Contents

Page S2-S4: Experimental Section.

Page S5-S11: Supplementary Tables and Figures.

Experimental Section

1. Materials

Iron chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, >99%) was purchased from Acros. Sodium hydroxide (NaOH), oleic acid (NF/FCC), and hydrochloric acid (HCl) were purchased from Fisher Scientific. Poly(acrylic acid), 1-octadece, tetraethylorthosilicate (TEOS), Igepal CO-520 (NP-5), ammonia (30%), copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$), sodium citrate dihydrate, sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$), germanium (IV) oxide (99.999%), copper (I) chloride (≥99.995%), sodium tartrate dibasic dihydrate, and cyclohexane (≥99.9%) were purchased from Sigma-Aldrich (St. Louis, MO). Hydrogen peroxide was purchased from Fluka Analytical. Diethylene glycol (DEG, 99%) was purchased from Alfa Aesar. PD-10 columns were purchased from GE Healthcare (Piscataway, NJ). Mal-PEG_{5k}-NH_2 was obtained from Creative PEGworks. High Q water was used throughout the research. All chemicals were used as received without further purification.

2. Generation of radioarsenic

The radioarsenic was produced by irradiating natural germanium oxide with 1.5-3 μA of protons from the University of Wisconsin GE PETtrace Cyclotron. Targets of 185 ± 10 mg GeO_2 powder were pressed at 150 MPa into a water-cooled stainless steel beam stop and covered with a 0.025 mm HAVAR containment foil. After irradiation and removal from the cyclotron, a polytetrafluoroethylene dissolution apparatus was fit over the irradiated target, allowing for the dissolution of the irradiated powder directly from the beam stop. Irradiated targets were dissolved in 2 mL of 4 M sodium hydroxide and bulk germanium over night to allow complete dissolution and radioactive decay of relatively short-lived ^{70}As. The next morning, the dissolved solution was transferred to a fritted column and bulk germanium was reprecipitated through neutralization with the addition of 630 μL of 12.1 M HCl. After filtration, the germanium precipitate was washed with 1 mL of 12.1 M HCl and the filtrates containing *As (*=71, 72, 74, 76), ^{67}Ga, and trace germanium were evaporated at 120 °C under flowing Ar with periodic additions of 100 μL of 30% H_2O_2 to maintain the *As(V) oxidation state. The residue was then reconstituted in 1 mL of 12.1 M HCl and loaded onto a ~0.7 mL BioRad AG1x8 anion exchange column that had been preconditioned with 10 M HCl. After eluting the *As(V) with 10 M HCl, the ^{67}Ga and trace germanium was eluted with 1 M HCl. The *As(V) eluant (2.9 ± 0.5 mL) was then reduced to *As(III) with the addition of 95 ± 7 mg of copper (I) chloride and the resulting arsenic trichloride was extracted twice into an equivalent volume of cyclohexane. The combined organic fractions were then contacted with 1 mL of 18 MOhm Milli-Q water, resulting in a nearly quantitative back extraction of $*As(OH)_3$.

3. Synthesis of superparamagnetic iron oxide nanoparticle (SPION)

Synthesis of iron-oleate complex. Iron-oleate complex was used as the precursor for the synthesis of SPION. $FeCl_3 \cdot 6H_2O$ (3.243 g, 12 mmol) and NaOH (1.44 g, 36 mmol) was each dissolved in methanol (40 mL) under magnetic stirring. Oleic acid (12 mL, 36 mmol) was added into the $FeCl_3$-methanol solution, followed by addition of NaOH-methanol solution using a separatory funnel. The mixture was stirred overnight at room temperature. Reddish-brown product could be observed at the bottom of the flask in the next morning. The product was washed with methanol twice, and then washed with deionized water twice before drying at room temperature for 48 h. The final iron-oleate complex was in a waxy solid form.

