Supporting Information

In Situ ^{111}In-doping for Achieving Biocompatible and Non-leachable ^{111}In-labeled Fe_3O_4 Nanoparticles

Jianfeng Zeng,[a] Bing Jia,[b] Ruirui Qiao,[a] Chao Wang,[b] Lihong Jing,[a] Fan Wang[b] and Mingyuan Gao*[a]

[a] *Institute of Chemistry, Chinese Academy of Sciences, Bei Yi Jie 2, Zhong Guan Cun, Beijing 100190;*

[b] *Medical Isotopes Research Center, Peking University, Xueyuan Road 38, Beijing 100191.*

Experimental Section

Chemicals. Ferric acetylacetonate ($Fe(acac)_3$, 14024-18-1, 97%) and cobaltous acetylacetonate ($Co(acac)_2$, 14024-48-7, 97%) were purchased from Sigma-Aldrich and used after two recrystallizations. Diphenyl ether was used after vacuum distillation. Indium chloride ($InCl_3$, 13465-11-7, 99.9%) and oleylamine (112-90-3, ≥70%) were purchased from Sigma-Aldrich and used as received. Other chemicals of analytical grade including hydrochloric acid, nitric acid, ethanol and ether were used as received. Indium-111 carrier free radionuclide ($^{111}InCl_3$ in 0.05 M HCl, NEZ304C) was purchased from Perkin-Elmer. Cobalt-57 carrier free radionuclide ($^{57}CoCl_2$ in 0.1 M HCl) was purchased from CYCLOTRON Co., Ltd. HOOC-PEG-COOH ($M_n \approx$ 2000) was synthesized according to a previous report.[1]

Synthesis of In-doped Fe_3O_4 Nanoparticles. The In-doped Fe_3O_4 nanoparticles were synthesized according to a previously reported method with slight modifications.[2] Taking the synthesis of 1‰ In-doped Fe_3O_4 as an example, 0.53 g (1.5 mmol) of $Fe(acac)_3$, 1.79 mL (6.0 mmol) of oleylamine, and 6.0 g (3.0 mmol) of HOOC-PEG-COOH (M_n = 2000) were dissolved in 25 mL of diphenyl oxide. Then, 1 mL of 0.05 M HCl containing 0.33 mg of $InCl_3$ was introduced under stirring. After that, the trace water in the system was removed by keeping the reaction system under vacuum at 100 °C for 40 min. Under atmospheric pressure, the finally formed reaction mixture was mechanically stirred and refluxed for 30 min under the protection of nitrogen. Then, the reaction system was cooled to room temperature. Upon addition of ether into the reaction mixture, the resultant In-doped Fe_3O_4 nanoparticles were

precipitated and isolated by a permanent magnet. By being redispersed in ethanol and subsequently precipitated with ether for three cycles, the nanoparticles were purified and dissolved in Milli-Q water for further experiments.

To show the impact of indium doping ratio on the size, size distribution, and crystal structure of the resultant nanoparticles, in the following preparations, the feeding molar ratios of In:Fe was tuned between 1:1,000 and 1:2, while the feeding concentration of Fe and other experiment variables remained unchanged.

Synthesis of ^{111}In-Doped Fe_3O_4 Nanoparticles. Following the aforementioned procedures for synthesizing nonradioactive In-doped Fe_3O_4 nanoparticles, ^{111}In-doped Fe_3O_4 nanoparticles were prepared by using 0.33 mg $InCl_3$ containing ~10 mCi $^{111}InCl_3$. The resultant nanoparticles were purified and isolated according to the procedures mentioned above, and then dialyzed against Milli-Q water for 8 h to remove the possible impurities.

Synthesis of Co-doped Fe_3O_4 Nanoparticles. Typically, 0.53 g (1.5 mmol) of $Fe(acac)_3$, 0.19 g (0.75 mmol) of $Co(acac)_2$, 1.79 mL (6.0 mmol) of oleylamine, and 6.0 g (3.0 mmol) of HOOC-PEG-COOH (M_n = 2000) were dissolved in 25 mL of diphenyl oxide. After being purged with nitrogen for 30 min, the reaction mixture was mechanically stirred and refluxed for 8 h under the protection of nitrogen. Then, the reaction system was cooled to room temperature. Upon addition of ether into the reaction mixture, the resultant Co-doped Fe_3O_4 nanoparticles were precipitated and isolated by a permanent magnet. By being redispersed in ethanol and subsequently precipitated with ether for three cycles, the nanoparticles were purified and dissolved in Milli-Q water for further experiments.

