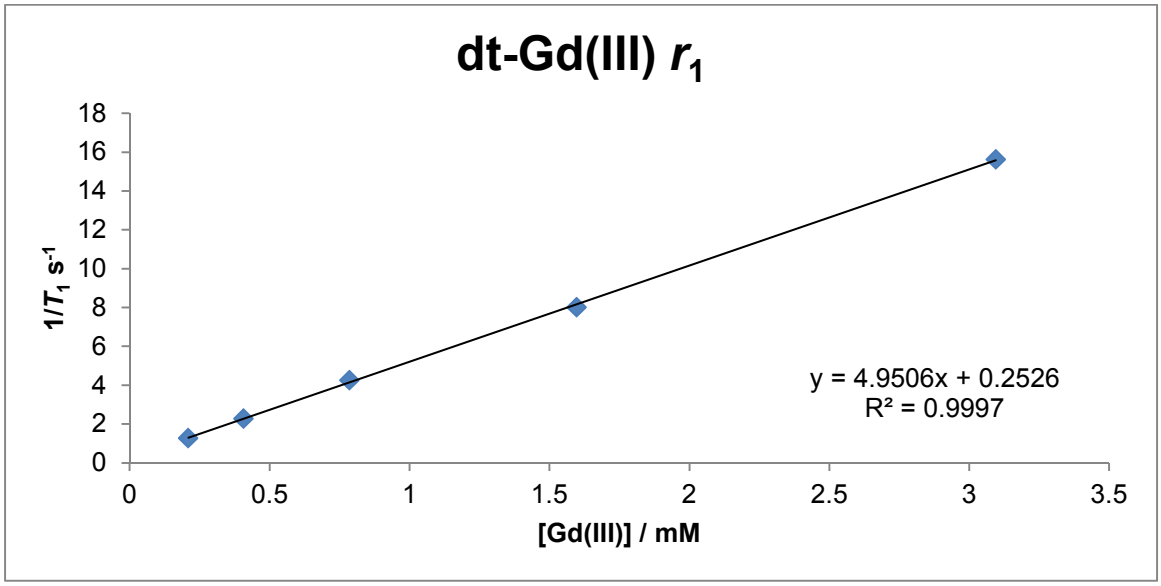


Figure S2. Example of r_1 relaxivity calculation for dt-Gd(III) (complex **4**)

Table S4. Measured values of T_2 and corresponding [Gd(III)] measured by ICP-MS for dt-Gd(III)

sample	[Gd] / mM	T_2 (ms)	T_2 (s)	1/ T_2
1	3.10	53	0.053	18.83
2	1.60	104	0.104	9.62
3	0.79	191	0.191	5.24
4	0.41	357	0.357	2.80
5	0.21	625	0.625	1.60

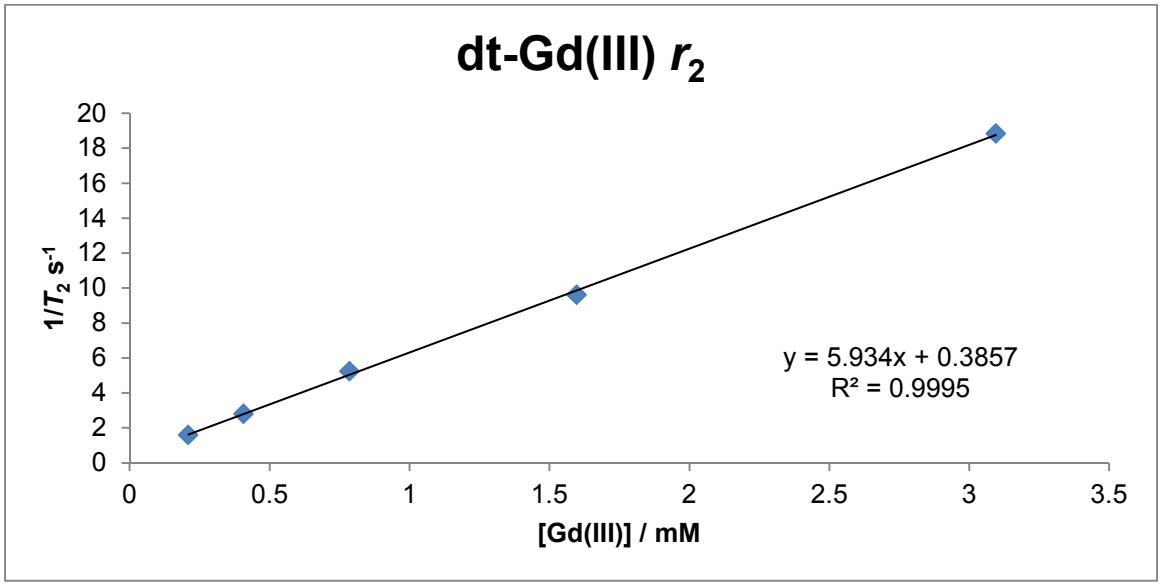


Figure S6. Example of r_2 relaxivity calculation for dt-Gd(III) (complex **4**)

***DNA synthesis**:*

Synthesis of oligonucleotides was performed on 3' disulfide C3 controlled pore glass beads (Glen Research). The 24mer poly-dT oligonucleotide were made using standard reagents with the single 5' modification added via the use of Cy3 phosphoramidite (Glen Research)

Deprotection of the oligonucleotide from the solid phase 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: 7998, calculated: 7991 $[M - H]^-$.

