SUPPORTING INFORMATION

Gd^{3+}-1,4,7,10-Tetraazacyclododecane-1,4,7-triacetic-2-hydroxypropyl-β-cyclodextrin/Pluronic Polyrotaxane as a Long Circulating High Relaxivity MRI Contrast Agent

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Synthesis of α, ω-Bis-tris(2-aminoethyl)amine Pluronic Triblock Copolymer. The typical synthetic procedure for the F127-TAEA derivative is described as follows. Dried Pluronic copolymer (0.400 mmol) was dissolved in 30 mL dry CH_2Cl_2. Triethylamine (1.5 equiv.) was slowly added over 30 min on an ice bath. The mixture was allowed to slowly warm up to 20 °C before addition of excess CDI (20.0 mmol). This mixture was then stirred under nitrogen for 24 h at 20 °C and concentrated with a rotary evaporator. The product was precipitated in 500 mL ether and filtered. The crude product was washed with ether three times, filtered, and vacuum dried to afford 70-98% of α,ω-bis-carbonylimidazole Pluronic F127 as a white powder. The CDI-activated Pluronic intermediate (3.53 g; 0.276 mmol) was dissolved in 30 mL dry CH_2Cl_2 before addition of tris(2-aminoethyl)amine (13.8 mmol). The mixture was then stirred under dried N_2 at 20 °C for 24 h. The product was precipitated in 300 mL ether and washed three times with diethyl ether by centrifugation. The final product was then dried under a 50 μm Hg vacuum for 72 h to yield α,ω-bis-tris(2-aminoethyl)amine Pluronic intermediate (Pluronic-TAEA) as a white powder. ^{1}H NMR (D_2O): δ = 1.00 ppm (m, CH_3 of PPG), 2.60-2.80 ppm (m, 16H, CH_2 of TAEA), 3.54-3.65 ppm (m, CH_2 of PEG, and PPG, CH of PPG).

Synthesis of Cholesterol-endcapped PR. Dried Pluronic-TAEA (0.08 mmol) and 2-hydroxypropyl-β-cyclodextrin (purchased from Sigma-Aldrich, 0.8 molar substitution), (i.e., ratio of CD:PPG unit = 1:2) was suspended in 60 mL hexane and the mixture vortexed for 3 min before vigorously stirring for 2 h. Next, bath sonication for 1 h at 20 °C, followed by 10 min probe sonication (Model W-350, 50 W, ½" probe), was performed to improve the threading efficiency of the Pluronic copolymer, followed by an additional 72 h stirring at 20 °C. The hexane was removed under reduced pressure and the intermediate re-dissolved in 20 mL of CH_2Cl_2 before addition of cholesteryl chloroformate (0.48 mmol). The mixture was stirred at 20

°C for 24 h. The unreacted reagents and unthreaded cyclodextrins were removed by twice dissolving the crude product in 10 mL of CH_3OH and precipitating the product in 500 mL diethyl ether. The product was purified by dialysis using 12,000-14,000 and 6,000-8,000MWCO regenerated cellulose membranes in DMSO first and progressively exchanged with deionized water for 5 d before lyophilization to generate a white powder of polyrotaxane (HPCD/Pluronic PR). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.92 ppm (S, H-NCO carbamate), 5.25 ppm (t, 1H, Chl-ethylene H), 5.0 ppm (b, C_1-H of CD), 4.5 ppm (b, OH propyl), 3.5-3.8 ppm (m, $C_{3,5,6}$-H of CD), 3.5 ppm (m, PEG-CH_2), 2.6-2.8 ppm (m, 16H, CH_2 of TAEA), 1.0 ppm (d, CH_3 of PPG), 0.8-0.6 ppm (m, Chl-CH_3).

