Supporting Information:

One-Pot Synthesis in Polyamines for Preparation of Water-Soluble Magnetite Nanoparticles with Amine Surface Reactivity

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General Information:

Synthesis medium triethylenetetramine and Fe(acac)$_3$ were purchased from SIGMA and used as received. FITC conjugated streptavidin and normal mouse IgG conjugated by PE-Cy5 were purchase from Millipore and Santa Cruz Biotechnology, respectively. Other chemicals and solvent were purchased from SIGMA and Alfa Aesar and used without further purification. Product structure and phase purity were determined by X-ray diffraction with a Phillips X`pert system equipped with a graphite monochromator (CuKα radiation, λ=1.54056 Å). Size distribution, particles morphology and crystallinity were studied on a JEOL 2010 transmission electron microscope (TEM). Surface composition was examined by Thermo Nicolet Nexus 670 FT-IR machine in 650-4000 cm^{-1} region and Kratos AXIS 165 X-ray Photoelectron Spectroscope. Thermogravimetric analysis (TGA, TA SDT Q600) and inductively coupled plasma atomic emission spectroscopy (ICP-AES, Varian Vista-MPX) were used to calculate the net weight of iron oxide. The magnetic properties were measured by Quantum Design SQUID magnetometer (MPMS XL-7) in the temperature range 5-300K. Photoluminescence spectra were collected on a PerkinElmer LS 55 Fluorescence spectrometer.

Preparation of 7 nm Fe_3O_4 nanoparticles.

Under a flow of argon, 0.353g (1 mmol) Fe(acac)$_3$ was mixed with 20 ml TETA. This mixture was first heated to 110 °C for 20 min to complete dissolved the solid. The resulting solution was gradually heated to 210 °C for two hours and then, heated to reflux for another 1 hour. After cooling to room temperature, the solid product was magnetically collected and washed with acetone for five times. The nanoparticles were dispersed in polar solvents such as water or alcohol.

Preparation of 10 nm Fe_3O_4 nanoparticles using 7 nm nanoparticles as seeds.

Under a flow of argon, 0.353 g (1 mmol) and 5 ml ethanolic dispersion of 7 nm Fe_3O_4 nanoparticles (10 mg/ml) were added to 20 ml TETA. The mixture was first heated to 110 °C for 20 min to remove ethanol. The mixture was heated to 210 °C for 2 h., then further to reflux for another 1h. After cooling to room temperature, the solid produce was collected and washed following same procedures described in the synthesis of 7 nm nanoparticles. By repeating this cycle, larger Fe_3O_4 nanoparticles can be made.

Preparation of Au-Fe_3O_4 nanocomposite.

The synthesis of 2-3 nm gold nanoparticles was based on Duff et al method. In a typical experiment, 0.5 ml 1M NaOH was diluted by 45 ml deionized water, then mixed with 1 ml tetrakis(hydroxymethyl)phosphonium chloride (THPC 80%) solution for 5 min. Then, 1.5 ml 1% $HAuCl_4$ solution was quickly added and the solution was further stirred for 5 min.

5 ml of colloidal gold solution was introduced into 2 ml water dispersion of Fe_3O_4 nanoparticles, and the mixture was stirred for 6 h. The solid product was collected by a magnetic field and washed with deionized water for five times to remove excess amount of gold particles. The final product was redispersed in ethanol or water by sonication.

Preparation of QDs- Fe_3O_4 nanocomposite.

Different sized CdSe-ZnS core-shell quantum dots are synthesized according to the literature and dried under vacuum.[1] 5 mg of CdSe/ZnS was dispersed in 2 ml chloroform and mixed with ethanol and magnetic nanoparticles water dispersion in a 2:2:1 ratio. The solution was stirred for 6 h. The solid product was isolated by a permanent magnet, washed with chloroform for 5 times to remove excess amount of quantum dots and redispersed in ethanol. In order to enhance the solubility of QDs/Fe_3O_4 nanocomposites, 30 mg 5-aminopentanol-1 was added and the solution was stirred for another 3 h. The solid part was collected, washed and redispersed in ethanol.

