Supporting Information for

A Class of Water-soluble Fe (III) Coordination Complexes as T_1-weghited MRI Contrast Agents

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Experimental section

Materials and Characterization

Solvents and raw materials are available without further process and purification. The successful synthesis of complexes was confirmed via Quadruple time-of-flight mass spectrometry (Q-TOF-MS). The absorption spectra of complexes **1**, **2** and **3** were measured using a UV/vis spectrophotometer (DU 730, Beckman Coulter). X-ray photoelectron spectroscopy (XPS) was recorded on AXIS. The IR spectra of complexes **1**, **2** and **3** were measured on an IR spectrophotometer (Nicolet Avatar 370). Fe content was confirmed by using an inductively coupled plasma atomic emission spectrometer (ICP-AES, Varian VistaMPX, USA).

Magneto Relaxivity Measurement

The *in vitro* and *in vivo* MRI performance was evaluated by measuring the relaxometry versus the iron concentration (0.3, 0.625, 1.25, 2.5 and 5 mM) on a 1 T magnetic resonance instrument (NMI20-Analyst, Shanghai Niumag Corporation). Complex **3** was dissolved in saline and injected into mice via the tail vein at a dose of 2.56 mg Fe kg^{-1}. For *in vitro* imaging, the parameters were TE = 0.04 ms and TR = 200 ms. For the 1.0 T T_1-weighted imaging, the parameters were set as TE = 100 ms and TR = 4500 ms. I*n vivo* MR imaging were estimated using the following parameters: slice thickness of 3 mm; a TE of 18.125 ms; a TR of 340 ms; field of view, 80 × 80 mm; matrix size, 256 × 192 mm.

Toxicity Analysis

The cytotoxicity of the coordination complexes toward both human umbilical vein endothelial cells (HUVECs), human breast cancer cells (BT474) and mouse breast cancer cells (4T1) was studied by the classic MTT assay. The cells were cultured in an incubator with a temperature of 37 ºC and a CO_2 content of 5% to grow adherently. The cells were incubated together for 12 h with different iron concentrations of complex **3**. The cell viability was calculated on the basis of the MTT absorption, as measured using a microplate reader (Varioskan Flash, Thermo Fisher Scientific).

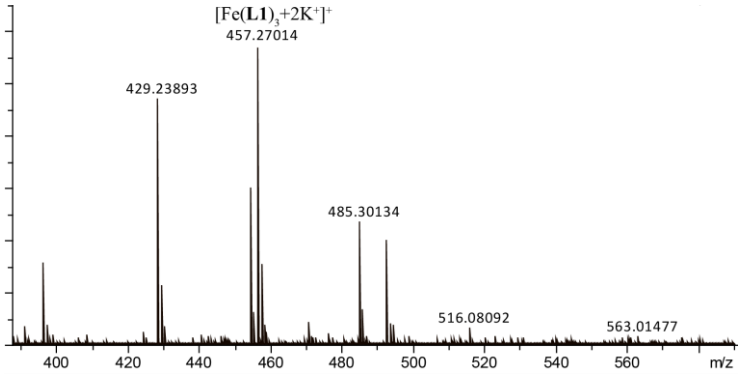
Hemolysis assays were carried out on red blood cells (RBCs, obtained from healthy BALB/c nude mice 5−6 weeks old; Shanghai Laboratory Animal Center). RBCs (0.4 mL, 2% volume ratio) was added to 1 mL deionized water, PBS, or PBS mixtures containing concentrations of compelx **3** (0.125, 0.25, 0.5, 1 mM of Fe), respectively. After 1 hour's incubation at 37°C, mixtures were centrifuged at 3,000 rpm for 5 minutes.

Further, to evaluate cytotoxicity of complex **3** to mice, histological analysis and blood routine examination were performed. The control group (healthy mice treated with PBS) and treatment group (injection of complex **3** at a dose of 2.56 mg Fe kg^{-1}) were humanely sacrificed and dissected after 7 days. For the blood routine examination, plateletcrit (PCT), mean platelet volume (MPV), platelet volume distribution widththe (PDW), white blood cell count (WBC), RBC, mean cell hemoglobin (MCH), mean cell volume (MCV), hematocrit (HCT), hemoglobin concentration (HGB), mean corpuscular hemoglobin concentration (MCHC) were determined. In addition, the main organs including the heart, liver, spleen, lung and kidney were collected, and the damage to the organs by complex **3** was evaluated from the H&E stained slices. At the same time, the weight for the two groups of mice was monitored every day.

