# Sensitive and Selective Label-Free DNA Detection by Conjugated Polymer-Based Microarrays and Intercalating Dye

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DNA microarrays are a unique and powerful tool in biomedical research for sequencing the human genome, understanding the gene expression, and developing diagnostic tests of genetic diseases by means of selective detection of specific DNA sequences.[1–5] Convenient solid-state, on-chip DNA synthesis has contributed significantly to the fast progress of DNA microarray development.[6–10] There has also been recent effort to improve sensitivity by applying the energy harvesting and signal transduction property of conjugated polymers to DNA detection.[11–16] A label-free detection strategy also has gained much interest because it can provide fast and cost-effective DNA detection. Among the label-free detection methods are protease-based detection, molecular beacon system, and the use of intercalating dyes.[15,17–20] Intercalating dyes are fluorescent molecules that preferably bind to the major groove of a double helix DNA over single strand DNA (ssDNA). SYBR green I, an intercalating dye, is an asymmetrical cyanine dye having a high quantum yield of 0.80 that is 100 times larger than that of ethidium bromide, a commonly used intercalating dye, and is also much less mutagenic than ethidium bromide. However, SYBR green I like other intercalating dyes can also stain ssDNA as the amount of SYBR green I required for double stranded DNA detection increases because its specificity toward double helix DNAs is not perfect. Hence, this nonspecific binding is a critical problem when only a trace amount of analyte DNA is available for detection. In this case, a large amount of the dye is required to produce a distinguishably strong signal, but the large amount of dye can reduce specificity.

We have developed a series of uniquely stable oxadizole-containing conjugated polymers toward photobleaching and chemical degradation and established an on-chip DNA synthesis strategy on thin-layers of these oxadiazole-containing conjugated polymers.[12,21] By achieving efficient fluorescence resonance energy transfer (FRET) from the polymer layer to dye-labeled DNAs we have shown a large signal amplification. In this communication, we present signal amplifying DNA microarrys having label-free DNA detection capability by combining the signal amplification scheme of the conjugated polymer (POX1)-based DNA microarray and the intercalating dye, SYBR green I. Because the emission signal from SYBR green I can be largely amplified by the FRET-based signal amplification mechanism, even a small amount of SYBR green I can produce a strong enough emission signal without losing the specificity as schematically illustrated in Scheme 1 a. Scheme 1 b,c shows the chemical structures, and the absorption and emission spectra of SYBR green I and POX1, respectively. As one can see, there is a large spectral overlap between the emission spectrum of POX1 and the absorption spectrum of SYBR green I, satisfying a requirement for efficient FRET. Moreover, the absorption $\lambda_{max}$ of POX1 is well separated from that of SYBR green I, allowing exclusive excitation of either the donor or the acceptor for the energy migration study between POX1 and SYBR green I.

We first studied the specificity of SYBR green I toward double stranded DNAs at three different concentrations: $5 \times 10^{-7}$ M, which is the manufacturer's recommended concentration, $5 \times 10^{-8}$ M, and $5 \times 10^{-9}$ M.[22] For the study 50 $\mu$L of the $1 \times 10^{-5}$ M (0.5 nmol) aqueous solution of the cDNA sequence (5′-ACA CAT CAC GGA TGT-3′), a 1-mismatch sequence (5′-ACA CAT CTC GGA TGT-3′) and

