DNA Microarrays

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# Signal-Amplifying Conjugated Polymer–DNA Hybrid Chips**

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Bio-/synthetic hybrid materials have recently received considerable attention owing to their potential biomedical applications.[1] The most reliable way of identifying any biological target is through its genetic code.[2] However, the current commercial DNA microarray requires costly and time-consuming PCR to multiply the number of analyte DNA molecules and label the analyte DNA with a fluorescent dye because of the low detection limit. In this context, devising self-signal-amplifying DNA microarrays can realize low-cost, fast, and reliable detection of nucleic acids. Herein, we report signal-amplifying DNA chips fabricated by on-chip DNA synthesis on a thin film of a newly developed conjugated polymer (Figure 1 and the chemical structure in Figure 2a).

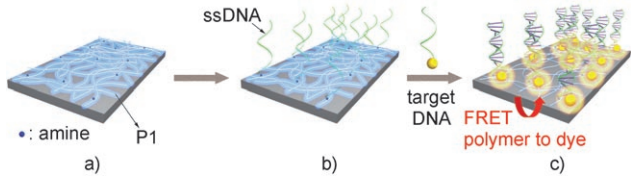


***Figure 1.*** Schematic representation of the signal-amplifying conjugated polymer-based DNA chip. a) **P1**-coated glass slide by covalent bonding; b) light-directed on-chip oligonucleotide synthesis; c) hybridization with a target DNA results in large emission enhancement of the fluorescent dye through efficient Förster resonance energy transfer.

Conjugated polymer-based biosensors are an attractive approach to improve the detection limit because an environmental change at a single site can affect the properties of the collective system, producing large signal amplification.[3] Therefore, if one devises a strategy combining the signal-amplification scheme of conjugated polymers and efficient on-chip DNA synthesis, signal-amplifying DNA microarrays can be conveniently prepared. On-chip oligonucleotide synthesis[2d,4] has the unique advantage of being performed in a parallel fashion, is flexible in sequence design, easy to manufacture, and has a high sequence fidelity compared with other recently developed methods, such as the pin microdotting method,[2c] the ink-jet microdropping method,[5] and the electrostatic addressing method.[6] Almost all the on-chip DNA synthesis technologies, however, require harsh conditions such as long exposure to UV light and/or to strong acids. Under these harsh conditions, conventional conjugated polymers will be photobleached or chemically degraded.

We have developed a novel conjugated polymer with a strong fluorescence emission and unique stability under the above-mentioned harsh conditions. Figure 2a shows the chemical structure of the poly(oxadiazole-co-phenylene-co-fluorene) **P1** with oxadiazole units and amine side chains. All monomer units of **P1** were designed to have their own contribution to the final property of **P1** and synthesized through multiple synthetic steps (see the Supporting Information). Oxadiazole is an electron-poor heterocyclic molecule that has been used in polymer design in which the improvement of electron transport and/or stability of the polymer is required.[7] We designed an oxadiazole-containing monomer (**M3**) and incorporated this unit into the conjugated polymer backbone by using a Pd-based Suzuki coupling method.[8] The oxadiazole-containing monomer unit **M3** of **P1** has an intense blue fluorescence emission at 413 nm in a chloroform solution and is stable when exposed to strong UV irradiation and a strong acidic environment. The amine groups on the phenylene unit (**M1**) of **P1** serve as functional groups for immobilization of **P1** on a glass substrate as well as linkers for direct on-chip synthesis of oligonucleotides on the resulting thin-layer film of **P1**. The fluorene unit (**M2**) of **P1** is incorporated to provide good solubility in organic solvents and to ensure a good spectral overlap with commonly used organic dyes for an efficient fluorescence resonance energy transfer (FRET). Figure 2b shows the absorption (UV) and photoluminescence spectra (PL) of **P1** in chloroform and incorporated in the film. The absolute quantum yield of **P1** solution in chloroform (1 mg $L^{-1}$), measured in an integrating sphere (PTI technologies, Inc.), was 94%. We investigated the stability of **P1** compared with commonly used conjugated polymers, such as poly(*p*-phenylene-ethynylene)s and poly(3-hexylthiophene), under strong UV irradiation and highly acidic conditions. None of the compounds except **P1** survived these tests (data not shown). The fluorescence of the conventional conjugated polymers was completely quenched by degradation of polymers under these harsh conditions. However, **P1** showed unique stability against the exposure to UV irradiation and acid treatments both in the solution and solid state. The unique stability of **P1** made possible on-chip DNA synthesis directly on a thin film of the conjugated polymer.

