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A flexible pentacene thin film transistors as disposable DNA hybridization sensor

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ABSTRACT

We fabricated a disposable DNA hybridization sensor using pentacene thin film transistors (TFTs) on flexible substrate. The analytes, 100-mer ss-DNA (poly A and poly T) or 100 bp ds-DNA were immobilized directly on the surface of the pentacene layer, thereby producing a dramatic change in the electrical properties of the devices. The electrical characteristics of devices were studied as a function of DNA immobilization conditions, DNA concentrations and between ss- and ds-DNA. Based on these results, we propose a disposable label-free sensor for DNA hybridization through direct measurement of electrical properties by the immobilization of DNA on flexible pentacene TFTs.

1. Introduction

The detection and quantification of nucleic acids through DNA hybridization is of great importance to many applications, such as medical diagnostics, forensic analysis, genotyping, and pathogen detection [1–5]. Traditional methods for detection of DNA are mainly based on radio labeled system or optical detection using fluorochrome tagging. These detection techniques have limitations due to involvement of complex and expensive optical instrumentation, along with difficulties in preparation of samples due to required pretreatments. Compared with these techniques, label-free electronic methods for sensing of DNA hybridization promise better sensitivity, selectivity, and low cost for fabricating the devices. These DNA hybridization sensors with "label-free" approach can adopt electrochemical detection [6], carbon nanotube network field-effect transistors [7], atomic force microscopy (AFM) [8], surface plasmon resonance (SPR) [9], genetic field effect transistor (FET) [10], and microcantilevers [11] or a combination of these as a transducer.

Among these methods, a DNA hybridization sensor using organic thin film transistors (OTFTs) is more suited for the application as disposable sensors, due to their potentially low-cost fabrication process and quicker response time [12]. Moreover, the structure and morphology of organic semiconductors can be carefully adjusted to enhance sensory sensitivity and selectivity. In comparison to chemiresistors, amperometric and potentiometric sensors, the detection limits and sensitivity of OTFTs-based biosensors also benefit from the signal amplification that is inherent in transistor structures. When compared to similar impedometric hybridization sensors, an OTFT based sensor has clear advantage in terms of reduced sensor noise, as the OTFT measurements are essentially carried out in dry state rather than wet. Moreover, due to their biocompatibility and flexibility, an organic semiconductor material offers great advantage of integration with biological systems.

Such DNA sensors based on OTFTs have been reported in past. Zhang and Subramanian [13] reported a DNA sensor based on pentacene TFT, in which DNA molecules are immobilized on the surface of pentacene layer and channel current increases due to the unambiguous doping effect. However, it was a misinterpretation of current phenomena as we can show on the basis of our own results [14]. In this work, we had demonstrated the application of pentacene TFTs as a DNA hybridization sensor.

The negatively charged DNA molecules upon immobilization on the hydrophobic pentacene surface by physical adsorption and hydrophobic interactions attracted holes from the OTFT channel region, thereby increasing the scattering of holes, while holes moved down from source to drain electrode. The life-time of hole (τ_{cp}) thus decreases due to increased scattering. This effect decreases field-effect mobility (μ_{FET}) which can be calculated as per Eq. (1).

$$\mu_{FET} = \frac{v_{dp}}{E} = \frac{e\tau_{cp}}{m_p^*} \quad (1)$$

where v_{dp} is the average drift velocity of the holes; E is electric field; e is the magnitude of the electronic charge; and m_p^* is the effective mass of the hole.

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Also, the OTFT channel current (I_{DS}) decreases in the process due to a decrease in field-effect mobility (μ_{FET}) and its magnitude can be given by Eq. (2).

$$I_{DS,sat} = \frac{WC_{PVP}\mu_{FET}}{2L}(V_{GS} - V_{TH})^2 \quad (2)$$

where W is the width and L is the length of the TFT channel; C_{PVP} is the capacitance per unit area of the PVP gate insulator; V_{GS} is the gate voltage and V_{TH} is threshold voltage. Therefore, we can expect an overall decrease in response current following DNA hybridization, as shown previously [14]. The magnitude of this change varied for single stranded DNA (ss-DNA) and double stranded DNA (ds-DNA), thereby allowing us to sense the extent of DNA hybridization.

