
Supplementary information

High-resolution T_1 MRI via renally clearable dextran nanoparticles with an iron oxide shell

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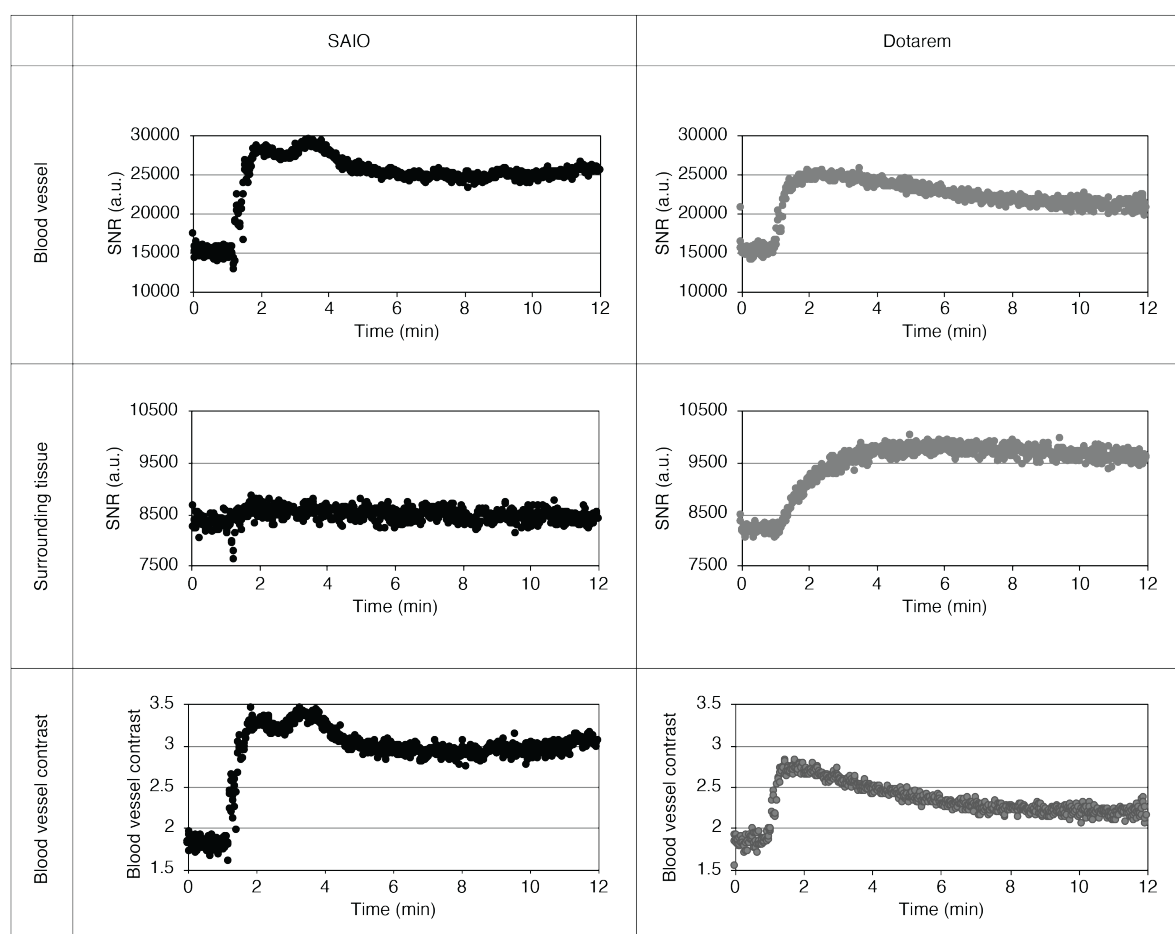
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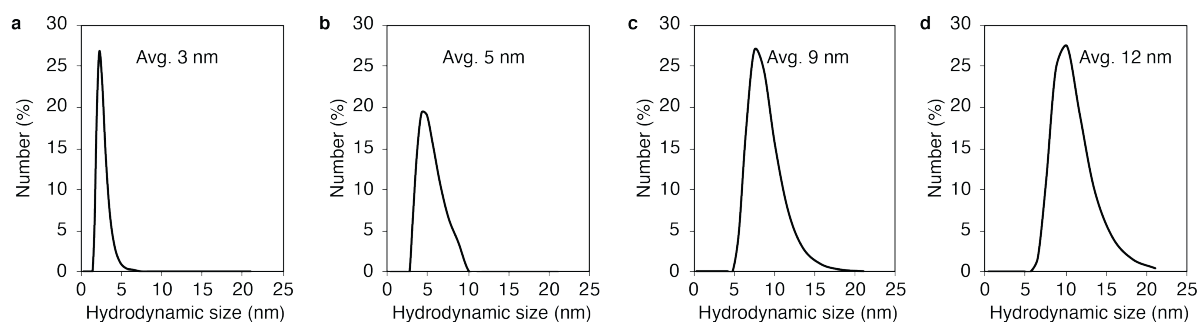
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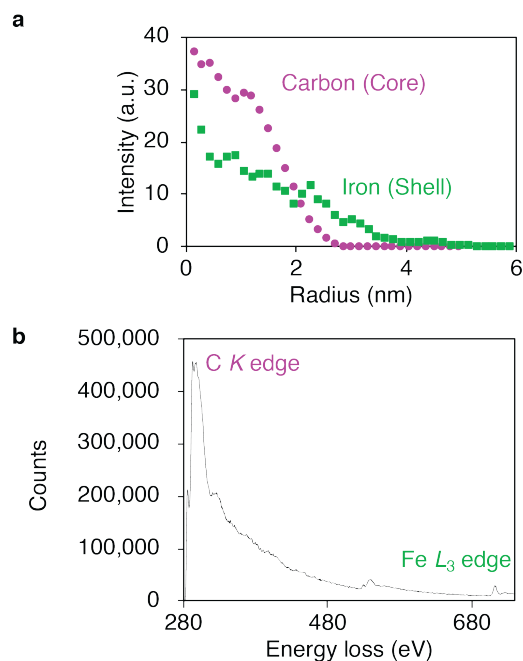
Supplementary Figures



