Electronic Supplementary Information (ESI)

Synthesis of Hollow Silica Nanospheres with Microemulsion as Template

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Experimental Section

Materials: Cyclohexane, *n*-hexanol (98%), ammonia hydroxide (28~30 wt%), tetraethylorthosilicate (TEOS, 98%), (3-aminopropyl)trimethoxysilane (APTS, 95%), polyoxyethylene(10)isooctylphenylether (TritonX-100), fluorescein isothiocyanate isomer I (FITC, 90%), Iron(III) acetyl acetonate ($Fe(acac)_3$, 99+%), and benzyl ether (99%) were purchased from Acrös. 1,2 dodecanediol (90%), oleylamine, and oleic acid (90%) were purchased from Aldrich. All reagents were used as supplied without further purification.

Synthesis of silica hollow nanospheres (process A): Hollow silica nanospheres (HS) were synthesized by a (W/O) reverse microemulsion method with APTS addition. The W/O reverse microemulsion was prepared by slightly modifying the method of Santra et al.[1] First, 29.65 g of cyclohexane, 10 g of Triton X-100, 8 mL of hexanol, and 1.7 mL of water were mixed at room temperature to generate the microemulsion system. Then, TEOS was added to the mixture. Two hours later, 500 μL of aqueous ammonia (28~30 wt %) was introduced to initiate the hydrolysis of TEOS. After 2-12 h, 200 μL of ethanolic solution of APTS was added and stirred continuously for 24 h. The mole ratio used was APTS: TEOS: EtOH: NH_3: H_2O: cyclohexane =1: 4.6: 22.5: 39.7: 1206: 3678. The reaction temperature was kept at 20 ^oC. Finally, ethanol was added to destabilize the microemulsion system. The as-synthesized nanospheres were isolated via centrifugation and washed with ethanol to remove surfactant and unreacted chemicals. After that, the product was soaked in water for a week to form the hollow spheres, HS. Dye containing hollow silica nanospheres, FITC-HS, were synthesized

by adding a mixture of APTS and an ethanolic solution of *N*-1-(3-trimethoxysilylpropyl)- *N*'-fluoresceylthiourea instead of APTS.

Synthesis of multifunctional silica hollow nanospheres (process A): The multifunctional hollow nanospheres (Fe_3O_4@FITC-HS) were obtained by adding 10 nm hydrophobic magnetic nanoparticles, Fe_3O_4 (1.4 mg) in cyclohexane and *N*-1-(3-trimethoxysilylpropyl)-*N*'-fluoresceylthiourea ethanolic solution and following the procedure described above of synthesis of silica hollow sphere. The hydrophobic Fe_3O_4 nanoparticles were synthesized by thermal decomposition method, e.g. reducing $Fe(acac)_3$ with 1,2-dodecanediol and protected by oleic acid and oleylamine in benzyl ether.[2]

Synthesis of silica yolk/shell nanoparticles (process B): The silica yolk/shell nanoparticles were synthesized by slightly modifying silica hollow sphere. Thus, 10 g of Triton X-100 was mixed with 29.65 g of cyclohexane, 8 mL of hexanol, and 1.7 mL of water at room temperature to generate the microemulsion system. Then, 100 μL of TEOS and variable amount of ethanolic solution of APTS were added to the mixture. The APTS ethanolic solution was prepared by 1.4 mL of 99.5% ethanol and 200 μL of APTS. Two hours later, 500 μL of aqueous ammonia (28~30 wt %) was introduced to initiate the hydrolysis of silanes, and the mixture was stirred for 36 h. The reaction was kept at room temperature. Finally, ethanol was added to destabilize the microemulsion system. The as-synthesized nanospheres were isolated via centrifugation and washed with ethanol and water to remove surfactant and unreacted chemicals, then soaked in H_2O for a week to form yolk/shell spheres.

Confocal fluorescence microscopy: HeLa cells were plated onto chambered slides 24 h prior to incubation with Fe_3O_4@FITC-HS at a concentration of 5×10^4 cells per well. After that, cells were treated with Fe_3O_4@FITC-HS (40 μg mL^{-1}) for 1 h at 37 °C in serum-free medium followed by phosphate-buffered saline (PBS) wash, and then further incubated in particle-free medium overnight. Treated cells were fixed with 4% paraformaldehyde and the cell skeleton and nucleus were stained with rhodamine phalloidin and DAPI, respectively. Finally, stained cells were mounted for confocal microscope (Leica TCS SP5) examinations.

