Green upconversion nanocrystals for DNA detection†

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By combining magnetic-field-assisted bioseparation and concentration technology with magnetite nanoparticles, novel green upconversion (UC) fluorescence nanocrystals ($NaYF_4$:Yb^{3+}/Er^{3+}) have been applied to the sensitive detection of DNA.

Current enhanced security and health concerns have attracted great interests on the developments of ultrasensitive fluorescence biodetection assays,[1] magnetic bioseparation,[2–7] fluorescence imaging[8–11] and magnetic resonance imaging (MRI) technology.[12,13] Among those nanobiotechnology developments, research into robust, sensitive and reusable luminescent and magnetic nanomaterials is very important. In order to enhance the detection sensitivity, many highly luminescent nanomaterials such as semiconductor quantum dots (QDs),[8,11,14] lanthanide doped particles,[15,16] and fluorescent SiO_2 nanoparticles[17–19] have been introduced in biodetection. Meanwhile, magnetic nanomaterials also have been used extensively in the development of highly efficient magnetic concentration and bioseparation[2–7,20,21] instead of time-consuming amplification procedures such as PCR. Although these phosphors work well among these outstanding researches, their inherent detection backgrounds in actual biological and environmental samples may substantially increase in the presence of interfering biomolecules, such as nucleic acids, green fluorescence proteins[22] and other fluorescent organic molecules, which can also be excited by UV rays. Therefore, the detection sensitivity would be influenced and the *in vivo* applications of these phosphors would be limited.

So, to search for appropriate luminescence nanomaterials which can overcome the limits suffered by the normal phosphors mentioned above is a necessary challenge in current biomedical and biotechnological studies. Photon upconversion is an alternative process for the generation of visible radiation by near-infrared (NIR) excitation.[16,22–26] Meanwhile, excitation in the NIR induces only a very weak autofluorescence background since the UV-excited biomolecules that previously interfered with normal phosphor luminescence can no longer be excited by IR radiation, and photodegradation in biological applications is thus avoided.[22,24,27] Therefore, water-soluble upconversion luminescent nanoparticles may be an efficient candidate for biolabels. The advantages of low autofluorescence background, high fluorescence quantum yield, high chemical stability, and tunable optical property by varying lanthanide dopants and host matrix,[22,24,26] make such nanoparticles suitable and ultrasensitive fluorescence biological labels.[16,22,27] All these favorable properties have indicated the great potential of UC nanoparticles in bioapplications. In this work, we describe the preparation of the novel upconversion luminescent nanoparticles and the bioconjugation of the as-prepared nanoparticles with DNA. Then these bioconjugated nanoparticles were applied to the sensitive detection of nucleic acids combining with the magnetic bioseparation and concentration technology using biofunctionalized magnetite nanoparticles.

The upconversion luminescent nanomaterial used in this work is Yb^{3+}/Er^{3+} ion-pair doped hexagonal phase $NaYF_4$ nanoparticles. As is already known, the luminescent efficiency of these upconversion materials is greatly dependent on the excited-state dynamics of the rare-earth ions and their interactions with the host matrix.[22,26] So, the upconversion performance of these materials can be significantly enhanced with a suitable selection of the host matrix and their phase. To the best to our knowledge, the hexagonal phase $NaYF_4$ is reported as one of the most efficient hosts for performing infrared to visible photon conversion when activated by Yb^{3+}/Er^{3+} ion-pairs.[22,26] In the near infrared to visible upconversion process of the upconversion luminescent materials, two (or more) low-energy photons from the excitation source are converted into one photon of higher energy. In these materials, the erbium ion (Er^{3+}) is ideally suited to convert infrared light to visible and it provides intermediate electronic energy levels ($^4I_{11/2}$) with long lifetimes, which are easily accessible with near-infrared radiation and can be conveniently pumped with low-cost commercial diodes. So, in this work, hexagonal phase $NaYF_4$ was chosen as the host matrix to prepare the monodispersed and water-soluble Yb^{3+}/Er^{3+} ion-pairs doped upconversion luminescent nanoparticles with our previous reported solvothermal technology.[22] These as-synthesized upconversion luminescent nanoparticles emit strong green upconversion fluorescence accompanied by very weak green and red emissions (Fig. 1(a)). From Fig. 1(a), it can be seen that the emission peaks of the fluorescence spectra are located at 525, 545 and 655 nm, corresponding to energy transfers from excited states $^2H_{11/2}$, $^4S_{3/2}$ and $^4F_{9/2}$ to the ground state $^4I_{15/2}$, respectively.[16,22,24,26] It is worth noting that the green emission is the main peak and it is so strong that the whole upconversion luminescence is nearly pure green (see the inset in Fig. 1(a)). The inset of Fig. 1(a) shows a photograph of naked-eye visible green upconversion luminescence of the $NaYF_4$:Yb^{3+}/Er^{3+} nanoparticles in a transparent 2 wt% aqueous colloid solution excited with a commercially available 980 nm laser. These nanoparticles have also been characterized with transmission electron microscopy (TEM) and X-ray diffraction (XRD) analysis. A TEM image

