Electronic Supplementary Information

Additional Acknowledgements
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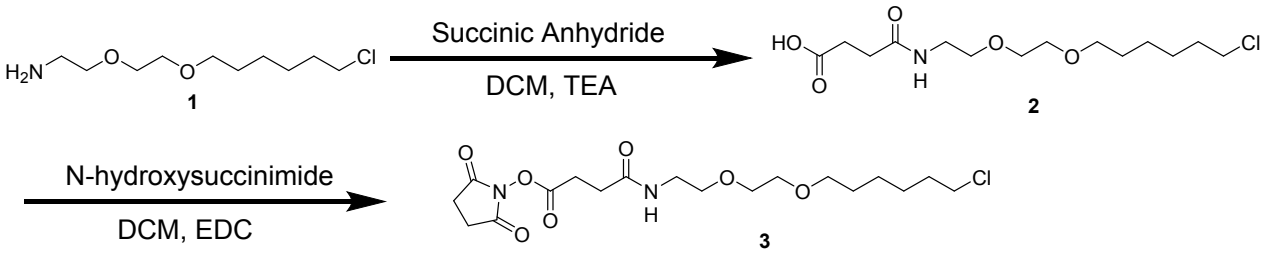
Materials and Methods
All reagents and solvents were purchased from Sigma-Aldrich unless otherwise noted. All chemical synthesis was performed under ambient conditions unless described explicitly. Chemical characterization was achieved using a Varian 500 MHz NMR, Bruker AutoFlex III MALDI spectrometer and a Bruker AmaZon SL LC-MS.

1. AuDNA-Gd(III)-HA synthesis:

1.1 DNA-Gd(III)-HA Synthesis:

Oligonucleotides were synthesized on solid phase controlled pore glass beads (CPGs) by standard techniques on a MerMade automated synthesizer. DNA purifications were performed on an Agilent dynamax 250 x 10 mm microsorb 300-10 C18 column using a linear gradient of 0% acetonitrile in 150 mM triethylammonium acetate buffer to 75% acetonitrile in 45 minutes on a Varian Prostar 500, with a flow rate of 3 mL/min.

Synthesis of the alkyne modified oligonucleotide was performed on 3' thiol modifier C3 disulfide controlled pore glass beads (Glen Research) using C8-alkyne dT phosphoramidite (Glen Research) for the five internal modifications, indicated as T*, and 5' amino modifier TEG phosphoramidite (Glen Research) indicated as NH_2. The synthesized oligonucleotide consisted of the sequence 3' – S-S-TTT-TTT-TTT-T*TT-T*TT-T*TT-T*TT-T*TT-T*TT–NH_2 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, purified by reverse phase HPLC, and characterized by MS-MALDI. (*m/*z) observed: 8244.8, calculated: 8248.0 $[M + H_3COO]^-$.

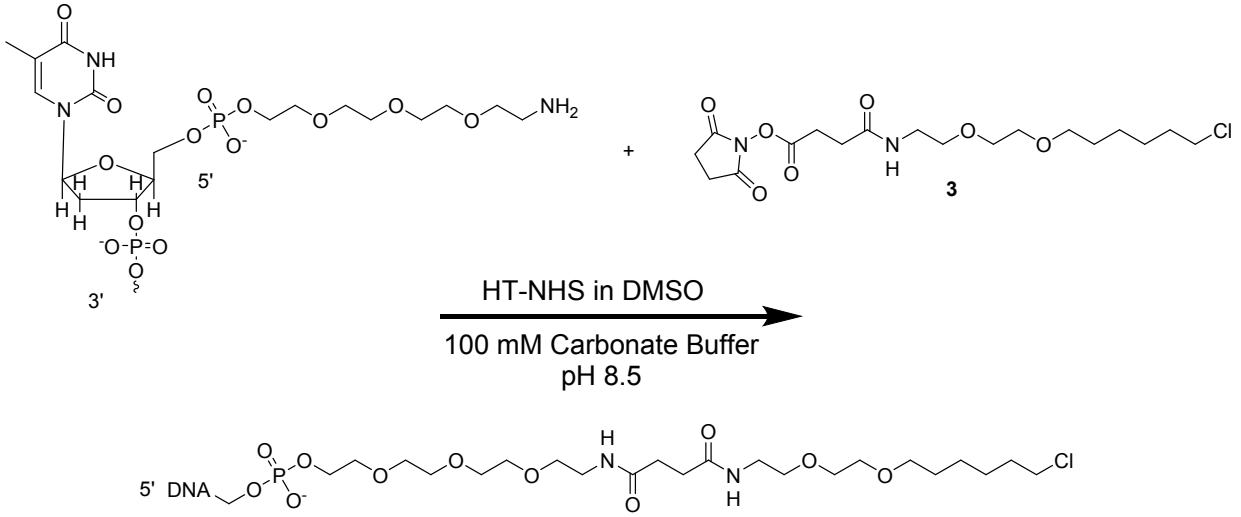


Scheme S1. Synthesis of HaloTag-targeting haloalkane

4-((2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)amino)-4-oxobutanoic acid (**2**) An amine terminated HaloTag-targeting group (**1**) was synthesized according to literature methods [1]. To a flame dried flask was added **1** (0.220 g, 0.983 mmol) and succinic anhydride (0.590 g, 5.90 mmol). The flask was then placed under vacuum for 60 minutes. The contents of the flask were dissolved in 50 ml dichloromethane (DCM) followed by the addition of triethylamine (0.121 g, 12.0 mmol). The solution was allowed to stir for 48 hours under nitrogen. The resulting mixture was concentrated *in vacuo*, diluted in 1 M HCl, and extracted into ethyl acetate (3 x 30 ml). The organic layer was dried over sodium sulfate and concentrated *in vacuo*. The resulting mixture was purified by reverse phase semi-preparative HPLC with an XBridge prep C18 column. The desired product

