# Supporting Information for:

# Tailored Dual Coating of Magnetic Nanoparticles for Enhanced Drug Loading

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## MATERIALS

$FeCl_3 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$, citric acid (CA), succinic anhydride, were purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). D, L-lactide was purchased from Yuan Shengrong (Beijing, China). mPEG (Mw: 2000) was sourced from Sigma-Aldrich (Beijing, China); Teteaethoxysilane (TEOS), 3-aminopropyltrimethoxysiane (APS), 4-dimethylamino pyridine (DMAP), stannous octanoate [$Sn(Oct)_2$], N-hydroxy succinimide (NHS), dicyclohexylcarbodiimide (DCC), and paclitaxel (PTX) were obtained from Aladdin (Tianijn, China). Ethanol, toluene, triethylamine, dichloromethane (DCM), ammonia (25%-28%) and other chemicals were purchased from Jiangtian Chemicals (Tianjin, China).

## METHODS

### Synthesis of $Fe_3O_4$ nanoparticles

The superparamagnetic $Fe_3O_4$ nanoparticles were generated by the co-precipitation method. In brief, $FeCl_3 \cdot 6H_2O$ (0.91 g) and $FeCl_2 \cdot 4H_2O$ (0.43 g) were mixed together in 100 mL deionized water under nitrogen atmosphere at 80$^{o}$C followed by the addition of 14 mL ammonia. The reaction was maintained for 2 h and then the product was cooled to ambient temperature followed by repeated wash with water and ethanol. The washed product (21 g) was then mixed citric acid (42 g) in aqueous media under sonication for 30 min followed by gentle stirring for 12 h to get citric acid-stabilized $Fe_3O_4$.

### Synthesis and activation of mPEG-PLA

Amphiphilic copolymer mPEG-PLA was synthesized via the ring opening polymerization of lactic acid at the presence of mPEG with $Sn(Oct)_2$ as the catalyst (**Fig. S1**). In detail, mPEG (5.0 g) and lactic acid (5.6 g) was mixed in a round-bottomed flask maintained at 60$^{o}$C followed by the addition of catalyst (5.6 mg). The temperature was then raised to 125$^{o}$C and the reaction lasted for 24 h under nitrogen protection. Afterwards, the crude product was dissolved in DCM, precipitated with cold diethyl ether, and then vacuum-dried to get mPEG-PLA. Then mPEG-PLA (7.0 g), succinic anhydride (1.1 g), and DMAP (0.5 g) were dissolved in 100 ml DCM followed by dropwise addition of triethylamine (300 μL). The reaction was maintained at ambient temperature for 24 h. The crude product was treated with hydrochloride solution (3%) to remove the remaining reactants and catalyst, then precipitated using cold diethyl ether, and vacuum-dried to get carboxyl-terminated mPEG-PLA (i.e. mPEG-PLA-COOH). Last, mPEG-PLA-COOH (3.44 g),

DCC (0.21 g), and NHS (0.17 g) were mixed in 50 ml DCM followed by slow addition of triethylamine (140 μL). The reaction was also maintained at ambient temperature for 24 h and the activated mPEG-PLA (mPEG-PLA-NHS) was purified using similar approach described above. The successful synthesis was verified by proton nuclear magnetic resonance ($^1$H NMR) with $CDCl_3$ as the solvent (**Fig. S2**).

