Silica nanoparticle assisted DNA assays for optical signal amplification of conjugated polymer based fluorescent sensors†

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A cationic conjugated polymer provided over 110-fold signal amplification for silica nanoparticle based assays, which allow detection of target DNAs at a concentration of 10 pM with a standard fluorometer.

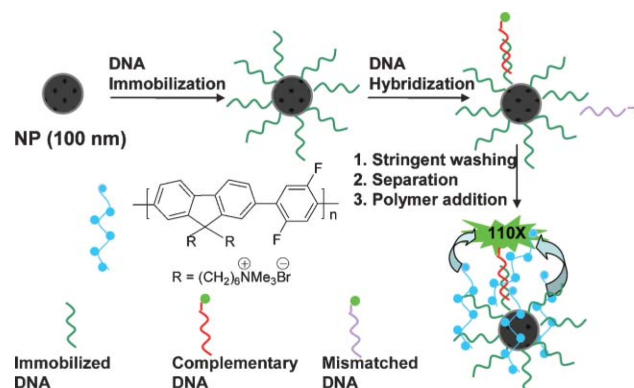
Methods for real-time, highly selective and sensitive polynucleotide detection are of vast scientific and economic importance because of their wide applications in identification of numerous biological pathogens and environmental contaminants.[1] Many DNA detection assays have been developed using different principles,[2] such as fluorescence,[3] electrochemical,[4] microgravimetric,[5] enzymatic,[6] electroluminescence,[7] and recently nanostructure based methods.[8] In general, the detection is constrained or limited by the levels of targets available in a particular sample. One has to rely on enzymatic sample replication (polymerase chain reaction) to increase the concentration of specific nucleic sequences to detectable levels or turn to complex labeling steps (dye multiplicity) or enhanced optical (or instrumentation) systems.[9] Such remedies are often reagent and instrumentation intensive, inciting higher levels of complexity and cost.

The unique optoelectronic properties of conjugated polymers (CPs) make them useful light-harvesting antennae for signal amplification in DNA sensor applications.[10] Their delocalized structure allows for electronic coupling between optoelectronic segments and efficient intra- and interchain energy transfer.[11] One specific approach that improves the sensitivity of homogeneous fluorescent DNA assays involves using a cationic conjugated polymer (CCP) and a chromophore (C*) labeled probe (*e.g.* DNA-C* or PNA-C*), which are selected to favor fluorescence resonance energy transfer (FRET) from the CCP to C*.[12] In addition to the factors such as spectral overlap, orientation and distance, recent studies reveal that the C* self-quenching upon CCP–DNA-C* complex formation and the mismatched energy levels between CCP and C* also affect the signal amplification.[13] Although this strategy has been proved successful for strand specific detection of DNA and RNA in aqueous media,[12] high selectivity is only observed with PNA-C* as the probe.[12a] Replacement of PNA-C* with DNA-C* leads to reduced selectivity as a result of the strong Coulomb attraction between the CCP and the negatively charged DNA probe.[12b,c]

Motivated by the wide biological applications of nanoparticles (NPs) and the high fluorescence quenching of polymers on the NP surface,[14] in this communication, we report a new strategy of using a probe-immobilized silica NP surface as a platform to simultaneously achieve high sensitivity and selectivity for DNA detection. We are particularly interested in silica NPs in the 100 nm size range since they are transparent in dilute solutions and their optical properties do not interfere with conjugated polymers.

Scheme 1 was designed to take advantage of the optical amplification of CCPs and easy separation of NPs. The approach begins with probe immobilized NPs in solution. If the added DNA is complementary to the probe, the NPs can capture the target and bring C* close to the surface. In the absence of the complementary sequence, no C* will be attracted to the NPs after stringent washing and separation steps. Only when the complementary sequence is present in the initial solution will CCP (donor) and C* (acceptor) be present in the final mixture, allowing intense acceptor emission upon polymer excitation.