In continuation to this proof-of concept, we fabricated in the present work, a biosensor for detecting DNA hybridization using pentacene OTFTs fabricated on flexible substrates. A biosensor based on DNA sensing principle for detection of pathogens and bioweapons in field applications should ideally be produced with cheap design, and hence the choice for flexible substrate such as polyethersulfone (PES) was obvious. Pentacene was the material of choice as organic semiconductor due to its excellent electrical properties and ease in immobilization of DNA over it. Moreover, its properties do no alter when fabricated over flexible substrates. Therefore, we tested the fabricated devices for sensing of single as well as hybridized double stranded DNA from synthetic sources to demonstrate the feasibility to use them in sensing the pathogens.

2. Experimental

2.1. Materials

ITO coated PES substrate, pentacene, poly(4-vinylphenol) (PVP), propylene glycol methyl ether acetate and ethylated poly(melamineco-formaldehyde) were purchased from Sigma Aldrich, Korea. Other chemicals and solvents were of analytical reagent grade and were used without further purification. The DNA (poly A/poly T/poly C) molecules were synthesized by Bionics Inc., Korea.

2.2. Fabrication of pentacene thin film transistor

The pentacene TFT devices in this study were fabricated with the process illustrated in Fig. 1. The top-contact pentacene TFTs devices were fabricated on a flexible PES substrate. Indium tin oxide (ITO) was used as gate electrodes. The gate insulator composed of poly (4-vinylphenol) (PVP) and was deposited over the ITO gate electrode to a thickness of 480 nm by spin coating and then baked at 200 °C for 1 h. The PVP solution was prepared by dissolving PVP (10 wt% of solvent) and methylated poly (melamine-co-formaldehyde) (5 wt% of solvent) as a cross-linking agent in propylene glycol methyl ether acetate as solvent. The pentacene active layer was patterned through the shadow mask by thermal evaporation at 0.1 Å/s rate to a thickness of 70 nm under high vacuum ($<5 \times 10^{-6}$ torr). The source and drain electrodes were made up of Au layer of 100 nm thickness, which were deposited by thermal evaporation using shadow mask. The pentacene TFTs thus obtained had a channel length (L) and width (W) of 100 and 1000 μm, respectively.

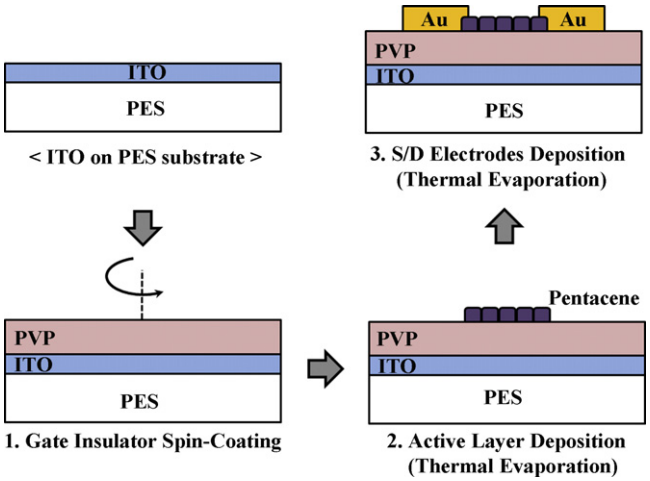

Fig. 1. Fabrication procedure of the pentacene TFTs.

2.3. DNA hybridization and sensing

As shown in Fig. 2, ss-DNA was first immobilized by pipetting a 1 μl drop of deionized (D.I.) water containing the DNA onto the pentacene TFTs channel region and then air-drying for 60 min. Subsequently, 1 ml of D.I. water was dropped slowly through slant onto the pentacene TFT surface for thorough washing of channel region. The devices were air-dried for 60 min and then characterized at room temperature. For validation of DNA hybridization on same substrate, ss-DNA (100 mer-poly A) immobilized pentacene TFTs were used for further immobilization of complementary ss-DNA (100 mer-poly T). The devices were air-dried for 60 min in a similar manner, washed again with D.I. water as before, and air-dried for 60 min before being characterized by Keithley 236 m. Similarly, the effects of different lengths and concentrations of DNA oligos were determined by characterization of different devices with different lengths or concentrations of DNA oligos immobilized onto them.