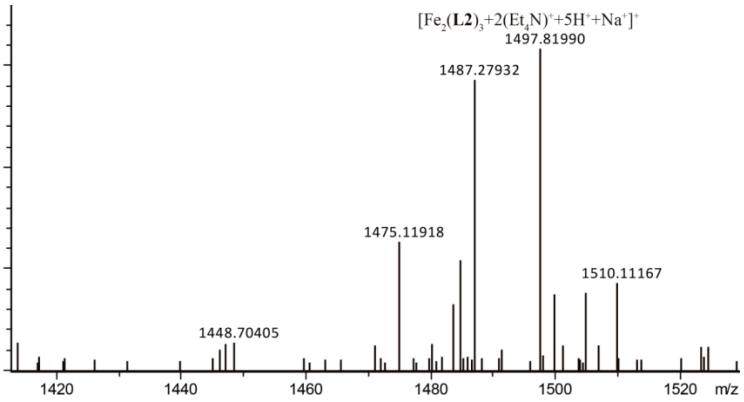
Synthesis

Complexes **2** and **3** were synthesized according to the reported procedure.[1,2] 1,2-Dihydroxybenzene (50 mg, 0.23 mmol) was resolved in 30 mL degassed methanol. A solution of KOH methanol solution (1.9 mL, 0.5 M) and $Fe(acac)_3$ (109 mg, 0.15 mmol) were added into the mixture. Coordination complex **1** was obtained after stirring overnight at R.T. L**2** (80.7 mg, 0.23 mmol) was resolved in 30 mL degassed methanol, methanol solution of KOH (0.9 mL, 0.5 M) and Et_4NCl (1.7 mL, 0.28 M) and $Fe(acac)_3$ (54.36 mg, 0.15 mmol) were added to the solution. The mixture was stirred at R.T. overnight afford to coordination complex **2**. L**3** (100 mg, 0.23 mmol) was resolved in 30 mL degassed methanol, methanol solution of KOH (0.9 mL, 0.5 M) and Et_4NCl (1.66 mL, 0.28 M), $Fe(acac)_3$ (54.36 mg, 0.15 mmol) were added to the solution. Coordination complex **3** was obtained after stirring overnight at R.T.

(a)



(b)



(c)

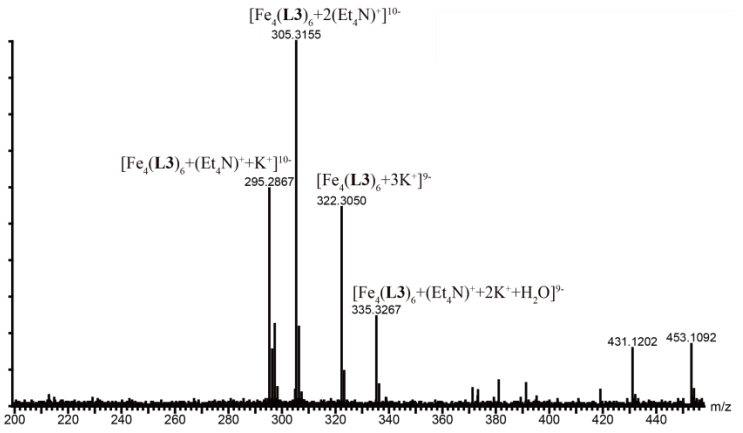
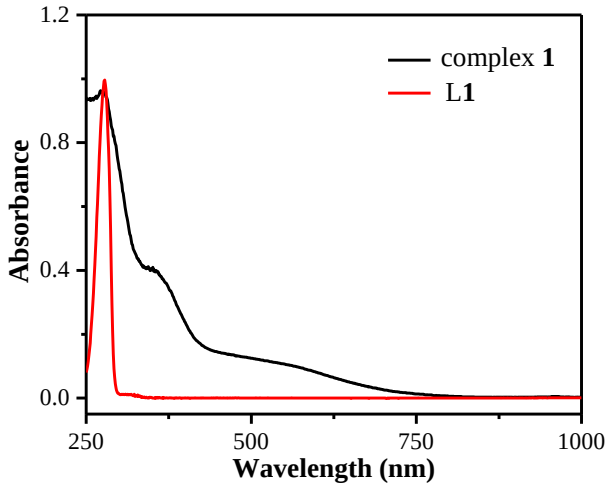


Figure S1. Mass spectra for the complex **1** (a), complex **2** (b) and complex **3** (c).