DNA used for synthesis of Gd(III)-DNA utilized the standard reagents from above, but included 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'. Further synthetic modification of this DNA was performed according to literature precedent.[2]

Nanoconjugate synthesis:

Poly-dT SNA conjugates were made by first deprotecting DNA using dithiothreitol, followed by purification on a GE NAP 5 column, and subsequent addition to spherical 13.0 ± 1.6 nm citrate capped gold nanoparticles. Nanocnojugates were salt aged and purified in an analogous procedure to that previously reported.[4]

Specifically, a standard synthesis was started by dissolution of 18.6 OD (260 nm) of DNA (corresponding to ~225 strands of DNA per nanoparticle) into 300 μL of 100 mM dithiothreitol in 180 mM (pH 8.0) phosphate buffer, and the solution was left to stir at room temperature for 1 hour. The DNA was then 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 32 mL of 13.3 nM nanoparticles in water is added 34.8 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 805, 824, 843, 862 and 882 μL, on per hour, with each addition followed by 30 seconds of sonication. Within the intervening time, the mixture was left to stir at room temperature. The final concentration of NaCl was 600 mM. The solution was left to stir a further 48 hours.

Purification of Poly-dT SNA conjugates was accomplished using three consecutive rounds of centrifugation (45 minutes at 15 x *g*) and resuspension until no further visible dye remained in the supernatant. Particle concentration was determined by ICP-MS by examination of Au content. DNA content per particle was quantified by dissolution of gold nanoparticle cores and UV/Vis analysis of Cy3 content according to a standard curve (see below). When not in use, particles are stored at 4 °C.

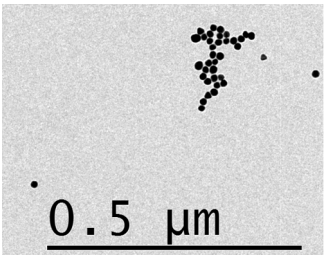
After synthesis and characterization, DLS and zeta-potential measurements of all nanoconjugates were conducted (Table S1). Zeta-potential of bare nanostars and spheres was measured in millipore water.

After Gd(III)-DNA conjugation, zeta-potential was measured in 1x DPBS (Gibco) to characterize the surface charge under physiological salt conditions.

Particle Synthesis and Characterization

Synthesis of Spherical Gold Nanoparticles: Gold nanoparticles were synthesized by citrate reduction of $HAuCl_4$, according to published procedures. The plasmon resonance wavelength was observed by UV/Vis spectroscopy and size was confirmed by DLS and TEM (Figure S3b).

Particle size was determined by analysis of over 200 particles using image J, and particle volumes and total gold content were approximated by the geometric formula for the volume of a sphere and the density of bulk gold, here resulting in a particle content of 67,881 gold atoms per AuNP.



Nanoconjugate relaxivity and Gd(III) loading:

Table S2. Measured values of T_1 and corresponding [Gd(III)] measured by ICP-MS for dt-Gd(III) pure particle conjugates

sample	[Gd] / mM	T_1 (ms)	T_1 (s)	1/ T_1
1	0.1218	424	0.424	2.36
2	0.0556	727.7	0.7277	1.37
3	0.0282	1242.7	1.2427	0.80
4	0.0140	1858.3	1.8583	0.54
5	0.0070	2527	2.527	0.40

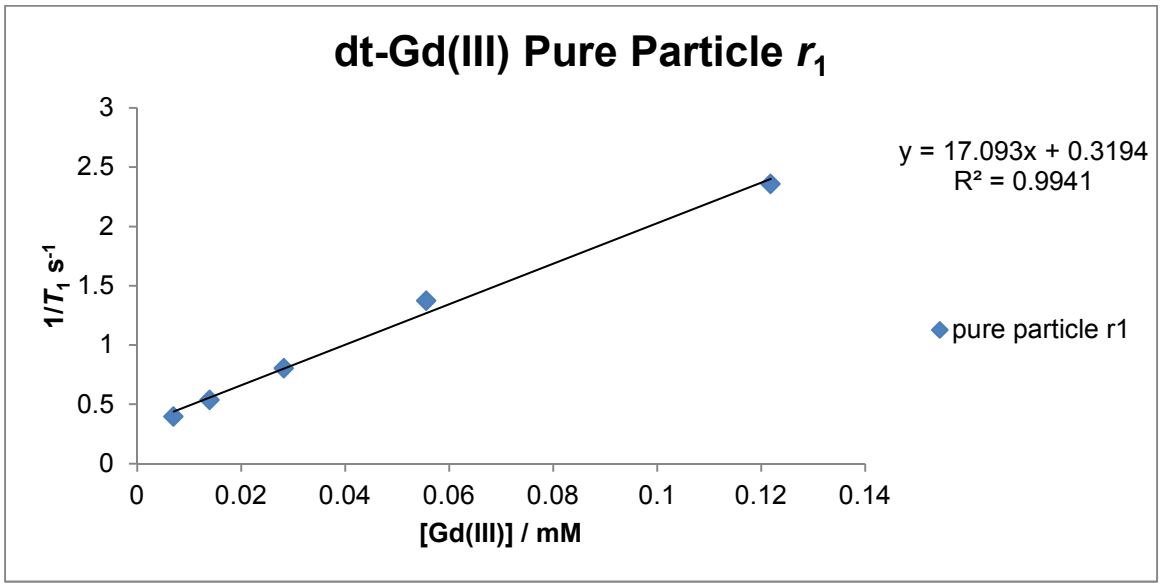


Figure S3. Example of r_1 relaxivity calculation for dt-Gd(III) pure particle conjugates

