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Research paper

NIR-responsive DNA hybridization detection by high efficient FRET from 10-nm upconversion nanoparticles to SYBR green I

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ABSTRACT

A near-infrared (NIR) responsive DNA probe has been developed for DNA hybridization detection with characteristics of high efficient Förster resonance energy transfer (FRET) from small (~10 nm) upconversion nanoparticles (UCNPs) to nucleic acid stain SYBR Green I (SG, a specific intercalator of double-stranded DNA). The positively charged UCNPs were prepared and facilely attached with single DNA strands by coordination interaction, which acted as probes to detect the DNA targets in the presence of SG. This probe possesses not only the superiority of the NIR-excitation nature of UCNPs which could minimize the autofluorescence background from biomolecules and the photodamage to biological specimens, but also the advantages of facile preparation and high FRET signals. The FRET efficiencies could increase significantly from 2.6% to 12.5% and then to 26% when the size of UCNPs reduced from 94 nm to 30 nm and to 10 nm, respectively. The 10 nm-UCNP-based DNA probe could reach a lower detection limit of complementary ssDNA2 at 3.2 nM and three-base mismatched ssDNA2-M3 at 7.6 nM. The high sensitivity and selectivity of the probe may endow the system with great potential in fluorescence-based biosensing under the irradiation of NIR light.

1. Introduction

In recent years, vast interests have focused on the DNA detection methodology for applications including clinical diagnostics and gene therapy [1]. Among diverse detection methods, fluorescent DNA detection is currently the primary analytical tool because of its convenient optical signal transduction, high sensitivity, and fast response [2,3]. Fluorescent DNA probes based on Förster resonance energy transfer (FRET) using organic fluorophore or inorganic quantum dot as marker have been used for identification of specific sequences and mutations [4–8]. However, conventional downconversion fluorescence DNA probes suffer from problems such as high autofluorescence background and inevitable photo damage to biological specimens associated with UV excitation [9].

To overcome the above problems, lanthanide-doped upconversion nanoparticles (UCNPs) are suitable alternatives to conventional fluorescence probes thanks to their superior physicochemical features such as large anti-Stokes shifts, low autofluorescence background, and high penetration depth [10]. Several UCNPs-based FRET DNA probes have been developed [9]. However, in most cases, the fluorescence signals of an energy acceptor from FRET are inconspicuous due to a low FRET efficiency, which may impair the assay sensitivity [11,12]. The low FRET efficiency is originated from two major aspects. First, the scale of UCNPs used in these probes is generally at tens of nanometers while the effective energy-transfer range is generally within 10 nm. A large part of emitting ions are out of the effective FRET range, remaining considerable fluorescence background of energy donor (UCNPs) which often overwhelms the relatively weak signal of energy acceptor [13,14]. Second, UCNPs used in these probes are usually coated by shell layers such as SiO_2 [15,16] or ligand [17,18], which increased UCNPs-to-acceptor distances and thus reduced the FRET efficiency. Besides the low FRET efficiency, these reported UCNPs-based FRET DNA probes suffered from laborious covalent attachments, in which UCNPs and DNA were mostly linked via –SiOCN/–NH_2 [15,16], –COOH/–NH_2 [19–21], and biotin/strepatividin [22,23].

Herein, we report a novel NIR-responsive DNA probe with high-efficiency FRET from 10 nm UCNPs ($NaYF_4$:Yb/Tm) to nucleic acid stain SYBR Green I (SG). The probe was facilely prepared from UCNPs and ssDNAs by coordination interaction, avoiding complicated covalent attachment procedures and shortening the UCNPs-to-acceptor distance which might improve the FRET efficiency. With the reduction of UCNPs size from 94 nm to 30 nm and then to 10 nm, the FRET efficiencies could increase significantly

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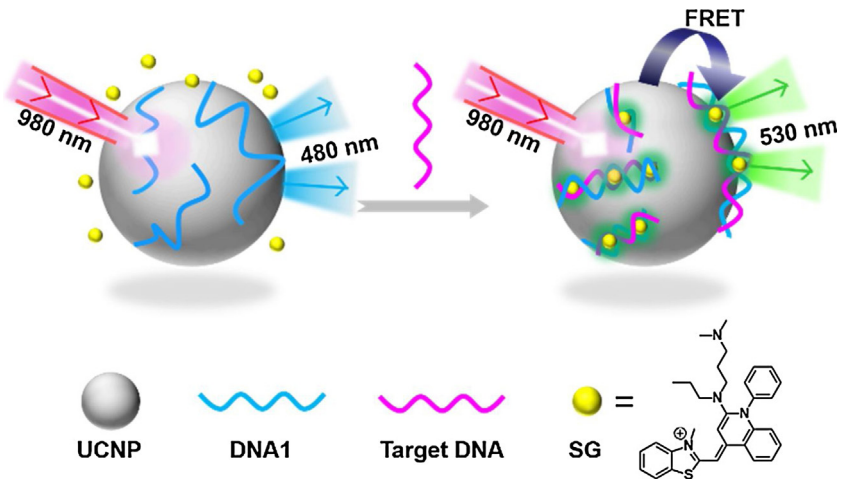

Scheme 1. Schematic illustration of the near-infrared responsive DNA probe based on FRET from UCNPs to SYBR Green I.

