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Label-Free, Real-Time Multiplexed DNA Detection Using Fluorescent Conjugated Polymers

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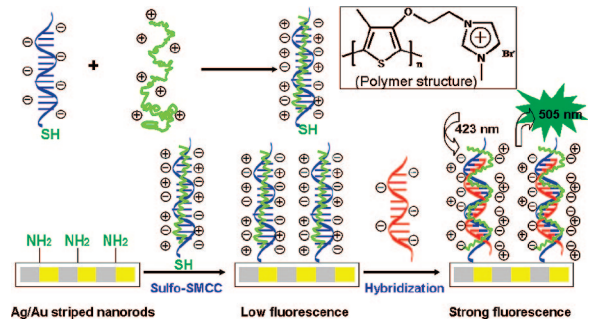
Biological multiplexing is one of the fastest growing areas in life science research because of its potential for extracting the most information from the smallest amount of sample volume at low cost.[1−3] Traditional multiplexed bioassay platforms, which are based on either planar microarrays or suspended encoding particles, often require an extra labeling step for the targets or the probes with reporter molecules, which prolongs the assay time and increases the assay cost. The need to overcome such hurdles has motivated research into the development of a label-free multiplexed assay system, where progress has been made in surface plasmon resonance (SPR)-based optical detection, nanowire-based electrical or electrochemical measurements, and mass spectrometry (MS)-based high-throughput screening.[4−7]

Conjugated polymers are novel materials with notable electrical and optical properties that have been successfully employed as label-free optical probes in biosensing applications.[8−10] For example, cationic conjugated polythiophene derivatives have been used in rapid and sensitive detection of DNA, where polymers bound to single-stranded DNA (ssDNA) undergo a major conformational change in comparison to when they bind to double-stranded DNA (dsDNA).[11−14] This conformational change transduces DNA hybridization into detectable colorimetric or fluorometric signals. Here we report the use of the same polythiophene derivatives in conjunction with metallic striped nanorods for multiplexed DNA detection that enables simultaneous monitoring of multiple biological recognition events in a label-free fashion.

Scheme 1 illustrates the overall detection strategy. Ag/Au-striped nanorods of different patterns were used as the array elements, where the particle identity was encoded by the difference in the reflectivities of adjacent metal strips.[3,15] The particles were precoated with 20 nm silica to reduce fluorescence quenching from the metal surface and provide a stable supporting layer for immobilization of DNA capture probes.[16] The thiolated DNA capture probes were premixed with a cationic conjugated polythiophene derivative {poly(1-methyl-3-[2-[(4-methyl-3-thienyl)-oxy]ethyl]-1*H*-imidazolium)} to form weakly fluorescent ssDNA−polymer duplexes through electrostatic interactions. The ssDNA−polymer duplexes were then covalently bound to the amino-modified silica-coated nanorods, where the particles of the same pattern carried the same DNA capture probes. Assorted nanorods carrying different DNA capture probes were then mixed before incubation with a mixture of target DNA sequences. Hybridization between the DNA capture probes and the target DNA led to formation of dsDNA−polymer triplexes, for which a strong fluorescence emission at 505 nm was observed. Both reflectance and fluorescence images of the nanorod mixture were collected, from which the identity of the target DNA was determined by the pattern of the nanorods (i.e., the corresponding capture probe) with strong fluorescence emission and the amount of the target DNA captured was quantified on the basis of the fluorescence intensity.

Scheme 1. Conceptual Illustration of Label-Free, Multiplexed DNA Detection Using Cationic Fluorescent Conjugated Polythiophene Derivatives and Ag/Au Striped Nanorods



The multiplexing assay concept was demonstrated using barcoded nanorods with three different patterns. Three different DNA capture probes, P1 (5′-TAACAATAATCCCTCA$_{20}$), P2 (5′-CACATCG-TATCCTAGT$_{20}$), and P3 (5′-GGCAGCTCGTGGTGAA$_{20}$), were mixed with conjugated polythiophene derivatives in stoichiometric quantities in separate solutions. The formed ssDNA−polymer duplexes were then coupled to the silica-coated nanorods with patterns of 000100, 01010, and 011110, respectively, where 0 refers to a Au strip and 1 to a Ag strip. These ssDNA−polymer duplex-bound particles were then mixed together and aliquoted into separate tubes before being incubated with target DNAs in different mixtures. It should be noted that similar assay performance was obtained when the DNA capture probes were immobilized on the corresponding particles before formation of the ssDNA−polymer complexes.

The left panel of Figure 1 shows reflectance and fluorescence images collected from four DNA hybridization assays. Nanorods with each of the three striping patterns were clearly distinguishable in the mixture in all of the reflectance images (upper panels). The assay results were determined on the basis of the fluorescence readouts (lower panels). In all cases, significant fluorescence intensity was observed only from the nanorod(s) with the capture probe(s) complementary to the target(s) in the incubation solution. A much weaker background was observed from particles having only ssDNA−polymer duplexes on the surface. For example, when the particle mixture was incubated with the solution containing target DNA T2, only P2-coated particles (01010) displayed strong fluorescence, whereas the other two nanorod patterns (000100 and 011110) showed little fluorescence (Figure 1A,C). Similarly, when the particle mixture was incubated with a solution containing targets DNA T2 and T3, both P2- and P3-coated particles (01010 and 011110) displayed strong fluorescence, whereas the P1-coated nanorods (000100) remained silent (Figure 1B,D). All three types of nanorods showed strong fluorescence signals when all three target DNAs were present in the hybridization solution, whereas no fluorescence signal was measurable when all three targets were absent (Figure S-4 in the Supporting Information). Quantitative

