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Supporting Information

One-pot synthesis of Gd^{3+}-functionalized gold nanoclusters for dual model (fluorescence/magnetic resonance) imaging

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1. Experimental Section

Materials and Apparatus.

Peptides were purchased from GL Biochem (Shanghai) Ltd. $GdCl_3 \cdot 6H_2O$ was from Alfa Aesar. $HAuCl_4 \cdot 3H_2O$ was from Chemical Reagent Company (Shanghai, China). All tissue culture materials were obtained from Invitrogen.

The size of the nanocluster was determined at 200 kV using a JEOL JEM-2010F low to high resolution transmission electron microscope (HRTEM). Fourier transform infrared (FT-IR) spectra were obtained using an IRPRESTIGE-21spectroscope (Shimadzu) with KBr pellets. Fluorescence spectra were recorded by a Varian Cary Eclipse fluorescence spectrophotometer. Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) was performed on a Thermo E. IRIS Duo ICP-OES instrument. The cell images were recorded on a TCS SP5 confocal microscopy system (Leica Co., Germany) with a tunable argon ion laser (458, 488, 514nm) and a separate photomultiplier tube for scanning.

Synthesis of Gd-AuNC

To synthesize Gd-AuNC, we first produced cyclodecapeptide-coated AuNCs by a slight modification to the previously reported protocol.[1] In a typical experiment, aqueous $HAuCl_4$ solution (100 μL, 25 mM) was added to a cyclodecapeptide solution (1 mL, 2 mM) under vigorous stirring, and then NaOH solution (75 μL, 0.5 M) was introduced 2 min later. The sample was sealed and stored at 37°C. The reaction was allowed to proceed for 15 h.

Next, to an as-prepared CP-AuNC solution (2 mL) was added $GdCl_3$ (340 μL, 10 mM) in 5 μL aliguots, while maintaining the pH at 8.5 with NaOH. The mixture was magnetically stirred at room temperature for 4 h, followed by dialysis against 25 mM HEPES buffer (pH 7.8, containing 0.5 mM sodium citrate) for 2 h. The buffer solution was next changed to a fresh solution of 12.5 mM HEPES (pH 7.8) for overnight dialysis. The product was stored at 4 °C for further use.

Synthesis of 7.8 nm Gd^{3+}-Functionalized Gold Nanoparticle (Gd-AuNP)

To prepare Gd-AuNP, we first synthesized CP-coated AuNP through reduction of $HAuCl_4$ with sodium borohydrate ($NaBH_4$). Briefly, aqueous $HAuCl_4$ solution (1 mL, 5 mM) was added to a cyclodecapeptide solution (1 mL, 2 mM) under vigorous stirring, followed by the addition of NaOH solution (120 μL, 0.5 M). After that, a fresh $NaBH_4$ solution of 10 mM was added drop by drop into the mixed solution to fully reduce the gold ions until the solution became dark red color. The solution was then centrifuged at 15000 rpm for 2 min to remove large particles. The supernatant was further centrifuged at 17000 rpm for 16 min to collect the pellets, which contained CP-AuNPs with core size of ~ 7.8 nm. The nanoparticles were washed with water twice and then redispersed in HEPES buffer (1mL, 12.5 mM).

Next, $GdCl_3$ (100 μL, 10 mM) was added to the as-prepared CP-AuNP solution (1 mL) drop by drop, while maintaining the pH at 8.5 with NaOH. The mixture was magnetically stirred at room temperature for 4 h. Following that, any remaining Gd^{3+} ion was removed by centrifugation and washing with HEPES buffer.

Synthesis of Gd^{3+}-Cyclodecapeptide Complex (Gd-CP)

To a freshly prepared cyclodecapeptide solution (316 μL, 2 mM) in HEPES buffer (12.5 mM, pH 7.8) was added 0.95 equiv. of $GdCl_3$ (30 μL, 10 mM) relative to the amount of cyclodecapeptide, and the resultant solution was allowed to incubate at room temperature for 4 h.

