Fe_3O_4-ZIF-8 assemblies as pH and glutathione responsive T_2-T_1 switching magnetic resonance imaging contrast agent for sensitive tumor imaging in vivo

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Supporting Information

Experimental section

Materials: Oleylamine (OA, 80−90%, ACROS ORGANICS), Tris(2,4-pentanedionato)iron(III) (>98.0%, Tokyo Chemical Industry Co., Ltd.), N-Methyl-2-Pyrrolidinone (NMP, Adamas Reagent Co., Ltd.), 3,4-dihydroxy-Hydrocinnamic acid (98+%, DHCA, Alfa Aesar (China) Chemicals Co., Ltd.), Zinc nitrate hexahydrate (98%, $Zn(NO_3)_2 \cdot 6H_2O$, Alfa Aesar (China) Chemicals Co., Ltd.), and 2-Methylimidazole (99%, Hmim, ACROS ORGANICS), Polyvinylpyrrolidone (PVP, molecular weight: 10000, K13~18, Adamas Reagent Co., Ltd.), Phosphate Buffered Saline (PBS, HyCione^{TM}), Glutathione (98%, GSH, ACROS ORGANICS), Ultrapure water (18.2 MΩ·cm, 25 ^{o}C). Analytical grade chemical reagents such as Sodium hydroxide (NaOH), Ethanol absolute, Hexane, and Tetrahydrofuran (THF) were purchased from Shanghai RichJoint Chemical Reagents Co., Ltd.. All the chemicals were used without further purification.

Characterization: Powder X-ray diffraction (PXRD) data with scan range of 5-70^{o} were collected with a Bruker D8 ADVANCE X-ray powder diffractometer (Cu Kα). Fourier-transform IR (FT-IR) spectra with wavenumber of 500-4000 cm^{-1} were recorded on a Nicolet Avatar 370 FT-IR spectrophotometer with potassium bromide pressed pellets. Hydration size and zeta potential studies were carried out on a Malvern Zetasizer Nano ZS. Iron-ion concentrations were measured through high-dispersion inductively coupled plasma atomic emission spectroscopy. Hysteresis loops were measured on a superconducting quantum interference device (Lake Shore). Magnetic resonance relaxometry studies were performed on an NMI20 Analyst (0.5 T, Niumag, Shanghai, China).

Preparation of the hydrophobic Fe_3O_4 nanoparticles: Fe_3O_4 nanoparticles were synthesized using a previous reported approach with a slightly modification. Specifically, in 100 mL a round-bottom flask, 30 mL of oleylamine (OA) was heated to 300 ^{o}C and stirred under nitrogen atmosphere for 2h to remove the residual oxygen and water. Then, a solution containing N-methyl-2-pyrrolidinone (4 mL), oleylamine

(6 mL) and iron(III) 2,4-pentanedionate (353.2 mg, 1 mmol) was injected into the thermos flask under stirred, resulting in a dark color of the solution immediately. After continuous heating and stirring for 10 min, the reaction mixture was cooled down to room temperature. The Fe_3O_4-OA nanoparticles were precipitated with 20 mL of ethanol, and then collected by centrifugation. The finally obtained Fe_3O_4-OA nanoparticles were re-dispersed in hexane for further experiments.

Ligand exchange: In a typical experiment, 100 mg of 3,4-dihydroxy-hydrocinnamic acid (DHCA) was dissolved in 28 mL of THF containing 20 mg of hydrophobic Fe_3O_4 nanoparticles, and then shaking on a shaker for 2.5 h at room temperature. After that, 600 μL of NaOH (0.5 mol/L) aqueous solution was added to the above solution, and the Fe_3O_4-DHCA nanoparticles were precipitated by ethanol, collected by centrifugation and finally re-dispersed in ultrapure water for further experiments.