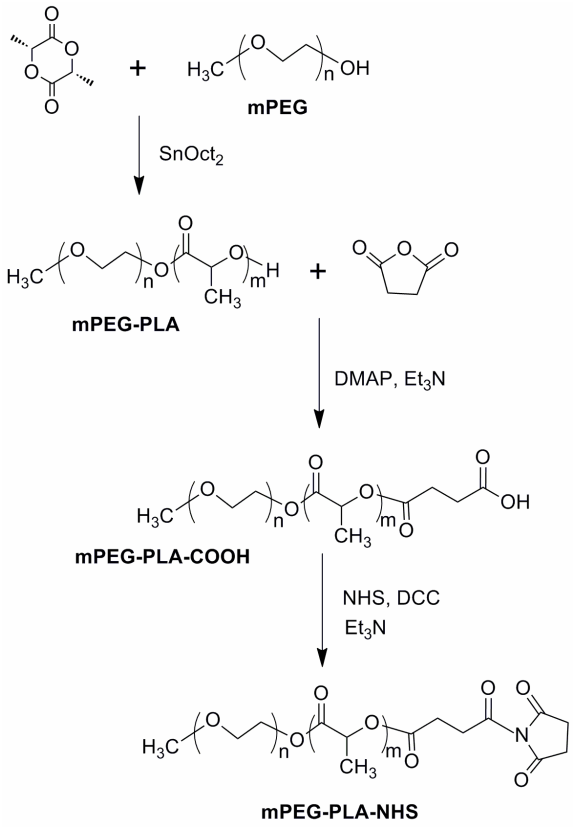


**Figure S1**. The synthesis and activation route of mPEG-PLA.

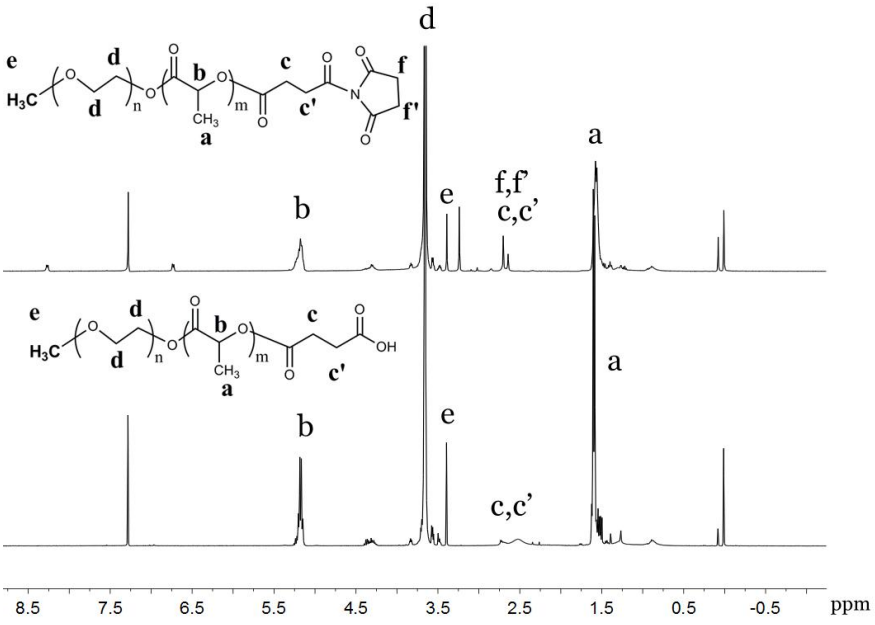


**Figure S2**. The $^1$H-NMR spectra of MPEG-PLA-COOH and MPEG-PLA-NHS in $CDCl_3$

**Synthesis of silica and polymer coated SPIONs**

Coating $Fe_3O_4$ with silica and polymer was a three-step process (**Fig. S3**). In the first step, citric acid-stabilized $Fe_3O_4$ nanoparticles (80 mg) were added to a mixture of ethanol (60 mL) and water (20 mL).TEOS (200 or 600 μL) dissolved in ethanol (20 mL) was carefully added to the above solution under nitrogen atmosphere followed by sonication for 30 min and additional stirring for 5 h. $Fe_3O_4$–$SiO_2$ was purified by washing the above product with ethanol and water in triplicate, and then freeze-dried ready for use. Secondly, hydroxyl-terminated $Fe_3O_4$–$SiO_2$ (80 mg), APS (2 mL), and triethylamine (300 μL) were mixed in toluene (50 mL) under nitrogen atmosphere and sonicated for 1h followed by reaction at ambient temperature for 12 h. The crude product was magnetically separated and washed with toluene and DCM to get amino-terminated $Fe_3O_4$–$SiO_2$ (i.e. $Fe_3O_4$-$SiO_2$-APS). Last, the coating of polymer was achieved via the formation of amide bond between $Fe_3O_4$-$SiO_2$-APS (40 mg) and mPEG-PLA-NHS (2.0 g). The reaction was carried out in DCM with nitrogen protection and lasted for 24 h. Afterwards the products were magnetically separated and washed with DCM to remove excess polymer, vacuum-dried to get silica and polymer coated SPIONs ($Fe_3O_4$-$SiO_2$-Polym).

