Electronic Supplementary Information (ESI) for

Gd^{3+}-Functionalized Near-Infrared Quantum Dots for *In Vivo* Dualmodal (Fluorescence/Magnetic Resonance) Imaging

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Synthesis

Materials

Cadmium oxide (CdO, 99.99 %), Selenium (Se, powder, 99. 999 %), and Tellurium (Te, shot, 1-2 mm, 99.99 %) were purchased from Sigma-Aldrich. Tri-octylphosphine oxide (TOPO), tri-octylphosphine (TOP), tri-butylphosphine (TBP), hexadecylamine (HDA, 90 %), and stearic acid were purchased from Tokyo Chemical Industry (Japan). Sulfur (S, crystalline, 99.9999%), glutathione (GSH, reduced form), gadolinium chloride (99.9 %) and potassium *t*-butoxide were purchased from Wako (Japan). DOTA-NHS ester (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid mono-*N*-hydroxysuccinimide ester, B-280) was purchased from Macrocyclics, USA. Other organic solvents used were of analytical reagent grades.

Precursor stock solutions were prepared under an argon atmosphere. A Se-Te stock solution was prepared by dissolving 24 mg (0.3 mmol) of Se and 13 mg (0.1 mmol) of Te in 1 ml of TBP at room temperature. A Cd-S stock solution was prepared as follows: 40 mg (1.25 mmol) of sulfur was added to 10 ml of TOP and heated at 100 ^{o}C. After sulfur was completely dissolved, the solution was cooled to room temperature. A mixture of 160 mg (1.25 mmol) of CdO and 2g of stearic acid was loaded into a 25 mL three-necked flask and heated at 300 ^{o}C. After CdO was completely dissolved, the mixture was cooled to 80 ^{o}C. At this temperature, the sulfur/TOP solution was added under stirring. The Cd-S stock solution was stored under argon at room temperature.

CdSeTe/CdS QDs

The mixture of 25 mg (0.2 mmol) of CdO and 250 mg of stearic acid was loaded into a 25 mL of three-necked flask and heated at 300 ^{o}C. After CdO was completely dissolved, the solution was cooled to room temperature. Then 2g of TOP and 2g of HDA was added to the flask and heated to 300 ^{o}C. At this temperature, 0.25 ml of the Se-Te stock solution was quickly injected using a syringe. Immediately, the solution changed from colorless to a deeply colored solution. By monitoring of the growth of QDs with their fluorescence spectra, the formation of QDs (ca. 780 nm emission) was checked. When the desired QDs were formed, the solution was cooled to 200 ^{o}C. At this temperature, the formation of CdS shell was performed. Additions of 0.5 ml of the Cd-S stock solution resulted in the formation of CdSeTe/CdS QDs that emit at 800 nm. Then, the QD solution was cooled to 60 ^{o}C and 20 ml of

chloroform was added. QD nanoparticles were precipitated by addition of methanol and separated by centrifugation. To remove excess TOPO, HDA, and stearic acid, the QDs were dissolved in chloroform again and precipitated by addition of methanol. This procedure was repeated three times. The resulting QDs were dissolved in 20 ml of tetrahydrofuran (THF) and stored in a dark place.

GSH-coated CdSeTe/CdS QDs

GSH-coated CdSeTe/CdS QDs were prepared by surface modification of the hydrophobic CdSeTe/CdS having an organic layer (TOPO, TOP and HDA). 0.2 ml of an aqueous solution (100 mg/ ml) of GSH was slowly added to 0.5 ml of the THF solution of the QDs at room temperature and the mixture was heated to 60 ^{o}C. The resulting precipitates of QDs were separated by centrifugation. To the QD precipitates, 1 ml of water was added and then 50 mg of potassium *t*-butoxide was slowly added. The mixture was sonicated for 5 min and filtered through a 0.2 μm membrane filter. Excess GSH and potassium *t*-butoxide were removed by the dialysis using PBS buffer (pH=7.4).