Quantum Yield

The quantum yield (QY) of AuNC and Gd-AuNC were obtained using Rhodamine 6G (Sigma 252433, Dye Content: 99%) as a reference. Based on the emission peak area and absorbance of the nanoclusters and Rhodamin 6G, the QY of AuNC and Gd-AuNC could be determined according to the following equation (1)[1]:

$$\varphi_{sample} = \varphi_{ref} \times \frac{F_{sample}}{F_{ref}} \times \frac{A_{ref}}{A_{sample}} \quad (1)$$

Where φ_{ref} is the known QY of reference compound, F_{sample} and F_{ref} are the integrated areas of fluorescence of the samples and reference at 550-850 nm following excitation at 510 nm, respectively. A_{ref} and A_{sample} are the absorbance of the reference and samples at excitation wavelength (510 nm). The data points were plotted in Fig. S6.

Gel Electrophoresis of Gd-AuNC

Gd-AuNC was first concentrated by lyophilization, then 20 μL of the concentrated solution (2.5 mM Gd) was analyzed by 2% agarose gel electrophoresis at 8.5 V cm^{-1} and 17 mA for 15 min.

To assess whether Gd-AuNC could resist protein adsorption, we analyzed the nanoclusters incubated in the presence or absence of FBS at pH 7.4 by agarose gel electrophoresis. Briefly, Gd-AuNC was dissolved in PBS at pH 7.4 containing 17% (v/v) FBS and then incubated at room temperature for 24 h. Three samples (1: Gd-AuNC + FBS (stained with Coomassie brilliant blue 250); 2: Gd-AuNC + FBS; 3: Gd-AuNC) were then analyzed by 2% agarose gel electrophoresis at 8.5 V cm^{-1} and 17 mA for 20 min. After that, pictures were taken under the irradiation of 365 nm UV excitation.

Relaxation Time Measurements and *In Vitro* MRI.

The relaxation times at low field strength were measured on a 0.55 T MRI instrument (MicroMRI, Shanghai Niumag Corp.) at 32°C. A quadrature coil with an inner diameter of 1.8 cm was used for RF transmission and reception. For T_1 and T_2 measurements, samples with various Gd^{3+} concentrations (determined by ICP-AES) were dispersed in water. T_1 measurements were performed using an inversion recovery (IR) sequence with various inversion times (*TI*), and T_2 was determined using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (τ = 800 μs, 2000 echoes, TR = 2000 ms). The relaxivity (r_i, i = 1, 2) of the contrast agents were calculated according to the equation (2)[2]:

$$(1/T_i)_{obs} = (1/T_i)_{dis} + r_i\,[M] \quad (2)$$

Where $(1/T_i)_{obs}$ and $(1/T_i)_{dis}$ represent the observed solvent relaxation rate and the intrinsic diamagnetic solvent relaxation rate, respectively.

T_1-weighted MR images of the samples were obtained on the same instrument with spin echo (SE) sequence (32°C, TR/TE = 208 ms/7.2 ms, NS = 2, field of view (FOV) = 25 mm × 25 mm, slice thickness = 5 mm, matrix = 256×256).

Cell Culture and Cytotoxicity.

HeLa and NIH/3T3 cells were provided by Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS, China). The HeLa cells were grown in Dulbecco's minimal essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C and 5% CO_2. NIH/3T3 cells were cultured using DMEM supplemented with 10% Bovine Calf Serum (BCS).

In vitro cytotoxicity was measured by performing methyl thiazolyl tetrazolium (MTT) assays on the HeLa and NIH/3T3 cells. Cells were seeded in a 96-well cell culture plate at 5×10^3 cells/well under 100% humidity and cultured at 37 °C and 5% CO_2 for 24 h. Samples with different concentrations of Gd^{3+} (final concentrations of 0, 12.5, 25, 50, 100 or 200 μM, diluted in PBS) were then added to the wells. The cells were subsequently incubated for 24 h at 37 °C with 5% CO_2. MTT (10 μL, 5 mg/mL) was added to each well and the plate incubated for another 4 h at 37 °C in 5% CO_2. After the addition of 150 μL DMSO, the assay plate was allowed to stand at 37 °C for 10 min. Colorimetric measurements were performed at 492 nm on a scanning multiwall spectrometer (SPR-960, Sunostik). The following formula was used to calculate the inhibition of cell growth: cell viability (%) = (mean of *Abs.* value of treatment group / mean *Abs.* value of control) × 100%.

