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Supporting Information

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Supporting Information

Multimodal Gadolinium-Enriched DNA Gold Nanoparticle Conjugates for Cellular Imaging

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Material and Methods

Scheme S1. Synthetic scheme of DNA-Gd(III) conjugate.

Figure S1. Characterization of DNA-Gd(III) conjugates by MALDI-MS.

Figure S2. Transmission electron micrographs of 13 nm AuNPs before and after conjugation to DNA-Gd(III). Scale bar is 20 nm.

Figure S3. Plots of relaxivities (r_1s) of DNA-Gd(III), 13 nm DNA-Gd(III)@AuNP, and 30 nm DNA-Gd(III)@AuNP at 60 MHz and 37 ºC in water. The slope of the linear line fit of $1/T_1$ (s^{-1}) versus mM Gd(III) is r_1.

Figure S4. T_1-weighted MR images of solution phantoms (in cell media) of 60 μM, 40 μM, and 20 μM [Gd(III)] DNA-Gd(III)@AuNPs and DOTA-Gd(III) at 600 MHz (14.1 T) and 25 °C. TE = 10.2 ms, TR= 1933.8 ms, FOV= 20 x 20 mm^2.

Table S1. T_1s of solution phantoms acquired in ***Supplementary Figure 4*** (14.1 T and 25 °C).

Figure S5. Plots of relaxivities (r_1s) of DOTA-Gd(III) and 13 nm DNA-Gd(III)@AuNPs at 14.1 T (600 MHz) and 25 °C. The slope of the linear line fit of $1/T_1$ (s^{-1}) versus mM Gd(III) is r_1.

Figure S6. T_1-weighted MR images of solution phantoms of 60 μM, 40 μM, and 20 μM [Gd(III)] DNA-Gd(III)@AuNPs and DOTA-Gd(III) in water at 3 T (127.6 MHz) and 25 °C. TE = 11 ms, TR = 500 ms, FOV = 75 x 100 mm^2. Accompanying table is the contrast to noise ratio (CNR) of the acquired image.

Table S2. T_1s of NIH/3T3 cell pellets (images from ***Figure 4***) incubated with 60 μM, 40 μM, and 20 μM [Gd(III)] DOTA-Gd(III) or DNA-Gd(III)@AuNPs for 24 hours. T_1s were taken at 14.1 T and 25 °C

Figure S7. Total number of nanoparticles (DNA-Gd(III)@AuNPs) taken up by NIH/3T3 and HeLa cells with varying time (**A**, incubation concentration was 6.5 μM) and incubation concentration (**B**, incubation time = 24 hours). Au ICP-MS data is acquired from samples in Figures 1 and 2. Conversion to # of nanoparticles was calculated as follows:

$$\frac{ng\ Au}{mL}\ x\ \frac{mole\ Au}{197\ g\ Au}\ x\ 3\ mL\ x\ \frac{10^{-9}g}{ng}\ x\ (dilution\ factor)\ x\ \frac{6.0221^{-23}}{moles\ Au}\ x\ \frac{AuNP}{65{,}800\ Au\ Atoms}$$

Figure S8. Analytical flow cytometry of NIH/3T3 and HeLa cells incubated with 0.15 nM (nanoparticle concentration) Cy3-DNA-Gd(III)@AuNPs for 1, 2, 4, and 24 hours. Error bars represent ± 1 standard deviation of the mean for duplicate experiments.

Figure S9. Percent cell viability of NIH/3T3 and HeLa cells incubated with 3.3, 6.5, 13, 26, and 52 μM [Gd(III)] DNA-Gd(III)@AuNPs and DOTA-Gd(III) for 24 hrs. Error bars represent ± 1 standard deviation of the mean for duplicate experiments.

Figure S10. Percent cell viability of NIH/3T3 and HeLa cells incubated with 6.5 μM DNA-Gd(III)@AuNPs and DOTA-Gd(III) for 1, 2, 4, 8, and 24 hours. Error bars represent ± 1 standard deviation of the mean for duplicate experiments.

Figure S11. Picture of DNA-Gd(III)@AuNPs in buffer after 7 months.

Table S3. Raw ICP-MS data and Au/Gd(III) ratio calculation for samples from ***Table 1***.

Table S4. Raw ICP-MS data for NIH/3T3 cell uptake of DNA-Gd(III)@AuNPS in *Figures 1 and 2*. Au/Gd(III) ratio was calculated via: $\left(\frac{\frac{ng}{mL}Au}{197\frac{g\,Au}{mole\,Au}}\right)/\left(\frac{\frac{ng}{mL}Gd(III)}{157.25\frac{g\,Gd(III)}{mole\,Gd(III)}}\right)$

Table S5. Raw ICP-MS data for HeLa cell uptake of DNA-Gd(III)@AuNPS in *Figures 1 and 2*. Au/Gd(III) ratio was calculated via: $\left(\frac{\frac{ng}{mL}Au}{197\frac{g\,Au}{mole\,Au}}\right)/\left(\frac{\frac{ng}{mL}Gd(III)}{157.25\frac{g\,Gd(III)}{mole\,Gd(III)}}\right)$

Material and Methods

Materials All reagents and solvents were purchased from Sigma-Aldrich unless otherwise noted. Citrate-stabilized AuNPs (13 ± 1.0 nm diameter) were prepared as described previously. AuNPs of 30 nm in diameter were purchased from Ted Pella Inc (USA). Oligonucleotides were synthesized on an Expedite 8909 Nucleotide Synthesis System (ABI) by standard solid-phase phosphoramidite synthesis techniques. All bases and reagents were purchased from Glen Research. The oligonucleotides were purified using reverse-phase high-performance liquid chromatography (RP-HPLC) using a Varian Microsorb C18 column (10 mm, 300 mm) with 0.03 M triethylammonium acetate (TEAA), at pH 7.0, and a 1.0% per min gradient of 95% CH_3CN/5% 0.03 M TEAA at a flow rate of 3 mL/min while monitoring the UV signal of DNA at 254 nm. After purification, the oligonucleotides were lyophilized and stored at -78 °C until use. Before nanoparticle conjugation, the 3-disulfide functionality was reduced with dithiothreitol (DTT) following published procedures.

Relaxivity (r_1)

A stock solution of DNA-Gd(III)@AuNPs was prepared in 200 μL of water, and diluted with 20 uL of water after each T_1 acquisition. T_1s were determined at 60 MHz (1.41 T) and 37 °C using an inversion recovery pulse sequence on a Bruker mq60 Minispec NMR spectrometer using 4 averages, 15 s repetition time, and 10 data points (Bruker Canada; Milton, Ontario, Canada). The starting and final Gd(III) concentrations of the solutions were determined using ICP-MS. The inverse of the longitudinal relaxation time ($1/T_1$, s^{-1}) was plotted against Gd(III) concentration (mM) and fitted to a straight line. Lines were fit with $R^2 > 0.99$.

General Cell Culture

NIH/3T3 and HeLa cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Media, Dulbecco's phosphate buffered saline (DPBS), and 0.25% trypsin/EDTA solutions were purchased from Invitrogen (Carlsbad, CA, USA). All Corning brand cell culture consumables (flasks, plates, and serological pipettes) were purchased from Fisher Scientific (Pittsburgh, PA). NIH/3T3 cells were cultured using DMEM (with 4 mM L-glutamine modified to contain 4.5 g/L glucose and 1.5 g/L sodium carbonate) supplemented with 10% CBS (ATCC). HeLa cells were cultured using EMEM (with Earle's balanced salt solution and 2.0 mM L-glutamine modified to contain 1.0 mM sodium pyruvate, 0.1 mM nonessential amino acids, and 1.5 g/L sodium bicarbonate) supplemented with 10% FBS (Mediatech, Manassas, VA, USA). All experiments were done in the aforementioned cell-specific media in a 5.0% CO_2 incubator operating at 37 °C. NIH/3T3 and HeLa cells were harvested using a 0.25% trypsin/EDTA solution. All compounds/nanoparticles incubation, leaching, and harvesting were carried out at 37 °C in a 5.0% CO_2 incubator unless otherwise specified.

