Supporting Information

Multifunctional Magnetic and Fluorescent Core-shell Nanoparticles for Bioimaging

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Materials. Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, Tianjin Fuchen Chemical Reagent Factory) and ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, Xilong Chemical Co., Ltd.), oleic acid (OA, Tianjin Fuchen Chemical Reagent Factory), carboxymethyl chitosan (CMCS, Shandong Aokang Biological Technology Co., LTD, molecular weight of 200000), N-(3-dimethylaminopropyl)- N′-ethylcarbodiimide (EDC, Alfa Aesar), and N-hydroxysuccinimide (NHS, Alfa Aesar) were used as purchased without further purification. Sodium chloride, (3-aminopropyl) triethoxysilane (APTES), tetraethyl orthosilicate (TEOS), cyclohexane, hexanol, ethanol, disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), ninhydrin and ammonia aqueous solutions (25%) were purchased from Beijing Chemical Plant and were used directly.

Characterizations. Morphology of NPs was observed with a JEOL JEM-3010 high resolution transmission electron microscope **(**HRTEM**)** manipulated at an accelerating voltage of 200 KV. Samples were prepared by placing a drop of the as-synthesized NPs dispersion in ethanol on a clean copper grid, and then evaporating at ambient temperature.

Powder X-ray diffraction (XRD) patterns were recorded on a D/max2500VB2+/Pc X-ray diffractometer (Rigaku) using Cu Kα radiation in the 2θ range 5^o-90^o.

X-ray photoelectron spectroscopy (XPS) analyses of dried powder samples were conducted on a VG ESCALAB MKII X-ray photoelectron spectrometer (VG Scientific Ltd., UK) with Al Kα radiation. Survey spectra were recorded for 0-1350 eV binding energy range.

Zeta-potential measurements were carried out on a Brookhaven 90 Plus/BI-MAS particle size analyzer with ultrasonically redispersed NPs in water at 25℃.

Magnetic characterization was carried out on a vibrating sample magnetometer (VSM, Jilin University JDM-13 VSM) at room temperature.

Fourier transform infrared (FTIR) spectra were recorded with a Nicolet-50 DXC FTIR spectrophotometer. Dry samples were prepared as KBr pellets at room temperature.

The ultraviolet-visible (UV-vis) and fluorescence (FL) spectra were used to characterize the optical properties, which were measured in open-sized 1 cm path-length quartz at room

temperature using a spectrophotometer (Cintra 20, GBC, and Australia) and a fluorescence spectrofluorimeter (Horiba Jobin Yvon FluoroMax-4 NIR, NJ, USA), respectively.

Isothermal titration calorimetry (ITC) analyses were carried out on an isothermal titration calorimeter (Nano ITC SV, TA instruments, USA).

Synthesis of Fe_3O_4 NPs. The preparation of OA coated Fe_3O_4 NPs was performed according to the literature[1]. 2.35 g $FeSO_4 \cdot 7H_2O$ and 4.1 g $FeCl_3 \cdot 6H_2O$ were dissolved into 100 mL deionized water in a flask. This solution was stirred for 30 min under nitrogen atmosphere, followed by fast adding 25 mL ammonia aqueous solutions (25%) at room temperature. The solution color changed from orange to black, resulting in a black precipitate. Then, with vigorous stirring, 1 mL OA was added into the dispersion dropwise at 80 °C in 1 h. The mixture was allowed to react another 1 h under nitrogen atmosphere. After cooling to room temperature, 0.5 g sodium chloride was added into the system. Then, the above mixture solution was transferred to an extractor. The Fe_3O_4 NPs were received in organic phase after extracting with cyclohexane. Under the protection of single layer of OA, the Fe_3O_4 NPs had good dispersibility in cyclohexane. After the cyclohexane was removed under reduced pressure, the further drying in vacuum for 12 h gave the Fe_3O_4 NPs as a black power.

