

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 2 a).

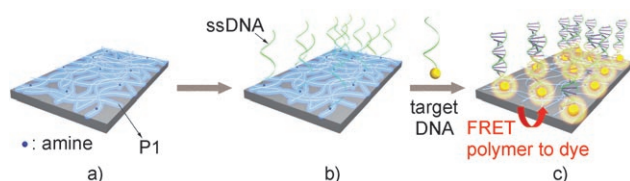


Figure 1. Schematic representation of the signal-amplifying conjugated polymer-based DNA chip. a) **P1**-coated glass slide by covalent bonding; b) light-directed on-chip oligonucleotide synthesis; c) hybridization with a target DNA results in large emission enhancement of the fluorescent dye through 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 3 a. We covalently linked **P1** to a glass substrate to

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[**] We appreciate financial support from the National Science Foundation (BES 0428010) and the equipment grant from the University of Michigan College of Engineering. J.K. and K.L. also acknowledge the Ilju Foundation for the Ilju scholarship.

Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

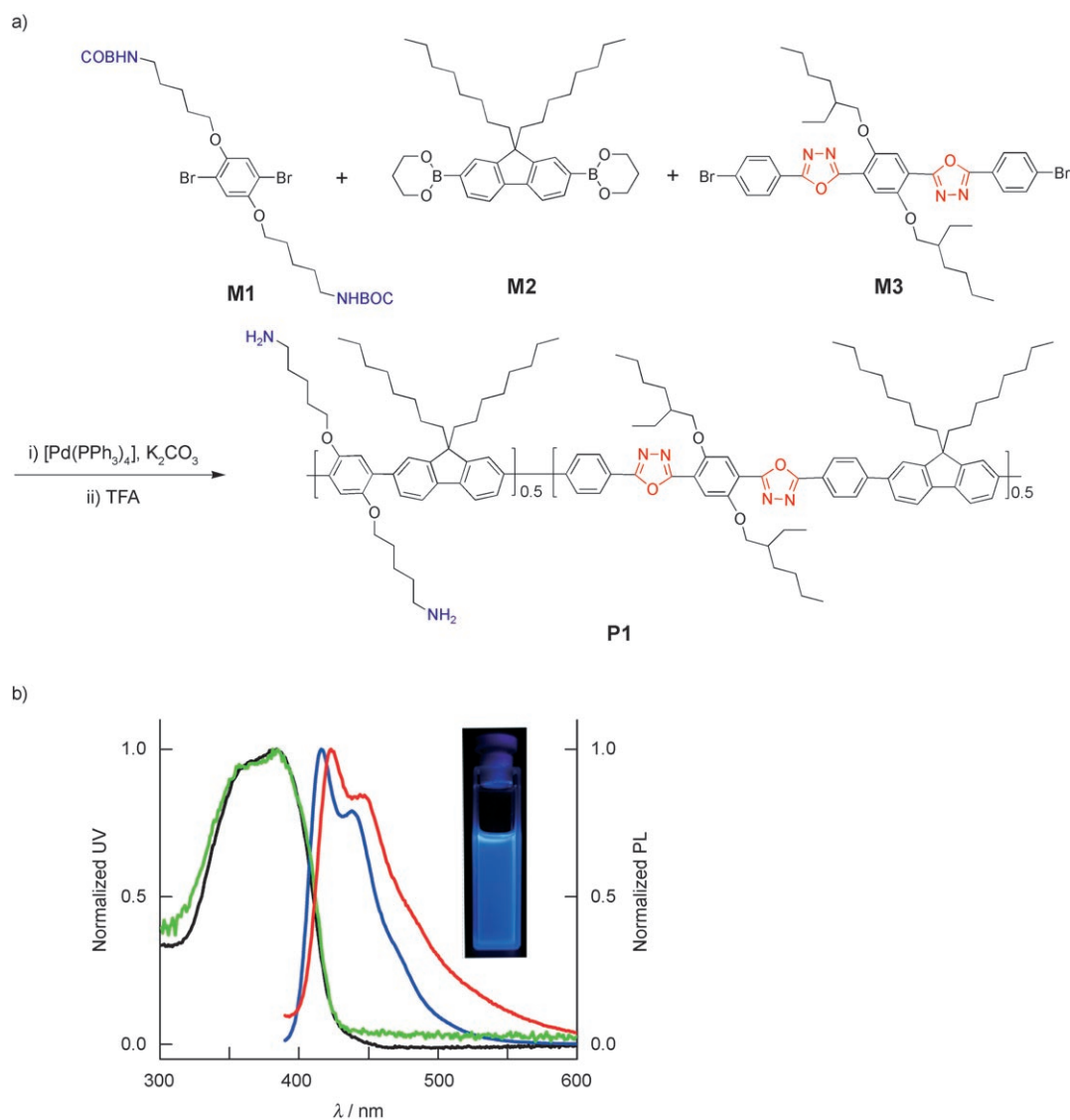


Figure 2. a) Chemical structure of **P1**. b) UV/PL spectra of **P1** in chloroform (black=UV, blue=PL) and solid film (green=UV, red=PL).