Flow Cytometry

Cell Counting and Percent Cell Viability Determination Using a Guava EasyCyte Mini Personal Cell Analyzer (PCA) Flow Cytometry System

Cells were counted and percent cell viability determined via a Guava EasyCyte mini personal cell analyzer (Guava Technologies, Hayward, CA, USA). Specifically, after cell harvesting an aliquot (10 or 20 µL) of the cell suspensions were mixed with Guava ViaCount reagent (final sample volume of 200 µL) and allowed to stain at room temperature for at least 5 minutes (no longer than 20 minutes). Stained samples were then vortexed for 5 seconds, after which cells were counted and percent cell viability determined via manual analysis using the ViaCount software module. For each sample, 1000 events were acquired with dilution factors that were determined based upon optimum machine performance (~ 50 – 200 cells/µL). Instrument reproducibility was assessed daily using GuavaCheck Beads and following the manufacturer's suggested protocol using the Daily Check software module.

Assess percentage of cell labeling with Cy3 labeled Gd@AuNPs by flow cytometry

The uptake Cy3-DNA-Gd(III)@AuNPs was assessed using flow cytometry (BD LSR, BD Biosciences, San Jose, CA). NIH/3T3 cells were incubated with 0.15 nM Cy3-DNA-Gd(III)@AuNPs for 4.0 hours. Cells were then washed with PBS three times, followed by incubation with 2.5 µg/ml of Hoechst 33342 (nuclear counterstain) for 20 min at room temperature in dark. Following another PBS wash to remove excess Hoescht, cells were trypsinized and centrifuged at 200 x g and 25 °C to remove excess trypsin/EDTA. Cells were then re-suspended in 0.5 mL of PBS and assessed using flow cytometry. Dot plots were gated on FSC/SSC properties of NIH/3T3 cells to exclude free fluorescent labeled nanoparticles. Data were analyzed using BD FACSDiVaTM based software. Quadrant markers were set accordingly with controls.

Confocal Laser Scanning Microscopy (CLSM)

NIH/3T3 and HeLa cells were grown to 30% confluence (using 100 µL working volumes) on 8 chamber Lab-Tek® II German coverglass systems (Nalge Nunc International, Naperville, IL, USA). Cells were then incubated with 0.25 nM AuNP (12.5 nM Cy3) for 4.0 or 24 hours in phenol red free medium supplemented with serum (as described above). After AuNP incubation, cells were rinsed two times with DPBS followed by addition of 100 µL of fresh medium. Cells were then either prepared for imaging or incubated with fresh medium for 24 hours (at 37 °C and 5.0% CO_2, leached) followed by two DPBS rinses and addition of 100 µL of fresh medium and then prepared for imaging. Cells were prepared for imaging via labeling with 10 µM CellTracker® Green and 5 µM DAPI (Invitrogen, Carlsbad, CA, USA) in complete medium for 30 minutes (at 37 °C and 5.0% CO_2), medium was then aspirated, cells were rinsed two times with DPBS, followed by addition of 100 µL of fresh medium. Images were acquired on a Zeiss LSM 510 inverted microscope (computer controlled using Zeiss Zen software) equipped with a mode-locked Mai Tai DeepSee® Ti:sapphire two-photon laser (Spectra Physics, Mountain View, CA, USA). Specifically, DAPI was excited using 780 nm excitation wavelength (2-photon) at

8.4% laser power through a HFT KP 660 beamsplitter and imaged through a 435 – 485 nm IR bandpass filter (no pinhole). CellTracker® Green was excited using the 488 nm wavelength of an argon ion laser at 3.0% laser power through a HFT 488/543 beamsplitter and imaged with a PMT through a 500 – 550 nm IR bandpass filter (140 µm pinhole). Cy3 (AuNPs) was excited using the 543 nm wavelength of an He/Ne laser at 4.0% laser power through a HFT 488/543 beamsplitter and imaged with a PMT through a 560 – 615 nm IR bandpass filter (140 µm pinhole). An Appochromat water immersion objective (40X, NA 1.2) was used for all measurements. All images were acquired at 1024 x 1024 resolution with 15 z-stack slices.

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

Quantitation of Au and Gd was accomplished using ICP-MS of acid digested samples. Specifically, 50 µL of TraceSelect nitric acid (> 69%, Sigma, St. Louis, MO) was added to cell suspensions or media and placed at 65 °C for at least 4 hours to allow for complete sample digestion. 50 µL of TraceSelect HCl (fuming 37%, Sigma, St. Louis, MO) was then added to each sample for long term sample stability and elimination of matrix effects. Nanopure H_2O and multi-element internal standard were added to produce a solution of 1.5% nitric acid (v/v), 1.5% HCl (v/v) and 5.0 ng/mL internal standard up to a total sample volume of 3 mL. Individual Au and Gd(III) elemental standards were prepared at 0.500, 1.00, 5.00, 10.0, 25.0, 50.0, 100, and 250 ng/mL concentrations with 1.5% nitric acid (v/v), 1.5% HCl (v/v) and 5.0 ng/mL internal standards up to a total sample volume of 10 mL.

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. 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).

MR imaging and T_1 analysis

14.1 T MR imaging and T_1 measurements were performed on a General Electric/Bruker Omega 600WB 14.1 T imaging spectrometer fitted with accustar shielded gradient coils at 25 °C. For solution phantoms, 50 µL of 60, 40 and 20 µM Gd(III) (DOTA-Gd(III) and DNA-Gd(III)@AuNP) in complete cell media were added to flame-sealed 5¾" Pasteur pipettes and centrifuged at 4.0 °C and 100 x *g* for 5.0 minutes. Capillaries were then placed in a custom-made glass capillary holder and imaged in a 20 mm birdcage coil. For cell phantoms, ~ 1.5 x 10^6 NIH/3T3 cells were incubated with 20 or 5.0 µM [Gd(III)] concentrations of Gd(III)@AuNP or DOTA-Gd(III) for 24 hours, rinsed two times with DPBS, and harvested with trypsin. After addition of complete media (1.0 mL total volume) cells were added to flame-sealed 5¾' Pasteur pipettes and centrifuged at 4.0 °C and 100 x *g* for 5.0 minutes. Capillaries were then placed in a custom-made glass capillary holder and imaged in a 10 mm birdcage coil. Spin lattice relaxation times (T_1) were measured using a saturation recovery pulse sequence with static TE (10.18 ms)

and variable TR (350, 500, 750, 1000, 1500, 2500, 4000, 7500, 15000 ms) values. Imaging parameters were as follows: field of view (FOV) = 10 x 10 mm^2 (20 x 20 mm^2 for solution phantoms), matrix size (MTX) = 256 x 256, number of axial slices = 4 (3 for solution phantoms), slice thickness (SI) = 1.0 mm, and averages (NEX) = 6 (2 for solution phantoms). T_1 analysis was carried out using the image sequence analysis tool in Paravision 3.0.2 software (Bruker BioSpin, Billerica, MA, USA) with monoexponential curve-fitting of image intensities of selected regions of interest (ROIs) for each axial slice.

3 T MR images were acquired on a Siemens 3 T TIM Trio imaging system using a 35 mm diameter mouse body coil. 200 uL samples of 60, 40 and 20 μM Gd(III) concentration of DOTA-Gd(III) and Gd(III)@AuNP solutions were placed in wells of a 96-well plate alongside 200 uL samples of unlabeled AuNP and water. Samples were imaged at ambient temperature (~25 ºC) using a T_1-weighted spin echo sequence with TR = 500 ms, TE = 11 ms, FOV = 27 x 100 mm^2, imaging matrix size = 192 x 259, slice thickness = 2 mm, and 4 signal averages.