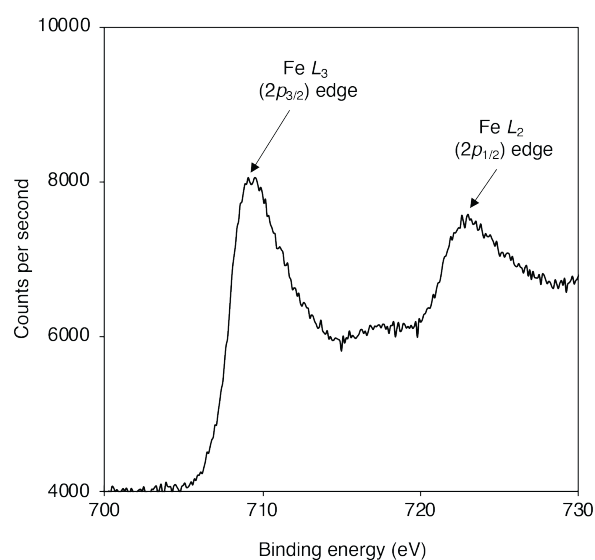
Supplementary Figure 1 | Signal-to-noise ratio (SNR) versus time plots of SAIO- and Dotarem-injected rats. SNR of the blood vessel (top row) and surrounding tissue (middle row). Dividing the SNR of blood vessel by that of the surrounding tissue gives the blood vessel contrast (bottom row).



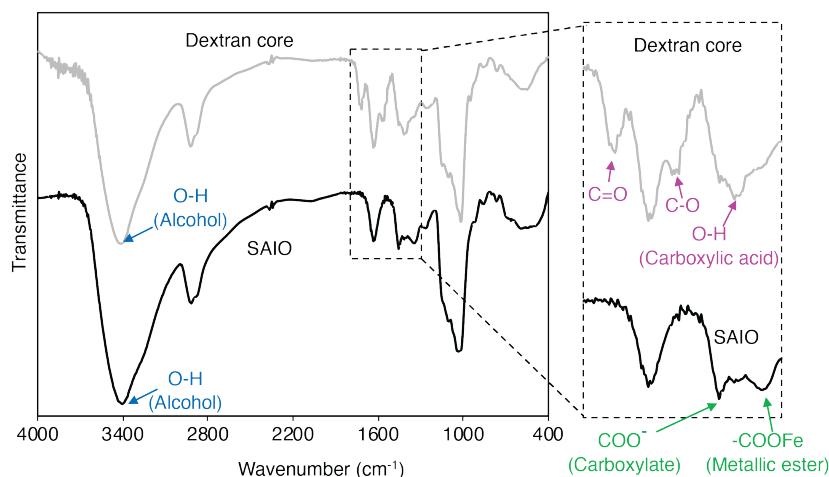
Supplementary Figure 2 | Size control of the supramolecular core. By varying the rate of cross-linking agent addition, the average hydrodynamic size can be controlled from 3 to 5, 9 and 12 nm.



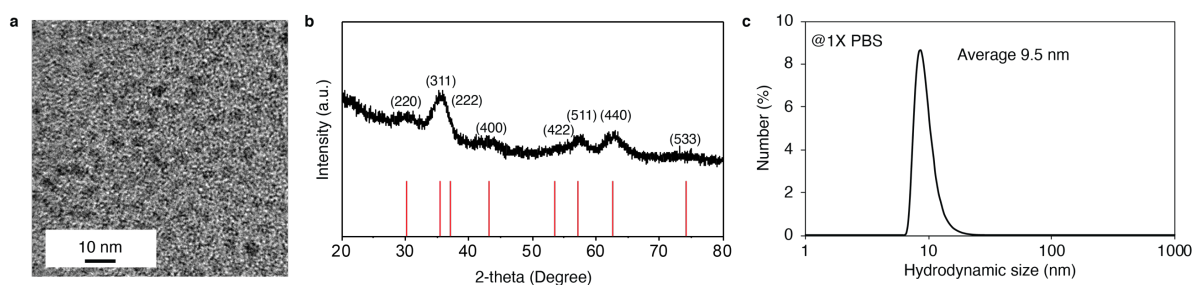
Supplementary Figure 3 | Electron microscopy analyses of SAIO. **a**, Radially averaged intensities of carbon and iron. **b**, Electron energy-loss spectra of SAIO. Peaks of carbon K edge and iron L₃ edge are shown.