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 piranha solution ($\text{H}_2\text{O}_2/\text{H}_2\text{SO}_4$ 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-

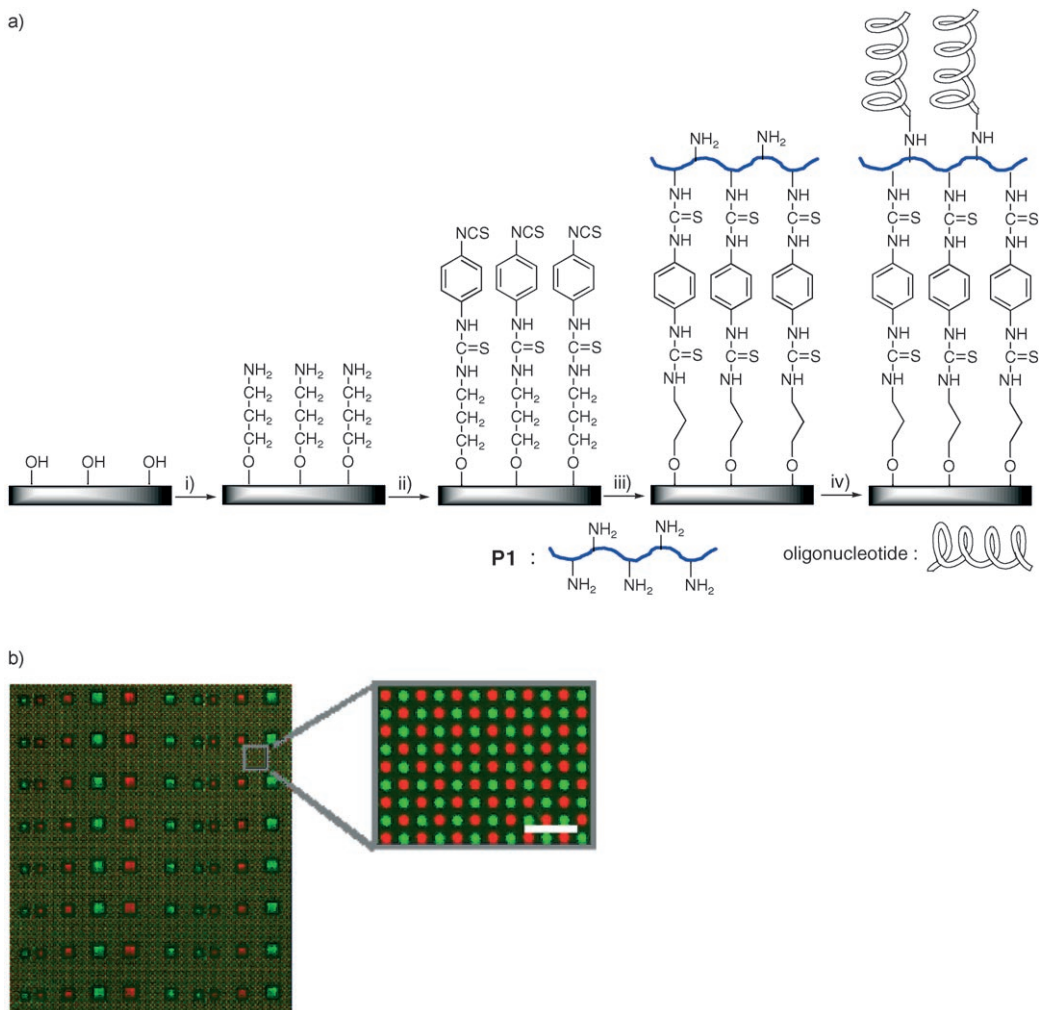


Figure 3. a) Schematic representation of the light-directed parallel on-chip DNA synthesis on **P1**-immobilized glass: i) APTMS, ii) 1,4-phenylene-diisothiocyanate, iii) polymer (**P1**), and iv) cyclic procedures of oligo synthesis. b) A fluorescence image of a patterned signal-amplifying DNA microarray 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 ($2.44 \text{ pmol cm}^{-2}$) 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 require-

ment 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 (λ_{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 noncomple-