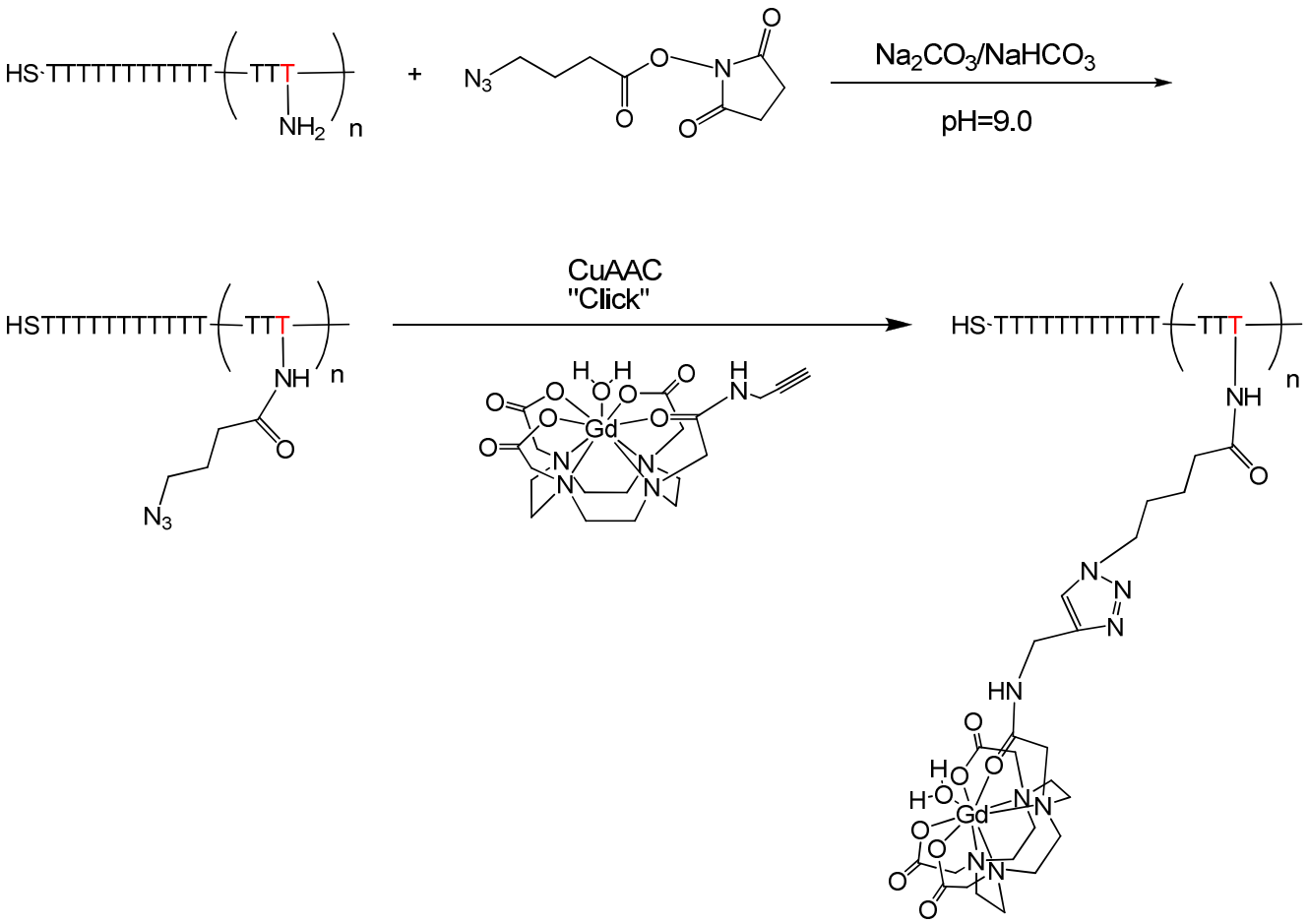
Synthesis of amine-modified oligonucleotides. Oligonucleotides (3' SH-T9TTTNH2 TTT NH2TTT NH2TTTNH2TTTNH2 5') were prepared by the conventional phosphoamidite method on 3'-thiol modifier C6 controlled pore glass supports (1.0 μmol) using an Expedite 8909 Nucleotide Synthesis System (ABI). To incorporate the amino group into the oligonucleotides, amino-modifier C6 dT phosphoamidite (TNH_2) (Glen research, USA) were used during the DNA synthesis. After automated synthesis, the glass supports were treated with a mixture of saturated 30% ammonia (aq.) at 55 °C for 16 hours. Detached and deprotected oligonucleotides were evaporated to dryness, dissolved in water, and purified by RP-HPLC. The oligonucleotides were characterized by MALDI-MS. The concentrations of oligonucleotides were determined by monitoring the absorbance at 260 nm with a UV- Cary 5000 spectrophotometer.

Synthesis of azido-modified oligonucleotides. Azido-modified oligonucleotide can be obtained by conjugating post-synthesis of an amino-modified oligonucleotide with an azide N-hydroxysuccinimide (NHS) ester, azidobutyrate NHS Ester (Glen Research, USA). Lyophilized amino-modified oligonucleotide (1 μmol) was dissolved in 0.5 mL of 0.1M Na_2CO_3/$NaHCO_3$ buffer (pH 8.5). To this solution, excess of azide N-hydroxysuccinimide (NHS) ester (5 mg) in 100 μL of DMSO was added. The resulting mixture was incubated overnight at room temperature, purified by RP-HPLC and characterized by MALDI-MS.

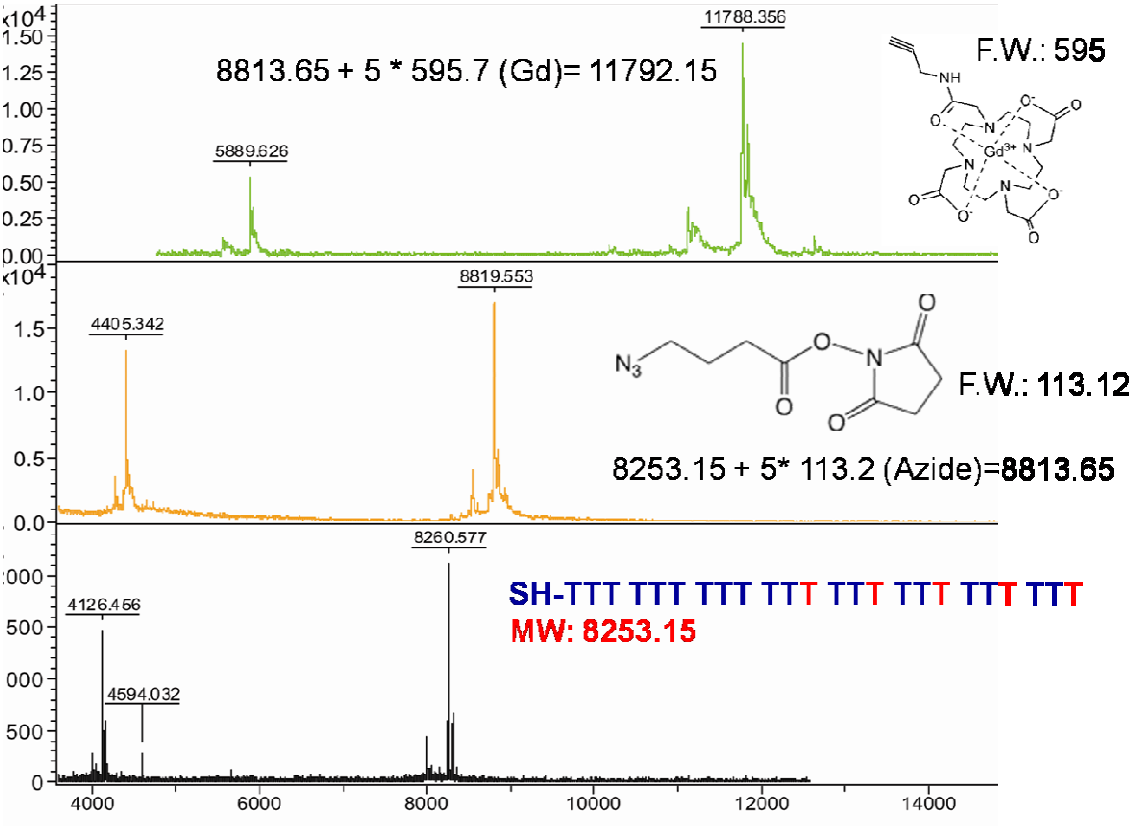
Synthesis of DNA-Gd(III) conjugates. The Gd(III)-modified oligonucleotides were synthesized by coupling an azido-modified oligonucleotide and an alkyne-modified Gd(III) chelate (**Gd595**) MRI contrast agent through a click chemistry approach. (**Gd595** was synthesized following procedures from Y. Song, E. K. Kohlmeir, T. J. Meade, *J. Am. Chem*. Soc. **2008**, *130*, 6662.) To 950 μL 0f 0.20 M aqueous NaCl Tris-hydroxypropyl triazolyl ligand (2.0 μmol), sodium ascorbic acid (2.0 μmol) and copper (II) sulphate pentahydrate (0.40 μmol), Gd(III)-chelate (10 mg) were added sequentially. The above solution was added to lyophilized azido-modified oligonucleotide (1.0 μmol) and incubated for 2.0 hours to allow for the click-chemistry ligation to occur. The oligonucleotides were purified using a NAP^{TM}-5 column (GE healthcare, U. S. A),