Preparation of Fe_3O_4-ZIF-8 assemblies: In a typical process, 2-methylimidazole (410.0 mg, 5 mmol) and PVP (26 mg) were mixed with the aforementioned water solution of Fe_3O_4 nanoparticles (1.5 mL, 5 mg/mL based on Fe_3O_4) by sonication and then stirred with a speed of 750 rpm at room temperature. Next, 1.5 mL aqueous solution of $Zn(NO_3)_2 \cdot 6H_2O$ (29.7 mg, 0.1 mmol) was rapidly poured into the above mixed solution, stirring for another10 min. The brown solution was centrifuged and washed with deionized water to obtain the brown Fe_3O_4-ZIF-8 assemblies.

Relaxivity measurements of Fe_3O_4 and Fe_3O_4-ZIF-8: All T values and *T*-weighted MR images were obtained using a magnetic resonance scanner (0.5 T; MiniMR-60; Shanghai Niumag). To determine the relaxation properties of Fe_3O_4 and Fe_3O_4-ZIF-8 nanoparticles, the longitudinal relaxation time (T_1) and transverse relaxation time (T_2) were measured with different iron ion concentrations that determined by inductively coupled plasma (ICP), using the following parameters: SF = 18 MHz, TW = 8000 ms, SW = 100 kHz, RG1 = 20 db, and DRG1 = 3. The longitudinal relaxation rate (r_1) and transverse relaxation rate (r_2) were obtained by a linear fitting of the iron ion concentration (as the abscissa) and $1/T_1$ and $1/T_2$ (as the ordinate). T_1- and T_2-weighted MR images were obtained with the parameters of TR = 300 ms and TE = 0.04 ms, and TR = 4500 ms and TE = 100 ms, respectively

In vitro MRI assessment of the pH- and GSH-responsive Fe_3O_4-ZIF-8: To explore the response of Fe_3O_4-ZIF-8 under different pH and GSH concentrations, the pH and GSH-responsive properties of Fe_3O_4-ZIF-8 were tested by adjusting the PBS pH with HCl to obtain values of 7.4, 6.2, and 5.0 and a consistent GSH concentration of 0-4 mM. To verify the feasibility of utilizing acidic conditions and GSH to trigger the disassembly of Fe_3O_4-ZIF-8 and form dispersed Fe_3O_4 nanoparticles, the hydrodynamic size change of Fe_3O_4-ZIF-8 in PBS buffer with different pH values (7.4, 6.2, and 5.0) and GSH concentrations (0, 1, 4 mM) were monitored by DLS. Additionally, to understand how the conversion of an MRI contrast agent of Fe_3O_4-ZIF-8 is triggered by pH and GSH, MRI scans were performed on a 0.5 T MRI scanner with Fe_3O_4-ZIF-8 assembies that were incubated in PBS buffer with different pH values (7.4, 6.2, and 5.0) and GSH concentrations (0 and 4 Mm) for 3 h.

Cell culture: Mouse breast tumor 4T1 cells were provided by the Shanghai Institutes for Biological Sciences. The 4T1 cell lines were cultured in Dulbecco`s modified eagle medium (DMEM: with the addition of 10% fetal bovine serum and 1% double-antibody (penicillin and streptomycin) solutions) at 37^{o}C with 5% CO_2 and 100% humidity for 24 h and then treated with trypsin-EDTA solution.

In vitro cytotoxicity assay of Fe_3O_4-ZIF-8: The cytotoxicity of the Fe_3O_4-ZIF-8 assembly was evaluated with MTT assays. With 96-well plates, HUVEC and 4T1 cells (5×10^4 cells/well) were first plated for 24 h, and then treated with different concentrations of Fe_3O_4-ZIF-8 (0, 5, 10, 20, 50, and 100 μg/mL) in DMEM for another 12 or 24 h at 37^{o}C with 5% CO_2. Thiazolyl blue tetrazolium bromide (20 μL, 5 mg/mL) was then added to each well and the well plates were incubated for 4 h with similar incubation conditions. Subsequently, the supernatant was removed slowly, and the remaining purple formazan crystals were lysed with 150 μL of dimethyl sulfoxide (DMSO). Ultimately, the optical absorption of the formazan was measured with a microplate reader (Multiskan MK3, Thermo Fisher Scientific, Waltham, MA, USA). The background was measured with a microplate reader (Thermo Fisher Scientific).