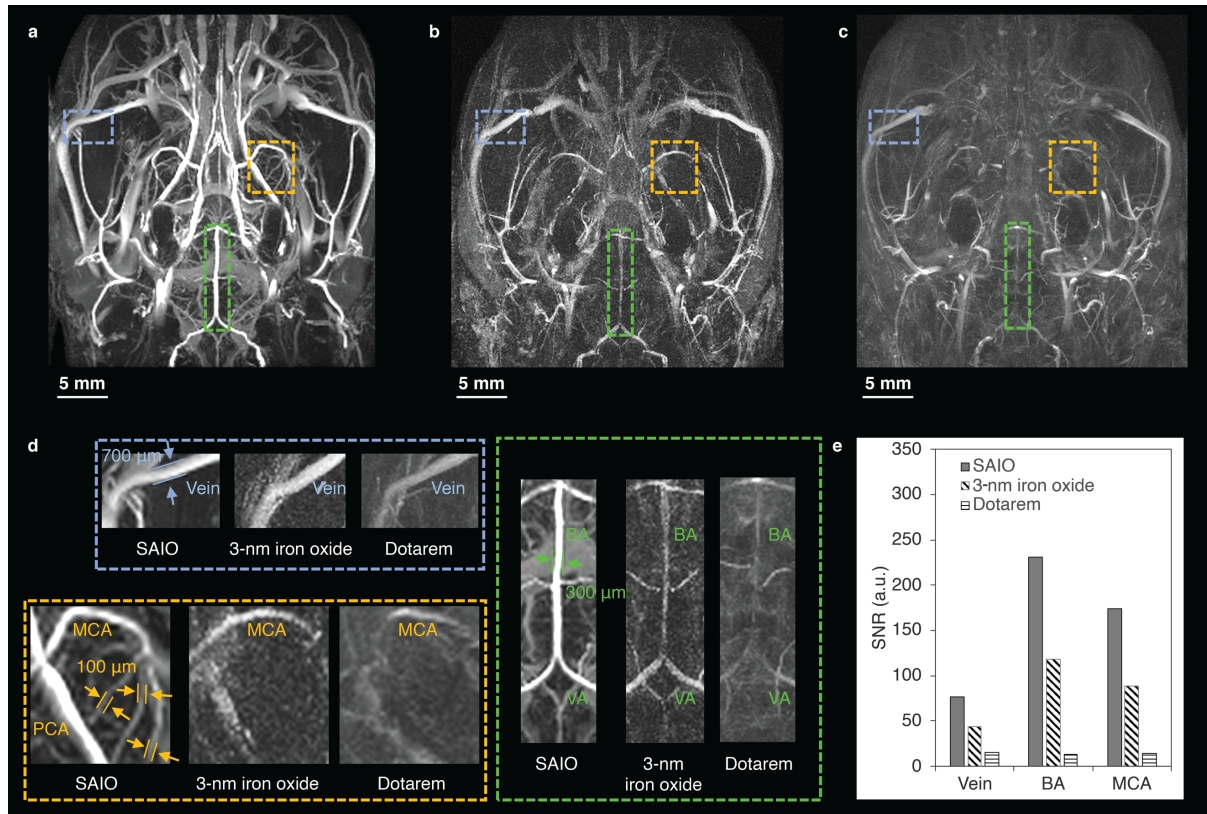
Supplementary Figure 4 | X-ray photoelectron spectroscopy (XPS) data. XPS spectra show two main peaks at binding energies of 709.5 eV (Fe L₃ (2p_{3/2}) edge) and 724.4 eV (Fe L₂ (2p_{1/2}) edge), indicating that Fe is in a ferric oxidation state.



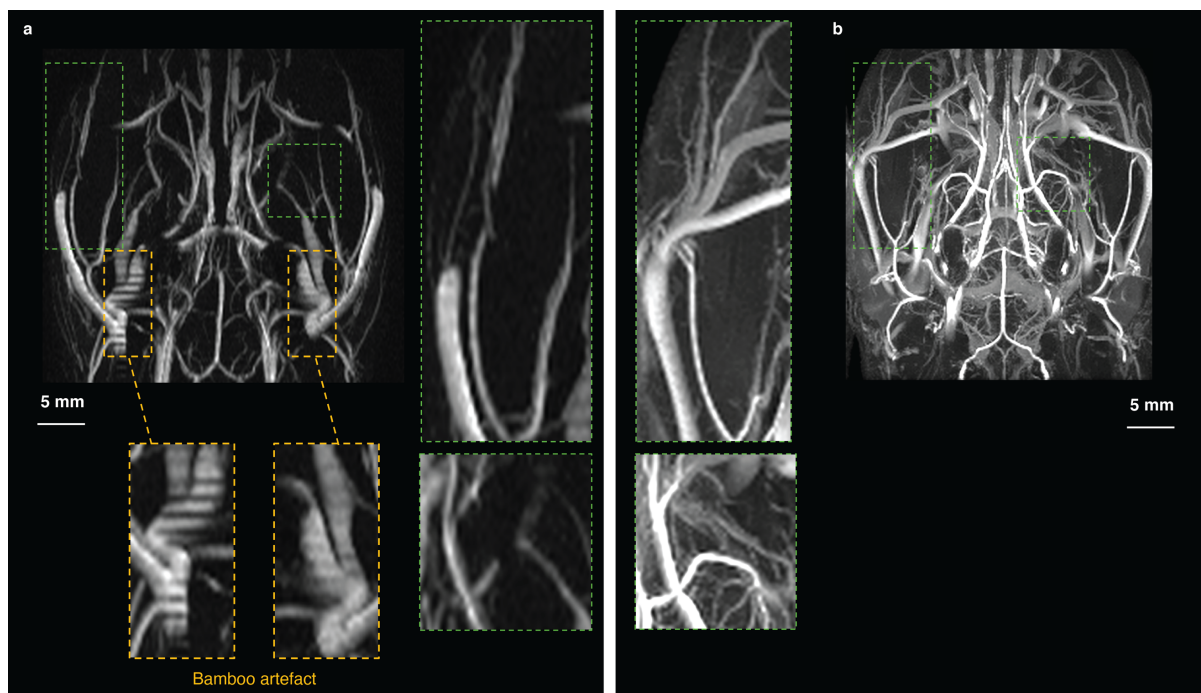
Supplementary Figure 5 | Fourier-transform infrared spectroscopy (FT-IR) analyses of dextran core and SAIO. The peak at $3,400\text{ cm}^{-1}$ corresponds to O-H (alcohol) and is shown for both dextran core and SAIO. The peaks at $1,730\text{ cm}^{-1}$, $1,560\text{ cm}^{-1}$ and $1,420\text{ cm}^{-1}$ correspond to C=O, C-O and O-H (carboxylic acid), respectively, which are observed only for the dextran core. In SAIO, those peaks are shifted to $1,460\text{ cm}^{-1}$ and $1,380\text{ cm}^{-1}$, which are attributed to carboxylate and iron-coordinating metallic ester, respectively.