and followed by running through a RP-HPLC. The final conjugates were characterized with MALDI-MS.

Preparation of DNA-Gd(III)@AuNP conjugates. The 13 nm AuNPs were synthesized and functionalized with oligonucleotides according to previously reported methods from our group. 30 nm AuNPs were purchased from Ted Pella (Redding, CA). AuNPs were functionalized with alkanethiol-modified oligonucleotides. Prior to use, the disulfide functionality on the oligonucleotides was cleaved by addition of DTT to lyophilized DNA and the resultant mixture incubated at room temperature for 2.0 hours (0.1 M DTT, 0.18 M phosphate buffer (PB), pH 8.0. The cleaved oligonucleotides were purified using a NAP^{TM}-5 column. Freshly cleaved oligonucleotides were added to AuNPs (1 OD/1.0 mL), and the concentrations of PB and sodium dodecyl sulfate (SDS) were brought to 0.01 M and 0.01%, respectively. The oligonucleotide/AuNPs solution was allowed to incubate at room temperature for 20 min. The concentration of NaCl was increased to 0.10 M using 2.0 M NaCl, 0.01 M PBS while maintaining an SDS concentration of 0.01%. The final mixture was brought to 0.10 M NaCl over 24 hours and shaken for an additional 24 hours to complete the process. The final conjugates was stored in buffer with excess DNA-Gd(III) strands at -4 ºC. Before use, the DNA-Gd(III)@AuNPs were span down and washed with fresh buffer.



Scheme S1. Synthetic scheme of DNA-Gd(III) conjugates.



C6-dT-NH2 458.41(NH2)

C6-SH CPG Reduced FW - 196.2 Unreduced FW - 328.1 (265.46)

C3-SH CPG Reduced FW - 154.12 Unreduced FW - 244.27 (181.3)

N3-NHS 113.12

dT 304.2

Gd595 595.7 (exact mass Maldi: 596.12)

SH-TTT TTT TTT TTT TTT TTT TTT TTT

MW=304.2*19+458.41*5+ 181.3 =5779.8+2292.05+ 181.3 =8071.85+181.3=8253.15

+N3-NHS

8253.15+113.12*5-5=8813.65

+Gd595

8813.65+595.7*5=11792.15

Figure S1. Characterization of DNA-Gd(III) conjugates by MALDI-MS.

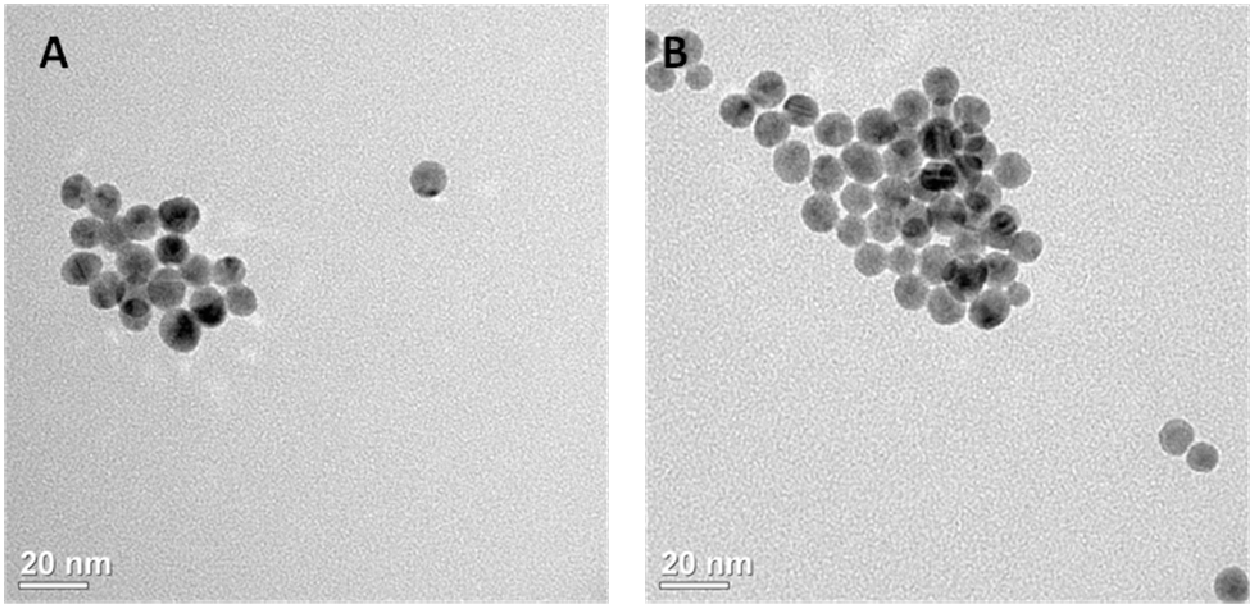


Figure S2. Transmission electron micrographs of 13 nm AuNPs before and after conjugation to DNA-Gd(III). Scale bar is 20 nm.