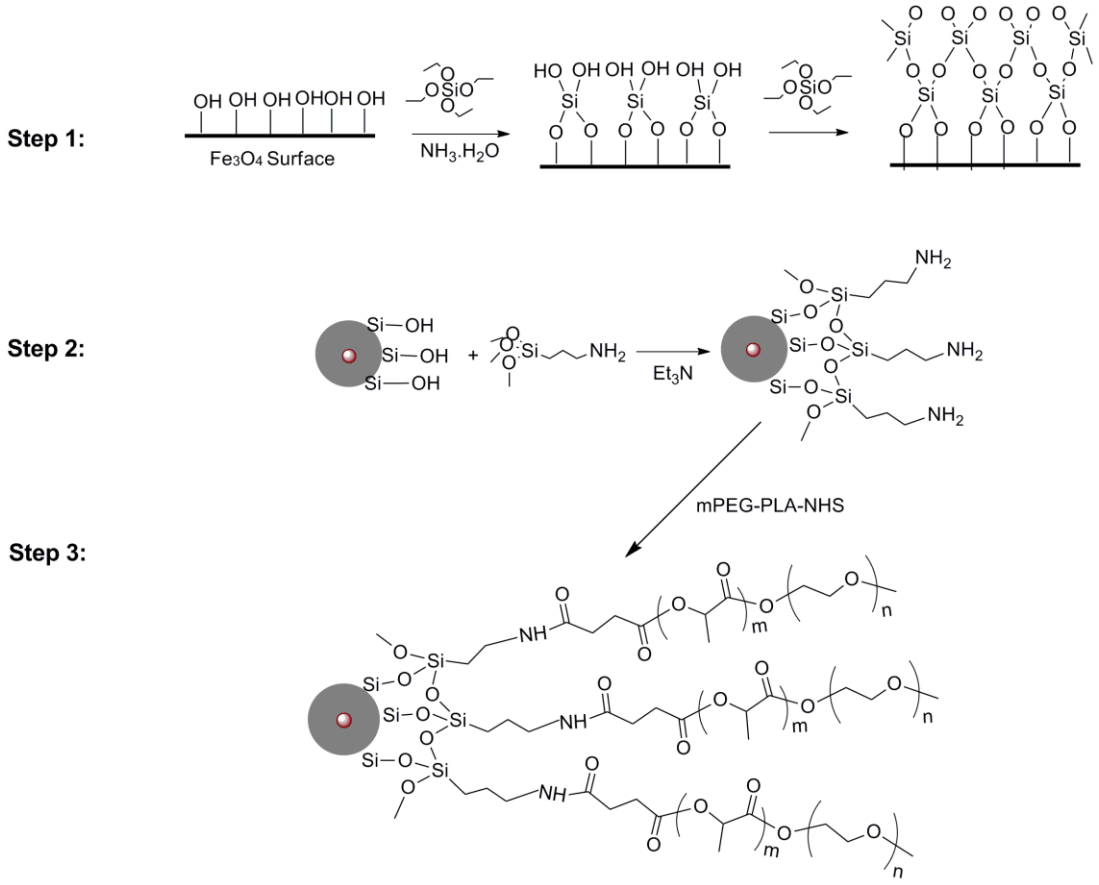


**Figure S3**. The illustration of coating $Fe_3O_4$ with silica and mPEG-PLA.

**Synthesis of polymer-coated SPIONs**

The citric acid-stabilized $Fe_3O_4$ (40 mg), APS (0.5 mL), and triethylamine (0.4 mL) were added to toluene (25 mL) under nitrogen protection and sonicated for 1 h followed by reacting at ambient temperature for 12 h. Then the APS-modified $Fe_3O_4$ (i.e. $Fe_3O_4$-APS) and polymer-coated SPIONs (i.e. $Fe_3O_4$-Polym) were obtained using a similar protocol described above (Figure S3, Step 2-3).

**SPIONs characterization**

The molecular weight of mPEG-PLA was obtained via $^1$H NMR analysis that employed a Bruker AVANCE III 400M NMR instrument with tetramethylsilane as the internal standard and $CDCl_3$ or as the solvent. FTIR analysis was performed using a Tensor 27 spectrometer via the KBr disc method. XRD was employed to examine the crystal structure of SPIONs; the testing was carried out in a Rigaku D/MAX—2500 instrument at 40 kV/25 mA with the Cu Kα (λ=0.154 nm) radiation. The content of Fe in SPIONs was quantified by inductively coupled plasma atomic emission spectroscopy (Varian VISTA-MPX). The magnetization investigation used a LDJ 9600-1 vibrating sample magnetometer (VSM) at 300 K under a magnetic field of 10 kOe. TEM (JEOL JEM-100CX II) was exploited to probe the morphology and size of SPIONs. The hydrodynamic diameters of particles were measured by dynamic light scattering using a Brookhaven Zeta plus instrument (n =3). As the control, the VSM profile and TEM image of citric-acid stabilized $Fe_3O_4$ without any additional coating was shown in **Fig. S4**. The hydrodynamic diameter and size measured by TEM of $Fe_3O_4$, $Fe_3O_4$-$SiO_2$, and $Fe_3O_4$-$SiO_2$-Polym were summarized in **Table S1**.

