Electronic supplementary information for

Redox-Triggered Aggregation of ESIONPs with T_1 to T_2 Switchable Contrasting Effect for T_2-weighted Magnetic Resonance Imaging

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Materials

$FeCl_3 \cdot 6H_2O$ (98%, Sigma-Aldrich), sodium oleate (97%, TCI), oleic acid (85%, TCI), oleyl alcohol (60%, TCI), diphenyl ether (99%, TCI) were all used without further purification. Other reagents and solvents were purchased from Sinopharm.

Synthesis and characterization of ESIONPs

The iron-oleate complex was obtained by reaction of metal chlorides and sodium oleate. In brief, sodium oleate (36.53 g, 120 mmol) and iron chloride (10.81 g, 40 mmol) were dissolved in 80 ml ethanol, 60 ml distilled water and 140 ml hexane. After reacting for 4 hours at 70 °C, the mixture was extracted to obtained organic phase, following washed with distilled water for 3 times. After that, the mixture was evaporated to obtain iron-oleate complex. To obtain 3nm sized ESIONPs, 2 mmol iron-oleate, oleyl alcohol (1.61 g, 6 mmol) and oleic acid (0.565 g, 2 mmol) were dissolved in diphenyl ether (10 g, 58 mmol). Then, the mixture was heated to 250 °C under argon atmosphere and maintained for 30 min. After that, the reaction was cooled rapidly to room temperature and added 50 ml acetone. The final product was obtained by centrifugation and dissolved in hexane for later use.

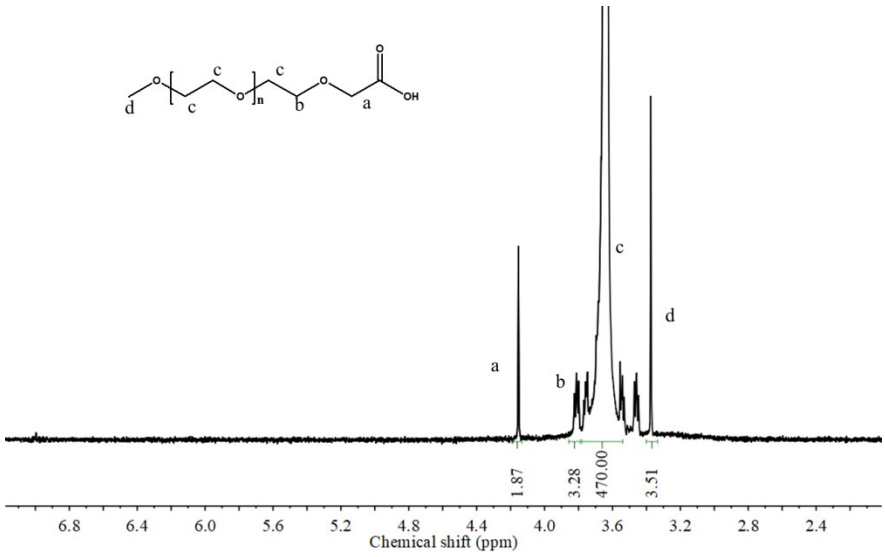

Fig. S1 ^{1}H NMR spectrum of mPEG-COOH (recorded in $CDCl_3$)

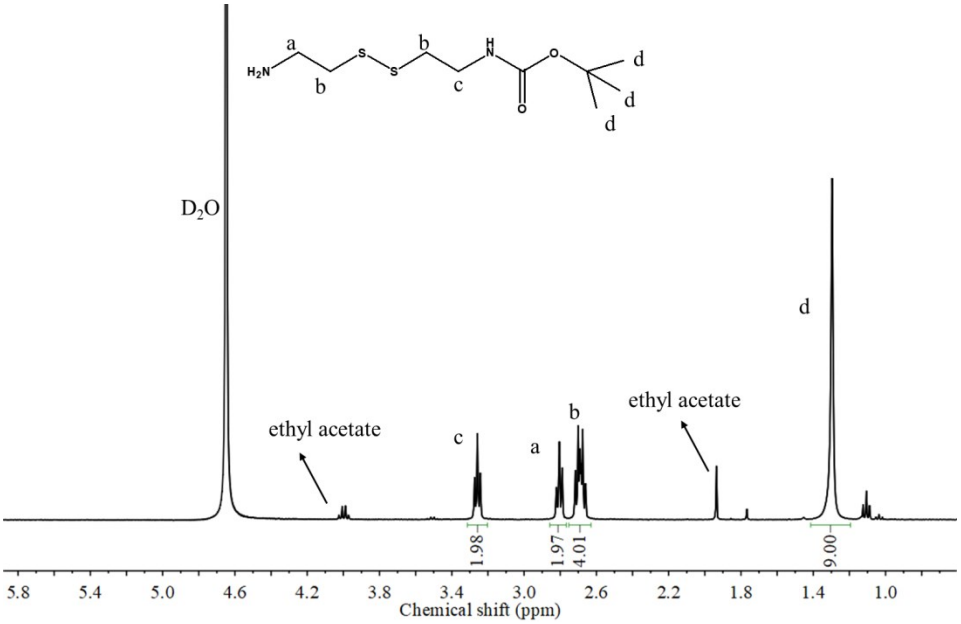

Fig. S2 ^{1}H NMR spectrum of mono-Boc-cystamine (recorded in D_2O)