Confocal Laser Scanning Microscopy (CLSM)

The cellular uptake of Gd-AuNC was determined by laser-scanning confocal fluorescence microscopy. For confocal microscopic study, HeLa and NIH/3T3 cells were seeded in a 24 well plate at 2×10^4 cell/well density in 0.5 mL of serum-free culture medium. After an incubation time of 4 h with 50 μL Gd-AuNC (1.2 mM Gd), cells were washed three times with PBS, fixed in a 4% paraformaldehyde in PBS, and then labeled with 4',6-diamidino-2-phenylindole (DAPI, Molecular Probes). The cell samples were examined by a TCS SP5 confocal spectral microscope at a 40×objective, using 405 nm Diode laser and 514 nm Argon laser as excitation source.

***In Vivo* MRI.**

In vivo experiments were performed on anesthetized white Kunming mice (50 g) with 10% chloral hydrate (150 μL) as authorized by the regional ethics committee for animal experiments. MRI was conducted on the Siemens TrioTim 3T MRI scanner, using a 3D-FLASH (TR = 5.7 ms, TE = 1.72 ms, FOV = 61 × 140 mm, matrix = 112 × 256, slice thickness = 0.8 mm). The average signal intensity of region of interest (ROI) was analyzed using the MRIcro image viewer (Version 1.40; http://www.cabiatl.com/mricro/). The mice were scanned before and after the administration of contrast agent. A total of 400 μL of Gd-AuNC solution (2.5 mM Gd) in PBS was injected intravenously, and the dose was calculated to be 0.02 mmol Gd kg^{-1}. For comparison, a control mouse injected with 100 μL of Gd-DTPA solution (10 mM Gd) was also scanned, and

the dose was 0.02 mmol Gd kg^{-1} as well.

Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) Analysis

To investigate the particle distribution in various organs, samples of Gd-AuNC (200 μL, 1.286 mM Au by ICP-AES) or Gd-AuNP (400 μL, 0.528 mM Au by ICP-AES) were intravenously injected through the tail vein into Kunming mice (n = 3). At 24 h post-injection, the mice were sacrificed via cervical dislocation. The organs, such as heart, liver, spleen, lung, and kidneys, were discretized, rinsed with sterile physiological saline, and then blotted dry with filter paper. After that, organs were further dehydrated at 80°C for 24 h, then digested and mineralized in aqua regia (~1 mL for each organ) at room temperature for 48 h. Gold content in the organs was next determined by ICP-AES analysis.

Urinary Excretion Investigation.

10 Kunming mice were randomly divided into two groups (n = 5), which were intravenously injected through the tail vein with solutions of Gd-AuNC (200 μL, 1.286 mM Au by ICP-AES) or Gd-AuNP (400 μL, 0.528 mM Au by ICP-AES). The mice were maintained under the same experimental conditions and kept surviving for 1 month. At several time points (2.5, 5.25, 9, 13, 24, 48, and 72 h post-injection) urine was collected. 0.5 mL of each urine sample was taken out for fluorescence and MR imaging after filtration. Then another 1 mL of each urine specimen was lyophilized and completely lysed in 0.5 mL aqua regia. Gold content was then determined by ICP-AES analysis.

