Supporting Information

Sensitive and Selective Label-free DNA Detection by Conjugated Polymer-based Microarrays and Intercalating Dye

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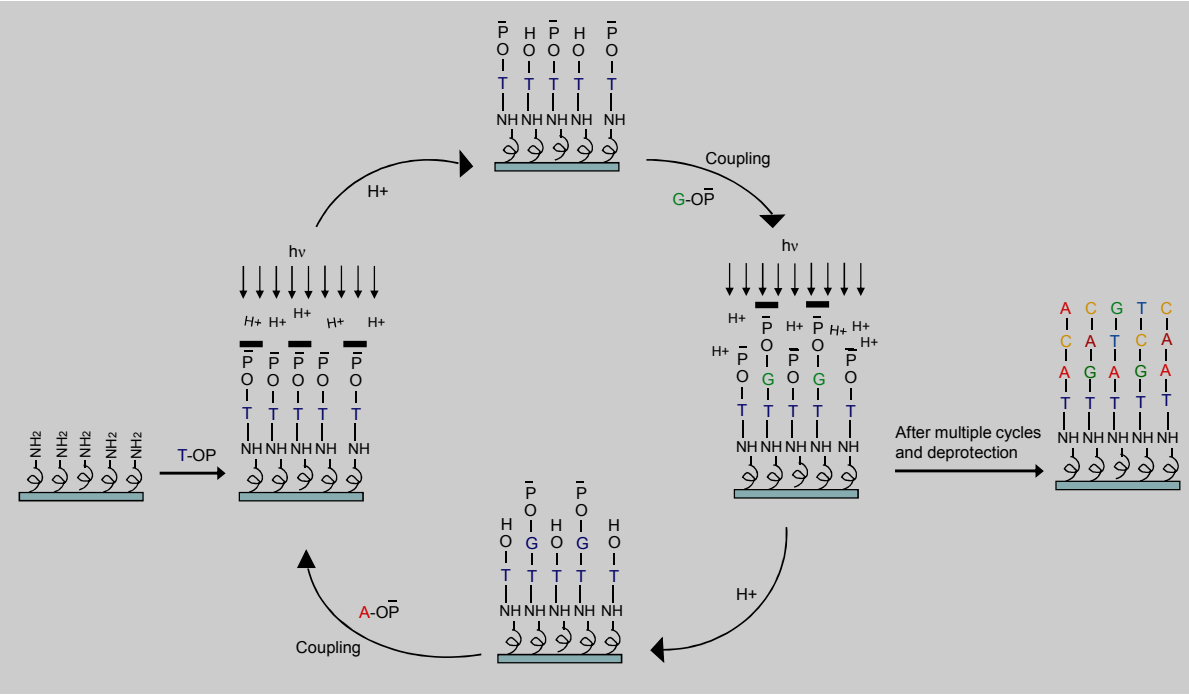
Materials and Methods

The chemicals and reagents from Sigma-Aldrich Chemical Co. and Glen Research were used without further purification. Varian Cary50 UV/Vis spectrophotometer was used to conduct the absorption study and PTI QuantaMaster™ Spectrofluorometer with an integrating sphere was used to find the quantum yields as well as photoluminescence spectra. SYBR green I, the intercalating dye was purchased from Invitrogen worldwide and also used without further purification. Detail synthetic route and characteristic data for conjugated polymers (POX1) was previously reported.[1]

Light directed on-chip oligonucleotide synthesis.

The glass slide was inserted into a holder attached to a DNA synthesizer. Standard phosphoramidite chemistry was used for all steps of oligonucleotide synthesis except for the deprotection step. In this step, the terminal dimethoxytrityl protecting group was deprotected using a photoacid generator (PAG), which was put into the holder containing the slide. The digital photolithographic projection unit from a Digital Light Project (Texas Instruments) was attached to the synthesizer and used to project a preset light pattern onto the slide to activate PAG. For each deprotection step, PGA and CH_2Cl_2 were filled into the slide holder and activated to remove the terminal dimethoxytrityl protecting group. The deprotection step followed by the

attachment of a base unit was repeated until the desired DNA sequence was obtained, as illustrated in Scheme S1. Patterned DNA synthesis was confirmed by GenePix 4000B microarray scanner (Molecular Devices Corp.) with dual lasers (532 nm/17 mW and 635 nm/10mW).



Scheme S1. Schematic illustration of the on-chip DNA synthesis.