***In-Vitro* T_2-weighted MR image:** *In-vitro* T_2- weighted MR images were acquired with a 4.7 T MR scanner (Bruker Biospec). To prepare the cells for MRI, 1×10^5 HeLa cells were incubated with Fe_3O_4@FITC-HS (40 or 80 μg mL^{-1}). After 1 h incubation, the cells were washed twice with PBS solution, trypsinized, and collected in 0.5 mL

centrifuge tubes by centrifugation. After centrifugation, the centrifuge tubes were placed in a water bath and were then scanned in a 4.7 T MR scanner (Bruker Biospec), repetition time (TR)/echo time (TE) = 4000 ms/80 ms, field of view (FOV) = 6 cm, number of excitations (NEX) = 4, spectral width (SW) = 41666.7 Hz, slice thickness (Slth) = 2.0 mm, matrix = 256×256.

Cell culture: HeLa, MCF-7, or NIH3T3 cells (American Type Culture Collection) were cultured in DMEM (GIBCO) containing fetal bovine serum (GIBCO, 10%) and penicillin/streptomycin (GIBCO, 1%) in a humidified atmosphere with CO_2 (5%) at 37 °C.

Cell proliferation assay: Typically, 1×10^5 cells per well were seeded in 24-well plates for proliferation assays. After incubation with different amounts of Fe_3O_4@FITC-HS suspension in serum-free medium for 1 h, cells were allowed to grow in regular growth medium for 24 h followed by incubation with fresh serum-free medium containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (0.5 mg mL^{-1}) for 4 h at 37 °C for proliferation assay. The dark blue formazan dye generated by the live cells was proportional to the number of live cells and the absorbance at 570 nm was measured using a microplate reader (Bio-Rad, model 680). Cell numbers were determined from a standard plot of known cell numbers versus the corresponding optical density. As shown in Figure 4S, cell proliferation was not hindered by the presence of Fe_3O_4@FITC-HS.

Supplementary Figures:

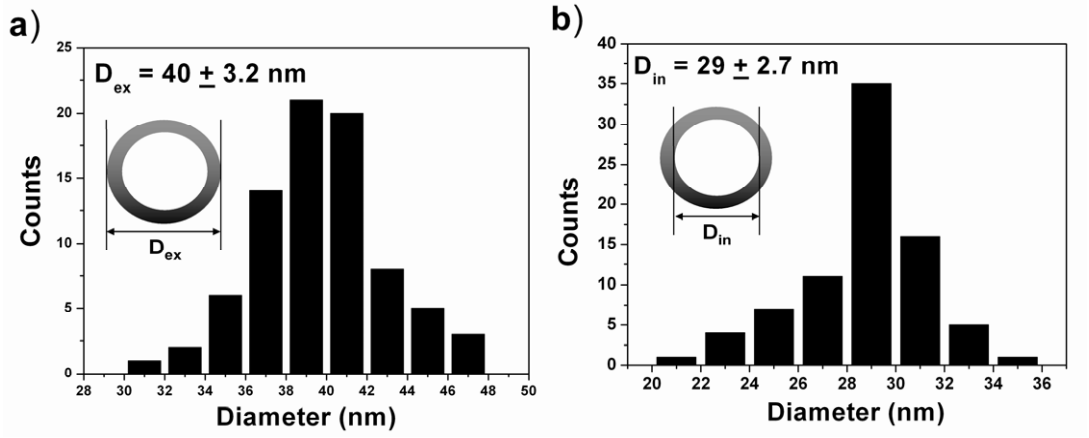

Fig. S1 Size distributions of a) external diameter and b) internal diameter of FITC-HS.