Synthesis of ^{57}Co-Doped Fe_3O_4 Nanoparticles. ^{57}Co-doped Fe_3O_4 nanoparticles were prepared according to the procedures mentioned above for synthesizing Co-doped Fe_3O_4 nanoparticles except that 90 μL of $^{57}CoCl_2$ in 0.1 M HCl (~ 0.38 mCi) was introduced prior to refluxing process. The trace water was removed by keeping the reaction system under vacuum at 100 °C for 40 min. Then, following the synthesizing and purifying procedures for Co-doped Fe_3O_4 nanoparticles, ^{57}Co-doped Fe_3O_4 nanoparticles were obtained.

Characterization. Transmission electron microscope (TEM) images of the nanoparticles were taken on a JEM-100CXII electron microscope at an acceleration voltage of 100 kV. The particle size was determined by averaging at least 300 particles per sample. Powder X-ray diffraction (XRD) pattern of the particle samples was recorded on a Regaku D/Max-2500 diffractometer under Cu Kα_1 radiation (λ = 1.54056 Å). Dynamic light scattering (DLS) measurements were carried out at 298.0 K with a Nano ZS (Malvern) equipped with a solid-state He-Ne laser (λ = 633 nm) for measuring the hydrodynamic size of the resultant nanoparticles. The concentration of the metal elements in different systems was determined by using inductively coupled plasma atomic emission spectrometer (ICP-AES, Jiangsu Skyray instrument Co., Ltd.) or inductively coupled plasma mass spectrometer (ICP-MS, Series X7, Thermo Electron). The ^{111}In-doped Fe_3O_4 nanoparticles were analyzed after the resultant samples decayed for 400 d.

Colloidal Stability of In-doped Fe_3O_4 Nanoparticles. The colloidal stability of the prepared In-doped Fe_3O_4 nanoparticles in aqueous mediums was studied by DLS method. The hydrodynamic sizes of In-doped Fe_3O_4 nanoparticles in both water and phosphate buffer saline (PBS) were monitored for one month. In addition, the temperature-dependent DLS experiments were also performed. In detail, a water solution of the In-doped Fe_3O_4 nanoparticles was heated from 20 to 80 °C by 5 °C intervals. Equilibration time of 5 min was adopted before the DLS measurements at each temperature point during the temperature increase. After the measurements performed at 80 °C were finished, the solution was cooled down to 20 °C, finishing one cycle. The whole measurements were repeated for five cycles in total.

Leaching Properties of In-doped and Co-doped Fe_3O_4 Nanoparticles in Milli-Q Water. To study the dopant leaching properties, In-doped and Co-doped Fe_3O_4 nanoparticles were dialyzed against Milli-Q water using dialysis bags with a cut-off molecular weight of 8-12 kDa. The concentrations of the released indium and cobalt ions were determined by ICP-MS and ICP-AES, respectively.

Leaching Properties of ^{111}In-Doped Fe_3O_4 Nanoparticles under Different pH. Five identical solutions of ^{111}In-doped Fe_3O_4 particles were prepared. By using

hydrochloric acid, their pH values were adjusted to 0.5, 1, 2, 3, or 5, respectively. For comparison, two solutions containing the same amount of particles in Milli-Q water and 1×PBS were also prepared. All solutions were incubated at room temperature for 125 h. Aliquots were extracted at 1 min, 10 min, 90 min, 18 h, 66 h, and 125 h for monitoring the release of ^{111}In by the instant thin-layer chromatography (ITLC) method. The eluent solution was 10 mM EDTA (ethylene diamine tetraacetic acid). The signal of ^{111}In was determined by using a radio-thin layer scanner (Bioscan AR2000, Washington, DC).

Leaching Properties of ^{111}In-Doped Fe_3O_4 Nanoparticles in Biological Media. The ^{111}In-doped Fe_3O_4 particles were dissolved in saline and fetal bovine serum (FBS), respectively, by 0.4 mg/mL. After being incubated for 24 h, the released ^{111}In was collected by ultrafiltration using 100 kDa MWCO centrifugal filter (Millipore YM-100). The radioactivities of the residue remaining in the filter and the filtrate were determined by a gamma counter (Wallac 1470-002, Perkin-Elmer). The integration time was 30 s. After the above measurements, the nanoparticle residue was redispersed in the corresponding media for the second round of incubation for 24 h, which was followed by the radioactivity measurements as mentioned above.

