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Surface Manganese Substitution in Magnetite Nanocrystals Enhances T_1 Contrast Ability by Increasing Electron Spin Relaxation

Zhenghuan Zhao,[1,2,‡] Chengjie Sun,[1,‡] Jianfeng Bao,[3] Lijiao Yang,[1] Ruixue Wei,[1] Jingliang Cheng,[3] Hongyu Lin,[1] and Jinhao Gao[1*]

[1]State Key Laboratory of Physical Chemistry of Solid Surfaces, The MOE Key Laboratory of Spectrochemical Analysis & Instrumentation, The Key Laboratory for Chemical Biology of Fujian Province, and iChEM, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China.

[2]College of Pharmaceutical Sciences, Southwest University, Chongqing 400716, China.

[3]Department of Radiology, First Affiliated Hospital, Zhengzhou University, Zhengzhou 450052, China.

‡These authors contributed equally to this work.

*Email: jhgao@xmu.edu.cn

Supplementary Information

Experimental Sections

Reagents. Oleic acid (tech 90%), manganese(II) chloride tetrahydrate (tech 90%), and 1-octadecene (90%) were purchased from Alfa Aesar. $FeCl_3$ (99%), NaCl (AR), hexane, sodium oleate, isopropanol, and ethanol were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All chemicals were used as received without further purification.

Characterizations. Transmission electron microscopy (TEM) images were captured on a JEM-2100 microscope at an accelerating voltage of 200 kV. The energy-dispersive X-ray (EDX) element mapping analysis was performed on a Tecnai F20 microscope at an accelerating voltage of 300 kV. The element analysis of Mn and Fe was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer nano ZS instrument. The MRI testing and T_1 relaxation time measurements were conducted on a 0.5 T NMR120-Analyst NMR Analyzing&Imaging system (Niumag Corporation, Shanghai, China) and a 1.5 T Siemens Avanto instrument. The τ_s values were calculated based on the line widths in electron paramagnetic resonance (EPR) spectrum (Bruker EMX-10/12).

Synthesis of Fe-oleate complex. The synthesis of iron oleate was carried out according the published procedure with minor modification. In a typical experiment, 4.56 g of sodium-oleate (15 mmol) and 0.81g of $FeCl_3$ (5 mmol) were dissolved in a mixture of 20 mL of distilled water and 10 mL of ethanol. The resulting solution was heated to 70 °C and kept at that temperature for 4 h under argon atmosphere. When the reaction was completed, the iron-oleate was washed three times with distilled water in a separatory funnel. Natural volatilization of hexane in the dish gave iron-oleate complex as a waxy solid, which was stored at room temperature.

Synthesis of Mn-oleate complex. The synthesis of manganese oleate was carried out according the

published procedure with minor modification. In a typical experiment, 3.04 g of sodium-oleate (15 mmol) and 0.99 g of $MnCl_2 \cdot 4H_2O$ (5 mmol) were dissolved in a mixture of 45 mL of distilled water and 15 mL of ethanol. The resulting solution was heated to 75 °C and kept at that temperature for 4 h under argon atmosphere. When the reaction was completed, the mixture was extracted with 60 mL of hexane. The organic phase was washed three times with distilled water in a separatory funnel. Natural volatilization of hexane in the dish gave manganese-oleate complex as a waxy solid which was stored at room temperature.

Synthesis of MnIO octapods with various substitution degrees. In a typical synthesis of MnIO octapods with a 6:1 Fe/Mn ratio, iron oleate (0.75 g, 0.83 mmol), Mn-oleate (0.17g, 0.28 mmol), NaCl (12.6 mg, 0.22 mmol), oleic acid (134 μL, 0.41 mmol), and distilled water (60 μL) were mixed together with 10 mL of 1-octadecene. The resulting solution was degassed in vacuum for 30 min and backfilled with argon to remove any low volatile impurities and oxygen at room temperature. After that the reaction solution was heated to reflux with a constant heating rate of 3.3 °C min^{-1}, and kept at the temperature for 2 h. Then the solution was cooled to room temperature and mixed with 30 mL of isopropanol to precipitate the nanoparticles. The nanoparticles were separated by centrifugation and washed 3 times with ethanol. The final product was dissolved in hexane for long-term storage at 4 °C. The preparation of MnIO octapods with Fe/Mn ratio of 17:1 and 20:1 are similar to the aforementioned procedure except for the quantity of Mn-oleate (0.12 g for 17:1 and 0.08 g for 20:1) and NaCl (11.8 mg for 17:1 and 11 mg for 20:1).

Synthesis of MnIO plates with various substitution degrees. In a typical synthesis of MnIO plates with a 8:1 Fe/Mn ratio, iron oleate (0.5 g, 0.55 mmol), Mn-oleate (0.11g, 0.18 mmol), NaCl (14 mg, 0.24 mmol), oleic acid (90 μL, 0.28 mmol), and distilled water (60 μL) were mixed together with 10 mL of 1-octadecene. The resulting solution was degassed in vacuum for 30 min and backfilled with

argon to remove any low volatile impurities and oxygen at room temperature. After that the reaction solution was heated to reflux with a constant heating rate of 3.3 °C min^{-1}, and kept at the temperature for 2 h. Then the solution was cooled to room temperature and mixed with 30 mL of isopropanol to precipitate the nanoparticles. The nanoparticles were separated by centrifugation and washed 3 times with ethanol. The final product was dissolved in hexane for long-term storage at 4 °C. The preparation of MnIO plates with Fe/Mn ratio of 11:1 and 18:1 are similar to the aforementioned procedure except for the quantity of Mn-oleate (0.08 g for 11:1 and 0.05 g for 18:1) and NaCl (13 mg for 11:1 and 12.4 mg for 18:1).

Synthesis of iron oxide (IO) cubes with an edge length of 17 nm. In a typical synthesis of IO cubes, iron oleate (1.2 g, 1.29 mmol), NaCl (13 mg, 0.22 mmol), oleic acid (200 μL, 0.65 mmol), and distilled water (60 μL) were mixed together with 10 mL of 1-octadecene. The resulting solution was degassed in vacuum for 30 min and backfilled with nitrogen to remove any low volatile impurities and oxygen at room temperature. Then the reaction solution was heated to reflux quickly, and kept at the temperature for 1.5 h. Then the solution was cooled to room temperature and mixed with 30 mL of isopropanol to precipitate the nanoparticles. The nanoparticles were separated by centrifugation and washed 3 times with ethanol. The final product was dissolved in hexane for long-term storage at 4 °C.

Synthesis of IO octapods with an edge length of 19 nm. In a typical synthesis of IO octapods, iron oleate (0.8 g, 0.86 mmol), NaCl (10 mg, 0.17 mmol), oleic acid (110 μL, 0.35 mmol), and distilled water (60 μL) were mixed together with 10 mL of 1-octadecene. The resulting solution was degassed in vacuum for 30 min and backfilled with argon to remove any low volatile impurities and oxygen at room temperature. After that the reaction solution was heated to 320 °C with a constant heating rate of 3.3 °C min^{-1}, and kept at the temperature for 1 h. Then the solution was cooled to

room temperature and mixed with 30 mL of isopropanol to precipitate the nanoparticles. The nanoparticles were separated by centrifugation and washed 3 times with ethanol. The final product was dissolved in hexane for long-term storage at 4 °C.

Synthesis of IO plates with a thickness of 2.5 nm. In a typical synthesis of IO plates, iron oleate (0.5 g, 0.54 mmol), NaCl (12 mg, 0.21 mmol), oleic acid (87 μL, 0.27 mmol), and distilled water (60 μL) were mixed together with 10 mL of 1-octadecene. The resulting solution was degassed in vacuum for 30 min and backfilled with argon to remove any low volatile impurities and oxygen at room temperature. After that, the reaction solution was heated to reflux with a constant heating rate of 3.3 °C min^{-1}, and kept at the temperature for 1 h. Then the solution was cooled to room temperature and mixed with 30 mL of isopropanol to precipitate the nanoparticles. The nanoparticles were separated by centrifugation and washed 3 times with ethanol. The final product was dissolved in hexane for long-term storage at 4 °C.