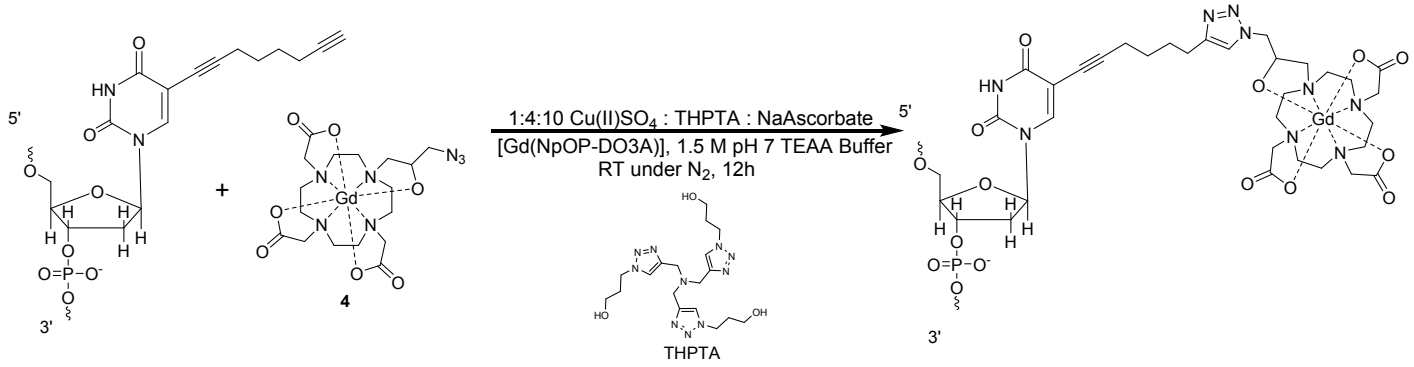
appears as a colorless oil. 1H NMR (500 MHz, Chloroform-d) δ 4.49 (s, 5H), 3.77 – 3.26 (m, 7H), 1.77 (s, 1H), 1.61 (s, 1H), 1.41 (d, J = 44.8 Hz, 2H). 13C NMR (126 MHz, CDCl3) δ 176.56, 173.09, 71.33, 70.10, 70.04, 69.64, 45.05, 39.34, 32.50, 31.68, 31.28, 29.29, 26.66, 25.35.

2,5-dioxopyrrolidin-1-yl 4-((2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)amino)-4-oxobutanoate (**3**) To a flame dried flask was added **2** (0.144 g, 0.445 mmol), n-hydroxysuccinimide (NHS) (0.153 g, 1.33 mmol), and 0.5 g sodium sulfate. The flask was then left under vacuum for 60 minutes and then placed under nitrogen. 30 ml of anhydrous DCM was added to the flask and allowed to stir for 30 minutes until the NHS was fully dissolved. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (0.104 g, 0.670 mmol) was injected into the flask and the solution was allowed to stir at room temperature for 16 hours. The resulting mixture was concentrated *in vacuo* yielding a dark brown oil. This oil was dissolved in a 1:1 suspension of water and ethyl acetate, extracted into ethyl acetate (3x3 ml), and characterized by ESI-MS. (m/z) observed: 443.21, calculated 443.17 $[M + Na]^+$. The resulting NHS-ester was concentrated *in vacuo* and used immediately.



Scheme S2. Attachment of HaloTag targeting functionality to 5' end of DNA

Addition of the HaloTag targeting functionality was performed off CPG and began with 1 μmol of 5x alkyne modifier-NH_2 poly dT DNA in 500 μL of 100mM pH 8.5 carbonate buffer (Scheme S2). To the mixture is added to 5 mg of freshly extracted HT NHS ester (**3**) in 100 μL of DMSO. The reaction is allowed to stir overnight and is observed to be complete by full reaction of starting DNA by MALDI-MS. Intermediate HT-DNA is purified by reverse phase HPLC. (*m/*z) observed: 8497.2, calculated: 8493.7 $[M - H]^-$.



Scheme S3. Click chemistry conditions for the synthesis of Gd(III) DNA

Covalent attachment of Gd(III) complex **4** to DNA begins with dissolution of 1 μmol of the 5x alkyne bearing HT-poly dT 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.) [2] is added 100 nmol Cu(II) sulfate, 500 nmol tris-hydroxypropyl triazolyl amine, 10 μmol of **4**, and 1 μmol sodium ascorbate. The reaction vessel is capped and allowed to stir overnight. The reaction mixture was analyzed by MS-MALDI to verify that the reaction had reached completion with 5 Gd(III) chelates per DNA strand. The resulting 5x Gd(III)-HT DNA complex is then purified by reverse phase HPLC and characterized by MS-MALDI. (*m/*z) observed: 11546.2, calculated: 11547.3 [M + $H_3COO]^-$.

1.2 Nanoconjugate Synthesis:

Citrate stabilized spherical gold nanoparticles were synthesized according to Frens [3] and measured by TEM. In consecutive batches, AuNP measured 14.1 ± 0.9 nm (batch 1) and 13.3 ± 1.7 nm (batch 2).

HaloTag Nanoconjugate Hydrodynamic Radius (nm)	
Citrate stabilized AuNP	34.7 ± 0.4
HT-DNA AuNP	36.0 ± 0.6

Table S1. Dynamic light scattering of AuNP before and after conjugation to DNA.

DNA was deprotected, and AuDNA-Gd(III)-HA were functionalized and purified in an analogous procedure as that reported for 13nm gold nanoparticles.[4]

Specifically, 29 OD (260 nm) of DNA (corresponding to ~200 strands of DNA per nanoparticle) 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 by UV/Vis at 260 nm.