Synthesis of Fe_3O_4@SiO_2 NPs. The Fe_3O_4@SiO_2 core-shell NPs were synthesized through a reverse microemulsion method[2]. Typically, 6 mg of dried Fe_3O_4 NPs was dispersed in 38 mL cyclohexane and then 10 g triton X-100, 8 mL hexanol and 1.7 mL H_2O were added under ultrasonication at room temperature. Subsequently, 0.2 mL TEOS was added to the above mixture solution. After 4 h of stirring, 0.6 mL ammonia aqueous solutions (25%) was added dropwise to initiate the TEOS hydrolysis and the reaction was continued to proceed at room temperature for 24 h under constant mechanical stirring. Ethanol was added to the solution to form dark precipitates, which were collected by magnetic separation. The dark precipitates were further purified by ultrasonication in ethanol for several times to remove surfactant and unreacted reactants, and then dried under vacuum for 12 h.

Synthesis of Fe_3O_4@SiO_2-NH_2 NPs. Amine-functionalized Fe_3O_4@SiO_2 NPs were prepared by introduction of APTES according to the reported method[3]. 10 mg of Fe_3O_4@SiO_2 NPs were dispersed in the mixture of ethanol (20 mL) and water (4 mL) under ultrasonication and a mechanical stirring. Subsequently, 0.48 mL of ammonium aqueous solutions (25%) and 0.4 mL of APTES were added to above solution and the mixture was stirred for 24 h at room temperature. The NPs were separated from the solution via centrifugation, purified by washing with ethanol several times, and then dried in vacuum for 10 h.

Synthesis of Fe_3O_4@SiO_2-NH-CMCS NPs. Briefly, 10 mg of Fe_3O_4@SiO_2-NH_2 NPs were dispersed in 30 mL water under ultrasonication. 5 mg of CMCS, 5 mg of EDC, and 6 mg of NHS were added into the mixture under ultrasonication for 10 min at 0 °C. Then, the mixed solution was further stirred for another 24 h at room temperature. Afterwards, the NPs were separated from the solution through centrifugation, washed with deionized water and ethanol, and then dried in vacuum for 10 h.

Synthesis of Fe_3O_4@SiO_2-CMCS-Cy5 NPs. 0.1 mg of the functional fluorescent dye (Cy5-NH_2), 5 mg of EDC and 6 mg of NHS were dissolved in 30 mL phosphate buffer solution (0.2 mol/L Na_2HPO_4-NaH_2PO_4, pH=6.0). 10 mg of the above prepared Fe_3O_4@SiO_2-NH-CMCS NPs were added into the buffer mixture under sonication at 0 °C for 10 min and then the mixture was warmed to room temperature. The mixed solution was continued for 24 h with mechanical stirring at room temperature. After the reaction, the NPs were separated from the solution through centrifugation, washed with deionized water and ethanol, and then dried in vacuum for 10 h. The final product was saved in refrigerator for biological application.

Cytotoxicity Assay. Cell viability was monitored using Tali™ viability kit-Dead Cell Green (Invitrogen, Catalog A10787) that was a green-fluorescent nuclear and chromosome stain. It does not penetrate intact membranes, but easily penetrate compromised membranes characteristic of dead cells. The measurement was performed at 48 h post-incubation of Fe_3O_4@SiO_2-CMCS-Cy5 NPs. Replace the fresh cell medium after 48 h of incubation and then adds 1 μL Dead Cell Green into 100 μL cell medium for 0.5 h incubation.

Cellular Uptake. Used cell line was mouse osteoblast cell. In a typical procedure, 7.5×10^4 cells were plated in a 35 mm petri dish for 4 h to allow the live cells to attach. Cells were washed with PBS and incubated with cell culture medium containing Fe_3O_4@SiO_2-CMCS-Cy5 NPs (40 μg mL^{-1}) for 24 h at 37 °C. After incubation, the cells were washed several times with PBS to remove the remaining particles and dead cells, and then observed under a fluorescent microscope.

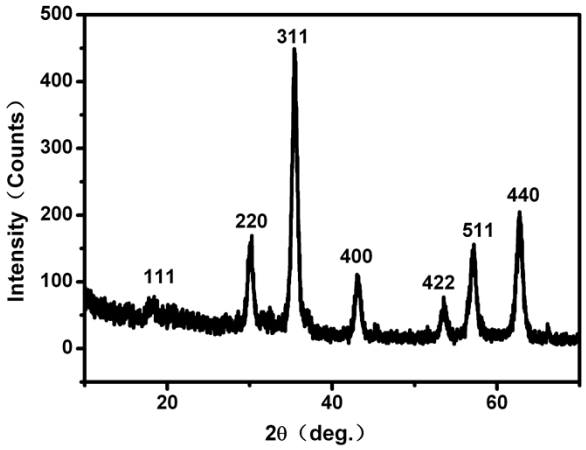

Figure S1. XRD pattern of Fe_3O_4 NPs.