Preparation of Fe_3O_4 nano-seed with a diameter of 13 nm. In a typical experiment, 0.8 g iron-oleate (0.88 mmol) synthesized as aforementioned and 142 μl oleic acid (0.44 mmol) were dissolved in 15 mL 1-octadecene at room temperature. The mixture was degassed in vacuum for 30 min and backfilled with argon to remove any low volatile impurities and oxygen at room temperature. After that the reaction solution was heated to reflux with a constant heating rate of 3.3 °C min^{-1}, and kept at that temperature for 1 h. The resultant solution was then cooled to room temperature and mixed with 30 mL isopropanol to precipitate the nanoparticles. The nanoparticles were separated by centrifugation and washed 3 times with ethanol. After washing, the nanoparticles were dissolved in hexane for long term storage at 4 °C.

Preparation of MnIO nano-seed with a diameter of 17 nm. In a typical experiment, 0.76 g iron-oleate (0.84 mmol), 0.17 g Mn-oleate (0.28 mmol), and 132 μl oleic acid (0.41 mmol) were dissolved in 15 mL 1-octadecene at room temperature. The mixture was degassed in vacuum for 30 min and backfilled with argon to remove any low volatile impurities and oxygen at room temperature. After that the reaction solution was heated to reflux with a constant heating rate of 3.3 °C min^{-1}, and kept at that temperature for 1 h. The resultant solution was then cooled to room temperature and mixed with 30 mL isopropanol to precipitate the nanoparticles. The nanoparticles were separated by centrifugation and washed 3 times with ethanol. After washing, the nanoparticles were dissolved in hexane for long term storage at 4 °C.

Preparation of Fe_3O_4@FeMnO nanoparticles. In a typical experiment, 100 mg Fe_3O_4 nano-seed, 0.1 g iron-oleate (0.11 mmol), 0.04 g Mn-oleate (0.64 mmol), and 50 μl oleic acid (0.16 mmol) were dissolved in 15 mL 1-octadecene at room temperature. The mixture was degassed in vacuum for 30 min and backfilled with argon to remove any low volatile impurities and oxygen at room temperature. After that the reaction solution was heated to reflux and kept at that temperature for 1.5 h. The resultant solution was then cooled to room temperature and mixed with 30 mL isopropanol to precipitate the nanoparticles. The nanoparticles were separated by centrifugation and washed 3 times with ethanol. After washing, the nanoparticles were dissolved in hexane for long term storage at 4 °C.

Preparation of MnIO@Fe_3O_4 nanoparticles. In a typical experiment, 100 mg MnIO nano-seed, 0.15 g iron-oleate (0.16 mmol) and 50 μl oleic acid (0.16 mmol) were dissolved in 15 mL 1-octadecene at room temperature. The mixture was degassed in vacuum for 30 min and backfilled with argon to remove any low volatile impurities and oxygen at room temperature. After that the reaction solution was heated to reflux and kept at that temperature for 1 h. The resultant solution was

then cooled to room temperature and mixed with 30 mL isopropanol to precipitate the nanoparticles. The nanoparticles were separated by centrifugation and washed 3 times with ethanol. After washing, the nanoparticles were dissolved in hexane for long term storage at 4 °C.

Measurement of MR relaxivities of nanoparticles. To measure the T_1 relaxivities, samples with different magnetic ion concentrations were dispersed in 1% agarose solution. The T_1 relaxation times for all the samples were measured on a 0.5 T NMI20-Analyst NMR system and a 1.5 T Siemens Avanto instrument. The T_1-weighted MRI images for the samples were acquired by 1.5 T siemens Avanto instrument using MSE sequence as following parameters: TR/TE=100/12 ms, 256 matrices, thickness =1mm, NS=2.

Cell culture. SMMC-7721 cells were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China) and cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) supplemented with 10% fetal bovine serum (FBS, Hyclone) and antibiotics (100mg/mL streptomycin and 100 U/mL penicillin). All cells were maintained in a humidified atmosphere of 5% CO_2 at 37 °C.

***In vitro* cytotoxicity evaluation.** The experiment was performed in quintuplicates. SMMC-7721 cells (1×10^4) were seeded in 96-well plates and incubated for 12 h in RPMI-1640 (containing 10% FBS and antibiotics). After cells were washed twice with PBS, fresh media containing MnIO and IO nanoparticles with different concentrations was added and the cells were incubated for 24 h. The growth media was replaced with RPMI-1640 containing 0.5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and incubated for another 4 h. After the media were discarded, 100 μL of DMSO was added to dissolve the precipitates and the absorbance at 492 nm of the resulting solution was measured using a MultiSkan FC microplate reader (Thermo scientific).

Statistical analysis. Statistical analysis was performed using the Student's t-test for unpaired data, *p* values of less than 0.05 were accepted as a statistically significant difference compared to controls.

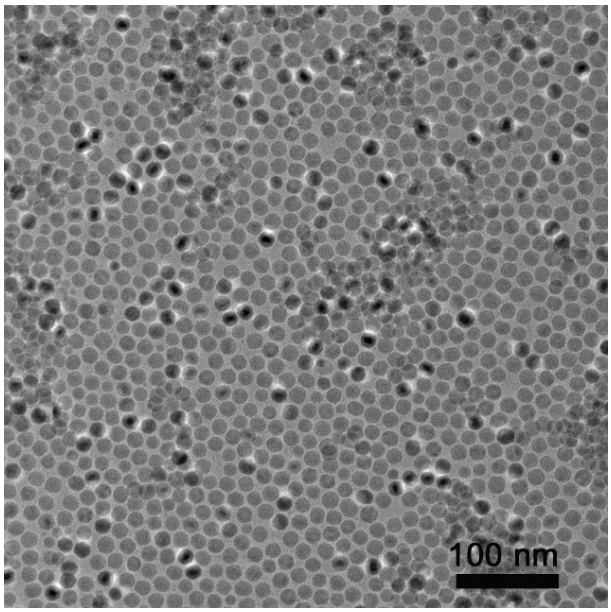

Figure S1. TEM image of spherical MnIO nanoparticles.

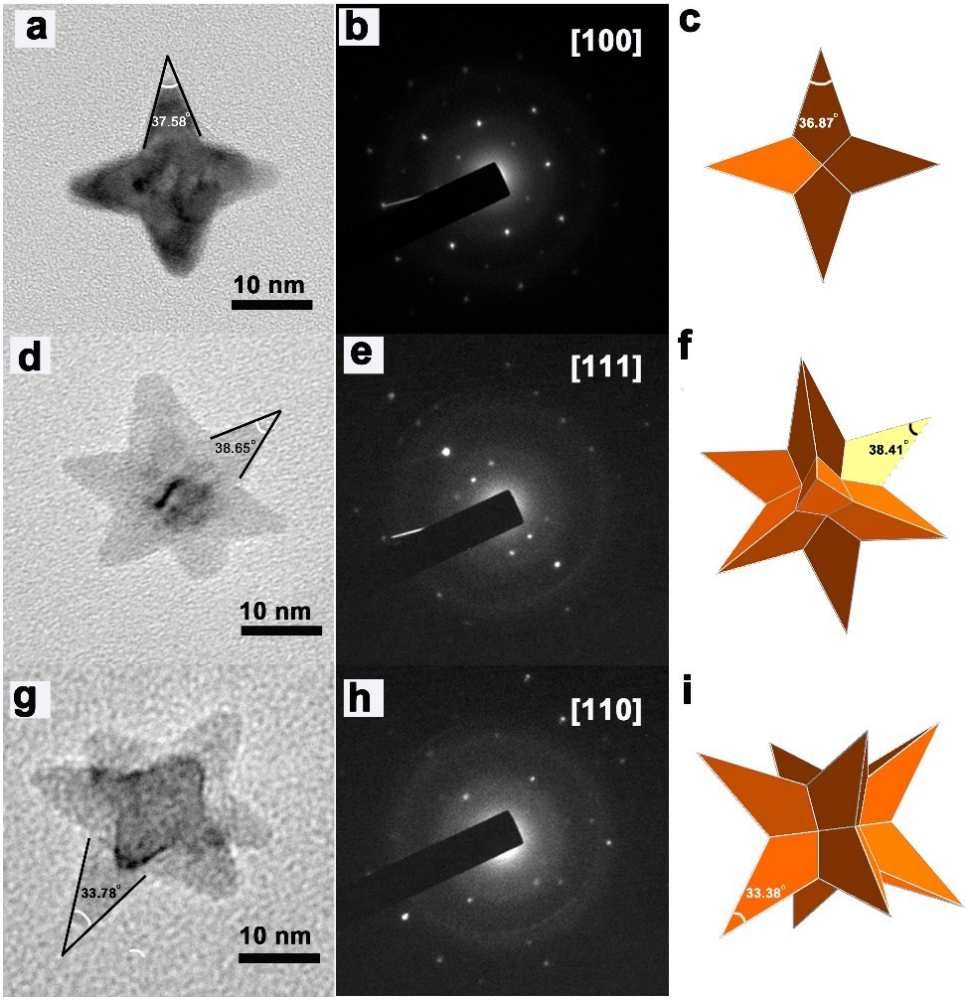

Figure S2. Geometric model of MnIO octapods. (a, d, g) High-resolution transmission electron microscopy (HRTEM) images, (b, e, h) selected-area electron diffraction (SAED) patterns, and (c, f, i) geometric models of individual MnIO octapods oriented along the [100], [111], and [110] directions, respectively.

