Supporting Information

Body Temperature Sensitive Micelle for MRI Enhancement

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1. General Experimental Section

Materials. Multiple block polymers Poly (N - isoprylacrylamide - b - methyl methacrylate) (PNIPAAm - b - MMA, purchased from Polymer Sources. Inc, Mn = 10000, N - isoprylacrylamide = 10 mol %; Mn = 50000, N - isoprylacrylamide = 80 mol %) was pre-treated with THF solution. 2 - (4, 7, 10 - tris (2 - (tert - butoxy) - 2 - oxoethyl) - 1, 4, 7, 10 – tetraazacyclododecan - 1 - yl) acetic acid (DOTA - tris - tBu ester) was purchased from TCI, Shanghai. N-Hydroxysuccinimide (NHS) and Europium (III) chloride hexahydrate was purchased from Sigma Aldrich. Ethanediamine, triethylamine and N - (3 - Dimethylaminopropyl) - N - ethylcarbodiimide hydrochloride (EDCI) were both from Sinopharm Chemical Reagent Co., Ltd. Trifluoroacetic acid (TFA) and glycine ethyl ester hydrochloride were purchased from Aladdin Reagent (Shanghai, China). All the other reagents and medical supplies were purchased from Sigma Aldrich and Sinopharm Chemical Reagent Co., Ltd. All reagents were of analytical grade, and used as received. Doubly deionized water and ultra-purified water used for all experiments were both obtained from a Millipore system.

Synthesis. The synthesis route of Eu - [PNIPAAm - MMA - DO3AmcE] was proceeding as follows in Fig.1. About 2g PNIPAAm-b-MMA (Mn = 10000, 10 mol % N - isoprylacrylamide) was treated in 1:5 DMF/water solution by adding DOTA-tBu (0.611 mmol, 350 mg), 12 equivalence EDCI (7.332 mmol, 1.405 g) and NHS (7.332 mmol, 844 mg) was added to the substrate at 4 °C for 1h activation time. 800 μl ethanediamine and 4ml triethylamine were then dropwise added to the flask with stirring overnight at room temperature, the small molecules were removed by dialysis at deionized water to obtain the

complex 1. Deprotect reaction was proceeded with TFA and vigorously stirred for 5 h at room temperature. The crude production 2 were prepared without further treatment, and then it was modified with a 10 fold dose of glycine ethyl ester hydrochloride (1 mmol, 139 mg) as the amidating reaction. The products 3 were washed in hot water repeatedly following by dialysis for 24 h. After lyophilization the products 3 were added to the aqueous solution of Europium (III) chloride hexahydrate and the pH was adjusted to 8 using potassium hydroxide[1]. Subsequently, the solution was stirred at 60 °C for 3 days. The xylenol orange indicator test was used to check for the presence of Free State Eu (III) ion. The solution was then dialyzed again and the 3.3 g crude product 4 was conserved in the FALCO tube after dialysis and lyophilization.