Gd^{3+}-DOTA- CdSeTe/CdS QDs

10 mg of DOTA-NHS ester (Mw. 761.5) was dissolved to 1 ml of 10 mM PBS buffer (pH=7.4). 20 μl of the DOTA-NHS ester solution was slowly added to 1ml of GSH-coated CdSeTe/CdS QDs (1 μM, 10 mM PBS) and the solution was stirred for 1 hr at room temperature. The concentration of GSH-coated QDs was determined by fluorescence correlation spectroscopy using Rhodamine 6G as a reference. After the conjugation reaction, the QDs solution was dialyzed using 10 mM PBS buffer to remove unreacted DOTA-NHS ester. After the dialysis, 0.1 ml of $GdCl_3$ aqueous solution (10 mM) was slowly added using a syringe under vigorous stirring. Excess Gd^{3+} was removed by dialysis using 10 mM PBS buffer.

An average number of Gd^{3+}-DOTA complexes per QD particle

Gd^{3+}-DOTA complexes are highly stable in aqueous solution and its stability constant (log K_{ML}) is reported as 25.3 (J. Huskens et al. *Inorg. Chem*. **1997**, *36*, 1495). Thus we can assume that the concentration of Gd^{3+}-DOTA complexes is almost the same as that of DOTA in the presence of excess Gd^{3+} ions. To determine an average number of Gd^{3+}-DOTA complexes per QD particle, we estimated the number of DOTA binding at a QD surface. Since DOTA-NHS ester has UV absorption around 260 nm that is arising from its imide groups, the concentrations of DOTA-NHS ester bound to QDs can be determined by comparison of its absorption spectrum before and after the conjugation reaction. We reacted 0.02 ml of DOTA-NHS ester (10 mg/mL) with 1 ml of GSH-coated QDs (1 μM). The amount of unreacted DOTA-NHS ester was determined from the concentration of the aqueous solution of DOTA-NHS ester separated by dialysis. Fig. S1 shows the UV spectra of DOTA-NHS ester used for the conjugation

reaction and unreacted DOTA-NHS ester. From the UV spectral data, an average number of Gd^{3+}-DOTA complexes per QD particle was calculated to be 77±18.

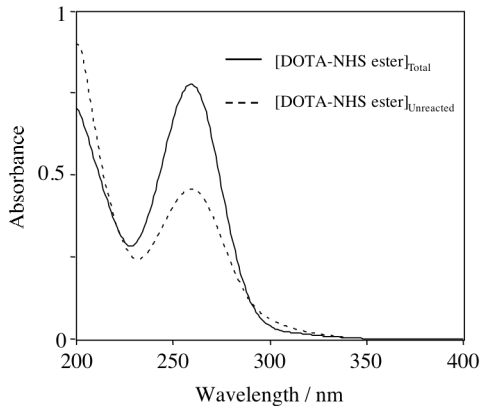


Fig. S1 UV spectra of total DOTA-NHS ester used for the conjugation reaction and unreacted DOTA-NHS ester separated by dialysis.

A determination method for hydrodynamic diameters of QDs using FCS

FCS uses the fluctuations of fluorescence intensity in a tiny excitation volume to determine the diffusion times of fluorescent particles. Fluctuations in the fluorescence intensities *I(t)* can be analyzed by using the autocorrelation functions $G(\tau)$:

$$G(\tau) = \frac{< I(t) I(\tau + t) >}{< I(t) >^2} \qquad (1)$$

where the symbol < > stands for the ensemble average.

If a three-dimensional Gaussian profile in the confocal volume of the lateral radius ω_o and axial radius ω_z was assumed, equation (1) for a one-component diffusion can be expressed as:

$$G(\tau) = 1 + \frac{1}{N}\left(1 + \frac{\tau}{\tau_d}\right)^{-1}\left(1 + \frac{\tau}{(\omega_z/2\omega_o)^2 \tau_d}\right)^{-1/2} \quad where \quad \tau_d = \frac{\omega_o^2}{4D} \qquad (2)$$

where N is the average number of fluorescent particles in the excitation volume and τ_d is the diffusion time of the fluorescent particles, depending on the diffusion constant D and ω_o. From the analysis of $G(\tau)$ using the equation (2),

the diffusion time τ_d of the fluorescent particles can be determined. Using the Stokes-Einstein relationship ($D=k_BT/6p\eta r$), the hydrodynamic diameter d_{QD} of QDs is related to the following equation:

$$d_{QD} = d_{ST} \times \frac{\tau_{QD}}{\tau_{ST}} \qquad (3)$$

where d_{ST} is the hydrodynamic diameter of a standard particle , τ_{QD} and τ_{ST} are the diffusion time of QDs and a standard particle, respectively. Using the diffusion time τ_{ST} of a standard fluorescent latex bead (20 nm in diameter, Molecular Probe. Inc.), hydrodynamic diameters of QDs are determined based on the equation (3).