Synthesis of SPION. SPION was synthesized following the previously reported procedure with slight modifications.[1] In a typical experiment for ~10 nm sized SPION, pre-prepared iron-oleate (2.9 g, ~3 mmol) was dissolved in 1-octadece (40 mL). No extra oleic acid was used. The mixture was first heated to 80 ºC to accelerate the dissolution of solid iron-oleate, and then heated to 120 ºC and maintained at this temperature for 2 h to remove air and water from the

system. The reaction mixture was then directly heated to 310-320 ºC and kept at this temperature for 30 min. The black-brown mixture was then cooled down to room temperature, washed with hexane and ethanol, and collected with magnetic separation. The final product could be well-dispersed in cyclohexane.

4. Poly(acrylic acid) (PAA) modification of SPION

The PAA modification of SPION was achieved by following the reported procedure with slight modifications.[2] PAA (560 mg) was added in DEG solution (20 mL) and heated to 110 ºC with vigorous stirring. No nitrogen flow was used in our case. A chloroform solution of oleic acid capped SPION (3 mL) was then injected into the hot solution. The mixture was kept at 110 ºC for 2 h. Afterwards, the system was further heated to 240 ºC and kept at this temperature for another 3 h until the solution became clear. As-synthesized SPION@PAA nanoparticles were washed with ethanol and cyclohexane, and collected by magnetic separation. The final sample could be well-dispersed in water (pH 7-8).

5. Coating of SPION with a layer of dense silica

The synthesis of SPION@dSiO_2 was carried out by a base-catalyzed silica layer formation from TEOS on as-prepared SPION in a water-in-oil reverse microemulsion.[1] Igepal CO-520 (NP-5, 2 mL) was dispersed in cyclohexane (40 mL) in a 100 mL three-necked flask and stirred for 30 min. Subsequently, 0.5 mL of oleic acid capped SPION-cyclohexane solution was injected into the cyclohexane/NP-5 mixture. The mixture was stirred at room temperature (22 ºC) for 2 h. Afterwards, ammonia (0.28 mL, 30%) was added, and the system was sealed and stirred for another 2 h. TEOS (250 µL) was delivered into the system at a precisely controlled rate of 200 µL/h using a Syringe Pump. The mixture was sealed and kept under magnetic stirring for 17 h at room temperature before addition of methanol to precipitate the nanoparticles. The as-synthesized SPION@dSiO_2 nanoparticles were washed with ethanol for more than 3 times to remove excess NP-5. The resulted SPION@dSiO_2 could be well-dispersed in water.

6. Synthesis of citrate capped CuS nanoparticles

The general procedures for the synthesis of citrate capped CuS nanoparticles were similar as reported by Zhou et al.[3] In a typical synthesis of CuS nanoparticles, 10 mL of $CuCl_2$ (0.8524 mg/mL) and 10 mL of sodium citrate (1 mg/mL) were added into 30 mL of 18 MOhm Milli-Q water. The mixture was stirred at room temperature for 30 min. Fifty µL of Na_2S (1 mmol/mL) was then added into the mixture and stirred for 5 min before reacting at 90 ºC in a water bath for 15 min. Citrate capped CuS with green color could be obtained and directly used for radioarsenic labeling.

7. Synthesis of *As-SPION@PEG

500 µL of SPION@PAA (3.1 mM Fe, dispersed in 1 mM sodium hydroxide solution, pH 7-8) was mixed with 150 µL (~6 MBq) of *As in an eppendorf tube. The mixture was kept under constant shaking at 500 rpm for 2 h at 37 ºC. Afterwards, suitable amount of EDC/S-NHS (62.5 µL, 2 mg/mL) was added and activated for 15 min at pH 4-5. Two mg of NH_2-PEG_{5k}-Mal and Na_2CO_3 (2 µL, 0.1 M) were then added and reacted at room temperature for 2 h (pH 7-8). To purify and collect *As-SPION (with either PAA or PEG at the surface) for further studies, PD-10 column was used to remove the free *As from *As-SPION. The *As-SPION was subsequently concentrated by using a centrifugal filter (molecular weight cut off: 10 kDa), and re-dispersed in HEPES buffer (pH 7.5, 0.25 mM) before injection into mice.