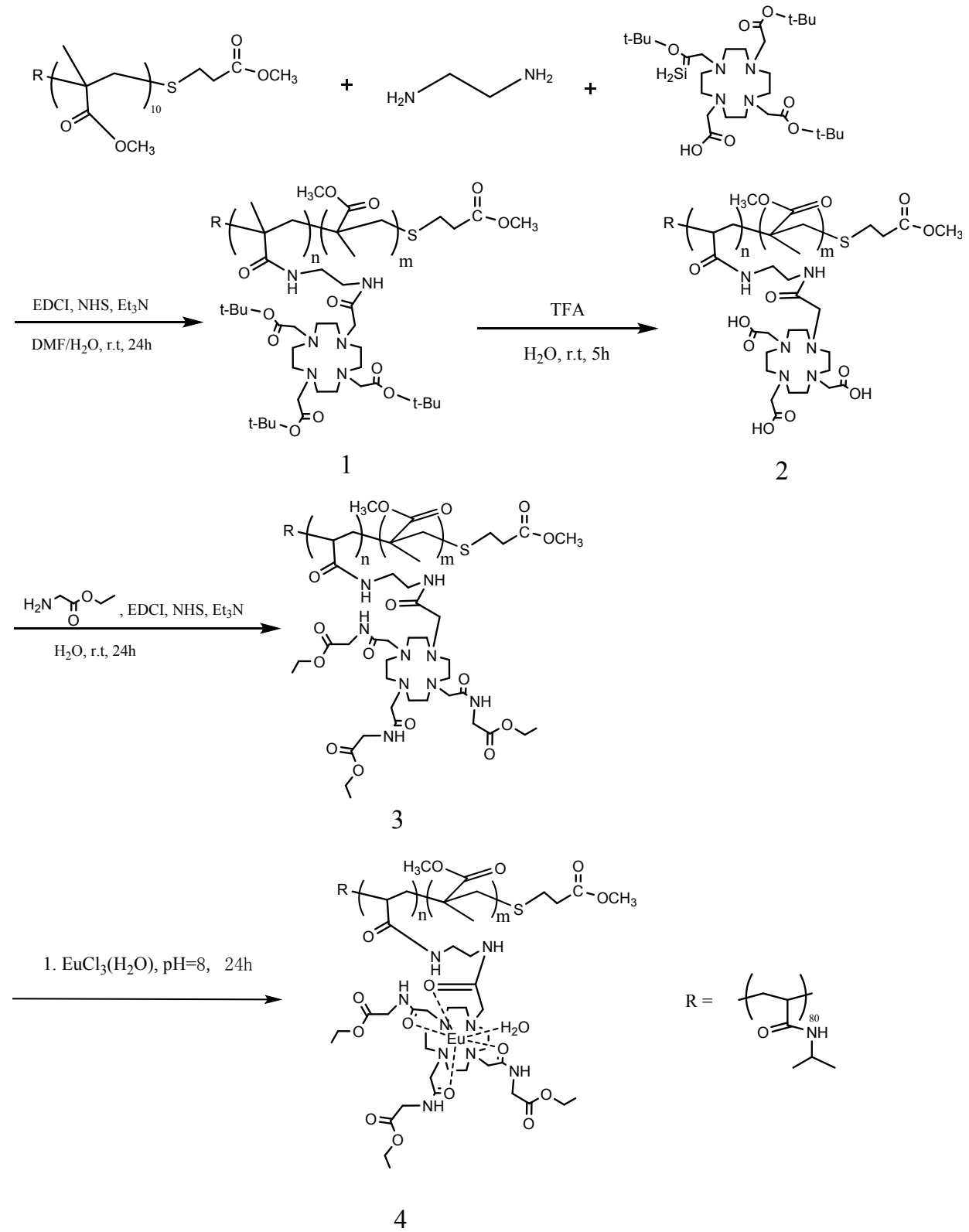

Figure S1. Synthesis of Eu - [PNIPAAm - MMA - DO3AmCE], block polymers were modified with 1, 4,7,10 - tetraazacyclododecane - 1, 4,7,10 - tetrakis (acetyl - glycine) (DOTA - 4Gly) by amidation reaction, and chelating of Eu ionic to obtain the Eu - [PNIPAAm - MMA - DO3AmCE] polymers.

Micellization. To obtain a core-shell micelle solution, 10 eq block polymers PNIPAAm - b - MMA

(Mn = 50000, N - isoprylacrylamide = 80 mol%) were dissolved in modicum DMF mixed with Eu-[PNIPAAm-MMA-DO3AmCE], which was then dropwise added to a 10 fold volume of water with vigorous stirring under ultrasonic for more than 30 min and then filtered using a 0.22 μm pore-sized syringe filter. The solution was injected into a dialysis tube (MwCo = 3500) and subjected to dialysis against 1000 ml ultra purified water for more than 24 h. Subsequently, the products were lyophilized before further examination.

2. Characterization section

Critical micelle concentration measurements. The formation of micelles from the block copolymers Eu-[PNIPAAm-MMA-DO3AmCE] was verified by the critical micelle concentration (CMC) value, which is a threshold concentration when unimers formed as a micelle. It can be measured by introducing the pyrene probe as a fluorescence sensor in the reported literature[2]. Briefly, aliquots of pyrene solutions (6×10^{-4} M in acetone, 6 μl) were diluted to a final concentration of 6×10^{-7} M. Those solutions were stored overnight to ensure sufficient dispersion of pyrene in the solutions and evaporation of acetone. The polymer solutions at different concentrations were added to the containers with pyrene probe, and the fluorescence spectra were recorded with the excitation wavelength of 340 nm to obtain the first peak (I_1) and the third peak (I_3) by fluorescence spectrometer (FluoroMax 4, HORIBA Instruments, Inc.). The intensity of I_1 and I_3 peaks evidently changed at a certain concentration range in the emission spectrum. From the plot of I_3/I_1 ratio versus concentration, the ratio of intensity was virtually a constant = 0.9 at low concentrations, due to the fact that pyrene in water has a very small absorption. The inflexion point of the curve at 10 μg/ml was the CMC value of Eu-[PNIPAAm-MMA-DO3AmCE].