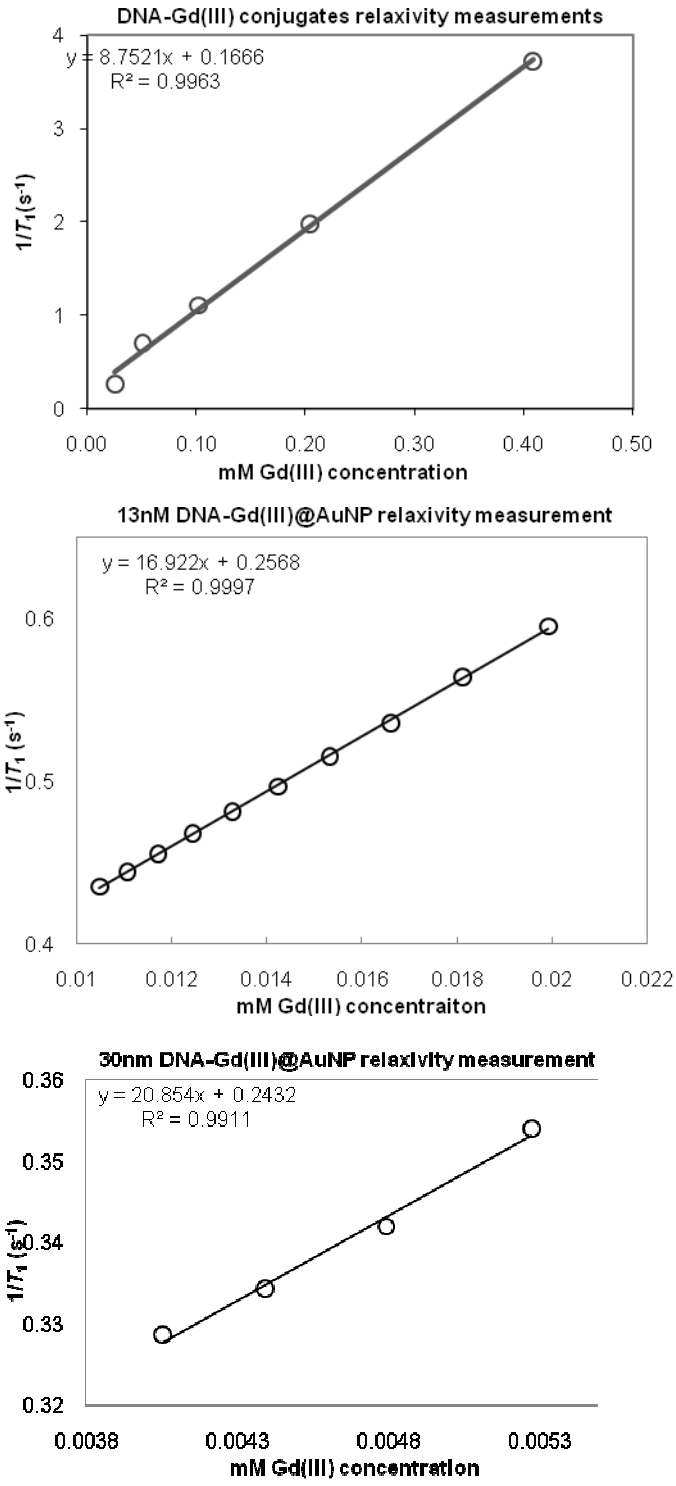


Figure S3. Plots of relaxivities (r_1s) of DNA-Gd(III), 13 nm DNA-Gd(III)@AuNP, and 30 nm DNA-Gd(III)@AuNP at 60 MHz and 37 ºC in water. The slope of the linear line fit of $1/T_1$ (s^{-1}) versus mM Gd(III) is r_1.

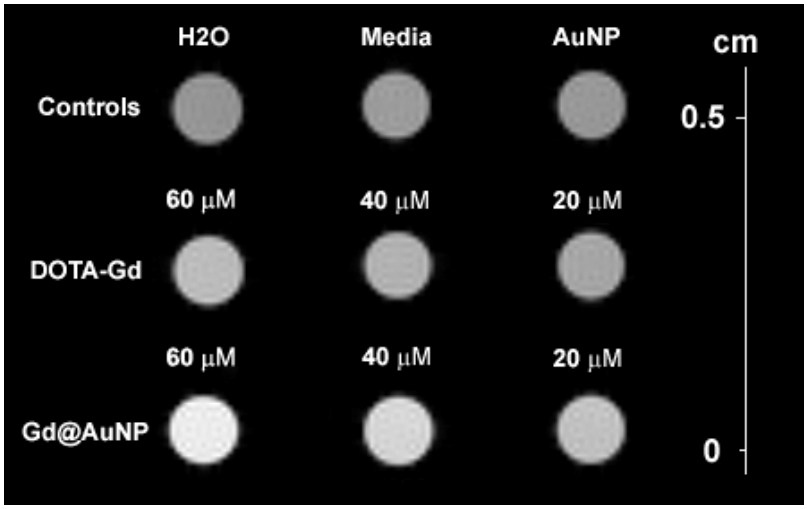


Figure S4. T_1-weighted MR images of solution phantoms (in cell media) of 60 μM, 40 μM, and 20 μM [Gd(III)] DNA-Gd(III)@AuNPs and DOTA-Gd(III) at 600 MHz (14.1 T) and 25 °C. TE = 10.2 ms, TR= 1933.8 ms, FOV= 20 x 20 mm^2.

Table S1. T_1s of solution phantoms acquired in ***Supplementary Figure 4*** (14.1 T and 25 °C).

				DOTA-Gd(III)			DNA-Gd(III)@AuNP		
	H_2O	media	AuNP	60 μM	40 μM	20 μM	60 μM	40 μM	20 μM
T_1 (ms)	3406.28	3297.32	3252.21	2353.63	2503.29	2932.95	1644	1984.82	2346.86
	3410.42	3288.79	3270.79	2361.03	2559.79	2953.92	1654.73	1995.24	2345.4
	3428.7	3330.03	3280.25	2357.62	2569.51	2948.33	1657.95	2003.59	2365.1
avg	3415.133	3305.38	3267.75	2357.427	2544.197	2945.067	1652.227	1994.55	2352.453
stvd	11.93003	21.76941	14.26505	3.703786	35.75802	10.8592	7.304152	9.404004	10.97664
%				69.0%	74.5%	86.2%	48.4%	58.4%	68.9%

		DOTA-Gd(III)		DNA-Gd(III)@AuNP	
mM	μM	T_1 (ms)	$1/T_1$ (s^{-1})	T_1 (ms)	$1/T_1$ (s^{-1})
0.06	60	2357.427	0.424191	1652.227	0.605244
0.04	40	2544.197	0.393051	1994.55	0.501366
0.02	20	2945.067	0.339551	2352.453	0.425088
0	0	3415.133	0.292814	3415.133	0.292814

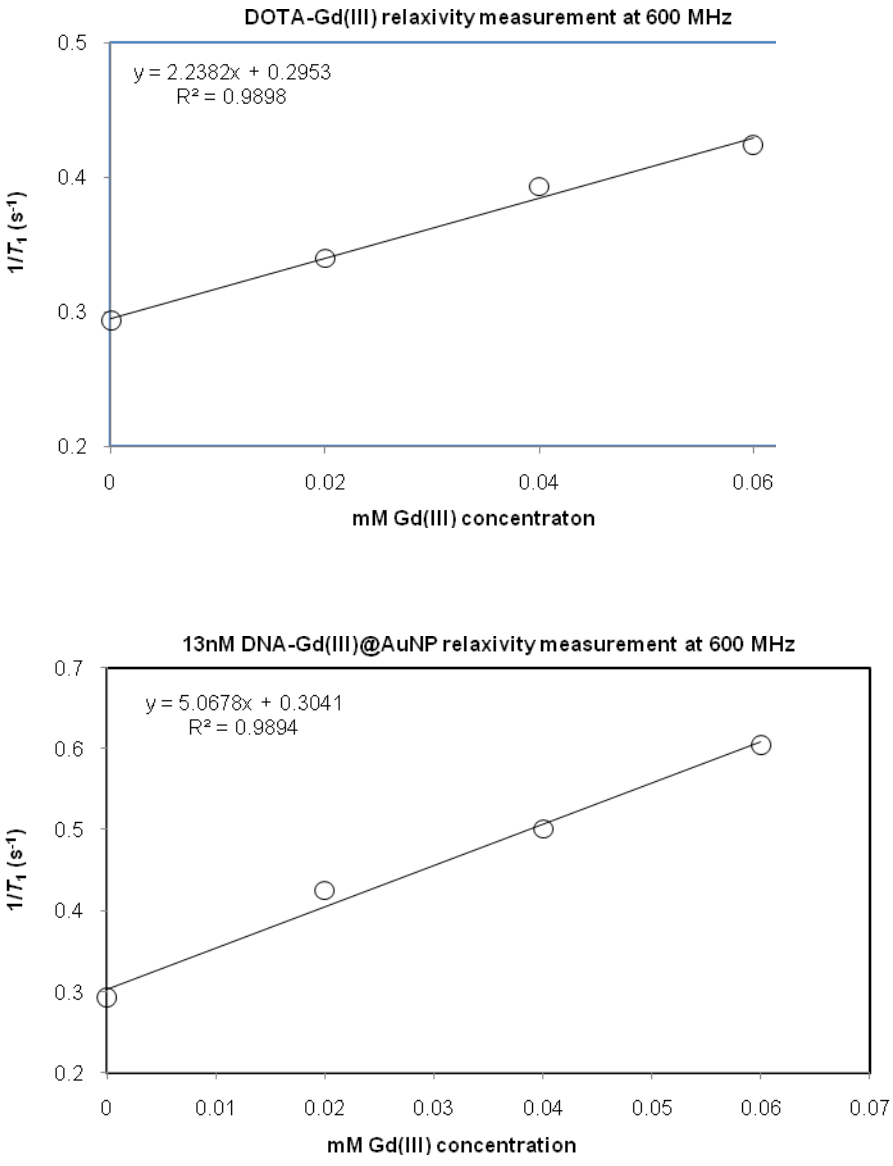
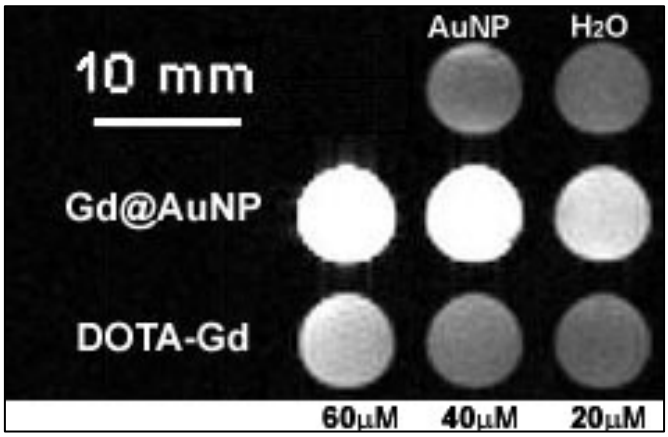


