Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information for:

Succinylated Heparin Monolayer Coating Vastly Increases Superparamagnetic Iron Oxide Nanoparticle T_2 Proton Relaxivity

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Section S1. T_2 relaxivity of SPIONs

Generally, there are two contributions to proton relaxation in paramagnetic systems: the inner- and outer-sphere relaxations, and the total relaxivity of a paramagnetic agent is given by Equation S1: [1]

$$\frac{1}{T_2} = \left(\frac{1}{T_2}\right)_{inter\ sphere} + \left(\frac{1}{T_2}\right)_{outer\ sphere} \qquad \text{(S1)}$$

But, the inner-sphere contribution of the superparamagnetic particles to the relaxation is minor and more often completely negligible as compared to the dominant outer-sphere contribution.[2] Hence, most studies have focused on the outer-sphere component,[3-5] and calculating the relaxation rate according the classical diffusion theory for outer-sphere when SPIONs fulfill $\tau_D > 1/\Delta w_r$: [6]

$$\frac{1}{T_2} = 2.25v\left(\Delta w_r\right)^2 \tau_{CP}^2/\tau_D \qquad \text{(S2)}$$

Where v, Δw_r and τ_{CP} are the volume fraction of magnetized material, the RMS frequency shift at the particle surface and the Carr-Purcell parameter (defined as half the echo time (TE) or, for a CPMG echo train, half the inter echo time), respectively; $\tau_D$is the time required for water to diffuse past a magnetized particle: [7]

$$v = \left(\frac{4}{3}\right)\pi r^3 N_0[M] \times 10^{-3}$$

$$\Delta w_r = \sqrt{\frac{4}{5}}\gamma B_{eq} = \frac{\sqrt{\frac{4}{5}}\gamma\mu}{r^3} = \frac{8\pi}{3\sqrt{5}}\gamma M \qquad \text{(S3)}$$

$$\tau_{CP} = \frac{TE}{2}$$

Where r and M are the radius and magnetization of iron oxide core; N_0, γ and TE are Avogadro's number, the proton gyromagnetic ratio and the echo time, respectively. Accordingly, Equation S2 can be reorganized into the following form:

$$\frac{1}{T_2} = \frac{2}{1875}\pi^3 N_0 \gamma^2 TE^2 \cdot [M] \cdot r^3 \frac{M^2}{\tau_D} \tag{S4}$$

In the absence of solute-solute interactions, the solvent relaxation rates are linearly dependent on the concentration of the paramagnetic species ([M]) and the r_2 is defined as the slope of this dependence in units of M^{-1} s^{-1} or, more commonly, mM^{-1} s^{-1} (eq S5):[1]

$$R_2 = \frac{1}{T_2} = \left(\frac{1}{T_2}\right)_0 + r_2[M] \tag{S5}$$

Combining Equation S4 and S5, we obtain:

$$r_2 = \frac{d(\frac{1}{T_2})}{d[M]} = \frac{2}{1875}\pi^3 N_0 \gamma^2 TE^2 \cdot r^3 \frac{M^2}{\tau_D} \tag{S6}$$

The magnetization M can be approximated by Equation S7, and the diffuse time τ_D is given by Equation S8, [1]

$$M = \frac{\mu}{\frac{4}{3}\pi r^3} \tag{S7}$$

$$\tau_D = \frac{r^2}{D} \tag{S8}$$

Where μ is the Curie moment of the magnetic nanoparticle, *D* is the diffusion coefficient of water molecules. Inserting Equation S7 and S8 into Equation S6, we obtain:

$$r_2 = \frac{3\pi}{5000} N_0 \gamma^2 \mu^2 TE^2 \cdot \frac{D}{r^5} \tag{S9}$$

It should be noted that the values of N_0, γ and TE are constant with the same iron oxide core. We set the quantities whose values remain unchanged as a constant: β, the Equation S9 can be expressed with Equation S10:

$$r_2 = \beta \cdot \frac{D}{r^5} = 5\beta \int_r^{+\infty} \frac{D}{r^6} dr \tag{S10}$$

$$\beta = \frac{3\pi}{5000} N_0 \gamma^2 \mu^2 TE^2 \tag{S11}$$

When D is not homogeneous, there are two contributions to D in superparamagnetic systems: the diffusion coefficient of water molecules in polymer coating D_1 and in pure water phase D_0:

$$\begin{aligned} r_2 &= 5\beta \left(\int_r^{r+d} \frac{D_1}{r^6} dr + \int_{r+d}^{+\infty} \frac{D_0}{r^6} dr \right) \\ &= \beta \left(\frac{D_1}{r^5} - \frac{D_1}{(r+d)^5} + \frac{D_0}{(r+d)^5} \right) \\ &= \beta \left[\frac{D_0}{r^5} - (D_0 - D_1) \left(\frac{1}{r^5} - \frac{1}{(r+d)^5} \right) \right] \end{aligned} \tag{S12}$$

