Supplementary Material (ESI) for Chemical Communications

Green Upconversion Nanocrystals for DNA detection

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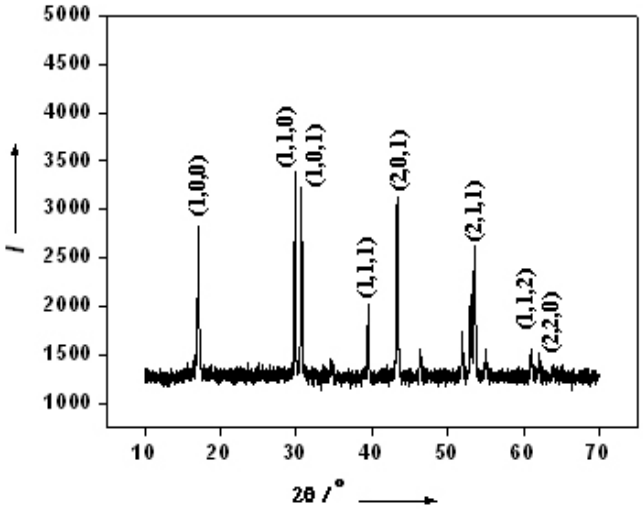

Figure S1. XRD pattern of the hexagonal phase $NaYF_4:Yb^{3+}/Er^{3+}$ nanoparticles.

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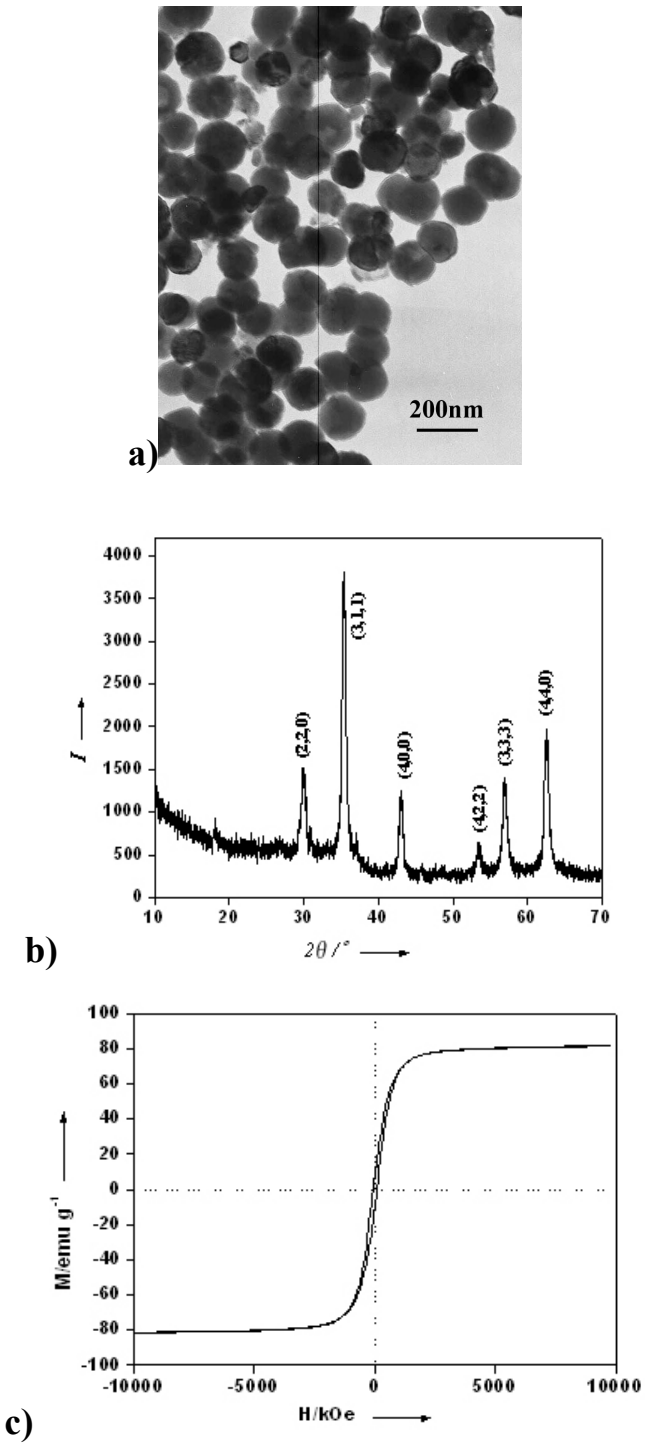