To 50 mL of 10 nM citrate stabilized gold nanoparticles in water is added 54 μL of tween 20 (for a total concentration of 0.01% v/v) and deprotected and purified DNA in 4 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 1.25, 1.29, 1.32, 1.35 and 1.38 mL 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 600 mM. The solution is left to stir for a further 48 hours.

Purification of AuDNA-Gd(III)-HA is conducted by three rounds of centrifugation at 4 °C (30 minutes at 21.1 x g), followed by resuspension in fresh DPBST (0.01% Tween20). This procedure yielded up to 4 nmol AuDNA-Gd(III)-HA from 5 nmol citrate stabilized gold nanoparticles.

1.3 AuDNA-Gd(III)-HA Characterization:

Nanoparticle characterization was performed on JOEL 1230 and Hitachi HD7700 TEMs. UV/Vis/NIR spectra of colloidal solutions were collected on a LAMBDA 1050 spectrophotometer (Perkin Elmer). DLS and zeta-potential measurements were done with a Brookhaven ZetaPals zeta potential and particle size analyzer. Relaxivity was measured on a Bruker minispec mq60 NMR spectrometer (60 MHz)**.** Particle concentration and

Gd(III) loading were determined by ICP-MS by examination of the Gd/Au ratio. When not in use, particles are stored at 4 °C.

The volume of AuDNA-Gd(III)-HA, and thus the number of gold atoms per particle were calculated assuming the particles to be perfect spheres, using the simple geometric approximation for the surface area of a sphere. 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).

Relaxivity (r_1): A stock solution of AuDNA-Gd(III)-HA conjugates was made (700 µL). Serial dilution was performed four times for a total of five solutions. Solutions were heated to 37 °C and 200 µL of each dilution was measured for 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}).

Table S2. Batch to batch characteristics of for AuDNA-Gd(III)-HA

1.41 T	**r_1 (mM^{-1} s^{-1})**	**r_2(mM^{-1} s^{-1})**	**Loading Gd/nanoparticle**	**r_2/r_1 ratio**	**Trials (ICP)**
Batch 1	18.4	29.9	589 ± 54	1.62	5
Batch 2	13.9	25.9	427 ± 19	1.86	1
Average	16.2	27.9	-	1.74	-
St Dev	3.2	2.8	-	0.2	-

Table S3. Values of experimental T_1 times and corresponding [Gd(III)] measured by ICP-MS for AuDNA-Gd(III)-HA

sample	[Gd] / mM	T_1 (ms)	T_1 (s)	1/ T_1
1	0.1384	460.2	0.46	2.17
2	0.0663	851.6	0.85	1.17
3	0.0338	1410	1.41	0.71
4	0.0171	2070	2.07	0.48
5	0.0084	2625	2.62	0.38

Table S4. Values of experimental T_2 times and corresponding [Gd(III)] measured by ICP-MS for AuDNA-Gd(III)-HA

sample	[Gd] / mM	T_2 (ms)	T_2 (s)	1/ T_2
1	0.1384	250.8	0.25	3.99
2	0.0663	465.6	0.47	2.15
3	0.0338	786.4	0.79	1.27
4	0.0171	1187.3	1.19	0.84
5	0.0084	1578.2	1.58	0.63

2. Cell culture

2.1 Culture methods

U-2 OS (ATCC) and U-2 OS HT-ECS (Promega) were cultured in McCoy's 5A media (Life Technologies) supplemented with 10% fetal bovine serum (VWR) (FBS) at 37°C and 5% CO_2. U-2 OS HT-ECS is stably transfected with a HaloTag expression vector that includes a neomycin resistance gene. Therefore, U-2 OS HT-ECS media included 800 μg/ml G418 (Life Technologies).

2.2 Cell labeling and flow cytometry

Cells were labeled by first treating them with trypsin, follow by inhibition with media, and collected into microcentrifuge tubes. The cells were then pelleted at 1000g for 5 minutes at 4°C. Media labeled with 1:1000 HaloTag-targeted AlexaFluor488 (Promega) was used to resuspend the cells. After a 15 minute incubation at 37°C, cells were pelleted at 1000g for 5 minutes at 4°C, resuspended in 400μL DPBS, spun down again, and finally resuspended in DPBS with 2% bovine serum albumin (Sigma-Aldritch) for flow cytometry. Analysis was performed on a LSR II (BD) flow cytometer and data were analyzed using FlowJo software (Treestar).

2.3 Electron microscopy

For transmission electron microscopy (TEM) imaging of cells, cell fixation was conducted in a Pelco Biowave microwave systems with cold spot and vacuum chamber. Cell pellets were fixed twice using Karnovsky's fixative. The cells were then washed three times with sodium phosphate buffered saline. The sample was then enrobed in a gel of 2% agarose (Amresco) to promote pellet integrity through the embedding process. 2% OsO4 in water was applied as an additional fixative and to promote image contrast. The enrobed pellet was then washed with water and dehydrated with acetone washes. EMBed 812 resin (Electron Microscopy Sciences) was used for embedding. The resin was polymerized at 60 degrees C. Sectioning was conducted using a Leica Ultracut S. The resin was sliced to 90 nm thickness and collected on copper mesh grids. TEM was conducted using a 1230 JOEL transmission electron microscope. All imaging was carried out at 100 keV accelerating voltage.

2.4 Gadolinium uptake

Cells were incubated with the indicated concentration of AuDNA-Gd(III)-HA for the indicated amount of time. They were then rinsed in the plate once with Dulbecco's phosphate buffered saline (DPBS, Life Technologies), trypsinized (Life Technologies), treated with trypsin inhibitor (Life Technologies), and collected into microcentrifuge tubes. The cells were then pelleted at 1000g for 5 minutes at 4°C. Cell pellets were washed twice by resuspending in 400μL DPBS and pelleting.