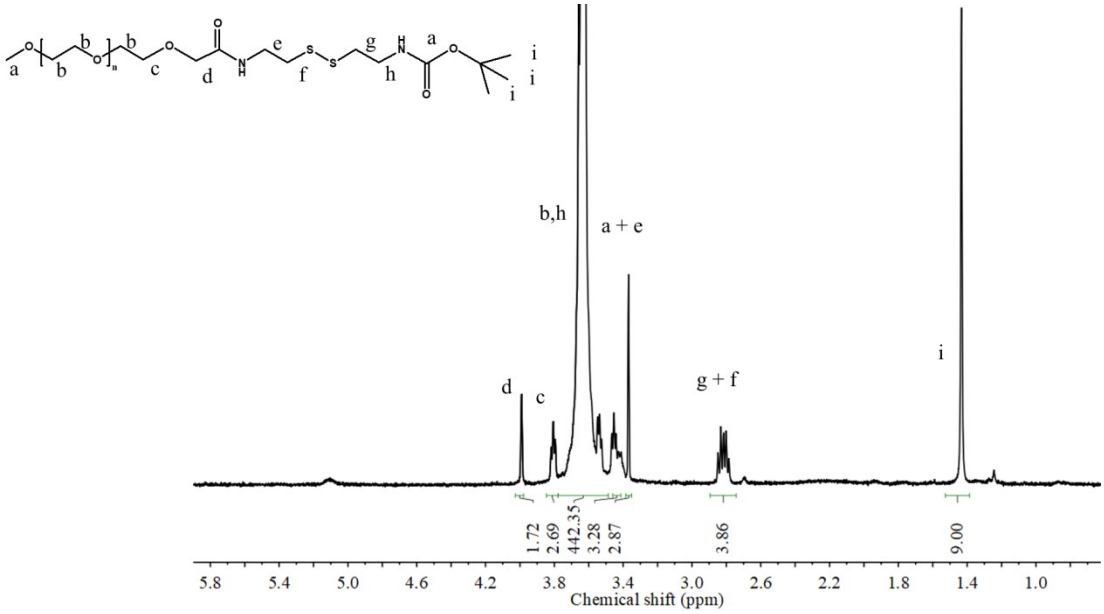

Fig. S3 ^{1}H NMR spectrum of mPEG-*s*-*s*-NH_2-Boc (recorded in $CDCl_3$)

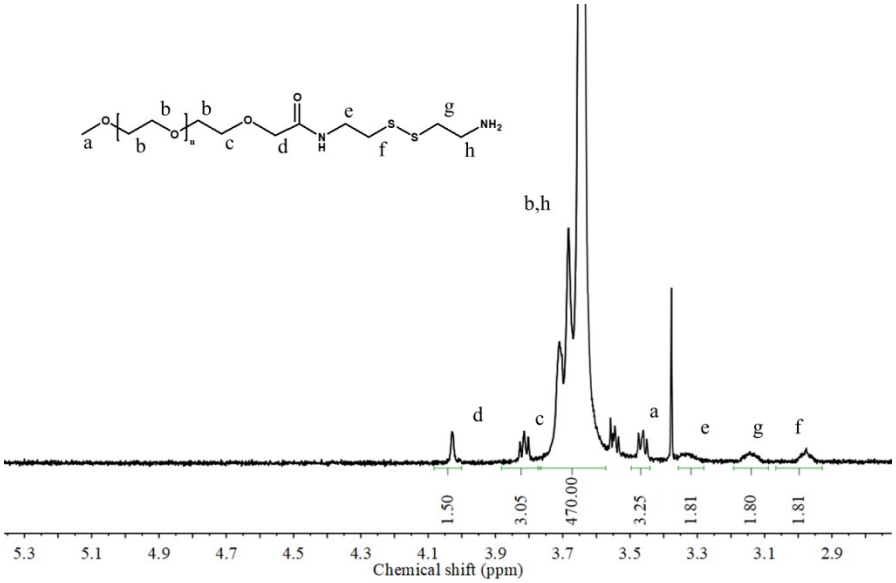

Fig. S4 ^{1}H NMR spectrum of mPEG-*s*-*s*-NH_2 (recorded in $CDCl_3$)

