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The effect of pH and DNA concentration on organic thin-film transistor biosensors

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ABSTRACT

Organic electronics are beginning to attract more interest for biosensor technology as they provide an amenable interface between biology and electronics. Stable biosensor based on electronic detection platform would represent a significant advancement in technology as costs and analysis time would decrease immensely. Organic materials provide a route toward that goal due to their compatibility with electronic applications and biological molecules. In this report, we detail the effects of experimental parameters, such as pH and concentration, toward the selective detection of DNA via surface-bound peptide nucleic acid (PNA) sequences on organic transistor biosensors. The OTFT biosensors are fabricated with thin-films of the organic semiconductor, 5,5′-bis-(7-dodecyl-9H-fluoren-2-yl)-2,2′-bithiophene (DDFTTF), in which they exhibit a stable mobility of 0.2 $cm^2 V^{-1} s^{-1}$ in buffer solutions (phosphate-buffer saline, pH 7.4 or sodium acetate, pH 7). Device performance were optimized to minimize the deleterious effects of pH on gate–bias stress such that the sensitivity toward DNA detection can be improved. In titration experiments, the surface-bound PNA probes were saturated with 50 nM of complementary target DNA, which required a 10-fold increase in concentration of single-base mismatched target DNA to achieve a similar surface saturation. The binding constant of DNA on the surface-bound PNA probes was determined from the concentration-dependent response (titration measurements) of our organic transistor biosensors.

1. Introduction

Improvements in biosensor technology have been realized through an improved understanding of the interface between biology and electronics. While opportunities for electronic detection of biological species using organic transistors are beginning to appear, analysis of biological systems is dominated by more elaborate conventional detection systems. Genetic disease diagnosis and personalized medicine design would benefit tremendously from a low-cost and fast detection tool for DNA hybridization. Hybridization between a dissolved DNA sequence and a surface-tethered complementary DNA (or PNA) sequence is currently evaluated by surface plasmon resonance (SPR) [1,2], surface plasmon fluorescence spectroscopy (SPFS) [3,4], ellipsometry [5] and microgravimetric sensors, including quartz crystal microbalances (QCM) [6] and cantilever based biosensors [7].

Optical measurements provide the bench-mark standard for biological detection applications; however, this method

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suffers from low throughput and relies on laborious labeling process involving radiolabeled tags or fluorophores and costly detectors [3]. The use of electronic systems for detecting biological species has garnered wide interest due to the simplicity in the detection signal [8,9]. Recent advances in biomolecular detection using OTFTs have shown great promise for a viable and low cost detection systems [10,11]. Despite the wide range of chemical sensing technologies, an inexpensive handheld or easily transportable system for detecting volatile or aqueous analytes with adequate sensitivity, selectivity and reliability still remains elusive. The device stability and biocompatibility for applications aimed at detecting low concentrations of biomolecules in blood or tissue presents significant challenges. Unstable device performance is caused by counterions from the electrolyte migrating into the organic film resulting in leakage current, and redox reactions occurring at the organic semiconductor–electrolyte interface, which irreversibly degrade the organic semiconductor [12].

Two approaches have been described to overcome these issues, which include incorporating thick encapsulation layers of appropriate polymer and using salt-free analyte solutions [13,14]. However, both of these issues directly affect the sensor sensitivity by either blocking the signal or decreasing the Debye screening length [14,15]. Two key challenges facing organic transistor technology must be overcome before these systems can be acceptable for biological detection, which includes: (1) stability of transistor in harsh media (or with variable pH), and (2) selectivity toward a particular analyte with high sensitivity. In our previous work, we improved organic transistor operation stability in water by using low voltage device with robust organic semiconductors [10,16]. We demonstrated the potential application of these transistors as label-free selective DNA sensors [17].

In this report, we characterize the electronic response of OTFT sensor to surface-bound PNA/DNA hybridization in buffer solutions with varying pH, target DNA concentration, and number of base mismatches in the target sequence. Surface titration experiments are used to show the surface saturation as a function of DNA target concentration base mismatches. PNA/DNA titration measurements are characterized by the Langmuir model [18] for the various studied DNA concentrations. The rate constants associated with DNA hybridization are comparable with previously published data [17].