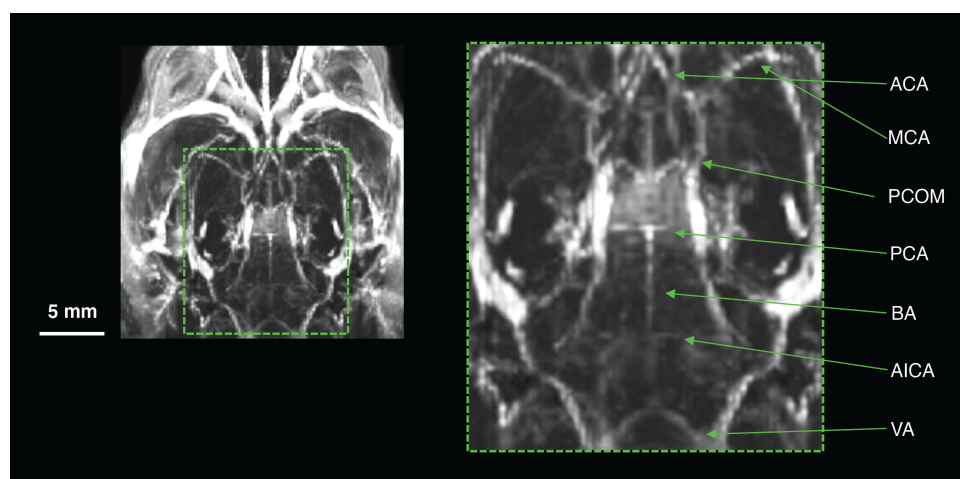
Supplementary Figure 6 | Transmission electron microscopy (TEM), x-ray diffraction (XRD) and dynamic light scattering (DLS) analyses of 3-nm iron oxide particles. **a**, TEM image of 3-nm iron oxide particles. The particles have an average diameter of 3 nm with a narrow size distribution of less than 10%. Three independent experiments were performed with similar results. **b**, XRD pattern of 3-nm iron oxide particles. All peaks are indexed with Joint Committee on Powder Diffraction Standards (JCPDS) reference card number 39-1346, indicating that the particles are crystalline maghemite. **c**, DLS measurements of phosphate-polyethylene glycol-modified 3-nm iron oxide particles at 1X phosphate-buffered saline (PBS).



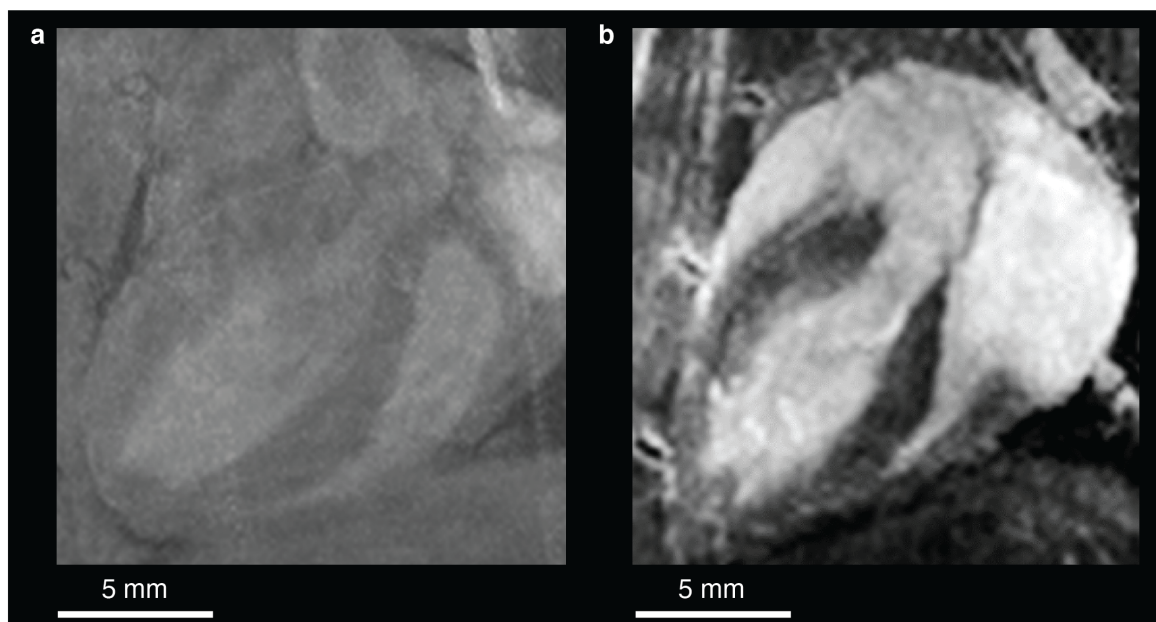
Supplementary Figure 7 | Comparison of magnetic resonance (MR) imaging performance of SAIO, 3-nm iron oxide particles and Dotarem. **a**, SAIO-enhanced, **b**, 3-nm iron oxide particles-enhanced and **c**, Dotarem-enhanced MR angiogram images of rat brain. **d**, Comparisons of enlarged vessel image from each MR angiogram. All of the tested contrast agents visualize large vessels ($\sim 700 \mu\text{m}$), such as vein (blue box). SAIO clearly shows vessels such as basilar artery (BA), vertebral artery (VA), middle cerebral artery (MCA) and posterior cerebral artery (PCA) while other contrast agents poorly visualize them (green and orange boxes). Smaller vessels ($\sim 100 \mu\text{m}$) are also clearly observed with SAIO but not by other contrast agents (orange box). **e**, Signal-to-noise ratio (SNR) of SAIO is substantially higher than that of other contrast agents. MRI images were taken by 9.4-T preclinical MRI scanner using a 3D flow compensated spoiled gradient echo (GEFC) pulse sequence. Dosage was 0.1 mmol of Fe or Gd per kg. The spatial resolution is 100 μm isovoxel. SNR was measured by the mean signal intensity of a region of interest divided by the standard deviation in air.