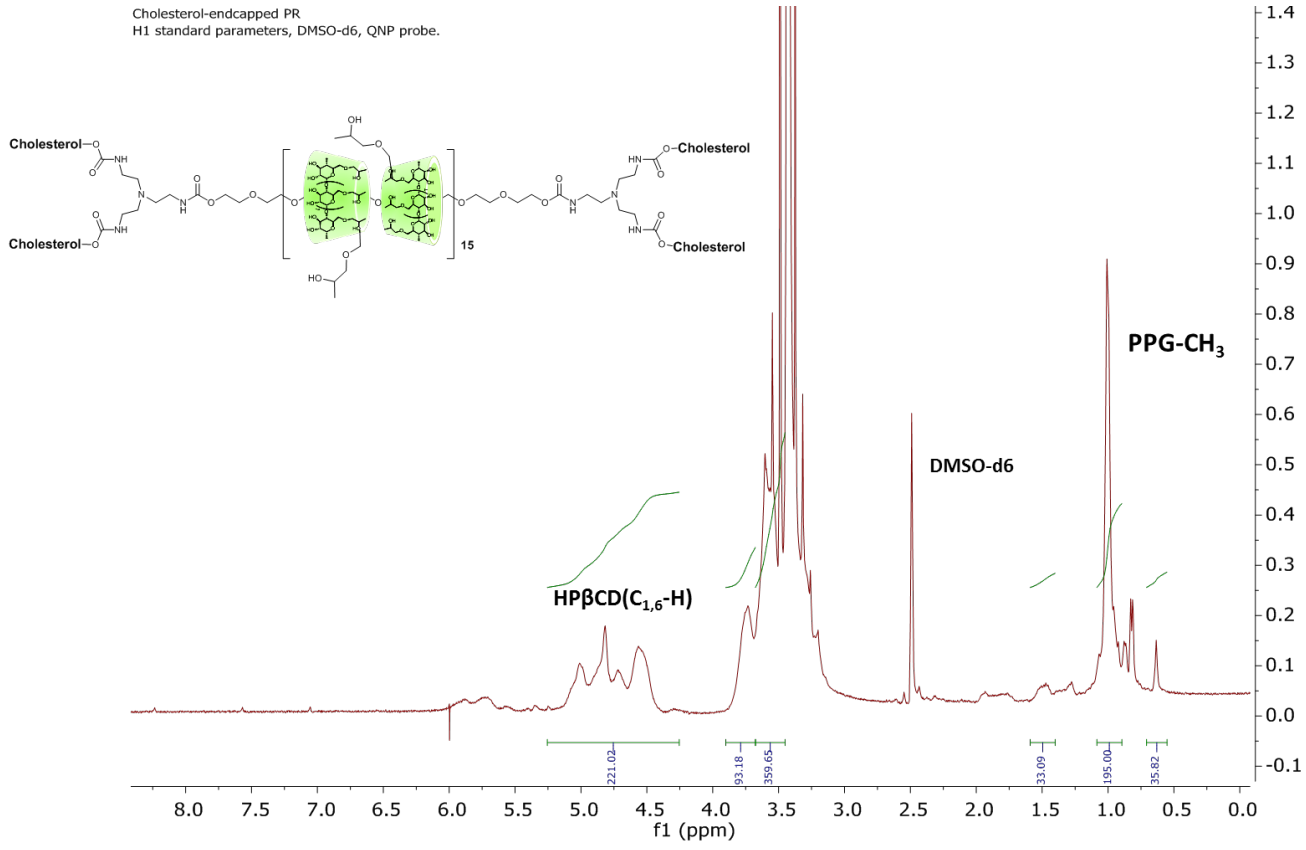

Figure S1: ^{1}H NMR of Cholesterol-endcapped HPCD/Pluronic PR.

Synthesis of 1,8-Diamino-3,6-dioxooctane-modified PR. Dried PR (100 mg) from the previous step was dissolved in 10 mL DMSO and stirred under a N_2 atmosphere. To this mixture, an excess of 1,1'-carbonyldiimidazole (100 mg) was added and allowed to stir for 4 h. At the end of 4 h, an excess of 1,8-diamino-3,6-dioxooctane (5 mL) was slowly added to the solution and the reaction mixture was allowed to stir for 16 h at 20 °C. At the end of 16 h, the reaction was stopped and the product was purified by dialysis using 12,000-14,000 and 6,000-8,000 MWCO regenerated cellulose membranes in DMSO first and progressively exchanged with deionized water for 5 d before lyophilization to generate 1,8-diamino-3,6-dioxooctane modified PR (DADO-HPCD/Pluronic PR) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.05 ppm (s, 1H-NH-CO), 5.25 ppm (t, 1H, Chl-ethylene H), 5.1-4.3 ppm (b, C1,6-H of CD), 3.8-3.5 ppm (m, $C_{3,5,6}$-H of CD), 3.5 ppm (m, PEG-CH_2), 3.4-3.2 ppm (m, $C_{2,4}$-H of CD) 2.6-2.8 ppm (m, 16H, CH_2 of TAEA), 1.0 ppm (d, CH_3 of PPG), 0.8-0.6 ppm (m, Chl-CH_3).