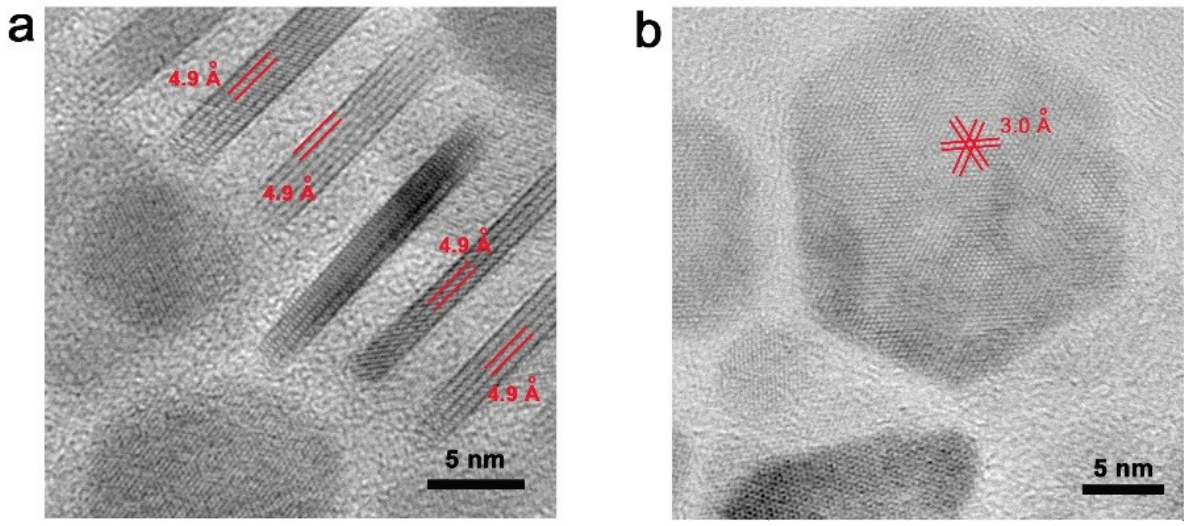

Figure S3. Characterization of MnIO plates. (a) HRTEM image of MnIO plates with a lattice spacing distance of 4.9 Å from the perpendicular view. (b) HRTEM image of MnIO plates with a cross lattice spacing distance of 3.0 Å. These results indicate that MnIO plates are terminated by the {111} basal facets.

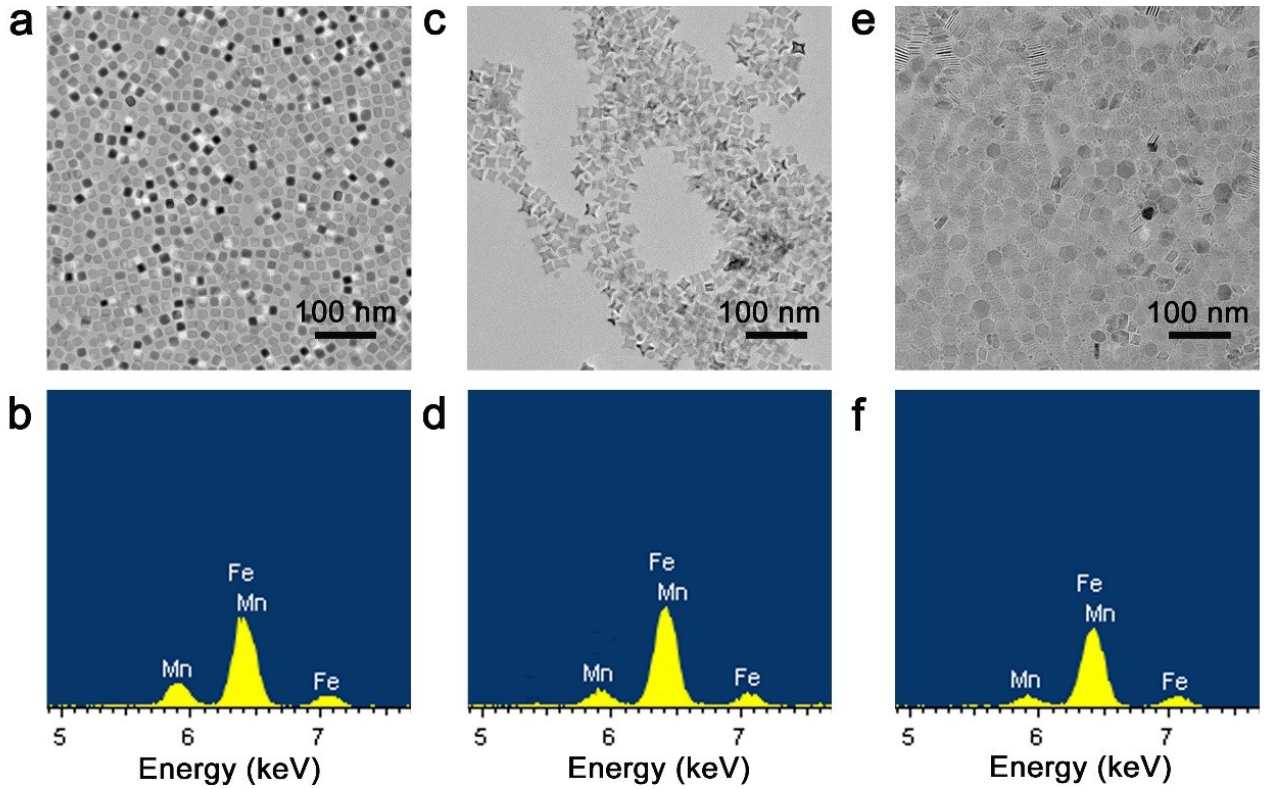

Figure S4. Analysis of anisotropic shaped MnIO nanoparticles by energy dispersive X-ray spectroscopy (EDS). TEM images of accumulative MnIO (a) cubes, (c) octapods, and (e) plates on copper mesh. The EDS results for accumulative MnIO (b) cubes, (d) octapods, and (f) plates on copper mesh.

