Label-free, Fluorescence Detection of DNA on Nanoparticle Scaffolds

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ABSTRACT

We have developed a nanoparticle-based DNA biosensor that can detect unlabelled ssDNA with good selectivity and decent sensitivity. The biosensor makes use of the optical changes of a cationic polymer interacting with ssDNA versus that of hybridized DNA to provide a fluorescence signal only when a specific hybridization event occurs (i.e. only when perfectly complementary DNA is present). Through monitoring this change in fluorescence intensity this nanoparticle-based biosensor can detect of fewer than 5 pmol of ss-DNA in a 1 mL sample (i.e. has a limit of detection of 5 nM ssDNA).

Keywords: biosensor, DNA, nanoparticles, label-free detection, polymeric transduction

1 INTRODUCTION

The identification of hybridization events is still an important challenge in the development of rapid, sensitive and specific methods for the detection of DNA. Currently many of the most useful DNA-based detection devices utilize polymerase chain reaction (PCR) to allow high-sensitivity detection. Recently however, Leclerc and his collaborators have demonstrated a new PCR-free method for the detection of DNA at concentrations as low as zeptomoles (10^{-21}).[1,2] This method involves the use of a fluorescent and positively-charged polythiophene which forms a duplex in the presence of a negatively-charged single-stranded (ss) DNA (Figure 1). The formation of this duplex leads to significant changes in the optical and fluorescence properties of the cationic polythiophene. Specifically, as the polymer "wraps around" the probe ssDNA, the backbone of the polymer becomes more rigid and planar, shifting the polymer absorbance band to higher wavelengths, and the polymer no longer absorbs light at the same wavelengths and as such becomes non-emissive. However, upon hybridization with a complementary ssDNA, the polymer backbone returns to a twisted structure, similar to that of the free polymer, the absorption maximum shifts back to a lower wavelength and the fluorescence signal "turns on", signaling that a hybridization event has occurred (Figure 1). Within this report we will describe the preparation of the ssDNA-modified nanoparticles and the subsequent preparation of the "duplex" structure capable of providing a fluorescence response in the presence of unlabelled complementary ss-DNA.

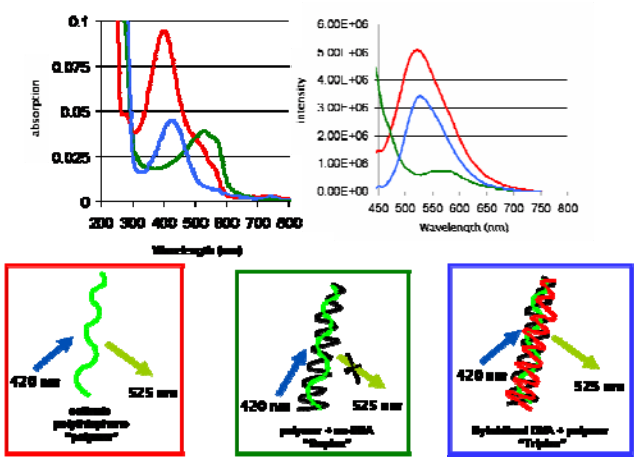

Figure 1. Absorption and emission spectrum of the cationic polythiphene alone (red curve, depicted in red box), the duplex formed between the polymer and ss-DNA (green curve and depicted in the green box) and the "triplex" formed between hybridized complementary ss-DNA and the polymer (blue curve, depicted in blue box). Note that the fluorescence emission is "turned on" (green versus blue curves) when the ss-DNA is hybridized.

2 RESULTS AND DISCUSSION

Single stranded (ss)-DNA-modified nanoparticles were prepared through the addition of 3-aminopropyltriethoxysilane (APTES) to add an amine group to the surface of the silica nanoparticles followed by the reaction of the amine-nanoparticles with succinic anhydride to produce carboxylic acid-modified nanoparticles. Subsequently the nanoparticles are reacted with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) in the presence of amine-modified ssDNA (25 mers) resulting in the covalent modification of the silica nanoparticles with ss-DNA (ss-DNA NP). Following isolation and purification of the ss-DNA NPs, hybridization of the nanoparticles in the presence of a Cy5-modified complementary strand of ssDNA demonstrated that there are between 150-300 ssDNA capable of undergoing hybridization on the surface of each ss-DNA