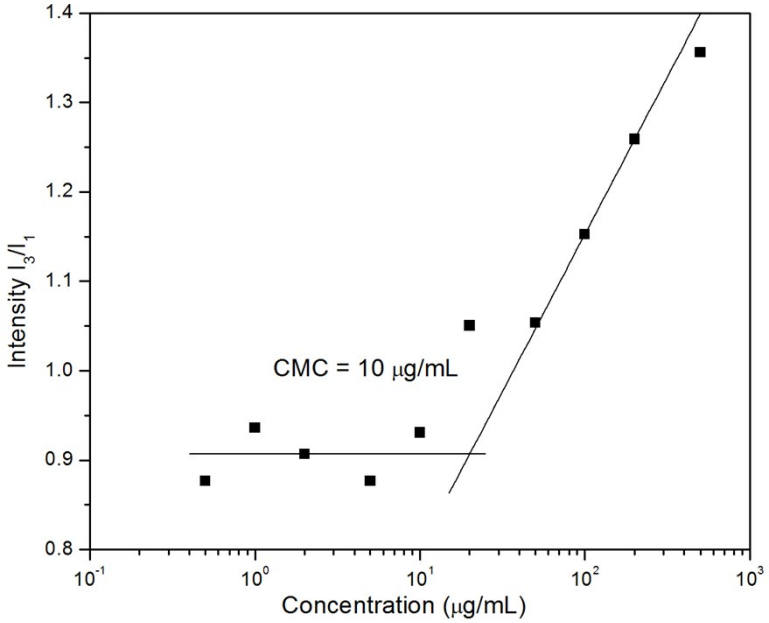

Figure S2. The characterization of critical micelle concentration (CMC) in aqueous solution. Intensities of I_3/I_1 bands by using fluorescence spectra of pyrene in various concentration of Eu-[PNIPAAm-MMA-DO3AmCE].

Characterization of Micelle Low critical solution temperature (LCST). The micelle morphology was measured by Malvern's dynamic light scattering (DLS) and transmission electron microscopy (TEM), FEI Tecnai G^2 system. Samples were prepared under suitable concentrations larger than the CMC, and then the water solutions were filtered for several times for characterization. To investigate the feasibility of the micelle's thermo-response, polymeric micelle's LCST were also characterized by variable temperature experiments. Due to its morphological change around the LCST, the DLS and ultraviolet-visible spectrophotometer analysis were used to measure it. The 0.5 mg/ml micelle solution with Eu-[PNIPAAm-MMA-DO3AmCE] were prepared by PBS buffer solution, pH = 7.1. The diameters of micelles at different temperatures were obtained also by the DLS. Its turbidity measurements at 280 nm, 450 nm, and 550 nm were measured at enzyme-labeling instrument, respectively. The solutions at 96 well plates were heated at a rate of 1 °C per 5 minute. The LCST was defined as the inflexion point both in the diameters curve and the turbidity curves, approximately equal to 37 °C

Europium's Characterization in the Micelle. The concentration of Europium (III) was determined by Inductive Coupled Plasma Atomic Mass Spectrometry (ICP-MS) (X Series 2, Thermo Electron, USA). The sample was preprocessed with hot concentrated nitric acid for 1 h. The amount of Eu(III) per micelle was estimated by the ratio of concentration of Eu(III) and the concentration of Eu-[PNIPAAm-MMA-DO3A] solution.

3. CEST experiments section

^{1}H NMR and Z-spectra experiment. The ^{1}H NMR and CEST experiments were implemented with a 5 mm diameter sample tube at Bruker Avance 500 MHz NMR spectrometer. 220 mg Eu-[PNIPAAm-MMA-DO3AmCE] was fully dispersed into 10 ml D_2O/H_2O = 2/8 (v/v) solution, in order to obtain a 20 mM micelle solutions. The Eu-DOTA-4AmC$^-$ salt with the same concentration was also dissolved in the same solution for the comparison. The CEST spectra were obtained by using a presaturation pulse sequence, with a 5 s hard saturation pulse at a power level optimized to 27.6 dB (equivalent to B_1 = 25 μT). The irradiation frequency of presaturation offsets range from 35,000 Hz to -35,000 Hz with an increment of 500 Hz.