Where d is the thickness of the coating layer. If the SPIONs are not coated with a polymer coating that decreases the diffuse coefficient of water molecules, r_2 have the maximum value: $\beta D_0 / r^5$.

r_2 scaling with radius

The formula derived here is best applied to consideration of varied coatings on a common nanoparticle core, although comparison across sizes is possible, but must consider the variation in μ with increasing particle size. More specifically, the magnetization M can be described as $M = M_s \cdot \rho$, in which M_s is specific saturation magnetization with the unit of emu/g and ρ is the density of the nanoparticle. Millan *et al.*[8] showed that the nonideality in M_s versus volume can be explained by an outer shell of the nanoparticle which does not contribute to M_s. They showed the equation:

$$M_s = M_{s0}\left(\frac{r - d_{NP}}{r}\right)^3 \tag{S13}$$

Where M_{s0} is the bulk saturation magnetization, r is the nanoparticle radius, and d_{NP} is the thickness of the nanoparticle shell which does not contribute.

While the relationship in not perfect, they showed that a d_{NP} of 1 nm was well fit to a broad selection of literature data.

For the purposes of understanding the scaling of r_2 with nanoparticle radius, we can combine equation S13 with S7 and rearrange to yield:

$$\mu = \frac{4}{3}\pi\rho M_{s0}(r - 1)^3 \tag{S14}$$

For r in nanometers, setting for convenience, and substituting S14 in S12 gives the relationship for the expected scaling of r_2 to r of:

$$r_2 = \frac{2\pi^3}{1875}N_0\gamma^2TE^2\rho^2M_{s0}^2(r - 1)^6\left[\frac{D_0}{r^5} - (D_0 - D_1)\left(\frac{1}{r^5} - \frac{1}{(r + d)^5}\right)\right] \tag{S15}$$

Considering only the case of the uncoated nanoparticle this gives:

$$r_2 \sim \left[\frac{(r-1)^6}{r^5}\right] \tag{S16}$$

Evolution of the $\tau_D > \frac{1}{\Delta w_r}$ criterion

As mentioned above, the characteristic diffusion time τ_D is r^2/D while Δw_r can be approximated by $\frac{8\pi}{3\sqrt{5}}\gamma M$. For the purpose of evaluating $\tau_D > 1/\Delta w_r$, we will approximate the magnetization M as $M = M_s \cdot \rho$, in which M_s is specific bulk saturation magnetization with the unit of emu/g. In this paper,

$$\rho = \frac{5.18 \times \frac{4\pi}{3}r^3 + 1 \times \frac{4\pi}{3}[(r+d)^3 - r^3]}{\frac{4}{3}(r+d)^3} = 4.18 \cdot \frac{r^3}{(r+d)^3} + 1$$

(S17)

Thus, $M = M_s \cdot \left(4.18 \cdot \frac{r^3}{(r+d)^3} + 1\right)$.

When the core of SPINO is 14 nm the corresponding M_s is 50 emu/g,

$\tau_D = \frac{r^2}{D_1} = 3.16 \times 10^{-8} s$ and $\frac{1}{\Delta w_r} = \frac{3\sqrt{5}}{8\pi\gamma M} = 5.34 \times 10^{-9} s$.

Thus, the SPIONs of 14nm fulfill $\tau_D > 1/\Delta w_r$.

When the core of SPINO is 8nm, the corresponding M_s is 45.2 emu/g, $\tau_D = 1.25 \times 10^{-8} s$ and $\frac{1}{\Delta w_r} = \frac{3\sqrt{5}}{8\pi\gamma M} = 7.87 \times 10^{-9} s$, which meets $\tau_D > 1/\Delta w_r$. However, τ_D and $\frac{1}{\Delta w_r}$ of the SPIONs for 6 nm were calculated as $8.6 \times 10^{-9} s$ and $1.25 \times 10^{-8} s$, which does meet the conditions $\tau_D > 1/\Delta w_r$

for our formula application. Further, the superparamagnetic limit for magnetite is ~20 nm. Therefore, the core size of SPION that meet the premise of our formula application is approximately 8~20 nm.