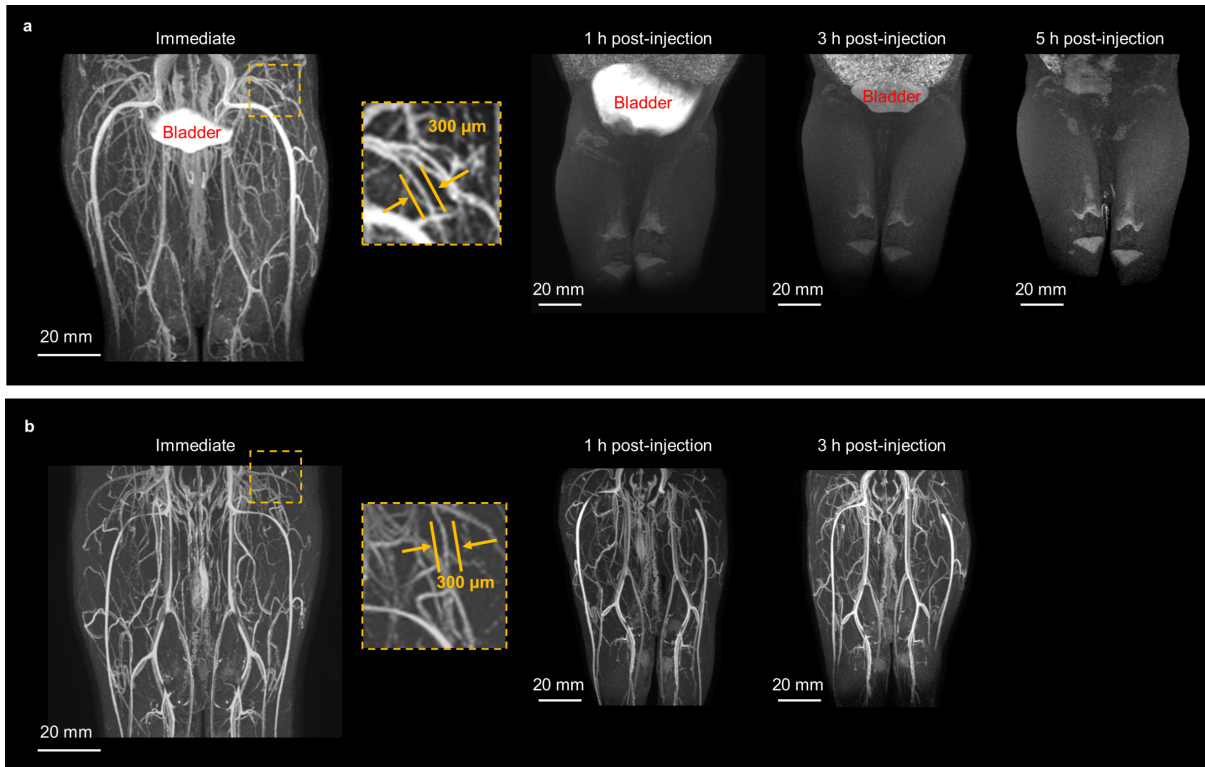
Supplementary Figure 8 | Comparison of time-of-flight (TOF) and SAIO for rat brain MR angiography. **a**, TOF MR angiogram and **b**, SAIO-enhanced MR angiogram images of rat brain. Several blood vessels are observed with TOF but a number of vessels are missed (green boxes). Bamboo artefacts are observed in TOF image (orange boxes). MRI images were taken by 9.4-T preclinical MRI scanner. Fast low angle shot (FLASH)-TOF-2D-flow-comp and 3D FLASH pulse sequences were utilized for panel (**a**) and (**b**), respectively. Dosage was 0.1 mmol of Fe per kg. Panel (**a**) and (**b**) have an imaging resolution of 100 μm isovoxel and $150 \times 150 \times 400 \mu\text{m}^3$, respectively.



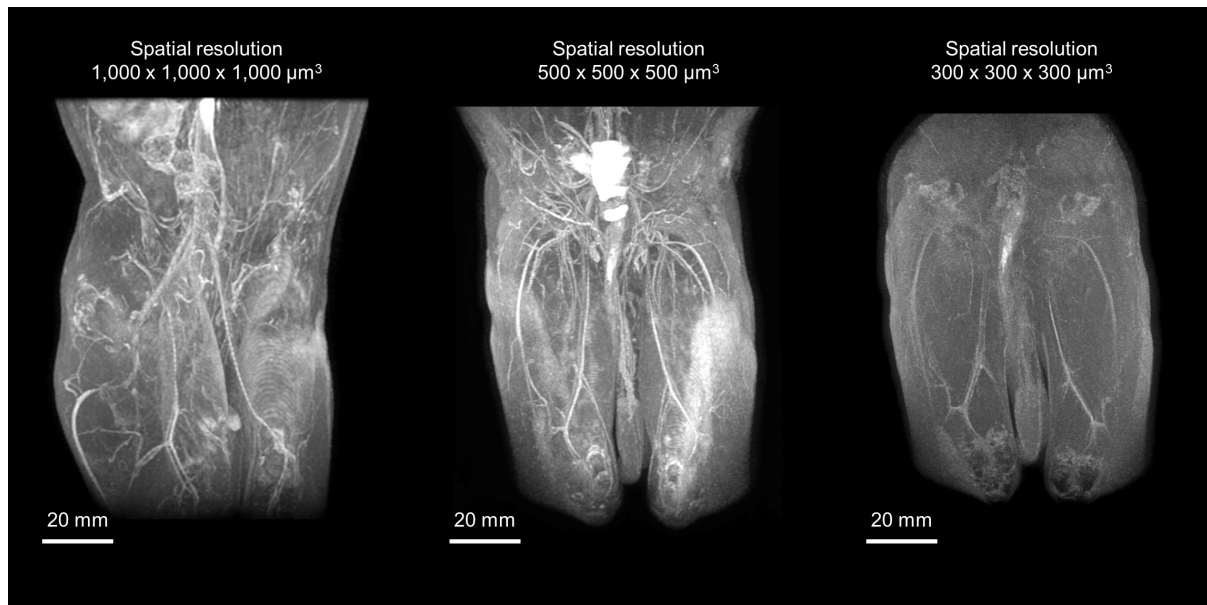
Supplementary Figure 9 | First-pass image of Dotarem-enhanced MR angiography of rat brain. Brain vasculatures, including the anterior cerebral artery (ACA), middle cerebral artery (MCA), posterior communicating artery (PCOM), posterior cerebral artery (PCA), basilar artery (BA), anterior inferior cerebellar artery (AICA) and vertebral artery (VA), are shown with bright contrast. MRI images were obtained by 9.4-T preclinical MRI scanner with a 3D FLASH pulse sequence. Dosage was 0.1 mmol of Gd per kg. The imaging resolution is $300 \times 300 \times 500 \mu\text{m}^3$.



