Supplementary Information

Mn(II) Chelate-Coated Superparamagnetic Iron Oxide Nanocrystals as High-Efficiency Magnetic Resonance Imaging Contrast Agents

Changqiang Wu, Tianwu Chen, Lihua Deng, Qian Xia, Chuan Chen, Mu Lan, Yu Pu, Hongjie Tang, Ye Xu, Jiang Zhu, Chengyi Shen, Xiaoming Zhang**

Experimental Section

1. Synthesis and characterization of catechol group modified Mn(II) Chelate ($[Mn(Dopa\text{-}EDTA)]^{2-}$)

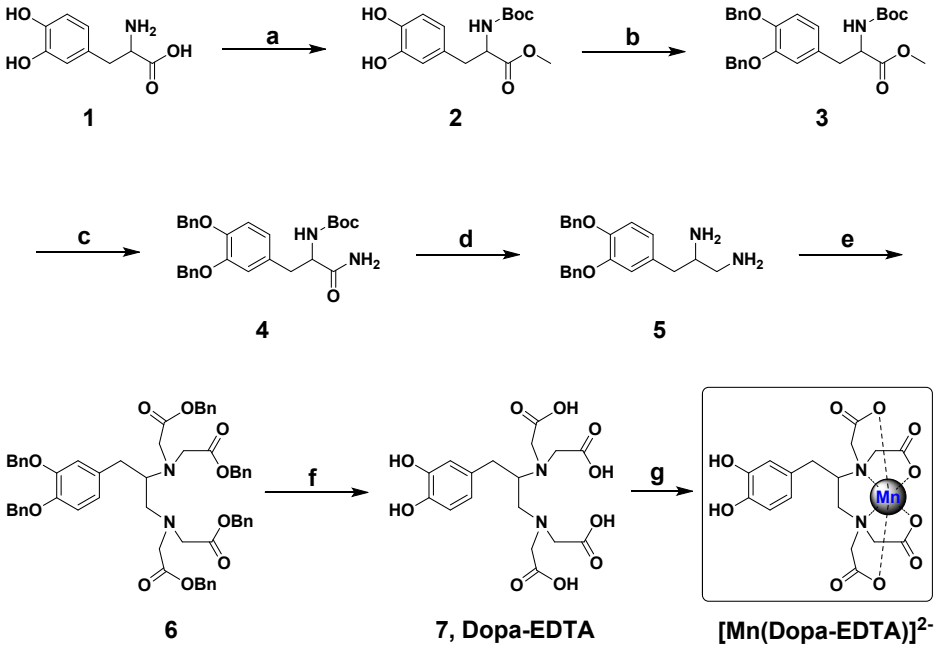


Figure S1. Graphical synthetic route of catechol group modified Mn(II) Chelate. **Reagents and conditions: a.** (Ⅰ) MeOH, $SOCl_2$, 95 ℃, 1 h; (Ⅱ) $(Boc)_2O$, $NaHCO_3$, THF/H_2O, rt, 12 h; **b.** Bn-Cl, K_2CO_3, KI, 90 ℃, 6 h; **c.** (Ⅰ) NaOH, ACN/H_2O, 50 ℃, 1 h; (Ⅱ) Isobutyl chloroformate, DIPEA, NH_3, THF, rt, 24 h; **d.** (Ⅰ) TFA, CH_2Cl_2, rt, 12 h; (Ⅱ) BH_3·THF, THF, 97 ℃, 24 h; **e.** Benzyl bromoacetate, DIPEA, KI, ACN, 50 ℃, 24 h; **f.** H_2, Pd-C, THF/MeOH, rt, 12 h; **g.** $MnCl_2·4H_2O$, pH 7.4.

1.1 methyl 2-((tert-butoxycarbonyl)amino)-3-(3,4-dihydroxyphenyl)propanoate (2)

Compound 1, L-Dopa (5 g, 25.36 mmol), dissolved in excess methanol (60 ml). Then, $SOCl_2$ (4 ml, 55.78 mmol) was added in under the ice bath. The mixture was heated to reflux and reacted for 1 h. The reaction was monitored by ^1H NMR (DMSO). After complete reaction, the solvent was removed by rotary evaporation and a yellow oily liquid product was obtained. Yield: 10 g (99%). ^1H NMR (400 MHz, DMSO) δ 6.67 (s, 1H), 6.59 (d, J = 1.8 Hz, 1H), 6.43 (s, 1H), 4.10 (s, 1H), 3.68 (s, 3H), 2.97 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.96, 145.94, 144.96, 120.73, 117.22, 116.19, 110.29, 79.24, 54.11, 52.82, 40.28, 35.74, 0.63; (m/z) for $C_{10}H_{13}NO_4$: Calcd, 212.21 $[M+H]^+$; found, 212.19 $[M+H]^+$.

The liquid product (10 g, 25.36 mmol) and $NaHCO_3$ (9.4 g, 111.58 mmol) were dissolved in THF (26 ml) and H_2O (12 ml), and $(Boc)_2O$ (6.6 g, 30.4 mmol) was slowly added in. The mixture was stirred for 12 h at room temperature. The reaction was confirmed by TLC. The solvent was removed, the raw product was washed with water, and extracted with ethyl acetate. The organic phase was collected, and washed with brine and dried over Na_2SO_4. After solvent removed, pure product was collected

as white solid (Compound 2). Yield: 10.5 g (85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.74 (t, J = 9.4 Hz, 1H), 6.64 (s, 1H), 6.50 (dd, J = 8.0, 1.9 Hz, 1H), 5.26-5.03 (m, 1H), 4.64-4.37 (m, 1H), 3.71 (s, 3H), 3.10 -2.82 (m, 2H), 1.47-1.31 (m, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.94, 171.18, 155.07, 144.49, 142.85, 127.85, 121.18, 115.74, 114.98, 77.01, 60.48, 54.57, 51.81, 37.84, 28.29, 27.64, 20.98, 14.17, 1.00; (m/z) for $C_{15}H_{21}NO_6$: Calcd, 312.33 $[M-H]^-$; found, 310.19 $[M-H]^-$.