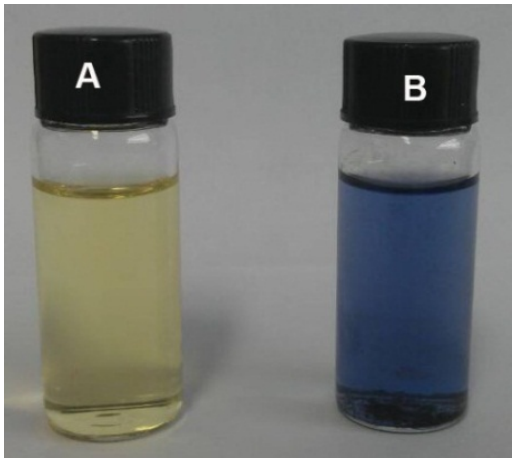

Figure S2. The solution of (A) Fe_3O_4@SiO_2 and (B) Fe_3O_4@SiO_2-NH_2 NPs after ninhydrin reaction.

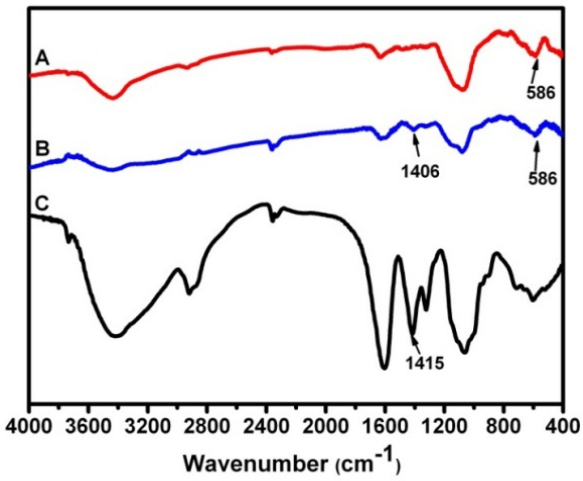

Figure S3. FTIR spectra of (A) Fe_3O_4@SiO_2-NH_2, (B) Fe_3O_4@SiO_2-CMCS and (C) CMCS.

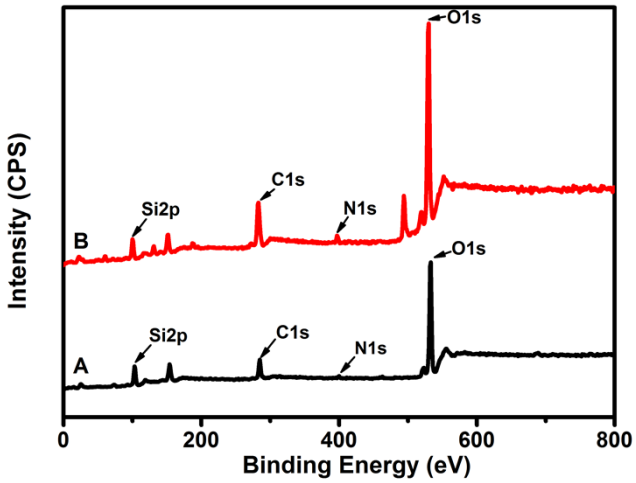

Figure S4. XPS pattern of (A) SiO_2-NH_2 NPs and (B) SiO_2-NH-CMCS NPs.

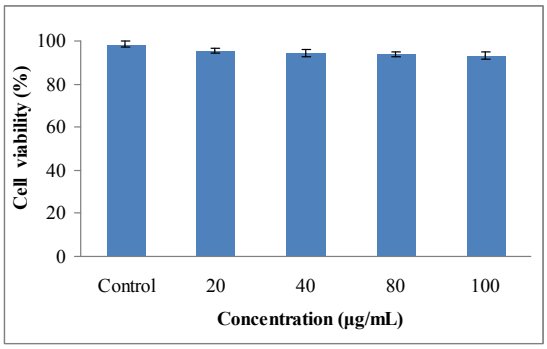

Figure S5. Cell viability assay of Fe_3O_4@SiO_2-CMCS-Cy5 NPs.