Preparation of biotin conjugated Fe_3O_4 nanoparticles.

In a 10 ml flask, 100 mg EDC, and 10 mg biotin were subsequently added to 3 ml buffer solution and stirred for 15 min. Separately, 10 mg TETA coated iron oxide nanoparticles were dispersed in 2 ml buffer by sonication and mixed with EDC activated biotin for 4 h at room temperature. The solid produce was washed with ethanol for five times and dried under vacuum.

Investigation of binding and separation of protein with biotin-Fe_3O_4 nanoparticles.

An amount of 0.1 mg biotin conjugated Fe_3O_4 nanoparticles were first dispersed in 100 µl PBS buffer solution. Then, 100 µl FITC conjugated streptavidin (50 µg/ml) or PE-Cy5 conjugated normal-mouse IgG was added to the nanoparticles dispersion. The mixture was incubated for 30 min with shaking. The nanoparticles were collected by a small permanent magnet and redispersed in PBS buffer solution. The separation of FITC-streptavidin from a mixture of proteins was carried out in a similar procedure.

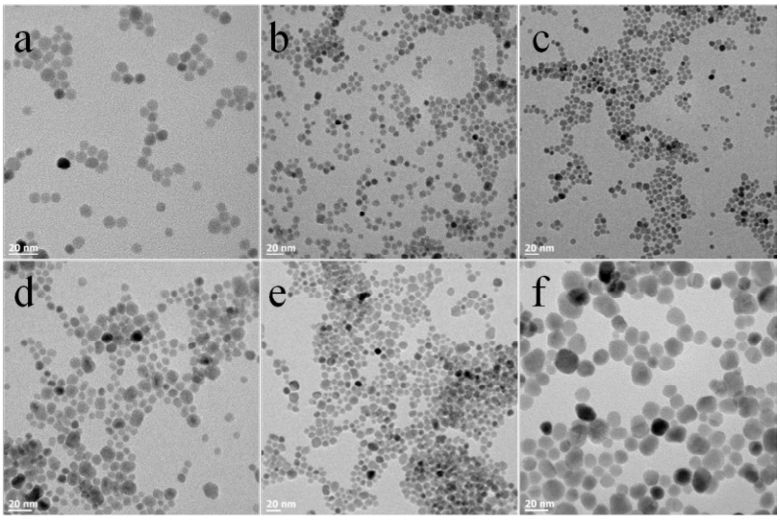

Fig. S1 TEM images of Fe_3O_4 nanoparticles prepared under varied condition a) normal condition with heating rate of 2°C/min, b) 5°C/min, c) 10 °C/min, d) 20 °C/min, e) prepared through a injection technique, f) doubled concentration of iron source.

From TEM studies of nanoparticles in various synthesis conditions, it is suggested that mild heating ramp is important to achieve narrow size distribution while rapidly heating the system from room temperature to reflux (20°C/min) would result in nanoparticles with broad size distribution. It is probably because that the decomposition and nucleation are not a fast process, and the system would enter growth phase before the formation of the nuclei is finished when applying a steep heating ramp. To further confirm this speculation, instead of heating the mixture gradually to reflux, we conduct the synthesis through an injection technique. The iron precursors are pre-dissolved in TETA under protection of argon and neat polyamine solvents were heated to reflux temperature. Then, the iron source was introduced to the hot solvents by a rapid single injection which induced an immediately color change from light yellow to black, indicating the formation of iron oxide nanoparticles. Under similar situation, nanoparticles prepared with an injection technique usually yield nanoparticles with very narrow distribution. This is because rapid addition of iron precursor above decomposition temperature would raise the concentration of iron source above nucleation threshold immediately and cause a short nucleation burst. Further nucleation does not occur because of the depletion from the burst, which minimize the percentage of growth during nucleation period. Assuming that the reaction system is uniform, the size distribution of the particles is largely determined by the time over which the nuclei are formed and begin to grow.[2] Therefore, slow growth of nuclei from a short nucleation produces nanoparticles with very high uniformity.[3] However, in our method, synthesis with an injection technique only produce nanoparticles with broaden size distribution compared to gradually heating method, implying that a controlled slow decomposition and nucleation process is crucial in receiving uniform product.