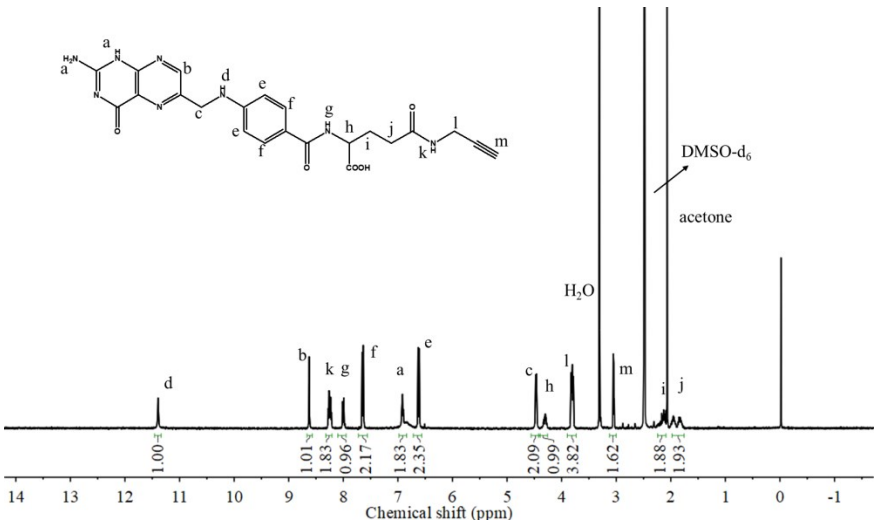

Fig. S5 ^{1}H NMR spectrum of alkynyl-FA (recorded in DMSO-d_6)

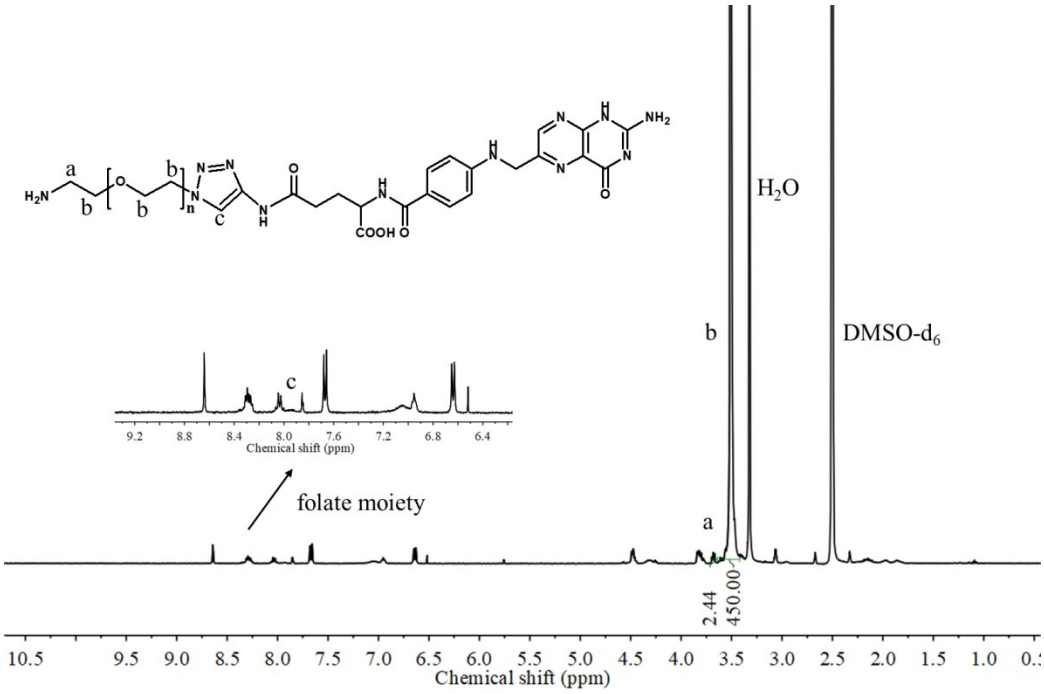

Fig. S6 ^{1}H NMR spectrum of FA-PEG-NH_2 (recorded in DMSO-d_6)