MRI measurements

All MRI experiments were performed on a horizontal bore 11.7 T AVANCE 500WB spectrometer (Bruker BioSpin, Germany) equipped with a 89 mm micro imaging gradient insert (150 gauss/cm). A standard volume coil (Bruker BioSpin, Germany) was used to transmit/receive RF at ^{1}H frequency (500MHz). The MRI measurements of phantom solutions were done at room temperature. T_1 weighted images of phantom solutions were obtained by a gradient echo method using following parameters: FOV, 20 mm; matrix dimensions, 256 x 256; thickness, 0.5 mm; TE (ms) /TR (ms), 5.3/47.0; flip angle, 30 degree; 32 averages. The T_1 values of phantom solutions were measured by a saturation recovery method with following parameters: FOV, 20 mm; matrix dimensions, 256 x 256; thickness, 0.5 mm; TE (ms) /TR (ms), 3.5/32, 3.5/62, 3.5/125, 3.5/250, 3.5/500, 3.5/1000, 3.5/2000, 3.5/4000; flip angle, 90 degree; 2 averages. The T_2 values of phantom solutions were measured by a spin echo method using following parameters: FOV, 20 mm; matrix dimensions, 256x256; thickness, 0.5mm; TE (ms) /TR (ms), 10/2000, 20/2000, 30/2000, 40/2000, 50/2000, 60/2000, 70/2000, 80/2000, 90/2000, 100/2000, 110/2000, 120/2000, 130/2000, 140/2000, 150/2000, 160/2000; flip angle 180 degree; 2 averages.

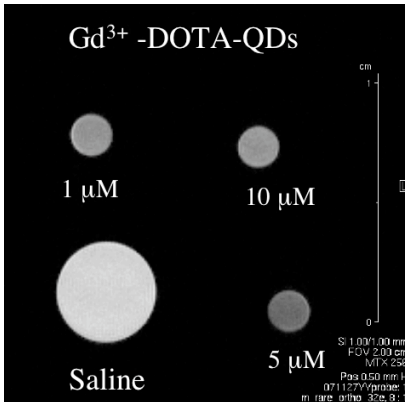
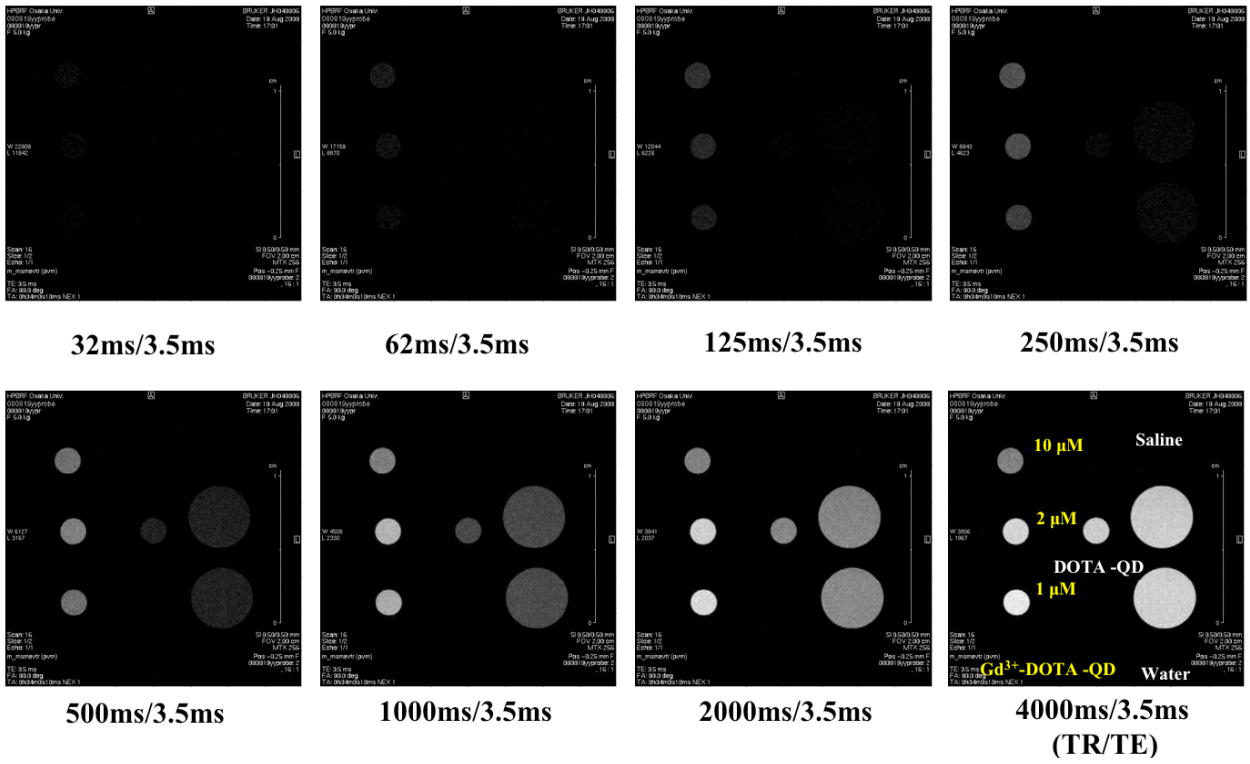