Under the same heating rate, double the concentration of iron precursor would not only result larger nanoparticles but also decrease the uniformity. In a concentrated system, nuclei saturate at early stage and start to grow while a large fraction of the reactant is under decomposition and forming new nuclei, resulting a mixed phase of nucleation and growth, which lead to larger size deviation.

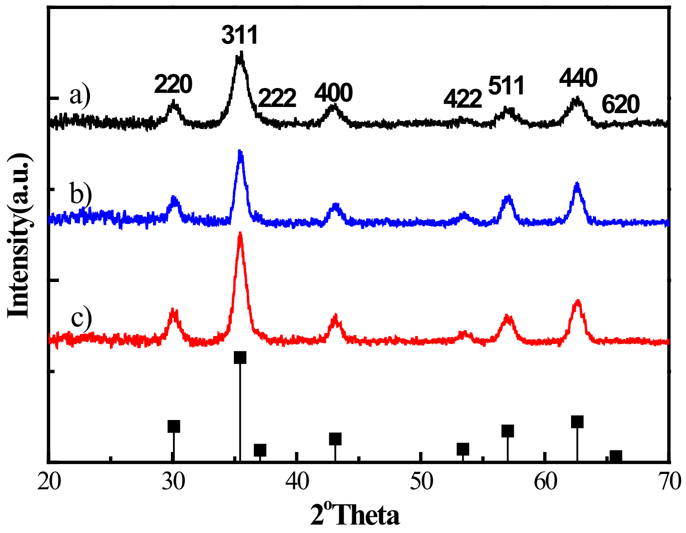

Fig. S2 Powder X-ray diffraction patterns for (a) 7.4, (b) 9.8, (c) 11.9 nm and standard reference pattern of magnetite (JCPDS No. 19-0629)

Due to the similar diffraction pattern of magnetite and maghemite, lattice parameters are calculated and compared with standard bulk materials. The calculated lattice parameters for 7.4 nm, 9.8 nm and 11.9 nm nanocrystals are 8.381, 8.392 and 8.394 Å, which are very close to the lattice parameter of standard bulk magnetite (8.396Å) and far from the one of maghemite (8.346Å). Along with the information from XPS measurement, the results strongly indicate that the as-prepared nanoparticles are in single phase of magnetite.

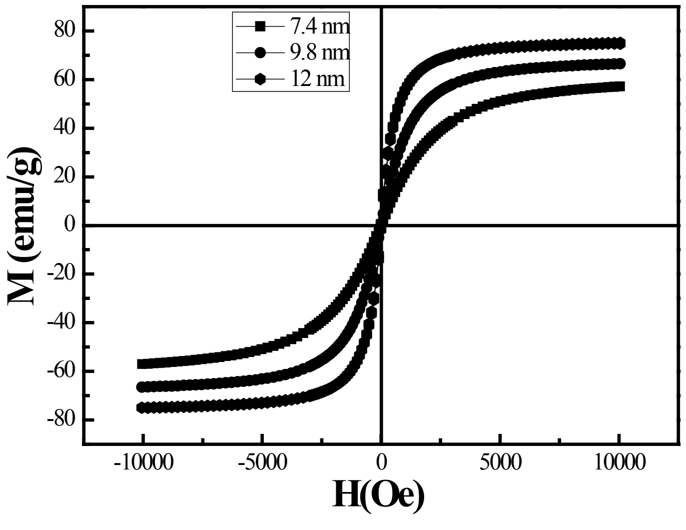

Fig. S3 Hysteresis loops of different sized TETA coated magnetite nanoparticles at 300K. After removing organic layer, the normalized saturation magnetizations are 75.8 emu.g^{-1}, 79.5 emu.g^{-1} and 82 emu.g^{-1} for 7.4 nm, 9.8 nm and 12 nm Fe_3O_4, respectively.