The CCP (structure shown in Scheme 1) and C* (Fluorescein: Fl) used in this study were selected to have matched energy levels so that photoinduced electron transfer process is minimized.[13] The CCP was synthesized according to the previous report.[13] The silica NPs were synthesized using a modified Stöber method through hydrolysis of tetraethoxysilane (TEOS) in a mixture of ethanol and water in the presence of ammonia.[15] After surface modification with (3-aminopropyl)trimethoxysilane, followed by activation with 2,4,6-trichloro-1,3,5-triazine, the modified NPs were reacted with amino labeled synthetic oligonucleotide (5′-NH_2-AGCACCCA-CATAGTCAAGAT-3′). After purification, the average number of DNA probes on each NP was calculated to be $\sim$400 based on the UV absorbance measurement. Field emission scanning electron microscope (FE-SEM) images showed that the NPs before and after DNA immobilization are spherical in shape with an average size of $\sim$100 nm (Fig. 1).



Scheme 1 CCP-assisted NP-based DNA assay.

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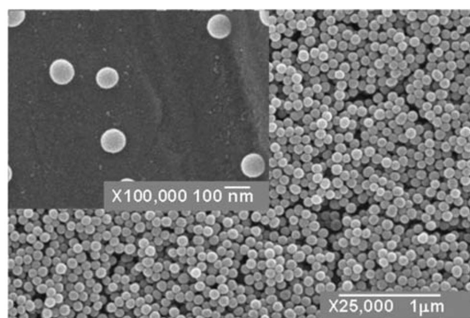

Fig. 1 FE-SEM images of the synthesized silica NPs. Inset: probe-modified NPs.

Hybridization of probe modified NPs (1.6 mg per 100 μL) with Fl labeled target DNA (DNA_T: 5′-Fl-ATCTTGACTATGTGG-GTGCT-3′) of varying concentrations has been studied in PBST buffer (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer and 0.1% Tween 20, pH = 7.0). The number of nanoparticles present was calculated as in the ESI.† There was a linear increase in the total number of DNA_T molecules captured by each NP when [DNA_T] was in a range of 0 to 3 μM, which was followed by saturation at higher [DNA_T]. From the fluorescence change in DNA_T solutions before and after hybridization and removal of NPs, the number of DNA_T strands captured by each NP was calculated to be on average $\sim 7 \pm 1$ for 0.18 μM, $\sim 24 \pm 2$ for 0.75 μM, $\sim 56 \pm 5$ for 2.0 μM, and $\sim 83 \pm 5$ for 3.0 μM, respectively. The representative fluorescence changes in DNA_T solutions before and after hybridization with NP removal for $7DNA_T$–NP is shown as Fig. S2 in the ESI.†

Upon excitation at 490 nm, the solution ([NP] = 0.2 mg per 2 mL) fluorescence increases almost linearly from 7 to $56DNA_T$–NP (Fig. 2 inset), indicating that there is almost no Fl self-quenching on the NP surface. We now examine how the number of DNA_T strands on each silica NP influences FRET. Measurements were carried out in PBST buffer, at a [NP] = 0.2 mg per 2 mL, upon CCP addition. Fig. 2 compares the Fl emission from NP solutions containing 7, 24 and $56DNA_T$–NP at a charge ratio (CCP/DNA probe and DNA_T, +/−) of 3.2, upon excitation at 370 nm. The most intense Fl emission was observed for CCP–$56DNA_T$–NP, which is approximately 40% more intense as compared to that for CCP–$24DNA_T$–NP and is about 4-fold greater than that for CCP–$7DNA_T$–NP. For $56DNA_T$–NP, the integrated Fl emission is approximately 40-fold greater than that obtained by excitation of Fl at 490 nm in the absence of the CCP, while over 75- and 110-fold enhancement is observed for 24- and $7DNA_T$–NP, respectively. The integrated fluorescence signals used for the calculation of signal amplification is shown in the ESI as Table 1.† Under similar experimental conditions, a solution of free $dsDNA_T$ with [Fl] the same as that of $7DNA_T$–NP generated only a 14-fold signal amplification (ESI†). Monitoring Fl emission upon direct excitation in the absence and presence of CCP showed a 25% decrease in fluorescence intensity for the free $dsDNA_T$ solution while there was no obvious intensity change observed for DNA_T–NP solutions. This indicates that Fl quenching upon DNA_T–CCP complex formation is minimized with NP based sensor design, which is due to the limited movement and the large distance of Fl molecules on the NP surface (for $7DNA_T$–NP, on average, there is one Fl molecule every 4500 nm^2 on the surface). The higher signal amplification in the presence of NPs could be attributed to the increased local concentration of donor units which is due to the complexation between the CCP and unhybridized ssDNA strands on the NP. This agrees well with the observation that with an increased number of DNA_T strands on each NP there is a decrease in signal amplification.