Figure S5. Plots of relaxivities (r_1s) of DOTA-Gd(III) and 13 nm DNA-Gd(III)@AuNPs at 14.1 T (600 MHz) and 25 $^{\circ}$C. The slope of the linear line fit of $1/T_1$ (s^{-1}) versus mM Gd(III) is r_1.

Table S2. T_1s of NIH/3T3 cell pellets (images from ***Figure 4***) incubated with 60 μM, 40 μM, and 20 μM [Gd(III)] DOTA-Gd(III) or DNA-Gd(III)@AuNPs for 24 hours. T_1s were taken at 14.1 T and 25 °C.

slices	T_1 (ms)	Contol 1	Control 2	20 μM DNA-Gd(III)@AuNP	5 μM DNA-Gd(III)@AuNP	20 μM DOTA-Gd(III)	5 μM DOTA-Gd(III)
2		3299.45	3236.97	1843.53	2344.72	3237.05	3240.71
3		3299.06	3253.54	1850.02	2349.75	3257.35	3225.72
4		3261.97	3243.30	2002.52	2361.09	3188.95	3188.44
	avg.	3286.827	3244.603	1898.69	2351.853333	3227.783333	3218.29
	std. dev.	21.52739	8.361533	89.97795119	8.385239015	35.12895292	26.91545838
	% reduction			57.8%	71.6%	98.2%	97.9%



	[Gd] in DOTA-Gd(III) samples				[Gd] in DNA-Gd(III)@AuNP samples		
	AuNP	20 μM	40 μM	60 μM	20 μM	40 μM	60 μM
CNR vs water	0	0	3	13	17	45	86

Figure S6. T_1-weighted MR images of solution phantoms of 60 μM, 40 μM, and 20 μM [Gd(III)] DNA-Gd(III)@AuNPs and DOTA-Gd(III) in water at 3 T (127.6 MHz) and 25 °C. TE = 11 ms, TR = 500 ms, FOV = 75 x 100 mm^2. Accompanying table is the contrast to noise ratio (CNR) of the acquired image.

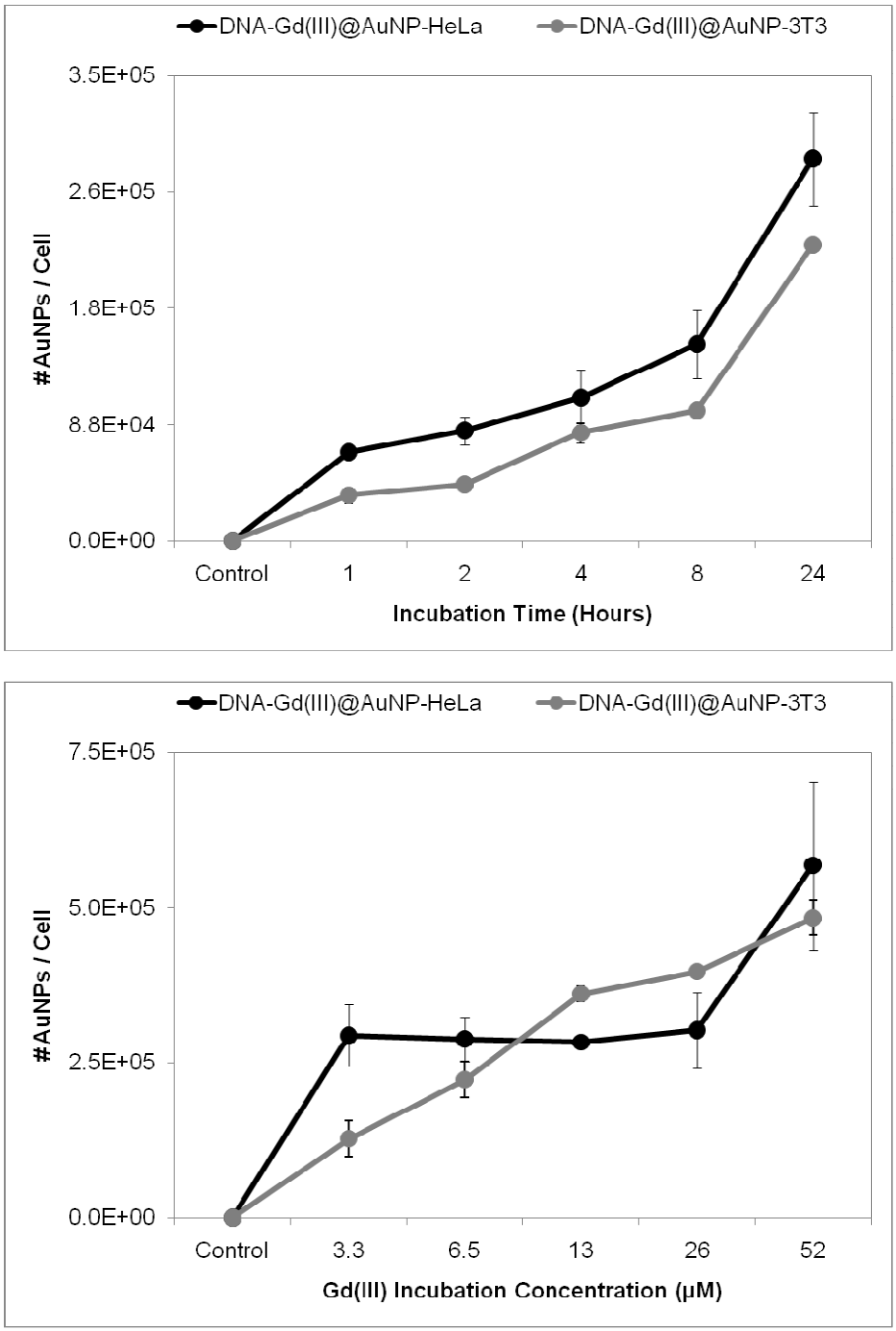


Figure S7. Total number of nanoparticles (DNA-Gd(III)@AuNPs) taken up by NIH/3T3 and HeLa cells with varying time (**A**, incubation concentration was 6.5 μM) and incubation concentration (**B**, incubation time = 24 hours). Au ICP-MS data is acquired from samples in Figures 1 and 2. Conversion to # nanoparticles was calculated as follows:

$$\frac{ng\ Au}{mL}\ x\ \frac{mole\ Au}{197\ g\ Au}\ x\ 3\ mL\ x\ \frac{10^{-9}g}{ng}\ x\ (dilution\ factor)\ x\ \frac{6.0221^{-23}}{moles\ Au}\ x\ \frac{AuNP}{65{,}800\ Au\ Atoms}$$