110 mg Eu-[PNIPAAm-MMA-DO3AmCE] was conducted on a Bruker AVANCE III 500 MHz NMR spectrometer with sw = 150 ppm, ns = 3200.^{1}H NMR (500 MHz, D_2O+drops of D_2O): δ=54 - 55 ppm (Bond water), 29.61 - 18.92 (m, ring CH_2N), 11.20 - 6.06 (m, $OCHCH_2N$), 4.80 (s, Bulk water), 4.04 - 3.51 (m, OCH_2CH_3), 2.77 (d, $3J_{H-H}$ = 57.3 Hz), 2.29 - 1.85 (m, $CH_2CH_2CH_2$), 1.71 - 1.34 (m, CH_2CH_2, polymer backbone), 1.34 - 0.90 (m, NHCO, polymer backbone), 0.45 - -0.40 (m, ^{1}H), -2.45 (s, ^{1}H), -4.79 (d, J_{H-H} = 307.5 Hz, ^{1}H), -8.54 (dd, J_{H-H} = 428.5, 179.5 Hz, ^{1}H), -10.76 (dd, J_{H-H} = 460.5, 204.7 Hz, ^{1}H), -12.97 (s, ^{1}H). Hydrogen on -NH were not found.

The CEST experiments were also conducted on a Bruker AVANCE III 500 MHz NMR spectrometer, with a selective saturation pulse optimized at 25 μT, for 5 s duration time and scanned MR frequencies ranging from +70 to -70 ppm with 1 ppm increments (where the water resonance is referenced to 0 ppm). The Z-spectrum of Eu-DOTA-4AmC$^-$ with the same concentration of Europium (III) was also acquired with the above procedure as control experiments.

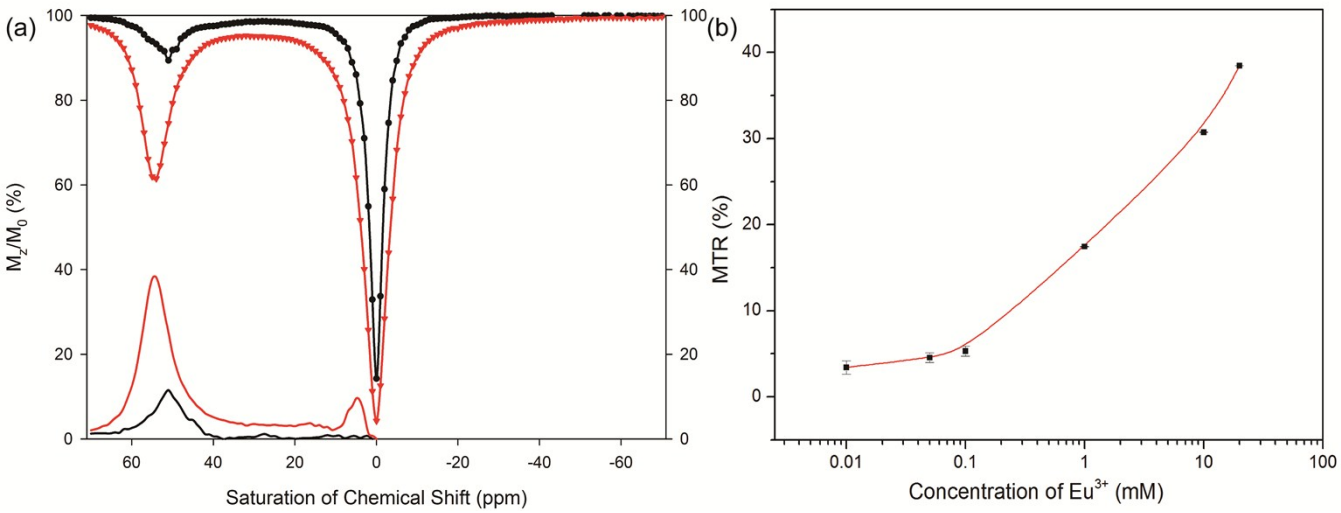

Figure S3. (a) Z-spectra and the plots of Asymmetric magnetization transfer ratio (MTR_{asym}) of Eu-[PNIPAAm-MMA-DO3AmCE] micelle(red) in comparison to the Eu-DOTA-4AmC$^-$ (black) with the same Eu^{3+} concentration of 20 mM. (b) The MTR_{asym} of the Eu-[PNIPAAm-MMA-DO3AmCE] micelle at different Eu^{3+} concentrations, the saturation pulse duration time is 5 s, B1 = 25 μT.