Table S3. Measured values of T_2 and corresponding [Gd(III)] measured by ICP-MS for dt-Gd(III) pure particle conjugates

sample	[Gd] / mM	T_2 (ms)	T_2 (s)	1/ T_2
1	0.1218	193.6	0.194	5.17
2	0.0556	344.8	0.345	2.90
3	0.0282	620.3	0.620	1.61
4	0.0140	965.5	0.966	1.04
5	0.0070	1396.2	1.396	0.72

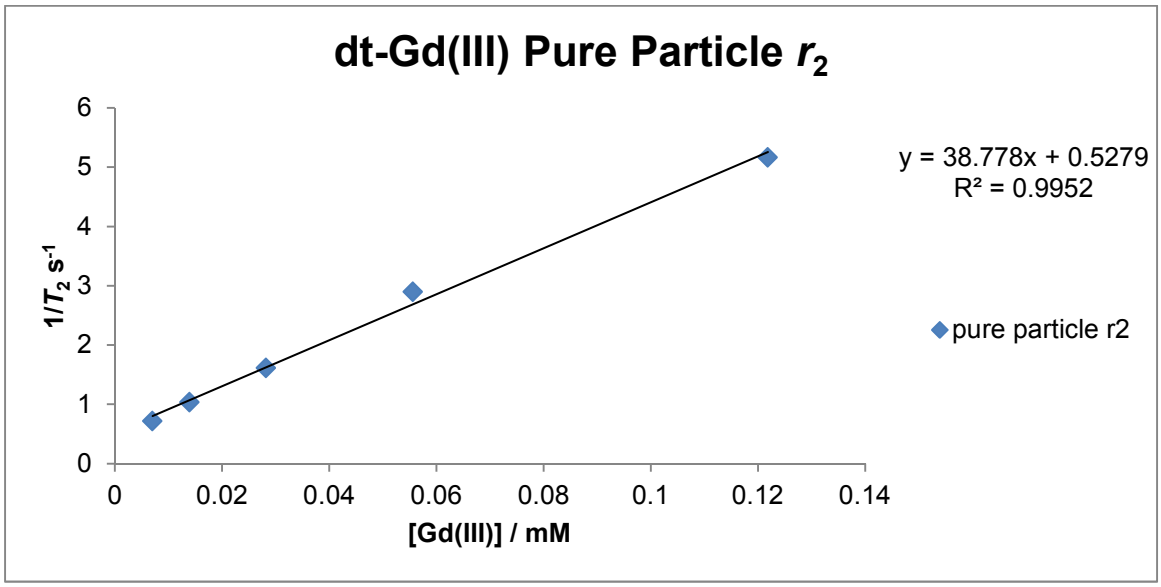


Figure S4. Example of r_2 relaxivity calculation for dt-Gd(III) pure particle conjugates

Table S4. 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	0.2741	265	0.265	3.77
2	0.1357	510.1	0.510	1.96
3	0.0639	909.7	0.910	1.10
4	0.0335	1487	1.487	0.67
5	0.0168	2188	2.188	0.46

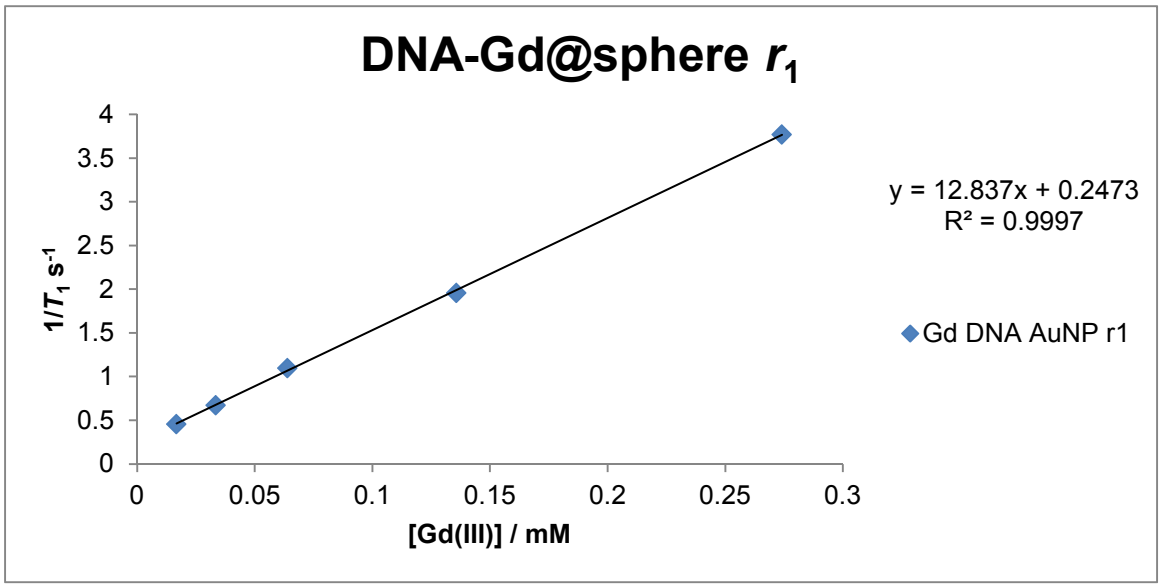


Figure S5. Example of r_1 relaxivity calculation for DNA-Gd@sphere conjugates

Table S5. 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	0.2741	126	0.126	7.94
2	0.1357	242.5	0.243	4.12
3	0.0639	454.1	0.454	2.20
4	0.0335	753.9	0.754	1.32
5	0.0168	1163	1.163	0.86