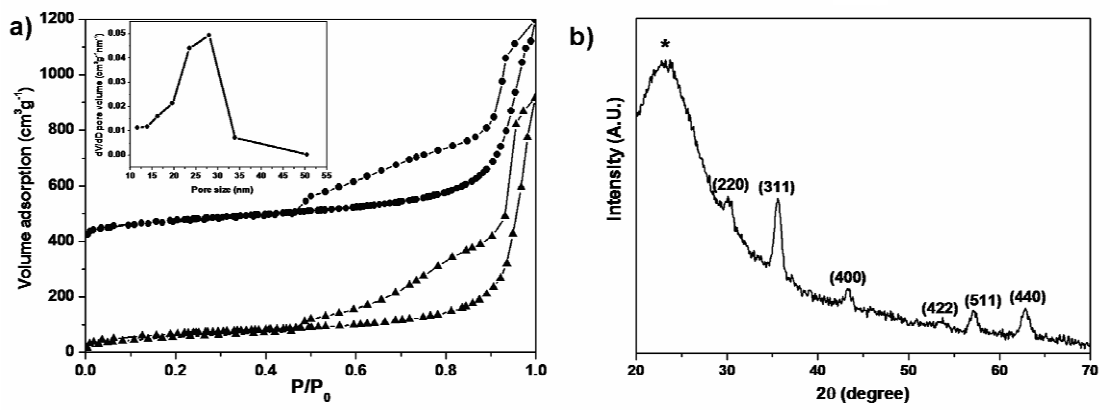

Fig. S2 a) N_2 adsorption and desorption isotherms of FITC-HS and Fe_3O_4@FITC-HS; b) XRD of Fe_3O_4@FITC-HS; * is originated from amorphous silica.

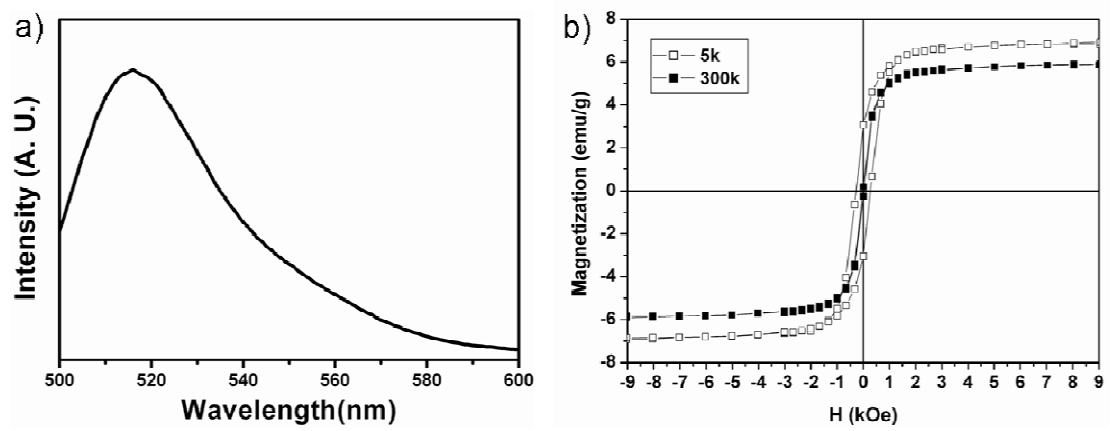

Fig. S3 a) Photoluminescence spectrum (λ_{ex}= 480 nm) of aqueous suspension of FITC-HS; b) field-dependent magnetization curves of Fe_3O_4@FITC-HS at 300 K and 5 K.

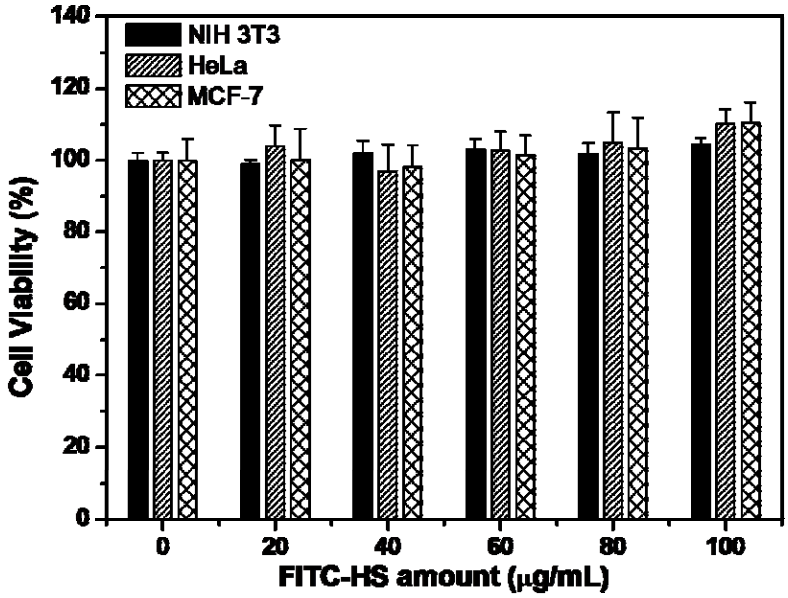

Fig. S4 The cytotoxic effect of Fe_3O_4@FITC-HS on cell proliferation.