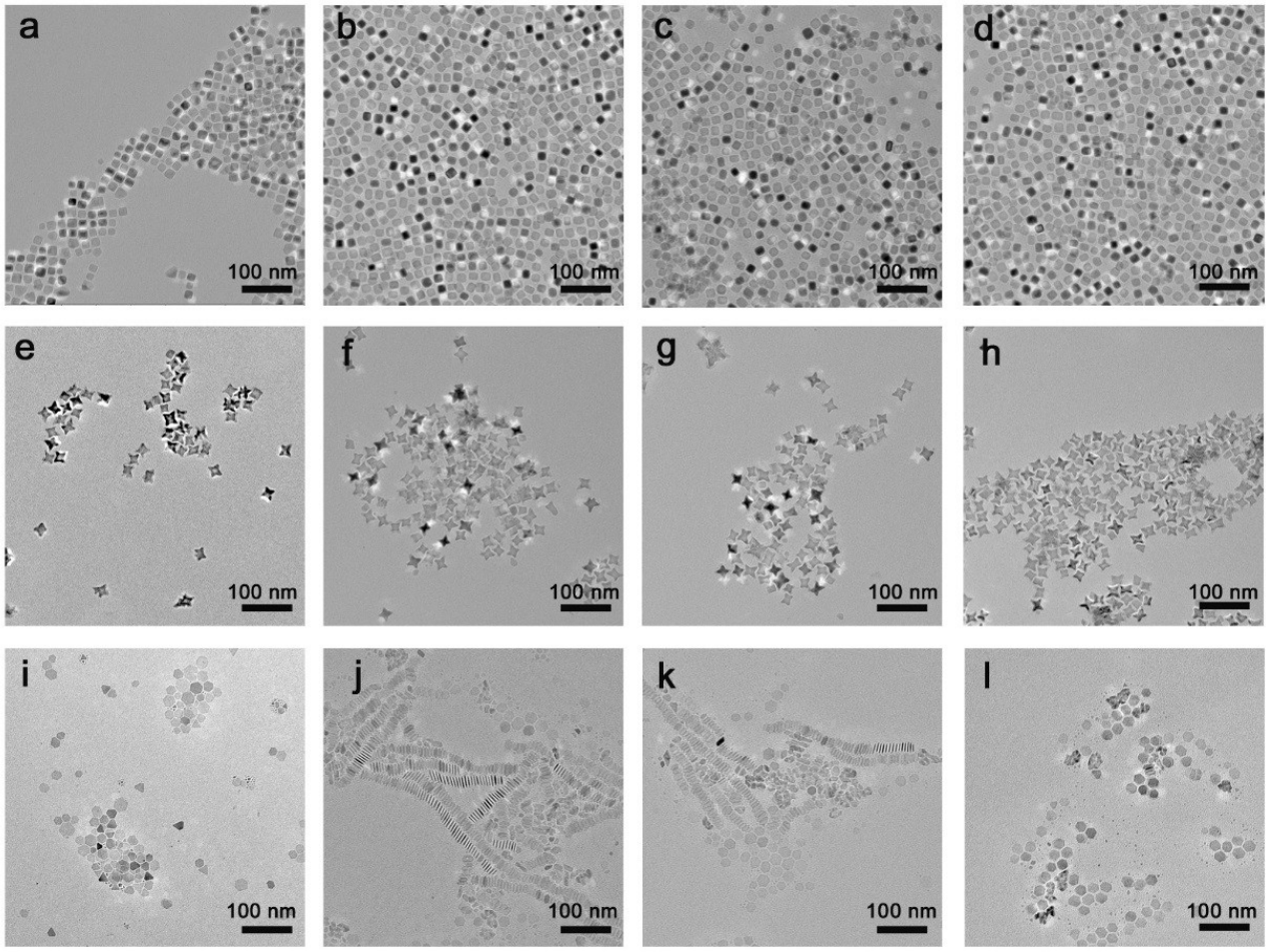

Figure S5. TEM analyses of anisotropic-shaped MnIO nanoparticles with different Mn/Fe ratios. TEM images of (a) IO and MnIO cubes with Mn/Fe ratios of (b) 1:22, (c) 1:12, and (d) 1:5. TEM images of (e) IO and MnIO octapods with Mn/Fe ratios of (f) 1:20, (g) 1:17, and (h) 1:6. TEM images of (i) IO and MnIO plates with Mn/Fe ratios of (j) 1:18, (k) 1:11, and (l) 1:8.

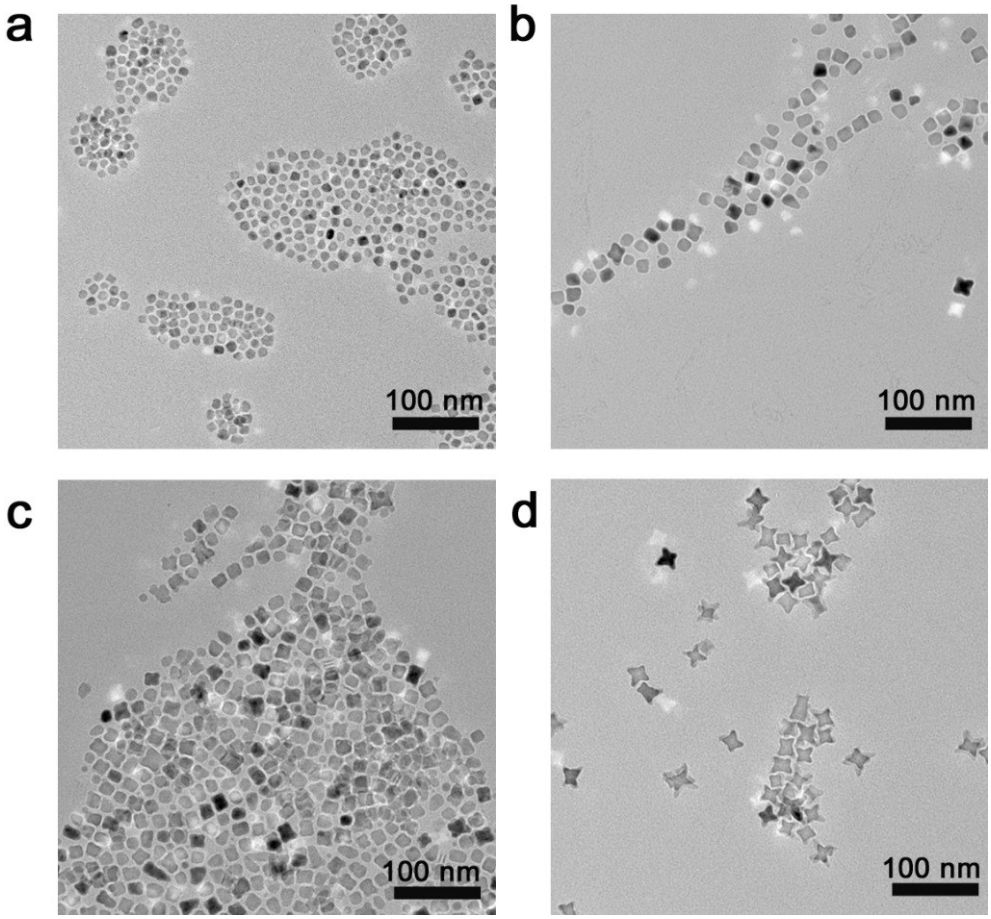

Figure S6. TEM analyses of the reaction solutions of MnIO octapods at certain time intervals. TEM images of the MnIO nanoparticles after (a) 10, (b) 30, (c) 45, and (d) 60 min reaction time in the presence of NaCl.