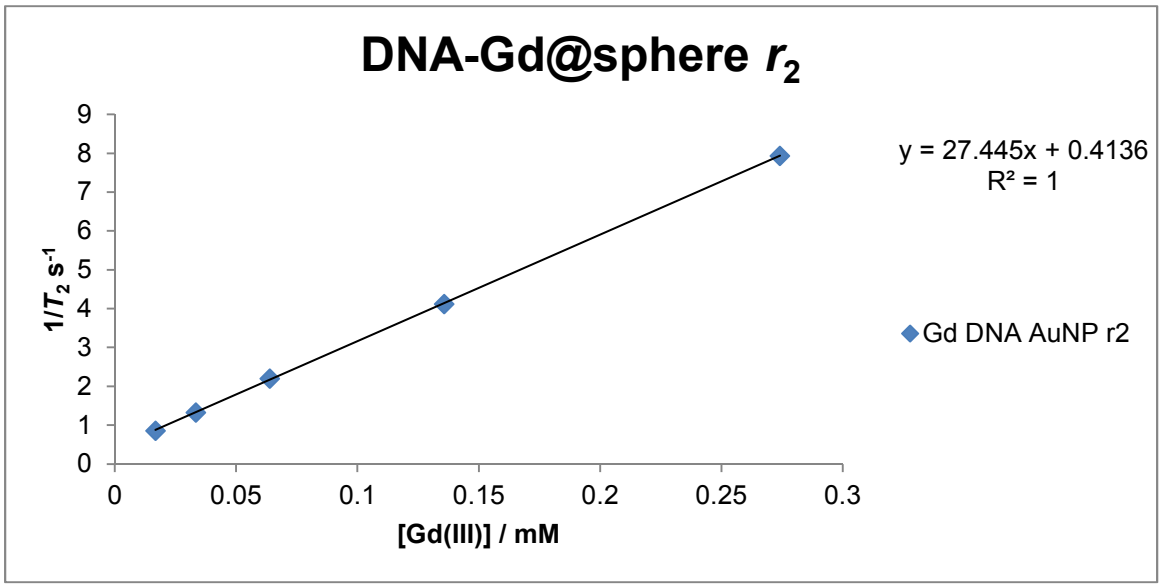


Figure S6. Example of r_2 relaxivity calculation for DNA-Gd@sphere conjugates

Table S6. Measured values of T_1 and corresponding [Gd(III)] measured by ICP-MS for dt-Gd(III) SNAs

sample	[Gd] / mM	T_1 (ms)	T_1 (s)	1/ T_1
1	0.405	108.9	0.109	9.18
2	0.200	214.1	0.214	4.67
3	0.098	423.3	0.423	2.36
4	0.049	726	0.726	1.38
5	0.026	1203	1.203	0.83

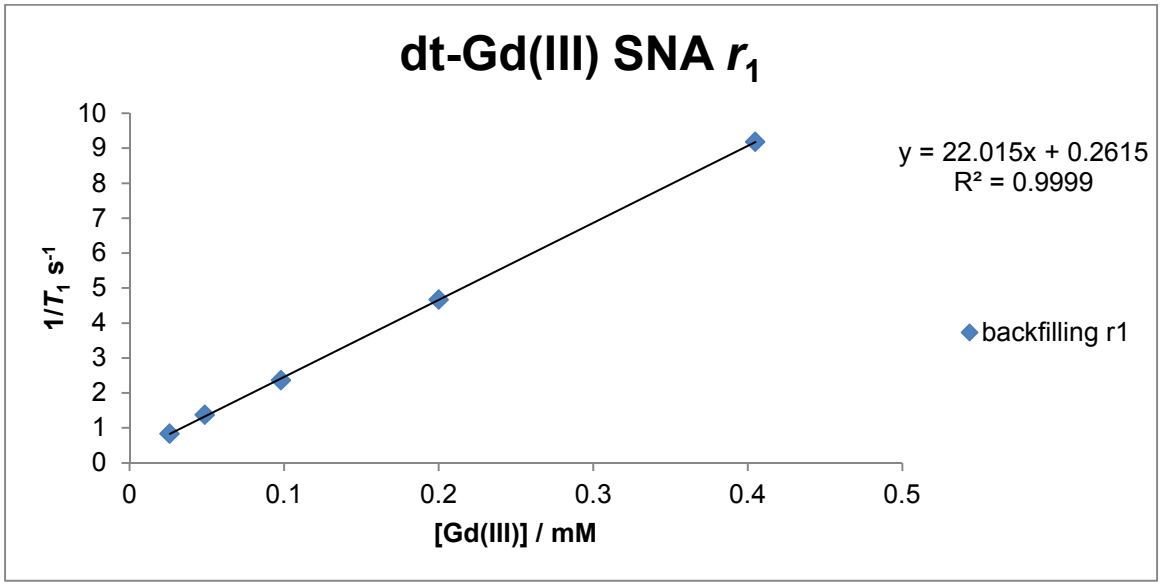


Figure S7. Example of r_1 relaxivity calculation for dt-Gd(III) SNA conjugates

Table S7. Measured values of T_2 and corresponding [Gd(III)] measured by ICP-MS for dt-Gd(III) SNAs

sample	[Gd] / mM	T_2 (ms)	T_2 (s)	1/ T_2
1	0.405	45	0.045	22.2
2	0.200	88	0.088	11.4
3	0.098	173.9	0.174	5.75
4	0.049	311.2	0.311	3.21
5	0.026	524.6	0.525	1.91

