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Fluorescence resonance energy transfer between $NaYF_4$:Yb,Tm upconversion nanoparticles and gold nanorods: Near-infrared responsive biosensor for streptavidin

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ABSTRACT

We represent a fluorescence resonance energy transfer (FRET) system using upconversion nanoparticles (UCNPs) and the gold nanorods (GNRs) as the energy donor–acceptor pair for directly determining streptavidin in near-infrared (NIR) region. $NaYF_4$:Yb,Tm UCNPs, which had a strong emission at 800 nm under 980-nm excitation, were adopted as the energy donor. The GNRs, which demonstrated strong surface plasmon absorption around 800 nm, were chosen as acceptor to quench the 800 nm emissions of the UCNPs. There had the spectral overlap between the emission of the donor nanoparticles (UCNPs) and the absorption of the acceptor nanoparticles (GNRs). This UCNP-based FRET system was then used to determine the amount of streptavidin. In this system, $NaYF_4$:Yb,Tm UCNPs conjugated with biotin, while GNRs conjugated with streptavidin. When added GNRs into UCNPs, the streptavidin were preferred to bind with biotin and decreased spacing between the donor and acceptor NPs. Consequently, FRET occurred and a linear relationship between the luminescence quenching efficiency and the concentration of streptavidin was obtained. Owing to the aforementioned merits of UCNPs as an energy donor and the strong quenching ability of GNRs, satisfactory analytical performances have been acquired.

1. Introduction

In recent years, fluorescence resonant energy transfer (FRET)-based assays have attracted a great deal of attention as powerful tools for biological detections because of their simplicity and high sensitivity [1–4]. FRET is a nonradiative process in which the energy is transferred from a donor at its excited state to a nearby acceptor molecule via long-range dipole–dipole interactions. In the FRET process, the efficiency of energy transfer (ET) is highly dependent of the donor and acceptor molecules in very close proximity, and the donor emission spectrum to overlap with the acceptor absorption, as well as relative dipole orientations between the donor and acceptor [5–8].

There are a number of conventional fluorescent biolabels used in FRET-based biological detections including organic dyes and fluorescent proteins or semiconductor quantum dots [9–11]. However, they are susceptive to photo-bleaching, exhibit broad absorption and emission bands and small Stokes shift, or inherent toxicity and chemical instability. In addition, these downconversion fluorescent materials have rather strong autofluorescence and scattering light, arising from biomolecules when the assay is conducted in biological sample matrixes, which limits the detection and long time observation [12,13]. In the past years, upconversion (UC) phosphors, especially rare-earth (RE) doped UC nanocrystals (NCs) have attracted tremendous amount of attention [14–16]. Basically, emissions of RE ions demonstrate sharp emission lines, long lifetime, large Stokes shifts and high photostability. And more, compared with down-conversion phosphors such as fluorescent dyes and quantum dots, UC-NCs based on lanthanide compounds exhibit excellent advantages, such as minimum photodamage to living organisms, weaker autofluorescence backgrounds, and deeper light-penetration in tissues because near infrared (NIR) excitation is used [17,18]. In addition, most of lanthanide compounds demonstrate nontoxicity, biocompatibility and high chemical stability. Up to now, UCNPs have been used as optical nanoprobes for various biosensing and bioimaging applications in protein detection and DNA sensing, which show significant advantage due to their unique anti-Stokes luminescence properties [19–21]. Recently, more attentions have been paid to the biological applications of $NaYF_4$:Yb,Tm NCs, because it has a near-infrared (NIR) UC emission at 800 nm under 980 nm excitation [22,23]. It means both the excitation and the emission of UCNPs locate in the NIR spectral range (NIR-to-NIR UC emission), the so-called biological window. These characters offer advantages

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for biological application, because both NIR excitation and emission lights will not be absorbed by biological samples and can penetrate deep in cells and tissues [24,25].

In FRET-based assays, it is also of great importance to choose suitable energy acceptors, which have as large as possible power to quench the emission of donors. Recently FRET from UCNPs to various types acceptors, such as organic dyes, quantum dots and noble metal NPs have been reported [26–28]. Among various acceptors, gold nanoparticles (GNPs) have been successfully employed as an energy acceptor of UCNPs [29,30]. GNPs have super-strong surface plasmon absorption (SPA), which can quench luminescence of nanophosphors or dye molecules effectively. And more, the size and morphology of GNPs can be easily controlled, which is promised to accurately adjust their optical properties. For instance, when control the ratio of length to diameter, the SPA of gold nanorods (GNRs) can be easily tuned from visible into the NIR region [31], overlapping with the NIR emissions of $NaYF_4$:Yb,Tm NCs around 800 nm and realizing effective FRET.

In this work, we have developed a FRET system using $NaYF_4$:Yb,Tm UCNPs and GNRs as the energy donor–acceptor pair for directly determining streptavidin in NIR region. This is an example of using a UCNPs- and GNRs-based donor–acceptor FRET system to realize accurate biological detection in the NIR region.

2. Experiment

2.1. Materials

Cetyltrimethylammonium bromide (CTAB) (99%), $HAuCl_4 \cdot 3H_2O$ (99.99%), $AgNO_3$ (99%), $NaBH_4$ (98%), ascorbic acid (AA, 99%), polystyrenesulfonate sodium (PSS), polyacrylamide (PAH), branched polyethylenimine (PEI, Aldrich, MW 25,000), and ethylene glycol (EG, Acros, 99%), streptavidin and activated biotin were obtained from Sigma. All the glassware was cleaned and thoroughly rinsed with Millipore water prior to the experiments. Hydrated rare earth nitrate ($RE(NO_3)_3$(RE=Y,Yb,Tm, 99.99%), were purchased from Baotou Ruike Functional Materials Co., Ltd. All the chemicals used were the analytical reagents.