8. Serum stability studies

For serum stability studies, *As-SPION@PAA and *As-SPION@PEG were incubated in complete mouse serum at 37 ºC for up to 24 h (the time period investigated for serial PET imaging). Portions of the mixture were sampled at different time points and filtered through 100

kDa cutoff filters. The filtrates were collected, and the radioactivity was measured. The percentages of retained (i.e., intact) *As on the SPION@PAA (or SPION@PEG) were calculated using the equation (total radioactivity - radioactivity in filtrate)/total radioactivity. Without PEGylation, over 75% of *As was found to desorb from SPION@PAA at 24 h post-treatment, whereas only ~45% of *As desorbed from SPION@PEG (i.e., ~55% of *As remained on the SPION@PEG surface) under the same experimental condition.

9. In vivo PET imaging and biodistribution studies

All animal studies were conducted under a protocol approved by the University of Wisconsin Institutional Animal Care and Use Committee. Data acquisition, image re-construction, and region-of-interest (ROI) analysis of the PET data were performed similar as described previously.[4] 200 μL of *As-SPION@PEG (~5.5 MBq) was intravenously injected into normal BALB/c mice (n = 4) to investigate the biodistribution of *As-SPION@PEG in vivo. After the last PET scans at 20 h post-injection, biodistribution studies were carried out to confirm that the %ID/g values based on PET imaging truly represented the radioactivity distribution in mice. For in vivo lymph node mapping with PET, 40 uL of *As-SPION@PEG (~3 MBq) was subcutaneously injected into the right footpad of mouse. The time points of 2.5 and 15 h post-injection were chosen for serial PET scans.

10. In vivo MRI

All MRI experiments were performed using a 4.7 T small animal scanner (Agilent Technologies, Santa Clara, CA). In vivo imaging was carried out before contrast agent administration and at several time points post-injection of 200 μL (or 40 μL) of SPION@PAA (7.77 mM Fe) in PBS (pH=7.4) solution for liver imaging or lymph node mapping. To detect SPION accumulation, in vivo T_2*-weighted images were collected using a multi-slice gradient echo sequence with the following parameters: TR = 500 ms; TE = 12 ms; flip angle = 20°; FOV = 40×40 mm^2; matrix 256×256; NEX = 8; slice thickness = 1 mm for axial liver images and 0.5 mm for coronal lymph node mapping.

Supplementary Tables and Figures

Table S1. Half-life and emission properties of different arsenic radionuclides.

Radionuclide	Emission	$t_{1/2}$	E_{max} of β^+ (MeV)	E_{max} of β^- (MeV)	β^+ (%)	β^- (%)
^{70}As	β^+, γ	52.6 m	3.04	-	91	-
^{71}As	β^+, γ	2.72 d	0.81	-	28.3	-
^{72}As	β^+, γ	26.0 h	3.34	-	87.8	-
^{74}As	β^+, β^-, γ	17.8 d	1.36	1.54	29	34
^{76}As	β^-, γ	1.08 d	-	2.96	-	100
^{77}As	β^-, γ	38.8 h	-	0.68	-	100

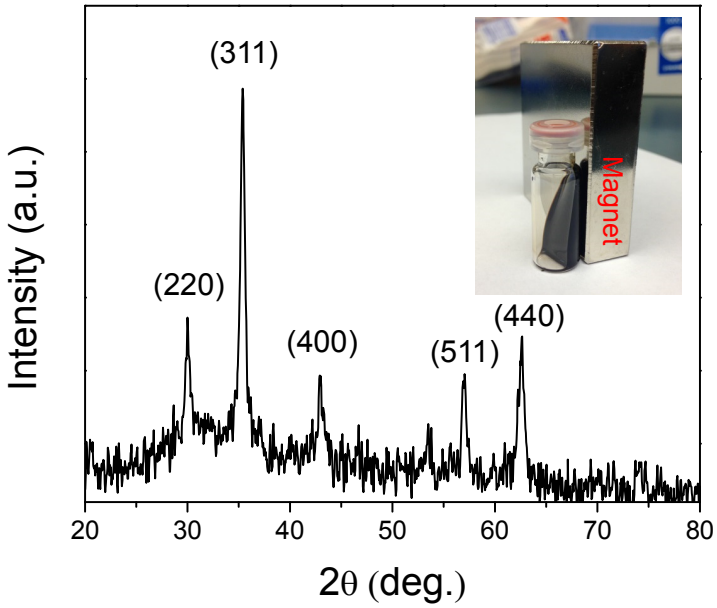

Figure S1. The X-ray diffraction pattern of as-synthesized SPION. Inset shows the ferrofluidic behavior of SPION in cyclohexane at room temperature under a magnetic field.