1.2 methyl 3-(3,4-bis(benzyloxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate (3)

Compound 2 (10.5 g, 25.36 mmol), K_2CO_3 (8.4g, 60.86 mmol) and KI (0.42g, 2.54 mmol) were dissolved in ACN (80 ml), and Bn-Cl (7.06g, 55.79 mmol) was slowly added in. The mixture was stirred for 6 h at 90 ℃ oil bath. The reaction was confirmed by TLC. Filter to remove the inorganic salts, the filtrate was evaporated by rotary evaporation, and crude product was recrystallized with ethanol and pure product (Compound 3) was collected as a white solid. Yield: 12 g (96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (dddd, J = 10.0, 9.5, 8.6, 4.9 Hz, 12H), 6.88 (d, J = 8.2 Hz, 1H), 6.75 (d, J = 1.9 Hz, 1H), 6.66 (d, J = 8.1 Hz, 1H), 5.14 (d, J = 1.5 Hz, 4H), 4.97 (d, J = 8.1 Hz, 1H), 4.54 (d, J = 7.1 Hz, 2H), 3.66 (s, 14H), 1.43 (d, J = 9.8 Hz, 31H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.59, 128.69, 127.96, 127.37, 122.37, 116.25, 76.40, 71.71, 52.25, 27.18; (m/z) for $C_{29}H_{33}NO_6$: Calcd, 492.58 $[M+H]^+$; found, 492.88 $[M+H]^+$.

1.3 tert-butyl (1-amino-3-(3,4-bis(benzyloxy)phenyl)-1-oxopropan-2-yl)carbamate (4)

Compound 3 (8 g, 16.27 mmol) and NaOH (1.63 g, 32.55 mmol) was dissolved in ACN (60 ml) and water (20 ml). The solution was stirred for 1 h at 50 °C. The reaction was confirmed by TLC. PH of the solution was adjusted to 2.0 by carefully addition of concentrated hydrochloric acid. Extracted with ethyl acetate, organic layer was collected and washed with brine and dried over Na_2SO_4. After remove of solvent, the product was collected as a white solid. Yield: 7.3 g (94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60-7.25 (m, 10H), 6.87 (d, J = 8.2 Hz, 1H), 6.78 (s, 1H), 6.70 (d, J = 8.0 Hz, 1H), 5.14 (s, 4H), 4.90 (s, 1H), 4.64-4.48 (m, 1H), 3.07 (d, J = 5.2 Hz, 2H), 1.52-1.32 (m, 7H). (m/z) for $C_{28}H_{31}NO_6$: Calcd, 476.55 $[M-H]^-$; found, 476.88 $[M-H]^-$. The product (4.2 g, 8.79 mmol) and N, N-diisopropylethylamine (2.27 g, 17.6 mmol) were dissolved in THF. Isobutyl chloroformate (1.32 g, 9.67 mmol) was slowly added in and the mixture was stirred for 1 h at room temperature. The reaction was confirmed by TLC. Then, ammonia was injected for 10 min and stirred overnight at room temperature. The reaction was confirmed by TLC. The solvent was removed by rotary evaporation and washed with water, and extracted with ethyl acetate. Organic layer was collected and washed with brine and dried over Na_2SO_4. After dried, crude compound was recrystallized with ethanol and pure product was collected as a white solid (Compound 4). Yield: 12 g (96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (dddd, J = 11.6, 10.0, 6.6, 3.9 Hz, 9H), 6.90 (d, J = 8.1 Hz, 1H), 6.84 (d, J = 1.8 Hz, 1H), 6.74 (dd, J = 8.1, 1.8 Hz, 1H), 5.17 (d, J = 7.1 Hz, 4H), 3.53 (dd, J = 9.1, 4.2 Hz, 1H), 3.13 (dd, J = 13.8, 4.1 Hz, 1H), 2.67 (dd, J = 13.8, 9.1 Hz, 1H); ^{13}C NMR (101 MHz, CDCl3) δ 173.41, 155.36, 148.78, 148.04, 137.25, 129.71, 128.55, 128.52, 127.85, 127.83, 127.42, 127.32, 122.27, 115.99, 115.12, 80.31, 77.36, 77.25, 77.05, 76.73, 71.28, 71.01, 55.45, 38.00, 28.32, 1.05, 0.03; (m/z) for $C_{28}H_{32}N_2O_5$: Calcd, 475.56 $[M-H]^-$; found, 475.88 $[M-H]^-$.

1.4 3-(3,4-bis(benzyloxy)phenyl)propane-1,2-diamine (5)

Compound 4 (7 g, 14.69 mmol) was dissolved in dichloromethane (120 ml), Trifluoracetic acid (12 ml) was carefully added in and stirred for 12 h at room temperature. The reaction was confirmed by TLC. Solvent was removed and saturated sodium bicarbonate solution was added in. Extracted with ethyl acetate, the organic layer was collected and washed with brine and dried over Na_2SO_4. After solvent removed, pure product was collected as a white solid. Yield: 4.7 g (85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63-7.23 (m, 10H), 6.95-6.84 (m, 1H), 6.80 (d, J = 1.6 Hz, 1H), 6.72 (dd, J = 8.1, 1.6 Hz, 1H), 5.17 (d, J = 7.8 Hz, 4H), 2.88 (dd, J = 7.7, 3.7 Hz, 1H), 2.72 (ddd, J = 18.3, 13.0, 4.4 Hz, 2H), 2.45 (ddd, J = 19.6, 11.8, 6.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.27, 148.86, 147.97, 137.31, 137.20, 130.87, 128.52, 127.84, 127.40, 127.30, 122.28, 116.03, 115.12, 101.39, 77.37, 77.26, 77.06, 76.74, 71.32, 71.12, 56.46, 40.41, 29.75, 1.42, 1.06, 0.68, 0.03; (m/z) for $C_{23}H_{24}N_2O_3$: Calcd, 377.45 $[M+H]^+$; found, 377.55 $[M+H]^+$.

The product (3 g, 8 mmol) was dissolved in THF, boron hydrogen reagent (48 ml, 48 mmol) was added in and the mixture was heat to reflux and reacted for 24 h. The reaction was confirmed by TLC. 10 ml of methanol was slowly added in under the ice bath, then the mixture was heat to reflux for 30 min. After the solvent was removed, pH of the solution was adjusted to 2.0 by carefully addition of concentrated hydrochloric acid. Then washed with ethyl ether and extracted with water, and water layer was collected. NaOH was added to adjust pH to 8 ~ 9, extracted with DCM. The organic layer was collected, washed with brine and dried over Na_2SO_4. After solvent removed, pure product was collected as a white solid (Compound 5). Yield: 2.4 g (83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63-7.23 (m, 10H), 6.95-6.84 (m, 1H), 6.80 (d, J = 1.6 Hz, 1H), 6.72 (dd, J = 8.1, 1.6 Hz, 1H), 5.17 (d, J = 7.8 Hz, 4H), 2.88 (dd, J = 7.7, 3.7 Hz, 1H), 2.72 (ddd, J = 18.3, 13.0, 4.4 Hz, 2H), 2.45 (ddd, J = 19.6, 11.8, 6.9 Hz, 2H); ^{13}C NMR (101 MHz, DMSO) δ 163.76, 128.88, 128.09, 127.94, 40.60, 40.39, 40.19, 39.98, 39.77, 39.56, 39.35; (m/z) for $C_{23}H_{26}N_2O_2$: Calcd, 385.46 $[M+Na]^+$; found, 385.40 $[M+Na]^+$.

