Supplementary Information

Relaxivity Control of Magnetic Nanoclusters for Efficient Magnetic Relaxation Switching Assay

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Chemicals. FeCl₃·6H₂O (> 97 %, Sigma-Aldrich), sodium acetate anhydrous (NaOAc, > 98.5 %, Samchun Chemicals), poly(acrylic acid) (PAA, 35 wt % in H₂O, Aldrich), ethylene glycol (EG, > 99.5 %, Samchun Chemicals), (+)-Biotin hydrazide (>97 %, Sigma), Streptavidin (Sigma), 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC, > 98.0 %, TCI), and *N*-hydroxysulfosuccinimide sodium salt (sulfo-NHS, >98.5 %, Aldrich) were obtained from commercial sources and used as received. Deionized (DI) water purified Milli-Q purification system was used for all the experiments.

Synthesis of magnetic nanoclusters (MNCs). In a typical synthesis of 100 nm MNC, FeCl₃·6H₂O (27 g, 0.1 mol), NaOAc (100g, 1.2 mol) and DI water (100 g, 5.6 mol) were completely dissolved in 1.5 L EG by vigorous mechanical stirring to form a yellow-brown turbid solution. Then, the solution was refluxed to 20 h. The reaction solution turned reddish-brown and then slowly black. After cooling down to room temperature, the black sediment was magnetically separated by attaching a strong permanent magnet onto the outside of the reaction flask and washed with ethanol and DI water several times to eliminate organic and inorganic byproducts. Finally, ~7.5 g (97 % yield) of dried powder of MNCs was obtained. The size of MNCs could be precisely and reproducibly controlled from tens to hundreds of nanometers by varying the initial reaction conditions. First, the size of the MNCs increased with increasing FeCl₃ concentration as the concentrations of all the other components were maintained constant. The size of the MNCs increased from 15 to 30, 50, and 100 nm as the amount of FeCl₃·6H₂O was increased from 37 to 55, 74, and 100 mmol, respectively, because the higher concentrations of iron precursor induced the formation of larger hydrous ferric oxides (HFOs), and consecutive transformation of iron oxides could result in larger MNCs. The second factor for determining the size of the MNCs was the ripening process of HFOs. The yellowish-brown HFOs, generated by the rapid hydrolysis and condensation of the iron (III) salt solution at room temperature in the presence of NaOAc always resulted in MNCs of the same size if they were rapidly heated to the refluxing condition. However, the HFOs were found to undergo the ripening process at 70 °C. After heating the solution of HFOs at 70 °C for appropriate times, the reaction temperature was increased to the reflux condition to complete the reaction and afford the formation of MNCs. The size of the generated MNCs increased from 100 nm to 200 nm and then to 300 nm as the heating time at 70 °C was increased from 1 h to 12 h and then to 22 h, respectively. It was expected that the small amount of ferrous ion Fe^{2+} generated by the reduction of Fe^{3+} with EG could trigger the ripening process through a dissolution-reprecipitation mechanism (i.e., Fe(II) adsorption and electron transfer to structural Fe(III) enhances the dissolution rate and subsequent reprecipitation as a thermodynamically more stable phase).¹

Preparation of PAA-coated MNCs (PAA-MNCs). As-prepared MNCs were collected, washed with DI water several times, and then redispersed into 1M HCl aqueous solutions under sonication for 10 minutes. The MNCs were magnetically separated. The supernatant was discarded, and the MNCs were rinsed twice with DI water. The resulting MNC solution (20 mL, 5 mg of MNC/mL DI water) was added into the PAA solution (20 mL, 50 mg of PAA/mL DI water) and incubated on a shaker for 1 hour at room temperature. The PAA-MNCs were centrifuged, rinsed twice with DI water to remove excess PAA, and then redispersed into DI water. After suspension in DI water, 4 mL of NH₄OH (28 ~ 30 wt %) aqueous solution was added to the PAA-MNC colloidal solution, resulting in an optically well-dispersed solution. The PAA-MNCs were centrifuged, after which the supernatant was removed and replaced with DI water.