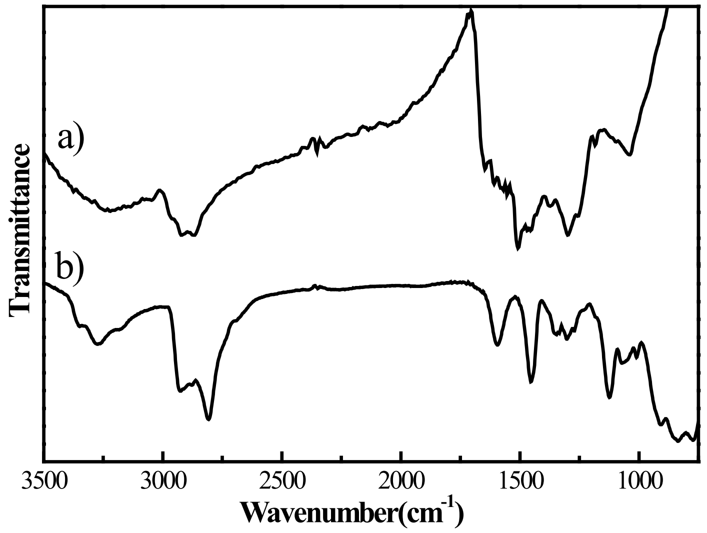

Fig. S4 FT-IR spectra of a) Polyamine coated Fe_3O_4. b) Neat TETA.

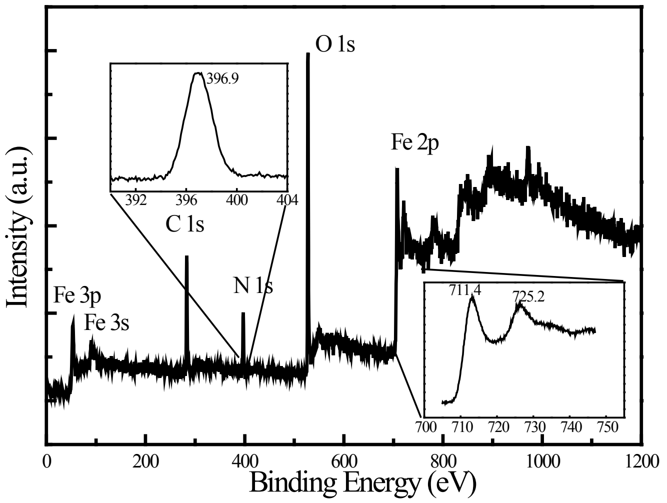

Fig. S5 XPS spectra of as-prepared TETA coated nanoparticles.

XPS was used to precisely indentify the composition of as-prepared NPs. From the scan, the two photoelectron peaks at 711.4 eV and 725.2 eV are very close to the previously reported binding energy value of core-level Fe 2p lines in magnetite.[4] Additionally, based on the fact that iron and oxygen element can only come from iron oxide (TETA contains only nitrogen and carbon), the calculated atomic ratio of iron and oxygen (Fe:O=1.33 for magnetite) also confirm the present of single phase magnetite of as-prepared NPs.