Fig. S2 T_2 weighted MR images for Gd^{3+}-DOTA-QDs in PBS. Saline (0.9 % of NaCl aqueous solution) is used as a reference.

T_1 weighted images



T_2 weighted images

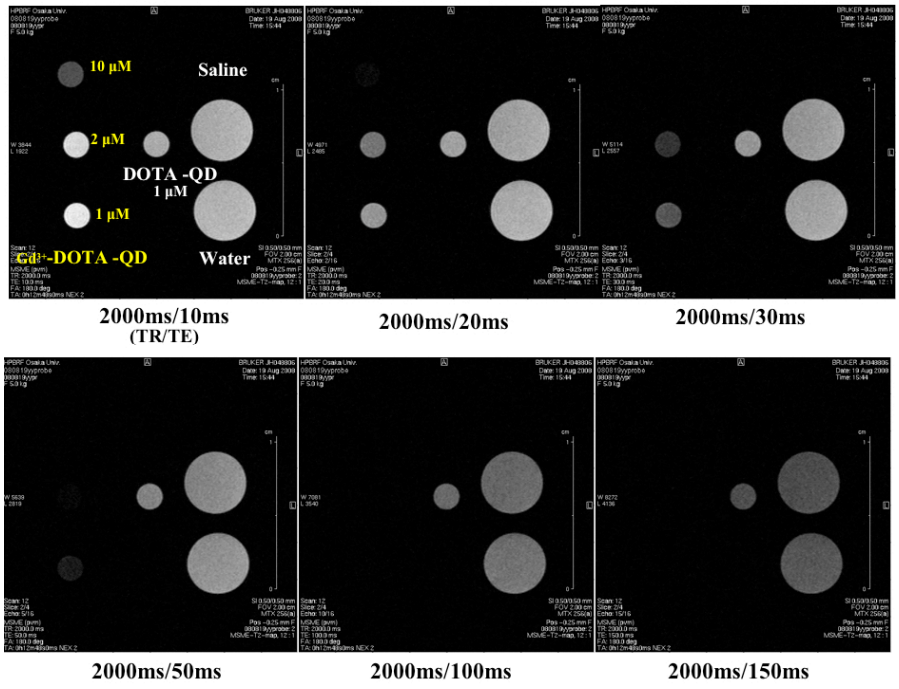


Fig. S3 Dependence of MR images of Gd^{3+}-DOTA-QDs (in PBS) on TE/TR. DOTA-QDs (1 μM), where saline (0.9 % of NaCl aqueous solution), and deionized water were used as reference solutions.