Section S2. Figures and tables

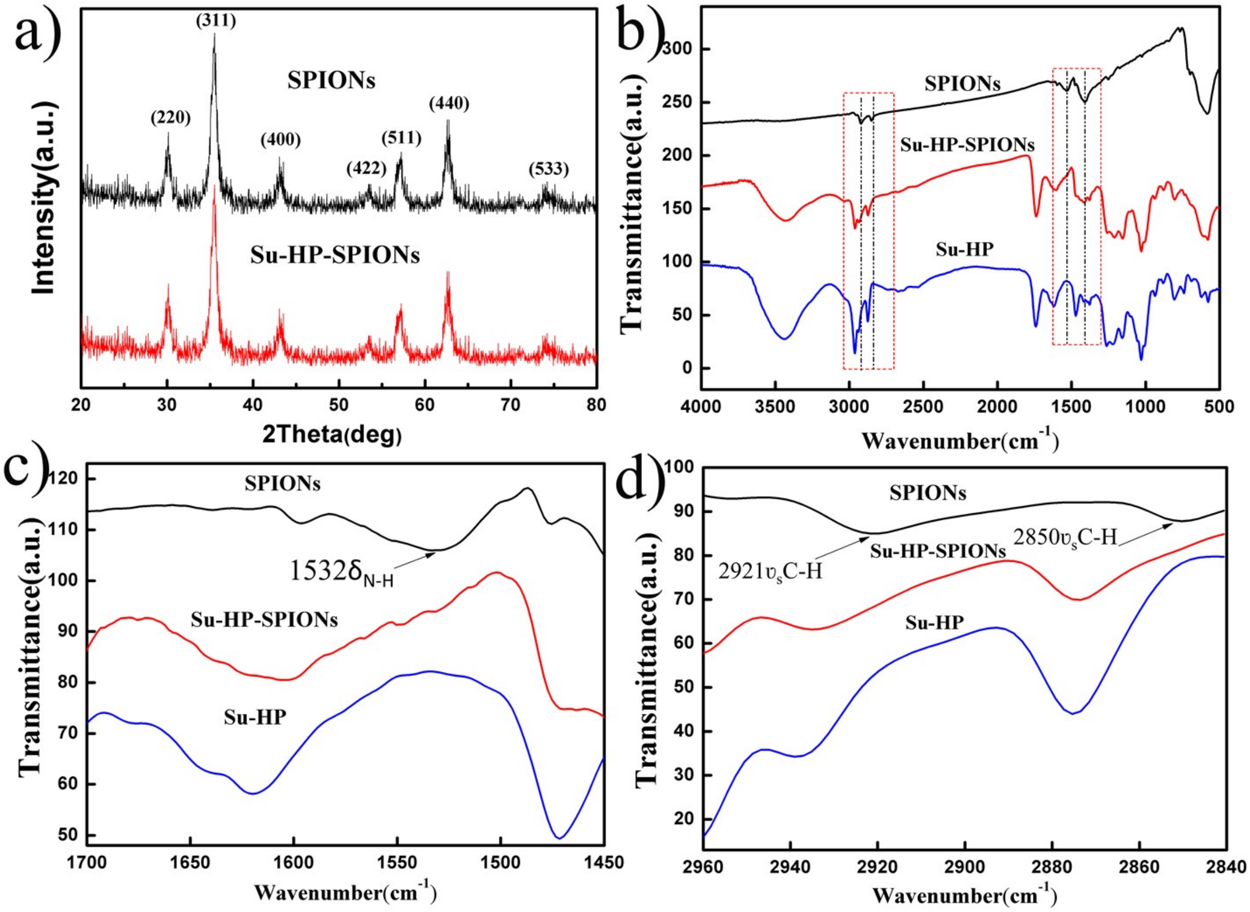

Figure S1. (a) X-ray diffraction (XRD) patterns of SPIONs and Su-HP-SPIONs. The XRD patterns of 14-nm nanoparticles gives the diffraction peaks nearly identical to that known for the commercial magnetite powder (Aldrich catalog No. 31, 006-9), and the average correlation size was estimated with Scherrer's formula to be 14.6 nm, close to the 14-nm average size determined by statistical analysis of the TEM images. (b) FTIR spectra SPIONs, Su-HP-SPIONs and Su-HP; (c) (d) drawing of partial enlargement. The disappearance of the C-H stretch bands of oleic acid and oleamine at 2921and 2850 cm^{-1} and the stretching vibration peak of amino N-H at 1532 cm^{-1}suggests the complete replacement of original hydrophobic capping ligands by Su-HP.[9]