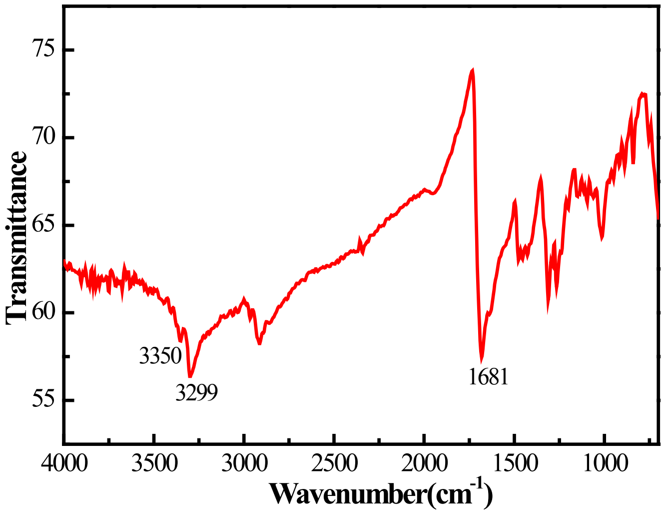

Fig. S6 FT-IR of biotin conjugated TETA coated Fe_3O_4 nanoparticles. After the conjugation, an intense peak appeared at 1681 cm^{-1} which is ascribed to the associated amide I band, and amide II band is located at 1550 cm^{-1} which is mainly N-H, mixed with C-N.

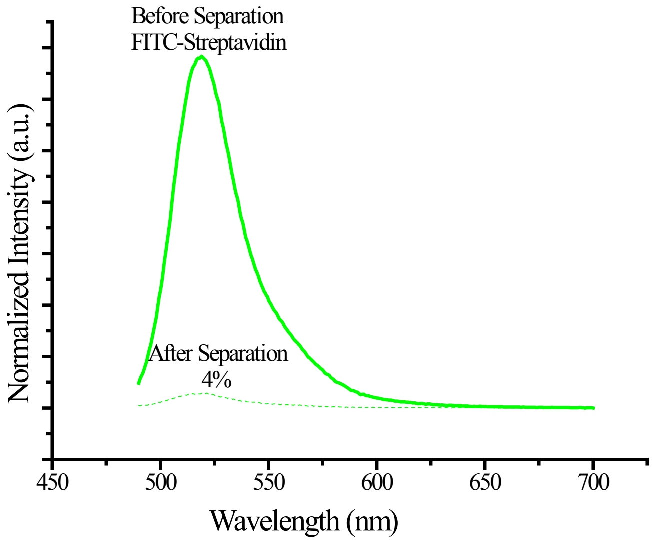

Fig. S7 Fluorescence spectra of biotinylated Fe_3O_4 nanoparticles dispersion before and after the mixing with FITC-streptavidin.

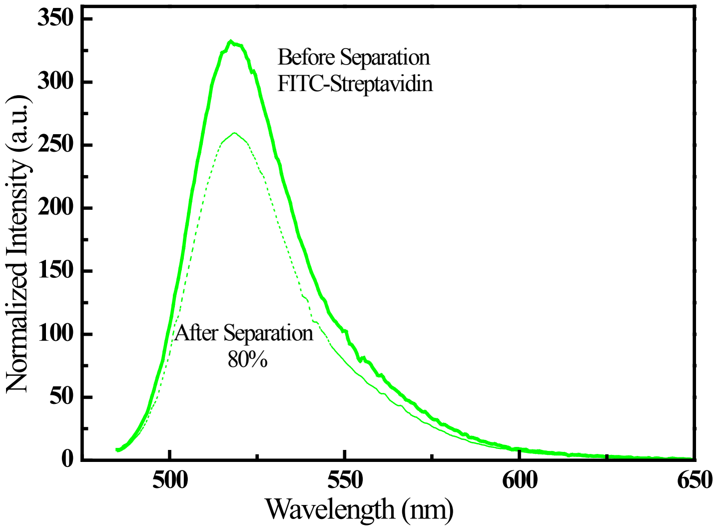

Fig. S8 Fluorescence spectra of as-prepared nanoparticles dispersion before and after the mixing with FITC-streptavidin.