1.5 {Benzyloxycarbonylmethyl-[2-(bis-benzyloxycarbonylmethyl-amino)-3-(3,4-bis-benzyloxy-phenyl)-propyl]-amino}-acetic acid benzyl ester (6)

Compound 5 (3.8 g, 10.48 mmol), N, N-diisopropylethylamine (8.13 g, 62.9 mmol) and KI (0.17g, 1.05 mmol) were dissolved in ACN (60ml). Then, benzyl bromoacetate (12.01 g, 52.45 mmol) was added in and stirred for 24 h at 50 ℃ oil bath. The reaction was confirmed by TLC. Filter to remove the inorganic salts, the filtrate was washed with water and extracted with ethyl acetate. The organic layer was collected and washed with brine and dried over Na_2SO_4. After solvent removed, crude product was collected and purified by column chromatography (silica, hexanes/EtOAc, 3:1). Yield: 8 g (80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65-7.06 (m, 36H), 6.82 (d, J = 1.8 Hz, 1H), 6.78 (d, J = 8.2 Hz, 1H), 6.62 (dd, J = 8.2, 1.8 Hz, 1H), 5.16 – 5.11 (m, 3H), 5.08 (t, J = 6.3 Hz, 8H), 4.73 (d, J = 4.9 Hz, 1H), 3.68 – 3.45 (m, 8H), 3.13 (s, 1H), 2.92 (s, 3H), 2.70 (d, J = 5.9 Hz, 1H), 2.66-2.56 (m, 1H), 2.53 (d, J = 7.6 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.24, 171.80, 171.23, 148.65, 140.85, 137.46, 135.75, 134.99, 128.73, 128.72, 128.61, 128.56, 128.54, 128.44, 128.35, 128.29, 127.72, 127.33, 127.03, 121.92, 115.11, 77.37, 77.25, 77.05, 76.73, 71.38, 71.01, 67.36, 66.29, 66.16, 65.45, 60.69, 55.29, 52.82, 1.05, 0.03; (m/z) for $C_{59}H_{58}N_2O_{10}$: Calcd, 956.10 $[M+H]^+$; found, 956.26 $[M+H]^+$.

1.6 Catechol group modified ethylene diamine tetraacetic acid (7, Dopa-EDTA)

Compound 6 (1 g, 1.05 mmol) was dissolved with proper amount of anhydrous THF (8 ml) and methanol (24 ml), 500 mg Pd-C (10%) was added in. Then hydrogen is introduced into the airtight reaction for 12 h at room temperature. The reaction was confirmed by TLC. Filter to remove the Palladium carbon, and the filtrate was collected and the solvent was removed. The product was dissolved in water, and then acetone was added in to obtained precipitate. The precipitates was collected and filtered, filter cake was dried to obtain pure product as a palm red solid. Yield: 0.35 g (80%). ^{1}H NMR (400 MHz, D_2O) δ 6.66 (d, J = 8.1 Hz, 1H), 6.59 (s, 1H), 6.50 (d, J = 8.0 Hz, 1H), 3.75 – 3.37 (m, 8H), 3.04 (d, J = 7.4 Hz, 2H), 2.81 (dd, J = 14.1, 5.4 Hz, 1H), 2.46 – 2.30 (m, 1H). ^{13}C NMR (101 MHz, D_2O) δ 174.58, 170.51, 144.84, 142.96, 128.19, 121.58, 116.23, 60.94, 55.46, 54.61, 52.78, 32.03; (m/z) for $C_{17}H_{22}N_2O_{10}$: Calcd, 415.36 $[M+H]^+$; found, 415.2 $[M+H]^+$.

1.7 catechol group modified Mn(II) Chelate ($[Mn(Dopa\text{-}EDTA)]^{2-}$)

Dopa-EDTA (63.2 mg, 0.15 mmol) was dissolved in ultrapure water and pH of the solution was adjusted to 7.0 by carefully addition of NaOH (1.0 M). $MnCl_2 \cdot 4H_2O$ (21.5 mg, 0.1 mmol) was slowly added in and resulting solution was stirred. The last pH of solution was readjusted to 7.4. After lyophilization product was collected as solid. ESI-MS (m/z) for $C_{17}H_{18}MnN_2O_{10}$: Calcd, 465 $[M+H]^-$; found, 466 $[M+H]^-$.

2. Preparation of SPIO@$[Mn(Dopa\text{-}EDTA)]^{2-}$

Tow size hydrophobic SPIO nanocrystals were synthesized via the high-temperature decomposition method, diphenyl ether and octadecylene were used as reactive solvents.

2.1 Synthesis of 4 nm SPIO nanocrystal.

$Fe(acac)_3$ (1 mmol), 1,2-hexadecanediol (5 mmol), oleic acid (3 mmol), oleylamine (3 mmol), and diphenyl ether (10 ml) were mixed and magnetically stirred under a flow of nitrogen after deoxidized in a two-necked flask. The mixture was heated to 200 °C for 30 min. Then, under a blanket of nitrogen, the mixture was heated to reflux (265 °C) for another 30 min. The reaction system was cooled to room temperature by removing the heat source. Ethanol was added in, and a black material precipitated and was separated via centrifugation (5000 rpm, 10 min). The black product was redissolved in hexane, centrifugation (12000 rpm, 20 min) was applied to remove any undispersed residue. The product was then precipitated with ethanol, centrifuged (6000 rpm, 10 min) to remove the solvent, and redispersed into hexane.

2.2 Synthesis of 7 nm SPIO nanocrystal.

$Fe(acac)_3$ (1 mmol), 1,2-hexadecanediol (5 mmol), oleic acid (3 mmol), oleylamine (3 mmol), and 1-octadecylene (10 ml) were mixed and magnetically stirred under a flow of nitrogen after deoxidized in a two-necked flask. The mixture was heated to 200 °C for 2 h. Then, under a blanket of nitrogen, the mixture was heated to reflux (~310 °C) for 1 h. The reaction system was cooled to room temperature by removing the heat source. Following the workup procedures described in the synthesis of 4 nm SPIO.