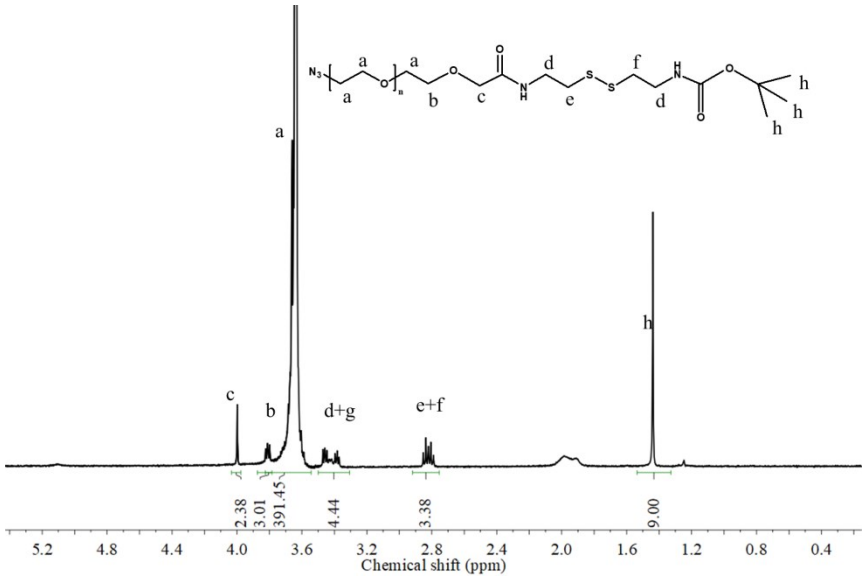

Fig. S7 ^{1}H NMR spectrum of N_3-PEG-*s*-*s*-Boc (recorded in $CDCl_3$)

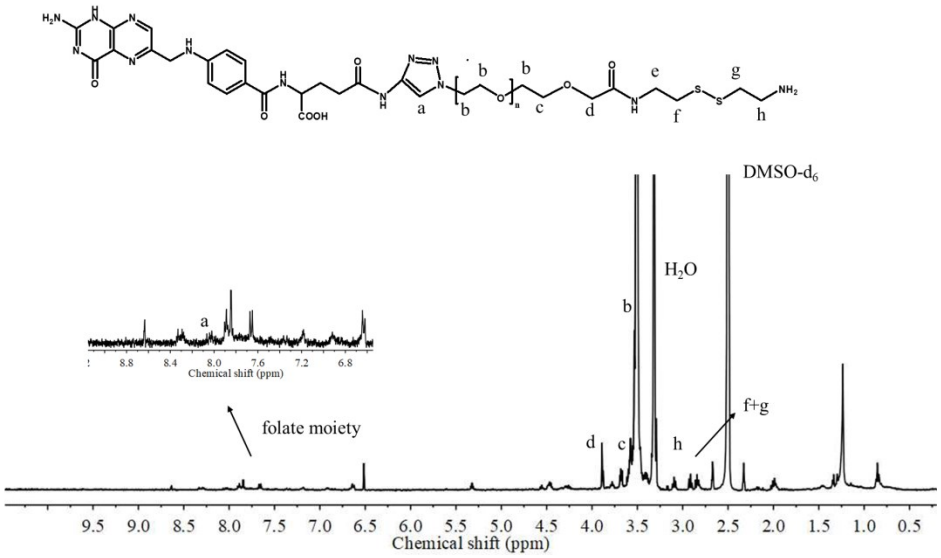

Fig. S8 ^{1}H NMR spectrum of FA-PEG-*s*-*s*-NH_2 (recorded in DMSO-d_6)

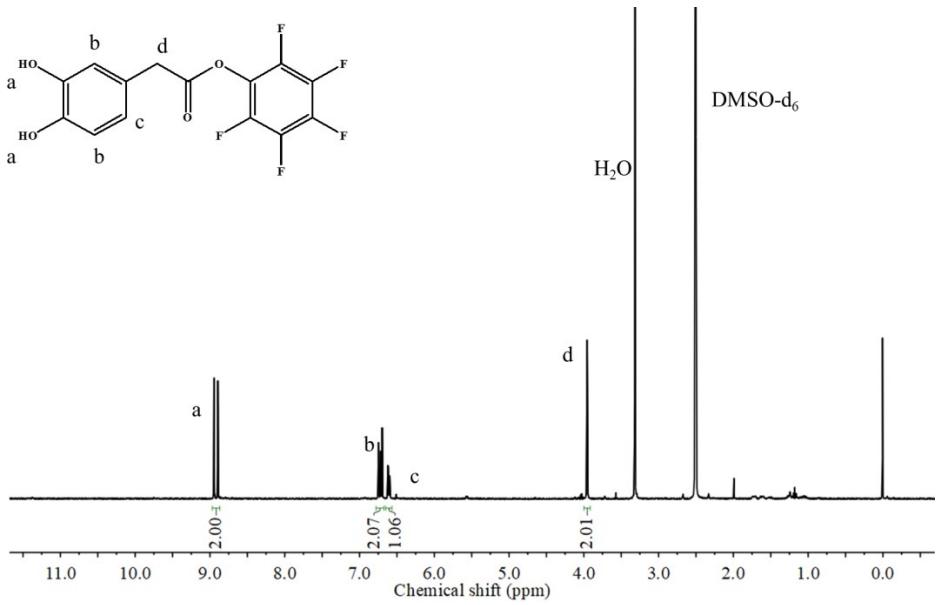

Fig. S9 ^{1}H NMR spectrum of DOPA-PFP (recorded in DMSO-d_6)