2.2. Synthesis and PAH coated GNRs

GNRs were prepared according to the seed-mediated growth method developed by EI-Sayed and co-workers [32]. Briefly, a seed solution used for the synthesis of the GNRs was prepared by mixing 7.5 mL of CTAB (0.1 M) and 2.5 mL of $HAuCl_4$ (1 mM) with 0.6 mL of freshly prepared $NaHB_4$ (10 mM) solution under vigorously stirring at 28 °C for 2 h.

In a flask, 132 mL of 0.1 M CTAB was mixed with 120 mL of 1 mM $HAuCl_4$, then 2.4 mL of 10 mM silver nitrate aqueous solution and 2.2 mL of 2 M hydrochloric acid were added to the flask. After gently mixing the solution, 1.92 mL of 0.1 M ascorbic acid was added. On continuously stirring this mixture, certain concentration of seed solution was added finally to initiate the growth of the GNRs. These GNRs were aged 5 h to ensure full growth at 28 °C. After preparation, excess CTAB molecules were removed by centrifuging twice at 10,000 rpm for 10 min, and then redispersed in deionized water.

Then, 2 mL of PSS solution (10 mg/mL prepared in 1 mM NaCl) and 1 mL NaCl solution (1 mM) were added simultaneously to the purified CTAB capped GNRs solution (10 mL) by stirred vigorously for 30 min. Surface-modified GNRs with PSS solution were centrifuged twice at 10,000 rpm to remove excess polyelectrolyte and redispersed in 1 × PBS (pH=7.4). 2 mL of PAH (10 mg/mL soluted in 1 mM NaCl) and 1 mL NaCl (1 mM) were simultaneously added to the purified PSS-capped GNRs solution (10 mL). After mixed gently for 3 h, the solution was centrifuged for 10 min at 10,000 rpm to remove excess PAH polymer. Then the pellet was redispersed in 10 mL 1 × PBS (pH=7.4).

2.3. Preparation of streptavidin-conjugated GNRs

The conjugation of streptavidin and GNRs was on electrostatic adsorption. PAH coated GNRs were taken with positive charge, which may adsorb protein through increased protein incubation concentration. In this work, 2 mL streptavidin (10 mg/mL in PBS) was added into 10 mL PAH-GNRs then incubated together at 4 °C overnight. NPs were separated by centrifugation and washed thoroughly by repeated resuspension and centrifugation in PBS.

2.4. Synthesis of water-soluble $NaYF_4$:Yb,Tm NCs

PEI-modified water-soluble NIR-to-NIR UC luminescent $NaYF_4$:Yb,Tm NCs were synthesized via a solvothermal method following a procedure reported previously [33,34]. NaCl (2.5 mmol), PEI (0.4 g), $Y(NO_3)_3$ (0.78 mmol), $Yb(NO_3)_3$ (0.20 mmol), and $Tm(NO_3)_3$ (0.02 mmol) was dissolved in EG (15 mL) under vigorous stirring. When the solution became transparent, NH_4F (4 mmol) in EG (10 mL) was added to the solution. After stirred for another 10 min, the whole mixture was transferred into a 25 mL Teflon-lined stainless steel. The autoclave was sealed and heated under 200 °C for 2 h hydrothermal treatment. After the autoclave cooled down to room temperature naturally, $NaYF_4$:Yb,Tm NCs were harvested by centrifugation and washed with deionized water thrice, and then redispersed in phosphate buffered saline (PBS).

2.5. Preparation of biotin-conjugated $NaYF_4$

100 mg of PEI-UCNPs was diluted in 10 mL of PBS buffer solution (pH 7.4, 10 mM), then added 800 μL of activated biotin (5 mg/mL in DMSO) to the mixture under gentle shaking for 4 h of reaction at room temperature. Biotin was covalently conjugated with UCNPs by amido bond. Then the mixture was harvested with centrifugation and washed three times with PBS buffer solution to remove excess biotin. The bio-UCNPs was diluted in PBS and stored at 4 °C for further use.

2.6. Characterization

The size and morphology of as-prepared PEI-coated UCNPs were recorded on a Hitachi (Tokyo, Japan) H-8100IV transmission electron microscope (TEM) under an acceleration voltage of 200 kV. Samples for TEM investigations were prepared by dispersing the particles in water then dropping one drop of the suspension on a copper TEM grid coated with a holey carbon film. The Fourier-transform infrared (FTIR) absorption spectra were measured using a Shimadzu DT-40 model 883 IR Spectrophotometer with the KBr pellet technique. The powders were mixed homogeneously and compressed at a pressure of 10 kPa to form transparent pellets. The absorption spectrum of GNRs was recorded with a UV-1800 UV/visible/NIR scanning spectrophotometer (Shimadzu, Japan), in the wavelength range from 350 to 1000 nm.

2.7. Detection of streptavidin based on the LRET system

To investigate the luminescence quenching degree, 1 mL of bio-UCNPs (0.1 mg/mL) was incubated with an increasing amount of streptavidin-GNRs (0, 2, 3, 5, 10, 20, 30, 40, 50, 60, 70 and 80 μL) at room temperature for 30 min and the mixtures were then subject to luminescence measurement. The upconversion luminescence (UCL) was measured with a Hitachi F-4500 fluorescent spectrometer from 350 nm to 850 nm. In UCL measurement, a continuous 980 nm diode laser (Beijing Hi-Tech Optoelectronic Co., Ltd.) was