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A paper-based resonance energy transfer nucleic acid hybridization assay using upconversion nanoparticles as donors and quantum dots as acceptors

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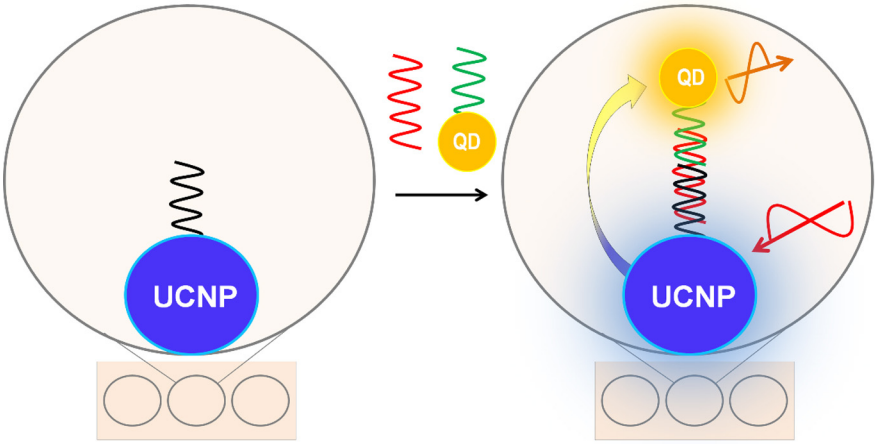
HIGHLIGHTS

- Covalent immobilization of upconversion nanoparticles on paper.
- LRET-based label free DNA detection using quantum dots as acceptors.
- Use of polyethylene glycol to eliminate non-specific adsorption of quantum dots.
- Improved analytical performance compared to analogous assays.

GRAPHICAL ABSTRACT



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ABSTRACT

Monodisperse aqueous upconverting nanoparticles (UCNPs) were covalently immobilized on aldehyde modified cellulose paper via reduction amination to develop a luminescence resonance energy transfer (LRET)-based nucleic acid hybridization assay. This first account of covalent immobilization of UCNPs on paper for a bioassay reports an optically responsive method that is sensitive, reproducible and robust. The immobilized UCNPs were decorated with oligonucleotide probes to capture HPRT1 housekeeping gene fragments, which in turn brought reporter conjugated quantum dots (QDs) in close proximity to the UCNPs for LRET. This sandwich assay could detect unlabeled oligonucleotide target, and had a limit of detection of 13 fmol and a dynamic range spanning nearly 3 orders of magnitude. The use of QDs, which are excellent LRET acceptors, demonstrated improved sensitivity, limit of detection, dynamic range and selectivity compared to similar assays that have used molecular fluorophores as acceptors. The selectivity of the assay was attributed to the decoration of the QDs with polyethylene glycol to eliminate non-specific adsorption. The kinetics of hybridization were determined to be diffusion limited and full signal development occurred within 3 min.

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1. Introduction

Lanthanide doped upconverting nanoparticles (UCNPs) have attracted much attention for applications in bioanalysis due to their unique optical properties. Upconversion is based on the sequential absorption of two or more photons in the IR region of the electromagnetic spectrum followed by the narrow band emission of radiation of higher energy within the UV to NIR region. IR excitation minimizes autofluorescence from biological material, and reduces optical background that is commonly associated with an excitation source that operates in the UV or visible wavelength range. In addition, lanthanide doped UCNPs emit multiple narrow and well-defined emission peaks suitable for multiplexed optical analysis [1]. UCNPs have been used in bioassays as passive labels, and as energy donors in luminescence resonance energy transfer (LRET) for the detection of nucleic acids [2–4], proteins [5–7] and metal ions [8–10]. While UCNP LRET-based assays offer access to a ratiometric approach that provides for good precision, they suffer from LRET efficiencies that are generally well below 50%. Improving the LRET efficiency provides higher sensitivity and lower detection limits of bioassays. Reported strategies to improve LRET efficiency include surface decoration of UCNPs [11], adjustment of donor and acceptor distance [12] and optimization of LRET acceptor properties [13]. We have previously demonstrated the use of a sandwich-based assay format for the detection of thrombin where a dense monolayer of UCNPs deposited onto a glass surface allows a single LRET acceptor to interact with multiple donors [5]. The multiple donor–acceptor interactions at the surface provided about 4 fold enhancement of the LRET ratio.