As the previous work has demonstrated that the size of the polymeric PARACEST agent could optimize the detectable concentration of the CEST MRI[3], the Eu-[PNIPAAm-MMA-DO3AmCE] polymeric micelle solution with a homogeneous diameter of 94 nm was optimized. The Z-spectrum of this micelle solution was obtained as shown in Figure S3 (a), in comparison to the conventional Eu-DOTA-4AmC$^-$ aqueous solution at the same concentration of Eu^{3+}. The Z-spectrum clearly showed that the chemical shift of bound water could be visualized as differences between Eu-[PNIPAAm-MMA-DO3AmCE] and Eu-DOTA-4AmCE (50 ppm relate to H_2O), and it demonstrated that the polymeric micelle system enhanced the CEST effect more than 3 fold compared with the conventional Eu contrast agent. However, the Eu-[PNIPAAm-MMA-DO3AmCE] micelle's linewidth of Z spectrum was broader than the conventional Eu^{3+} contrast agent. Such phenomena might be attributed to polymeric micelle's size and morphology, which was similar to the magnetization transfer effect triggered by macromolecules.

Table S1. Metal concentrations analysis vs the micelle solutions obtained by ICP-MS

Micelle concentration (μg/ml)	Eu^{3+} concentration (mmol/l)
22.10×10^3	20
11.05×10^3	10

1.10×10^3	1
110	0.1
55	0.05
10	0.01

The CEST features of Eu-2 and the three Eu-poly2 samples were compared on a per agent basis (Figure 2), and

The lower detection limit of micelle agents, based on the assumption that a 5% CEST effect change is easily detected, was determined from the asymmetric magnetization transfer ratio (MTR_{asym}) of different micelle concentrations (Table S1). The concentration of Eu(III) in the stock polymeric micelle agents was determined by the inductively coupled plasma mass spectrometry (ICP-MS). The experiments were conducted on a Bruker AVANCE III 500 MHz NMR spectrometer, with a selective saturation pulse at 25 μT, for 5 s duration time. The selective pulse irradiate the ±54 ppm site to obtain the MTR_{asym} = ((Ms^- - Ms^+)/Ms^-), and the plot of Eu(III) concentration vs the MTR_{asym} was obtained as shown in Figure S3 (b). The detection limits for the micelle agents is 10 μg/ml. Since that even the concentration of micelle is approaching the critical micell concentration (10 μg/ml micelle solution with 0.01 mM Eu^{3+}), the CEST effect of polymeric micelle could still be kept at 5% $MTR_{asym.}$

To measure the proton exchange rate of the Eu-[PNIPAAm-MMA-DO3AmCE] micelle solution, The equation 1 was derived from the Bloch-McConnell equations using identical assumptions..

$$\frac{M_Z}{M_0 - M_Z} = \frac{C_{agent}}{C_{water}} \times K_b R_1^a \left(\frac{1}{K_b^2} + \frac{1}{\omega_1^2} \right) \qquad [1]$$

ω is the amplitude of RF field (B1) magnetic strength of the saturation RF pulse in radians/second, and R_1 is the T_1 relaxation rate (T_1 = 3.2 s) of the spin pool. C_{agent} and C_{water} (55.5 mM) are contrast agent and water's concentration, respectively. The Plots of CEST effect = M_z/ (M_o-M_z) versus ($1/\omega^2$) (Eq. 1, henceforth referred to as omega plots) provide a direct readout of the exchange rate, $1/k_b$. Three different samples of Eu-[PNIPAAm-MMA-DO3AmCE] micelle solution (1, 10, and 20 mM) collected at 25 °C are shown in Fig. S7. The equation's slope or intercepts of X-axis of these three plots provide a third independent measure of $1/k_b$ or, equivalently, τ_m. It gave an average value τ_m = 120 ± 40 μs. The variable temperature experiments also demonstrate that, when the temperature was increased, the

τ_m value decreases.