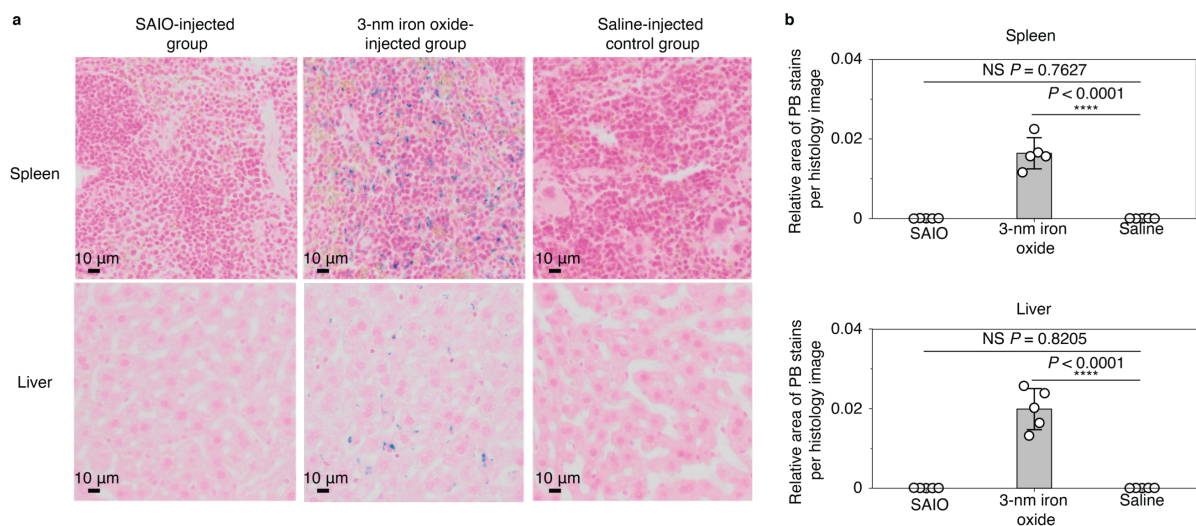
Supplementary Figure 10 | MRI images of non-contrast agent-injected and Dotarem-injected rat heart. a, An MRI image obtained without contrast agents. No coronary arteries are visible. Anatomical structures of the heart are also not easily distinguishable. **b,** Dotarem-enhanced MRI image. Anatomical structures of heart are observed but coronary arteries are hardly shown. Fast clearance of Dotarem and resulting low T_1 contrast make the MRI image coarse. MRI images were obtained by 9.4-T preclinical MRI scanner using a 3D FLASH pulse sequence with respiratory and electrocardiography gating. The imaging resolution is $188 \times 156 \times 200 \mu\text{m}^3$. Dosage was 0.1 mmol of Gd per kg.



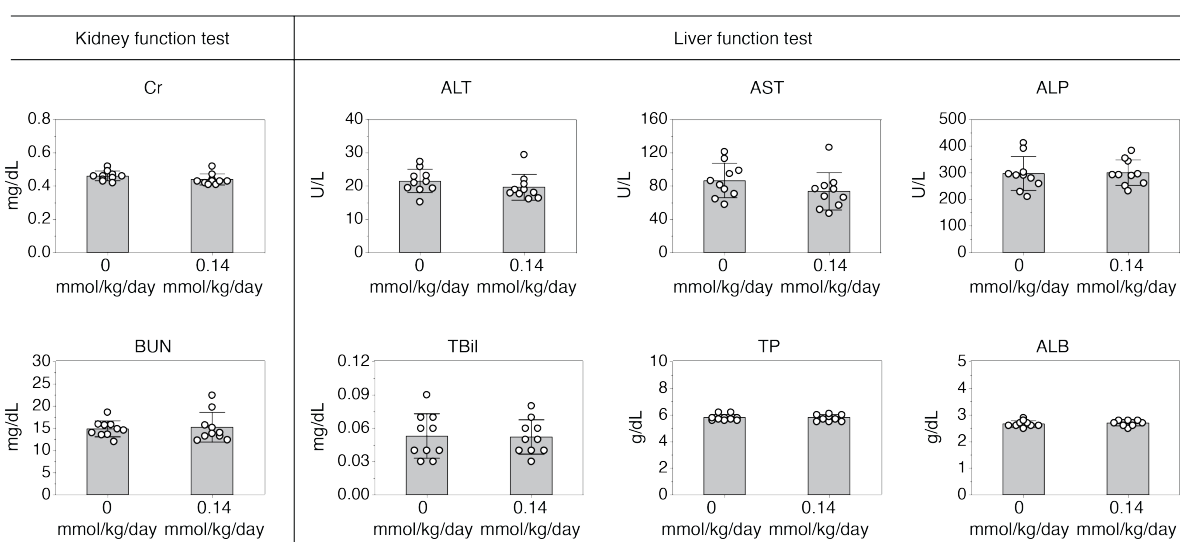
Supplementary Figure 11 | SAIO- and 3-nm iron oxide particles-enhanced MR angiography of rabbit lower extremity. **a**, SAIO-enhanced MR angiogram images. Lower extremity vessels of up to 300 μ m are shown at immediate post-injection. At 1 h and 3 h post-injection, contrast enhancement is only shown in bladder. At 5 h post-injection, no contrast is observed in bladder indicating that SAIO is excreted through urine. **b**, 3-nm iron oxide particles-enhanced MR angiogram images. Image quality is comparable to that of SAIO but renal clearance is not observed even at 3 h post-injection. MRI images were obtained by 3-T clinical MRI scanner using a fast spoiled gradient echo (FSPGR) pulse sequence. The imaging resolution is 300 μ m isovoxel. Dosage was 0.1 mmol of Fe per kg.