(a)



(b)

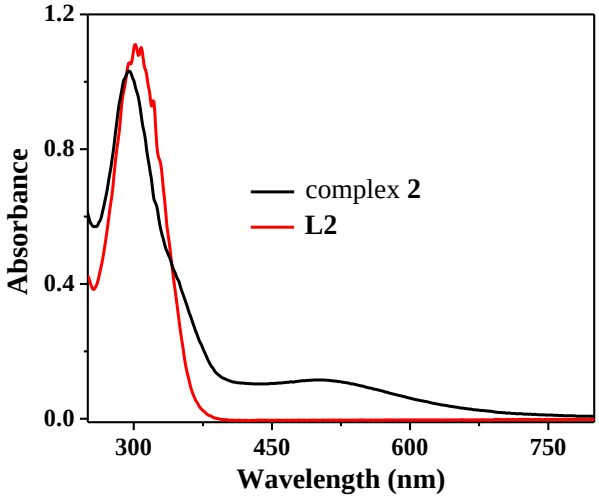
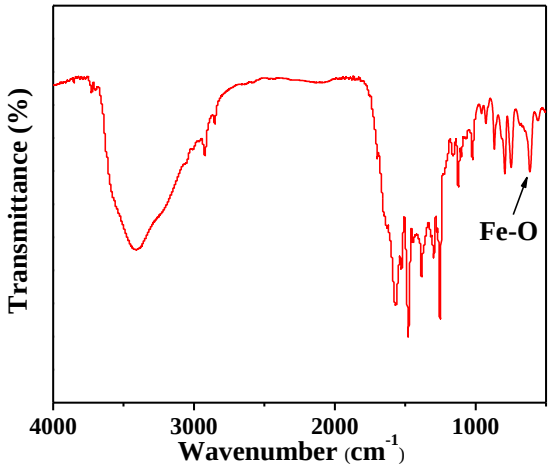
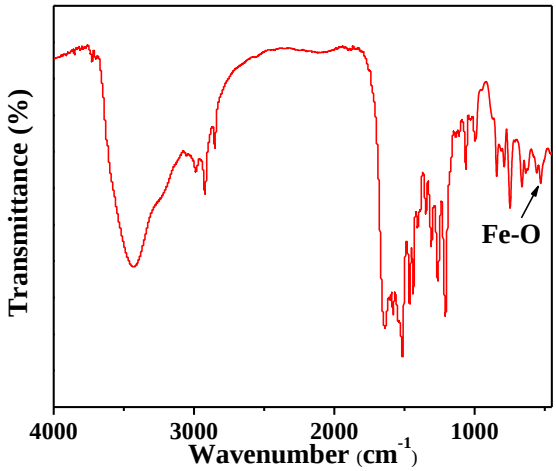


Figure S2. UV-vis spectra for complex **1** and L**1** (a) and complex **2** and L**2** (b) in methnol.

(a)



(b)



(c)