Biodistribution. For the biodistribution study, male Kunming mice (~ 6-week old) weighing approximately 20 g were anesthetized by intraperitoneal injection of sodium pentobarbital at a dose of 45.0 mg/kg. Then, the ^{111}In-doped Fe_3O_4 (~ 16 μCi per mouse, corresponding to a dose of 10 mg Fe/kg body weight) was intravenously injected into the tail vein. Mice (n = 5 per time point) were sacrificed by cervical dislocation at 10 min, 12 h, 24 h, 4 d, 6 d, 14 d, and 22 d postinjection, respectively. Tissues and organs of interest were harvested, weighed, and assessed for radioactivity by gamma counter. In parallel, the radioactivity of particles with the same radioactive dose serving as reference was measured at the same time points for calculating the biodistribution of the particles in different organs and tissues.

Biological Half-life. Mice (n = 5) were intravenously injected with ^{111}In-doped Fe_3O_4 nanoparticles, the injection dose was the same as that used for the biodistribution study. Then, each mouse was scanned at different time points

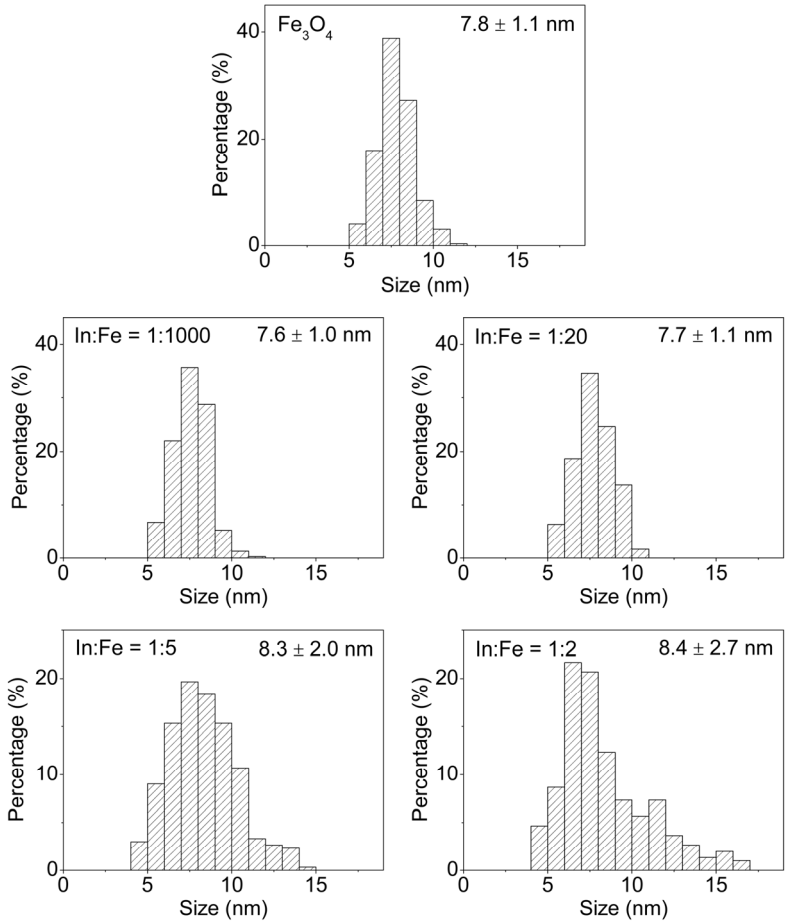

Fig. S1. Size distributions of Fe_3O_4 and In-doped Fe_3O_4 nanoparticles obtained by different In:Fe feeding ratios shown in Fig. 1.

post injection by using the radio-thin layer scanner. The integration time was 5 min. The effective half-life of ^{111}In-doped Fe_3O_4 nanoparticles in mice was obtained by fitting the experiment curves using the following equation

$$\frac{C_t}{C_0} = \left(\frac{1}{2}\right)^{t/T_e} \qquad \text{(S1)}$$

where C_0 and C_t are the counts detected at time point of 0 and t, respectively, T_e is the effective half-life.