2.3 Synthesis of SPIO@[Mn(Dopa-EDTA)]$^{2-}$.

SPIO@[Mn(Dopa-EDTA)]$^{2-}$ was prepared through a ligand exchange process (**Figure S2**). In brief, [Mn(Dopa-EDTA)]$^{2-}$ (10 mg) was dissolved in 4 mL of ultrapure water. 4 mL of acetone and 4 mL hexane dispersed SPIO (5 mg) was added to the flask. The solution was then heated to 70 °C for 12 h in nitrogen. The nanoparticles were precipitated, and then were collected by centrifugation and redispersed in distilled water. The product was dialyzed 1 days against water (MWCO 10 kDa membrane) to ensure no free [Mn(Dopa-EDTA)]$^{2-}$. Mn and Fe concentration of SPIO@[Mn(Dopa-EDTA)]$^{2-}$ solution was measured by inductively coupled plasma-mass spectrometry (ICP-MS).

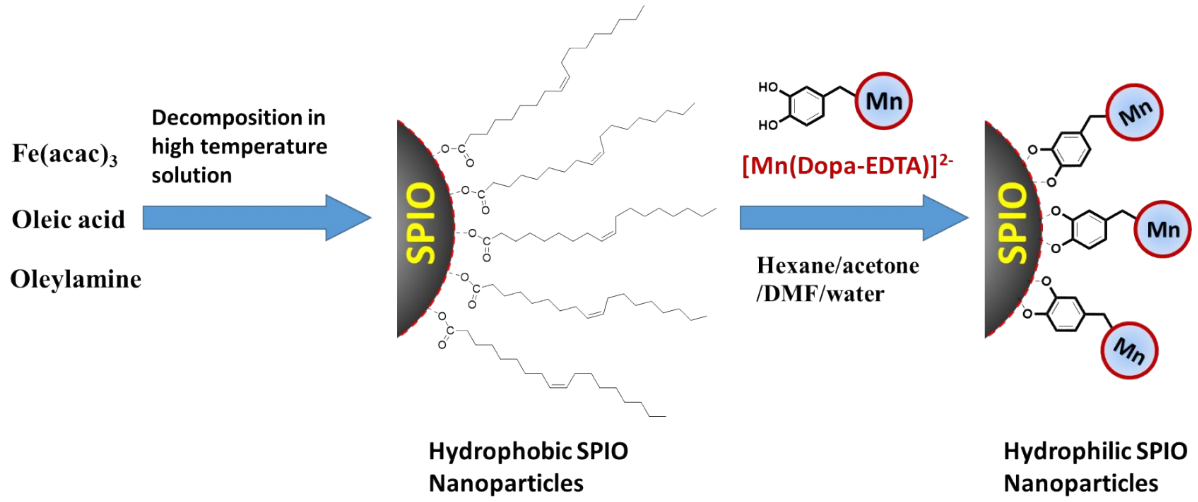


Figure S2: Schematic preparation of SPIO@[Mn(Dopa-EDTA)]$^{2-}$ nanoparticle.

3. Relaxivity and MR Phantom Study

T_1 and T_2 relaxivities of SPIO@[Mn(Dopa-EDTA)]$^{2-}$ was measured in different magnetic intensities (0.5 T, 1.5 T and 3.0 T). The SPIO@[Mn(Dopa-EDTA)]$^{2-}$ aqueous solution with different concentrations (0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 mM Fe and Mn) were prepared firstly. Longitudinal and transverse relaxation times were measured respectively and used for calculating the relaxivities. T_1-weighted and T_2-weighted MR images of SPIO@[Mn(Dopa-EDTA)]$^{2-}$ aqueous solution were acquired with a conventional spin-echo (SE) sequence under the following parameters: T_1 weighted images (TE = 9 ms, TR = 200 ms, slice thickness = 3 mm, FOV = 220mm, Flip angle = 90°); T_2 weighted images (TE = 100 ms, TR = 2500 ms, slice thickness = 3 mm, FOV = 220mm, Flip angle = 90°). T_1-MAP images was simulated from a series of phantoms acquired with inversion recovery (IR) sequence with TI values ranging from 50 to 1500 ms. T_2-MAP images was simulated from a series of phantoms acquired with SE sequence with TE values ranging from 9 to 500 ms.

4. Serum stability and cytotoxicity assay

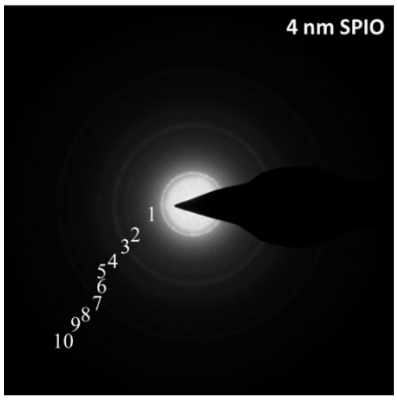
SPIO@[Mn(Dopa-EDTA)]$^{2-}$ and fetal bovine serum (FBS) were mixed in PBS to reach the final concentrations of 20% FBS (v/v) and 8 mM Fe+Mn. The obtained solution were incubated at 37 °C. 200 μl of samples were drawn for DLS analysis at 5 min, 1 h, 5 h and 3 h, respectively. Cytotoxicity was investigated by Cell Counting Kit-8 (CCK-8) assay. RAW 264.7 was cultured in RPMI-1640 medium containing 10% FBS. After digestion by Trypsin–EDTA, cells were inoculated in 96 well plates at a density of 1×10^4 per well and cultured in a humidified atmosphere containing 5% CO_2 at 37 °C for 24 h. Followed

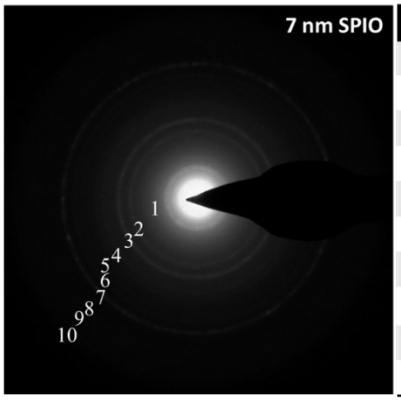
cells were incubated for 24 h in culture medium that included SPIO@[Mn(Dopa-EDTA)]$^{2-}$ nanoparticles with different concentrations of 0, 5, 10, 15, 20 Fe μg/mL. 10 μL per well CCK-8 reagent was added in and incubated for 2 h. Absorbance of samples was measured at 450 nm in a Microplate Reader (BIO-RAD, model 550). Cell viability was determined by the following equation: Cell viability (%) = (Ni/N0) × 100, where Ni and N0 are the absorbance of surviving cells treated with or without SPIO@[Mn(Dopa-EDTA)]$^{2-}$ nanoparticles respectively.