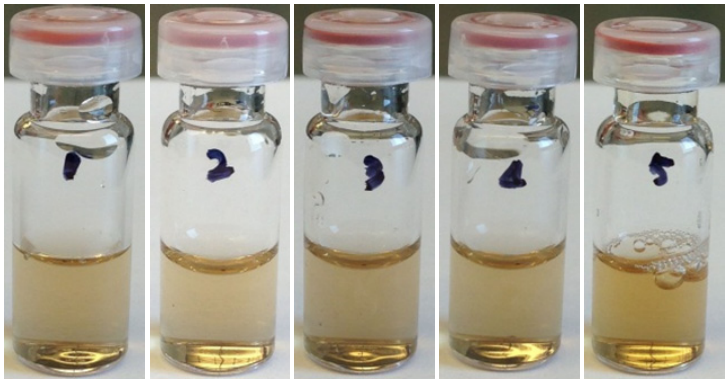

Figure S2. The stability of SPION@PAA nanoparticles in different solutions. (1) High Q water; (2) Phosphate-buffered saline (PBS); (3) Saline (0.9 % NaCl solution); (4) DMEM medium; (5) Fetal bovine serum (FBS). No obvious aggregations was observed within 6 months.

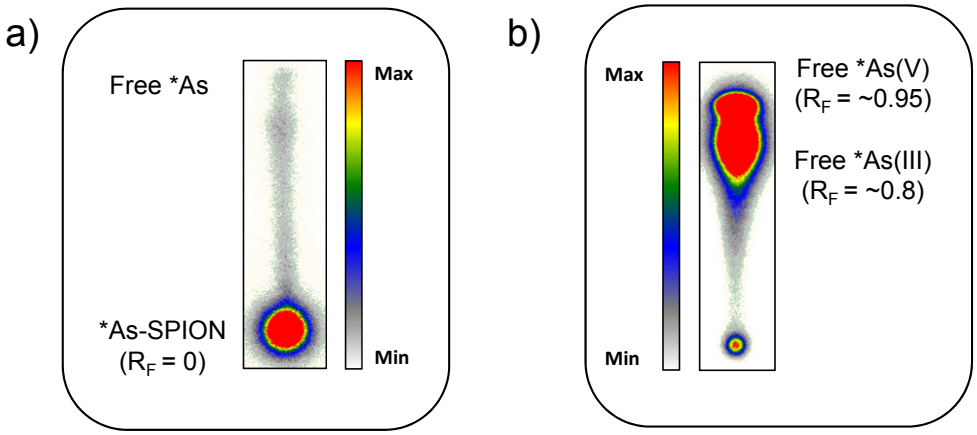

Figure S3. Autoradiograph of TLC plates. (a) *As-SPION nanoparticles after purification with PD-10 column. The vast majority of radioactivity came from *As-SPION (R_F = 0). (b) Mixture of *As(III) and *As(V) with R_F of ~0.8 and ~0.95, respectively.