2.5 Cell counting and viability

Cells were counted and viability was measured as part of cell uptake experiments using a Guava EasyCyte Mini Personal Cell Analyzer (EMD Millipore). After cell resuspension, an aliquot (50 μL) of the suspension was diluted in Guava ViaCount reagent (150 μL). Stained samples were vortexed for 10 s and then cells were counted using a Guava EasyCyte Mini Personal Cell Analyzer (PCA) using the ViaCount software module**.** Viability was measured using the EasyFit software module.

2.6 Inductively-coupled plasma mass spectrometry

Quantification of gadolinium for cell uptake experiments was accomplished using ICP-MS of acid digested samples. Specifically, aqueous samples were digested in concentrated nitric acid (> 69%, Sigma) and hydrochloric acid (37% BDH) and heated to 75 °C for overnight. Samples were then diluted with ultra-pure H_2O (18.2 MΩ·cm) and multi-element internal standard (CLISS-1, Spex Certiprep) to 3.0% nitric acid (v/v) and 5.0 ng/mL internal standard in a total sample volume of 3 mL. Individual Gd elemental standards were prepared at 0.78125, 1.5625, 3.125, 6.25, 12.5, 25.0, 50.0, 100, and 200 ng/mL concentrations with 3.0% nitric acid (v/v) and 5.0 ng/mL internal standards up to a total sample volume of 10 mL.

ICP-MS was performed on a computer-controlled (Plasmalab software) Thermo X series II ICP-MS (Thermo Fisher Scientific) operating in standard mode equipped with a CETAC 260 autosampler. A survey run and 3 main runs were acquired for each sample. For data interpolation and machine stability ^{157,158}Gd, and ^{115}In and ^{165}Ho were analyzed.

2.7 Cell pellet MRI

Adherent cells were incubated with 52 nM nanoparticles for 8 hours in T25 flasks, or with 100 μM Prohance or HTGd for 30 minutes in T25 flasks. After incubation the labeled media was removed and cells were washed once with DPBS. Cells were then trypsinized and collected into 1.5 ml microcentrifuge tubes. Cells were pelleted at 1000 g for 5 m, resuspended in 1 ml DPBS, and spun down again. This was repeated one more time. Cells were finally resuspended in 1 ml DPBS, placed into flame-sealed pipettes, pelleted at 100 g for 5 minutes, and imaged.

In order to determine Spin-lattice relaxation times (T_1), a rapid-acquisition rapid-echo (RARE-VTC) T_1-map plus sequence with static TE (11 ms) and variable TR (150, 250, 500, 750, 1000, 2000, 4000, 6000, 8000, and 10000 ms) values was used. The imaging parameters were: 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 regions of interest (ROIs) for each axial slice.

Supplementary Figures

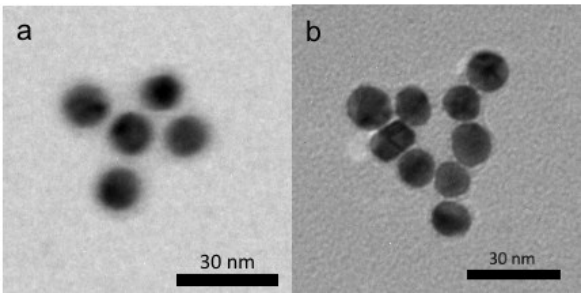


Figure S1. TEM images of a) batch 1 and b) batch 2 of citrate stabilized AuNP.