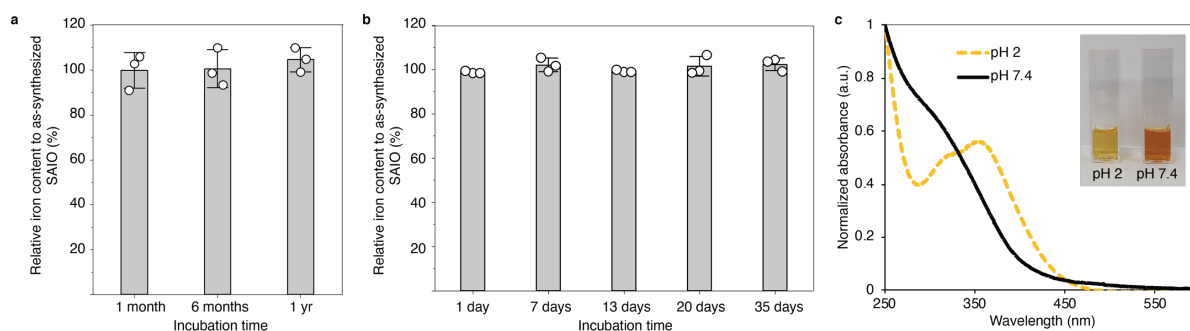
Supplementary Figure 12 | Dotarem-enhanced MR angiogram images of rabbit lower extremity obtained with varying spatial resolution. Blood vessel enhancement is shown when spatial resolution is $1,000 \times 1,000 \times 1,000 \mu\text{m}^3$ or $500 \times 500 \times 500 \mu\text{m}^3$, but vessels are poorly enhanced when spatial resolution is $300 \times 300 \times 300 \mu\text{m}^3$. MRI images were obtained by 3-T clinical MRI scanner using a FSPGR pulse sequence. Scanning time was 0.5 min, 5 min and 15 min for respective spatial resolution of $1,000 \times 1,000 \times 1,000 \mu\text{m}^3$, $500 \times 500 \times 500 \mu\text{m}^3$ and $300 \times 300 \times 300 \mu\text{m}^3$. Dosage was 0.1 mmol of Gd per kg.



Supplementary Figure 13 | Analyses of iron in liver and spleen after injection of contrast agents. **a**, Perls' blue (PB)-stained images of liver and spleen tissues taken at 24 h post-injection of contrast agents. SAIO-injected group (**a**, left panel) and saline-injected negative control group (**a**, right panel) show no iron staining in either the liver or the spleen. 3-nm iron oxide particles-injected positive control group (**a**, middle panel) shows blue staining in both the liver and spleen. Five independent animals were analysed for each group and showed similar results. **b**, Relative area of PB staining per histology image. The region of interest is $850,000 \mu\text{m}^2$. Data are presented as mean $\pm$ standard deviation ($n=5$ biologically independent animals for each group). All tests were performed using unpaired two-tailed Student's *t*-tests; **** $P < 0.0001$; not significant (NS), $P > 0.05$.



Supplementary Figure 14 | Kidney and liver function blood chemistry assays. For kidney function, creatinine (Cr) and blood urea nitrogen (BUN) were measured. For liver function, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total-bilirubin (TBil), total protein (TP) and albumin (ALB) were quantified. No statistically significant differences are observed in any of the tests of kidney or liver. Data are presented as mean $\pm$ standard deviation ($n=10$ biologically independent animals for each group). All tests were performed using unpaired two-tailed Student's t -tests; not significant (NS), $P > 0.05$.



Supplementary Figure 15 | Stability of SAIO. **a**, Stability of SAIO in 1X PBS and **b**, in blood plasma. Relative iron content compared to as-synthesized SAIO is plotted against time ($n=3$ independent samples). Data are presented as mean $\pm$ standard deviation. No iron leaching is observed within tested period of time. Iron was quantified using inductively coupled plasma mass spectrometry after extensive purification steps using a size-exclusion membrane filter with a molecular weight cut-off of 3 kDa. The dextran concentration was measured using a phenol–sulfuric acid assay. **c**, Absorption spectra of SAIO incubated at either pH 7.4 or pH 2. Absorption of SAIO at pH 7.4 gradually increases in the ultraviolet region. SAIO at pH 2 shows characteristic absorption peaks at 310 nm ($[\text{Fe}(\text{OH})(\text{OH}_2)_5]^{2+}$) and 350 nm ($[\text{Fe}_2(\text{OH})_2(\text{OH}_2)_8]^{4+}$), which may result from the hydrolysed iron oxide shell¹. The colour of the SAIO changes from brown (pH 7.4) to yellow (pH 2).

Supplementary Tables

Supplementary Table 1 | r_1 , r_2 and r_2/r_1 of SAIO, Dotarem and other small-sized iron oxide particles.

@ 3-T, 298 K				
	r_1 (mM ⁻¹ s ⁻¹)	r_2 (mM ⁻¹ s ⁻¹)	r_2/r_1	M (emu per g of Fe or Gd)
SAIO*	3.8	4.4	1.2	3.0
Dotarem	3.9	4.9	1.3	3.0
5-nm iron oxide	28	150	5.4	70
ref. [2]	4.8	29.2	6.12	30
3-nm iron oxide	4.1	19.2	4.1	N/A
exp.*	3.6	17.0	4.7	25

* MRI data are measured at 3-T and M values are measured at 1.5-T, 298 K.