The preparation of **P1**-coated glass substrates is described in Figure 3a. We covalently linked **P1** to a glass substrate to

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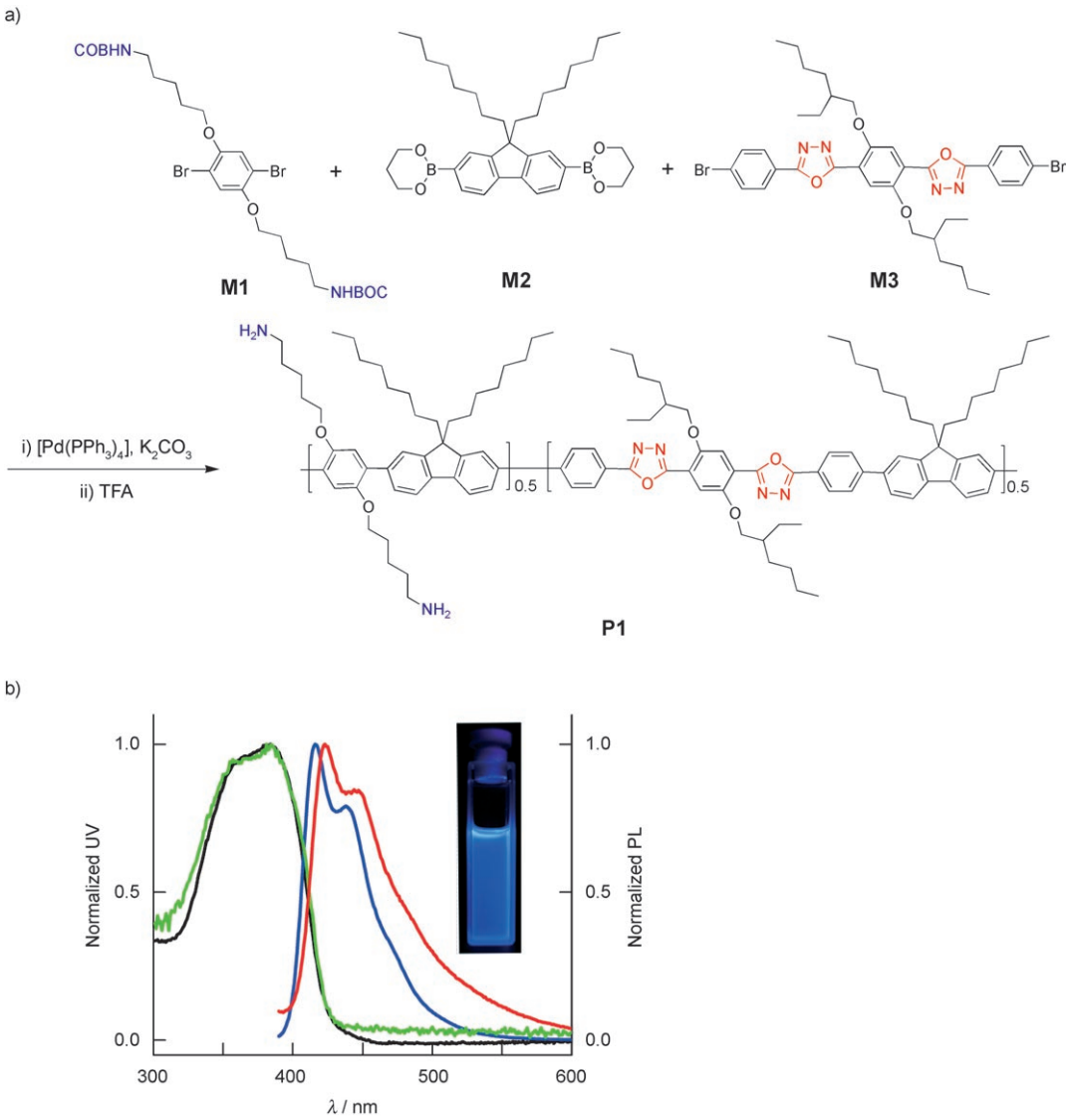