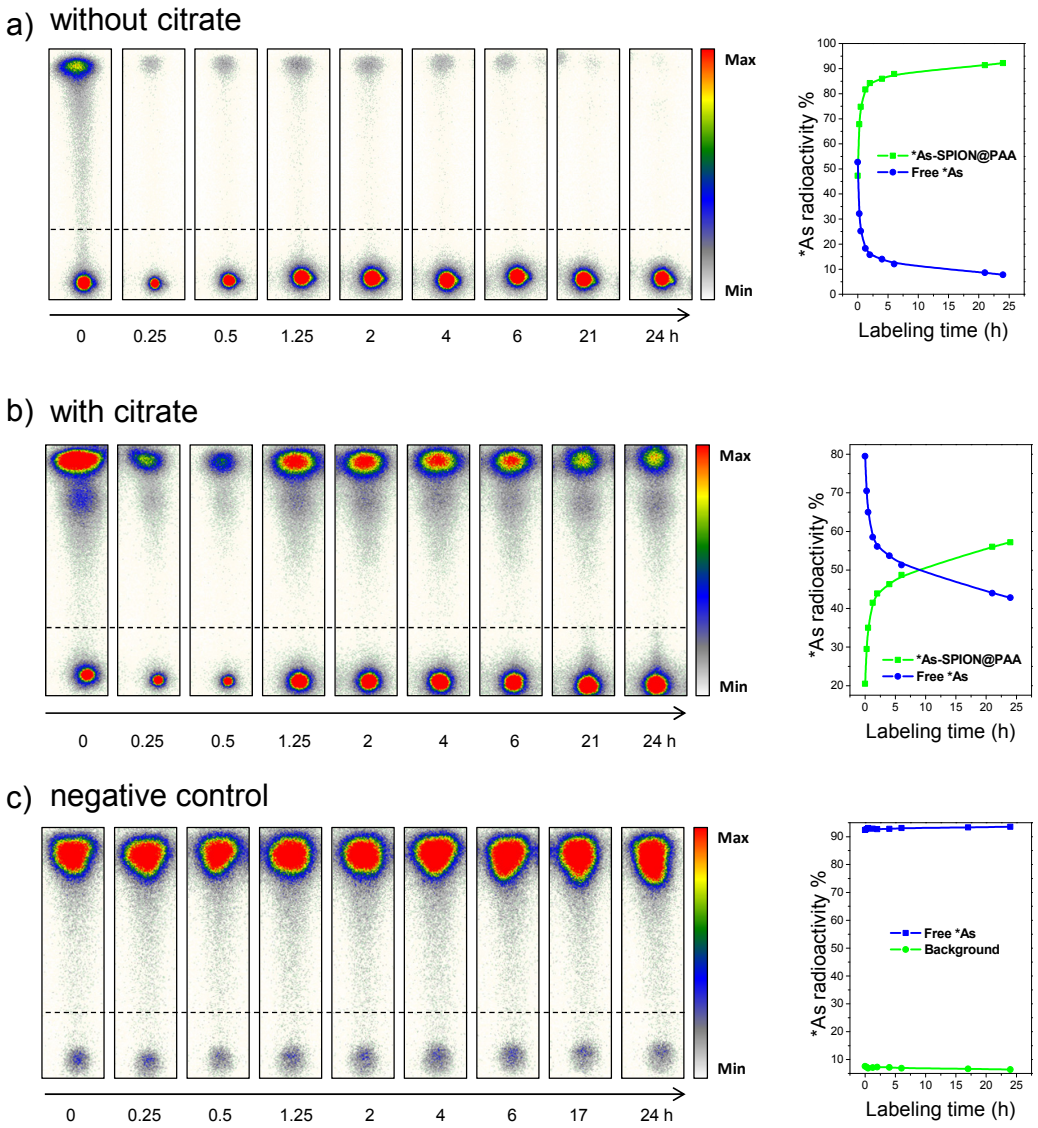

Figure S4. Autoradiograph of TLC plates (left) and corresponding radioactivity measurement of *As-SPION@PAA and free *As (right) of different samples. (a) SPION@PAA with *As but no citrate; (b) SPION@PAA with *As and sodium citrate (17 mM). (c) Negative control without any SPION@PAA or sodium citrate. 3:1 0.01 M sodium tartrate/methanol solution was used as the mobile phase.