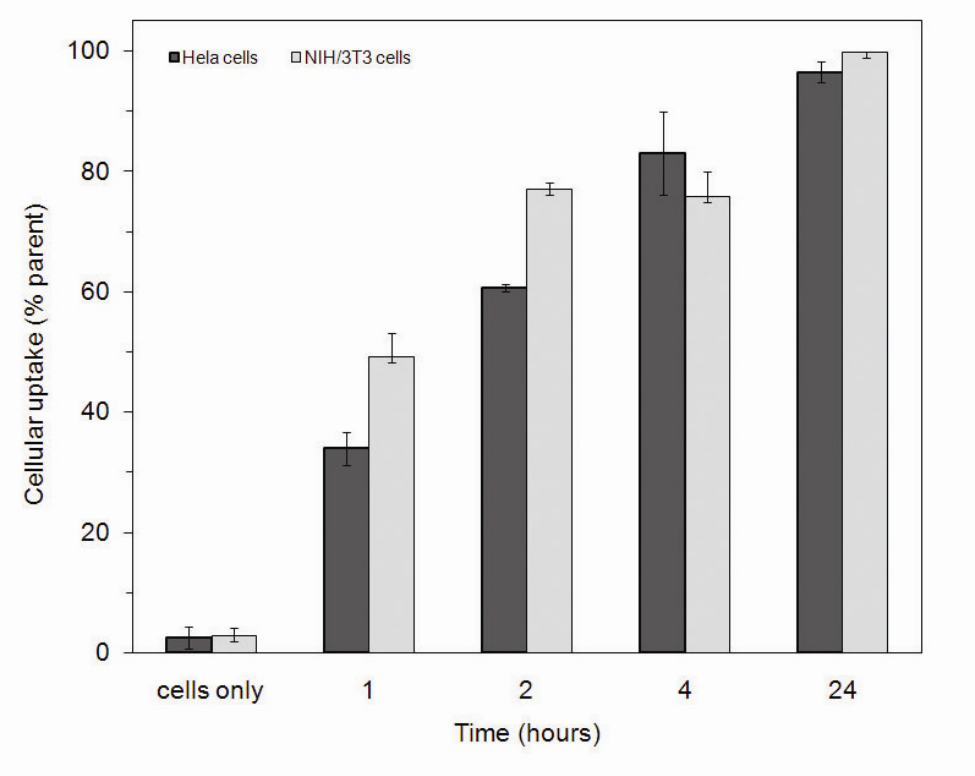


Figure S8. Analytical flow cytometry of NIH/3T3 and HeLa cells incubated with 0.15 nM (nanoparticle concentration) Cy3-DNA-Gd(III)@AuNPs for 1, 2, 4, and 24 hours. Error bars represent ± 1 standard deviation of the mean for duplicate experiments.

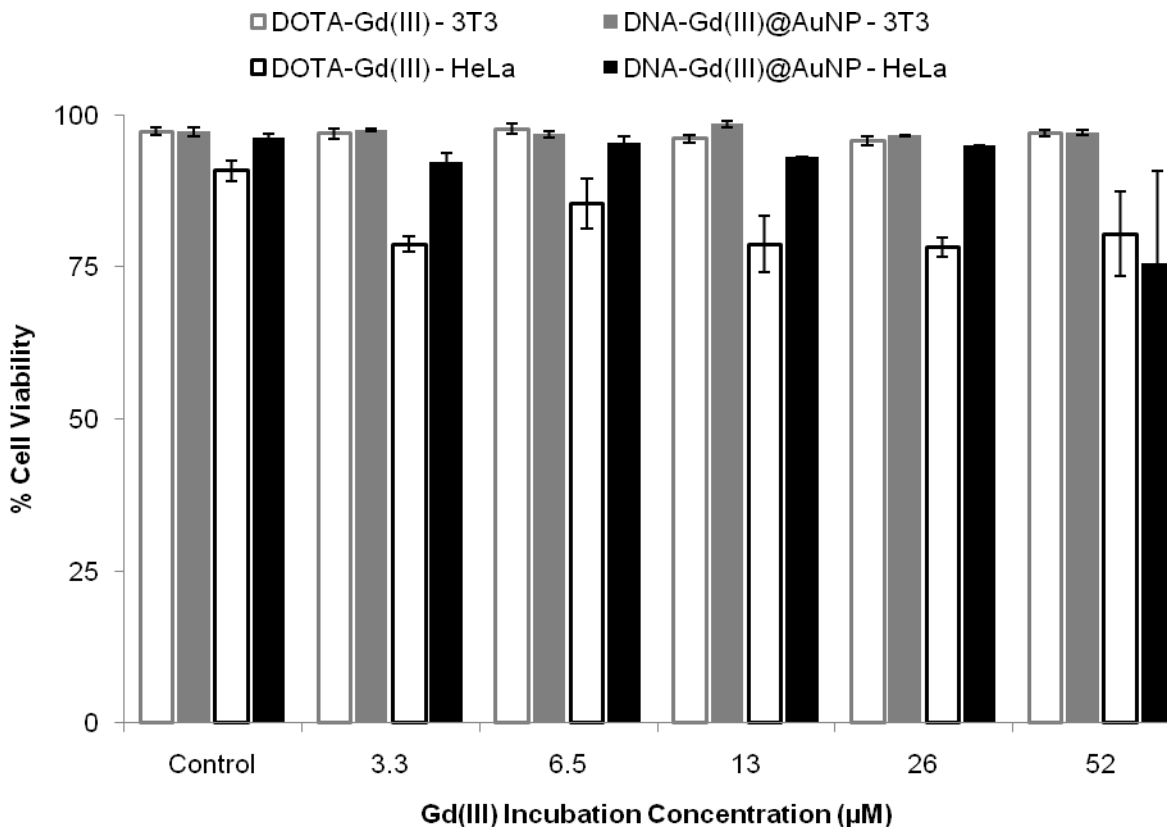


Figure S9. Percent cell viability of NIH/3T3 and HeLa cells incubated with 3.3, 6.5, 13, 26, and 52 μM [Gd(III)] DNA-Gd(III)@AuNPs and DOTA-Gd(III) for 24 hrs. Error bars represent ± 1 standard deviation of the mean for duplicate experiments.