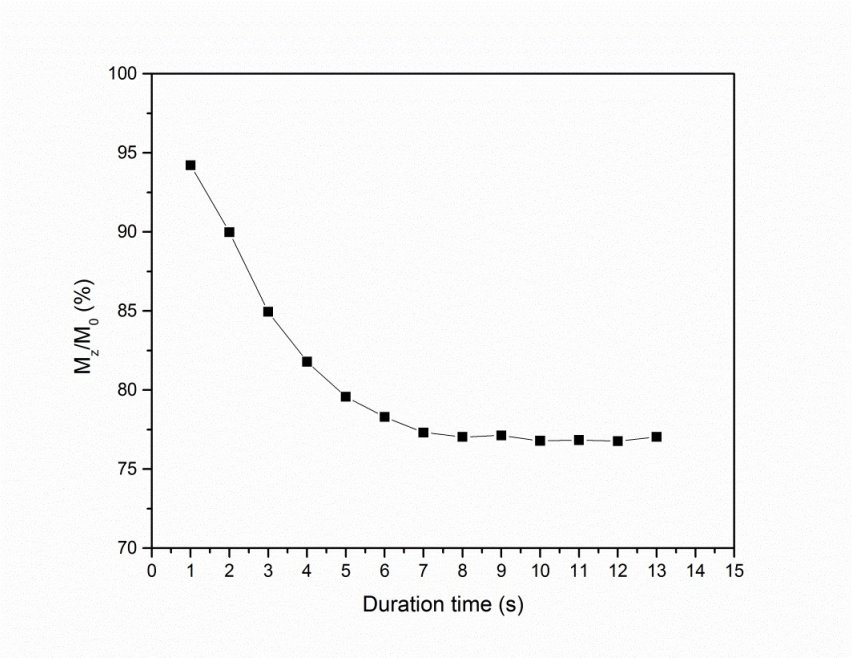

Figure S4. M_z/M_0 of Eu-[PNIPAAm-MMA-DO3AmCE] micelle at different duration times, the concentration of polymeric micelle is 10 mM, pH = 6.8, duration time from 1 s to 13 s with 1 s increment.

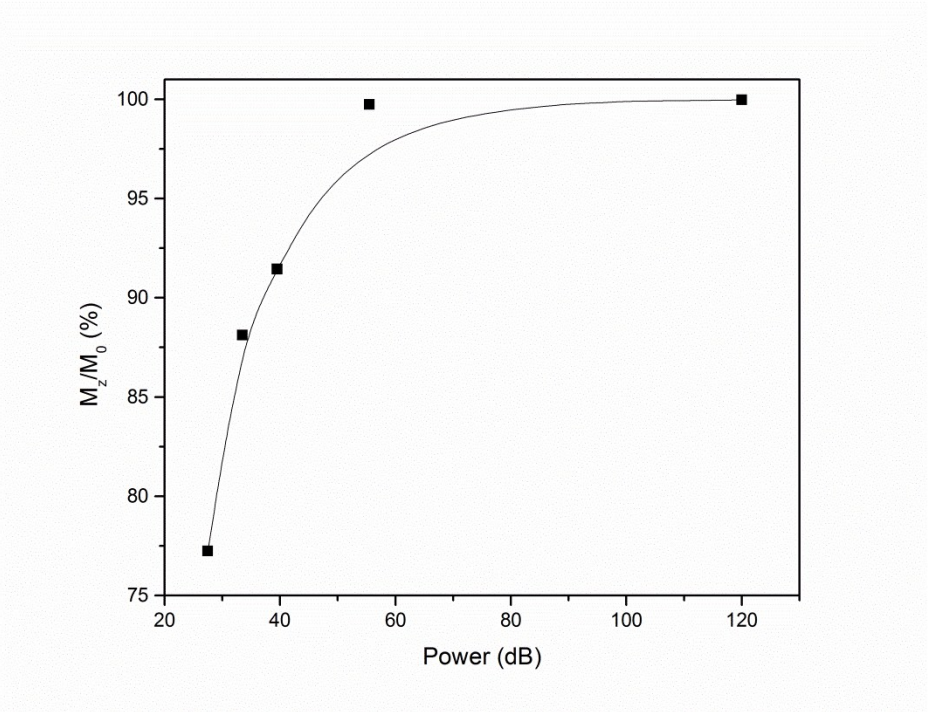

Figure S5. M_z/M_0 of Eu-[PNIPAAm-MMA-DO3AmCE] micelle versus various pulse power, 10 mM polymeric micelle solutions, pH = 6.8, pulse power at B1 = 25 μT (27.5 dB), 12.5 μT (32 dB), 7.5 μT (38 dB), 3 μT (54 dB) and 0 μT (120 dB).