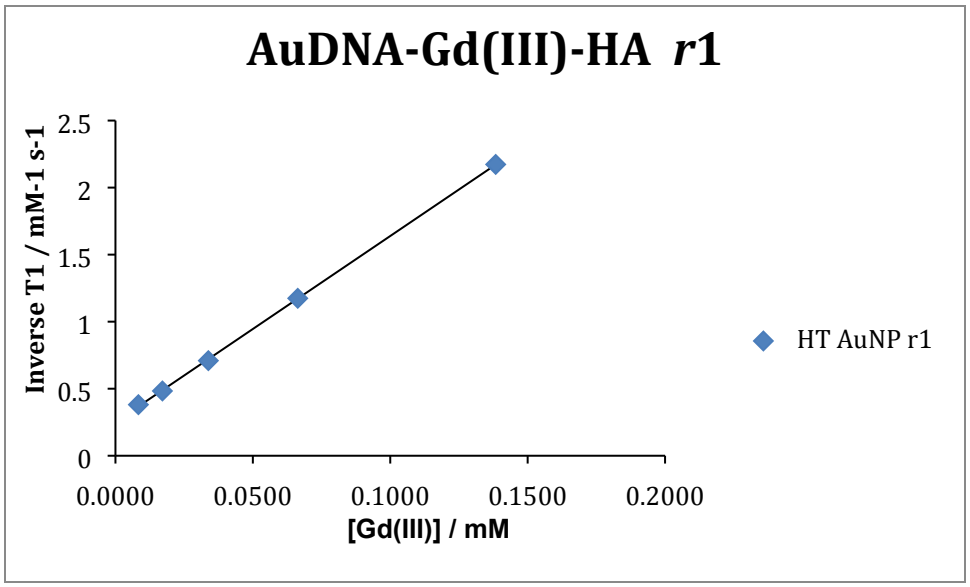


Figure S2. Example of r_1 relaxivity calculation for Batch 2 AuDNA-Gd(III)-HA

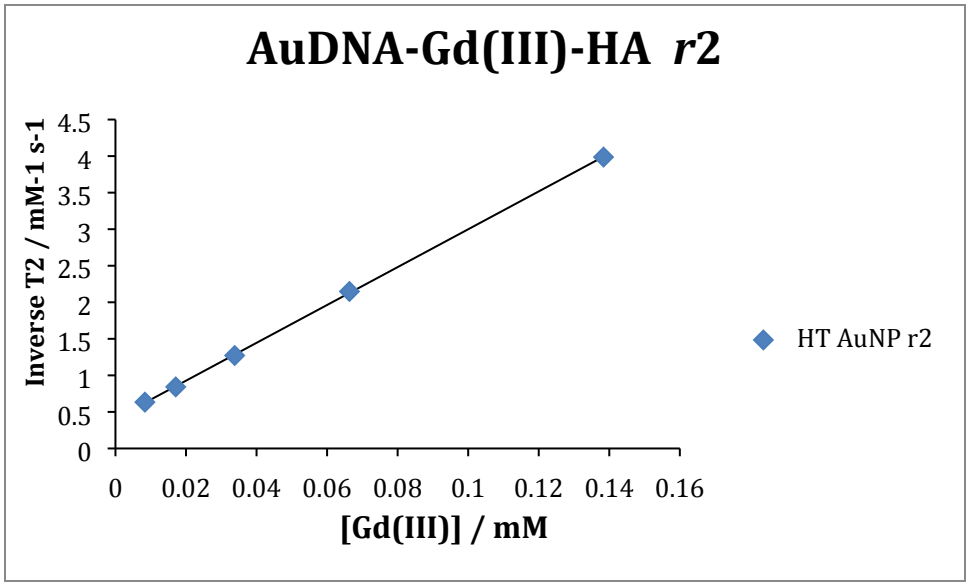


Figure S3. Example of r_2 relaxivity calculation for Batch 2 AuDNA-Gd(III)-HA

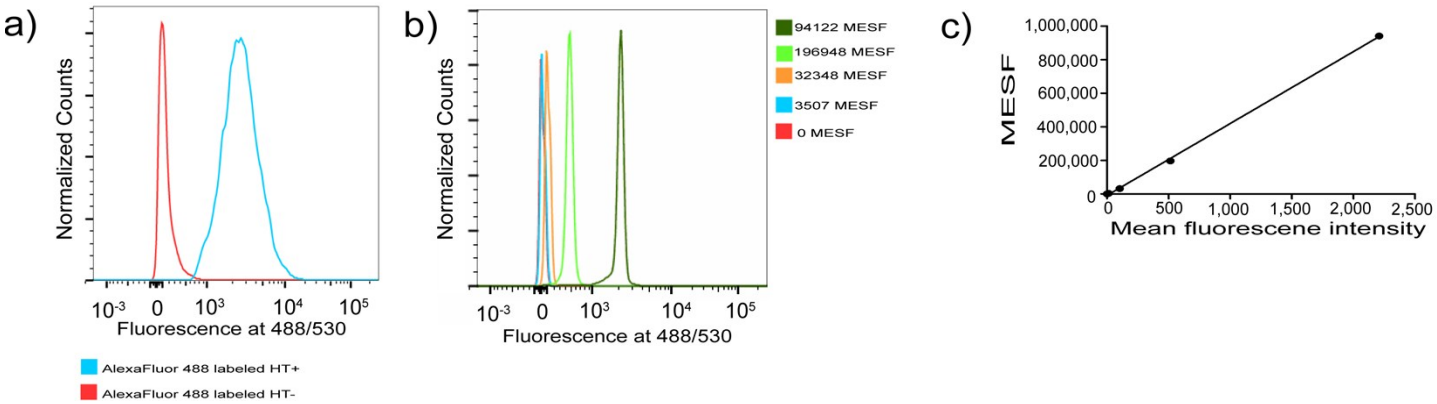


Figure S4. Procedure for quantifying the expression level of HaloTag on the surface of U-2 OS HT cells. a) Example flow cytometry data showing the fluorescence shift from HT+ cells compared to the fluorescence from the non-specific binding of AlexaFluor488-HT to U-2 OS cells. b) Example of the fluorescence intensity measured from beads with known molecular equivalents of soluble fluorochrome (MESF) for AlexaFlour488 (Bang's Lab) and c) the corresponding standard curve.