Animal tumor model: The tumor models were established by injecting 4T1 cells into

BALB/c mice (Shanghai Laboratory Animal Center, 5-6 weeks old）in the region of the right hind legs. Tumor-bearing mice, with a tumor size of about 5 mm, were used for MR imaging in vivo.

In vivo MRI: On a 0.5 T MRI instrument, *in vivo* T_1-weighted MR scanned images were obtained before and after the injection of Fe_3O_4-ZIF-8 for 0.25, 0.5, 1.75, 5, and 6 h, respectively, and with specific imaging parameters of (1) matrix size, 256 × 192 mm, (2) field of view, 80 × 80 mm, (3) repetition time (TR), 500 ms, (4) slice thickness, 3 mm, and (5) echo time (TE), 18 ms. The BALB/c 4T1 tumor-bearing mice were injected with 100 μL of Fe_3O_4-ZIF-8 nanoparticles (C_{Fe}: 1.137 mM) via the tail vein. During imaging, the mice were anesthetized by an intraperitoneal injection of 8% chloral hydrate.

The 3.0 T MRI imaging was performed on Verio 3.0 T MRI scanner, Siemens Medical, Germany. *In vivo* T_1-weighted MR scanned images were obtained before and after the injection of Fe_3O_4-ZIF-8 for 0.25, 0.5, and 1.7 h, respectively, and with specific imaging parameters of (1) matrix size, 102 × 128 mm, (2) repetition time (TR) = 800 ms, (3) echo time (TE) = 22 ms, (4) slice thickness = 1 mm, and (5) field of view (FOV) = 60 mm × 60 mm.

All animal operations were performed in accordance with the requirements of the Animal Ethics Committee of the Shanghai Normal University and the Institutional Animal Care and Use Committee.

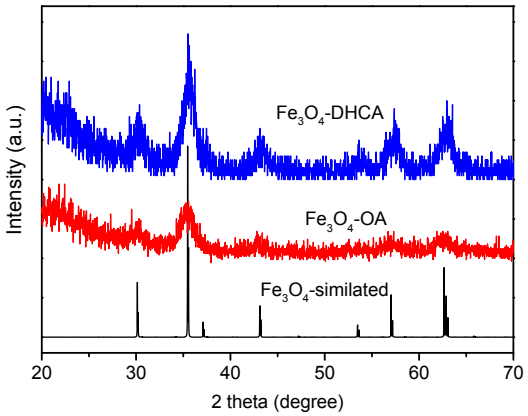

Fig. S1 PXRD patterns of hydrophobic Fe_3O_4-OA and hydrophilic Fe_3O_4-DHCA.

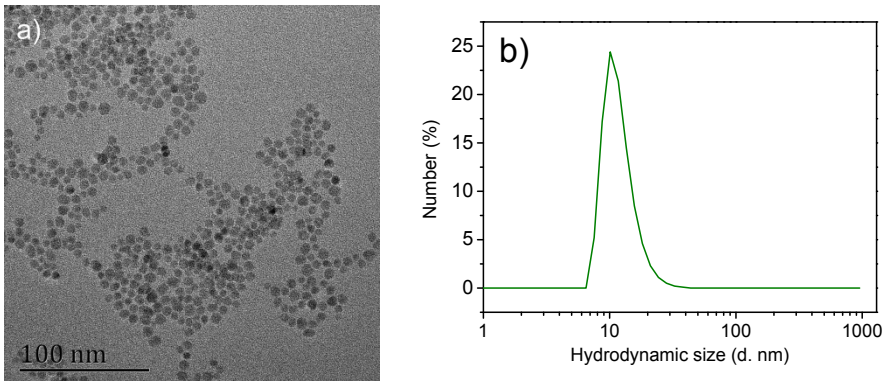

Fig. S2 a) TEM image and b) hydrodynamic size profile of Fe_3O_4 nanoparticles.