5. In vivo MRI Studies

All studies involving animals were approved by the Animal Care and Use Committee of the Institute. MRI was performed on a 3.0 T clinical scanner (GE Discovery MR 750 3.0 T) using rat or mice coil to acquire signals. Sprague-Dawley (SD) Rats (180~200 g) or BALB/c mouse (20~30 g) were anaesthetized with inhalational anaesthesia system and placed within a scanning coil. After intravenous injection (via tail vein) of SPIO@[Mn(Dopa-EDTA)]$^{2-}$ with the dosages of 0.06 mmol (Fe+Mn)/kg body weight, dynamic CE-MRA images (TR = 6 ms, TE = 2 ms, field of view = 140 mm× 140 mm, slices = 72, slice thickness = 0.8 mm, Flip Angle = 30°) of SD rats were acquired. Immediately after the MR imaging at 24 h, all rats were sacrificed, and main organs were extracted for pathological exams. All organs were fixed in 4% buffered paraformaldehyde overnight, and then embedded in paraffin. Adjacent slides were prepared for histological analysis using either hematoxylin-eosin (HE) staining or Perls' stain (iron staining). Sections were observed under an Eclipse 80i Microscope. Hepatic T_1WI (GR sequence: TR = 9.9 ms, TE = 3.5 ms, field of view = 60 mm× 60 mm, matrix= 512 × 512, slice thickness = 1.8 mm, flip angle = 30°) and T_2WI (SE sequence: TR = 4000 ms, TE = 101 ms, field of view = 60 mm× 60 mm, matrix= 512 × 512, slice thickness = 1 mm, flip angle = 142°) of mouse were acquired in tandem before and after intravenous injection (via tail vein) of 4 nm SPIO@[Mn(Dopa-EDTA)]$^{2-}$ with the dosages of 0.03 mmol (Fe+Mn)/kg body weight.

Supplementary Figures and Tables





ring	hkl	d	Fe_3O_4
1	111	4.62	4.86
2	220	3.00	2.97
3	311	2.58	2.53
4	400	2.15	2.10
5	422	1.75	1.71
6	511	1.66	1.62
7	440	1.51	1.48
8	620	1.33	1.33
9	533	1.25	1.28
10	444	1.12	1.21

Figure S3: SAED pattern of SPIO nanoparticles and Measured Lattice Spacing, d (Å), based on the rings and standard atomic spacing along with respective hkl indexes of Fe_3O_4 from the PDF Database.

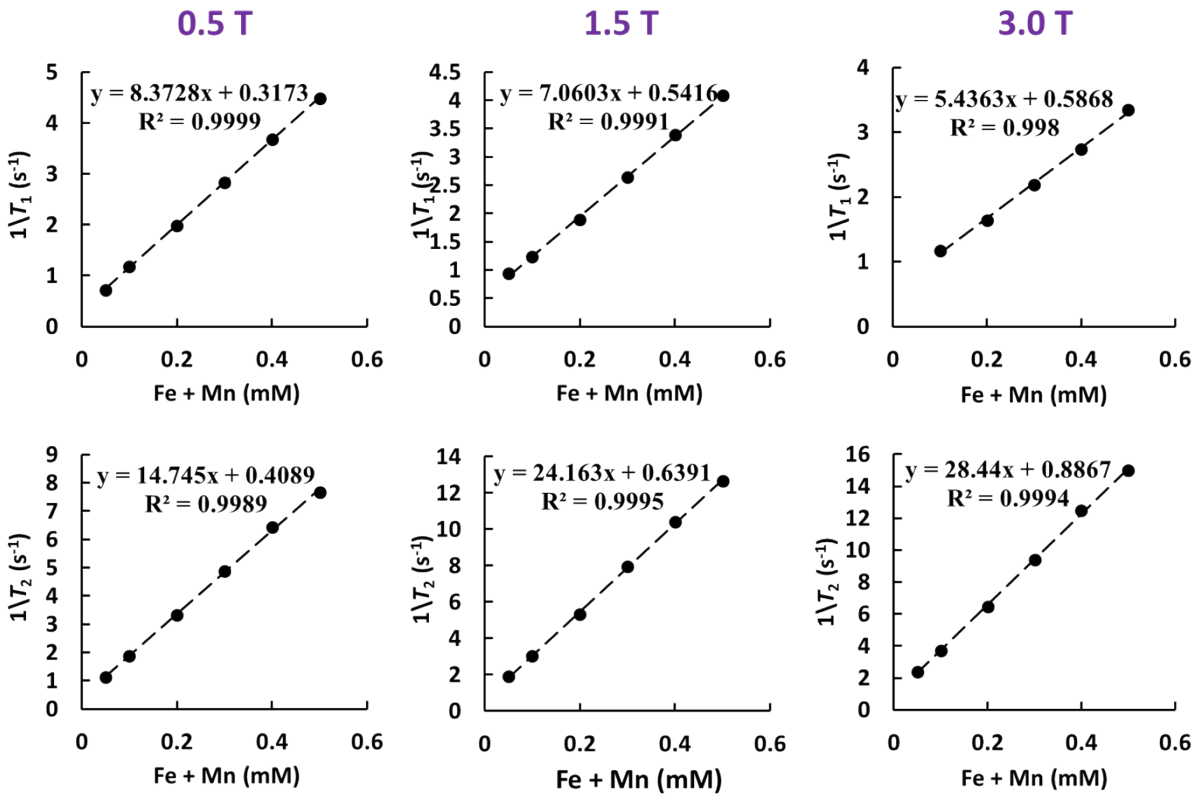


Figure S4: Relaxation rate ($1/T_1$ or $1/T_2$, s^{-1}) of 4 nm SPIO@[Mn(Dopa-EDTA)]$^{2-}$ aqueous solution as a function of metallic ion concentration (Fe + Mn, mM) at room temperature at diferent magnetic intensities (0.5, 1.5 and 3.0 T).