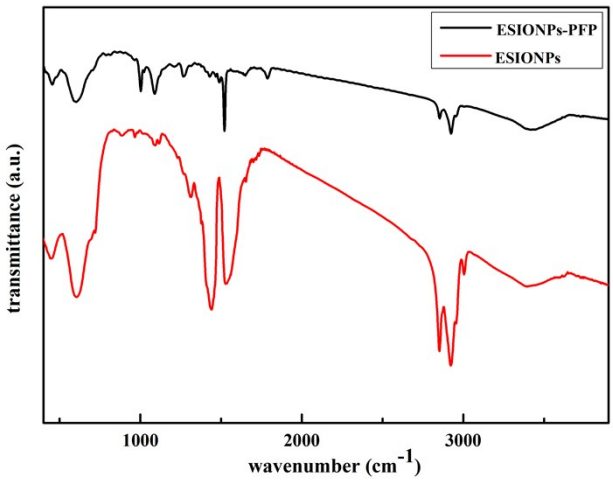

Fig. S10 FTIR spectra of ESIONPs, ESIONPs-PFP.

As shown in this figure, the peak assigned to the activated carbonyl bond of the ester at 1750 cm^{-1} confirming the PFP ester group on the surface of ESIONPs.

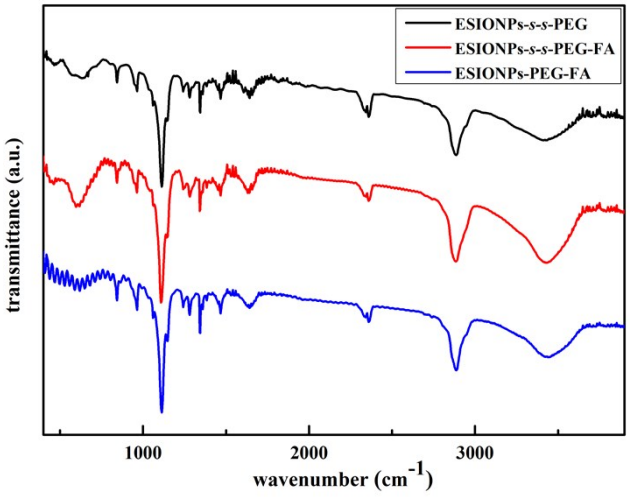

Fig. S11 FTIR spectra of ESIONPs-*s*-*s*-PEG, ESIONPs-*s*-*s*-PEG-FA, ESIONPs-PEG-FA.

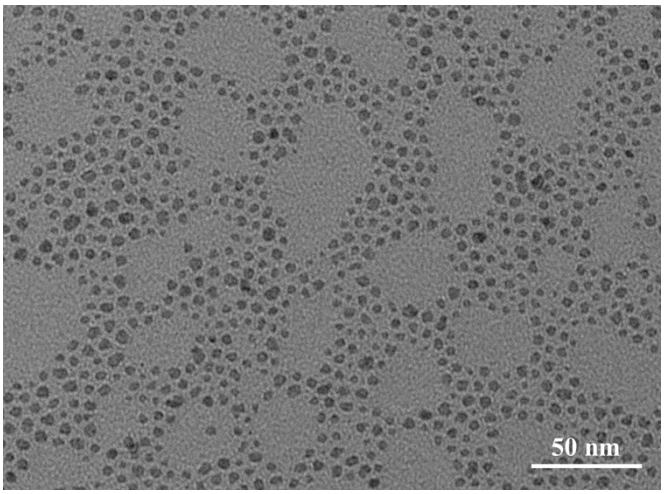

Fig. S12 TEM image of ESIONPs-OA.

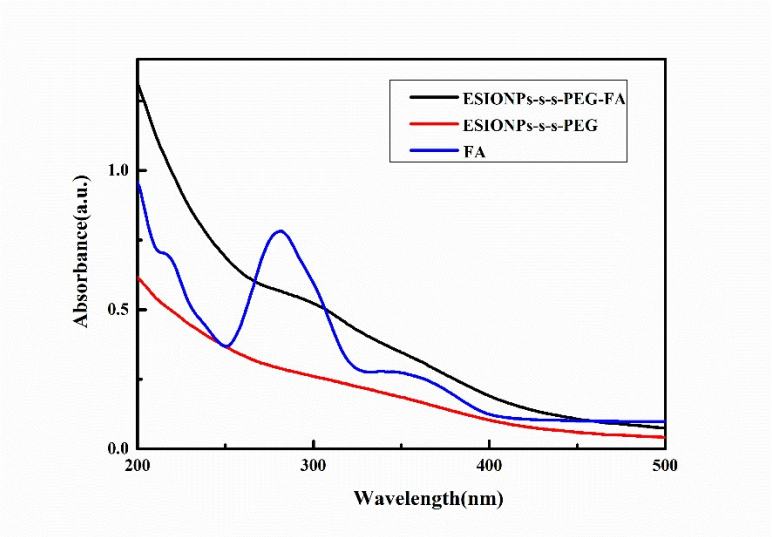

Fig. S13 UV-vis spectra of ESIONPs-*s*-*s*-PEG, ESIONPs-*s*-*s*-PEG-FA and FA molecule