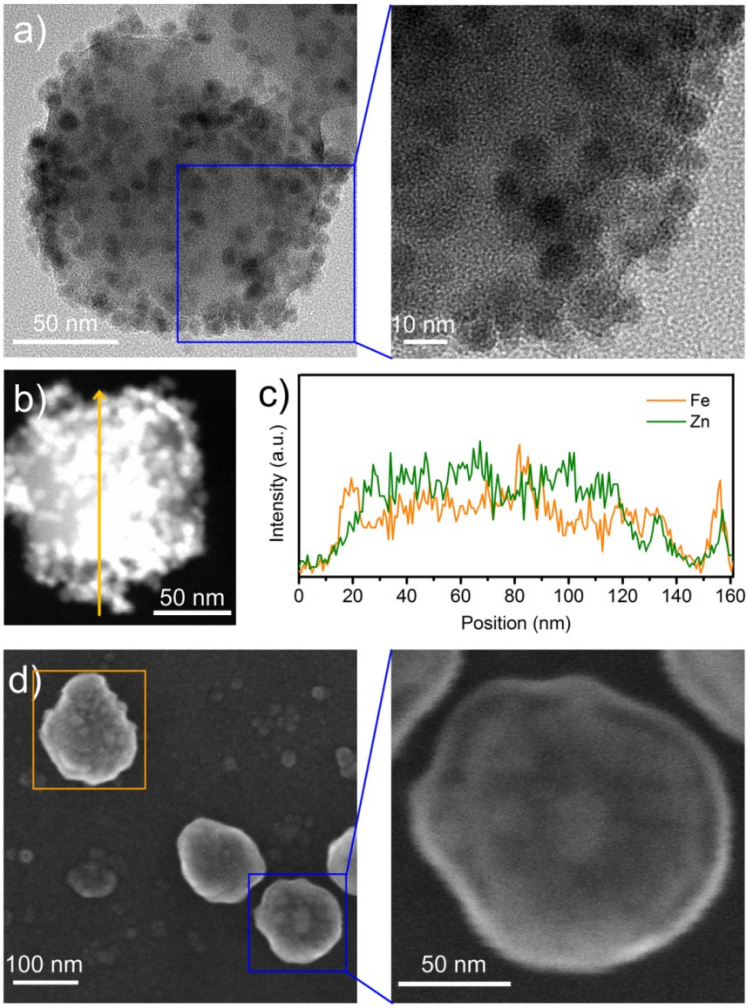

Fig. S3 a) High magnification TEM image of Fe_3O_4-ZIF-8. b) TEM image for element line scan along the direction of the yellow arrow and c) corresponding signal intensity of Zn and Fe elements. The similar signal intensity fluctuation of Fe and Zn proves that Fe_3O_4 and ZIF-8 are together. d) SEM image of the section of Fe_3O_4-ZIF-8 that were embedded into paraffin (the Fe_3O_4-ZIF-8 particle was rough (yellow square), and that was sliced should be smooth (blue square)). Due to the small size of the Fe_3O_4 nanoparticles, it is difficult to ascertain whether the Fe_3O_4 nanoparticles have been encapsulated into the ZIF-8 or just physically adsorbed at the surface.

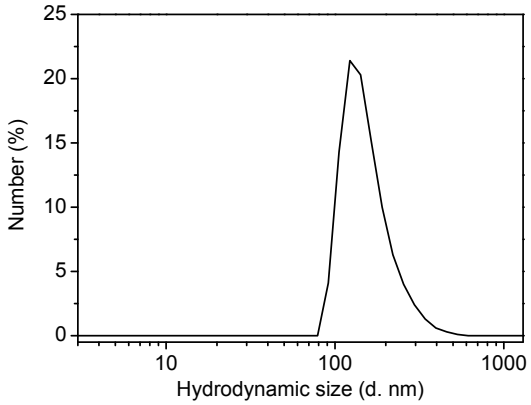

Fig. S4 Hydrodynamic size profile of Fe_3O_4-ZIF-8 assemblies.