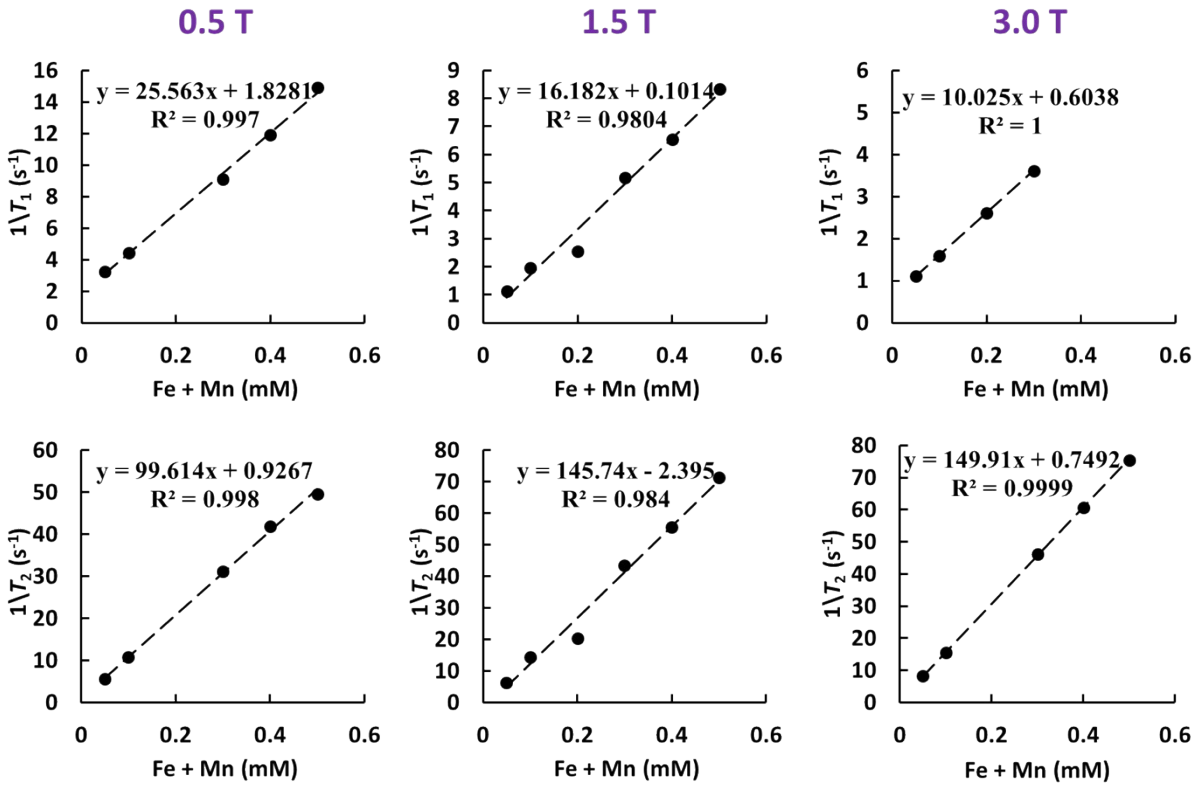


Figure S5: Relaxation rate ($1/T_1$ or $1/T_2$, s^{-1}) of 7 nm SPIO@[Mn(Dopa-EDTA)]$^{2-}$ aqueous solution as a function of metallic ion concentration (Fe + Mn, mM) at room temperature at diferent magnetic intensities (0.5, 1.5 and 3.0 T).

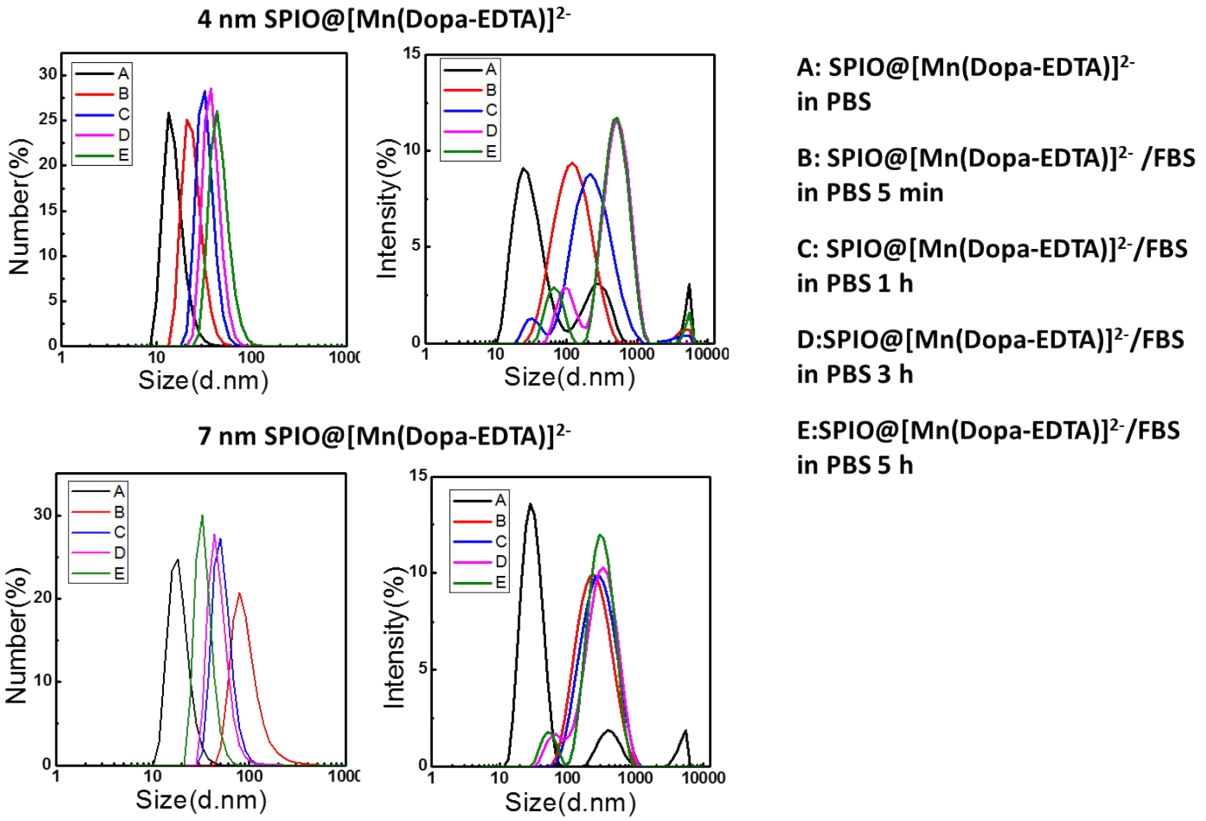


Figure S6: DLS size changes of SPIO@[Mn(Dopa-EDTA)]$^{2-}$ with time in 20% FBS solution at 37 ºC.