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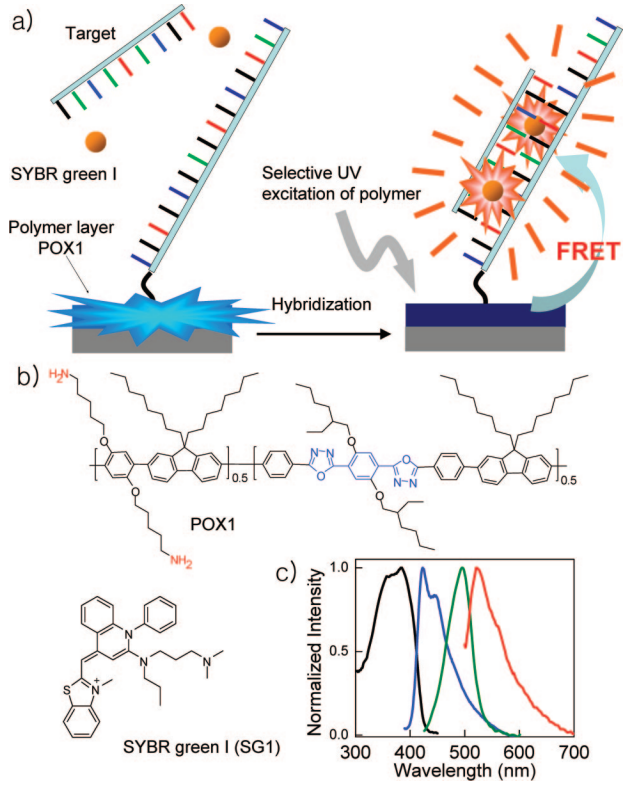
**Scheme 1**[a]




[a] (a) Schematic representation of a label-free conjugated polymer−DNA hybrid miroarray, (b) the chemical structures of POX1 and SYBR green I, and (c) their UV−vis/PL spectra (black/blue for POX1 and green/red for SYBR green I) in the solid film (POX1) and in 0.5 $\mu$M 6 × SSPE solution at pH = 7.4 (SYBR green I).

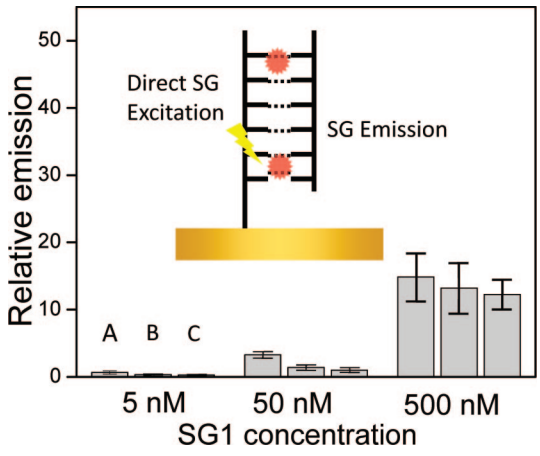



**Figure 1.** Selectivity test of conventional control slides without POX1. A: perfect match (5′-ACA CAT CAC GGA TGT-3′). B: 1-mismatch (5′-ACA CAT CTC GGA TGT-3′). C: random sequence (5′-TGT GTA GTG CCT ACA-3′).

a random sequence (5′-TGT GTA GTG CCT ACA-3′) were spread, respectively, onto a DNA microarray without POX1 and incubated at 37 °C together with SYBR green I. As shown in Figure 1, $5 \times 10^{-8}$ M and $5 \times 10^{-9}$ M concentrations gave a good specificity, but the signal was very weak and not well distinguishable due to the relatively large error range. As the concentration of SYBR green I increased to $5 \times 10^{-7}$ M, the emission intensity became much stronger. However, the specificity of the intercalating

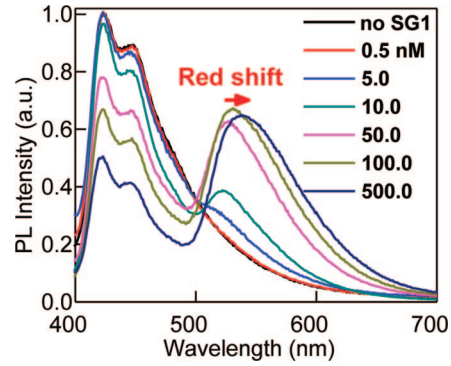



**Figure 2.** Emission profile in various SYBR green I concentrations. The excitation wavelength was 380 nm.

dye toward the double strand DNA significantly decreased, and we could hardly distinguish the target, 1-mismatch, and even the random seqeunce.