***Figure 2.*** a) Chemical structure of **P1**. b) UV/PL spectra of **P1** in chloroform (black = UV, blue = PL) and solid film (green = UV, red = PL).

prevent any loss of **P1** during the on-chip DNA synthesis. To do so, isothiocyanate-functionalized glass substrates were prepared by using a slightly modified literature procedure.[3e] First, aminopropyl groups were introduced onto a glass substrate by first cleaning with pirahna solution ($H_2O_2$/$H_2SO_4$ 3:7 (v/v)) followed by an aminopropyltrimethoxysilane (APTMS) coating. 1,4-Phenylenediisothiocyanate was then reacted with the amine of APTMS to form a reactive linker for **P1**. Finally, **P1** was chemically bound onto the glass substrate. After immobilization of **P1**, the derived UV spectrum of the glass substrate showed a new broad band at 350–400 nm, which corresponds to **P1** absorption. Fluorescence spectroscopy also showed a well-defined fluorescence emission spectrum of **P1** from the glass substrate.

The on-chip DNA synthesis[9] on the **P1**-coated glass substrate was conducted by using a modified automatic oligosynthesizer equipped with a UV patterning device. The synthesis is carried out by using 5′-(4,4′-dimethoxytrityl) (DMT) nucleophosphoramidite monomers as the building blocks and each synthesis cycle consists of a deprotection step by using photogenerated acids, coupling of a DMT-protected monomer, capping of unreacted terminal OH groups, and oxidation of the phosphite to phosphatetriester at internucleotide linkages.[4b,10] Various sequences of DNA can be synthesized at different locations on the chip by generating a strong acid at the desired locations by UV-induced decomposition of a photoacid generator (PAG). The photogenerated acid (PGA) then catalyzes the deprotection reaction, producing a 5′-OH group, which is available for the next monomer. We synthesized two different sequences. The first sequence was 5′-ACA TCC GTG ATG TGT T-glass-3′ (the 3′ T is a spacer), which was used for hybridization with the complementary sequence with hexachlorofluorescein (HEX) dye, and the second sequence was 5-ACAG AAG CAT TAT TTC T-glass-3′ for the Cy5-labeled complementary sequence.

Figure 3b shows the fluorescence image of the synthesized DNA on the **P1**-coated glass substrate after hybrid-

  