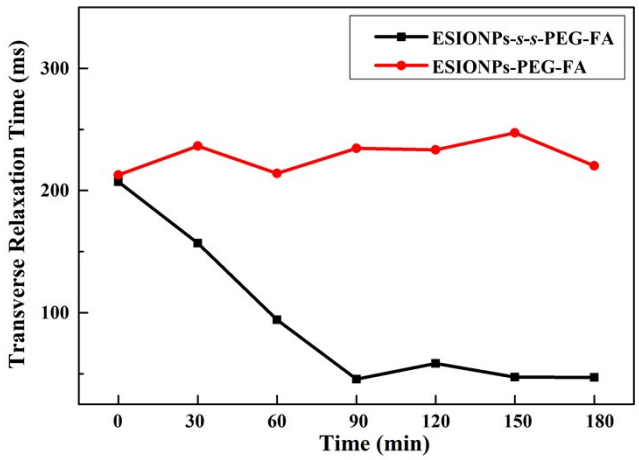

Fig. S14 Variation of transverse relaxation time (T_2) for ESIONPs-*s*-*s*-PEG-FA with an iron concentration of 0.35 mM incubated with 10 mM DTT over time.

Aggregation Profile of ESIONPs-*s*-*s*-PEG-FA

The variation of transverse relaxation time (T_2) was recorded to demonstrate the conversion from a T_1 CA to T_2 one. ESIONPs-*s*-*s*-PEG-FA was incubated in 10 mM DTT at an iron concentration of 0.35 mM. As shown in Fi. S14†, during the first 120 min of incubation, T_2 gradually decreased from 207.15 s to 58.5 ms and maintained

until 180 min. DTT-treated ESIONPs-PEG-FA served as control group and T_2 was stayed at about 220.19 ms in the whole time range, confirming that the redox-responsive assembly was depended on disulfide bonds.

Confirming the Targeting Capacity and Redox-Triggered T_1/T_2 MRI Switch of ESIONPs-s-s-PEG-FA on Cells

Human oral epidermoid carcinoma cells (KB cells) and human umbilical vein endothelial cells (HUVEC cells) were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). KB cells were cultured in RPMI 1640 FA free media containing 10% FBS and 100 units per mL of streptomycin and penicillin. HUVEC cells were cultured with DMEM containing 10% FBS and 100 units per mL of streptomycin and penicillin. The KB and HUVEC cells were all maintained at 37 °C and 5% CO_2.

The MRI effectiveness of ESIONPs-*s*-*s*-PEG-FA on the cellular level was observed through three samples, ESIONPs-*s*-*s*-PEG-FA and ESIONPs-PEG-FA, and ESIONPs-*s*-*s*-PEG. KB cells were seeded into culture dishes with 7 mL of culture medium. After reaching 80% gathering, the culture medium was replaced with fresh one containing the ESIONPs-*s*-*s*-PEG-FA and ESIONPs-*s*-*s*-PEG samples at Fe concentration of 0.1 mM respectively. The medium was removed and gently washed with 2 mL PBS for six times after incubation for 2 h. Using trypsin and centrifugation to obtain KB cells, the KB cells in 200 μL eppendorf tubes were measured to obtain T_1-Weighted MR images. Besides, KB cells were also incubated with ESIONPs-*s*-*s*-PEG-FA and ESIONPs-*s*-*s*-

PEG samples at an iron concentration of 0.1 mM respectively and collected by centrifugation for prussian blue staining study (Fig. S15†).

The imaging parameters were set as follows: TR = 500 ms, TE = 134 ms and NS = 1. Additionally, when the KB cells achieved 80% confluence, the culture medium was replaced with fresh one containing the ESIONPs-*s*-*s*-PEG-FA and ESIONPs-PEG-FA samples at 0.2 mM Fe concentration respectively. After incubation for 24 h, the KB cells were obtained by trypsin and centrifugation for T_2-Weighted MR images. The cellular MR images were performed on a 0.5T NMR-analyzer (GY-PNMR-10). The imaging parameters were set as follows: TR = 2000 ms, TE = 334 ms and NS = 1.