To evaluate probe immobilized NPs in DNA hybridization detection, 1.5 nmol of a DNA_T, a two-base mismatched sequence (5′-Fl-ATCTTGACTTTCTGGGTGCT-3′) and a random sequence (5′-Fl-CGTACGTACGTACGTACGTA-3′) were added separately to three probe-immobilized NP solutions in PBST buffer ([NP] = 1.6 mg per 2 mL), followed by hybridization and stringent washing steps.[16] Fig. 3 shows the normalized fluorescence spectra for NP hybridization with the three sequences. Strong Fl emission is observed when in the presence of the complementary sequence, while there is almost no Fl signal from solutions containing random or two-base mismatched sequences. With the amplification provided by the NP assay, it allows detection of Fl emission for [DNA_T] = 10 pM with a standard fluorometer.

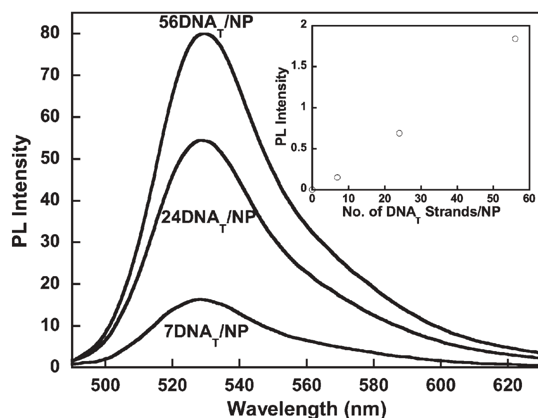

Fig. 2 Fl emission spectra of DNA_T–NP solutions in the presence of CCP upon excitation at 370 nm. Inset: Fl intensity of DNA_T–NP solutions upon excitation at 490 nm in the absence of CCP. [NP] = 0.2 mg per 2 mL and the charge ratio (+/−) is 3.2.

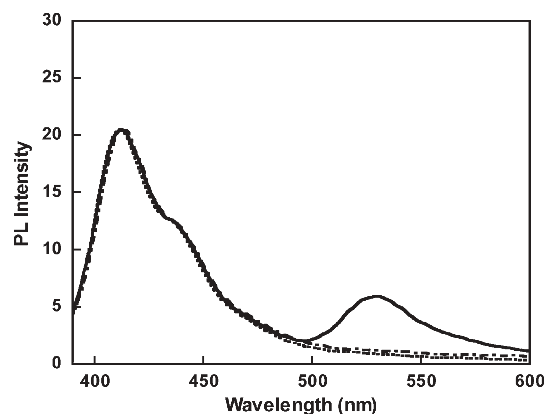

Fig. 3 Normalized emission spectra for NP hybridization with DNA_T (solid line), a random sequence (dotted line) and a two-base mismatched sequence (dash-dotted line) upon excitation at 370 nm. [NP] = 0.2 mg per 2 mL and [CCP] = 0.15 μM.

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In summary, we have developed a silica NP based assay for DNA hybridization detection with high sensitivity and selectivity. The NP based sensor strategy minimizes C* self-quenching within DNA_T–CCP complexes, and excess DNA probes on the NP surface could complex with CCP to increase the local concentration of donor units and deliver excitations to C*, resulting in over 110-fold signal amplification. The nearly homogeneous solution of silica NPs facilitates DNA hybridization and meanwhile provides the advantage of solid state sensors that allow separation and base mismatch detection. Further optimization of CCP structure–optical properties will likely yield practical platforms for real time detection.

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