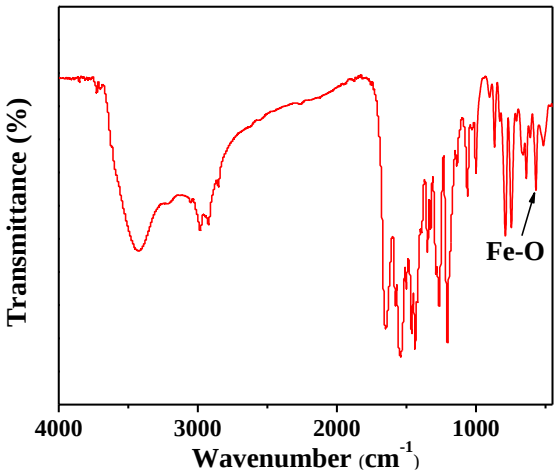
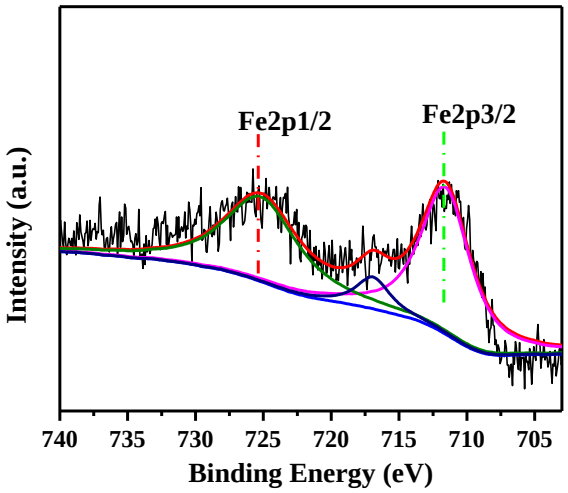


Figure S3. FT-IR spectra for complex **1** (a), complex **2** (b) and complex **3** (c).

The peaks at 613 cm^{-1} in the FT-IR spectra could be assigned to the formation of Fe-O coordination bond in complexes **1**. The peaks at 531 cm^{-1} in the FT-IR spectra could be assigned to the formation of Fe-O coordination bond in complexes **2**. The peaks at 568 cm^{-1} in the FT-IR spectra could be assigned to the formation of Fe-O coordination bond in complexes **3**.

(a)



(b)

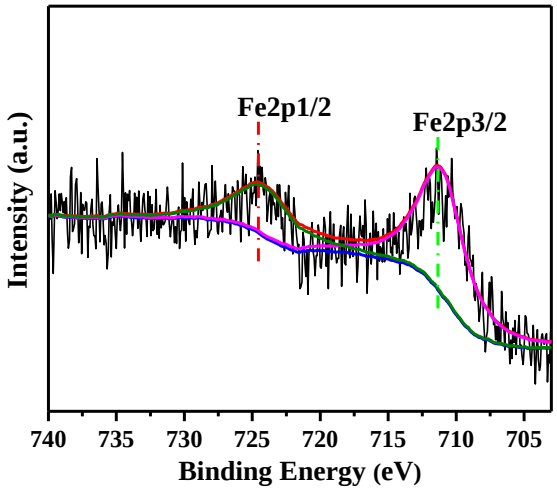


Figure S4. XPS spectra and fitted curves for complex **1** (a) and complex **2** (b).