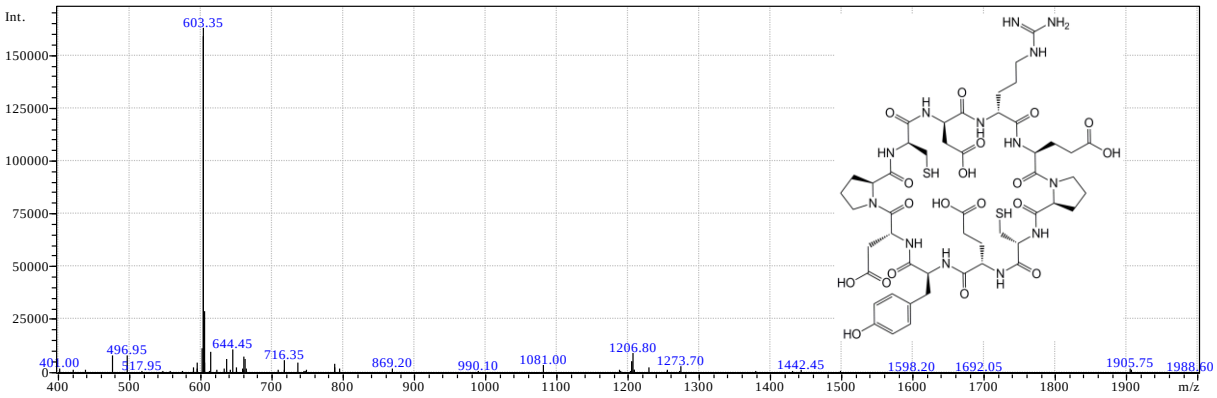

Fig. S1 ESI-MS spectrum and molecular structure of CP. ESI-MS: m/z $[M-2H]^{2-}$, calcd. 603.15, observed: 603.35; m/z $[M-H]^-$, calcd.1207.30, observed: 1206.80

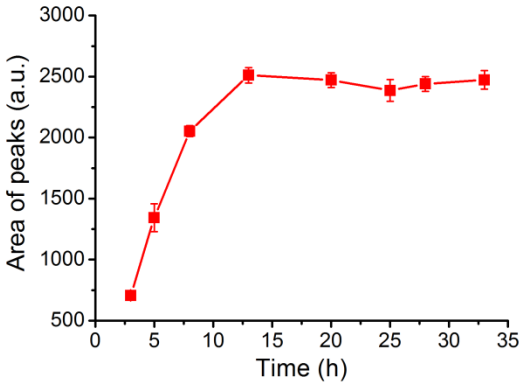

Fig. S2 Relative fluorescence intensity (area of the photoemission peak) as a function of incubating time at 37°C.

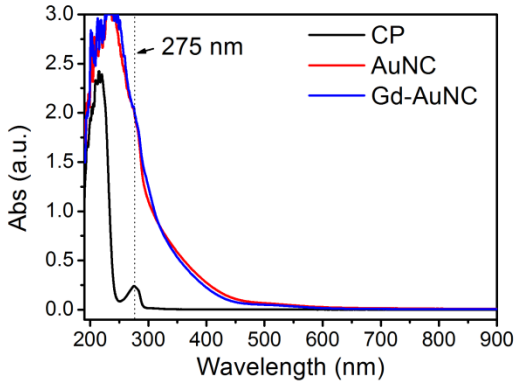

Fig. S3 UV-vis absorption spectra of CP, AuNC and Gd-AuNC.

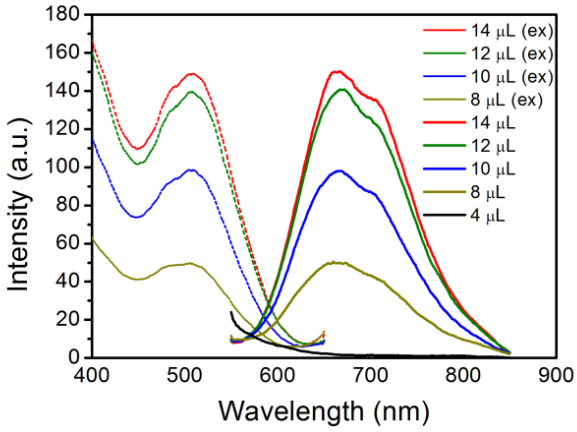

Fig. S4 Emission spectra of AuNC synthesized with different amount of NaOH. The experiment was performed as follow: CP (85 μL, 2 mM) was first mixed with $HAuCl_4$ (16 μL, 25 mM), then different amount (4 -14 μL) of 0.5 M NaOH aqueous solution was introduced 2 min later, and then water was added to make a final volumn of 150 μL. The samples were stored at 37 °C for 15 h, and emission spectra were recorded finally.