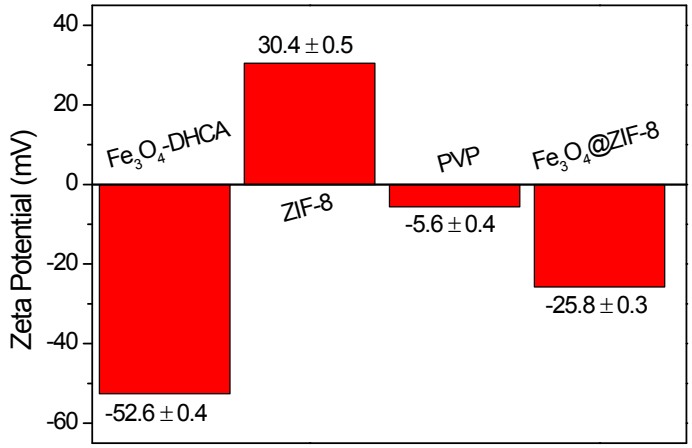

Fig. S5 Zeta potential of Fe_3O_4-DHCA, ZIF-8, PVP and Fe_3O_4-ZIF-8 assemblies in H_2O.

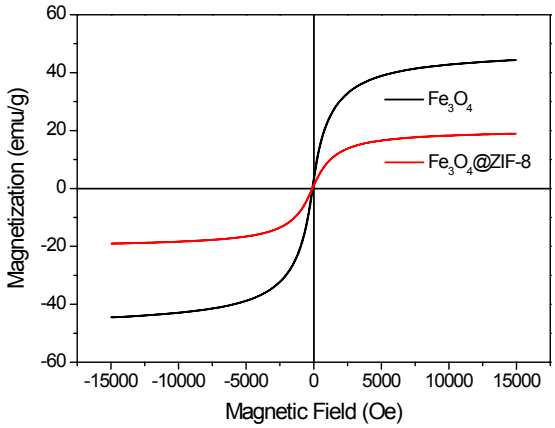

Fig. S6 Field-dependent magnetization curves for Fe_3O_4 nanoparticles and Fe_3O_4-ZIF-8 assemblies at 298 K.

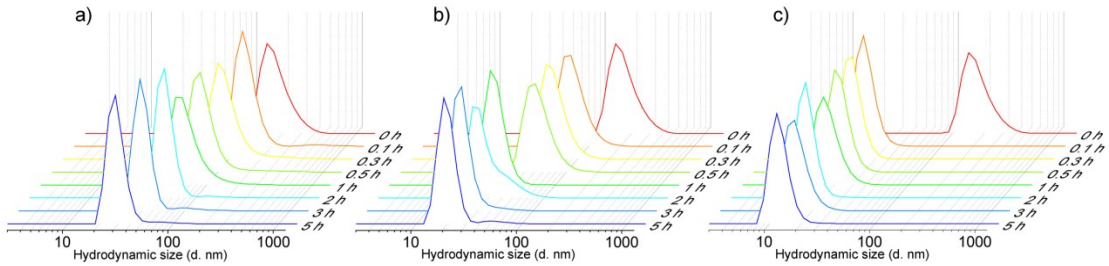

Fig. S7 Temporal hydrodynamic size profiles of Fe_3O_4-ZIF-8 in phosphate buffer solution with pH of a) 6.2, b) 5.0 and c) 4.0.