Synthesis of DO3A-Bn-SCN-modified PR. Dried PR (50 mg) from the previous step was dissolved in 5 mL DMSO and stirred under a N_2 atmosphere. To this mixture, an excess of S-2-(4-Isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane tetraacetic acid (DO3A-Bn-SCN) (50 mg) was added and allowed to stir for 6 h. At the end of 6 h, the reaction was stopped and the product purified by dialysis using 12,000-14,000 and 6,000-8,000 MWCO regenerated cellulose membranes in DMSO first and progressively exchanged with deionized water for 5 d before lyophilization to generate DO3A-Bn-SCN modified PR (DO3A-HPCD/Pluronic PR) as a yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.5-7.0 ppm (d, 4H-Ar), 5.25 ppm (t, 1H, Chl-ethylene H), 5.1-4.3 ppm (b, $C_{1,6}$-H of CD), 4.0 ppm (s, NH-CS), 3.8 ppm (s, 6H, CH_2-CO_2H), 3.5-3.8 ppm (m, $C_{3,5,6}$-H of CD), 3.5 ppm (m, PEG-CH_2), 3.4-3.2 ppm (m, $C_{2,4}$-H of CD), 3.5-2.46 ppm (m, 16H, CH_2-N-DO3A), 2.6-2.8 ppm (m, 16H, CH_2 of TAEA), 1.0 ppm (d, CH_3 of PPG), 0.8-0.6 ppm (m, Chl-CH_3).