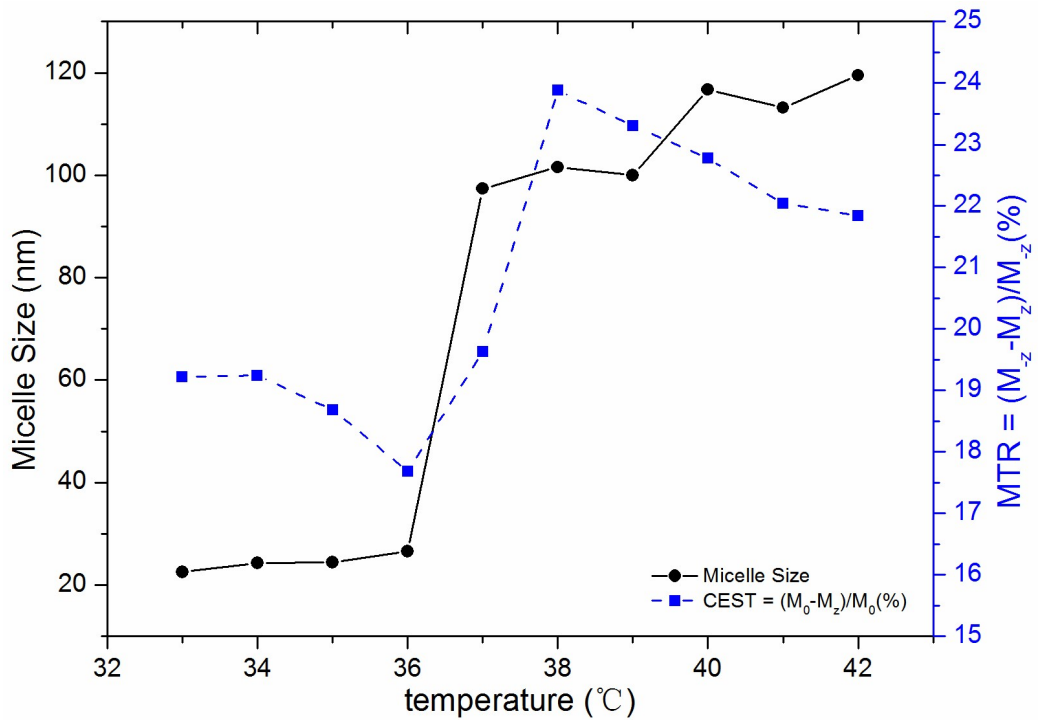

Figure S6. The CEST intensity (%) and the hydrate particle size (nm) for the Eu-[PNIPAAm-MMA-DO3AmCE] micelle versus temperature, 20 mM Eu (III) ions of Eu-[PNIPAAm-MMA-DO3A] micelle solution samples were measured from 33 to 42 ℃