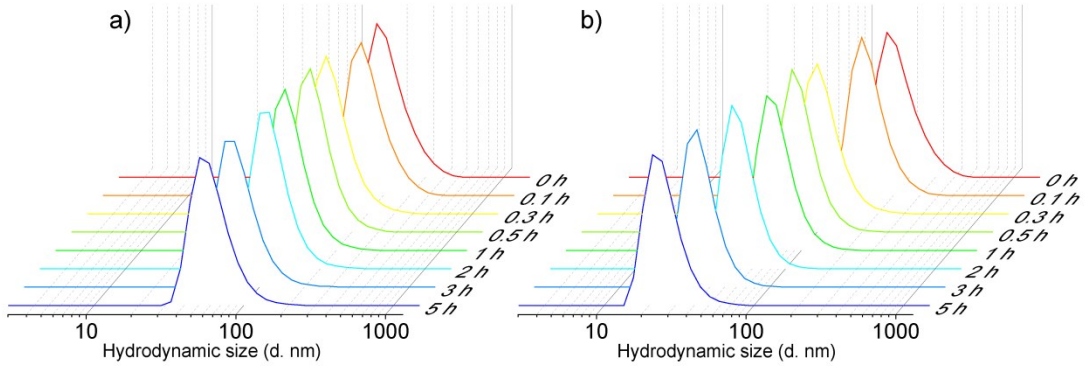

Fig. S8 Temporal hydrodynamic size profiles of Fe_3O_4-ZIF-8 in phosphate buffer solution with pH of 7.4 and a) 1 mM GSH, and b) 4 mM GSH.

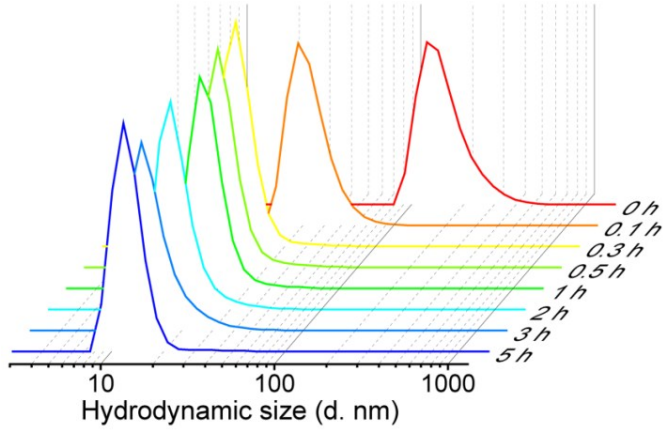

Fig. S9 Temporal hydrodynamic size profiles of Fe_3O_4-ZIF-8 in phosphate buffer solution with a pH of 5 and 4 mM GSH.

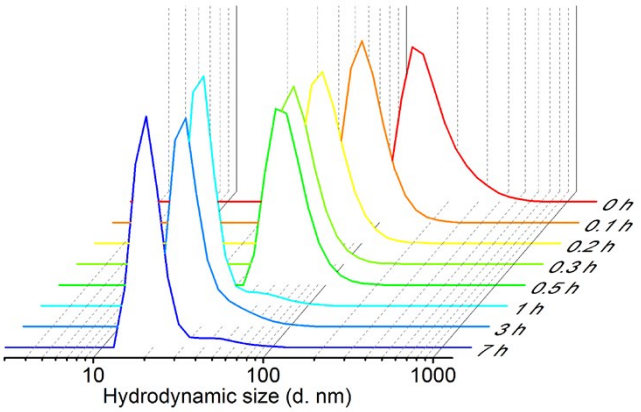

Fig. S10 Temporal hydrodynamic size profiles of Fe_3O_4-ZIF-8 in phosphate buffer solution with a pH of 6.2 and 1 mM GSH.