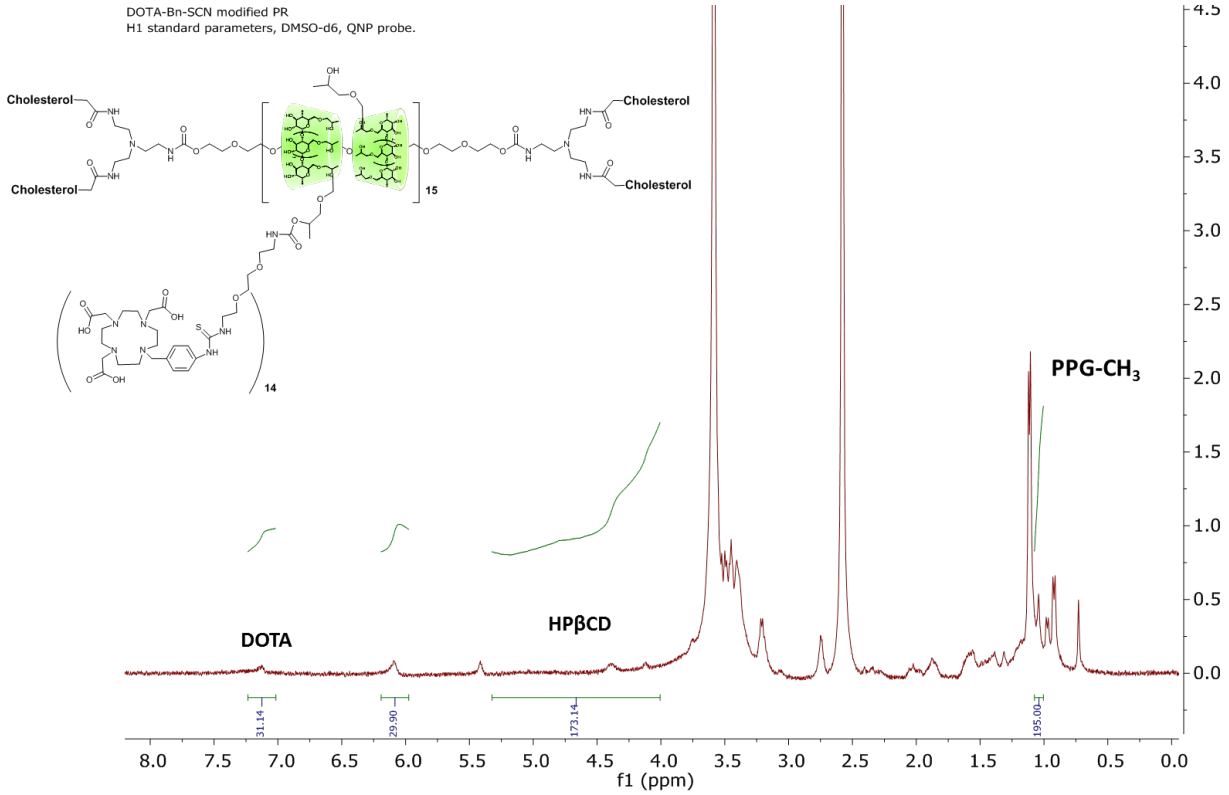

Figure S2: ^{1}H NMR of DO3A modified HPCD/Pluronic PR.

Synthesis of Polyrotaxane Gadolinium Complexes (Gd^{3+}-DO3A-HPCD/Pluronic PR). Gd^{3+} complexation was performed as follows: $GdCl_3.6H_2O$ (2 equiv of one DO3A) was dissolved in H_2O (2 mL) and mixed with the DO3A-HP-β-CD/F127-PR intermediate (50 mg) previously dissolved in H_2O (5 mL) and the pH of the solutions was maintained around 7.0 using NaOH and HCl solutions. The reaction mixtures were stirred at 20 °C for 72 h and the products were dialyzed against water (pH 7.0) for 3 days using membrane with MWCO of 12.0-14.0 kDa.

Nuclear Magnetic Resonance, NMR. ^{1}H NMR spectra were collected on Bruker AV-III-500-HD with a CryoProbe. Spectra were recorded at 25 °C using DMSO-d_6 as solvent unless otherwise indicated, at a concentration of approximately 10 mg of polyrotaxane/1 mL of solvent.

Gel Permeation Chromatography. Absolute mass of the polyrotaxane contrast agents were obtained from an Agilent Technologies 1200 series chromatograph equipped with a Shodex SB-803-HQ column with DMSO as eluant at a flow rate of 0.1 mL/min using RI and light scattering detections. Pullulan (MW 12.0 kDa), and three dextrans (MW 11.6; 48.6; and 667.8 kDa) were used as standards. The samples were dissolved in DMSO (2 mg/mL) and eluted for 150 min.