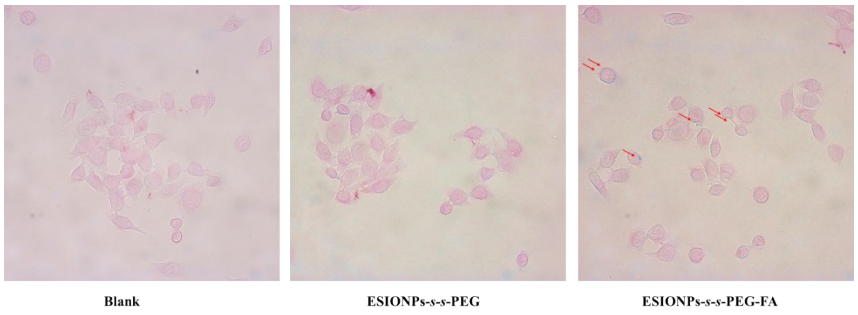

Fig. S15 Prussian blue staining of KB cells after incubated with ESIONPs-*s*-*s*-PEG-FA at an iron concentration of 0.1 mM for 2 h. KB cells treated with ESIONPs-*s*-*s*-PEG at an iron concentration of 0.1 mM for 2 h and KB cells without treatment were chosen as the control group. Red arrows indicate the staining nanoparticles in KB cells.

In vivo MRI study

Female athymic nude mice (4 weeks old, about 20 g) were obtained from Nanjing Sikerui Biological Technology Co. Ltd. and raised under Specific Pathogen Free (SPF) conditions for 1 week. The culture medium of KB cells was removed by centrifugation.

Then, KB cells were washed with PBS for 3 times. After counting by blood counting chamber, the PBS suspension containing 2×10^6 KB cells/mL was obtained. The armpit of nude mice was subcutaneously injected with the suspension mentioned above. After about 10 days, the tumor diameter reached about 5mm and the nude mice were used for in vivo MRI study. All animal experiments were approved by the Animal Ethics Committee of the Chinese Academy of Sciences and were conducted according to the relevant laws and institutional guidelines of the U.S. National Institutes of Health.

To further investigated the T_1/T_2 switch, T_1-weighted MR images of tumor-bearing mice within 24 h at different time points have been monitored. The mice were anesthetized and the preinjected MR images were obtained. Then, three groups of samples, ESIONPs-*s*-*s*-PEG-FA, ESIONPs-*s*-*s*-PEG and ESIONPs-PEG-FA, were intravenously injected to the mice at a dose of 0.1mmol/kg, respectively. The T_1-weighted MR images were collected at special time points (Fig. S16†). The detailed imaging parameters were set as follows: TR/TE = 100 ms/16.8 ms, matrix = 512 × 256, and slice thickness = 0.3 mm.

