Signal Amplifying Conjugated Polymer-Based Solid-State DNA Sensors

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DNA microarray technology has had a significant impact on the field of molecular biology and plays an important role in the diagnosis of diseases,[1] drug development,[2] and identifying gene expression.[3] Low cost, high sensitivity, high selectivity, fast detection process are the desired characteristics and are also the demanding challenges in the development of DNA microarrays. However, the current detection method of the conventional DNA microarrys cannot provide sufficiently sensitive detection because it relies on the fluorescence emission of the dye on the analyte DNA. Thus, the sensory signal is simply proportional to the number of dye-labeled analytes recognized by the probe DNAs on the microarry.[4] Therefore, it is challenging to detect small amounts of target DNA and target molecules always need to be duplicated to certain amounts by polymerase chain reaction (PCR). Furthermore, costly and time-consuming labeling and subsequent stringent purification of the target molecules are also required. This makes current DNA microarray not suitable for the real-time organism detection and fast diagnosis of gene-related diseases.

Conjugated polymers are emerging materials for biological sensor applications because of their signal amplification property and environmental sensitivity.[5] Moreover, controlled assembly of fluorescent sensory polymers expands the dimensionality of the energy transport properties from 1-D to 2-D and to 3-D efficiently, augmenting the intrinsic high sensitivity even further.[6] We have been developing self-signal amplifying DNA microarrays. In this contribution we present the design principle of conjugated polymer-based signal amplifying DNA sensory films. We designed and synthesized a conjugated poly(*p*-phenyleneethynylene) (PPE) which has carboxylic acid side chains for bioconjugation with DNAs and alternating hydrophobic and hydrophilic side chains for Langmuir−Blodgett (LB) film fabrication. Scheme 1 illustrates the entire processes including the LB deposition, bioconjugation with probe DNAs, and the amplified detection principle.

The PPE for this study was synthesized by the Sonogashira−Hagihara coupling reaction.[7] Scheme 2 shows the polymerization process and the chemical structure of the PPE. We designed the PPE to have an amphiphilic property so that it can form a well-defined thin layer by the LB method. The first repeating unit has hydrophilic triethylene glycol side chains while the other repeat unit has hydrophobic side chains of ethyl-protected carboxylic acid, which are linked to the PPE backbone via the hydrophobic hexyl unit. After polymerization the ethyl protection group was removed to give free carboxylic acid groups for bioconjugation with amine-modified DNA sequence.

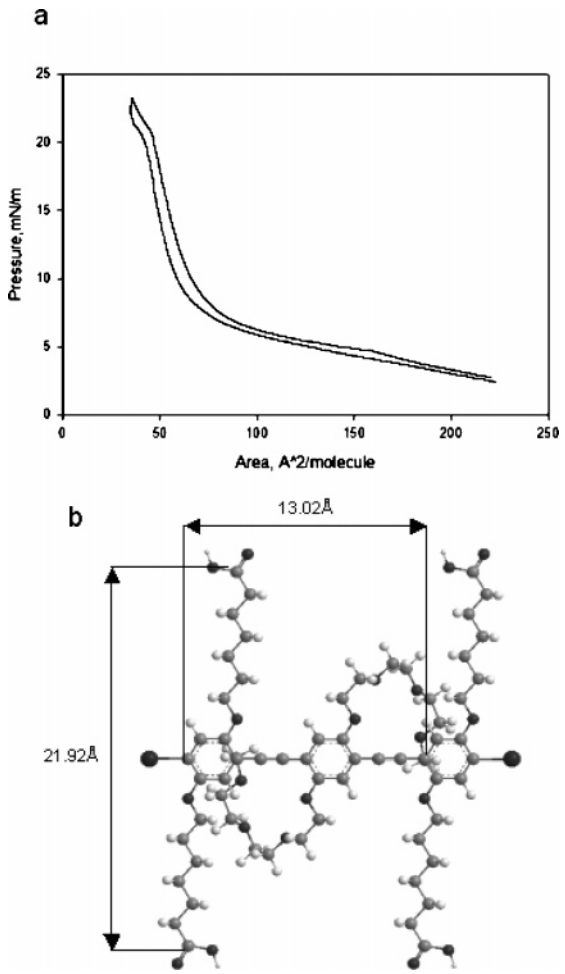

Figure 1. (a) The pressure−area isotherm and (b) the corresponding molecular model of the PPE.