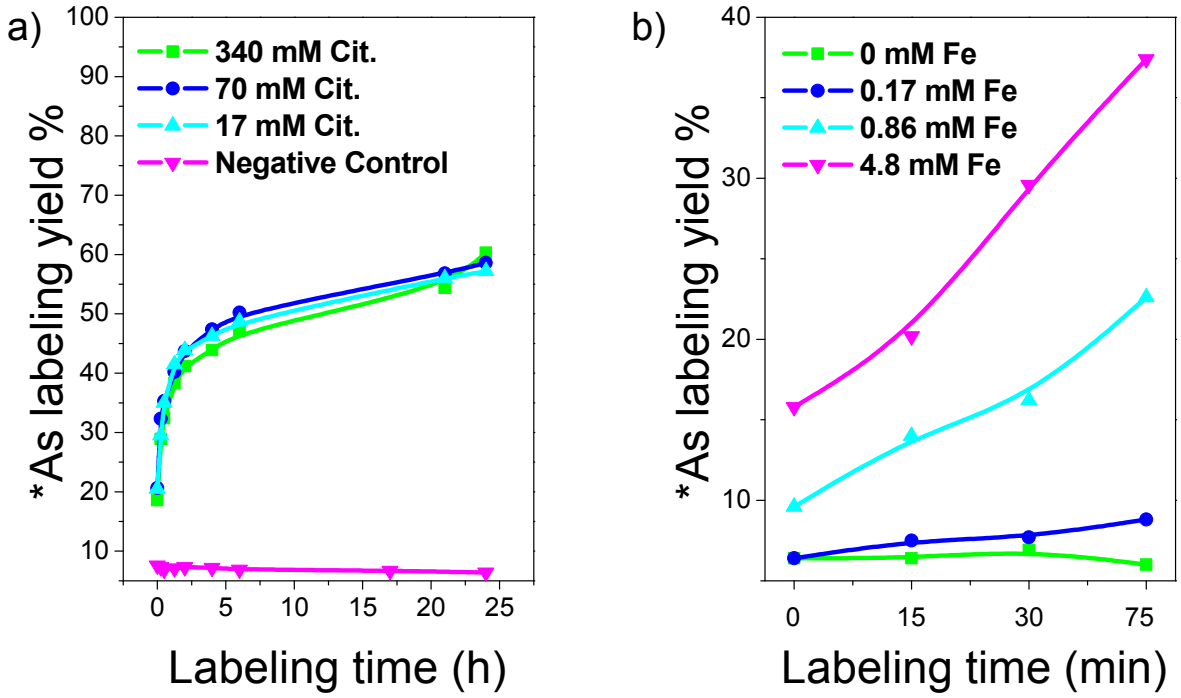

Figure S5. (a) Time-dependent *As-labeling yield of *As-SPION in the presence of sodium citrate (Cit.) at various concentrations ranging from 17 to 340 mM. A group without SPION@PAA was also used as a negative control. (b) Time-dependent *As-labeling yield of *As-SPION with varied Fe concentrations, from 0 to 4.8 mM. The labeling experiments were performed with the presence of sodium citrate (170 mM).