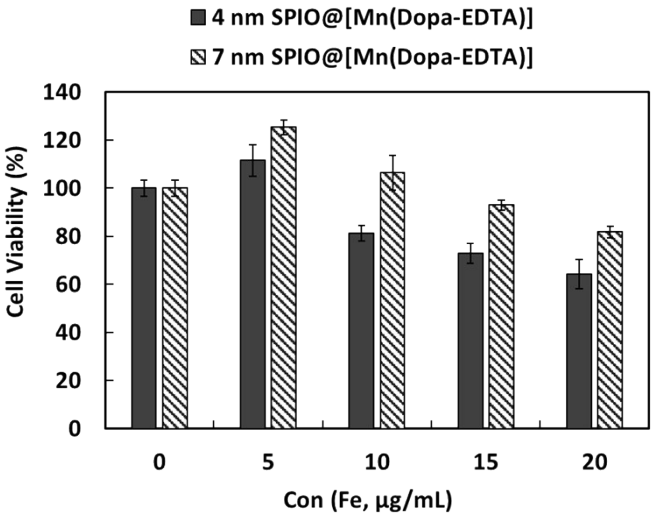


Figure S7: Raw 264.7 cells were incubated with different concentration of 4 nm and 7 nm SPIO@[Mn(Dopa-EDTA)]$^{2-}$ for 24 h. Cells growth was promoted at low concentration, and restrained with increasing of SPIO@[Mn(Dopa-EDTA)]$^{2-}$ nanoparticles in both groups. In addition, 4 nm SPIO@[Mn(Dopa-EDTA)]$^{2-}$ shown more toxic effects than 7 nm SPIO@[Mn(Dopa-EDTA)]$^{2-}$ at high concentration.

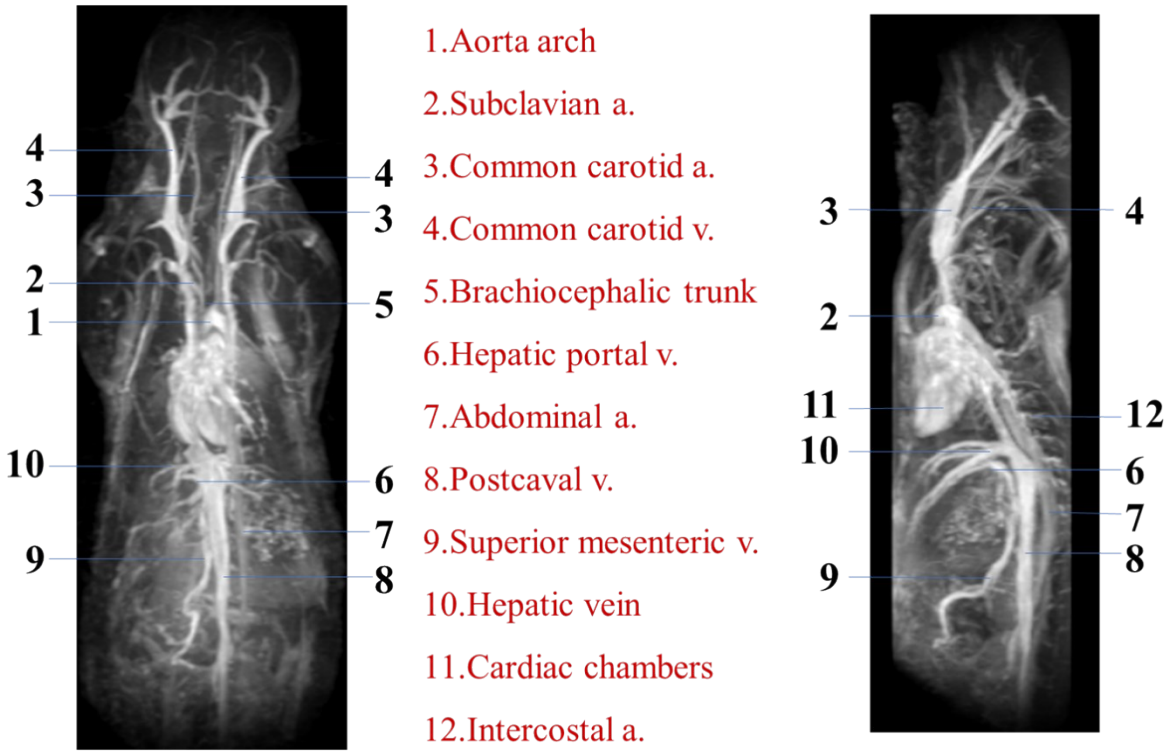


Figure S8: Main vascular anatomical annotation in CEMRA image of SD rat.

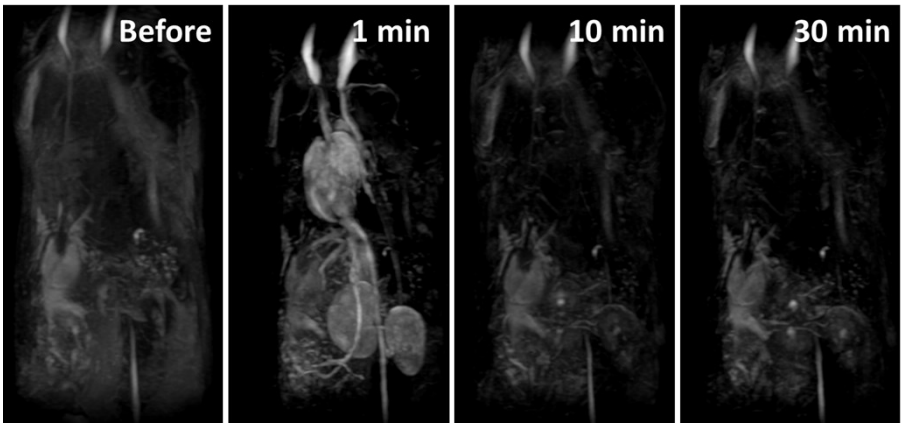


Figure S9: MRA images (TR = 6 ms, TE = 2 ms, field of view = 140 mm× 140 mm, slices = 72, slice thickness = 0.8 mm, Flip Angle = 30°) of SD rat before and after intravenous injection of GdDTPA (*Magnevist*) with the dosages of 0.1 mmol Gd/kg body weight.