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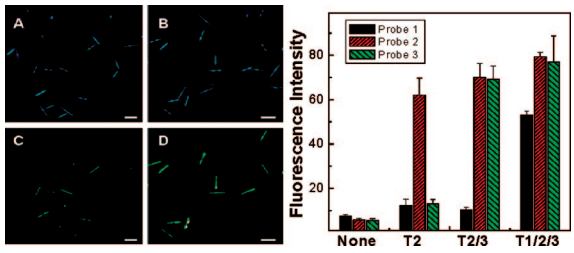

Figure 1. DNA detection in a label-free multiplexed format on barcoded nanorods using conjugated polymers. (left) Reflectance (A, B) and fluorescence (C, D) images showing the mixture of three DNA-coated nanorods included with (A, C) only target DNA T2 and (B, D) target DNAs T2 and T3. Scale bar = 5 μm. (right) Quantitative fluorescence readouts in multiplexed DNA detection. DNA targets in the incubation solutions are labeled on the *x* axis and the corresponding fluorescence readouts on the *y* axis. The colors of the columns indicate the sequences of the capture probes on different particles. See the text for details.

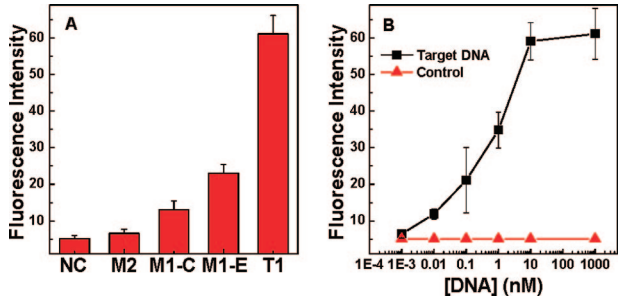

Figure 2. (A) Specificity of label-free DNA detection on barcoded nanorods. (B) Plot of fluorescence signal intensities detected using conjugated polymers on barcoded nanorods versus the concentration of the target DNA, T1.

readouts of the fluorescence intensities from each particle pattern after incubation with solutions containing different DNA targets are summarized in the right panel of Figure 1. Unambiguous detection of the presence of target DNA is clear.

Assay specificity was examined by mixing ssDNA−polymer duplex-bound nanorods with target DNA sequences having mutations at various sites. As Figure 2A shows, the DNA target with the perfectly matched sequence (T1: 3′-ATTGTTATTAGGGAG) produced the strongest fluorescence signal over background. Without extensive optimization, the fluorescence intensity was 12-fold stronger than that observed from the sequence with two mutations (M2: 3′-ATTGTTA**G**TAGG**A**AG) and the noncomplementary sequence (NC: GGTTTGTGTGGTTGG), 4-fold stronger than the one with a single mismatched base at the center (M1-C: 3′-ATTGTTA**G**TAGGGAG), and 3-fold stronger than the one with a single mismatch at one extremity (M1-E: 3′-ATTGTTATTAG-G**A**AG). This demonstrated assay specificity is comparable to that of other methods reported in the literature, including the recently published research in which DNA mutation was profiled using molecular beacons[17] or SPR.[18] The shared efficiency in differentiating point mutations among different methodologies is not unexpected, given that all of the aforementioned methods rely on DNA hybridization to distinguish complementary from noncomplementary sequences.

Sensitivity-wise, the described method using the conjugated-polymer-based, label-free DNA assay on nanorods is also comparable to conventional multiplexed DNA assays.[19,20] As Figure 2B shows, the fluorescence intensities from dsDNA−polymer-coated nanorods were logarithmically correlated with the concentration of target DNA.[20] The calculated limit of detection was ~5 pM. For a typical assay volume of 10 μL, this translates to detection of 50 amol (i.e., 3×10^7 molecules) with further improvement achievable (e.g., by reducing the amount of barcoded nanorods during incubation). The fluorescence signal leveled off at 10 nM, showing a dynamic range of 3 orders of magnitude. It should be noted that this dynamic range is tunable to suit different application needs through adjustment of the number of nanorods incubated with the target DNA molecules.

In conclusion, a label-free, multiplexed DNA assay in a solution-array format using conjugated polymers as the detection transducer for hybridization has been described. The striped metallic particles provide a means of differentiating the immobilized capture probes, while the changes in the optical signatures of conjugated polythiophene derivatives when they bind to ssDNA or dsDNA allow highly sensitive and specific detection of DNA hybridization events. Detection sensitivity at the attomole level has been successfully demonstrated, and single-base mutation in the target DNA sequence has been differentiated with more than 3-fold differences in fluorescence intensities. The same multiplexed label-free assay concept can be expanded to monitor other molecular interactions where DNA binding is involved.

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Supporting Information Available: Sequences of DNA used in the assay, a TEM image of a 20 nm thick silica-coated nanorod, fluorescence spectra of ssDNA−polymer and dsDNA−polymer complexes, and additional optical images from multiplexing assays and mutation studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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