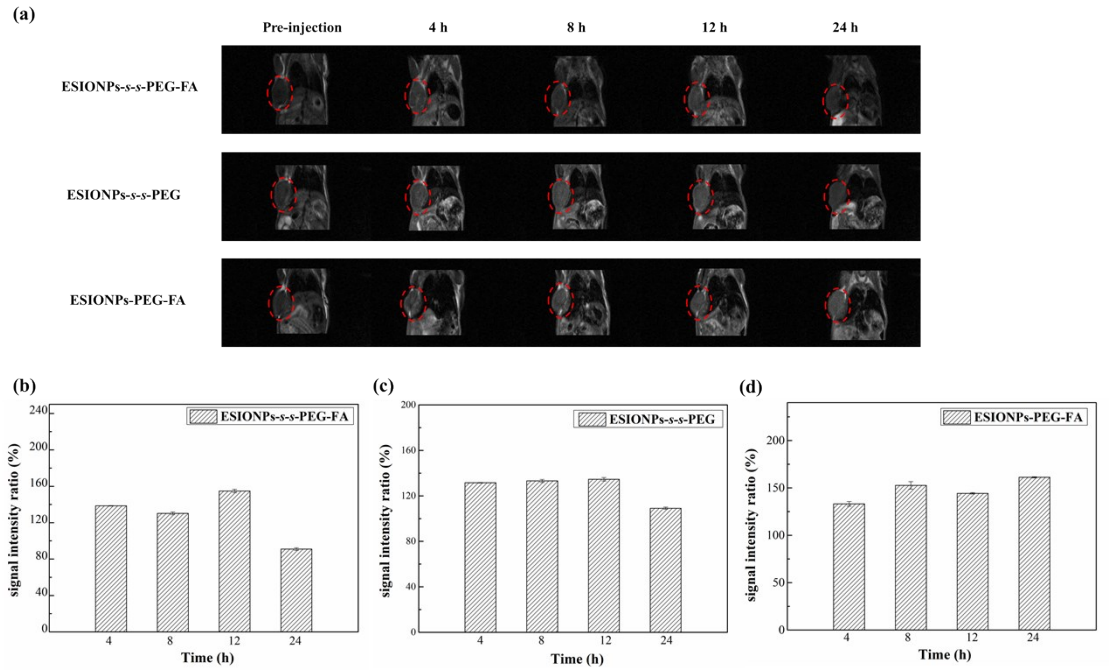

Fig. S16 (a) T_1-weighted MRI images of tumor-bearing mice before and after intravenous injection with ESIONPs-*s*-*s*-PEG-FA at an iron dose of 0.1 mmol/kg. The time points after injection for investigation are 4, 8, 12 and 24 h. Red dotted circles indicate the location of tumor tissue. Quantitative analysis of average signal intensity of T_1-weighted MRI of tumor-bearing mice intravenously injected with (b) ESIONPs-*s*-*s*-PEG-FA, (c) ESIONPs-*s*-*s*-PEG and (d) ESIONPs-PEG-FA.

Redox-Responsive Aggregation study

The redox-responsive aggregation property was observed by the TEM images of KB cells at first. KB cells were incubated with ESIONPs-*s*-*s*-PEG-FA and ESIONPs-PEG-FA samples for 24 h at an iron concentration of 0.1 mM, respectively. Then, the KB cells was collected to obtain TEM images. Besides, the tumor-bearing mice were intravenously injected with ESIONPs-PEG-FA and ESIONPs-*s*-*s*-PEG-FA at an iron dose of 0.1 mmol/kg. The mice were sacrificed and the tumor tissues were obtained for

TEM images and Prussian blue staining.

Cell and tissue toxicity

WST assay was used to evaluate the cytotoxicity of ESIONPs-*s*-*s*-PEG-FA against on KB cells and HUVEC cells. At first, KB cells were seeded in 96-well plates at a density of 8000 cells/well. After culturing for 24 h, the cell culture medium was replaced with fresh complete medium containing ESIONPs-*s*-*s*-PEG-FA with various Fe concentrations following incubation for 24 h. Then, the cell culture medium was replaced 100 μL with fresh medium each well and added 10 μL WST-1 solution to incubate for another 2 h. The cell viability was obtained at 450 nm of absorbance with a PerkinElmer microplate reader.

Tissue toxicity of ESIONPs-*s*-*s*-PEG-FA was evaluated by hematoxylin−eosin (H&E) staining. Nude mice (4 weeks old, about 20 g) were obtained from Nanjing Sikerui Biological Technology Co. Ltd. and raised under Specific Pathogen Free (SPF) conditions for 1 week. After that, the nude mouse intravenously injected with 200 μL physiological saline containing ESIONPs-*s*-*s*-PEG-FA at Fe concentration of 0.1 mmol/kg. The nude mouse injected with 200 μL physiological saline served as the control group. After raising 3 days, the mice were dissected to obtain main organs.

The tumor-bearing mice were intravenously injected with ESIONPs-*s*-*s*-PEG-FA with an iron dose of 0.1 mmol/kg and raised for 24 h and 7 days. Afterward, the mice were sacrificed to obtain the main organs. The iron concentration in main organs and tissues (including heart, liver, spleen, lung and kidney) was determined by ICP-MS and

presented as the percentage of the injected dose per gram of tissue (%ID/g) (Fig. S17†).

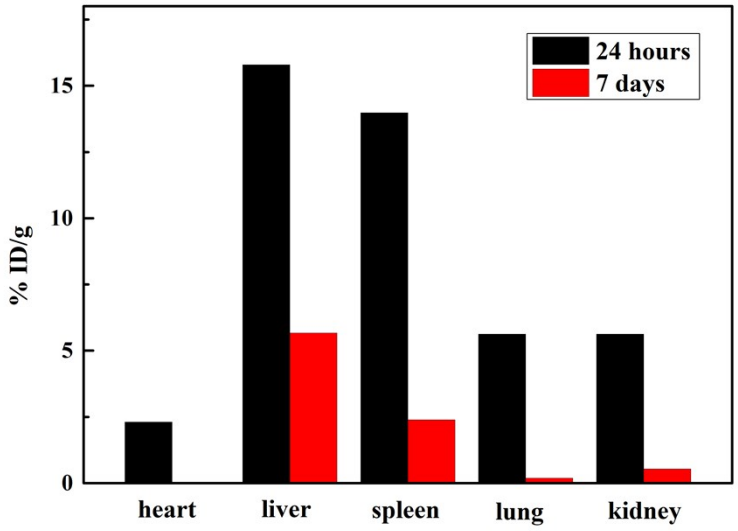

Fig. S17 Biodistribution of iron ion in main organs and tissues, including heart, liver, spleen, lung and kidney, at 24 h and 7 days after intravenous injection of ESIONPs-*s*-*s*-PEG-FA with an iron dose of 0.1 mmol/kg.