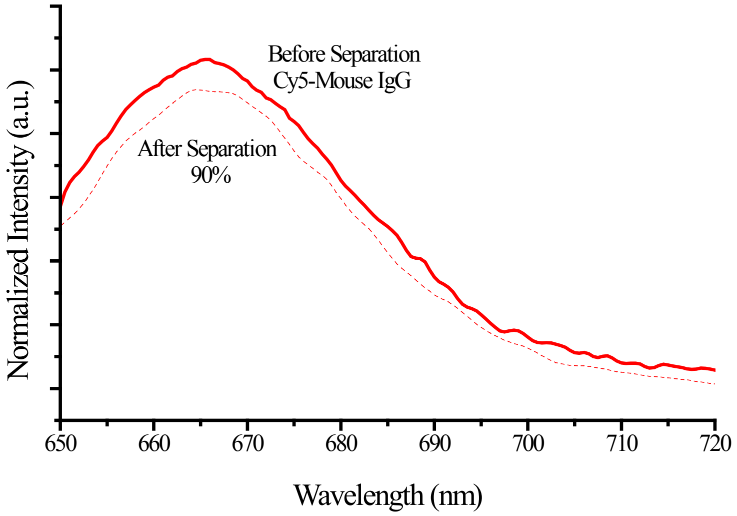

Fig. S9 Fluorescence spectra of biotin-conjugated nanoparticles dispersion before and after the mixing with Cy5-mouse IgG. Note: Relatively smaller decrease in the fluorescence intensity after the separation indicates weaker non-specific interactions. The conjugation of biotin consume a portion of amine groups which decrease the amount of positive charge on the surface of nanoparticles and lead to weaken electrostatic interactions. Therefore, the nonspecific interaction between protein and Fe_3O_4 nanoparticles is smaller when biotin is coupled to the surface.

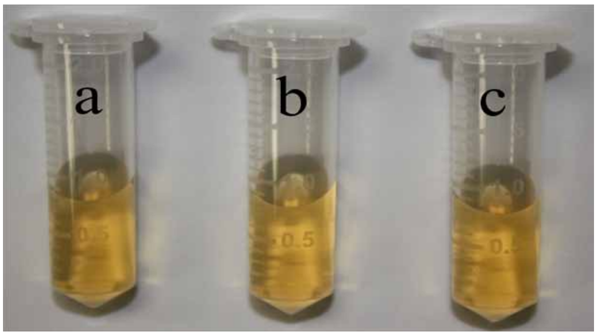

Fig. S10 Colloidal solution of 7.4 nm as-prepared magnetite nanoparticles stored under ambient condition for 2 months (a) water (b) Phosphate buffer, pH=5.8 (c) DMSO

Diameter (nm)	Zeta Potential	Hydrodynamic Radius (nm)	Polydispersity
7.4	45.2	14.5	0.105
9.8	49.5	17.9	0.102
11.9	55.7	19.8	0.116

Table S1. Colloidal properties of different sized as-prepared magnetite nanoparticles

	7.4 nm	9.8 nm	11.9 nm
Capping ligand per particle	1120	1840	2310

Table S2. The number of surface capping ligand molecules with respect to each functionalized nanoparticle. From XPS measurement, the atomic ratio between Fe and N for 7.4 nm particles is 1.9. This suggests that there are roughly 1120 TETA molecules on the surface of nanoparticles. This results were also confirmed by the data from TGA and ICP-AES studies.

Reference:

1. D. Wang, J. He, N. Rosenzweig and Z. Rosenzweig, *Nano. Lett.*, 2004, **4**, 409-413.
2. C. B. Murray, C. R. Kagan and M. G. Bawendi, *Annual Review of Materials Science*, 2000, **30**, 545-610.
3. S. Liu, G. Chen, P. N. Prasad and M. T. Swihart, *Chem. Mater.*, 2011, **23**, 4098-4101.
4. X. Teng, D. Black, N. J. Watkins, Y. Gao and H. Yang, *Nano. Lett.*, 2003, **3**, 261-264.