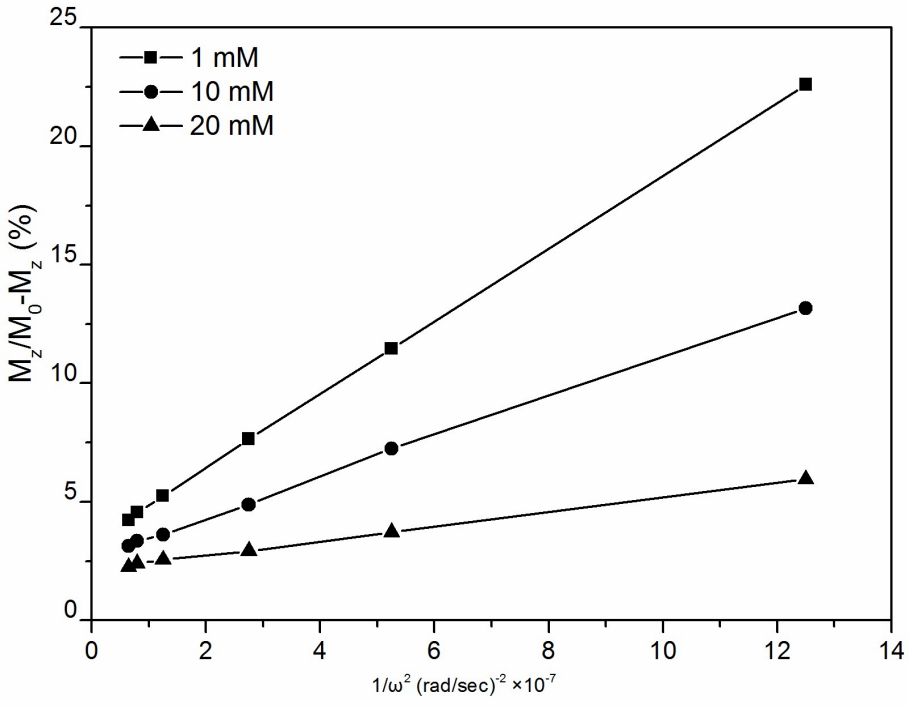

Figure S7. A fitting plot, $M_z/(M_0 - M_z)$ versus $1/\omega^2$ for 1 mM, 10 mM, and 20 mM Eu-[PNIPAAm-MMA-DO3A] in water at pH 6.8 at 25 ℃, using a 10 s saturation pulse at the indicated power levels.

In vitro CEST imaging. All the CEST images were measured at 9.4 T on a Bruker AVANCE III 400 MHz wide bore micro imaging system. The 5 mm tubes were heated from 33 °C to 42 °C with a

variable temperature control unit (BVT 3000) to measure the viariable temperature experiments. The concentration dependence of CEST experiments were measured with 6 different concentrations from Table S1. Before the experiments, the RF field and center frequency were calibrated in pre-scan. A Fast Low-Angle Shot (FLASH) pulse sequence (Flip angle = 10°, TR= 5012 ms, TE= 6 ms, matrix 128×128, field of view 20×20 mm^2, slice thickness 4 mm, one average) was used after a 8 μT selective saturation pulse at 54 ppm centered on the bond water frequency for 8 s. Images with saturation at -54 ppm were also acquired as a reference. The CEST images were obtained as ratios among the images of selective saturation experiments, which were calculated in MATLAB**.** The percent CEST was calculated by comparing the images acquired with selective saturation at +54 ppm with images acquired with selective saturation at -54 ppm. Because we used the FLASH pulse sequence, the contrast of the original MR image deteriorated to a certain degree. However, as the CEST MR image was obtained as the ratio of two different images, the contrast loss in the CEST images was small.

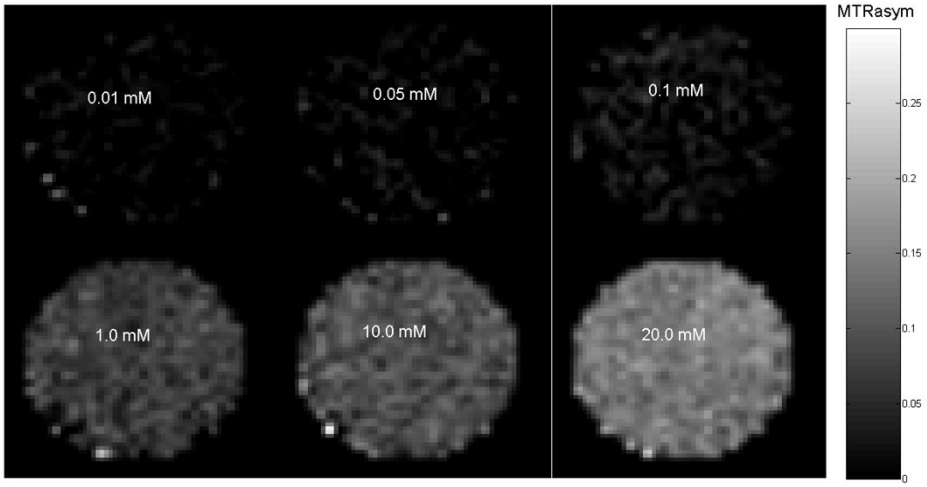

Fig S8. The Eu concentration dependence of the CEST imaging. All the phantom imaging were measured around pH 7 at 9.4 T, B_1 = 8 μT, saturation time = 8 s.

Reference

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