2.4. Microscopy

The immobilization of ss-DNA on the pentacene surface and subsequent hybridization of complementary strand was verified using fluorescent microscopy (Fig. 3). For this purpose, fluorescent intercalator ethidium bromide (EtBr) was used. A 5 μl drop of (1 mg/ml) aqueous solution of EtBr was placed on the channel area containing immobilized ss-DNA or hybridized sample and left for 5 min before being washed with D.I. water. The fluorescent images of devices with labeled DNA was obtained using a fluorescence microscope (Olympus BX50, Japan) with excitation and emission wavelengths as 510–490 and 590 nm, respectively. This intercalator could bind only to the hybridized DNA molecules and thus produced distinctive images than the devices with ss-DNA or control.

3. Results and discussion

3.1. Sensing mechanism

The DNA molecules were immobilized on the pentacene surface by physical adsorption via hydrophobic interactions. These molecules also have negatively charged phosphate groups on their backbone, which eventually affects the electrical performance of the pentacene TFTs. Fig. 4(a) shows the device characteristics of an OTFT with an applied gate voltage (V_{GS}) such that $V_{GS} < 0$ V. In this case, a hole accumulation layer is created at the interface of pentacene and gate insulator, so that, when a drain-source voltage (V_{DS}) is applied, the holes present in the accumulation layer will flow from the drain to the source electrode. In this ideal case, there is no interruption though the drain to the source electrode. When the DNA molecules are immobilized on the pentacene surface, negative charge of DNA molecules attract holes from the channel region, which increases

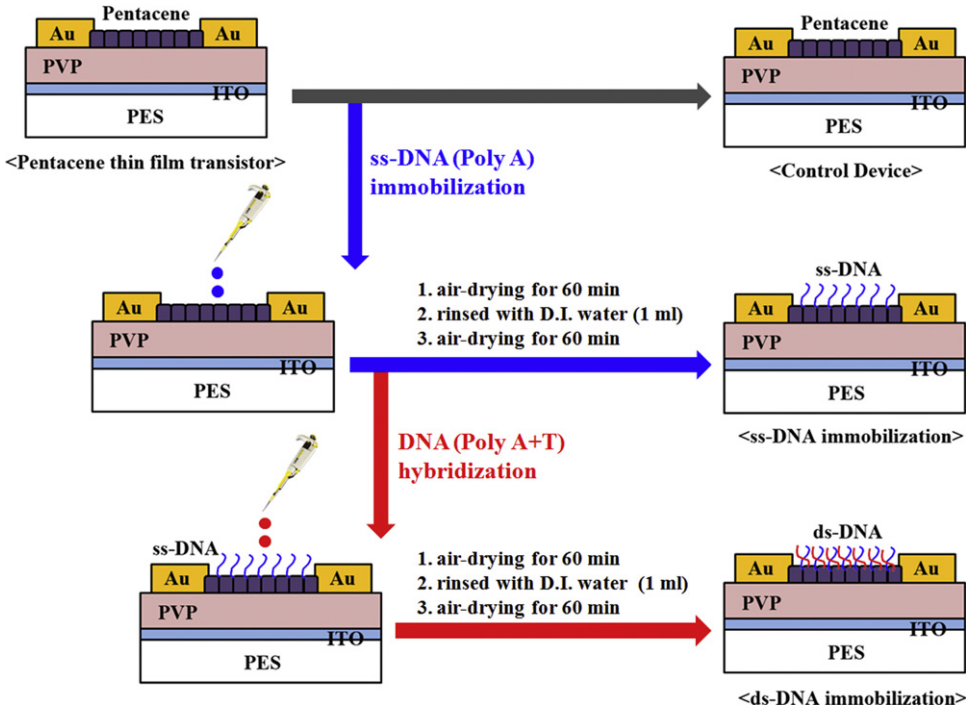

Fig. 2. Schematics showing the DNA immobilization and hybridization on the OTFTs.

the scattering of holes during their movement between source to drain electrodes (Fig. 4(b)). In this scenario, we expected the mean time between collisions or scattering (τ_{cp}) to decrease, thus decreasing field-effect mobility, as given by Eq. (1).