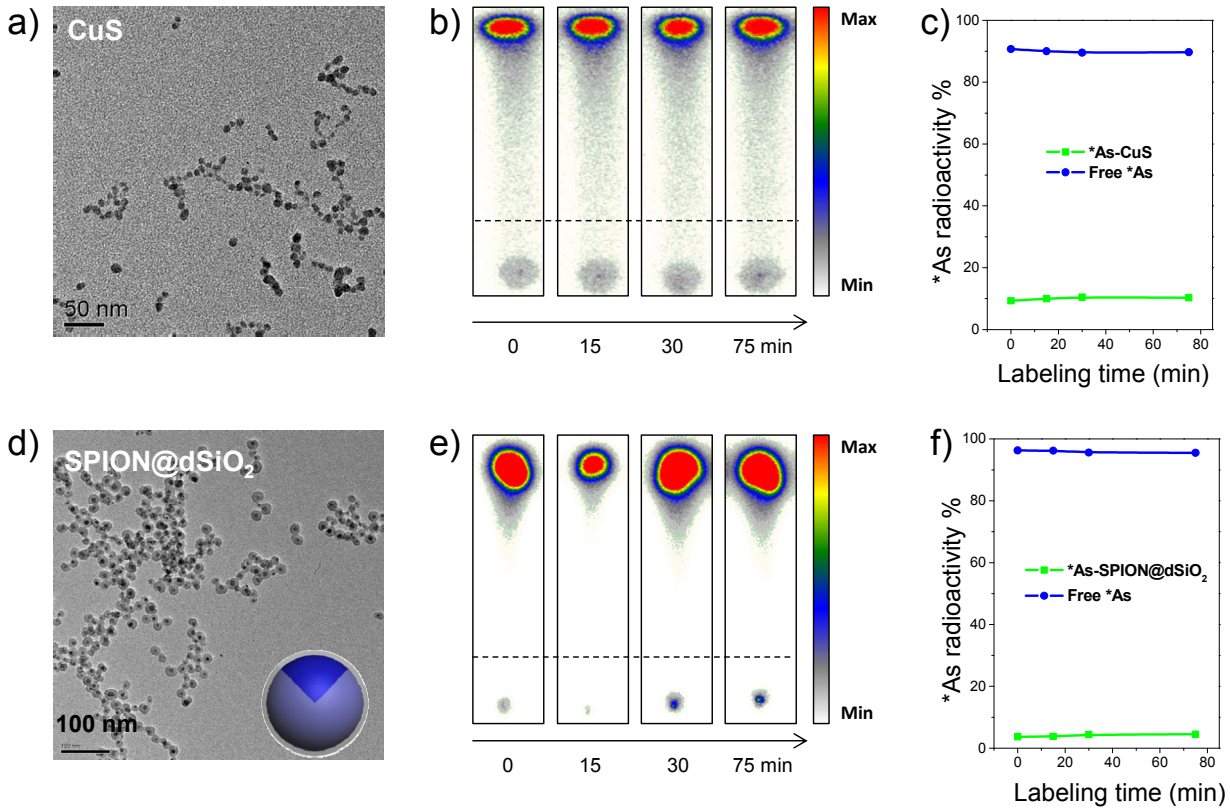

Figure S6. (a) TEM image of citrate capped CuS nanoparticles. (b) Autoradiograph of TLC plates and (c) corresponding radioactivity measurement of *As-CuS and free *As. (d) TEM image of SPION@dSiO$_2$ nanoparticles. (e) Autoradiograph of TLC plates and (f) corresponding radioactivity measurement of *As- SPION@dSiO$_2$ and free *As.

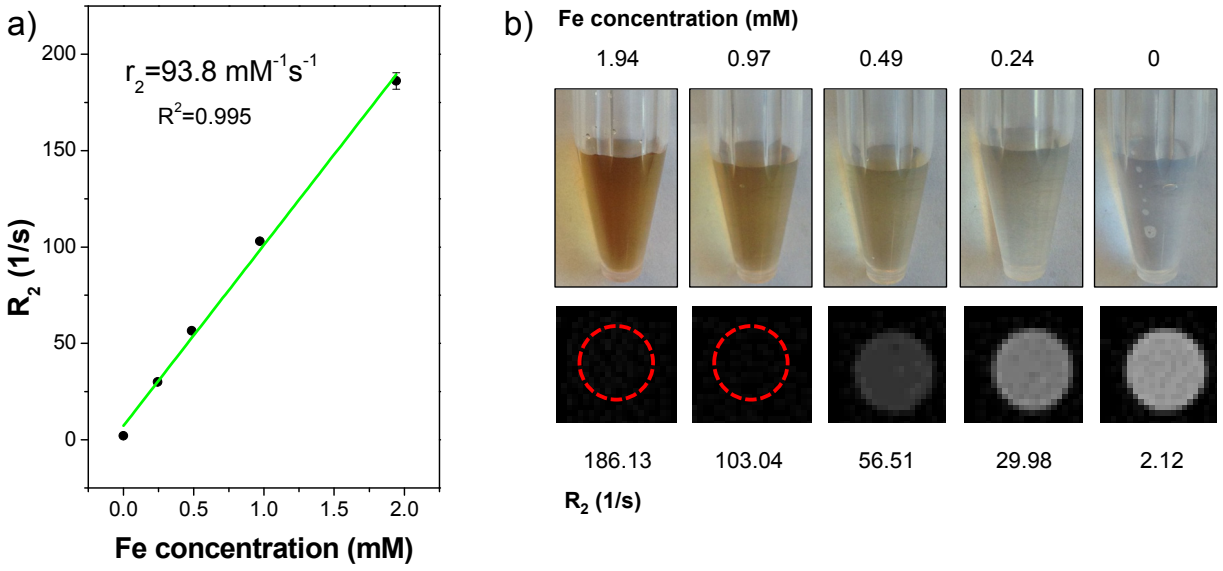

Figure S7. (a) Plot of inverse transverse relaxation times (R_2) versus Fe concentration of water soluble SPION@PAA. The slope indicates the T_2 relaxivity (r_2). (b) Digital photos of SPION@PAA with varied Fe concentrations and corresponding T_2*-weighted MR images and R_2 values.