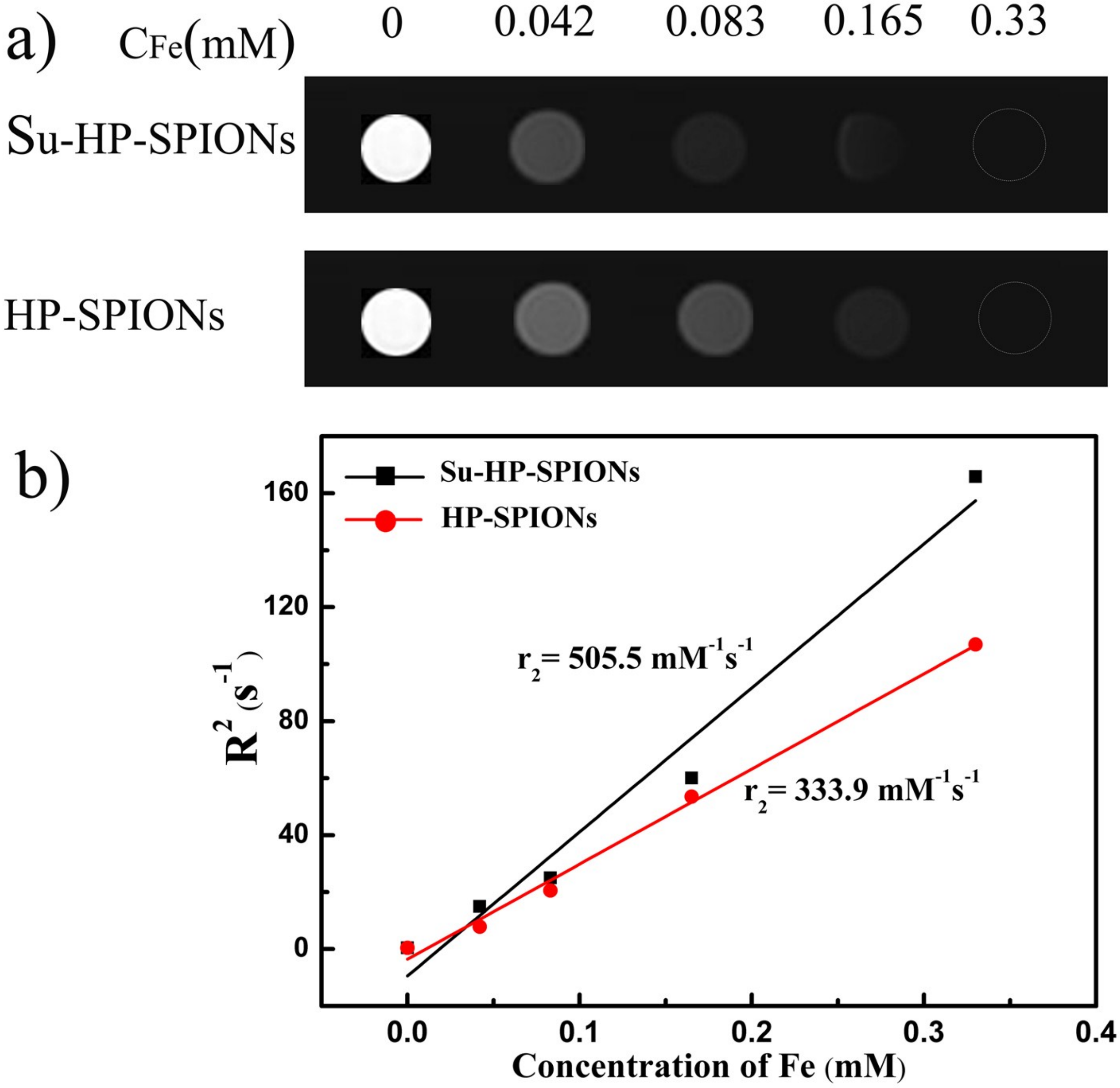

Figure S2. (a) T_2 weighted MRI images and (b) T_2 relaxation rates (r_2) of 19 nm core size for Su-HP-SPIONs and HP-SPIONs with different Fe concentrations measured at 3 T.