Figure S2. TEM image (a), XRD pattern (b) and room-temperature magnetization curve (c) of the as-prepared Fe_3O_4 nanoparticles. The XRD pattern can be easily indexed to Fe_3O_4 (JCPDS 82-1533).

LbL Fabrication and Identification of the Luminescent and Magnetic Nanoparticles

As we demonstrated before,[1] the $NaYF_4:Yb^{3+}/Er^{3+}$ nanoparticles have been fabricated by alternatively deposition of positively charged polymer (Poly-allylamine hydrochloride, PAH, Mw, 8000-11000, Aldrich) and negatively charged polymer (Poly-sodium 4-styrenesulfonate, PSS, Mw 13400, Fluka). According to the same procedure, the magnetite nanoparticles have also been coated with the functional PAH/PSS/PAH multilayer at pH 8.5. Both the zeta-potential and Fourier-transform infrared (FTIR) technology have been conducted to identify the functionalized PAH/PSS/PAH multilayer. The characterization of functional $NaYF_4:Yb^{3+}/Er^{3+}$ nanoparticles have been reported in our previous work[1] and here we only demonstrate the identification of the functional magnetite nanoparticles. At pH 7.0, the acidity adopted in the following DNA detection, the zeta-potential of the fabricated magnetic nanoparticles correspondingly changed from positive to negative with the alternative deposition of PAH and PSS on the surfaces of the nanoparticles, which indicated that the LbL fabrication is successful. The FTIR results further identified the surface chemical structure of the LbL fabricated nanoparticles. The appearance of the N-H vibrational band at 741 cm^{-1} and the strong N-H stretching bands at 3437 cm^{-1} are attributed to the existence of $–NH_2$ group resulted from PAH. Accordingly, the weak bands at 1641 cm^{-1} and 1421 cm^{-1} can also be assigned to the skeleton C-C vibrations of the benzene ring in PSS. Therefore, the results of both zeta-potential and FTIR demonstrated that the LbL fabrication is successful. The bioconjugation of the functionalized nanoparticles with nucleic acids was achieved through the $–NH_2$ groups on the functionalized nanoparticles according to the reported protocol.[2, 3]

Functionalization of Magnetic and Luminescent Nanoparticles with SMPB

Both amine-modified magnetite nanoparticles and luminescent nanoparticles were further functionalized with succinimidyl-4-(p-maleimidophenyl)butyrate (SMPB, Sigma).[2] In a typical experiment, 100 mg (0.024 nmol) magnetite nanoparticles were added into 10 mL DMSO/ethanol (v/v, 4:1) mixture solvent and ultrasonicated for 10 min. In order to allow the amino group on the outer surface of the nanoparticles to sufficiently react with SMPB, 3.5 mg SMPB was added and vortexed overnight at 30 ^oC. And then SMPB functionalized magnetite nanoparticles were purified with magnetic separation technology. In the case of luminescent nanoparticles, 27.5 mg (0.8 nmol) of amine-functionalized nanoparticles were modified with SMPB following the same procedure.

Preparation of DNA Functionalized Magnetic and Luminescent Nanoparticles

All DNA oligomers were purchased from Bioasia Biotechnology Company (Shanghai, China). The following DNA sequences were employed in our experiments:

DNA1 (probe): 3′ propylthiol- terminated DNA,
5′ TCC-ATG-CAA-CTC-TAA-A_{10}-$(CH_2)_3$-SH,
DNA2 (capture): 5′ propylthiol- terminated DNA,
3′ AAT-TGA-GGA-GAA-AGA-A_{10}-$(CH_2)_3$-SH,