Relaxivity, R_1 and R_2 of Gd^{3+}-DOTA-QDs

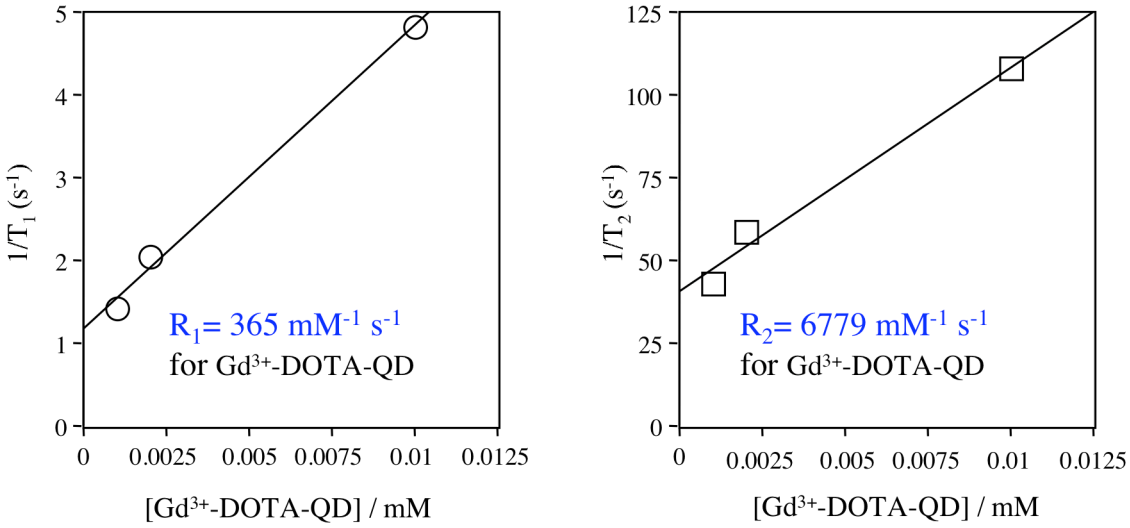


Fig. S4 The relaxivity of Gd^{3+}-DOTA-QDs in 10 mM PBS at 11. 7 T.

Cellular uptake

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich, St. Louis, MO) with 10% fetal bovine serum, 100 U/ml penicillin, and 10 mg/ml streptomycin at 37 ^{o}C (5% CO_2), and grown in 96-well LabTek chambers (Nalge Nunc International, Rochester, NY). After 24 hrs, the medium was replaced by 100 ml DMEM containing Gd^{3+}-DOTA-QDs, and cells were incubated at 37 ^{o}C (5% CO_2). For cell imaging we used an Olympus confocal inverted microscope FluoView1000 equipped with a UPlanSApo 40x 0.95 numerical aperture objective and a differential interference contrast (DIC) system. Gd^{3+}-DOTA-QDs were excited with a LD pumped solid laser (405 nm) and detected at 655-755 nm.