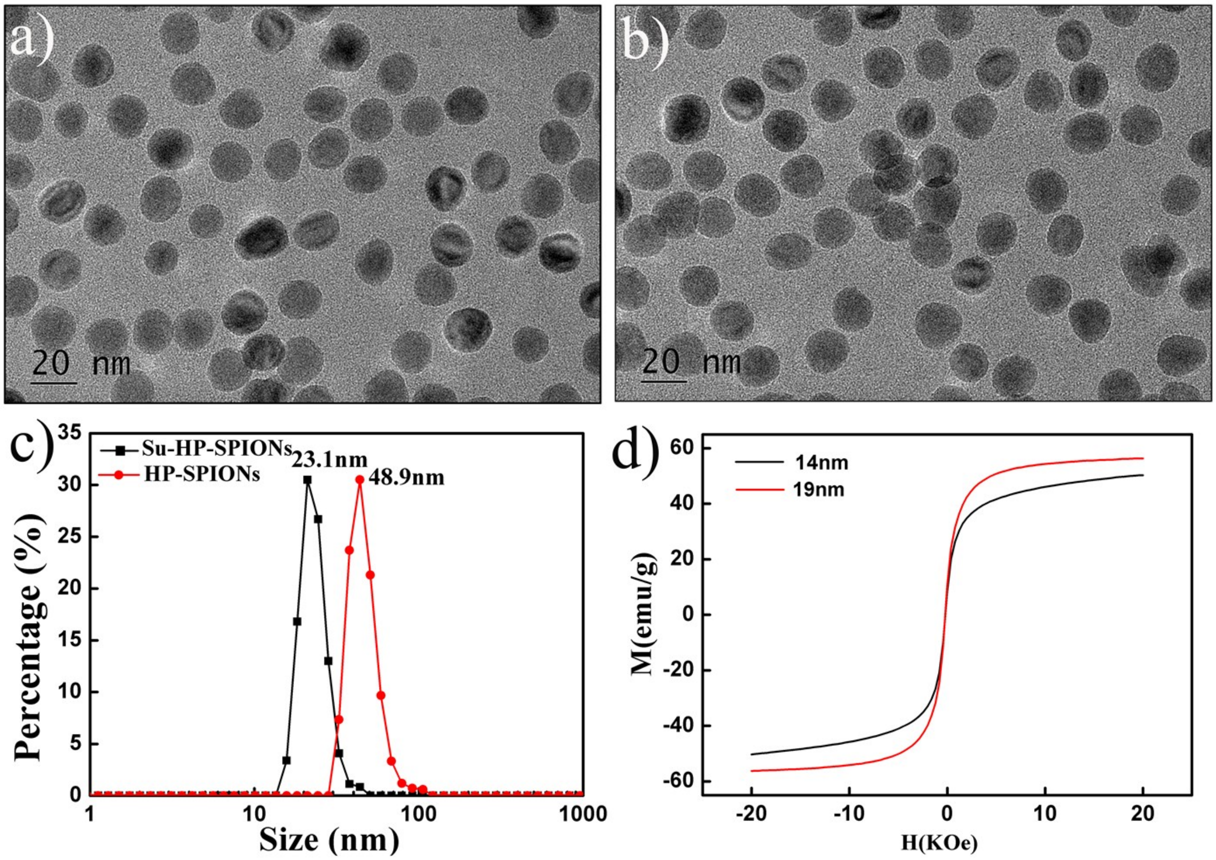

Figure S3. TEM images of (a) Su-HP-SPIONs and (b) HP-SPIONs with 19 nm core size in water. (c) Hydrodynamic size of the corresponding nanoparticles as measured by dynamic light scattering (DLS). (d) Magnetometric curves for SPIONs with different core size (14 nm and 19 nm).