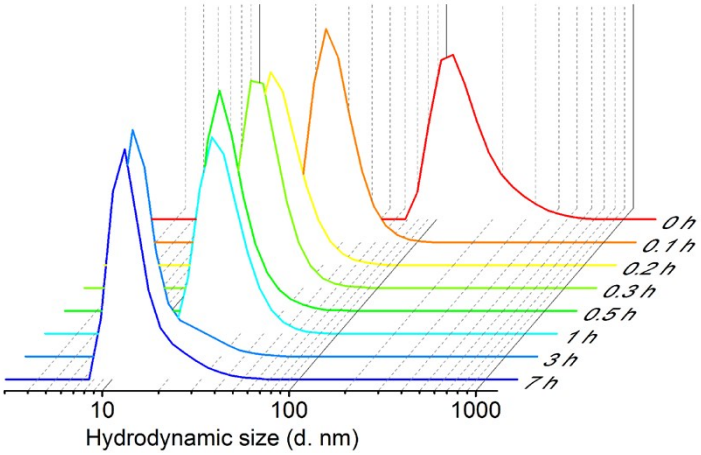

Fig. S11 Temporal hydrodynamic size profiles of Fe_3O_4-ZIF-8 in phosphate buffer solution (PBS) with a pH of 5.0 and 1 mM GSH.

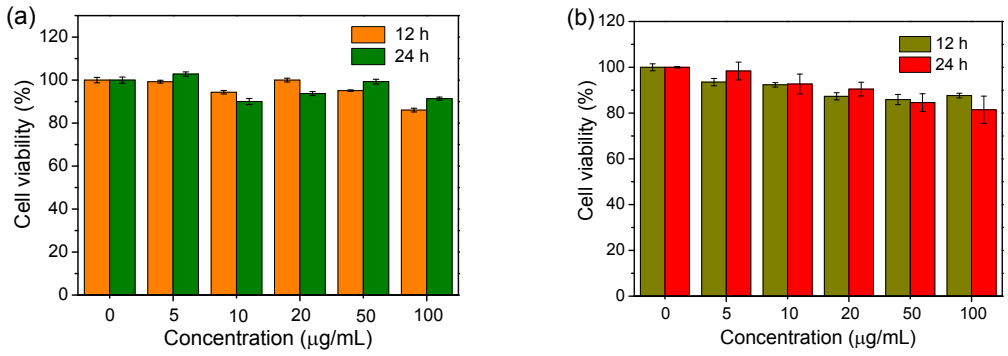

Fig. S12 Cell viability of (a) HUVEC and (b) 4T1 cell lines after incubation with different concentrations of Fe_3O_4-ZIF-8 for 12 and 24 h.

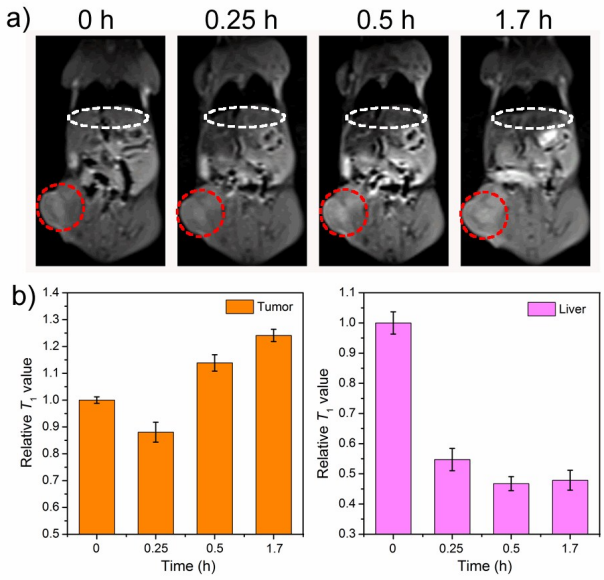

Fig. S13 a) In vivo T_1-weighted magnetic resonance images of tumor liver acquired on a 3T MRI scanner before (0 h) and after the intravenous injection of Fe_3O_4-ZIF-8 assemblies. b) The corresponding relative T_1 signals extracted from tumor (red circle) and liver (white circle) sites.