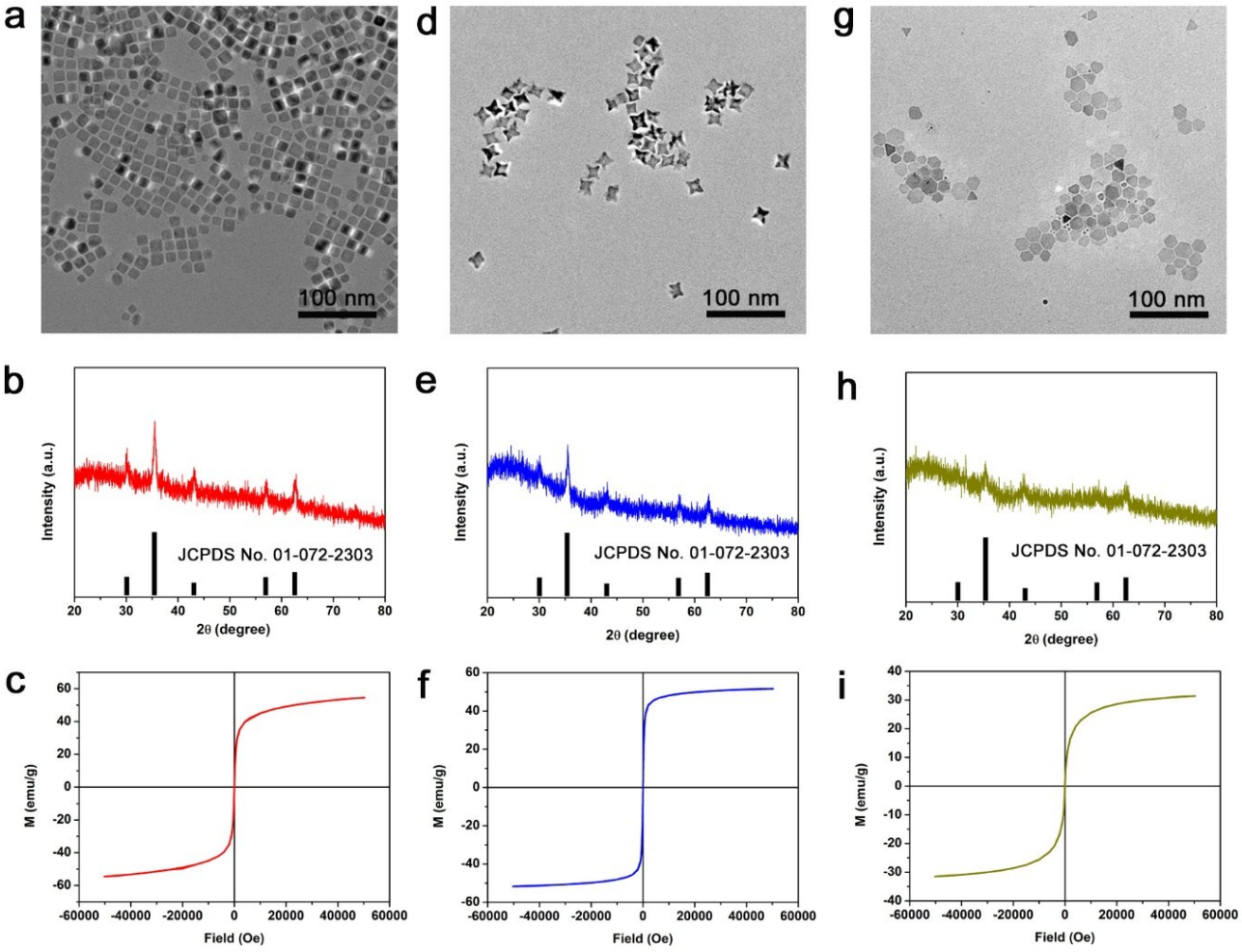

Figure S7. Characterization of anisotropic shaped IO nanoparticles. TEM images of IO (a) cubes, (d) octapods, and (g) plates. The XRD pattern images of IO (b) cubes, (e) octapods, and (h) plates. The XRD patterns are consistent with the magnetite reference values. The smooth *M-H* curves of IO (c) cubes, (f) octapods, and (i) plates.

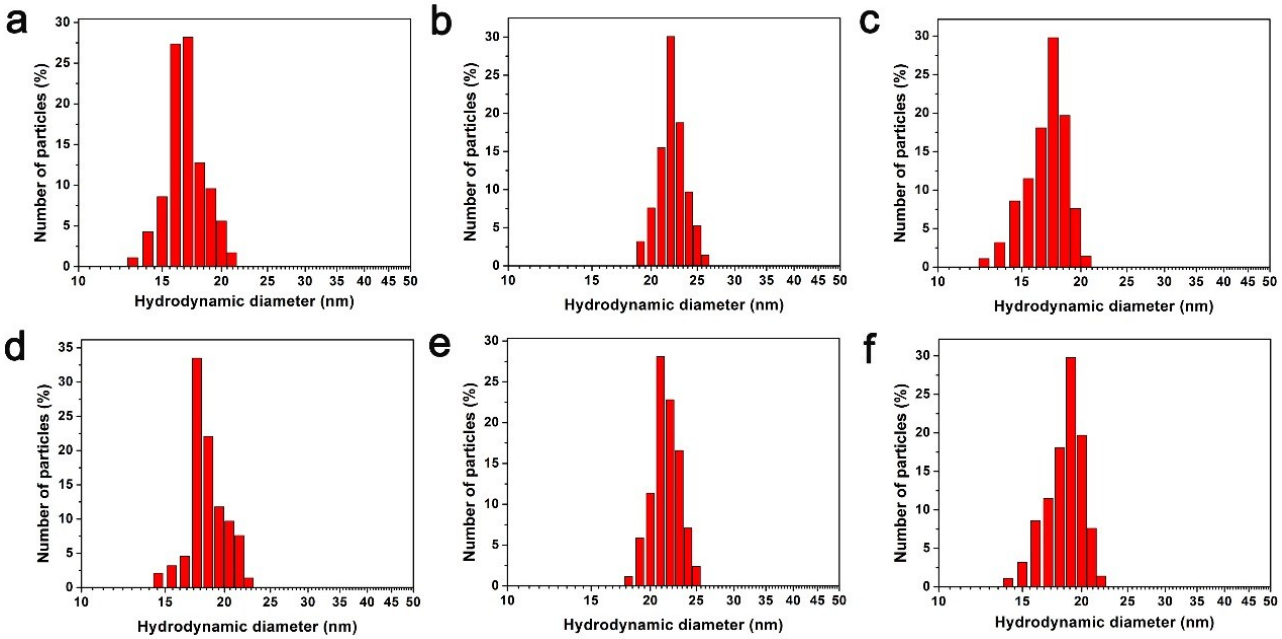

Figure S8. Dynamic light scattering (DLS) analyses of water-dispersible nanoparticles in PBS buffer. DLS analysis of water-dispersible (a) MnIO cubes, (d) IO cubes, (b) MnIO octapods, (e) IO octapods, (c) MnIO plates, and (f) IO plates.