The polymer was dissolved in chloroform at the concentration of 1 mg/mL. The polymer solution was spread at the air−water interface of a NIMA 112D trough. We studied the pressure−area isotherm to understand the conformation and the molecular packing of the PPE in the Langmuir monolayer. This PPE is believed to have the face-on structure due to its alternating hydrophobic and hydrophilic side chains because the symmetrically attached hydrophilic and hydrophobic side chains will position the mainchain phenyl ring co-facial to the air−water interface.[8] Figure 1 shows the pressure−area ($\pi-A$) isotherm and a molecular model of the PPE. The limiting area (A_0) of the $\pi-A$ isotherm that can be obtained by extrapolating the slope of the $\pi-A$ isotherm is about 270 Å^2/repeat unit, which is the surface area the repeat unit of the PPE occupies at the air−water interface and matches well with the molecular model.[8] As the polymer is compressed, the surface pressure increases until the polymer folds into multilayers at the area per repeat units of about 50 Å^2. Because of the surfactant design, the $\pi-A$ isotherm is completely reversible during the compression and expansion cycles even after the monolayer folds into multilayers.

The Langmuir monolayer of the PPE was transferred to a 1,1,1,3,3,3-hexamethyldisilazane (HMDS)-treated hydrophobic

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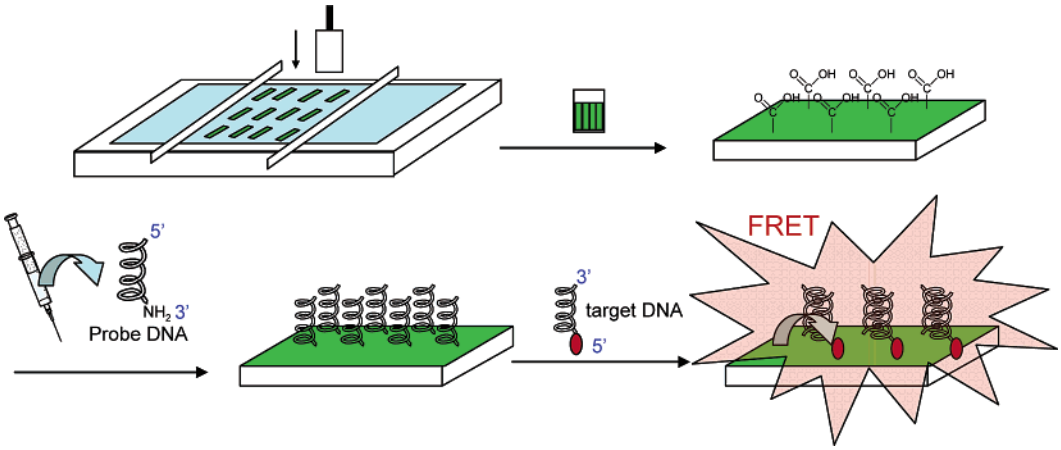
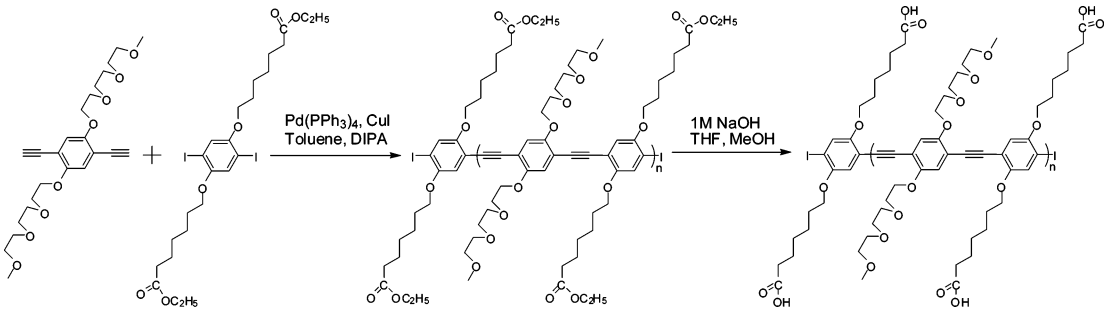
Scheme 1. Schematic Representation of the Fabrication and Amplified Detection