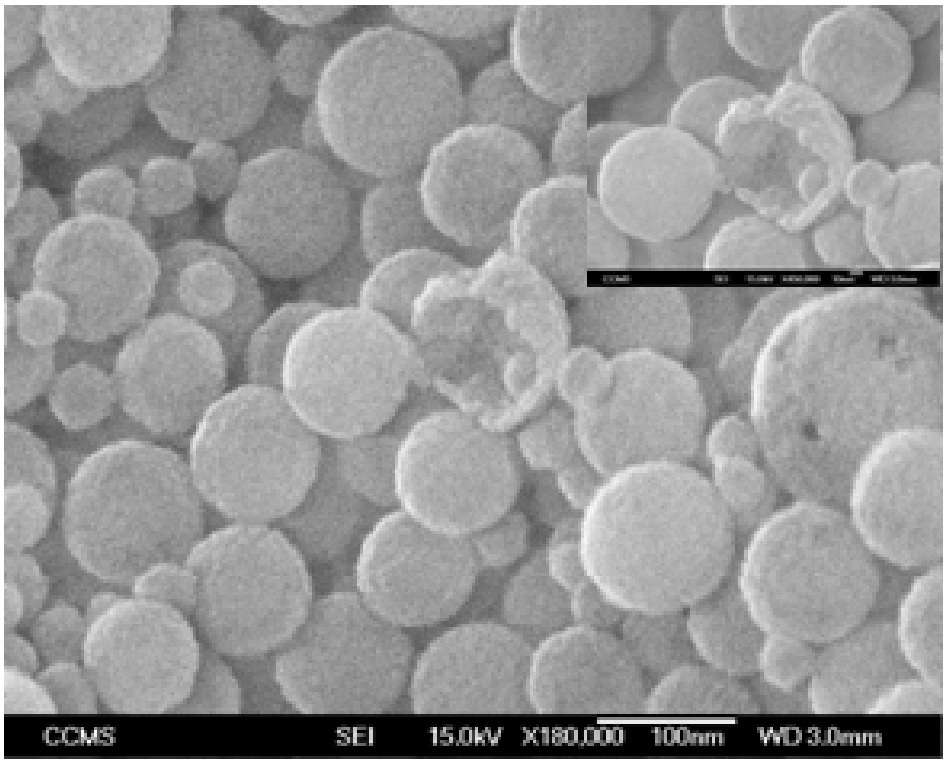

Fig. S5 SEM image of ball-in-sphere, a broken shell shows the hollow interior having a ball inside.

For examining the correlation between microemulsion droplet size and the size of the hollow spheres, we made additional samples with different particle sizes. In Table S1, we show the correlation between the droplet sizes of the W/O microemulsion (by DLS) and the sizes of the resulting silica particles (measured both by both DLS and TEM). The four samples of different sizes are produced by procedure A and procedure B of three different amounts of APTS. One can see that there is roughly a linear correlation between the droplet sizes of microemulsion and the product particle size as measured by TEM, with the former always greater than the later. This result quantifies our hypothesis that that the nanoparticles are formed inside the water domain in the W/O microemulsion. Also shown is that the mean particle sizes as measured in suspension by DLS are somewhat bigger than the sizes measured by TEM. This indicates a small degree of aggregation for the smaller particles (A_200) while for the bigger ones (B_200) the aggregation is pretty minor. Even for the A_200 sample, the particle sizes in solution (DLS) are small enough that they are well-suspended. Also shown in Table S1 is the zeta potential of the four samples. They can be divided into two groups. The smaller particles have small zeta potential and they tend to stick to each other to some extent. On the other hand, the two samples with larger particle sizes also have high zeta potential and they do not tend to aggregate. The surface charge determines the degree of aggregation which is quite reasonable.