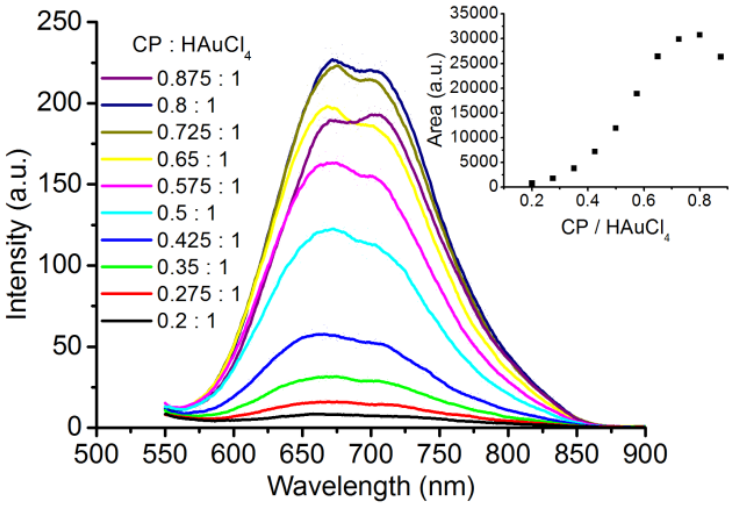

Fig. S5 Emission spectra of AuNC synthesized from different ratios of CP and $HAuCl_4$. The inset shows the relative fluorescence intensity (area of the photoemission peak) as a function of CP/$HAuCl_4$ ratio.

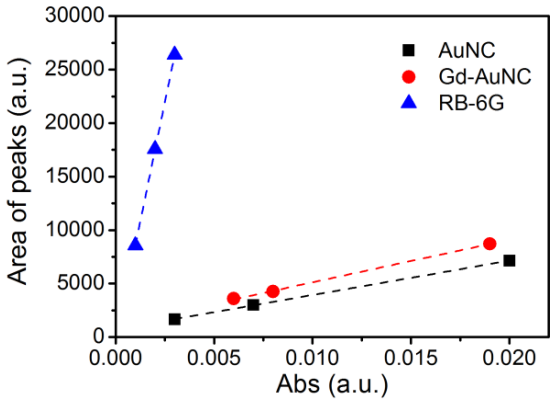

Fig. S6 Determination of the quantum yields (QY) of AuNC and Gd-AuNC by using Rhodamine 6G as a reference.

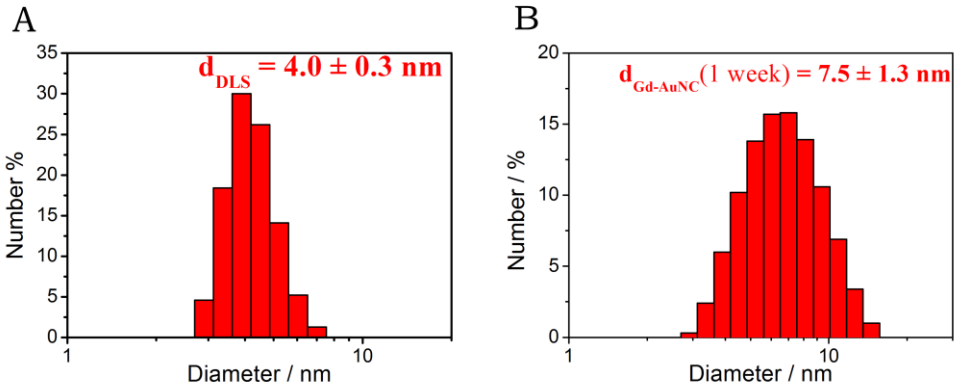

Fig. S7 Hydrodynamic diameters of (A) as-prepared Gd-AuNC and (B) Gd-AuNC that has been stored at room temperature for a week.

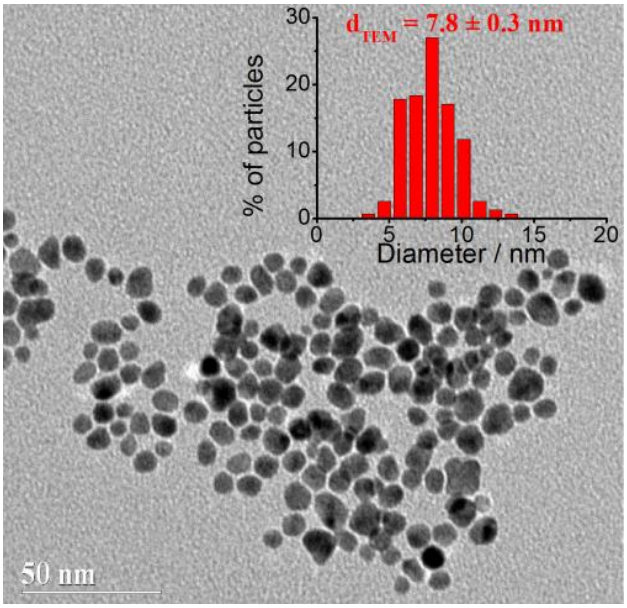

Fig. S8 Transmission electron micrograph (TEM) of Gd-AuNP, and the nanoparticles have an average core size of (7.8±0.3) nm, as indicated in the inset.