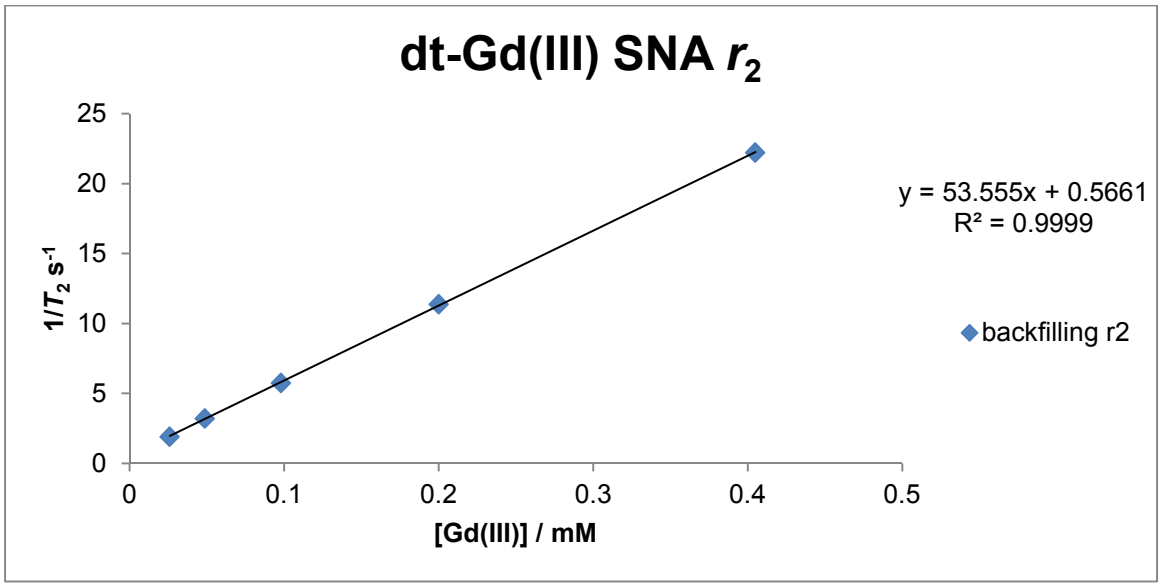


Figure S8. Example of r_2 relaxivity calculation for dt-Gd(III) SNA conjugates

Table S8. Particle loading data for dt-Gd(III) pure particle conjugates

Nanoconjugate Loading		
[Gd(III)][a]	**[AuNP]**[a,b]	**Gd(III)/AuNP**
0.121841	1.06E-04	1149.3
0.055599	5.02E-05	1107.4
0.028238	2.50E-05	1128.1
0.013956	1.25E-05	1114.6
0.007034	6.20E-06	1134.4
average loading		1126.8
standard deviation		16.5

[a]ICP results report concentrations in mM of metal [b]values include geometric approximation of gold atoms per spherical nanoparticle volume

Table S9. Particle loading data for DNA-Gd@sphere conjugates

Nanoconjugate Loading		
[Gd(III)][a]	**[AuNP]**[a,b]	**Gd(III)/AuNP**
0.27407	5.12E-04	535.2
0.13571	2.60E-04	521.9
0.06393	1.29E-04	494.1
0.03347	6.55E-05	511.2
0.01679	3.22E-05	522.0
average loading		516.9
standard deviation		15.3

[a]ICP results report concentrations in mM of metal [b]values include geometric approximation of gold atoms per spherical nanoparticle volume

Table S10. Particle loading data for dt-Gd(III) SNA conjugates

Nanoconjugate Loading		
[Gd(III)][a]	**[AuNP]**[a,b]	**Gd(III)/AuNP**
0.40500	5.46E-04	741.2
0.20007	2.70E-04	739.8
0.09789	1.33E-04	734.5
0.04866	6.81E-05	714.7
0.02592	3.57E-05	726.5
average loading		731.3
standard deviation		11.0

[a]ICP results report concentrations in mM of metal [b]values include geometric approximation of gold atoms per spherical nanoparticle volume

Surface coverage density analysis:

Table S11. Comparative surface densities of gold nanoparticle conjugates

	Surface Density (/nm^2)	
	Gd(III)	**DNA**
dt-Gd(III) pure particle	2.12	NA
Gd-DNA@spheres	0.97	0.19
dtGd(III) SNAs	1.38	0.38

Ligand density calculations use the geometric approximation of surface area based on the size of particles determined by TEM analysis (see above).

Poly dT particle loading:

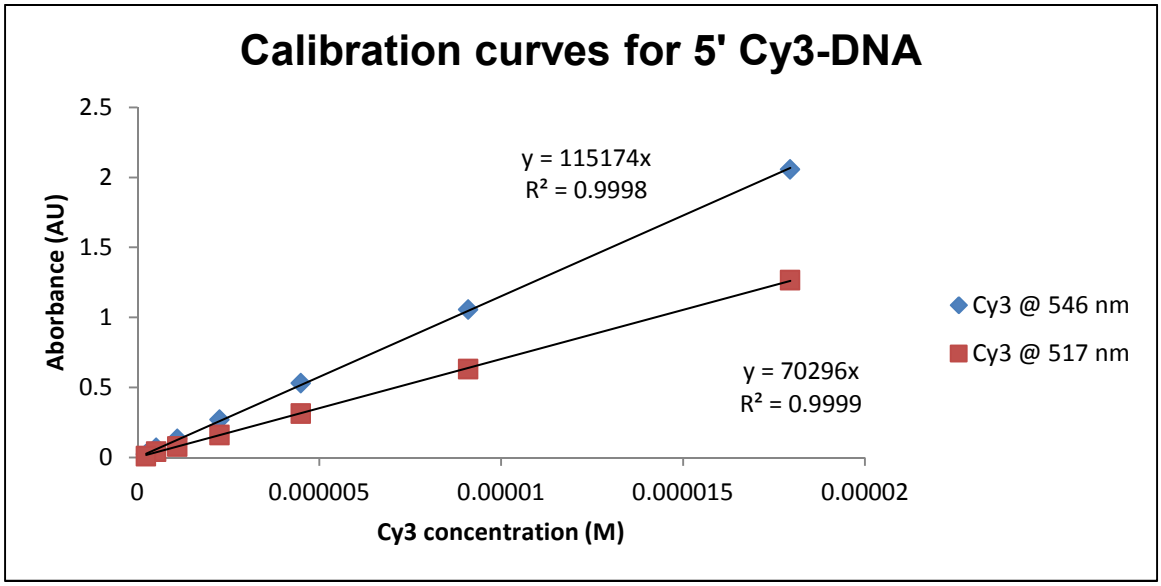


Figure S9. Calibration curves used to calculate DNA loading

For the purposes of quantifying fluorescently labeled DNA loading on SNAs bearing without Gd(III), a calibration curve was made which was standardized to Cy3-Gd(III)-DNA, where the stoichiometric ratio of Gd(III) to DNA is 5:1 (as synthesized and characterized prior, and used for the synthesis of DNA-Gd@spheres)[2] Specifically, an aliquot of DNA-Gd@spheres containing 677.5 nM particles (with a concentration 331.5 μM Gd(III) was diluted into 70 uL of solution containing 3.6 equivalents of potassium cyanide (roughly double of the molar ratio necessary for where 2 cyano groups coordinate per gold). Particles were allowed to digest at room temperature overnight until gold nanoparticle cores were completely digested. The concentration of KCN that should remain after complete dissolution of nanoparticle cores was calculated to 25 mM. All blanks, dilutions and subsequent unknowns were prepared such that the background concentrations amounted to 25 mM for standardization of conditions. From this stock of digested particles, 50 uL of solution was measured for UV/Vis absorbtion at 517 and 546nm, and 50 uL were used to serially dilute for a further 6 concentrations. Aliquots (5 μL) of each

solution used for UV/Vis absorbance were subsequently removed and digested for Gd(III) ICP analysis. Results were plotted as $1/5^{th}$ of Gd(III) concentration (due to the 5:1 stoichiometry between Gd(III) and Cy3) and analyzed by linear regression where the y-intercept is forced through zero. Using a 38.9 nM aliquot of post purified poly-dT SNAs, a similar 3.6 equivalents of KCN was added from an aqueous 450 mM KCN stock (such that remaining KCN totaled 25 mM), and sample was left to digest overnight. The dilution factor applied to the initial gold concentration by addition of KCN was recorded and the absorbances observed for 517 and 546 nm were used to calculate the concentration of DNA per gold core (see below).

Table S12. Calibration data and calculation of Cy3-labled oligonucleotide loading of poly-dT SNAs

Calibration Curve Raw Data			
[Gd(III)] / mM	**[Cy3] / mM**	**517 nm Absorbance**	**546 nm Absorbance**
0.0897	0.0179	1.266	2.058
0.0455	0.0091	0.632	1.056
0.0225	0.0045	0.313	0.530
0.0113	0.0023	0.160	0.270
0.0055	0.0011	0.077	0.133
0.0026	0.0005	0.041	0.068
0.0012	0.0002	0.009	0.022
poly-dT SNAs	**0.0072**	**0.507**	**0.834**

Kinetics of conjugation:

The particle loading resultant from the rate of backfilling were determined by performing a standard backfilling procedure while taking aliquots at specific time points. For accuracy, the standard purification procedure was used for each time point collected. Specifically, to 1 mL of 92.7 nM stock poly-dT SNAs DPBS was added 0.15 mg of complex **4** in aqueous 0.01% tween20. After 60 minutes, an aliquot of 100 μL was removed and diluted into 900 μL of DPBST (0.01% tween20). This solution was centrifuged for 45 minutes, (4 °C, 15 x *g*), and the supernatant removed. This process was repeated once more before the final diluted solution was analyzed by ICP for Gd(III)/Au content. This procedure was repeated as described for each of the remaining time points.