Biotinylation of PAA-MNCs. To conjugate 50 nm PAA-MNCs with (+)-biotin hydrazide, amide bonds were formed using the carboxylic group of PAA and the amine groups in biotin derivative through EDC coupling agent. The PAA-MNCs (20 mg) were dispersed in 10 mL phosphate-buffered saline (PBS; pH 7.4) buffer, followed by the addition of 2 mM EDC and 4 mM sulfo-NHS. After incubation for 10 min, 10 mg (+)-biotin hydrazide was added to the solution. The mixture was shaken for 2 hours at room temperature. The conjugated MNCs were then precipitated down (16,000 rpm, 20 minutes) and washed with DI water. The biotinylated MNCs were stored at DI water prior to use.

Characterizations. The morphologies of the materials were examined using transmission electron microscopy (TEM, Hitachi-7600), operated at an accelerating voltage of 100 kV. The crystal structure and crystallite size of the MNCs were determined using powder X-ray diffraction (XRD, MAC Science M18XHF-XRA) with Cu Ka radiation ($\lambda = 1.5406$ Å). An accelerating voltage of 40 kV and an emission current of 200 mA were used. Scans were recorded with a scanning speed of 1 $^{\circ}$ ·min⁻¹ for determination of the average crystallite size. The average crystallite size was calculated by the Debye-Scherrer equation, $D_{xrd} = 0.9 \cdot \lambda / \beta \cdot cos\theta$, where D_{xrd} is the average crystal diameter, λ the wavelength of the X-ray employed, β the full width at half maximum (FWHM) of the strongest peak (3 1 1) in radians, and θ the Bragg angle. The magnetic properties were analyzed in the powder samples. The M (H) curves were measured by a vibrating sample magnetometer (VSM, MicroSense EV9). M (H) was measured at 300 K, with an applied field up to 20 kOe. The concentration of metal ions in the samples was obtained by inductively coupled plasma atomic emission spectroscopy (ICP-AES; ICP-730 ES, Varian). The hydrodynamic sizes and zetapotentials of PAA-MNCs in aqueous solution were determined using a dynamic light scattering (DLS) instrument (Nano-ZS, Malvern Instruments). The T_2 relaxation times (ms) of the samples were measured for different concentrations of Fe₃O₄ using a 0.47 T magnetic relaxometer (mq-20, Bruker). Samples with different concentrations of PAA-MNCs with various diameters were prepared by diluting them in DI water. The r_2 values $(\mathbf{m}\mathbf{M}^{-1}\cdot\mathbf{S}^{-1})$ were calculated from the slope of the linear plots of $1/\mathbf{T}_2$ versus the Fe concentrations.

Detection of Streptavidin. Streptavidin was dissolved in PBS. Subsequently, samples were prepared by mixing various amounts of streptavidin to solutions of biotinylated MNCs. Following 30 minutes of incubation at 40 °C, the turbidity, hydrodynamic sizes, and T_2 values of the samples were measured on 1 mL samples using UV-Vis spectrophotometer, DLS instrument, and 0.47 T magnetic relaxometer, respectively. All measurements were taken in triplicate and the data were displayed as mean \pm standard error.

References

1. C. M. Hansel, S. G. Benner and S. Fendorf, *Environmental Science & Technology*, 2005, **39**, 7147-7153.



Fig. S1 TEM images of MNCs with different sizes. (a) 15 nm, (b) 30 nm, (c) 50 nm, (d) 100 nm, (e) 200 nm, and (f) 300 nm.



Fig. S2 XRD patterns of MNCs with different sizes. Scans were recorded for 2θ values between 28° and 45° with a scanning speed of $1^{\circ} \cdot \text{min}^{-1}$.



Fig. S3 Typical HR-TEM image of 100 nm MNC.



Fig. S4 Magnetic hysteresis curves of as-prepared MNCs with different sizes.





Fig. S5 Dispersibility changes of PAA coated MNCs with time in the wide range of pHs of aqueous solution.



Fig. S6. The relaxation rates, $1/T_2$, of PAA-MNCs as a function of [Fe] concentration with various diameters; 15 nm (black), 30 nm (red), 50 nm (green), 100 nm (blue), 200 nm (sky), and 300 nm (pink).



Fig. S7 The changes of (a) transmission at 600 nm of solutions and (b) the sizes of biotinylated MNC aggregations induced by streptavidin at different [Fe] concentrations; 271 μ M (black), 161 μ M (red), 89 μ M (blue), and 48 μ M (pink).



Fig. S8 The detection limits of SA were determined at threshold (5 % ΔT_2)



Fig. S9 The changes of T_2 relaxation times of biotinylated MNC with various amounts of streptavidin (SA) as the concentration of [Fe] decreased.