Supporting Information for

Magnetosome-inspired synthesis of soft ferrimagnetic nanoparticles for magnetic tumor targeting

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Experimental Section

Materials. Iron acetylacetonate (Fe(acac)₃), diphenyl ether, and oleylamine were purchased from Sigma Chemical Ltd (USA). The preparation of Mms6 protein and AMB-1 magnetotactic bacteria was described in the previous work (1, 2). The Cell Counting Kit-8 and Live/Dead Cell Viability Assay Kit were purchased from Biovision (USA). Dodecyl phosphocholine (DPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-mPEG) were purchased from Avanti Polar Lipids, Inc (USA). The Prussian Blue - Nuclear Fast Red Staining Kit was purchased from Sangon Biotech (Shanghai, China) Co., Ltd.

Synthesis of Magnetosome-like MNPs. Typically, Fe(acac)₃ (0.05 mmol) and 1,2-hexadecanediol (0.25 mmol) were mixed in 5 mL of diphenyl ether (containing 0.1% ultrapure water). Then 0.5 mmol of oleylamine and 3 mg Mms6 were added to the reaction mixture in 60 °C water bath for 3 hours to make a homogeneous solution. The solution was then transferred to a Teflon-lined stainless steel autoclave, and heated at 200 °C for 6 hours. After cooling to room temperature, the synthesized nanoparticles were precipitated and washed by ethanol for several times. The samples were stored in the chloroform for further experiments. Control MNPs were obtained at the same conditions as the magnetosome-like MNPs in the absence of Mms6 protein.

Purification of natural magnetosome MNPs. AMB-1 magnetotactic bacteria were cultured as previously reported (2), and then harvested by centrifugation at 8000 g for 20 min. After ultrasonic treatment, neodymium magnets were used to collect the magnetosome components in the supernatant. The skeleton and membrane molecules of magnetosome chains were dissolved with 1 % SDS (90 °C for 2 hours) and separated with neodymium magnet again. Then the purified magnetosome nanoparticles were washed 10 times with deionized water and used for the following experiments.
**Preparation of Water-soluble Magnetosome-like MNPs.** To render the nanoparticle hydrophilic, 2 mL of the organic dispersible ferrimagnetic iron oxide nanoparticles in chloroform (5 mg/mL) was mixed with 1 mL of chloroform solution containing 10 mg DSPE-mPEG. The solvent was removed by rotary evaporation after reaction in 60 °C water bath for 2 hours. Subsequently, 2 mL of ultrapure water was added and sonicated to disperse magnetosome-like MNPs in aqueous solution. The excess DSPE-mPEG was removed by dialysis. The same modification method was carried on the control MNPs.

**Characterization of Mms6 in Different Synthesis Stages.** The self-assembly behavior of nanoreactor was analyzed by DLS. After reactions, the reaction intermediate at 60 °C and reaction product at 200 °C were collected for DLS measurement. Reaction buffer without oleylamine surfactant was used as a control. Protein in the reaction intermediate at 60 °C were precipitated by adding 10 mL of ice acetone (4 °C) and collected by high-speed centrifugation. MNP product after reaction at 200 °C was accumulated by neodymium magnet, and washed twice with alcohol and then deoxidized ultra-pure water. Protein samples after reaction at 60 °C and 200 °C were analyzed by SDS-PAGE after boiled in SDS loading buffer for 5 min, staining with Coomassie brilliant blue. The protein band of SDS-PAGE was cut off and confirmed by LC-MS (liquid chromatography-mass spectrometry).

**1H,15N-HSQC Experiments.** The samples for HSQC experiments were obtained by adding the above Mms6 proteins into TBS buffer (pH 7.5, 20 mM Tris-HCl, 50mM NaCl, 3mM DPC). All 1H,15N-HSQC experiments were accomplished at 25 °C on a NMR spectrometer (600 MHz, Bruker) equipped with a CryoProbe. 1H,15N-labeled Mms6 was expressed and purified from E. coli using M9 cultures (containing 15NH4Cl). More detailed methods were described previously.

**Characterization of Magnetosome-like MNPs.** The TEM and HRTEM images were obtained on a 200 kV field emission TEM (Philips, CM200-TEM). The hydrodynamic size
and zeta potential of PEGylated magnetosome-like MNPs were measured in a Zetasizer Nano ZS analyzer (Malvern, UK). The XRD data were collected on a Philips XPert X-ray powder diffractometer. The FTIR data were measured using an ESCALab220i-XL spectrometer (VG, Thermo). Magnetometry measurements were conducted by a SQUID magnetometer (Model XP-5XL). For SQUID measurements, MNP samples were dried, loaded into gelatin capsules with wax, sealed, and fixed in a clear diamagnetic plastic straw. All metal ion concentrations in this work were measured by inductively coupled plasma mass spectrometry (ICP-MS, Thermo).

**Cytotoxicity Assay.** DSPE-mPEG coated magnetosome-like MNPs with various Fe concentrations (0, 25, 50, 100 μg/mL) were incubated with HepG2 cell lines for 24 h. The cell viability was tested using the cell counting kit-8 (CCK-8) by detecting the 450 nm absorbance. The live and dead stain assay was studied with a live/dead cell staining kit. The red color refers to dead cells with the green color refers to living cells.