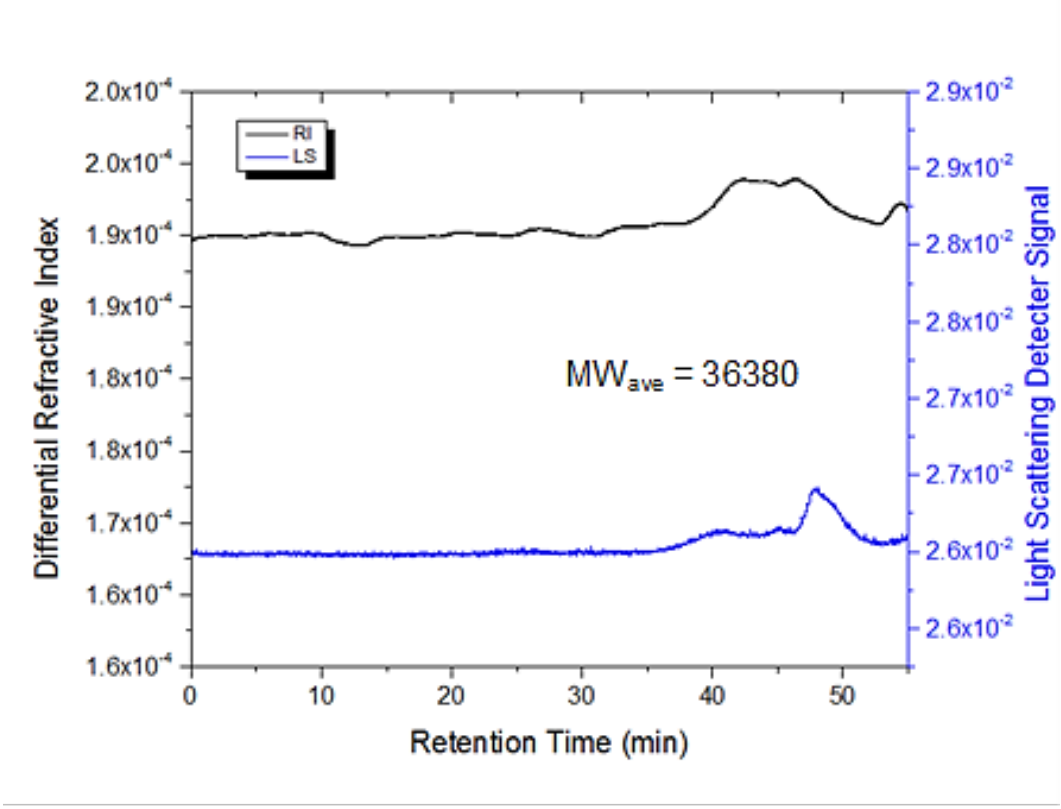

Figure S3: GPC-MALS/RI chromatogram of Chol endcapped Gd^{3+}-DO3A-HPCD/Pluronic PR.

Matrix Assisted Laser Desorption Ionization Time-Of-Flight, MALDI-TOF. MALDI-MS spectra were acquired over a mass range of 1500 – 35000 Da in positive-ion reflector mode on an Applied Biosystems/MDS Sciex 4800 MALDI-TOF/TOF Analyzer with 4000 Series Explorer v3.5 software using a laser power of 6000 and 6500 laser shots in linear mode. The matrix consisted of a freshly prepared ionic liquid matrix (ILM) made using a previously described

protocol with some modifications.[1] Briefly, 2',4',6'-trihydroxyacetophenone monohydrate (THAP) and 1,1,3,3-tetramethylguanidine (TMG) were mixed at a molar ratio of 1:2 in CH_3OH. The solution was then sonicated for 15 min at 40 °C. After removal of CH_3OH by centrifugal evaporation in a SpeedVac for 3 h at 20 °C, ILMs were left under a 50 μm Hg vacuum overnight. Final ILM solutions were then prepared at a concentration of 90 mg/mL in DMF for use as a matrix. The polyrotaxanes samples were prepared at 3 mg/mL in DMF and then mixed in a 1:80 polyrotaxane:ILM ratio for MALDI-MS analysis. Then, 0.6 μL of the polyrotaxane:ILM mixture was deposited onto a mirror-polished stainless steel MALDI target and allowed to dry at 20 °C under atmospheric pressure overnight before analysis.