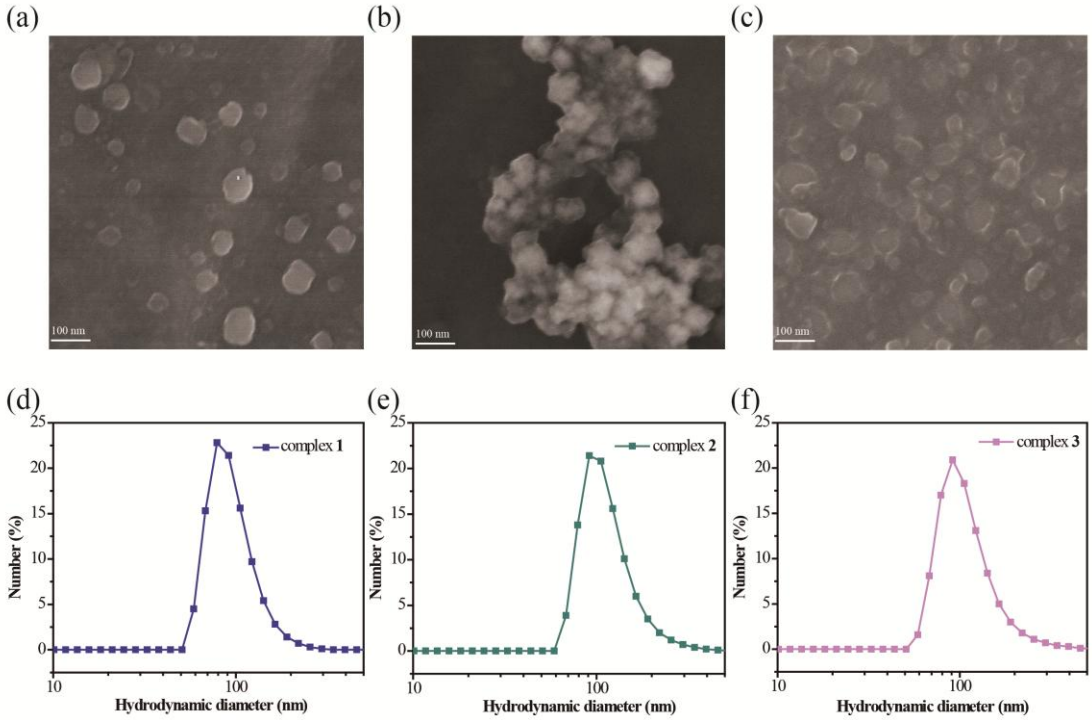
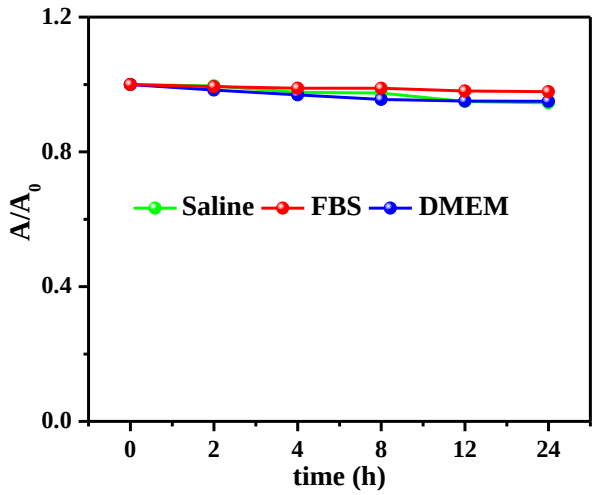


Figure S5. SEM image for complex **1** (a), complex **2** (b) and complex **3** (c). Hydrodynamic diameter profile for complex **1** (d), complex **2** (e) and complex **3** (f).

(a)



(b)

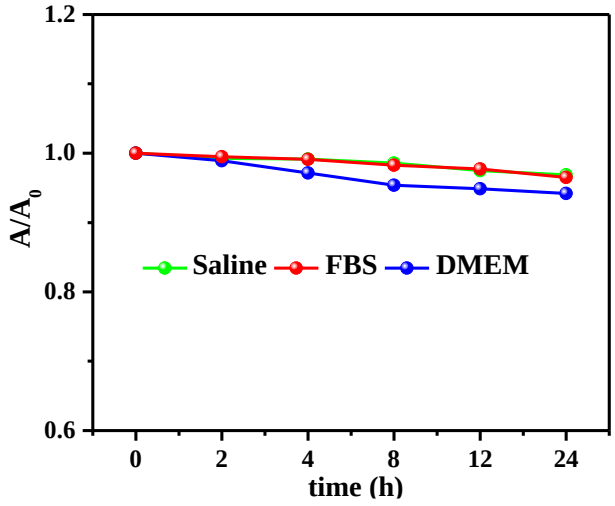
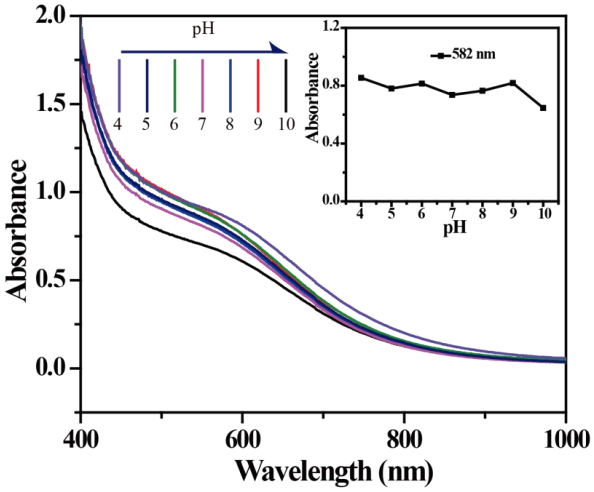
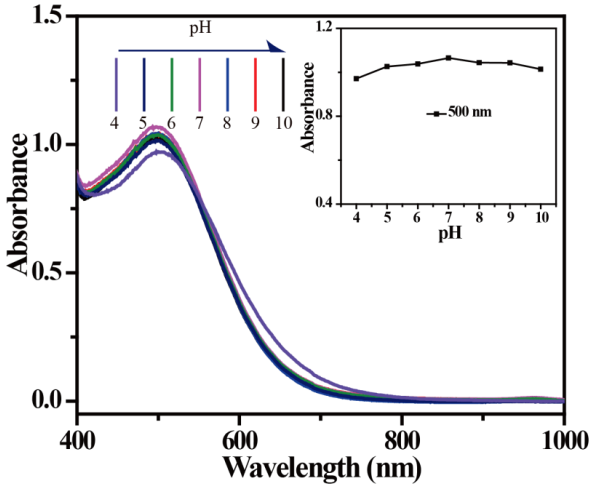