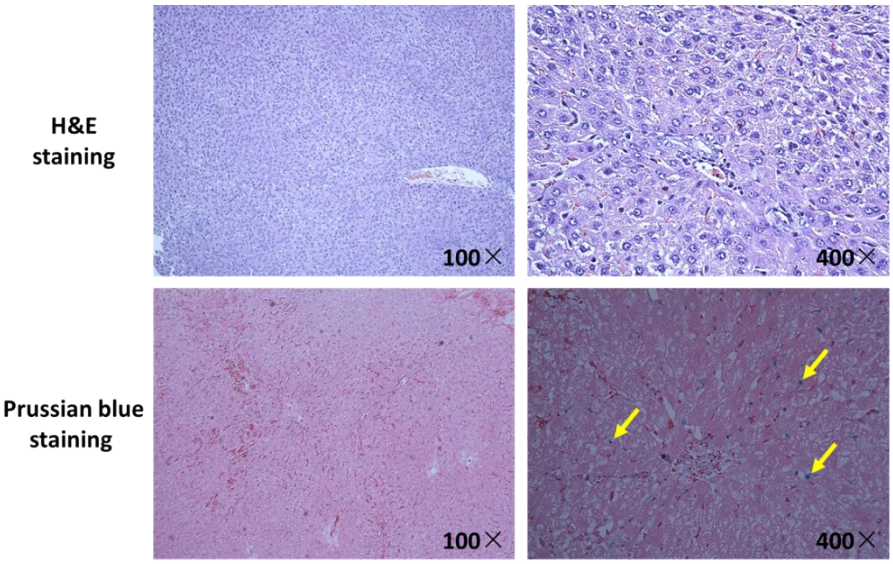


Figure S10: H&E staining and Prussian blue staining of the liver at 24 h after administration of 4 nm SPIO@[Mn(Dopa-EDTA)]$^{2-}$.

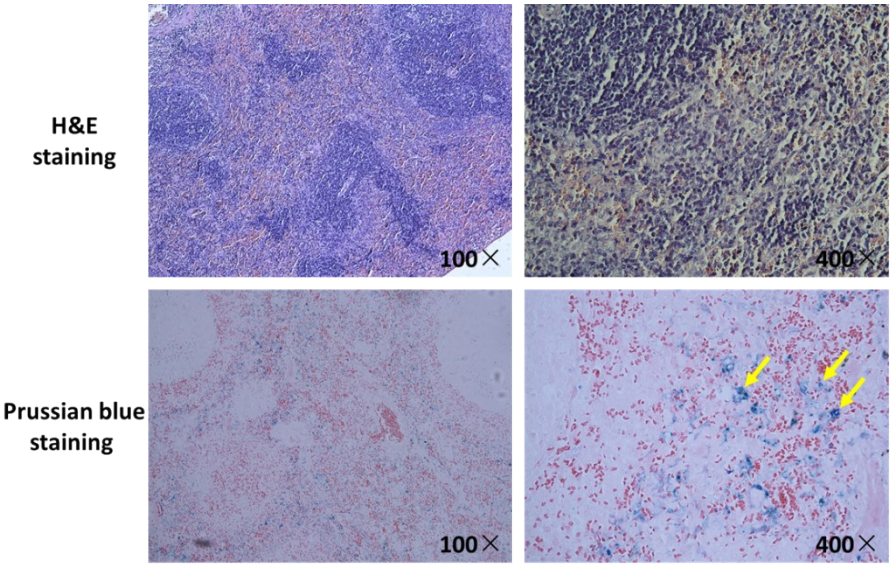


Figure S11: H&E staining and Prussian blue staining of the spleen at 24 h after administration of 4 nm [Mn(Dopa-EDTA)]$^{2-}$.

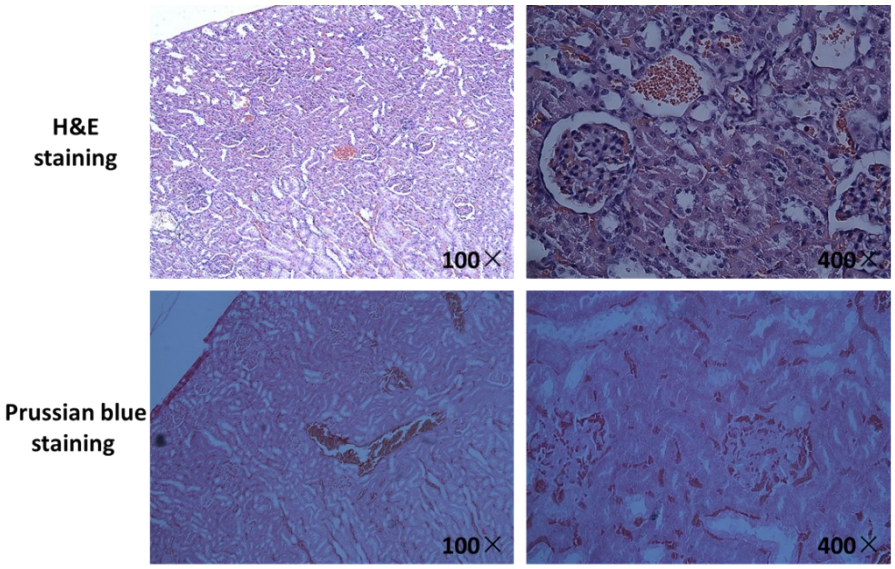


Figure S12: H&E staining and Prussian blue staining of the kidney at 24 h after administration of 4 nm SPIO@[Mn(Dopa-EDTA)]$^{2-}$.

Table S1: Fe and Mn mole ratio of SPIO@[Mn(Dopa-EDTA)]$^{2-}$ nanoparticles

Sample Name	Size [d. nm]	Fe/Mn	Fe/SPIO	Mn/SPIO
4 nm SPIO@[Mn(Dopa-EDTA)]$^{2-}$	3.7 ± 0.7	5.3	1071	202
7 nm SPIO@[Mn(Dopa-EDTA)]$^{2-}$	6.6 ± 1.0	17.4	6079	349

Table S2: The relaxivities of [Mn(Dopa-EDTA)]$^{2-}$ (Room temperature)

B_0 (T)	r_1 (mM^{-1} s^{-1})	r_2 (mM^{-1} s^{-1})	r_2/r_1
0.5	3.8	5.4	1.40
1.5	3.9	6.4	1.64
3.0	4. 5	8. 6	1. 91