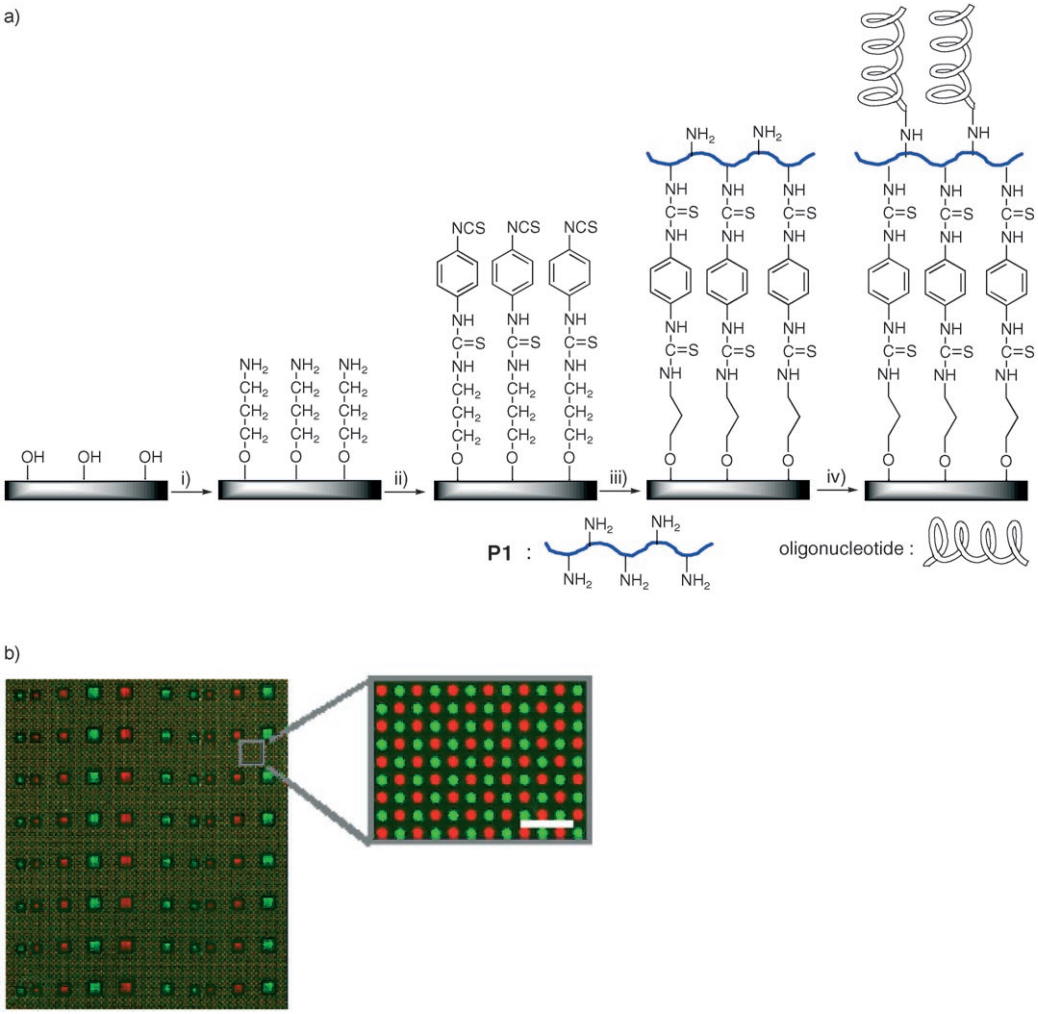


***Figure 3.*** a) Schematic representation of the light-directed parallel on-chip DNA synthesis on **P1**-immobilized glass: i) APTMS, ii) 1,4-phenylenediisothiocyanate, iii) polymer (**P1**), and iv) cyclic procedures of oligo synthesis. b) A fluorescence image of a patterned signal-amplifying DNA microarray with two different DNA sequences after hybridization with a mixture of c-DNA-HEX (green) and c-DNA-Cy5 (red; scale bar: 200 μm).

ization with two different dye-labeled complementary DNA molecules. Selective fluorescent patterns of green (HEX) and red (Cy-5) dots are clearly shown in Figure 3b. This result demonstrates that direct on-chip DNA synthesis onto a **P1**-coated glass slide was macroscopically accomplished. Moreover, during the harsh DNA synthesis procedures, the emissive property of **P1** was maintained. We prepared a control sample to conduct quantitative analysis of signal amplification by **P1**. The control sample had the same 16-base DNA sequence (5′-ACA TCC GTG ATG TGT T-glass-3′) as was synthesized on an amine-functionalized glass slide, but without **P1**. The density of the synthesized oligonucleotide (2.44 pmol $cm^{-2}$) on the conventional control slide was the same as that of the oligonucleotide on the **P1**-coated slide. This was confirmed by UV absorption at 410 nm (see the Supporting Information).