NP. With the number of ss-DNA molecules known, an appropriate concentration of the cationic polythiophene (hereby referred to as polymer) was added to an aliquot of the ss-DNA NP. This sample was incubated for 15 minutes at 50ºC at which time a fluorescence spectrum was recorded (data shown in Figure 2). Upon excitation of the sample at 420 nm (the absorption maximum of the polymer, Figure 1), the intensity of the emission at 525 nm (the emission maximum of the polymer, Figure 1) was recorded. The sample was then returned to the bath set to 50ºC and an aliquot of the complementary ssDNA was added. Following incubation for 15 minutes, the emission spectrum was again recorded (λ_{ex}=420 nm, λ_{em}=525 nm). As expected, the fluorescence intensity of the complex increases significantly (Figure 2)). Specifically, when the ss-DNA and the polymer are in the "duplex" or "fluorescence off" state, the fluorescence intensity is quite low. In contrast, when a complementary strand of ss-DNA is added and generating a "triplex" (i.e. hybridized complementary ss-DNA and the polymer) the fluorescence intensity increases dramatically (i.e. there is a 6.5 fold increase in intensity). This "turn on" of fluorescence can be used to signal the presence of a complementary strand of ss-DNA with excellent sensitivity. That is, when an identical concentration of ss-DNA with one or two mismatches is added to the ss-DNA NP "duplex", excitation at 420 nm results in significantly smaller increases in fluorescence intensity at 525 nm (Figure 2). That is, when two mismatches are in the added ss-DNA, the intensity increases only 2.9-fold, and when a ss-DNA with a single mismatch is added, the intensity only increases ~ 3.2 fold. As such, this nanoparticle bound biosensor provides the ability to distinguish between even a single nucleotide polymorphism with at good selectivity. However, the limit of detection for this nanoparticle-based sensing mechanism is 0.1 uM, which is not nearly sensitive enough for practical purposes. As such, to improve sensitivity, the ssDNA NP biosensor was altered such that a fluorophore capable of acting as a FRET acceptor was incorporated into the ss-DNA anchored to the surface of the silica nanoparticle. This allows the "turned on" fluorescence from the polymer to be transferred to the extremely bright fluorophore (Alexafluor546, AF546)). This is advantageous because AF546 has both a much higher extinction coefficient and a much greater quantum yield than the polymer. To take advantage of these properties, the resulting emission that will be recorded ans utilized in the sensing mechanism will be from the FRET acceptor, Af546, which has an emission mximum at 575 nm.

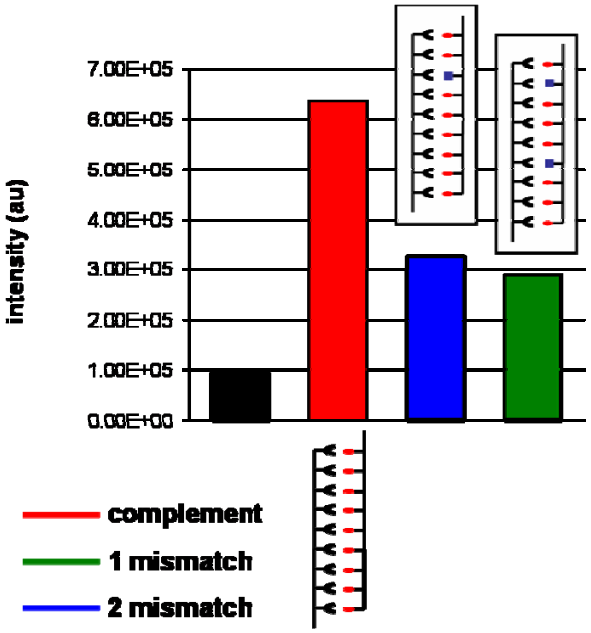

Figure 2. Plots demonstrating the differences in luminescence intensity from when the duplex structure is on the nanoparticle surface (Black bar) as compared to when a perfectly complementary strand of ssDNA is added (Red bar). Note that the increase in fluorescence intensity for the perfectly complementary strand is significantly different than when ssDNA with a single nucleotide polymorph (SNP) and two mismatches (Green bar) is added to the duplex-bearing nanoparticle. Note the nanoparticles were excited at 420 nm (the absorption maximum of the polymer) and the emission at 525 nm is plotted)