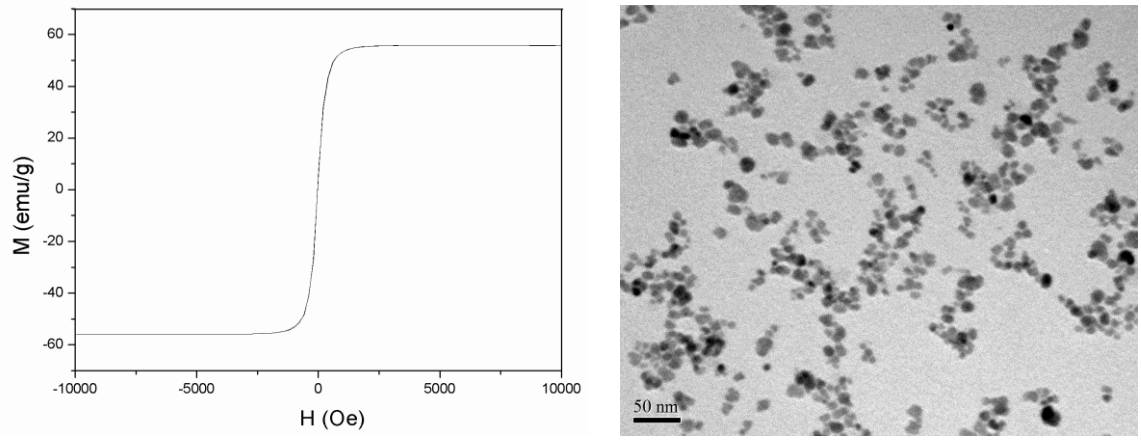


**Figure S4**. The VSM profile (*left*) and TEM image (right) of citric acid stabilized $Fe_3O_4$.

**Table S1**. The size of five different SPIONs by TEM and DLS analysis. For TEM data, more than 200 particles from 3 batches of samples were analysed. The thickness of coating for type II sample is larger than that of type I.

| SPIONs Type | TEM size (nm) | DLS size (nm) |
|---|---|---|
| $Fe_3O_4$ | 10 ± 1 | — |
| $Fe_3O_4$-$SiO_2$ (I) | 63 ± 11 | 82 ± 1 |
| $Fe_3O_4$-$SiO_2$ (II) | 86 ± 14 | 107 ± 1 |
| $Fe_3O_4$-$SiO_2$-Polym (I) | 117 ± 14 | 180 ± 3 |
| $Fe_3O_4$-$SiO_2$-Polym (II) | 156 ± 15 | 218 ± 4 |

**Drug loading and release**

Defined amount (50 mg) of SPIONs ($Fe_3O_4$-$SiO_2$-Polym (II)) was put to 10 mL DCM followed by addition of excess paclitaxel (100 mg) with stirring for 2 h. Then the organic solvent was evaporated under vacuum and the residue was transferred to 5 mL water with stirring. Eventually the drug-loaded nanoparticles were obtained post centrifugation and supernatant lypophilization. Drug quantification was achieved by high performance liquid chromatography (Dionex ULTIMATE 3000 HPLC) using a reverse-phase Kromasil $C_{18}$ column (250 mm x 4.6 mm, 5 μm). The mobile phase was a mixture of methanol, acetonitrile and water (35:40:25, v/v); the UV detection limit was 229 nm and the flow rate was set at 1 mL $min^{-1}$ with a 20 μl injection volume. Drug release study was carried out by a simple dialysis method (n = 3). Regenerated cellulose membrane (molecular weight cut-off: 14,000 Da) was used to separate the drug-loaded SPIONs and release media (phosphate buffered saline/PBS, pH 7.4). The temperature was set at 37$^{o}$C. Sink conditions were maintained by increasing the PBS volume due to the limited aqueous solubility of paclitaxel. At defined time points, the drug concentration was analysed by HPLC and the cumulatively released drug was plotted against the time to get the release profile.