Supplementary Table 2 | Pharmacokinetic parameters of intravenously injected SAIO.

	AUC _{last} (ng h/mL)	C _{max} (ng/mL)	T _{max} (min)	t _{1/2} (min)
Mean	68.75	90.64	1.20	24.6
S.D.	24.79	30.72	0.00	3.00

Supplementary Table 3 | Time profile of urinary concentration of SAIO.

	Amount of Fe (mg) in urine			Total amount of Fe in urine (mg)	Blank	Total amount (mg) (0 – 24 h)
	Collection period (h)					
	0 – 8	8 – 16	16 – 24			
Mean	2.5	0.18	0.042	2.7	0.3	2.4
S.D.	0.33	0.066	0.013	0.3	0.0	0.3

Supplementary Table 4 | MR imaging sequence parameters and hardware specifications utilized in this study.

	Figure 1d	Figure 1e	Figure 1f	Figure 3 Supplementary Figure 7, 8b	Figure 4a	Figure 4b	Supplementary Figure 8a	Supplementary Figure 9	Figure 5 Supplementary Figure 10	Figure 6 Supplementary Figure 11, 12
Magnetic field strength	3-T	9.4-T	9.4-T	9.4-T	9.4-T	9.4-T	9.4-T	9.4-T	9.4-T	3-T
Manufacturer	MR Solutions	Bruker Biospin	Bruker Biospin	Bruker Biospin	Bruker Biospin	Bruker Biospin	Bruker Biospin	Bruker Biospin	Bruker Biospin	GE Healthcare
MRI system model	MRS3000	BioSpec 94/20	BioSpec 94/20	BioSpec 94/20	BioSpec 94/20	BioSpec 94/20	BioSpec 94/20	BioSpec 94/30	BioSpec 94/20	Discovery MR750
MRI software	Preclinical Scan	ParaVision 5.1	ParaVision 5.1	ParaVision 5.1	ParaVision 5.1	ParaVision 5.1	ParaVision 5.1	ParaVision 6.0.1	ParaVision 5.1	DV25.0_R02
Transmitter coil	65 mm Volume	86 mm Volume	86 mm Volume	40 mm Volume	86 mm Volume	72 mm Volume	40 mm Volume	40 mm Volume	86 mm Volume	18ch T/R Knee
Receive coil	65 mm Volume	86 mm Volume	86 mm Volume	40 mm Volume	4ch Mouse Brain surface	72 mm Volume	40 mm Volume	40 mm Volume	4ch Rat Heart surface	18ch T/R Knee
Sequence	3D FLASH	3D GEFC	2D FLASH	3D GEFC	3D GEFC	3D FLASH	FLASH-TOF-2D-flow-comp	3D FLASH	3D FLASH	FSPGR
K-space trajectory	Cartesian	Cartesian	Cartesian	Cartesian	Cartesian	Cartesian	Cartesian	Cartesian	Cartesian	Cartesian
TR [ms]	20.0	7.00	7.00	10.0	8.78	6.40	18.0	14.3	7.13	13.5
TE [ms]	1.20	2.34	2.68	3.12	1.93	2.00	4.00	2.52	0.970	3.73
FA [deg]	16.0	20.0	20.0	20.0	20.0	20	80	20.0	23.0	25.0
Number of average	2	1	1	1	1	1	1	1	2	1
FOV [mm]	25.6 x 25.6 x 0.800	60.0 x 60.0 x 40.0	50.0 x 50.0	38.4 x 30.4 x 20.0	50.0 x 25.0 x 10.5	80.0 x 40.0 x 20.0	30.0 x 40.0	40.0 x 32.0 x 20.0	36.0 x 30.0 x 20.0	150 x 150 x 96.0
Voxel size [mm]	0.400 x 0.400 x 0.0250	0.200 x 0.200 x 0.200	0.260 x 0.260 x 1.50	0.100 x 0.100 x 0.100	0.190 x 0.190 x 0.350	0.312 x 0.312 x 0.312	0.150 x 0.150 x 0.400	0.300 x 0.300 x 0.500	0.188 x 0.156 x 0.200	0.290 x 0.290 x 0.300
Recon matrix	128 x 128 x 32	300 x 300 x 200	192 x 192	384 x 304 x 200	256 x 128 x 30	256 x 128 x 64	192 x 256	131 x 105 x 40	192 x 192 x 100	512 x 512 x 320
Receiver bandwidth [kHz]	50	50	50	75	100	75	100	75	100	41.67
ECG gating	None	None	None	None	None	None	None	None	Yes	None

Respiratory gating	None	Yes	Yes	Yes	None	None	None	None	Yes	None
Acquisition window	None	Exhalation phase	Exhalation phase	Exhalation phase	None	None	None	None	End-systolic phase	None

Supplementary Equation

Supplementary Equation (1)

$$\text{Signal intensity} = M_0 \sin(\alpha) \frac{1 - e^{-R_1 \cdot TR}}{1 - \cos(\alpha) \cdot e^{-R_1 \cdot TR}} e^{-R_2^* \cdot TE}$$

MRI signal intensity (SI) is determined by the gradient strength (M_0), flip angle (α), repetition time (TR), echo time (TE), R_1 and R_2^* ^{4,5}. The factors M_0 , α , TR and TE can be controlled by the MRI scanner, and R_1 and R_2^* can be adjusted by contrast agents. Notably, R_2^* can be considered an effective R_2 and is always larger than or equal to R_2 .

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3. Huang, J., Wang, L., Zhong, X., Li, Y., Yang, L. & Mao, H. Facile non-hydrothermal synthesis of oligosaccharide coated sub-5 nm magnetic iron oxide nanoparticles with dual MRI contrast enhancement effect. *J. Mater. Chem. B* **2**, 5344-5351 (2014).
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