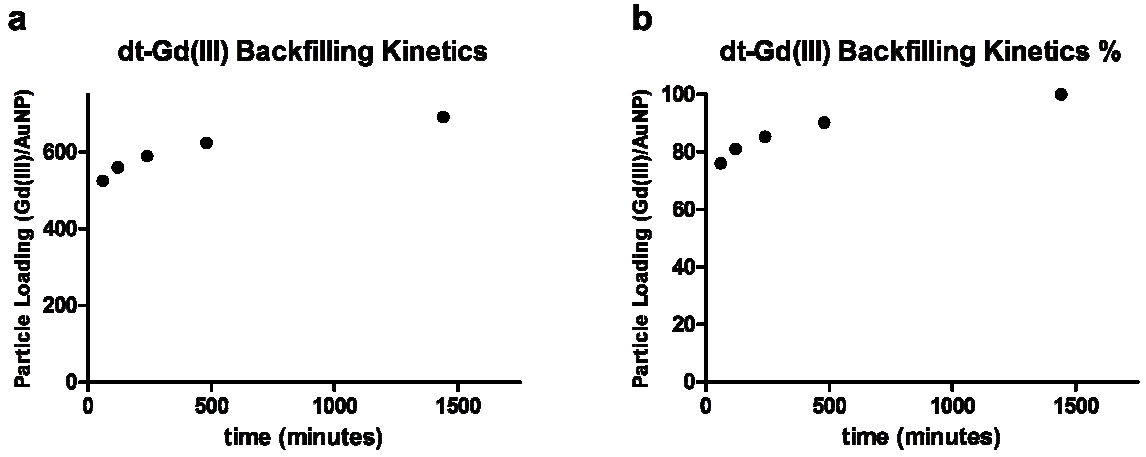


Figure S10. Observed loading of dt-Gd(III) over 24 hours by **a**) Gd(III)/AuNP and **b**) % of total.

Table S13. Backfilling kinetics for dt-Gd(III) SNA conjugates

Backfilling Kinetics			
Minutes	**[Gd(III)] / mM**	**[AuNP] / mM**	**Gd(III)/AuNP**
60	0.0107	2.04E-05	525.4
120	0.0112	1.99E-05	560.4
240	0.0121	2.05E-05	589.9
480	0.0110	1.76E-05	624.2
1440	0.0144	2.08E-05	691.3

Nanoconjugate stability:

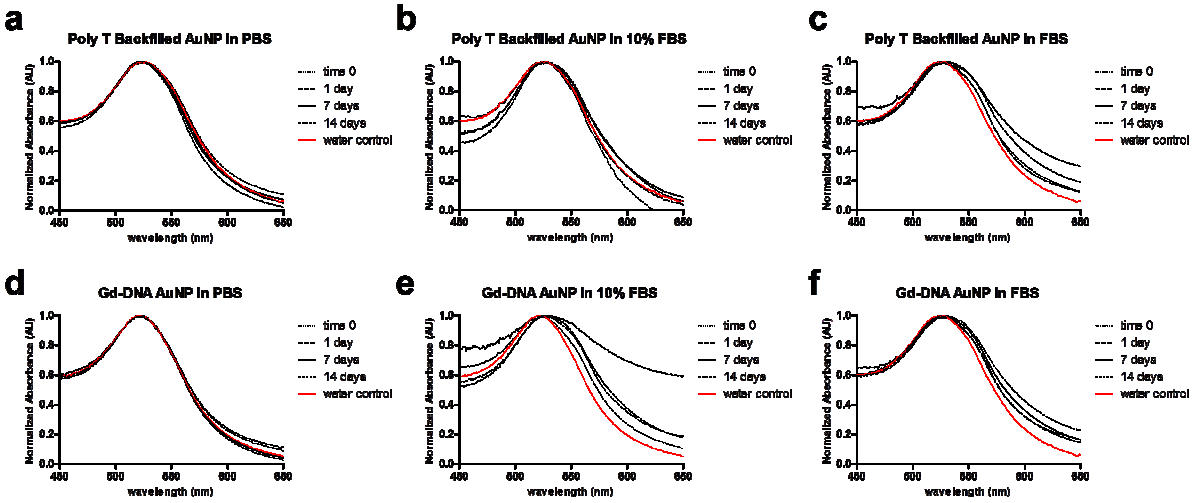


Figure S11. Colloidal stability of dt-Gd(III) SNAs and DNA-Gd@spheres measured under various conditions over 14 days at 37 °C. dt-Gd(III) SNAs were examined in (a) PBS, (b) 10% FBS and (c) FBS. DNA-Gd@spheres were examined in (d) PBS, (e) 10% FBS, and (f) FBS.

Table S14. Maximum surface plasmon resonance absorbance peaks for dt-Gd(III) SNAs over 14 days

Colloidal stability of dt-Gd(III) SNAs (λ_{max})					
Timepoint	**PBS (nm)**	**DMEM (nm)**	**10% FBS (nm)**	**FBS (nm)**	**water (nm)**
time 0	523	528	529	528	527
day 1	526	-	526	526	-
day 7	521	-	529	529	-
day 14	520	-	529	533	-

Table S15. Maximum surface plasmon resonance absorbance peaks for DNA-Gd@spheres over 14 days

Colloidal stability of DNA-Gd@spheres (λ_{max})					
Timepoint	**PBS (nm)**	**DMEM (nm)**	**10% FBS (nm)**	**FBS (nm)**	**water (nm)**
time 0	525	522	523	528	521
day 1	522	-	526	528	-
day 7	521	-	529	529	-
day 14	521	-	531	531	-

The colloidal stability of the dt-Gd(III) SNAs and DNA-Gd@spheres were measured under a range of cell culture related conditions over a period of 14 days at 37 °C. The experiment was conducted by diluting stocks of dt-Gd(III) SNAs and DNA-Gd@spheres into 1 mL of each of PBS, DMEM, 10%

FBS/DMEM and 100% FBS in a sterile environment. Each set of conditions was run in duplicate. At the time point specified, aliquots were taken from each tube and analyzed for colloidal stability by measurement of the maximum surface plasmon absorbance peak (Figure S11 and Tables S14, S15), and then centrifuged to remove gold. From these tubes was then removed an aliquot of supernatant to analyze Gd(III) content which was lost from the surface of the particles using ICP-MS. Gd(III) particle loading was quantified at time 0 for each condition, and the supernatant analysis data is reported as a percentage of Gd(III) present therein (necessarily lost from the particle surface) over the course of 14 days. Through the course of the experiment, each of the samples was stored in an incubator set to 37 °C.