We built the signal amplifying and self-signaling DNA microarray by means of covalent immobilization of POX1 on a glass substrate having isothiocyanato groups as a linker and the subsequent light-directed on-chip DNA synthesis (detailed procedure is in the Supporting Information). After the DNA synthesis, the resulting DNA patterns were confirmed by a UV scanner. The spot diameter was 55 $\mu$m, and the density of the synthesized DNA was 0.243 nmol/cm$^2$. We systematically investigated the signal amplifying property of our DNA microarrays by hybridizing 50 $\mu$L of $1.0 \times 10^{-5}$ M of the cDNA sequence (5′-ACA CAT CAC GGA TGT-3′) to the DNA microarrays together with SYBR green I at various concentrations. POX1 was selectively excited at 380 nm, and the emission of POX1 at 425 nm and the emission of SYBR green I at 525 nm were monitored. As shown in Figure 2, as the concentration of SYBR green I increased, the emission from POX1 at 425 nm decreased and instead the emission from SYBR green I at 525 nm gradually increased due to efficient energy transfer from POX1 to SYBR green I. One can clearly see the discrete SYBR green I emission when 5 nM or higher concentration of SYBR green I was used. However, when SYBR green I concentration increased from 50 nM to 100 nM, the emission of SYBR green I was broadened and bathochromic shifted. We found from the binding study of SYBR green I to ssDNAs that the peak broadening and bathochromic shift indicate nonspecific binding of SYBR green I to ssDNA, its aggregation, and the resulting fluorescence quenching. The fluorscence emission band of SYBR green I complexed with ssDNA was significantly broader, and the emission maximum was shifted to a longer wavelength (525 to 560 nm) as the SG1/ssDNA ratio increased (Supporting Information). It is known that the flourescence intensity of SYBR green I when attached to ssDNA is significantly lower than that of the dye complexed with a double strand DNA due to aggregation-induced self-quenching. Vitzthum and co-workers reported a bathochromic shift of the emission maximum of SYBR green I when it binds to ssDNA.[22] They reported that the emission maximum of SYBR green I was at 525 nm when the dye/base pair ratio was 1. However, the emission maximum shifted to 535 and 552 nm when the dye/base pair ratio increased to 2 and 10, respectively. Therefore, the

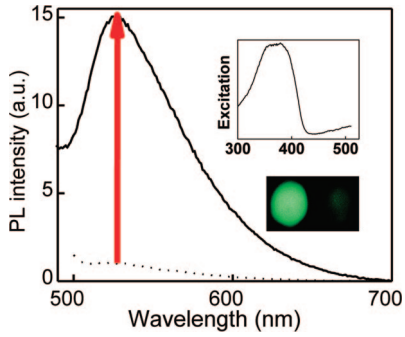



**Figure 3.** PL emission spectra of SYBR green I after hybridization with a target DNA ([c-DNA] = $1.0 \times 10^{-5}$ M, 5′-ACA CAT CAC GGA TGT-3′, [SYBR green I] = 50 nM) when excitated at 380 nm (solid) and 490 nm (dotted). Inset: (top) excitation spectrum for the SYBR green I emission at 525 nm and (bottom) fluorescence microscope images of a microarray spot upon excitation of POX1 at 405 nm (left) and SYBR green I at 500 nm (right).

bathochrimic shift of SYBR green I emission in the higher concentrations (100 nM or 500 nM) and the negligible increase in its emission intensity in the experiment are direct evidence of nonspecific binding of SYBR green I to ssDNA.