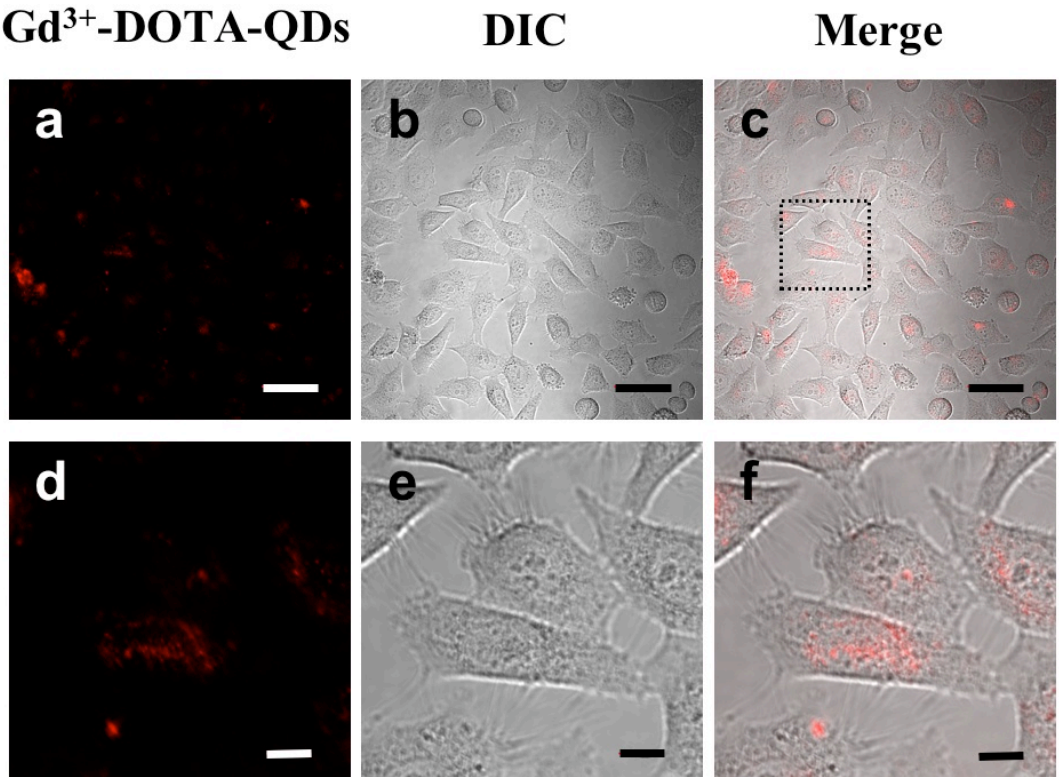


Fig. S5 Confocal microscopy images of HeLa cells in 7 hrs after treating with 20 nM of Gd^{3+}-DOTA-QDs: (top row: a-c); high magnification images at a black dashed box in (c): (bottom row: d-f). Fluorescence images indicating intracellular distribution of QDs are shown in (a) and (d). Differential interference contrast (DIC) images are shown in (b) and (e). Overlay of fluorescence and DIC images are shown in (c) and (f). Scale bars in (a-c) and (d-f) are 50 and 10 mm, respectively. Gd^{3+}-DOTA-QDs were uptaken by the cells through endocytosis or macropinocytosis after an incubation period. The majority of the QDs were distributed in the cytoplasm and around the perinuclear region.

Cytotoxicity

Cytotoxicity test was performed according to the procedure of a QMTT Cell Viability Assay Kit (BioChain, USA). The solutions of QMTT reagent were added to each well, and cells were incubated at 37 ^{o}C (5% CO_2) for 3 and 24 hrs in the presence of Gd^{3+}-DOTA-QDs. After the incubation, the absorbance at 570 nm of a formazan dye was measured with an absorption spectrometer (U-1900, Hitachi high-technology corporation, Japan) with 1-nm resolution in a 1-cm path length quartz cell.

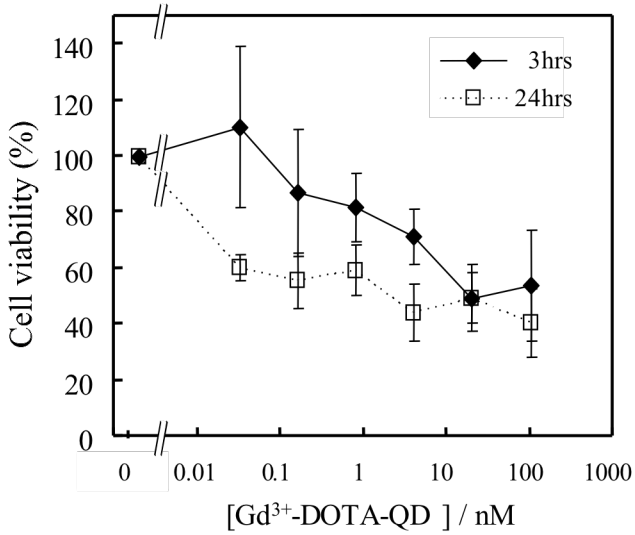


Fig. S6 The viability of HeLa cells in 3 hrs (◆) and 24 hrs (□) after treating with from 32 pM to 100 nM of Gd^{3+}-DOTA-QDs. Error bars indicate the standard deviations of three independent experiments. The viability decreased in dose-related manner and reached 55% at 100 nM of the Gd^{3+}-DOTA-QDs after 3hrs incubation. The viability further decreased in HeLa cells in the case of 24 hrs incubation.