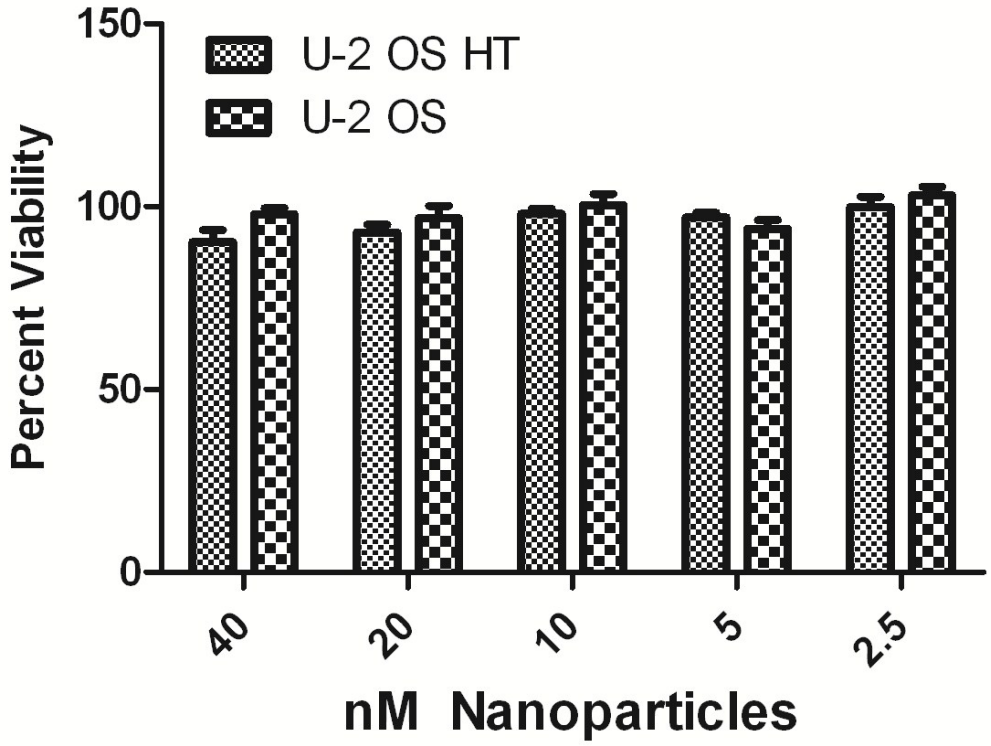


Figure S5. Percent viability of both U-2 OS HT and U-2 OS is measured after incubation with AuDNA-Gd(III)-HA. After a 24 hour incubation no significant loss of viability is measured at concentrations as high as 40 nM AuDNA-Gd(III)-HA.

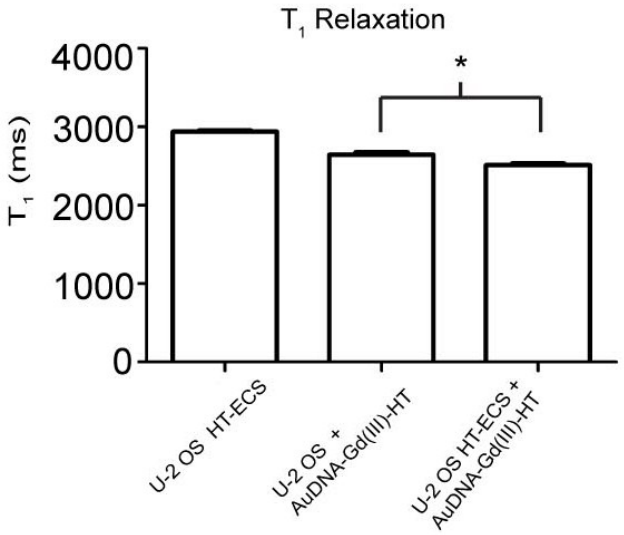


Figure S6. The T_1 values measured from cell pellets images averaged over 4 slices. * indicates $p<0.05$

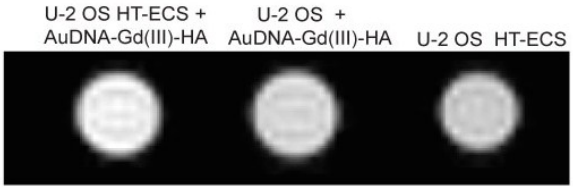


Figure S7. Greyscale image of the cell pellet shown in Figure 2c.

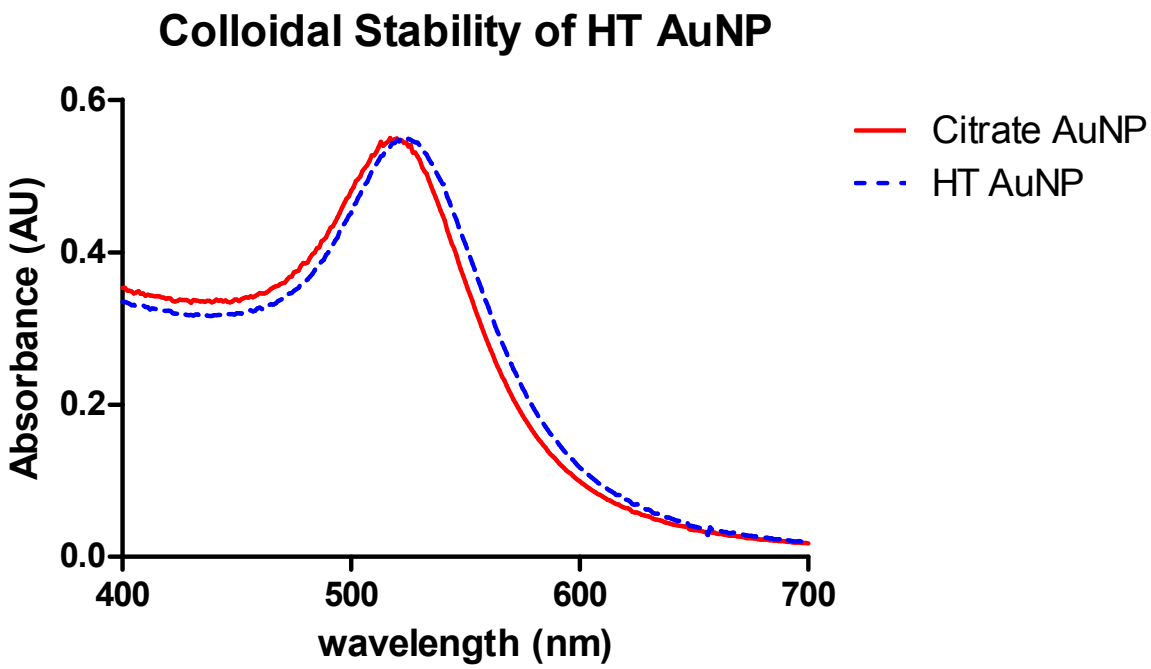


Figure S8. UV-Vis spectra of nanoparticles before and after conjugation to DNA.