from 2.6% to 12.5% and then to 26% measured from the fluorescence lifetime experiments. The 10 nm-UCNP-based FRET DNA probe could detect and distinguish the complementary ssDNA2 and three-base mismatched ssDNA2-M3 at nanomolar concentration. As far as we know, this work demonstrates for the first time the substantial promotion of FRET efficiency of 10 nm-UCNPs-based FRET system measured from fluorescence lifetime experiments and the great superiority of 10 nm-UCNP-based FRET system for DNA detection. The prepared UCNP-based DNA probe possesses not only the advantages of the NIR-excitation nature of UCNPs, which can minimize the autofluorescence background from biomolecules and the photodamage to biological specimens, but also the advantages of facile preparation and high FRET signals, which has great potential in fluorescence-based biosensing.

Scheme 1 presents the simple and general protocol of DNA detection by FRET. The NIR-responsive DNA probe is composed of UCNPs, DNA single strands, and nucleic acid stain SYBR Green I (SG). Oleate-capped $NaYF_4$:20%Yb,0.5%Tm UCNPs were synthesized according to the literature methods [24,25]. Considering the absorption of SG is centered at 490 nm, Tm^{3+} was chosen as the emitting ion for UCNPs to ensure the spectral match between energy donor and acceptor. After removing the oleate ligands of UCNPs via acid treatment [26], single-stranded DNA1 (ssDNA1) could be directly linked to oleate-free UCNPs via the coordination effect between abundance of negatively charged backbones of DNA and the exposed Ln^{3+} ions on UCNPs [27,28]. In the presence of complementary target DNA, the two DNA strands can form into a double-stranded DNA (dsDNA) where the stain SG would be intercalated and then the emission signal of SG at 530 nm would be enhanced, since the SG quenching occurs in the free state and its fluorescence is enhanced in the presence of dsDNA by a dampening of its intra-molecular motions [29].

2. Experimental

2.1. Materials

All DNA molecules, fetal bovine serum and SYBR Green I were obtained from the Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. and were used as received. All other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. and were used as received. The following DNA oligonucleotides are used in this work. ssDNA1 (5′-AAA GAG AGA GAG AGA GGG-3′) is used to form a complex probe. ssDNA2 (5′-CCC TCT CTC TCT CTC TTT-3′) is complementary with ssDNA1. ssDNA2-M3 (5′-CCC TCT CTG AAT CTC TTT-3′) and ssDNAa (5′-AAA AAA AAA AAA AAA AAA-3′) are three-base mismatched and noncomplementary with ssDNA1, respectively.

2.2. Methods

Transmission electron microscopy (TEM) images were taken on the JEOL JEM1200-EX transmission electron microscope with an accelerating voltage of 120 kV. UV–vis spectra were recorded on a Shimadzu UV-3100 UV–vis spectrophotometer. The emission spectra was obtained by a Hitachi F-4500 fluorescence spectrophotometer coupled with a commercial CW IR laser (980 nm, 60 W/cm^2, fiber-coupled semiconductor laser, Beijing Hi-tech Optoelectronic Co., China). The fluorescence lifetimes of the UCNPs were measured with a customized UV to mid-infrared steady-state and phosphorescence lifetime spectrometer (FSP920-C, Edinburgh Instrument) equipped with a digital oscilloscope (TDS3052B, Tektronix) and a tunable mid-band optical parametric oscillator (OPO) pulse laser as the excitation source (410–2400 nm, 10 Hz, pulse width $\leq$ 5 ns, Vibrant 355II, OPOTEK). Fourier transform infrared (FTIR) spectra were acquired on a PerkinElmer (USA) spectrometer. XRD measurements were carried out on a SmartLab X-ray diffractometer. Zeta potential of the oleate-free UCNPs and the UCNPs-DNA were determined using a DelsaNano C zeta potential analyzer.

2.3. Synthesis of lanthanide oleate complex ($Ln(oleate)_3$)

In a typical synthesis, aqueous solutions of $YCl_3{\cdot}6H_2O$ (3.18 mL, 0.5 M), $YbCl_3{\cdot}6H_2O$ (4 mL, 0.1 M), and $TmCl_3{\cdot}6H_2O$ (100 ul, 0.1 M) were mixed under agitation with oleic acid (12 mL) and 1-octadecene (30 mL) into a 250 mL three-neck flask. The mixture solution was heated to 160 °C under argon atmosphere to exclude water and oxygen and obtain a clear yellow solution, and then cooled down to room temperature. Thus the $Ln(oleate)_3$ complex for the preparation of UCNPs was obtained. The $Ln(oleate)_3$ complexes for preparation of 10 nm, 30 nm, 94 nm × 55 nm UCNPs were synthesized in the same way.

2.4. Synthesis of oleate-capped 10 nm UCNPs

Two methanol solutions of NaOH (15 mL, 5 mmol) and NH_4F (25 mL, 8 mmol) were mixed and oscillated for 10 s, and then added into the as-prepared $Ln(oleate)_3$ solution quickly. The mixed solution was stirred for 2 h at room temperature under argon atmosphere to exclude methanol and obtain a dark yellow solution. Then the solution was heated to 295 °C at a heating rate of 10 °C/min

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