**In Vitro MRI.** The MR relaxation rate was measured on 3.0 T scanner (Philips Medical systems) and 14.1 T NMR MicroImaging System (Bruker Biospec, Karlsruhe, Germany). The T₁-weighted MR images on 3 T were acquired using a turbo spin echo (TSE) sequence with the following parameters: TR/TE = 150, 300, 600, 1000, 2000, 4000, 8000/11 ms, slice thickness is 3 mm, flip angle is 90°, the average number of signal is 2. Field of view (FOV) = 120 mm × 120 mm, and matrix size = 240 × 240. T₁-weighted MR images on 14.1 T were acquired using a rapid acquisition with relaxation enhancement (RARE) sequence, details are referred in the following *in vivo* MRI section.
Establishment of Xenografted Tumor Model. The $1 \times 10^7$ 4T1 cell suspension (50 µL) was mixed with matrix gel (50 L) and then inoculated into the right leg of the mice in which the average body weight is 20 g. The volume of the tumor was measured accurately and in vivo MRI was started when it increased to 125 mm³. For in vivo studies, the mice were randomly assigned and 4 mice were used in each experimental group. All mice were cared in obedience to the guidelines outlined in the Guide for the Care and Use of Laboratory Animals.

In Vivo MRI. The in vivo MRI effect of magnetosome-like MNPs were performed on the xenografted tumor model mice. The DSPE-mPEG coated MNPs were injected into mice through the tail vein. The dose of the MNP samples was 0.15 mmol Fe/kg mice body weight in this work. The tumor area of the mice was immediately placed in a permanent magnet (about 0.5 T) for magnetic targeted adsorption, which was accumulated to be treated for 30 min, 60 min and 120 min. Representative MRI slices of the same mouse at different time points of magnetic treatment were presented. Then the mouse body was centered into a 14.1 T NMR MicroImaging System (Bruker Biospec, Karlsruhe, Germany), and then MRI study was performed at different treatment time. T₁-weighted MR images were acquired using a RARE sequence with the following parameters: TR/TE = 3500/10 ms, field of view (FOV) = 25 mm × 25 mm, matrix = 256 × 256, slice thickness = 0.5 mm, number of average = 2. The CNR in the MR images was calculated using the following equation:

$$\text{CNR} = \frac{\text{SI}_{\text{tumor}} - \text{SI}_{\text{muscle}}}{\text{SI}_{\text{noise}}}$$  \hspace{1cm} (1)

$$\Delta \text{CNR} = \frac{\text{CNR}_{\text{post}} - \text{CNR}_{\text{pre}}}{\text{CNR}_{\text{pre}}}$$  \hspace{1cm} (2)

where SI means signal intensities, and post or pre means post- or pre-treatment of magnet. The noise was estimated from the air background.
In Vivo Magnetic Targeting Efficiency Analysis. The mice were sacrificed after magnet treatment in different time, and then collected the tumor and organ tissues. Mice injected with normal saline were used as control. The wet weight of body and tissue from each mouse were measured accurately. The content of iron element in each tissue was figured out by ICP-MS measurement after acid digestion. Four mice were used as replicates in each group, and each nitratred tissue sample was measured 3 times. We selected the tumor tissue, liver, spleen, heart, lung and kidney after magnetic targeting treatment for 1 hour to calculate the targeting efficiency of nanoparticles. The enrichment efficiency is expressed as the percentage of the injection dose (% ID), which is calculated as follows:

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\% \text{ ID} = 100\% \times \left( \frac{\text{IC}_{\text{sample}} - \text{IC}_{\text{control}}}{\text{W}_{\text{tissue}}} \right) \times \frac{\text{W}_{\text{tissue}}}{\text{ID}}
\]

where ID means injected dose, which is the total amount of the iron injected; IC_{sample} means the iron concentration in the tissue of mouse injected with MNP samples; IC_{control} means the average iron concentration in the tissue of mouse injected with physiological saline; W_{tissue} means the wet weight of the tissue.

Half-life in blood. Mice were randomly divided into two groups, i.e. magnetic treatment group and control group. In the first group, the mouse tumor was placed in the external magnet (0.5 T) immediately after injection. Blood samples at 0 min, 10 min, 30 min and 60 min were collected in this period. The external magnetic field was removed after 60 min of magnetic treatment. Then continued the blood collection at 2 h, 6 h and 24 h after the injection. In the control group, the blood samples were collected at 0 min, 10 min, 30 min, 60 min, 2 h, 6 h and 24 h after injection. Each sample was 100 μL from the tail of each mouse, and mixed with heparin sodium to prevent blood clotting. The iron content in each blood sample was measured by ICP-MS after digested with concentrated nitric acid. The concentration of Fe in blood was calculated by subtracting reference value, which
was provided from the mice injected with normal saline. The half-life time was then concluded by the blood clearance profile.