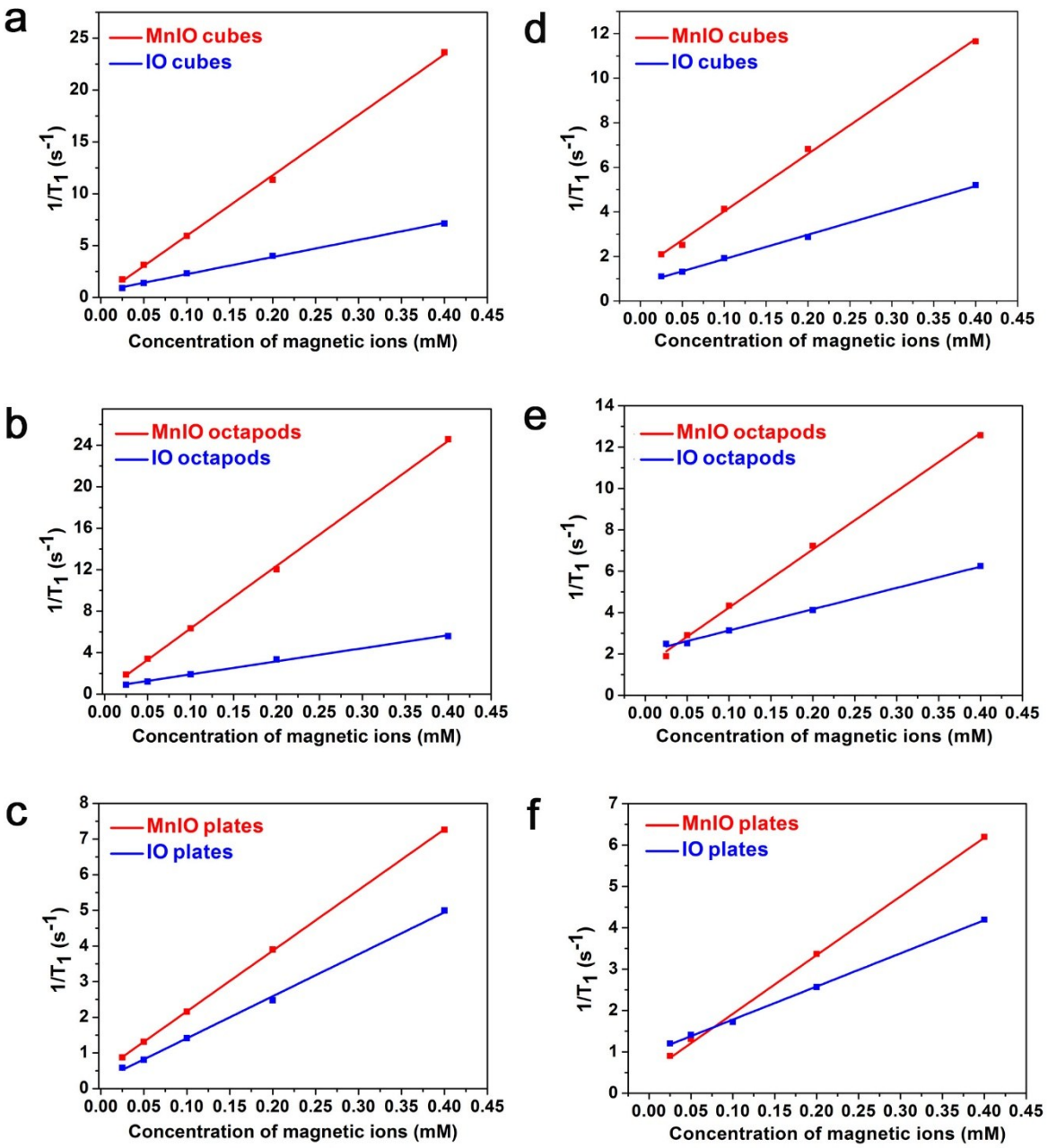

Figure S9. MR relaxivities of anisotropic shaped MnIO and IO nanoparticles at 0.5 and 1.5 T. Relaxation rate R_1 ($1/T_1$) *vs.* magnetic ion concentration for (a) MnIO and IO cubes, (b) MnIO and IO octapods, and (c) MnIO and IO plates measured on a 0.5 T MRI scanner. Relaxation rate R_1 ($1/T_1$) *vs.* magnetic ion concentration for (d) MnIO and IO cubes, (e) MnIO and IO octapods, and (f) MnIO and IO plates measured on a 1.5 T MRI scanner. These results indicate that MnIO nanoparticles exhibit significantly stronger T_1 contrast effects than IO nanoparticles.

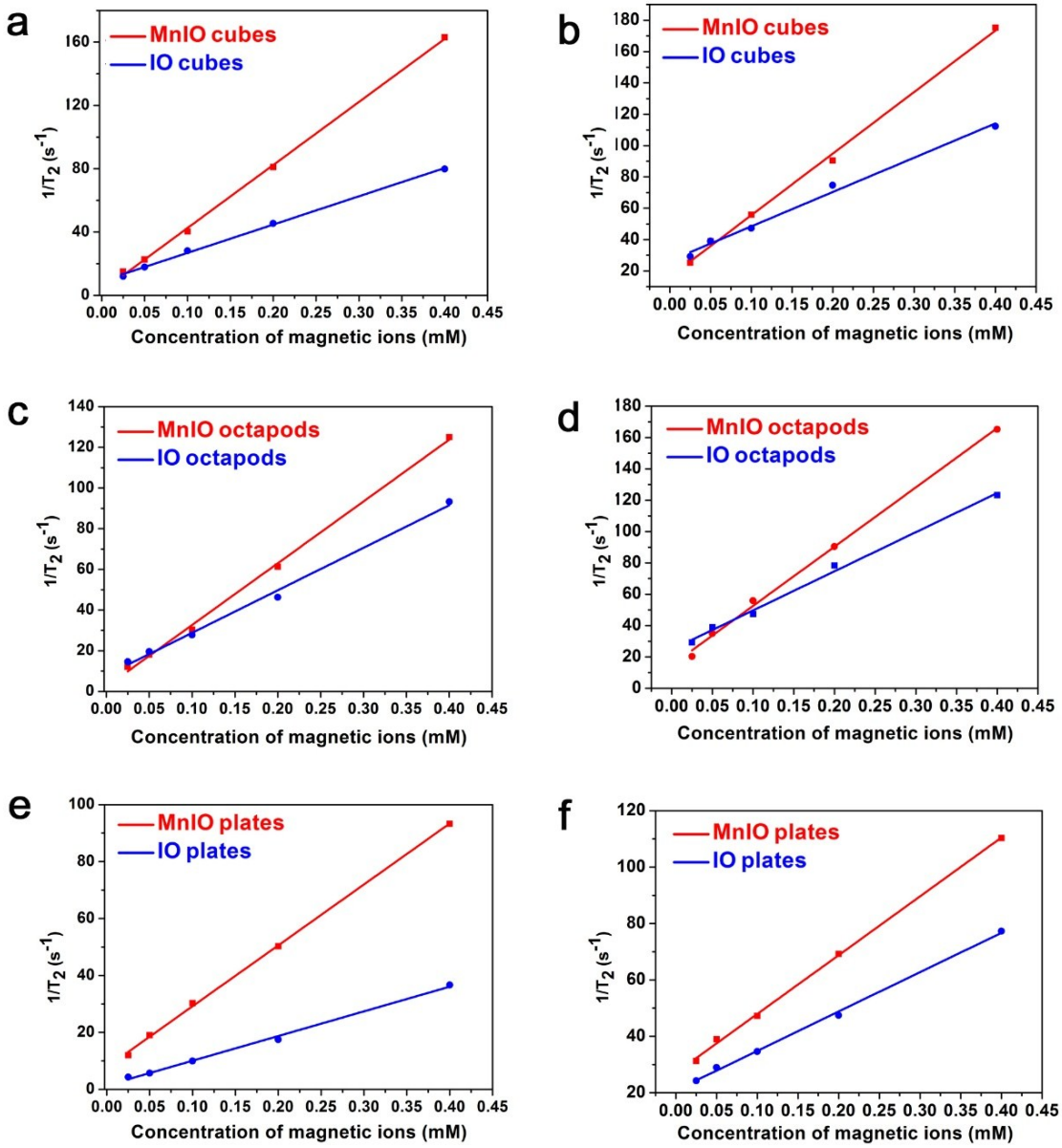

Figure S10. MR relaxivities of anisotropic-shaped MnIO and IO nanoparticles at 0.5 and 1.5 T. Relaxation rate R_2 ($1/T_2$) *vs.* magnetic ion concentration for (a) MnIO and IO cubes, (b) MnIO and IO octapods, and (c) MnIO and IO plates measured on a 0.5 T MRI scanner. Relaxation rate R_2 ($1/T_2$) *vs.* magnetic ion concentration for (d) MnIO and IO cubes, (e) MnIO and IO octapods, and (f) MnIO and IO plates measured on a 1.5 T MRI scanner.

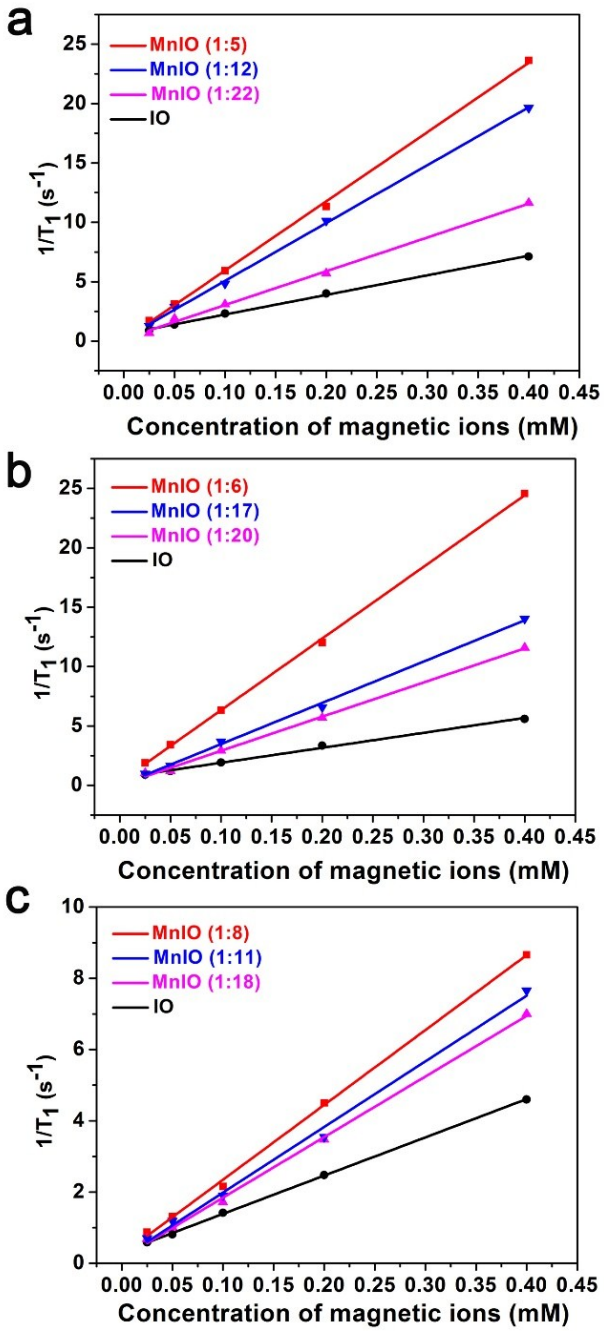

Figure S11. T_1 relaxivities anisotropic shaped MnIO nanoparticles with different Mn/Fe ratios at 0.5 T. Relaxation rate R_1 ($1/T_1$) *vs.* magnetic ion concentration for MnIO (a) cubes, (b) octapods, and (c) plates with different Mn/Fe ratios.