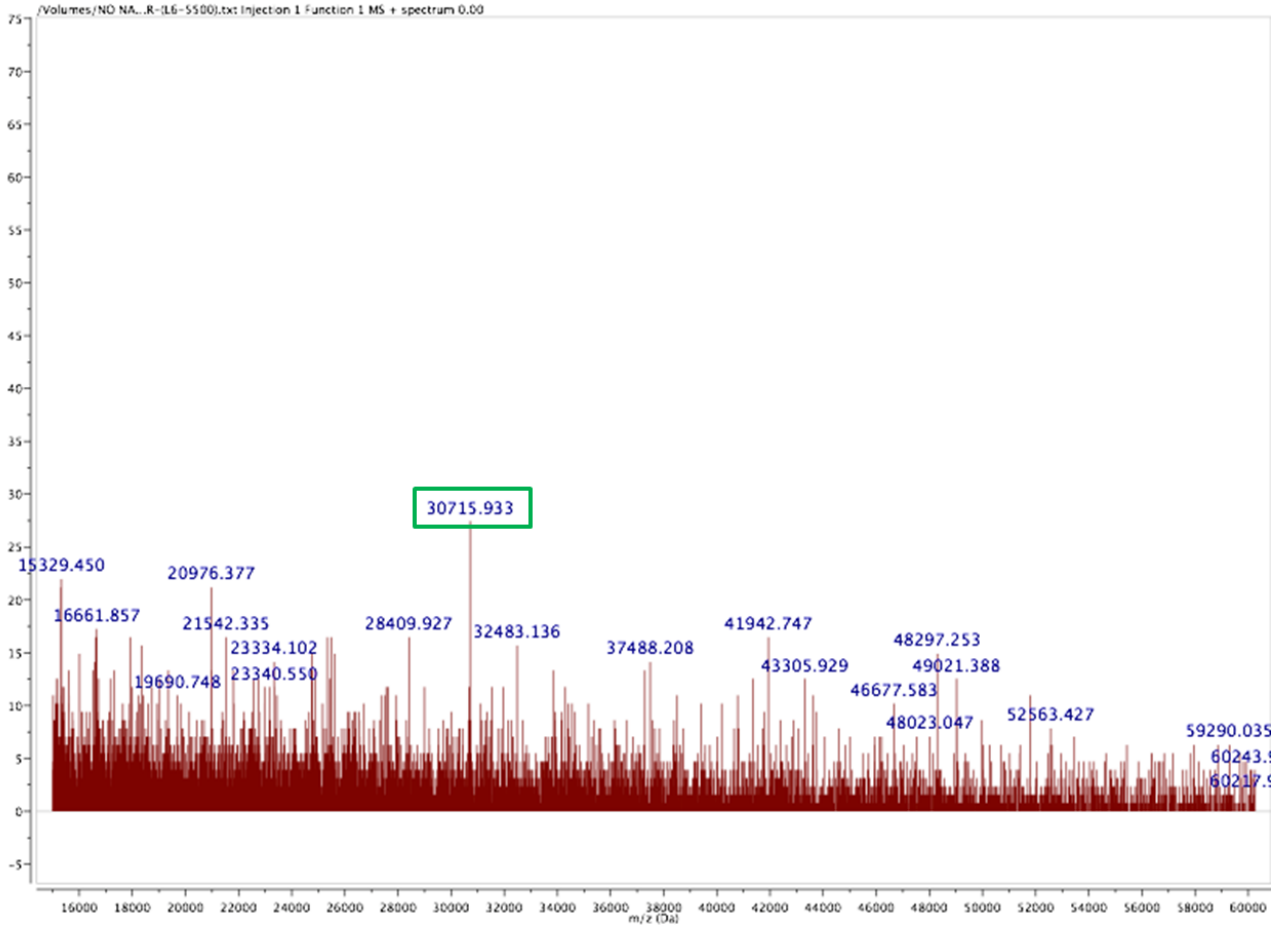

Figure S4: MALDI-TOF MS of Chol endcapped Gd^{3+}-DO3A-HPCD/Pluronic PR.

Inductively Coupled Plasma Mass Spectroscopy. ICP-MS (Quadrapole ICP-MS, Agilent Technologies) was utilized to analyze gadolinium content in the polyrotaxanes. Samples were digested with 2% nitric acid (TraceMetal Grade, Fisher Scientific) and diluted from a gadolinium, 2% HNO_3 Certiprep ME 1 standard solution of 1 mg/mL (SPX CertiPrep, Metuchen, NJ) using a standard additive method and introduced into a temperature controlled spray chamber with a MicroMist Nebulizer.

Atomic Force Microscopy. The topology (diameter, height) of the polyrotaxane particles was determined in air at 22 °C by tapping-mode atomic force microscopy using a Multimode AFM equipped with Nanoscope IIIa controller (Veeco Instruments, USA), an uncoated probe tip of 10 nm of less (NSC15/A1BS, MikroMasch, USA), and cantilevers having a spring constant of 40 N/m. In a typical measurement, 5.0 µL of the polyrotaxane sample (1.0 x 10^{-4} mg/mL in DMSO) was deposited onto a mica surface and air dried for 72 h prior to imaging.