**Prussian Blue Staining Assay.** The tumors were collected after magnet treatment for 60 minutes. All tumor specimens were fixed with 4% paraformaldehyde, and then embedded in paraffin. The Prussian blue staining assay was carried out with a Prussian Blue - Nuclear Fast Red Staining Kit.

**Histopathological Examination.** The mice organs were harvested at the 14th day after tail intravenous injection of DSPE-mPEG coated magnetosome-like MNPs. Mice injected with normal saline were used as control. All organ specimens were fixed with 4% paraformaldehyde, and the fixed organs were subsequently subjected to paraffin-embedded hematoxylin and eosin (H&E) histopathological examination.
**Fig. S1.** The SDS-PAGE analysis after reaction at 200 °C. lane 1, purified Mms6 protein; lane 2, the degraded hydrophobic fragment dissolved in organic phase, with a molecular weight lower than that of protein marker (4.1 kDa).
**Fig. S2.** The MS data corresponding to lane 6 of SDS-PAGE. (a) positive mode; (b) negative mode.
Fig. S3. FTIR analysis of control MNPs (gray), Mms6 protein (brown), natural magnetosome MNPs (green) and magnetosome-like MNPs (blue).
**Fig. S4.** More HRTEM images of magnetosome-like MNPs. (a)-(c) High-resolution electron micrographs from zone axis [100], [111], [-110], respectively. (d)-(f) Top views from zone axis [100], [111], [-110], respectively. (g)-(i) Three-dimensional morphologies.
Fig. S5. XRD pattern of the control MNPs (gray), natural magnetosomes MNPs (green) and magnetosome-like MNPs (blue).
Fig. S6. The (a-b) TEM pictures and (c) size distribution of magnetosome-like MNPs.
Fig. S7. The (a) TEM pictures and (b) size distribution of natural magnetosome MNPs.
Fig. S8. More HRTEM images of MNPs from control reaction. (a)-(b) TEM pictures of control MNPs. (c) The size distribution of control MNPs. (d) High-resolution electron micrograph of a representative crystal with tetrahedron shape. (e) Top view from zone axis [1-10]. (f) Three-dimensional morphology of the tetrahedron shaped magnetic crystal.
Fig. S9. Field-dependent magnetization curves ($M$-$H$) with the magnetic coercivity ($H_c$) and remanence ($M_r$) (see zoomed-in inset) at 5K.
Fig. S10. Fast response of magnetosome-like MNPs to a weak magnetic field (0.05 T).
Fig. S11. $M-H$ curve of DSPE-mPEG coated magnetosome-like MNPs at 300 K. The inset shows the $H_c$ and $M_r$. 
Fig. S12. MRI relaxation data at 3 T and 14.1 T. (a) $r_1$ value at 3 T; (b) $r_2$ value at 3 T; (c) $r_1$ value at 14.1 T; (d) $r_2$ value at 14.1 T.
Fig. S13. Cytotoxic analysis of DSPE-mPEG coated magnetosome-like MNPs. (a) The CCK-8 cell viability assays of HepG2 cells. (b) Live and dead stained fluorescence images of HepG2 cells. Green color: live cells; red color: dead cells; scale bar: 100 μm.
Fig. S14. Zeta potential of the DSPE-mPEG coated magnetosome-like MNPs.
Fig. S15. Hydrodynamic size of the DSPE-mPEG coated magnetosome-like MNPs after being incubated in PBS and serum at 37 °C for different time.
Fig. S16. The T1-weighted MRI gray-scale images corresponding to Figure 5(d).
Fig. S17. The biodistribution of MNPs in heart, lung and kidney after 60 min of magnetic field treatment.
**Fig. S18.** Blood clearance profile of magnetosome-like MNPs.
Fig. S19. H&E staining images of major organs of the mice after 14 days' injection. Scale bar: 100 μm.
Fig. S20. The mutant information of Mms6 protein. (a) SDS-PAGE analysis. lane 1, purified Mms6 protein; lane 2, purified Mms6 mutant protein. (b) Protein sequences of Mms6 (Mms6^{WT}) and Mms6 mutant (Mms6^{MT}).
Fig. S21. The TEM picture of the MNPs regulated by Mms6 mutant.
**Fig. S22.** The enrichment efficiency of the MNPs in liver and spleen tissues. % ID means the percentage of the injected dose. # p < 0.05 compared to the magnetosome-like MNPs treated with a 0.5 T magnetic field.

**Movie S1.** Rapid movement of magnetosome-like MNPs was recorded under an external magnetic field (0.05 T).

**SI References**

1. K. Ma et al., NMR studies of the interactions between AMB-1 Mms6 protein and magnetosome \( \text{Fe}_3\text{O}_4 \) nanoparticles. *J. Mater. Chem. B* **5**, 2888-2895 (2017).
2. L. Le Nagard, V. Morillo-Lopez, C. Fradin, D. A. Bazylinski, Growing magnetotactic bacteria of the genus *Magnetospirillum*: strains MSR-1, AMB-1 and MS-1. *J. Vis. Exp.* **140**, 58536 (2018).