Figure S6. (a) Stability of the complexes **1** in saline, FBS and DMEM: plots of UV-vis absorption ratio versus time, A_0 and A is the adsorption at 582 nm. (b) Stability of complex **2** in saline, FBS and DMEM: plots of UV-vis absorption ratio versus time, A_0 and A is the adsorption at 500 nm.

(a)



(b)



(c)

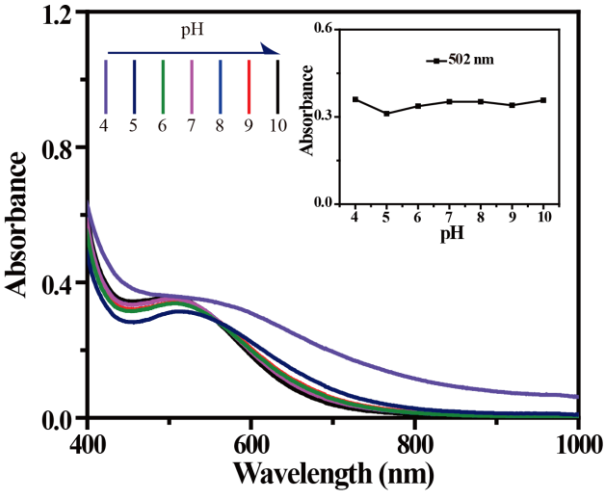
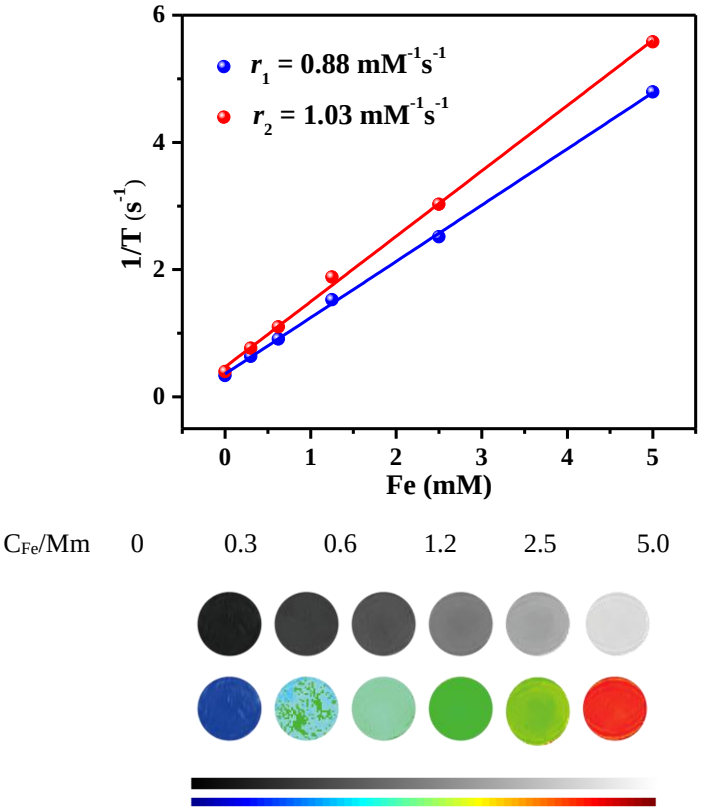


Figure S7. UV-vis spectra for complex **1** (a), complex **2** (b) and complex **3** (c) in different pH conditions.