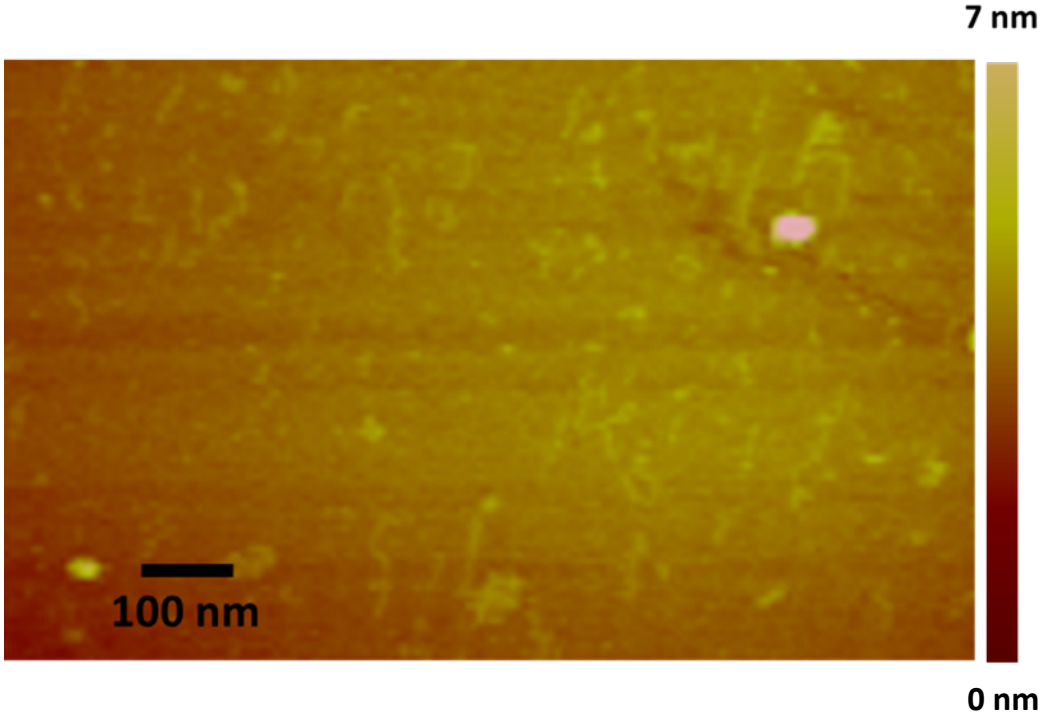

Figure S5: AFM of Chol endcapped Gd^{3+}-DO3A-HPCD/Pluronic PR. The sample was cast onto mica as a dilute DMSO solution and air dried before imaging.

Relaxivity Measurements. The T_1 and T_2 molar relaxivities of Gd^{3+}-HP-β-CD and Gd^{3+}-HP-β-CD/F127-PR was determined using Bruker minispec relaxometer (1.5T, 60 Hz). T_1 and T_2 relaxation times of the aqueous solution of each contrast agent at different concentrations (0.1, 0.25, 0.5, 1.0, 1.5, 2.0 mM) were measured using a saturation-recovery pulse sequence at 37 °C. The Gd(III) content of each sample was confirmed by ICP-OES after the MR scanning. T_1 data acquisition was completed at a fixed echo time (T_E = 11 ms) and different repetition times (TR = 100, 200, 400, 800, 1600, and 3200 ms). For T_2, T_R = 2000 ms, T_E = 15, 30, 45, 60, 75, 90, 105 ms. The net magnetization (M) of each sample was measured using the software Osirix (http://www.osirix-viewer.com). The relaxivities were calculated as the slope of the plot of $1/T_1$ and $1/T_2$ versus the concentration of Gd(III).

In Vivo MR Imaging. ***Animal Models.*** Balb/c nude homozygous mice of weeks, 20 g (Charles River) were cared for at the Animal Core Facility of Case Western Reserve University according to an animal protocol approved by the CWRU Institutional Animal Care and Use Committee (IACUC). ***MR Imaging.*** MRI studies were performed using a Bruker Biospec 7T MRI scanner with a radio frequency (RF) coil. Anesthetization was performed in an isoflurane induction chamber with a 2% isoflurane-oxygen mixture. Subsequently, the tail vein of the mouse was catheterized with a 27-gauge needle connected to 1.6 m tubing flushed with heparinized saline. The mouse was moved into the MR scanner, where anesthetization was maintained with 2% isoflurane-oxygen via nose cone. Respiration rate was monitored using a respiratory sensor placed under the abdomen of the mouse. Body temperature was maintained at 35 °C using a feedback control system. After pre-injection baseline MR images had been taken, the imaging agent was injected at a dose of 0.03 mmol Gd/kg mouse into the catheter, and was subsequently flushed with 100 μL of saline and the post injection images were

collected at 5, 15, and 30 min using the following conditions: Flash 3D T_1-weighted gradient echo, high resolution, T_R = 50 ms, T_E = 2.8 ms, FA = 15°, FOV = 80 x 40 x 40 mm^3, matrix = 256 x 256 x 21, slice thickness = 21 mm, N_{av} = 3, resolution = 0.0234 x 0.0234 cm/pixel, resolution = 0.0117 x 0.0234 cm/pixel. ***Data Analysis.*** MR image analysis was performed using Paravision 4.0 software. Regions of interest (ROI) were set in the tumor, in a non-tumor region, and in the background. To quantify the contrast in the tumor, contrast to noise ratios (CNR) was calculated at each time point. CNR is defined as:

$$ER = \frac{S_{post}}{S_{pre}}$$

Where S_{pre} and S_{post} are the signal intensities calculated from tissues pre-injection and post-injection images, respectively.