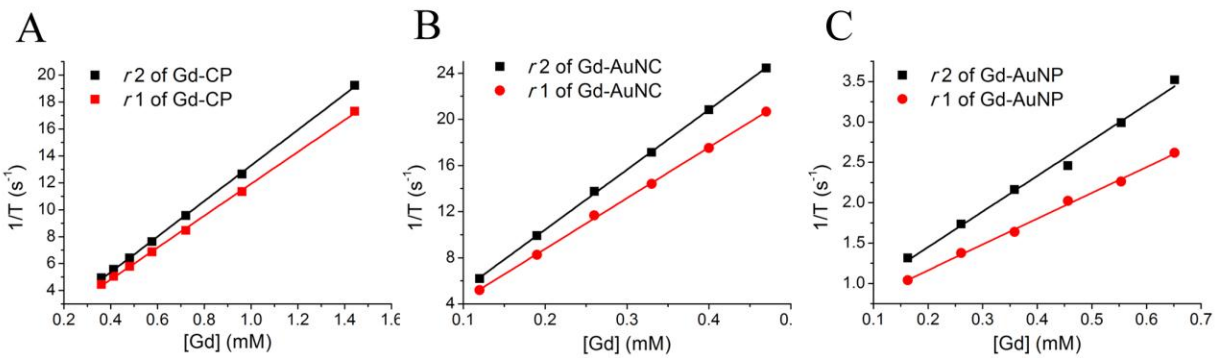

Fig. S9 Relaxivities of (A) Gd-CP, (B) Gd-AuNC and (C) Gd-AuNP at 0.55 T.