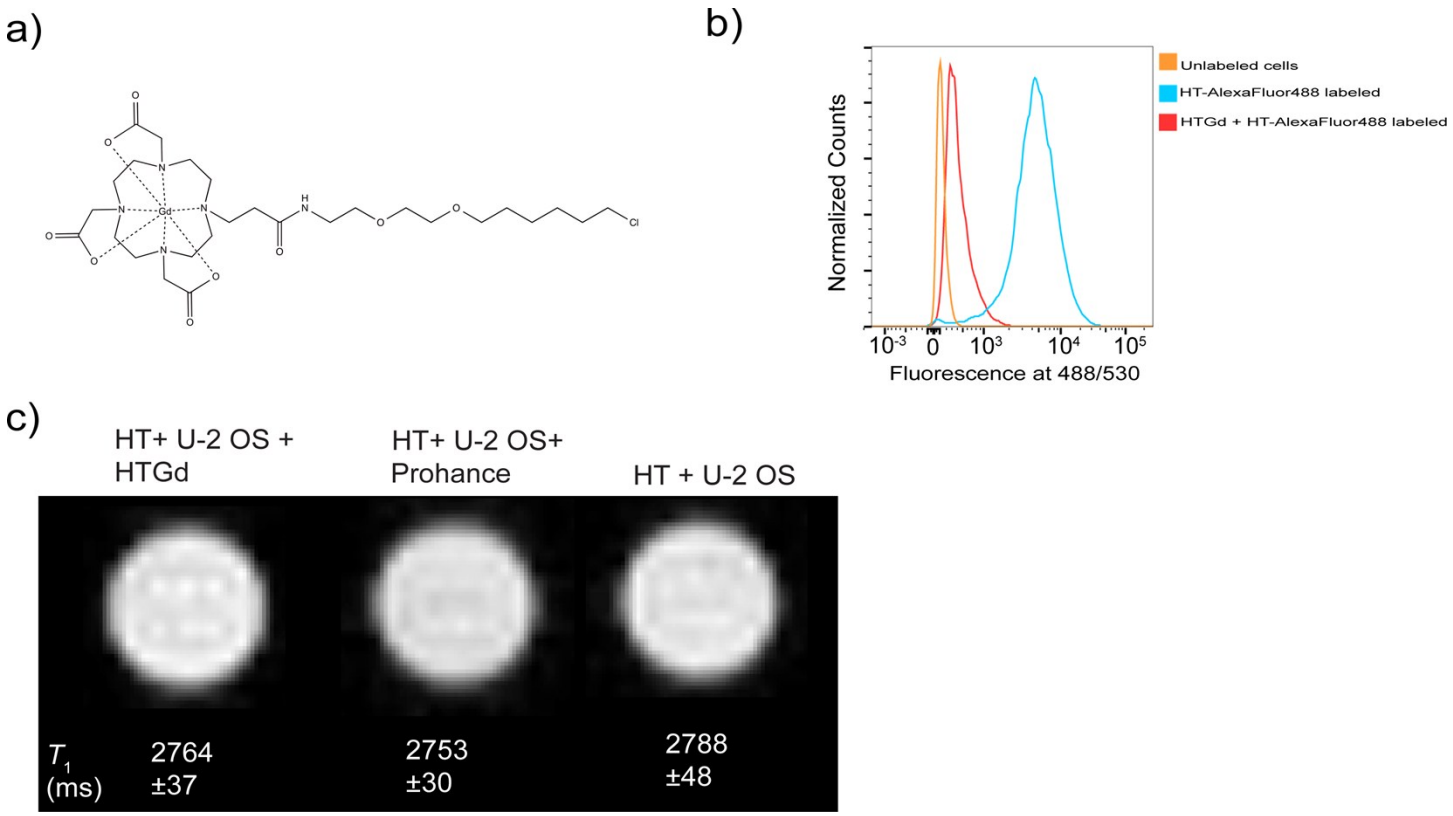


Figure S9. HaloTag expression is not detectable using a HT-targeted Gd(III) chelate. a) HTGd, a previously reported HaloTag-targeted Gd(III) chelate.[5] b) Flow cytometry data showing that HTGd binds to HT on U-2 OS HT-ECS cells. Unlabeled U-2 OS HT-ECS cells (orange line) are compared to U-2 OS HT-ECS cells after incubation with HT-targeted AlexaFluor488 (blue line), and to cells first incubated with 100 μM HTGd for 30 minutes and then labeled with HT-targeted AlexaFluor488 (red line). Similar to nanoparticles, the loss of fluorescence for HTGd + HT-AlexaFluor488 indicates HT binding. c) Cell pellet images prepared in the same manner as in Figure S7. Unlabeled HT+ cells are compared to cells that have been incubated with 100 μM HTGd for 30 minutes and to cells incubated with 100 μM Prohance for 30 minutes. No change in T_1 is observed in any comparison.