The FRET efficiency calculated by the equation 1 − (intensity of donor with acceptor)/(intensity of donor without acceptor) was 0.04, 0.22, 0.33, and 0.55 for 10, 50, 100, and 500 nM of SYBR green I, respectively. With the SYBR green I concentration of 50 nM, which has the best FRET efficiency without nonspecific binding to ssDNA, we achieved 15 times signal amplification from our signal amplifying DNA microarray as shown in Figure 3. The SYBR green I emission was largely amplified when POX1 was excited at 380 nm compared to the SYBR green I emission from the direct excitation of the intercalating dye at 490 nm. This large signal amplification stems from a much larger absorption intensity of the POX1 layer (0.015 at 380 nm) compared to that of SYBR green I (0.001 at 490 nm). Therefore, the POX1 layer absorbs a much larger amount of photon than SYBR green I can absorb and gives its energy as the FRET donor to the FRET acceptor, SYBR green I. Figure 3, inset, shows fluorescence microscope images of a DNA microarray spot for comparison. There is large contrast difference between the two images, confirming the efficient signal amplification. When 50 nM SYBR green I on the signal amplifying microarray was directly excited at 500 nm (right image) the spot was too dim. Conventional microarrays without the POX1 layer also showed the same dim spots. On the contrary, the POX1-coated signal amplifying DNA microarray showed strong emission with high contrast when POX1 was excited at 405 nm. The detection limit of the POX1-coated signal amplifying DNA microarray was in the subpicomolar regime. The excitation spectrum of the amplified SYBR green I emission at 525 nm in the Figure 3 inset clearly presents that the origin of the 525 nm emission is POX1.

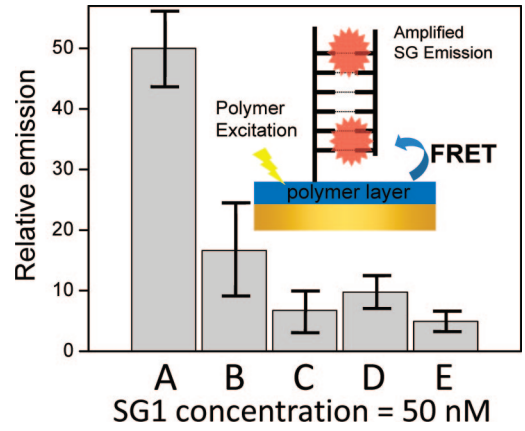



**Figure 4.** Selectivity test of the signal amplifying DNA microarray having the POX1 layer. A: perfect match (5′-ACA CAT CAC GGA TGT-3′), B: 1-mismatch (5′-ACA CAT CTC GGA TGT-3′). C: random sequence (5′-TGT GTA GTG CCT ACA-3′). D: prehybridized control. E: only POX1-coated slide. Hybridization condition: incubation in 6 × SSPE at 37 °C for 20 min, each [DNA] = $1.0 \times 10^{-5}$ M, [SYBR green I] = 50.0 nM.

Selectivity tests were conducted by using $1.0 \times 10^{-5}$ M one-mismatch DNA (5′-ACA CAT CTC GGA TGT-3′) and random mismatch DNA (5′-TGT GTA GTG CCT ACA-3′). We also tested nonspecific binding of SYBR green I (50 nM) to the ssDNA probes on the microarrays without having the cDNA and to the glass slide having only POX1 without ssDNA synthesis, respectively. Figure 4 shows the relative fluorescence intensity of each case and demonstrates the good specificity of the DNA microarrays. One can clearly see that the signal intensity of the 50 nM SYBR green I on our signal amplifying DNA microarray (Figure 4) is largely amplified compared to the signal intensity of the same 50 nM SYBR green I on the conventional DNA microarry (Figure 1) due to an efficient FRET.

In summary, we have demonstrated a label-free and signal amplifying DNA microarray using a conjugated polymer and an intercalating dye SYBR green I. Efficient FRET from the conjugated polymer to the dye produced large signal amplification so that without losing good selectivity, sensitive detection of subpicomolar concentrations of the target DNA was achieved.

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**Supporting Information Available:** Details for on-chip DNA synthesis, hybridization, fluorescence experiment, determination of SG1 concentration, and PL profile of SG1 in ssDNA (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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