Scheme 2. Synthesis and the Chemical Structure of the PPE



glass slide at a surface pressure of 20 mN/m. The carboxylic acid group of the side chains was then activated by 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC) and *N*-hydroxysulfosuccinimide (Sulfo-NHS) chemistry and subsequently reacted with an amine-modified single strand, 15-base DNA sequence. After the coupling reaction the 3′ end of the probe sequence is located close to the PPE surface. Once the probe DNA sequence was chemically coupled on the PPE side chain, hybridization tests were performed. The analyte DNAs were labeled with hexachlorofluorescein (HEX) at the 5′ end. Therefore, successful hybridization will place HEX close to the PPE surface, and efficient fluorescence resonance energy transfer (FRET) from the PPE to HEX is expected to occur (see Scheme 1). The adsorption spectrum of HEX and the emission spectrum of the PPE have a good overlap for efficient fluorescence energy transfer.

Figure 2 shows the fluorescence emission of the solid-state DNA sensor before and after the coupling of the probe DNA to the PPE and after hybridization of the complementary and noncomplementary DNA sequences. The sequence of the probe DNA, the complementary DNA, and a noncomplementary DNA are shown in Figure 2. Interestingly, we consistently observe a significant fluorescence emission enhancement after the coupling of the probe DNA to the PPE LB film while we did not observe any fluorescence emission enhancement when we did the same coupling reaction in solution. We believe that the emission enhancement is likely due to the alteration of the intermolecular packing state of the PPE. It has been demonstrated that intermolecular packing has a large influence on the emissive properties of conjugated polymer films.[8b,9] When the complementary DNA sequence was applied to the solid-state DNA sensor, the fluorescence emission of the PPE (excited at 440 nm) was quenched and the emission of HEX (557 nm) was significantly amplified. Direct excitation of HEX at 535 nm produced only weak fluorescence. This clearly demonstrates that the fluorescence energy transferred efficiently from the PPE to HEX and amplified the fluorescence sensory signal. When we tested the noncomplementary DNA sequence, the fluorescence of the PPE did not change and there was no HEX emission, demonstrating that the signal amplifying conjugated polymer-based DNA sensor maintains good selectivity as well. The DNA sensor is possibly reusable because after rinsing of the bound complementary sequence above its melting point the emission spectrum of the PPE film was recovered.

Probe sequence : 5'-ACATCCGTGATGTGT-3'-NH_2

Analyte sequence : HEX-5'-ACACATCACGGATGT-3' (complementary)

HEX-5'-TGTGTAGTGCCTACA-3' (non-complementary)

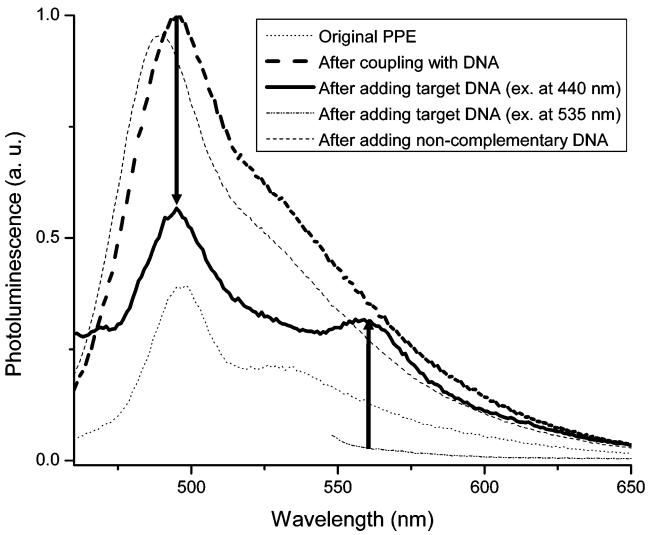


Figure 2. Fluorescence emission spectra of the solid-state signal amplifying DNA sensor. Efficient FRET amplifies the sensory signal only when the complementary sequence is introduced onto the DNA sensor.

In summary, we have developed a new PPE for signal amplifying solid-state DNA sensors. Thin layer films of the PPE were fabricated by the LB technique. A probing DNA sequence was bioconjugated to the PPE film to form a conjugated polymer-based signal amplifying sensor film. DNA hybridization tests showed good selectivity and the devised signal amplification by means of effective FRET from the underlying PPE layer to the dye of the target DNA sequence. Signal amplifying DNA microarray can be conveniently developed by applying the molecular design principle and the signal amplification principle presented in this contribution and the inkjet printing technique.

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Supporting Information Available: Synthetic procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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