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† Electronic supplementary information (ESI) available: XRD pattern of UC luminescent nanoparticles, TEM, XRD and magnetization line of the magnetite nanoparticles, Experimental details mentioned in text. See DOI: 10.1039/b604871d

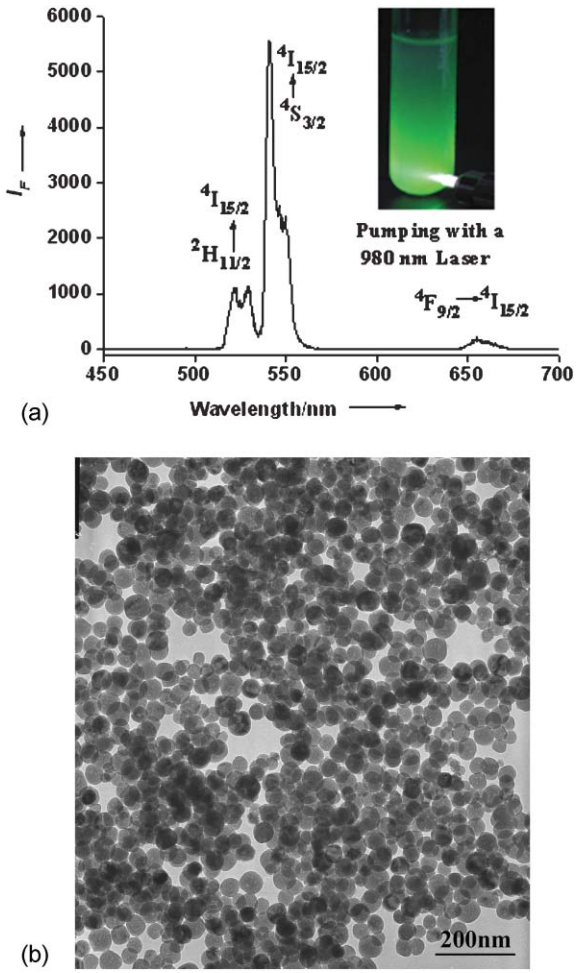

Fig. 1 Upconversion luminescence fluorescence spectrum (a) and photograph (inset in (a)), and TEM image (b) of the hexagonal phase $NaYF_4$:Yb^{3+}/Er^{3+} nanoparticles. The luminescent peaks are located at 525 nm (green), 545 nm (green) and 655 nm (red), respectively. The naked eye-visible green upconversion fluorescence pattern and fluorescence spectrum were acquired from the as-prepared upconversion nanoparticle aqueous solution (2 wt%) excited by a commercially available 980 nm laser at room-temperature.

(Fig. 1(b)) of the as-prepared nanoparticles clearly shows their uniform size and morphology, and the XRD pattern (ESI,† Fig. S1) can be readily indexed to that of the hexagonal phase $NaYF_4$ with unit cell parameters a = 5.96, b = 5.96, c = 3.53 Å (JCPDS no. 16-0334).[22]

The magnetite nanoparticles applied here were prepared with the reported protocol.[28] TEM (Fig. S2(a)), XRD (Fig. S2(b)) and magnetization curve measurements (Fig. S2(c)) (see ESI†) were demonstrated to characterize the as-synthesized magnetic nanoparticles. From Fig. S2(a), it can be seen that the magnetic nanoparticles have an average size of about 150 nm with a smooth surface. In addition, these magnetic nanoparticles have good dispersibility and morphology. Meanwhile, the XRD (Fig. S2(b)) results can be easily indexed to Fe_3O_4 (JCPDS 82-1533). The magnetic property measurements reveal that these magnetite nanoparticles have excellent saturation magnetization up to 81.3 emu g^{-1}, which make them excellent magnetic bioseparation materials.

For the subsequent bioconjugation, both the magnetic and luminescent nanoparticles were amino-group functionalized by polyelectrolyte using layer-by-layer (LbL) technology (see ESI†).[29] 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.[30,31]

With magnetic-field-assisted biochemical separation and concentration technology, these magnetic and upconversion luminescent nanoparticles bioconjugated with nucleic acids were used for the sensitive detection of trace amounts of DNA according to the procedure schematically depicted in Fig. 2. In a typical experiment for DNA detection, a three-component sandwich assay was used (Fig. 2). The polyelectrolyte functionalized upconversion luminescent nanoparticles were covalently linked to the 3′-propylthiol-terminated DNA, 5′-TCC-ATG-CAA-CTC-TAA-A10-$(CH_2)_3$-SH (DNA1), and used as probes to monitor the presence of specific target DNA strands. Meanwhile, the polymer fabricated magnetite nanoparticles were bioconjugated with the 5′-propylthiol- terminated capture DNA, 3′-AAT-TGA-GGA-GAA-AGA-A10-$(CH_2)_3$-SH (DNA2). In order to diminish the nonspecific absorption of DNA, both the bioconjugated magnetic and luminescent nanoparticle solution were treated with 10% bovine serum albumin (BSA) solution for 4 h at room temperature. At 37 °C, the capture DNA modified magnetic nanoparticles were reacted with target DNA, 5′-TTA-GAG-TTG-CAT-GGA-TTA-ACT-CCT-CTT-TCT-3′ (DNA3) and washed three times combining with magnetic separation technique with 0.3 M NaCl phosphate buffer solution (PBS, 10 mM, pH 7.0) to remove the unreacted and nonspecifically bound targets. Then, the magnetic nanoparticles (0.12 nM) were treated with a 0.3 M NaCl PBS solution of nanoparticle probes (4 nM) for 12 h to effect