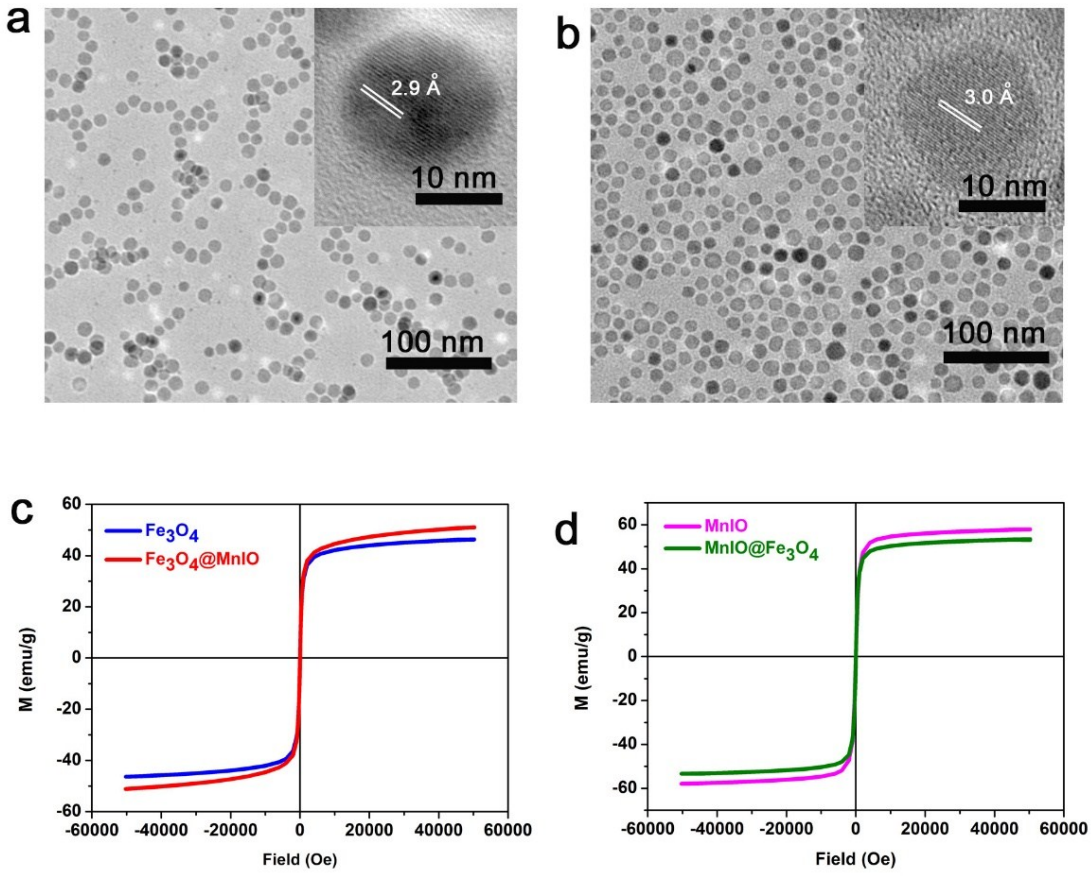

Figure S12. Characterization of Fe_3O_4 and MnIO nano-seeds. TEM images of (a) Fe_3O_4 nano-seeds and (b) MnIO nano-seeds. Insets are the corresponding HRTEM images. The smooth *M-H* curves of (c) Fe_3O_4 and Fe_3O_4@MnIO nanoparticles and (d) MnIO and MnIO@Fe_3O_4 nanoparticles.

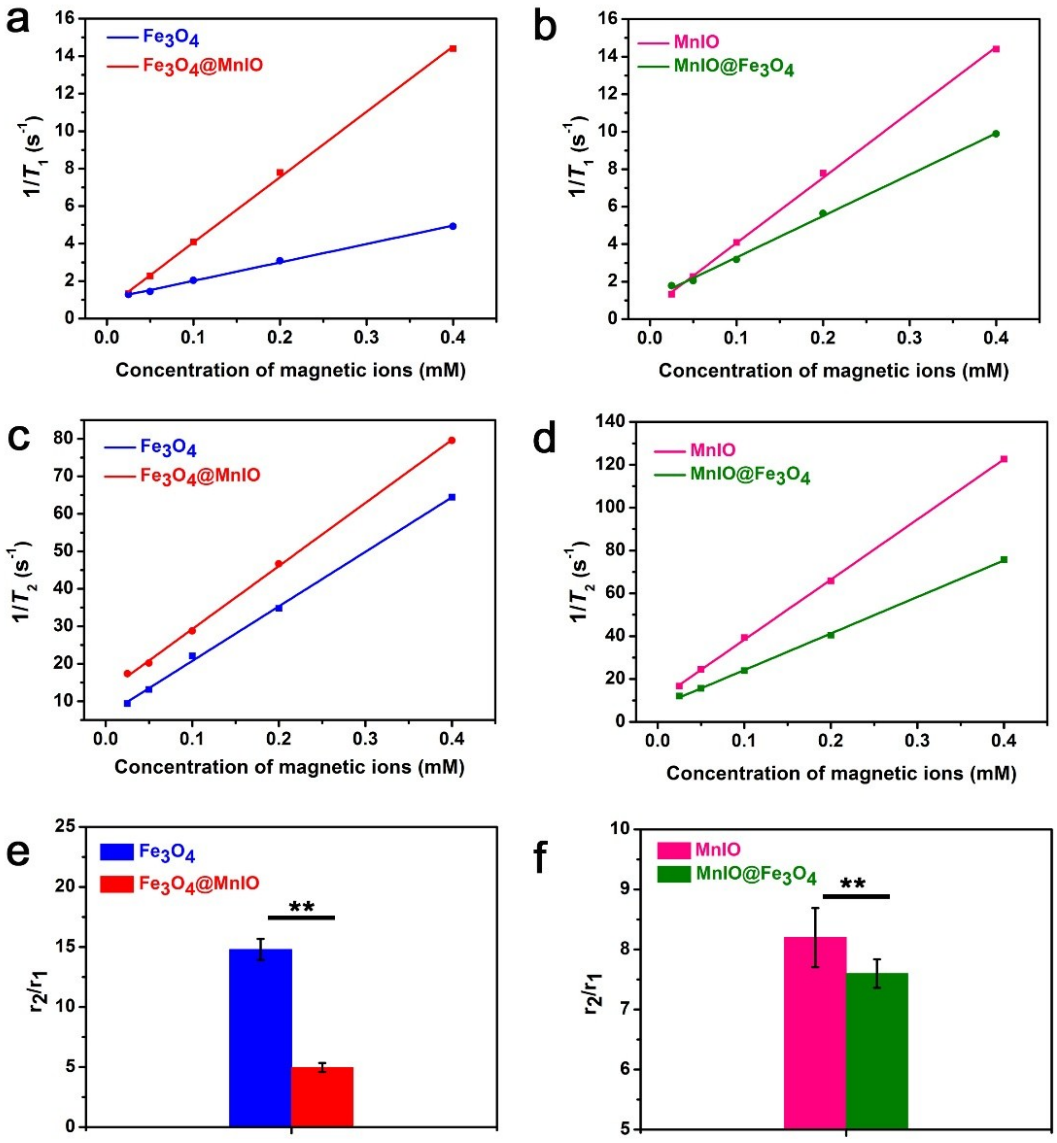

Figure S13. MR relaxivities analyses of Fe_3O_4, Fe_3O_4@MnIO, MnIO, and MnIO@Fe_3O_4 nanoparticles at 0.5 T. Relaxation rate R_1 ($1/T_1$) *vs.* magnetic ion concentration for (a) Fe_3O_4 and Fe_3O_4@MnIO nanoparticles and (b) MnIO and MnIO@Fe_3O_4 nanoparticles. Relaxation rate R_2 ($1/T_2$) *vs.* magnetic ion concentration for (c) Fe_3O_4 and Fe_3O_4@MnIO nanoparticles and (d) MnIO and MnIO@Fe_3O_4 nanoparticles. r_2/r_1 ratio analysis of (e) Fe_3O_4 and Fe_3O_4@MnIO nanoparticles, and (f) MnIO and MnIO@Fe_3O_4 nanoparticles. (**$p < 0.01$).