DNA3 (target): complementary "linker",
5′ TTA-GAG-TTG-CAT-GGA-TTA-ACT-CCT-CTT-TCT 3′,
Our strategy to bioconjugate nanoparticles with thiol-modified oligonucleotides followed a published procedure.[2, 3] The luminescent nanoparticles were derivatized with modified ssDNA oligomers (probe, DNA1) by incubating ~80 nM of nanoparticle solution overnight with 780 nM of oligonucleotides in 0.3 M PBS (0.3 M NaCl, 10 mM phosphate buffer saline, pH 7.0) overnight at 37 ^{o}C. Following removal of the supernatant, the modified nanoparticles were washed twice with 0.3 M PBS by successive centrifugation and redispersion and then finally redispersed in fresh 0.3 M PBS. Passivation of areas of the nanoparticle surrounding the oligonucleotide spots was carried out by redispersing the nanoparticles in a solution of cysteine (0.1mM) for 2 h to cap off the remaining SMPB sites. Finally, these nanoparticles were washed with ultrapure water. In the case of magnetic nanoparticles, bioconjugated magnetic nanoparticles were prepared by adding alkanethiol oligonucleotides (capture, DNA2) to aqueous magnetite nanoparticle solution (particle concentration ~2.4 nM) to a final oligonucleotide concentration of 780 nM. Then the solution was buffered at pH 7.0 (10 mM phosphate), and NaCl solution was added (final concentration of 0.3 M). The solution was allowed to "age" under these conditions for 12 h. Unbound oligonucleotides were subsequently removed by three magnetic separation and resuspension of pellet cycles. To cap off the remaining SMPB sites, the DNA modified magnetic nanoparticles were passivated with cysteine as above. It should be mentioned that both the DNA modified magnetic and luminescent nanoparticles should be treated with 10% bovine serum albumin (BSA, Sigma) solution for 4h at room temperature to diminish the nonspecific absorption before use to the detection of target oligonucleotides. Finally, the DNA modified nanoparticles were stored at 4 ^{o}C for use.

Detection of DNA

Based upon the hybridization of target DNA strand to phosphor nanoparticle probes with surface immobilized alkanethiol-capped oligonucleotides, DNA detection schemes have been reported in this work. In a typical experiment, target oligonucleotide of different concentration was added to DNA2 modified magnetic nanoparticle solution (0.12 nM) and annealed at 37 ^{o}C for 12 h. Then the unreacted target DNA (DNA3) was removed by magnetic separation and washing with 0.3 M PBS (0.3 M NaCl, 10 mM PBS, pH 7.0) buffer solution (three times) and redispersed. Afterwards, DNA probe (DNA1 modified UC phosphor colloidal solution, 4 nM) was added and aged for another 12 h to effect hybridization with the overhanging region of the target sequence. And then the resultant nanocomposites were treated with magnetic separation and rising to remove the unhybridized probes. During each rising step, the nanoparticles were separated from the supernatant by using magnetic force. And then, the nanocomposite was redispersed in 0.3 M PBS to fluorescence analysis. The upconversion luminescence spectra and intensities were followed at 545 nm with a F-4500 fluorescence spectrophotometer (Hitachi, Japan), equipped with a 980 nm laser as the excitation light.

Chemicals and Instruments

All the chemicals were of analytical grade and used as received without further purification. Deionized water was used throughout. The phase purity of the nanoparticle products was examined via XRD by using a Brucker D8-advance X-ray diffractometer with Cu Kα radiation (λ = 1.5418 Å), keeping the operation voltage and current at 40 kV and 40 mA, respectively. The 2θ range used was from 20 to 70° in steps of 0.02° with a count time of 2s. The size and morphology of the nanoparticles were observed by using a Hitachi Model H-800 transmission electron microscope. Samples were prepared by placing a drop of a dilute dispersion of the nanocrystals on the surface of a copper grid. Fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer with a LS-50B potable 980 nm laser (Beijing Hi-Tech Optoelectronic Co., China) as the excitation source, instead of the xenon source in the spectrophotometer, and with a fiber optic accessory. Excited by the potable 980 nm laser, the eye-visible green upconversion fluorescence photograph was obtained with a CCD camera.

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