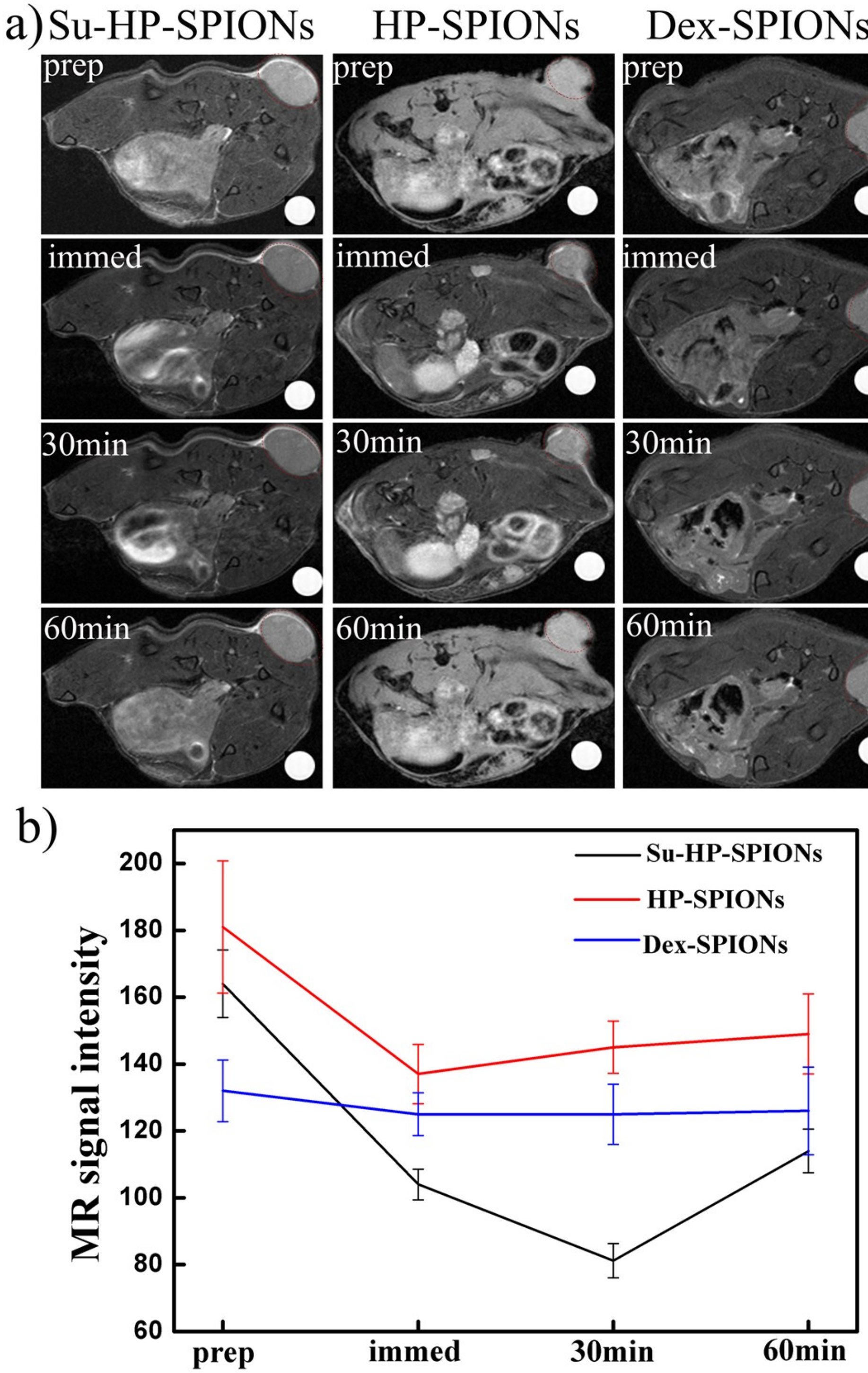

Figure S4. In vivo tumor imaging. (a) T_2 weighted MRI images of the tumor in mice before, immediately, 30 min and 60 min after the injection of Su-HP-SPIONs, HP-SPIONs, Dex-SPIONs with 14 nm cores and (b) the corresponding T_2-weighted MRI signal intensity decrease.

Table S1. The core size of hydrophobic SPIONs prepared, the heparin origin used in different batches, and the hydrodynamic size, zeta potential and r_2 value of the corresponding Su-HP-SPIONs.

Batch	**Core (nm)**	**HP origin**	**Hydrodynamic sizes(nm)**	**Zeta potential (mv)**	**r_2(mM^{-1}s^{-1})**
1	14	Celsus Laboratory (Cincinnati, U.S.A.)	18.71	-32.2	460.4
2	13.8	Hepalink Co. Ltd. (Shenzhen, China)	18.42	-33.6	466.2
3	14.2	Aladdin Co. Ltd. (Shanghai, China)	19.28	-31.6	459.2

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