2. Experimental

All materials were purchased from Sigma–Aldrich and used as received unless otherwise stated. The synthesis of the organic semiconductor, 5,5′-bis-(7-dodecyl-9H-fluoren-2-yl)-2,2′-bithiophene (DDFTTF), has previously been reported [10] and is used here as the active organic semiconductor. Thin-films of poly(4-vinylphenol) (PVP) (MW 20,000 g/mol) cross-linked with 4,4′-(hexafluoroisopropylidene) diphthalic anhydride (HDA) were spin-coated according to a previous method [16] and used here as the gate dielectric layer for low-voltage operation. The base sequence of the peptide nucleic acid (PNA) probe (BIO-SYNTHESIS, USA) and target DNA sequences (Eurofins MWG, Germany) are given in Fig. 1a. *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Fluka used to activate the carboxylic acid groups on the sensor surfaces. To prepare phosphate buffer solution (10 mM, pH 7), one phosphate buffered saline tablet was dissolved in 200 mL of deionized water. Eventually one tablet composed of 8.01 g/L NaCl and 0.2 g/L KCl. (calibrated by sigma). Sodium acetate buffer solution (ABS) (stock solution from sigma, 3 M, pH 7) composed of acetic acid and sodium acetate, was diluted in deionized water to achieve 10 mM concentrated solution prior to biosensing experiments.

The fabrication of OTFTs and their use as selective DNA sensors in aqueous media is schematically depicted in Fig. 1b and is given in Supporting information (SI) method [17]. Pulse plasma polymerization of maleic anhydride (ppMA) was used to functionalize the OTFT surface to facilitate the attachment of the PNA molecular probes, more detail can be seen in Supporting information [17]. The density of functional groups, was evaluated using Fourier Transform-Infrared Spectroscopy (FT-IR) (Nicolet 850 Spectrometer) at different input powers, on–off time ratios and depositions times. More detail on FT-IR results described in Supporting information (Fig. S1). Electrical measurements were performed with a Keithley 4200 Semiconductor Characterization System. Electrical sensing measurements were performed under constant bias conditions (V_{DS} = −0.5 V and V_G = −1 V). To ensure a constant analyte concentration, a peristaltic pump (NovoChem) was used at a constant flow rate of 300 μL min^{-1} [17].

3. Results and discussion

Electronic sensors based on OTFTs are evaluated in aqueous media for their performance as selective DNA sensors. Our previously reported aqueous-stable OTFT is used as the sensor platform with a 15 nm film of DDFTTF as the organic semiconductor on a thin polymer dielectric film (20 nm) in PVP–HDA [10,17]. Top-contact OTFTs are fabricated with gold electrodes in a geometry comprising a channel width (W) = 4 mm and length (L) = 50 μm (Fig. 1b). These OTFTs exhibited p-type transistor characteristics at low operating voltages (−1 V) in ambient air and showed an average mobility of 0.45 $cm^2 V^{-1} s^{-1}$ at V_{DS} = −1 V, on/off ratio of 1.5×10^3 and threshold voltage (V_{th}) of 0.045 V (Fig. 2). Moreover, these devices exhibit mobility of 0.2 $cm^2 V^{-1} s^{-1}$ at V_{DS} = −0.5 V in aqueous media (Fig. S2).

PNA/DNA hybridization was carried out between the surface-bound PNA strand and target DNA sequences with varying base mismatches. The designation of the target strands are as follows: fully complementary (**T2-MM0**), one-base mismatch (**T1-MM1**), and two-base mismatch (**T3-MM2**). The use of a PNA probe instead of DNA is an advantageous due to neutral backbone, it can be immobilize on both p-, n-type semiconductor based transistor surface [4,19]. Upon hybridization with the surface-bound PNA, the negative charge in the DNA backbone can therefore influence the current through the p-channel organic semiconductor film during OTFT operation providing a