Table S1

	Microemulsion DLS (Average nm)	Products DLS (Average nm)	Products Zeta (Average mV)	Products TEM (nm)
A_200	243	132	5.5	40±3
B_50	285	144	-5.2	56±27
B_100	308	152	12.2	100±41
B_200	351	156	8.0	129±20

*Sample ID reflects the synthesis process used (A or B) and the amount of ethanolic APTS added (50, 100, or 200μL).

*The average diameter of water droplet in W/O microemulsion determined by DLS is 7 nm.

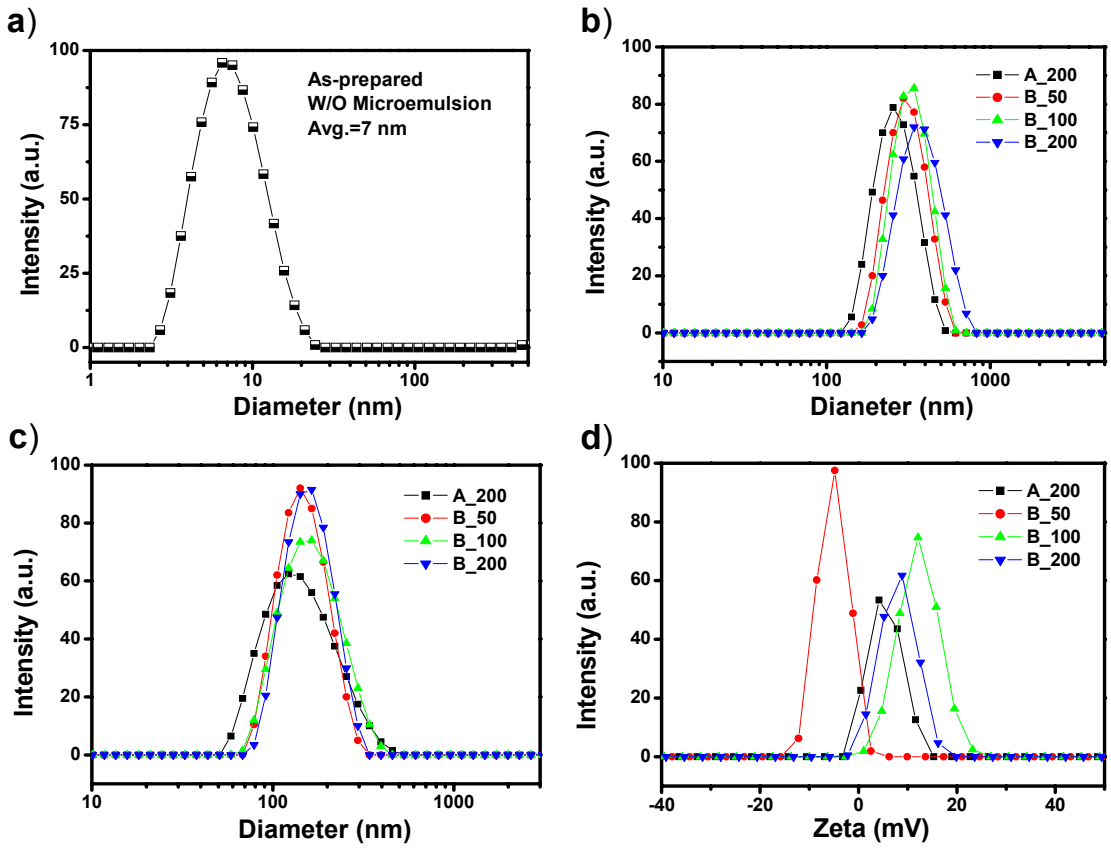

Fig. S6 Hydrodynamic diameter distribution of a) as-prepared W/O microemulsion (water, oil, surfactant, and co-surfactant); b) W/O microemulsion during synthesis process A and B; c) hollow silica nanospheres and yolk/shell silica nanospheres suspended in deionized water. d) Zeta potential measurements of hollow silica nanospheres and yolk/shell silica nanospheres.

1. S. Santra, R. P. Bagwe, D. Dutta, J. T. Stanley, G. A. Walter, W. Tan, B. M. Moudgil, R. A. Mericle, *Adv. Mater.* 2005, **17**, 2165.
2. S. H. Sun, H. Zeng, *J. Am. Chem. Soc.* 2002, **124**, 8204.