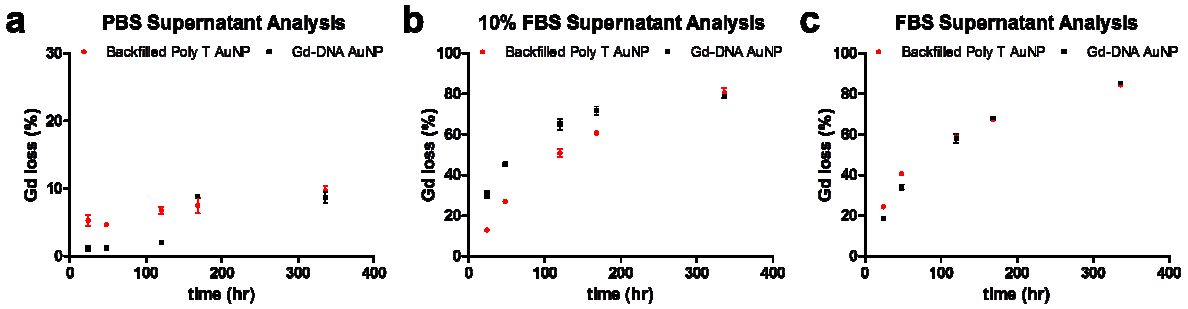


Figure S12. Complete supernatant analysis of dt-Gd(III) SNAs and DNA-Gd@spheres in (**a**) PBS, (**b**) 10% FBS and (**c**) FBS

Particle loading density analysis:

Table S16 Comparative surface densities of gold nanoparticle conjugates

	Surface Density (/nm^2)	
	Gd(III)	**DNA**
dt-Gd(III) particle	2.12	NA
Gd-DNA@spheres	0.97	0.19
dtGd(III) SNAs	1.38	0.38

Cell culture methods and additional cell uptake:

General Cell Culture. Dulbecco's modified phosphate buffered saline (DPBS), media, and dissociation reagents were purchased from Life Technologies (Carlsbad, CA). CorningBrand® cell culture consumables (flasks, plates, *etc.*) and sera were purchased from VWR Scientific (Radnor, PA). HeLa cells (ATCC® CCL-2) were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in phenol red-free minimum essential media (MEM) supplemented with 10% fetal bovine serum (FBS). Prior to all experiments, cells were plated and allowed to incubate for 24 hours before dosing. Cells were harvested with 0.25% TrypLE for 5 minutes at 37 °C in a 5.0% CO_2 incubator. For sterilization, all nanoconjugate solutions were filtered with 0.2 μm sterile filters prior to dosing. Cells were grown in a humidified incubator operating at 37 °C and 5.0% CO_2. Cell counting via Guava ViaCount Assay was performed as previously reported.[5]

Cellular Delivery Studies. Cellular delivery studies were performed with HeLa cells. For 24 hour uptake studies, HeLa cells were plated at a cell density of approximately 20,000 cells per well in a 24-well plate as counted by a hemocytometer. Stock solutions of dt-Gd(III) SNA and Gd-DNA@spheres (600 nM AuNP) were prepared by diluting with media to give a final concentration of 100 nM AuNP. This solution was serially diluted further with media to prepare incubating solutions of 180 μL per well. Cells were incubated with either dt-Gd(III) SNA and Gd-DNA@spheres for 24 h. To harvest, cells were rinsed in-plate three times with 500 μL PBS and trypsinized using 100 μL 0.25% TrypLE. Following trypsin treatment, 100 μL of media was added to each well. Subsequently, each well was transferred to a 1.5 mL Eppendorf tube, and centrifuged at 1000 × g at 4 °C for 5 minutes. After the cells were pelleted, the supernatant was decanted and 200 μL of media was added to each tube and mixed by a pipette to ensure that all cells were lifted into suspension. To determine cell uptake of Gd and Au, 50 μL of the cell suspension was used for cell counting and 130 μL was used for Gd and Au content analysis *via* ICP-MS.

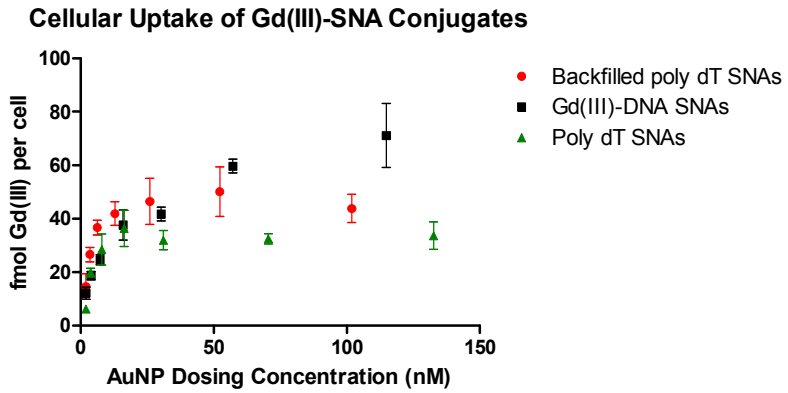


Figure S13. Cellular uptake studies performed standardized to gold nanoparticle uptake for DNA-Gd@spheres (**squares**), dt-Gd(III) SNAs (**circles**) and poly dT SNAs (**triangles**).

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