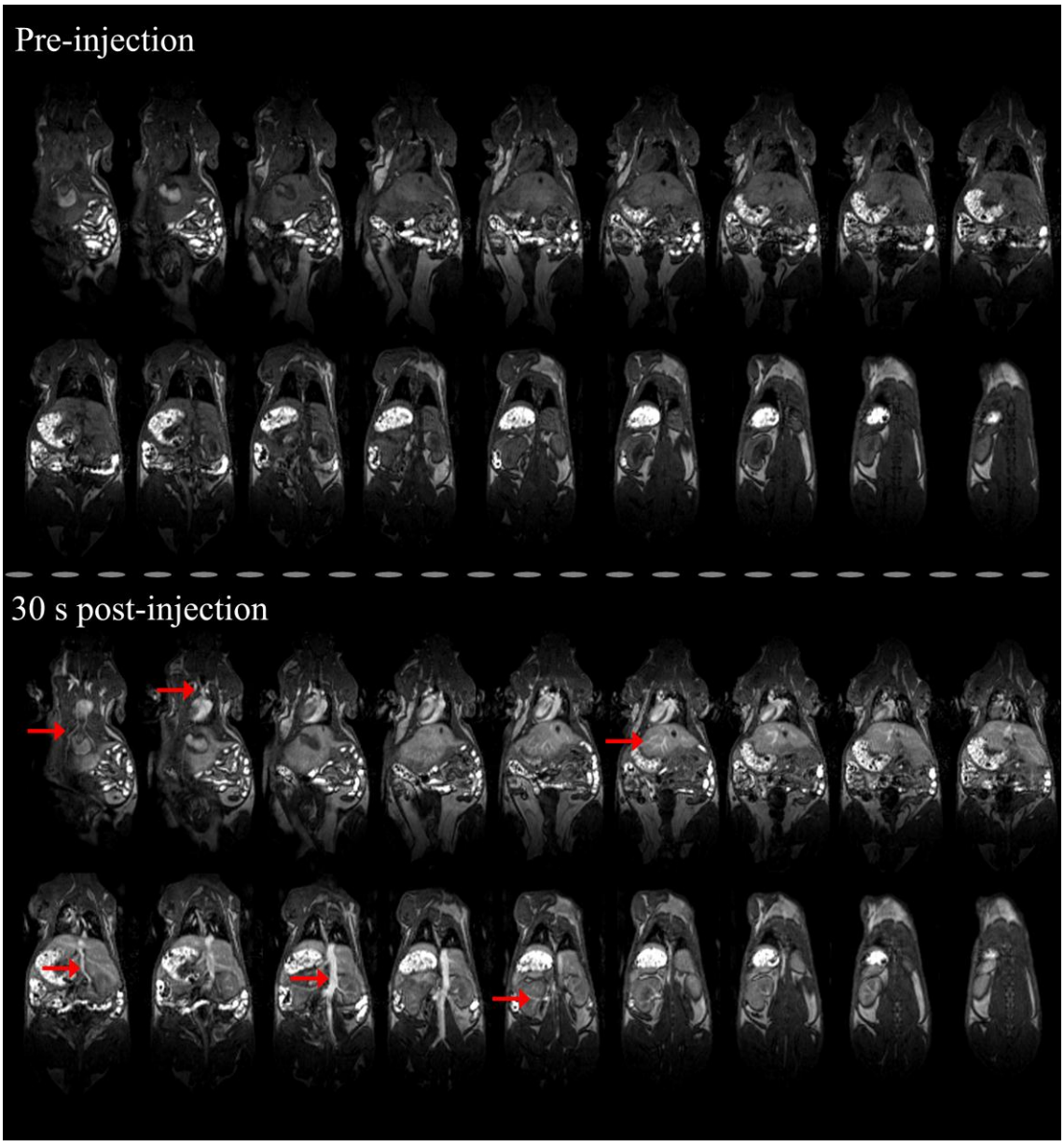

Fig. S10 Slices of the MRI scan of mice before and at 30 s after intravenous injection of Gd-AuNC (0.02 mmol Gd kg^{-1}). Slice thickness = 0.8 mm. Vessels that obviously enhanced after injection of Gd-AuNC were marked with arrows.