The performance of the pentacene TFT devices were evaluated in terms of their output and transfer characteristics. In order to find the output characteristics of devices, the channel current (I_{DS}) was measured as a function of the V_{DS} at a constant V_{GS}. Evaluation of transfer characteristics was carried out by measuring the I_{DS} between the source and drain as a function of the V_{GS} under a constant V_{DS}. One of the important parameters of OTFT was the field-effect mobility of carriers in its channel region. The field-effect mobility of hole (μ_{FET}) was determined while using the saturation drain current ($I_{DS,sat}$), which is given by Eq. (2).

3.2. Immobilization of DNA on pentacene surface

The influence of the immobilized DNA on pentacene surfaces was studied by fabricating the pentacene TFTs with 10 pmoles ss-DNA (poly A) for 60 min (immobilization time). Fig. 5(a) shows the I_{DS} as a function of V_{DS} under different V_{GS} (the output characteristic), whereas, Fig. 5(b) shows I_{DS} as a function of the V_{GS} measured at a constant V_{DS} (the transfer characteristic). At the same applied V_{GS}, the original pentacene TFTs (without ss-DNA immobilization) showed higher I_{DS} than the pentacene TFTs with

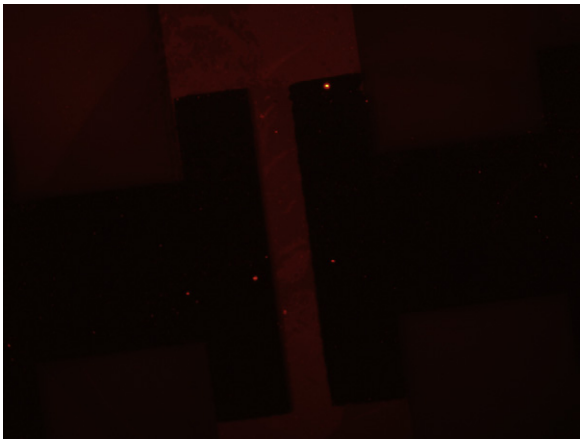

Fig. 3. Fluorescence images of pentacene TFT after hybridization of DNA.

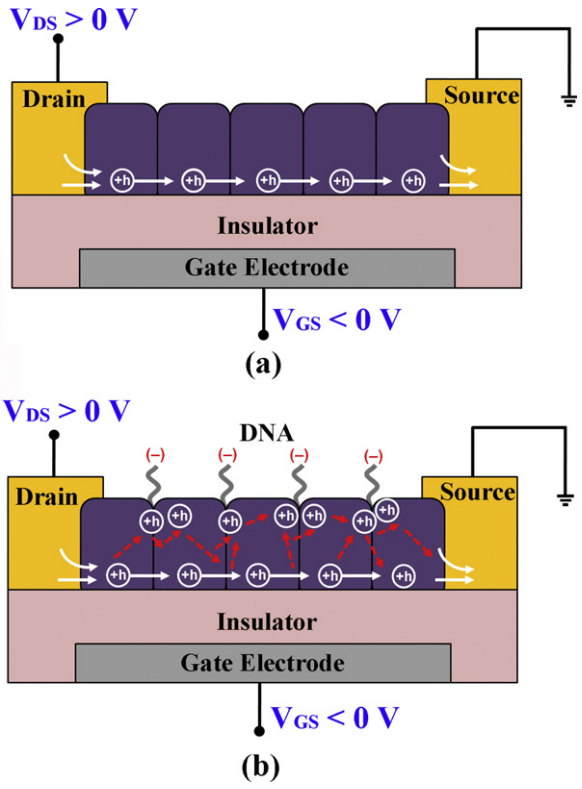

Fig. 4. DNA sensing mechanism on the pentacene TFTs. The device characteristics of an OTFT (a) w/o DNA immobilization and (b) w/DNA immobilization.