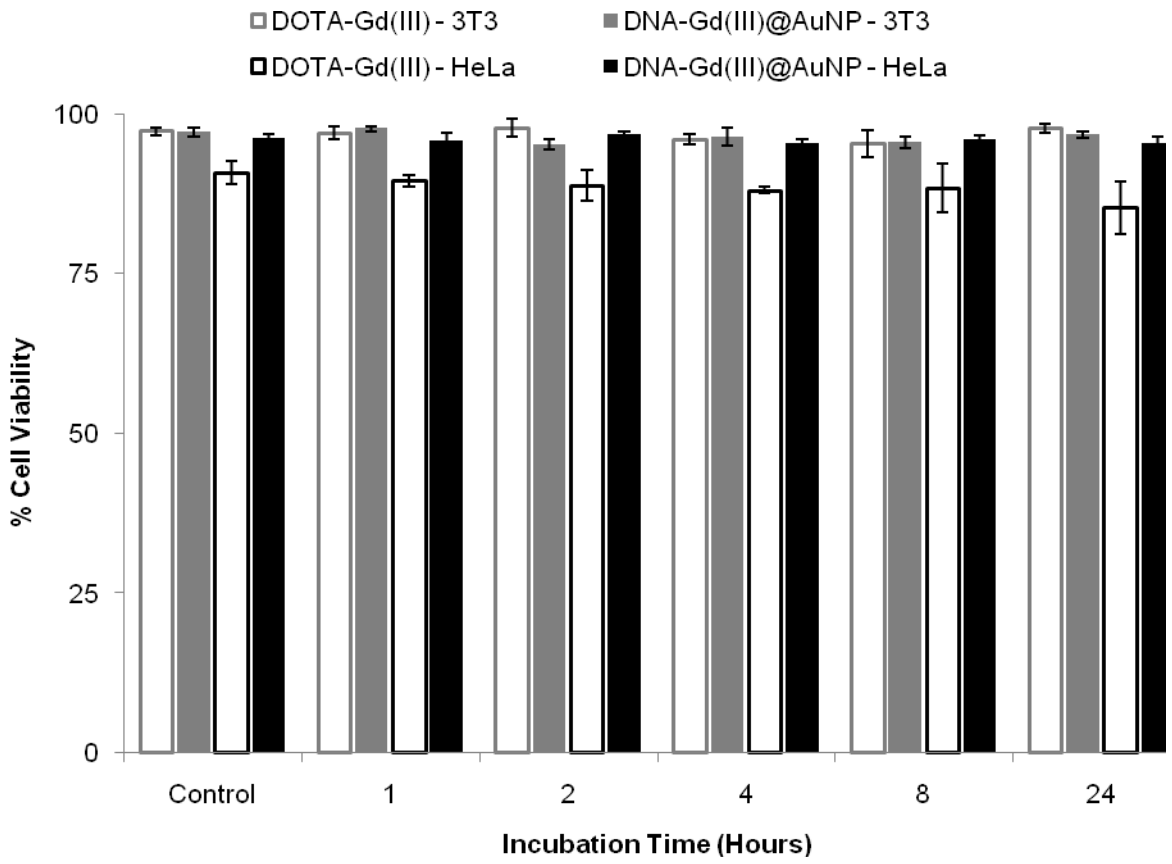


Figure S10. Percent cell viability of NIH/3T3 and HeLa cells incubated with 6.5 μM DNA-Gd(III)@AuNPs and DOTA-Gd(III) for 1, 2, 4, 8, and 24 hours. Error bars represent ± 1 standard deviation of the mean for duplicate experiments.

Figure S11. Picture of DNA-Gd(III)@AuNPs in buffer after 7 months.

Table S3. Raw ICP-MS data and Au/Gd(III) ratio calculation for samples from ***Table 1***.

13nm	1	2	
Gd ICP (ng/mL)	2.845	1.611	
Au ICP (ng/mL)	702.1	399.6	
Gd (nmol/mL)	0.018121019	0.010261146	
Au/ (nmol/mL)	5.27994E-05	3.00508E-05	Avg
# of Gd/AuNP	343.20503	341.4604493	342.3327

30 nm	1	2	
Gd ICP (ng/mL)	0.825	0.498	
Au ICP (ng/mL)	1237	778.9	
Gd (nmol/mL)	0.005254777	0.003171975	
Au/ (nmol/mL)	7.84321E-06	4.93862E-06	Avg
# of Gd/AuNP	669.9778446	642.2791609	656.1285

Table S4. Raw ICP-MS data for NIH/3T3 cell uptake of DNA-Gd(III)@AuNPS in *Figures 1 and 2*. Au/Gd(III) ratio was calculated via: $\left(\frac{\frac{ng}{mL}Au}{197\frac{g\,Au}{mole\,Au}}\right)/\left(\frac{\frac{ng}{mL}Gd(III)}{157.25\frac{g\,Gd(III)}{mole\,Gd(III)}}\right)$

Sample	Gd(III) ICP-MS (ng/mL)	Avg. Gd(III) ICP-MS (ng/mL)	Au ICP-MS (ng/mL)	Avg. Au ICP-MS (ng/mL)	Au/Gd(III) Ratio
6.5 µM DNA-Gd(III)@AuNPs 1 hr (1)	0.713	0.793	118.8	137	138.0
6.5 µM DNA-Gd(III)@AuNPs 1 hr (2)	0.872		155.2		
6.5 µM DNA-Gd(III)@AuNPs 2 hr (1)	1.024	1.089	161	170.4	124.8
6.5 µM DNA-Gd(III)@AuNPs 2 hr (2)	1.155		179.8		
6.5 µM DNA-Gd(III)@AuNPs 4 hr (1)	1.966	2.014	312	319.9	126.8
6.5 µM DNA-Gd(III)@AuNPs 4 hr (2)	2.061		327.8		
6.5 µM DNA-Gd(III)@AuNPs 8 hr (1)	2.379	2.837	395	469.9	132.2
6.5 µM DNA-Gd(III)@AuNPs 8 hr (2)	3.294		544.9		
6.5 µM DNA-Gd(III)@AuNPs 24 hr (1)	5.248	5.323	941.3	968.05	145.2
6.5 µM DNA-Gd(III)@AuNPs 24 hr (2)	5.397		994.8		
3.3 µM DNA-Gd(III)@AuNPs 24 hr (1)	3.625	3.380	680.6	636.7	150.4
3.3 µM DNA-Gd(III)@AuNPs 24 hr (2)	3.134		592.8		
13 µM DNA-Gd(III)@AuNPs 24 hr (1)	8.779	9.052	1593	1633	144.0
13 µM DNA-Gd(III)@AuNPs 24 hr (2)	9.324		1672		
26 µM DNA-Gd(III)@AuNPs 24 hr (1)	7.772	9.791	1360	1722	140.4
26 µM DNA-Gd(III)@AuNPs 24 hr (2)	11.81		2083		
52 µM DNA-Gd(III)@AuNPs 24 hr (1)	12.21	11.51	2162	2060	143.0
52 µM DNA-Gd(III)@AuNPs 24 hr (2)	10.81		1957		

Table S5. Raw ICP-MS data for HeLa cell uptake of DNA-Gd(III)@AuNPS in *Figures 1 and 2*. Au/Gd(III) ratio was calculated via: $\left(\frac{\frac{ng}{mL}Au}{197\frac{g\,Au}{mole\,Au}}\right)/\left(\frac{\frac{ng}{mL}Gd(III)}{157.25\frac{g\,Gd(III)}{mole\,Gd(III)}}\right)$

Sample	Gd(III) ICP-MS (ng/mL)	Avg. Gd(III) ICP-MS (ng/mL)	Au ICP-MS (ng/mL)	Avg. Au ICP-MS (ng/mL)	Au/Gd(III) Ratio
6.5 µM DNA-Gd(III)@AuNPs 1 hr (1)	0.241	0.247	38.55	39.57	127.9
6.5 µM DNA-Gd(III)@AuNPs 1 hr (2)	0.253		40.59		
6.5 µM DNA-Gd(III)@AuNPs 2 hr (1)	0.279	0.269	50.64	48.63	144.3
6.5 µM DNA-Gd(III)@AuNPs 2 hr (2)	0.259		46.61		
6.5 µM DNA-Gd(III)@AuNPs 4 hr (1)	0.306	0.453	60.42	60.52	106.6
6.5 µM DNA-Gd(III)@AuNPs 4 hr (2)	0.599		60.62		
6.5 µM DNA-Gd(III)@AuNPs 8 hr (1)	0.416	0.432	89.39	92.05	170.1
6.5 µM DNA-Gd(III)@AuNPs 8 hr (2)	0.448		94.71		
6.5 µM DNA-Gd(III)@AuNPs 24 hr (1)	0.561	0.558	150.2	150.3	215.0
6.5 µM DNA-Gd(III)@AuNPs 24 hr (2)	0.555		150.3		
3.3 µM DNA-Gd(III)@AuNPs 24 hr (1)	0.494	0.551	151.8	167.7	243.0
3.3 µM DNA-Gd(III)@AuNPs 24 hr (2)	0.607		183.6		
13 µM DNA-Gd(III)@AuNPs 24 hr (1)	0.647	0.677	153.9	155.4	183.2
13 µM DNA-Gd(III)@AuNPs 24 hr (2)	0.707		156.9		
26 µM DNA-Gd(III)@AuNPs 24 hr (1)	0.955	0.948	177.7	176.0	148.2
26 µM DNA-Gd(III)@AuNPs 24 hr (2)	0.94		174.2		
52 µM DNA-Gd(III)@AuNPs 24 hr (1)	1.57	1.627	277.8	287.1	141.0
52 µM DNA-Gd(III)@AuNPs 24 hr (2)	1.683		296.3		