To further improve sensitivity and dynamic range, in this work UCNPs were immobilized on paper to make use of the large surface area associated with the three dimensional matrix. Paper based assays have attracted much attention due to their low cost, fluid transport via capillary action, and easy modification and patterning [14]. More importantly, the three dimensional nature of paper was reported by Noor and Krull to be capable of providing more than a 10 fold enhancement in fluorescence resonance energy transfer (FRET) ratio for immobilized quantum dots (QDs) and dye acceptors in a label free nucleic acid hybridization assay [15,16]. The enhancement was attributed to the large available surface area, in combination with the contraction of wet paper upon drying that brought neighboring donors and acceptors into closer proximity [16]. Zhou et al. adsorbed UCNPs on paper for the detection of dye labeled oligonucleotides, however, the assay was limited in sensitivity and selectivity [17,18]. Herein, we report a novel design for the sensitive and selective detection of unlabeled target oligonucleotide on paper using covalently immobilized UCNPs as donors and QDs as acceptors. QDs are more photostable, offer higher extinction coefficients and wider absorption spectra than molecular dyes, and are known as excellent LRET acceptors [19]. However, their broad spectral absorption profiles has limited their use as acceptors. This is primarily because the wavelengths that are used to excite donors will often concurrently directly excite QDs, making it impossible to excite the QDs only by resonance energy transfer from the donor [19]. Use of QDs as acceptors is typically limited to chemiluminescence energy transfer (CRET) [20] and bioluminescence energy transfer (BRET) [21] where no excitation source is used. QDs have also been used as acceptors with lanthanide complexes as donors in time gated measurements [22]. Herein, QDs are effectively used as LRET acceptors without the need for time gated measurements. An epifluorescence microscope equipped with a continuous 980 nm laser provides for the excitation of UCNPs that in turn can excite QDs, where the intensity of acceptor emission is measured using a photomultiplier tube in conjunction with appropriate band pass filters. The narrow and well defined emission peaks of both donor and acceptor makes it possible to collect LRET sensitized QD photoluminescence in the absence of any donor background.

This work presents the first account of use of covalently immobilized UCNPs on paper as LRET donors for the optical detection of unlabeled nucleic acid targets (Fig. 1). Oligonucleotide probes decorating the UCNPs capture HPRT1 housekeeping gene fragments. An unhybridized segment of the HPRT1 target in turn hybridizes with an oligonucleotide reporter that is conjugated to QDs. This results in localization of QDs in close proximity to the UCNPs for LRET. The kinetics of hybridization are optimized, and non-specific adsorption by QDs is eliminated to build a hybridization assay that offers speed and high selectivity [17].

2. Experimental

A full list of materials and instrumentation can be found in the Supporting information.

2.1. Synthesis of $NaYF_4$:0.5% Tm^{3+}, 30% Yb^{3+}/$NaYF_4$ core/shell UCNPs

Core $NaYF_4$:0.5% Tm^{3+}, 30% Yb^{3+} UCNPs were synthesized according to previous reports [23]. In short, 0.4562, 0.2534, and 0.0042 g of $Y(CH_3CO_2)_3{\cdot}xH_2O$, $Yb(CH_3CO_2)_3{\cdot}4H_2O$ and $Tm(CH_3CO_2)_3{\cdot}xH_2O$ were stirred gently in 30 mL octadecene and 12 mL oleic acid (OA) under vacuum at 115 °C for 30 min. The mixture was then cooled to 50 °C under argon before a 20 mL methanol solution containing 0.20 g NaOH and 0.30 g NH_4F was

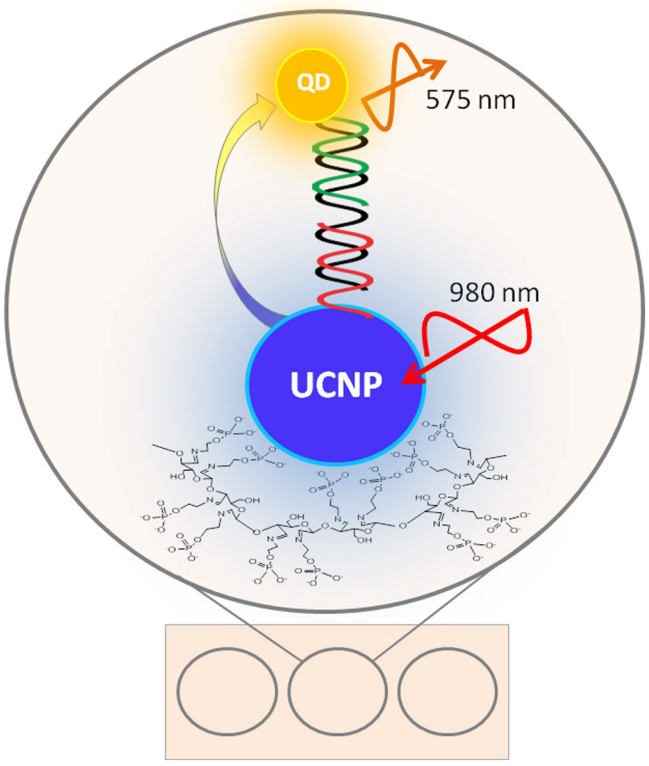

Fig. 1. The strategy for the detection of target DNA. Probe oligonucleotide is conjugated to an UCNP, which is immobilized on a paper substrate. Target oligonucleotide serves to bridge probe oligonucleotide on the UCNP and reporter oligonucleotide on the QD. Excitation of the UCNP at 980 nm provides for luminescence that excites the QD. The paper substrate is prepared to localize 3 reaction spots that are defined by wax printing. The orange, black and green nucleic acid strands represent the probe, target and reporter strands, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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