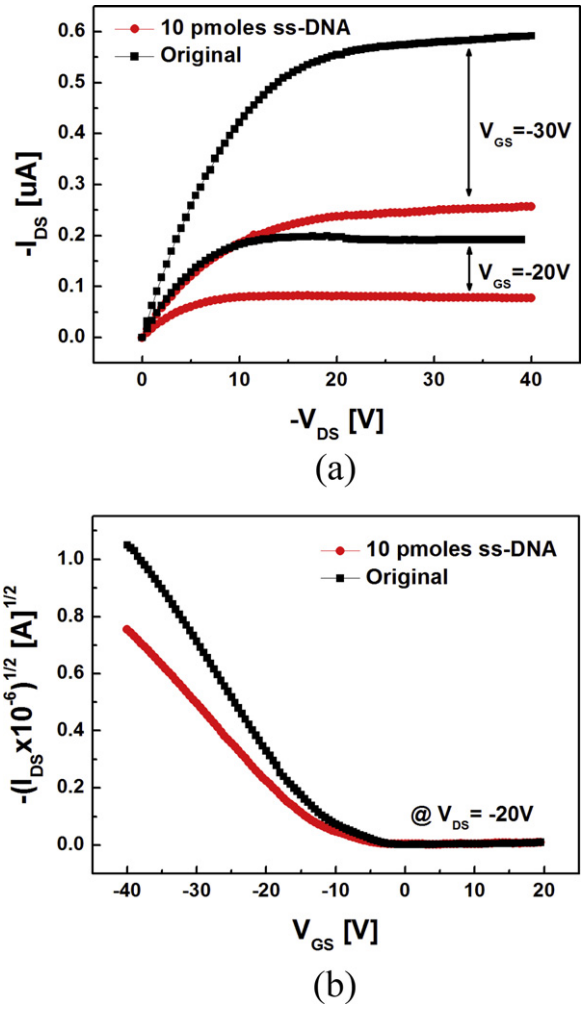

Fig. 5. Performance of the pentacene TFTs with immobilized DNA on pentacene surface: (a) output characteristics and (b) transfer characteristics of two pentacene TFTs (original, ss-DNA).

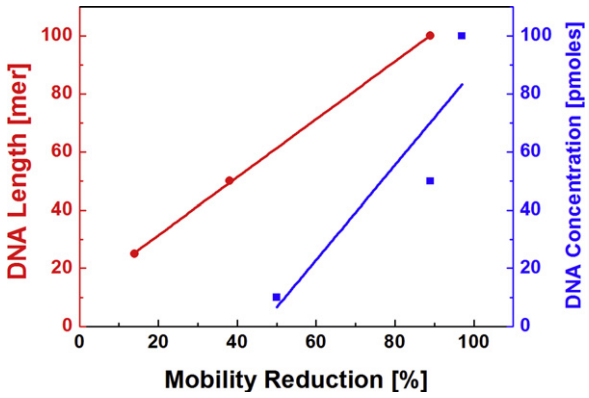

Fig. 6. Reduction ratio of field-effect mobility for the pentacene TFTs with immobilized ss-DNA, with respect to original device, as a function of DNA length and DNA concentration.