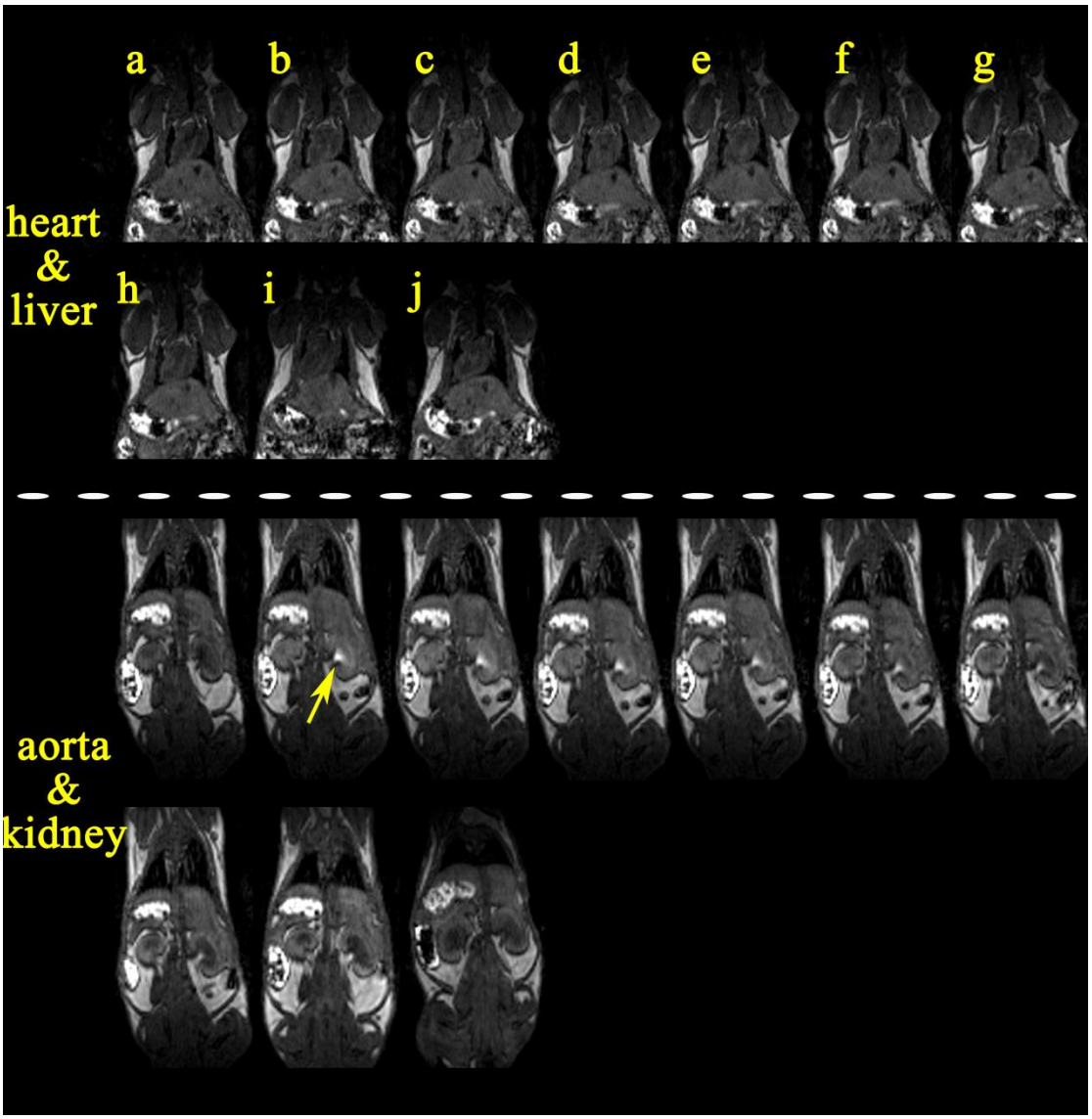

Fig. S11 T_1-weighted MR images of mice acquired before (a) and at (b) 30 s, (c) 1 min, (d) 2.5 min, (e) 5.5 min, (f) 10.5 min, (g) 30.5 min, (h) 1 h, (i) 2 h, and (j) 24 h after intravenous injection of Gd-DTPA (0.02 mmol Gd kg^{-1}). The injected contrast agent excrets fast through kidney filtration (marked with arrow in the image).

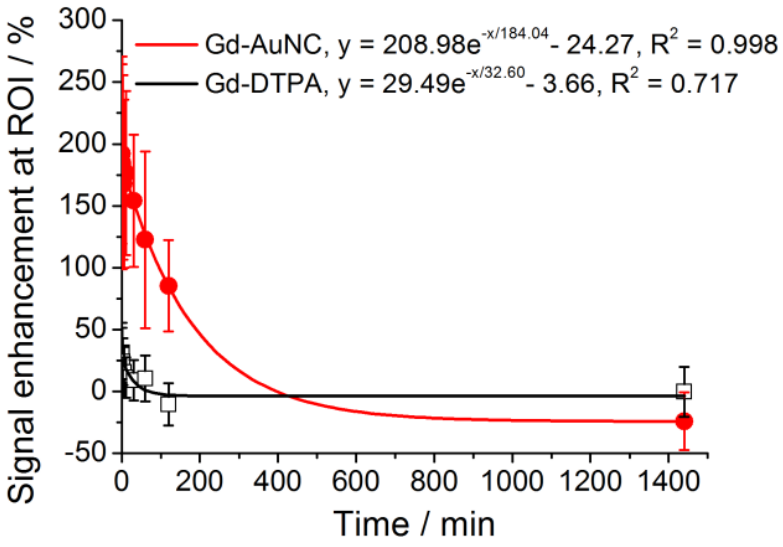

Fig. S12 Determination of blood circulation half-life ($t_{1/2}$) of Gd-AuNC and Gd-DTPA based on the relative signal enhancement in the heart. Exponential curves are fitted to the data points.

Tumbling time of Gd-AuNCs

The tumbling time of Gd-AuNCs (τ_R) was calculated using the following formula[3]:

$\tau_R = 4\pi\eta a^3/3k_BT$

Where, η dynamic viscosity = 10^{-3} pa.s, $k_BT = 4.2\times10^{-21}$ J, a is the hydrodynamic radius of Gd-AuNCs, which is 2.0 nm, T = 305 K.

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