We used a 15-base HEX-labeled complementary DNA sequence to observe the FRET effect from **P1** to HEX dye. FRET involves a nonradiative transmission of fluorescence energy from a donor molecule to the acceptor molecule. **P1** has a good spectral overlap with HEX, satisfying the requirement for efficient FRET. Figure 4a shows the fluorescent emission spectrum of the **P1**-coated DNA chip and the control slide before and after hybridization with the HEX-labeled complementary DNA (c-DNA-HEX). Upon hybridization tests with c-DNA-HEX on the signal-amplifying **P1**-immobilized DNA chip, one can observe a large signal amplification. The fluorescence emission of **P1** was decreased when excited at 380 nm, whereas the emission of HEX was significantly amplified. Direct excitation of HEX at 535 nm produced only a weak fluorescence emission as shown in Figure 4a. This large signal amplification clearly indicates an efficient fluorescence resonance energy transfer from **P1** to HEX. The detection limit of our signal-amplifying DNA microarray is $10^{-10}$ M (see the Supporting Information). We conducted the same hybridization test on the control slide. Direct excitation of HEX at its absorption maximum ($\lambda_{max}$) of 535 nm produced the same weak fluorescence emission as obtained from the direct excitation of the **P1**-immobilized DNA chip at 535 nm. A selectivity test was also done with HEX-labeled one-mismatch DNA (5′-HEX-ACA CAT CTC GGA TGT-3′) and HEX-labeled noncomplemen-

  

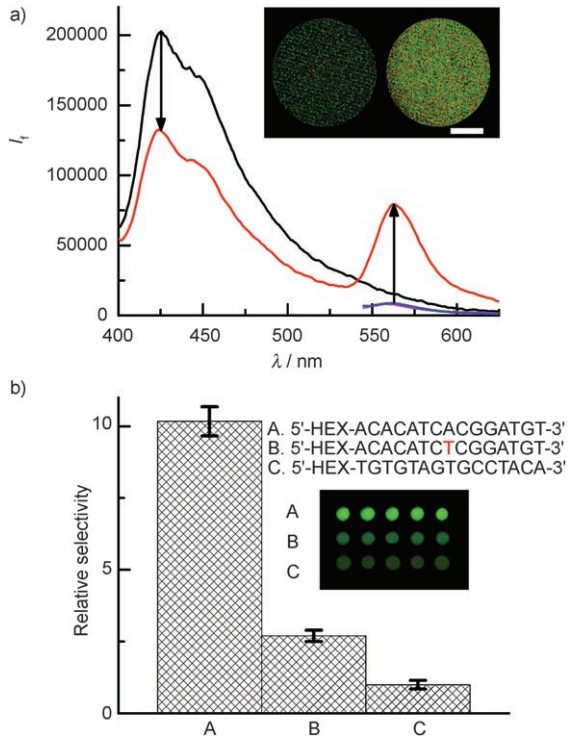


***Figure 4.*** a) PL emission spectra of **P1** substrate before (black) and after hybridization when excited at 380 nm (red) and 535 nm (blue); PL emission of the control (pink, excited at 535 nm). Note that the blue and pink lines are essentially superimposed and appear around 550–625 nm. Inset: Comparison of fluorescence intensity upon excitation at 535 nm (left) and 405 nm (right). Scale bar: 25 μm. b) Selectivity test: A) perfect match, B) one mismatch, and C) random sequence. Inset: A microscanned image, from the top row down: perfect match, one mismatch, and random sequence. The spot diameter is 55 μm. $I_f$ = fluorescence intensity.

tary DNA (5′-HEX-TGT GTA GTG CCT ACA-3′). Figure 4b shows the relative fluorescence intensity of HEX on the complementary and one-mismatch DNA compared with that of the noncomplementary DNA, demonstrating the selectivity of the signal-amplifying conjugated polymer-based DNA microarray.

In summary, we have established a fast and readily applicable strategy to make a signal-amplifying DNA microarray by developing a novel conjugated polymer and combining it with an efficient and convenient on-chip DNA synthesis. The newly developed conjugated poly(oxadiazole-co-phenylene-co-fluorene) is highly emissive and has unique stability in harsh environments. DNA hybridization tests showed a good selectivity and a large signal amplification achieved by efficient FRET from the emissive conjugated polymer to the dye-labeled target DNA. The results provide a design principle for further development of self-signal-amplifying DNA microarrays that possibly allow PCR-free DNA detection through a large signal amplification.

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