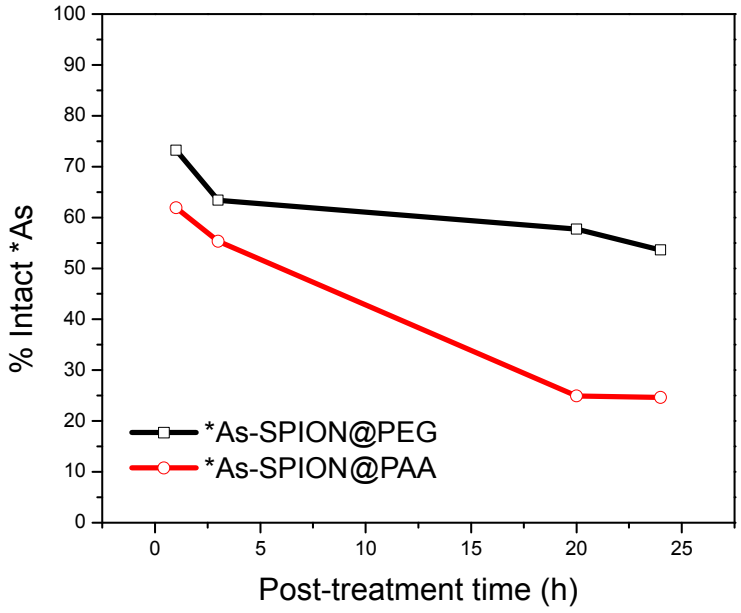

Figure S8. Serum stability of *As-SPION@PAA and *As-SPION@PEG. Samples were incubated in complete mouse serum at 37 ºC for 24 h.

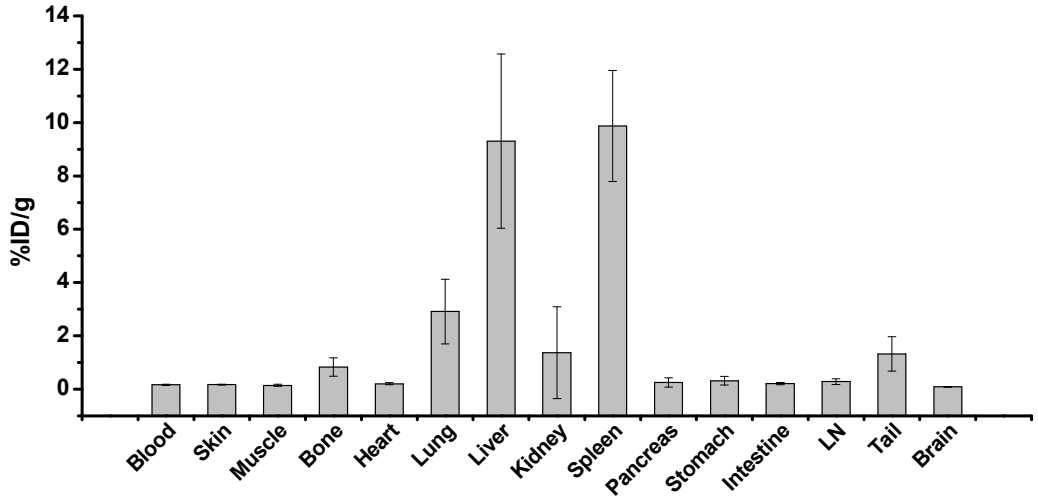

Figure S9. Biodistribution of *As-SPION@PEG at 20 h after intravenous injection in normal BALB/c mice (n = 4).

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[1] F. Chen, W. B. Bu, Y. Chen, Y. C. Fan, Q. J. He, M. Zhu, X. H. Liu, L. P. Zhou, S. J. Zhang, W. J. Peng, J. L. Shi, *Chem. Asian. J.* **2009**, *4*, 1809-1816.

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