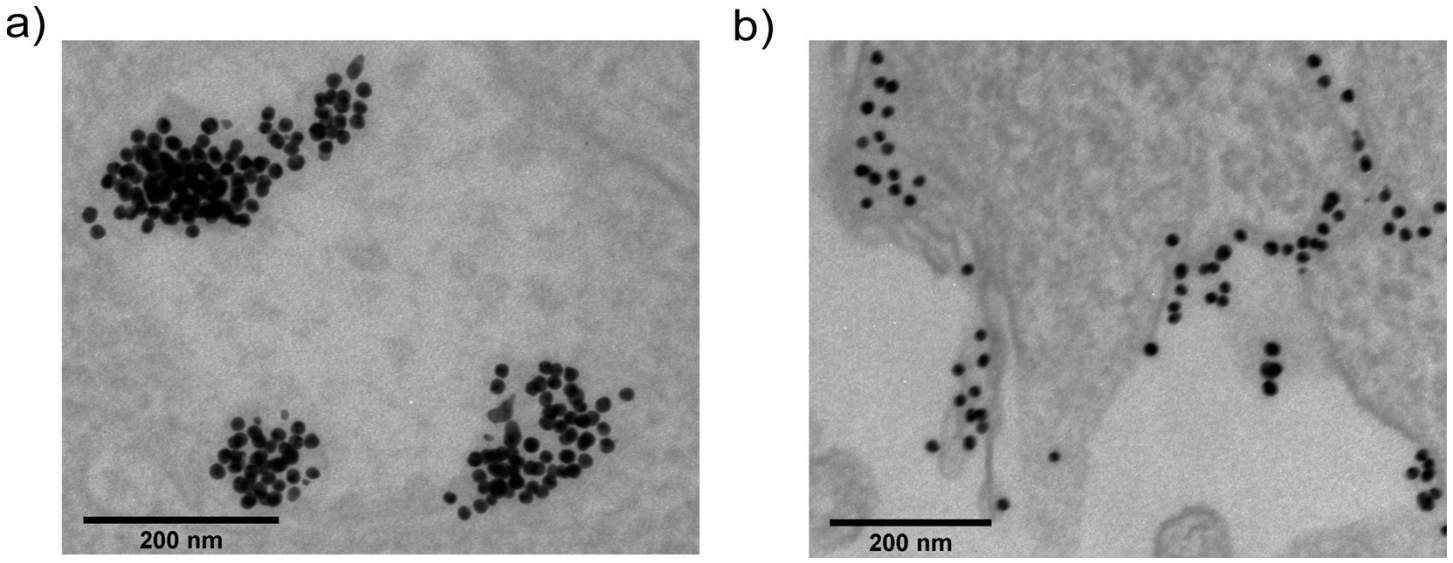


Figure S10. Close ups of the TEM images shown in figures 1a and 1b.

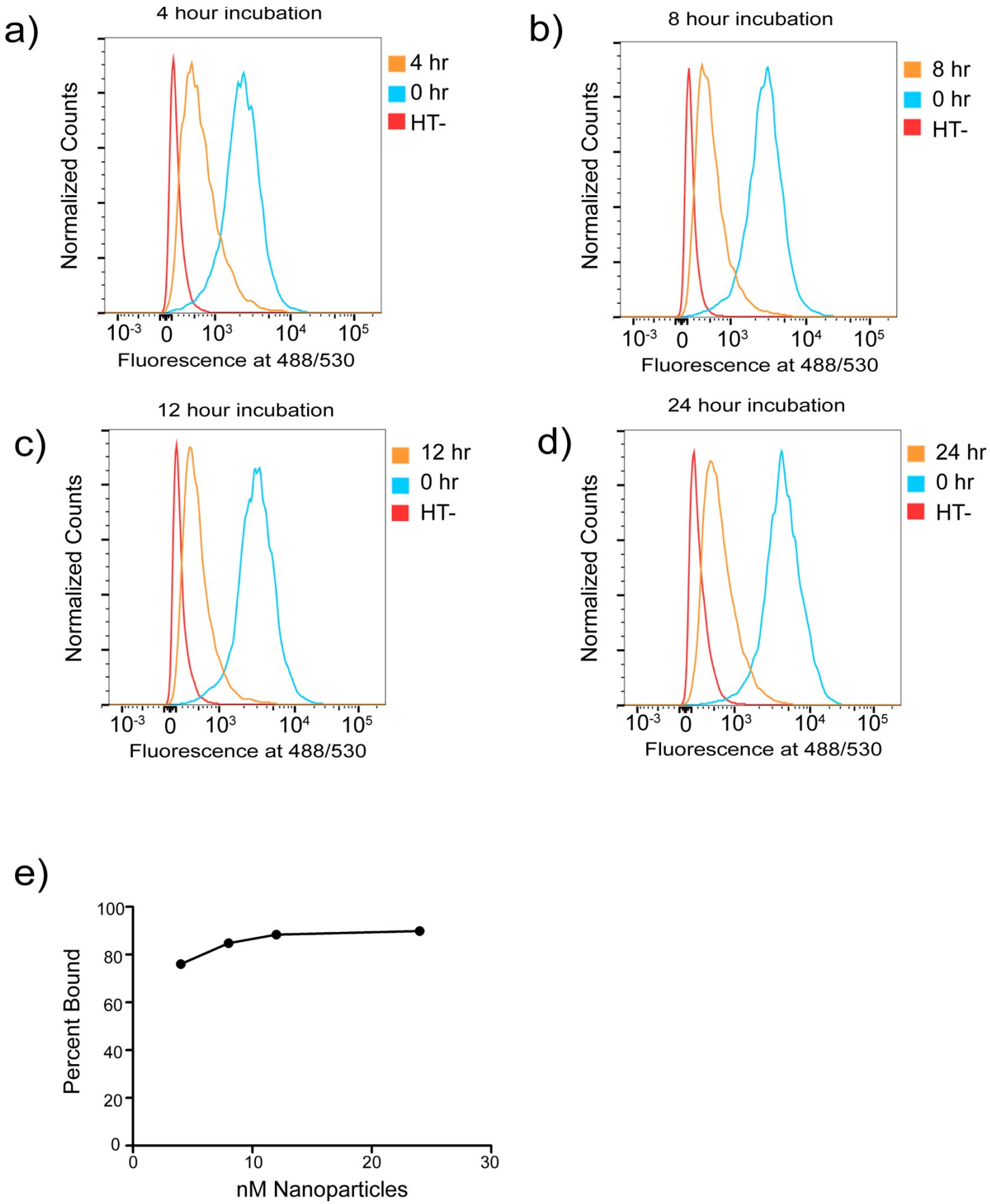


Figure S11. Flow cytometry data showing an extended time course experiment for AuDNA-Gd(III)-HT binding. a)-d) Cells were incubated with 40 nM nanoparticles for the indicated amount of time and treated with HT-targeted AlexaFluor488. Each time point is compared to cells that either do not express HT (HT-) or were

not treated with AuDNA-Gd(III)-HT (0 hr), after incubation with HT-targeted AlexaFluor488. e) Percent of HT that is bound to AuDNA-Gd(III)-HT for each time point.

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