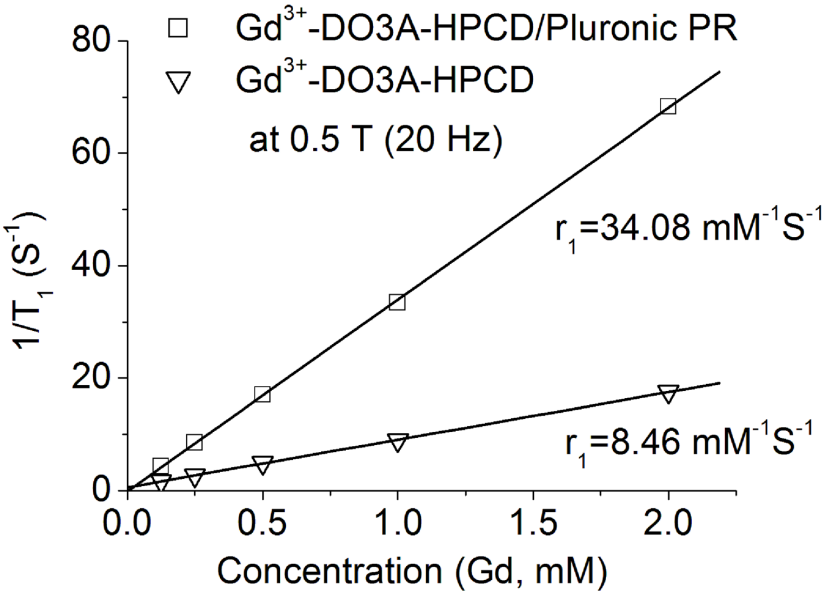

Figure S6: r_1 relaxivity measurements of Gd^{3+}-DO3A-HPCD/Pluronic PR and Gd^{3+}-DO3A-HPCD.

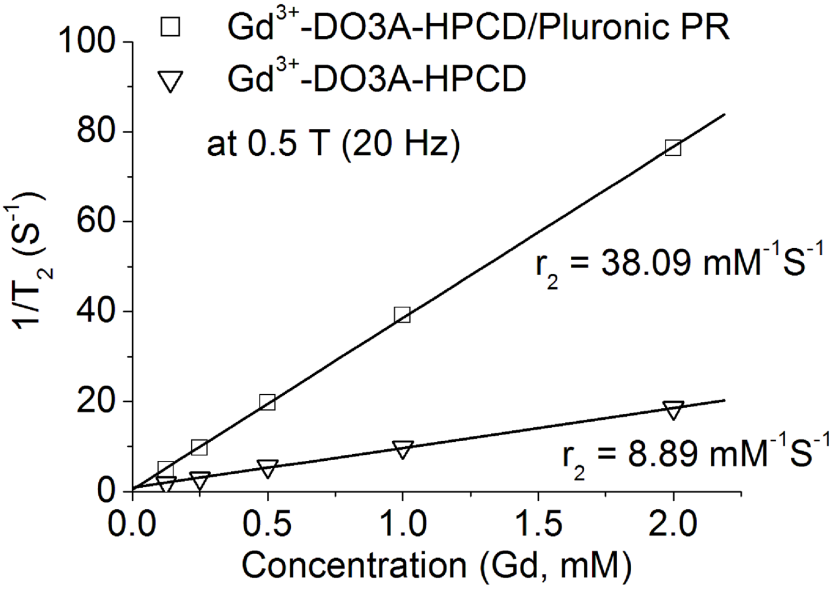

Figure S7: r_2 relaxivity measurements of Gd^{3+}-DO3A-HPCD/Pluronic PR and Gd^{3+}-DO3A-HPCD.

Reference

1. Michels, J. J.; O'Connell, M. J.; Taylor, P. N.; Wilson, J. S.; Cacialli, F.; Anderson, H. L. Synthesis of Conjugated Polyrotaxanes Chem. Eur. J. 2003, 9, 6167-6176