(a)



(b)

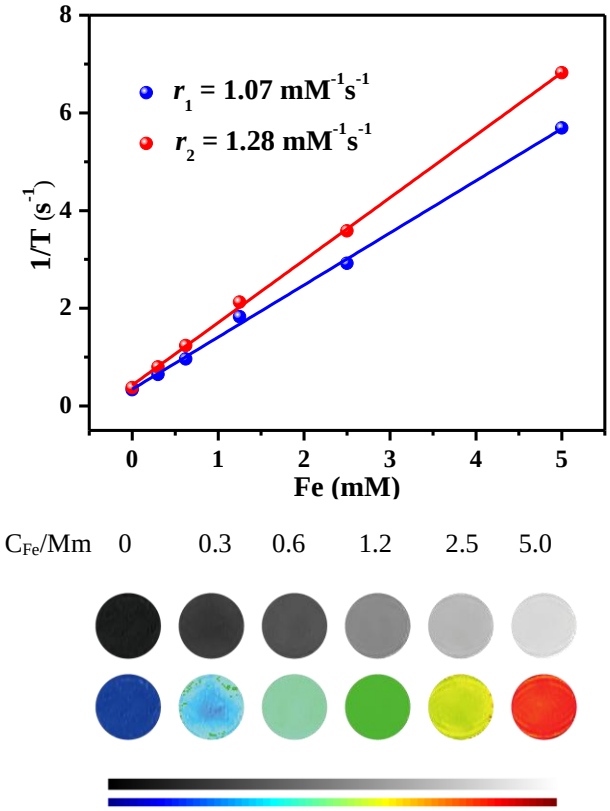


Figure S8. Plots of 1/T versus Fe(III) concentration and corresponding MRI images forcomplex **1** (a) and complex **2** (b).

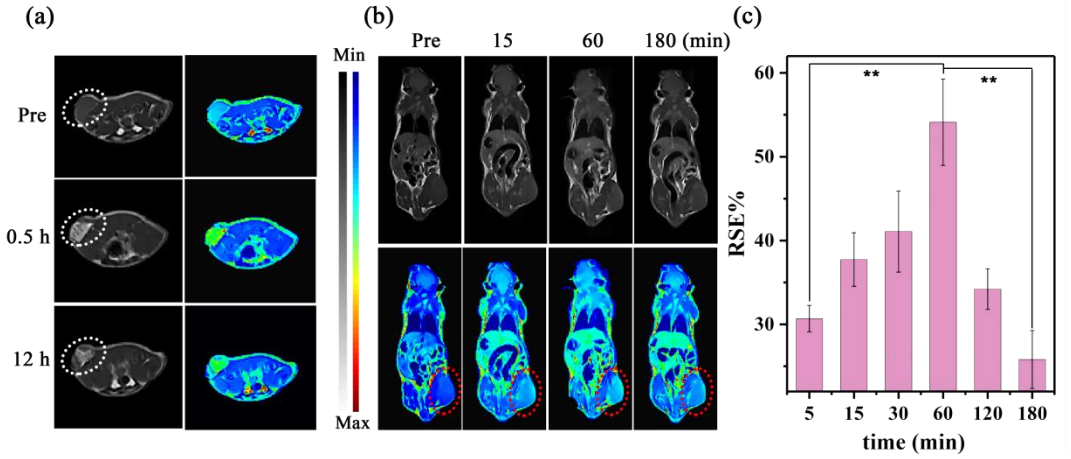


Figure S9. In vivo magnetic resonance images of BALB/c mice bearing 4T1 tumours after (a) intratumour injection (white ellipse); (b) intravenous injection (red ellipse) of complex 3 at different time intervals and (c) corresponding data analysis of the tumour in (b) (*$p < 0.05$,**$p < 0.001$, and ***$p < 0.0001$).

References

1. D. L. Caulder, R. E. Powers, T. N. Parac and K. N. Raymond, *Angew. Chem. Int. Ed.* 1998, **37**, 1840-1843.
2. B. Kersting, M. Meyer, R. E. Powers and K. N. Raymond, *J. Am. Chem. Soc.* 1996, **118**, 7221-7222.