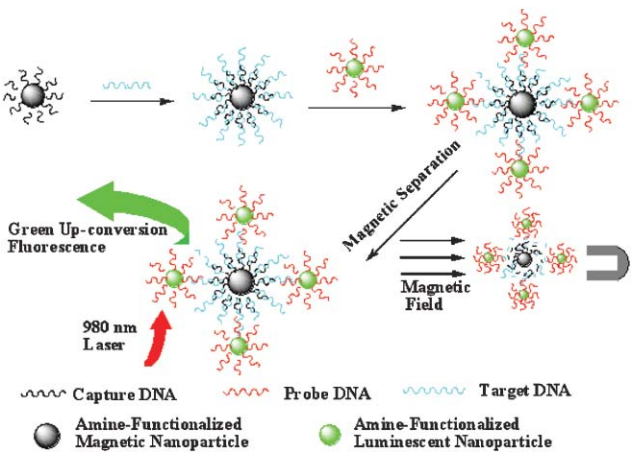

Fig. 2 Scheme of the DNA assay method combining UC fluorescence with magnetic separation. Magnetic nanoparticles were modified with capture DNA and phosphor nanoparticles were modified with probe DNA, respectively. Capture DNA modified magnetite nanoparticles were hybridized with target DNA and separated with an assistant magnetic field. The probe DNA modified luminescent nanoparticles were then conjugated to the magnetic nanoparticles through the hybridization with the overhanging region of the target sequences. The binary nanoparticles were purified with magnetic separation and detected with upconversion fluorescence technology. The excitation light is a commercially available 980 nm laser.

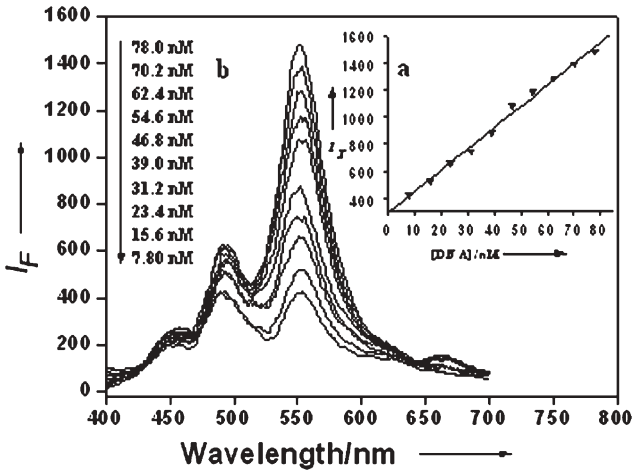

Fig. 3 Fluorescence spectra of binary nanocomposite in the presence of different concentration of target DNA (b) and the linear relationship (a) between luminescence intensity and target DNA content according to (b).

hybridization with the overhanging region of the target sequence (Fig. 2). The nanocomposite consisting of magnetic and luminescent nanomaterials through matched DNA was then washed with 0.3 M NaCl PBS and magnetically separated. Within each purifying circle, the binary nanocomposite of UC phosphors bioconjugated to the magnetic nanoparticles through the base-match interaction of the oligonucleotides, were pulled to the left side-wall and purified by the assistant magnetic field added to the left side wall of the reaction tube. Without the implementation of PCR technology, trace amounts of nucleic acids were detected by recording the upconversion luminescence intensity of the nanocomposite solution. Both the UC fluorescence spectra (Fig. 3(b)) and the calibration plot (Fig. 3(a)) of the enhancement of the UC fluorescence intensity with concentration of target DNA are depicted in Fig. 3. From Fig. 3, it can be seen that the concentration of the target DNA in the range of 7.8–78.0 nM is linear to the UC luminescence intensities. The linear calibration equation, $F_{UC} = 292.4 + 15.7C$ (F_{UC} is the upconversion fluorescence intensity and C is the concentration of the target DNA) of the detection was obtained according to the general detection procedure. Accordingly, the correlation coefficient is 0.9978 ($n = 10$).

In summary, combining magnetic-field-assisted biochemical separation and concentration technology, novel green upconversion luminescent nanoparticles have been applied to the sensitive detection of DNA. From these preliminary results, we can envision that the low background, the improved photostability and chemical stability of these UC phosphors, could allow real-time observations of ligand–receptor interactions and of molecular trafficking in living cells. It is also conceivable that further synthetic efforts may produce sufficiently monodispersed and homochromous upconversion luminescent nanoparticles, which should be important toward developing multiplexed ultrasensitive detection and multicolor imaging in both biomedical and biotechnological applications with the magnetic-field-assisted bioseparation and concentration.

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