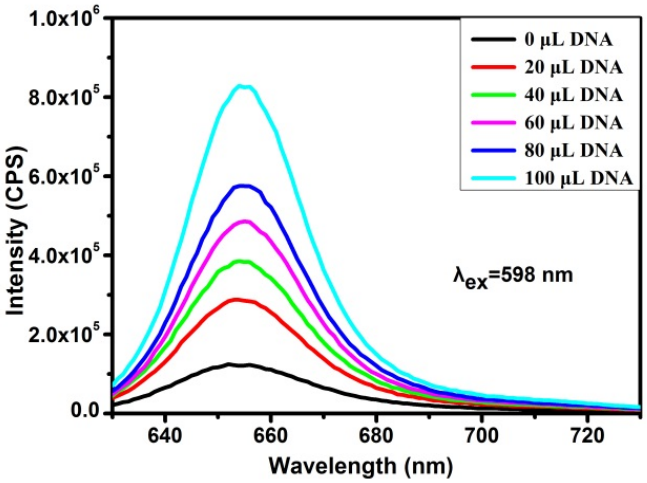

Figure S6. Fluorescence spectra of Fe_3O_4@SiO_2-CMCS-Cy5 NPs NPs/DNA in water with DNA concentration from 0 to 100 μL (excitation at 598 nm).

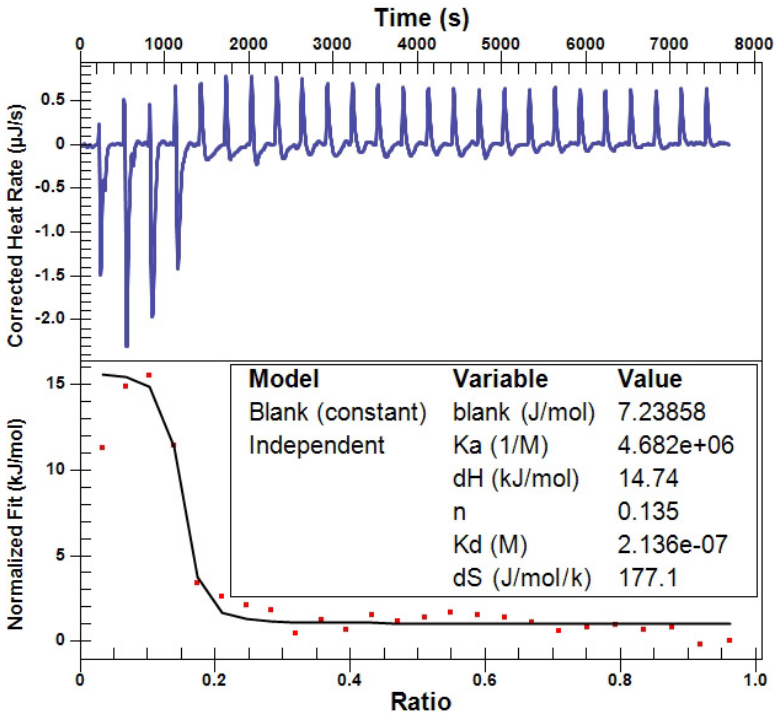

Figure S7. ITC titration of Fe_3O_4@SiO_2-CMCS-Cy5 NPs with DNA.

1. Y. Sun, X. Ding, Z. Zheng, X. Cheng, X. Hu and Y. Peng, *Eur. Polym. J.*, 2007, **43**, 762-772.
2. A. Z. M. Badruddoza, M. T. Rahman, S. Ghosh, M. Z. Hossain, J. Shi, K. Hidajat and M. S. Uddin, *Carbohydr. Polym.*, 2013, **95**, 449-457.
3. Y. Zhang, S. Pan, X. Teng, Y. Luo and G. Li, *J. Phys. Chem. C*, 2008, **112**, 9623-9626.