The biological half-life of ^{111}In-doped Fe_3O_4 nanoparticles in mice was calculated by the following equation

$$T_b = \frac{T_p \times T_e}{T_p - T_e} \qquad \text{(S2)}$$

where T_b and T_p represent the biological half-life and the half-life of radioactive decay

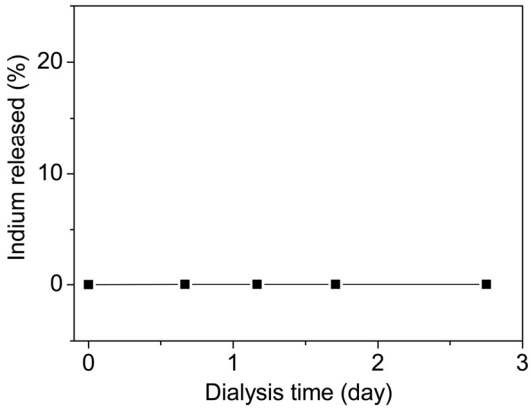

Fig. S2. Temporal In release of 1‰ In-doped Fe_3O_4 nanoparticles in Milli-Q water.

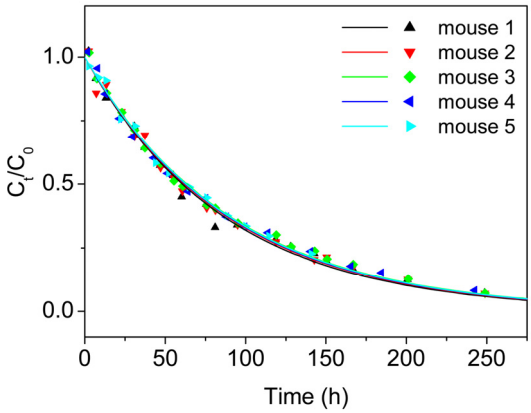

Fig. S3. The normalized whole body radioactivity counts against postinjection time after the administration of ^{111}In-doped Fe_3O_4 nanoparticles, overlaid with theoretical fitting curves based on single exponential decay.

of ^{111}In-doped Fe_3O_4 nanoparticles, respectively.

All animal experiments reported herein were carried out according to a protocol approved by Peking University Institutional Animal Care and Use Committee.

Supplementary Results

Fig. S1 shows the size distributions of In-doped Fe_3O_4 nanoparticles prepared by different In:Fe feeding ratios such as 1:1000, 1:20, 1:5, and 1:2. The Fe_3O_4 particles prepared in the absence of In was used as reference.

Fig. S2 shows the release of indium when 1‰ In-doped Fe_3O_4 nanoparticles were dialyzed against Milli-Q water. The amount of released In was determined by ICP-MS.

Fig. S3 shows the experimental data of the whole body radioactive counts against

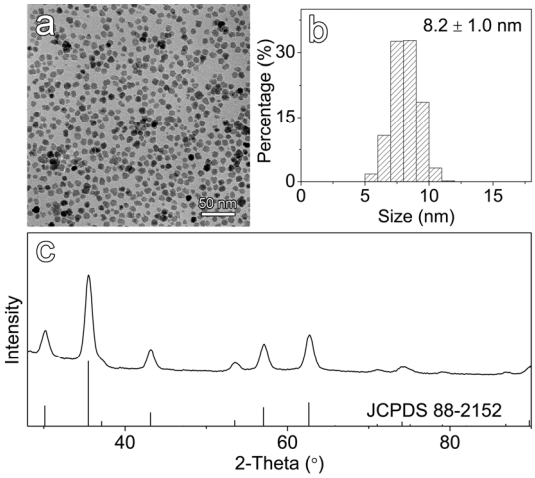

Fig. S4. TEM image (a), size distribution (b), and X-ray diffraction pattern (c) of the Co-doped Fe_3O_4 nanoparticles prepared by Co:Fe feeding ratio of 1:2. The JCPDS card data of $CoFe_2O_4$ are shown at the bottom of frame c.

post injection time. By fitting the experimental data using Equation S1, the effective half-life of ^{111}In-doped Fe_3O_4 nanoparticles in mice was determined to be 62.4 ± 1.0 h. Taking 67.3 h as the half-life of the radioactive decay of ^{111}In,[3] the biological half-life of the resultant ^{111}In-doped Fe_3O_4 nanoparticles in mice was estimated to be 33 days based on Equation S2.

Fig. S4 presents the TEM and XRD results of the Co-doped Fe_3O_4 nanoparticles.

References

1. Z. Li, L. Wei, M. Y. Gao and H. Lei, *Adv. Mater.*, 2005, **17**, 1001.

2. Q. Jia, J. Zeng, R. Qiao, L. Jing, L. Peng, F. Gu and M. Gao, *J. Am. Chem. Soc.*, 2011, **133**, 19512.