Determination of SG1 concentration.

SG1 concentration was calculated by using the Beer's law and the molar absorptivity of 73, 000 $L \cdot mol^{-1} \cdot cm^{-1}$ reported previously by Vitzthum and co-workers.[2] The absorbance of SG1 dissolved in a TE buffer (pH = 7.5) was measured at 495 nm.

$$A = \varepsilon \cdot b \cdot c$$

where A is the absorbance (no units, since $A = \log_{10} P_0/P$)
ε is the molar absorptivity with units of $L \cdot mol^{-1} \cdot cm^{-1}$
b is the path length of the sample (cm, 1 cm cuvette)
c is the concentration of the DMT in acetonitrile, expressed in $mol \cdot L^{-1}$.

Photoluminescence profile of SG1 in ssDNA

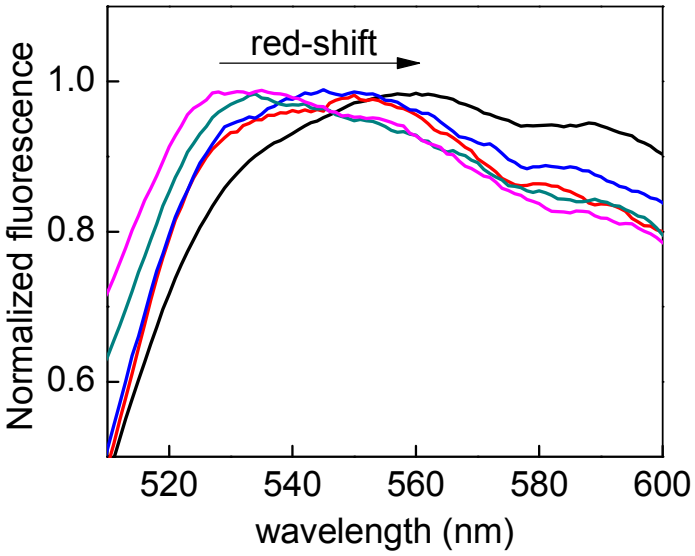


Figure S2. Normalized photoluminescence of SG1 solution (5 uM) in the presence of 15 base-pair ssDNA (0.1 nM; pink, 0.05 nM; green, 0.02 nM; blue, 0.01 nM; red, noDNA; black) in 6×SSPE buffer (pH = 7.4).

Hybridization

After the light directed on-chip oligonucleotide synthesis, the glass slides were washed with 5 mL of 6×SSPE. Prehybridized solution (30 µL, 20×SSPE, 15 µL acetylated bovine serum albumin (ac-BSA), 90 µL water) was then added to the glass slides and washed away after 3 minutes. Hybridization solution (15µL 20×SSPE, 28.5 µL water, 5 µL ac-BSA, 0.5 µL target DNA with sequence 5'-ACA CAT CAC GGA TGT-3', and 1 µl of SG1 with various concentration) was heated to 95 ºC. To hybridize the DNA, we added the hybridization solution and covered the slides with a second glass slide, not allowing any air to be trapped between the two glass slides. Hybridization was induced by slowly decreasing temperature. We incubated the slides for one hour at 37 ºC. After removing the solution from the slides, the slides were rinsed with 6×SSPE and iced water and dried with a stream of air. The quality of the slides was examined by using a fluorescence scanner and the PL intensity of the slides before and after hybridization was investigated by using a fluorescent spectrophotometer. Alternatively we

conducted the dye intercalating separately after the hybridization. This method also gave the same results that we obtained from the simultaneous hybridization and dye intercalation method. Selectivity tests with a single-mismatch DNA (5'-ACA CAT CTC GGA TGT-3') and a random target sequence (5'-TGT GTA GTG CCT ACA-3') were also performed by using the same condition. The fluorescence images in Figure 2(b) inset were obtained by using BX41 Fluorescence microscope, DP71 digital camera (Olympus), and Microsuite 5 Biological Suite Software (Olympus). The background (prehybridization) was subtracted. Direct excitation of SYBR green I and **POX1** excitation for amplification were carried out at 500 nm and 405 nm, respectively.

References

1. Lee, K.; Rouillard, J.-M.; Pham, T.; Gulari, E.; Kim, J. Angew. Chem. Int. Ed. 2007, 46, 4667.
2. Zipper, H.; Brunner, H.; Bernhagen, J.; Vitzthum, F. Nucleic Acids Res. 2004, 32, e103.