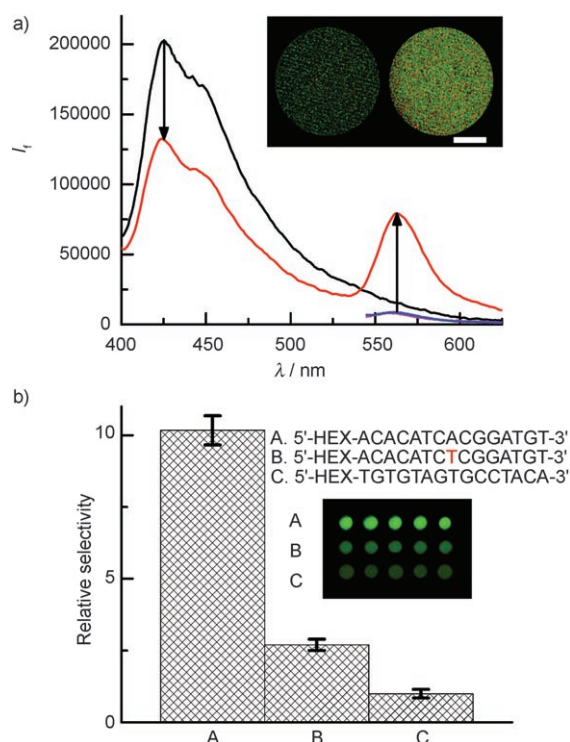


Figure 4. a) PL emission spectra of P1 substrate before (black) and after hybridization when excited at 380 nm (red) and 535 nm (blue); PL emission of the control (pink, excited at 535 nm). Note that the blue and pink lines are essentially superimposed and appear around 550–625 nm. Inset: Comparison 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.

Received: January 30, 2007

Revised: April 6, 2007

Keywords: conjugated polymers · DNA structures · FRET (fluorescence resonant energy transfer) · polyoxadiazoles · signal amplification

- [1] a) R. Ranger, D. A. Tirrell, *Nature* **2004**, *428*, 487–492; b) J. J. Storhoff, C. A. Mirkin, *Chem. Rev.* **1999**, *99*, 1849–1862; c) A. B. Sanghvi, K. P.-H. Miller, A. M. Belcher, C. E. Schmidt, *Nat. Mater.* **2005**, *4*, 496–502.
- [2] a) R. B. Breaker, *Nature* **2004**, *432*, 838–845; b) R. F. Service, *Science* **1998**, *282*, 396–399; c) M. Schena, D. Shalon, R. W. Davis, P. O. Brown, *Science* **1995**, *270*, 467–470; d) G. McGall, J. Labadie, P. Brock, G. Wallraff, T. Nguyen, W. Hinsberg, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 13555–13560.
- [3] a) D. T. McQuade, A. E. Pullen, T. M. Swager, *Chem. Rev.* **2000**, *100*, 2537–2574; b) P. S. Heeger, A. J. Heeger, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 12219–12221; c) K. Peter, R. Nilsson, O. Inganäs, *Nat. Mater.* **2003**, *2*, 419–424; d) H.-A. Ho, M. Boissinot, M. G. Bergeron, G. Corbeil, K. Doré, D. Boudreau, M. Leclerc, *Angew. Chem. Int. Ed.* **2002**, *41*, 1548–1551; e) B. Liu, G. C. Bazan, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 589–593; f) L. Chen, D. W. McBranch, H.-L. Wang, R. Helgeson, F. Wudl, D. G. Whitten, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 12287–12292; g) K. Haskins-Glusac, M. R. Pinto, C. Tan, K. S. Schanze, *J. Am. Chem. Soc.* **2004**, *126*, 14964–14971; h) C. C. Pun, K. Lee, H.-J. Kim, J. Kim, *Macromolecules*, **2006**, *39*, 7461–7463.
- [4] a) S. P. Fodor, J. L. Read, M. C. Pirrung, L. Stryer, A. T. Lu, D. Solas, *Science* **1991**, *251*, 767–773; b) X. Gao, E. LeProust, H. Zhang, O. Srivannavit, E. Gulari, P. Yu, C. Nishiguchi, Q. Xiang, X. Zhou, *Nucleic Acids Res.* **2001**, *29*, 4744–4750; c) K. Komolpis, O. Srivannavit, E. Gulari, *Biotechnol. Prog.* **2002**, *18*, 641–646; d) J.-M. Rouillard, M. Zuker, E. Gulari, *Nucleic Acids Res.* **2003**, *31*, 3057–3062; e) X. Gao, X. Zhou, E. Gulari, *Proteomics* **2003**, *3*, 2135–2141.
- [5] A. P. Blanchard, R. J. Kaiser, L. E. Hood, *Biosens. Bioelectron.* **1996**, *11*, 687.
- [6] J. Cheng, E. L. Sheldon, L. Wu, A. Uribe, L. O. Gerrue, J. Carrino, M. J. Heller, J. P. O'Connell, *Nat. Biotechnol.* **1998**, *16*, 541–546.
- [7] a) O. J. Bolton, J. Kim, *J. Mater. Chem.* **2007**, *17*, 1981–1988; b) X.-C. Li, G. C. W. Spencer, A. B. Holmes, S. C. Moratti, F. Cacialli, R. H. Friend, *Synth. Met.* **1996**, *76*, 153–156; c) Z. Peng, Z. Bao, M. E. Galvin, *Adv. Mater.* **1998**, *10*, 680–684.
- [8] N. Miyaoura, A. Suzuki, *Chem. Rev.* **1995**, *95*, 2457–2483.
- [9] L. J. McBride, M. H. Caruthers, *Tetrahedron Lett.* **1983**, *24*, 245–248.
- [10] X. Gao, P. Yu, E. Proust, L. Sonigo, J. P. Pellois, H. Zhang, *J. Am. Chem. Soc.* **1998**, *120*, 12689–12699.