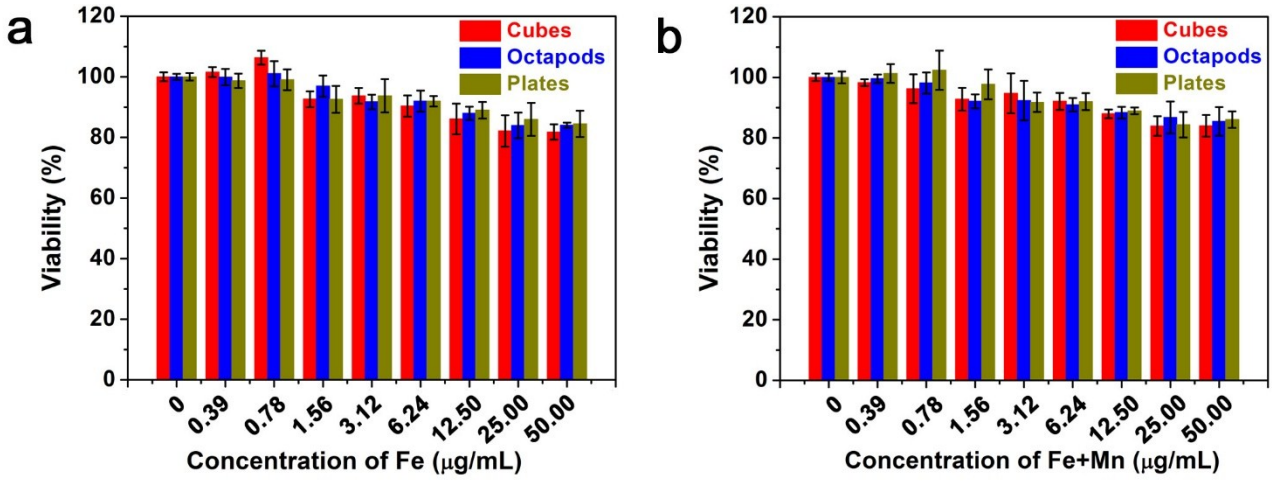

Figure S14. *In vitro* cytotoxicity analysis. MTT assay of SMMC-7721 cells incubated with (a) IO and (b) MnIO (n = 5/group).

Table S1. r_1 and r_2 values of MnIO and IO at different magnetic fields. The relaxivities are referred to the concentration of total magnetic ions. The error bars represent the standard deviation of three independent experiments.

Nanoparticles	H_0 (T)	r_2 ($mM^{-1}s^{-1}$)	r_1 ($mM^{-1}s^{-1}$)	r_2/ r_1
MnIO cubes		306.3 ± 15.2	57.8 ± 6.5	5.3
IO cubes		234.7 ± 11.3	16.3 ± 2.6	14.4
MnIO octapods	0.5	354.0 ± 14.6	62.1 ± 11.2	5.7
IO octapods		155.5 ± 8.8	14.4 ± 3.1	10.8
MnIO plates		152.3 ± 5.6	22.4 ± 2.5	6.8
IO plates		82.9 ± 3.3	11.2 ± 1.1	7.4
MnIO cubes		383.0 ± 17.1	25.2 ± 2.1	15.2
IO cubes		204.0 ± 12.3	10.7 ± 0.7	19.1
MnIO octapods	1.5	386.2 ± 16.7	28.4 ± 1.5	13.6
IO octapods		242.2 ± 11.5	10.4 ± 0.6	23.3
MnIO plates		201.6 ± 10.8	14.3 ± 1.1	14.1
IO plates		141.9 ± 9.3	8.2 ± 0.5	17.3

Table S2. Comparison of ΔH_{pp}, τ_s, and r_1 values of MnIO and IO. The relaxivities are referred to the concentration of total magnetic ions.

Nanoparticles	ΔH_{pp} (G)	τ_s (ns)	r_1 ($mM^{-1}s^{-1}$)
MnIO cubes	249.97	0.26	57.8
IO cubes	562.55	0.12	16.3
MnIO octapods	348.88	0.19	62.1
IO octapods	844.74	0.08	14.4
MnIO plates	281.46	0.23	22.4
IO plates	416.42	0.16	11.2

Table S3. r_1 and r_2 values of Fe_3O_4, Fe_3O_4@MnIO, MnIO, and MnIO@Fe_3O_4 nanoparticles at 0.5 T. The relaxivities are referred to the concentration of total magnetic ions. The error bars represent the standard deviation of three independent experiments.

Nanoparticles	r_2 ($mM^{-1}s^{-1}$)	r_1 ($mM^{-1}s^{-1}$)	r_2/ r_1
Fe_3O_4	143.1 ± 3.7	9.8 ± 1.7	14.6
Fe_3O_4@MnIO	152.1 ± 6.1	33.8 ± 3.1	4.5
MnIO	278.0 ± 9.7	33.9 ± 4.2	8.2
MnIO@Fe_3O_4	170.2 ± 5.3	22.3 ± 2.6	7.6

Table S4. MR SNR changes of liver regions after intravenous injection of MnIO or IO octapods in coronal plane (n = 3/group). We calculated the SNR changes of ROI by the equation: $\Delta SNR = |SNR_{post} - SNR_{pre}|/ SNR_{pre}$.

	MnIO octapods	IO octapods
SNR_{pre}/SNR_{pre} (%)	100	100
$SNR_{30\ min}/SNR_{pre}$ (%)	146.27 ± 8.77	129.12 ± 3.72
$\Delta SNR_{30\ min}$ (%)	46.27 ± 8.77	29.12 ± 3.72
$SNR_{1\ h}/SNR_{pre}$ (%)	173.31 ± 6.91	131.97 ± 6.06
$\Delta SNR_{1\ h}$ (%)	73.31 ± 6.91	31.97 ± 6.06
$SNR_{2\ h}/SNR_{pre}$ (%)	164.30 ± 1.64	122.43 ± 4.15
$\Delta SNR_{2\ h}$ (%)	64.30 ± 1.64	22.43 ± 4.15

Table S5. MR SNR changes of liver regions after intravenous injection of MnIO or IO octapods in transverse plane (n = 3/group). We calculated the SNR changes of ROI by the equation: $\Delta SNR = |SNR_{post} - SNR_{pre}|/ SNR_{pre}$.

	MnIO octapods	IO octapods
SNR_{pre}/SNR_{pre} (%)	100	100
$SNR_{30\ min}/SNR_{pre}$ (%)	151.10 ± 6.04	124.26 ± 4.23
$\Delta SNR_{30\ min}$ (%)	51.10 ± 6.04	24.26 ± 4.23
$SNR_{1\ h}/SNR_{pre}$ (%)	169.29 ± 7.43	129.88 ± 7.79
$\Delta SNR_{1\ h}$ (%)	69.29 ± 7.43	29.88 ± 7.79
$SNR_{2\ h}/SNR_{pre}$ (%)	161.84 ± 6.30	129.45 ± 7.62
$\Delta SNR_{2\ h}$ (%)	61.84 ± 6.30	29.45 ± 7.62