The ss-DNA-AF546-modified NPs are prepared in an analogous fashion to that of the nonlabelled DNA described above, and the nanoparticle coverage is quite similar (~200 ssDNA-AF546 per nanoparticle). Again, as highlighted in Figure 3, when the polymer is added to the ss-DNA-AF546 NP to make the "duplex", the "fluorescence off" state is achieved. As highlighted in Figure 3, the spectrum shows an emission maximum at ~575 nm stemming from the direct excitation of the AF546 fluorophore at 420 nm. Following the addition of a complementary strand of ss-DNA and incubation of the nanoparticle at 50ºC for 15 minutes, the fluorescence intensity at 575 nm increases ~2.5 fold in intensity. The increase in intensity at 575 nm is attributed to the generation of the "triplex" on the NP surface, which results in the fluorescence emission of the polymer being "turned on". When the polymer emission is turned on, the polymer becomes a FRET donor and the AF546 molecules appended to ss-DNA-AF546 become the FRET acceptor. As a result the emission intensity of AF546 (at 575 nm) increases in intensity, and this increase

in emission intensity can be used to signal the hybridization event between the complementary DNA. In contrast, if a noncomplementary ss-DNA is added to the "duplex" bearing nanoparticle under the same conditions, there is no change in the fluorescence intensity at 575nm. This demonstrates good selectivity for complementary ss-DNA.

As highlighted above, the limit of detection is an important factor in the development of new DNA biosensors. The development of this FRET based biosensor provides a significant enhancement in sensitivity over the first generation, allowing concentrations as low as ~5 nM to be detected. We believe that the nanoparticle scaffold can provide a number of significant advantages where sample isolation is concerned. For example, nanoparticles can be centrifuged or designed with magnetic components that will allow for a magnetic confinement of the biosensor (i.e. the sample volume could be 1 mL, but the nanoparticles can be concentrated into tens of microliters). Such a label-free detection scheme (only the probe DNA is labeled, the complementary DNA that produces the signal is unlabelled) provides a valuable new tool that may allow for the sensitive detection of DNA without the use of PCR.

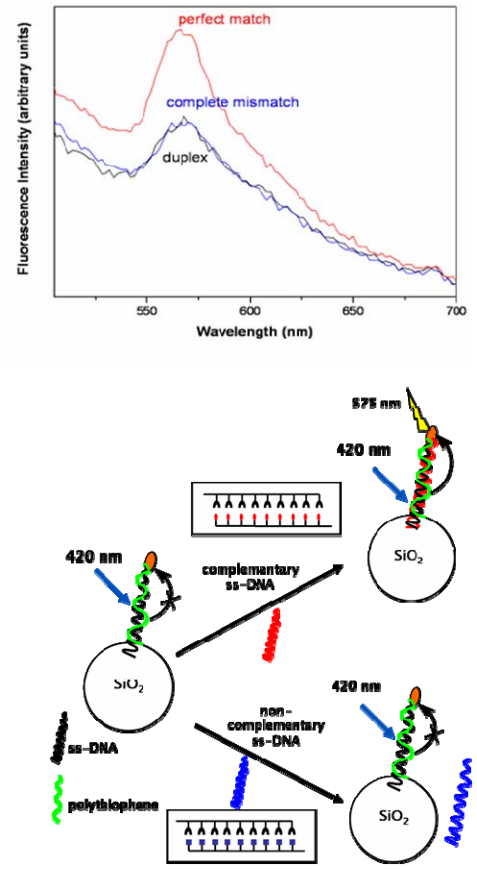

Figure 3. A cartoon representation of the FRET-based sensor on the nanoparticle surface. When the duplex structure is excited at 420 nm, there is no FRET to the fluorophore on the probe DNA. However, when a complementary ssDNA is added, the polymer becomes fluorescent and acts as a FRET donor for the fluorophore. As a result, there is an increase in fluorescence intensity for the fluorophore on the probe DNA attached to the nanoparticle. Note there is no change when a non-complementary ssDNA is added.

3 REFERENCES

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