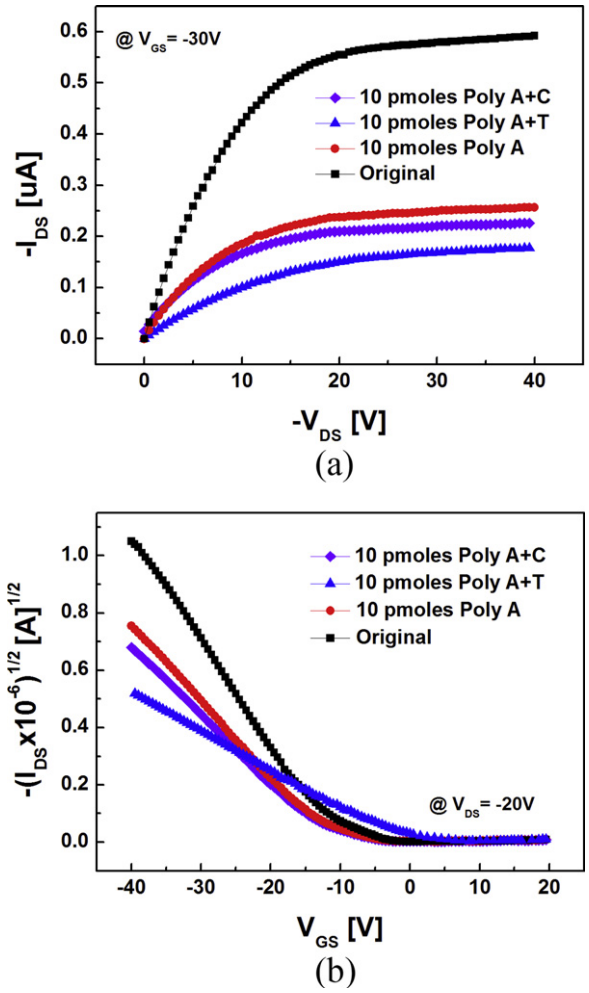

Fig. 7. Performance of the pentacene TFTs with DNA immobilized on pentacene surface: (a) output characteristics and (b) transfer characteristics of three pentacene TFTs (original, ss-DNA, ds-DNA).

the immobilized ss-DNA. The DNA molecules influenced the field-effect mobility in the pentacene TFTs as given by Eq. (2). Original pentacene TFTs (without ss-DNA immobilization) had a field-effect mobility of μ_{FET} = 0.038 cm^2/V s. Conversely, the pentacene TFTs with ss-DNA immobilization had a field-effect mobility of μ_{FET} = 0.019 cm^2/V s. After immobilizing ss-DNA, field-effect mobility of the device reduced approximately to 50%. The electrical characteristic of the pentacene TFTs with the ss-DNA immobilization gives a lower I_{DS} and field-effect mobility due to the ss-DNA immobilization. Such dramatic changes of electrical properties were well expected, since the phosphate group on the DNA backbone imparts a net negative charge in the DNA molecules, which attracts holes from the channel region and decreases the I_{DS} and field-effect mobility.

In order to derive correlation between device response and DNA length and concentration, the output and transfer characteristics of the devices were also measured by varying the concentration (10, 50, 100 pmoles) and the length (25, 50, 100 mer) of the ss-DNA on the pentacene surface (Fig. 6). As DNA concentration was increased, the field-effect mobility decreased from a value of 50, 89.47 and 97.37% for 100 mer 10, 50 and 100 pmoles ss-DNA, respectively. Such a drastic reduction in field-effect mobility values were due to increase in the concentration of immobilized DNA on the pentacene's surface, by which they attracts more holes from the channel region. As DNA length was increased, a decrease in the field-effect mobility was observed to approximately 14.35, 38.06 and 89.47% for 50 pmoles 25, 50 and 100 mer DNA, respectively. Such a reduction in field-effect mobility was due to the fact that longer DNA length carries more net negative charge. This result was indicative of the possibility of dynamic response from devices at low concentration and shot length of DNA.

In addition, we also found a dramatic difference in the I_{DS} and the pattern in field-effect mobility upon exposure to either ss-DNA (10 pmoles poly A) or ds-DNA (10 pmoles poly A + T). Fig. 7 shows the difference in the sensor output and the transfer characteristics for the original pentacene TFTs (without ss-DNA) with the pentacene TFTs with immobilized ss-DNA or ds-DNA. The ds-DNA resulted in a higher ΔI_{DS} compared to what caused by the ss-DNA, as the ds-DNA carry more net negative charge. The values for field-effect mobility reduced approximately to 50 and 86.84% for ss-DNA and ds-DNA, respectively. On the other hand, during a negative control experiment (10 pmoles poly A + C), the pentacene TFT device did not show significant change in electrical properties. In this device, poly C did not immobilize on the pentacene channel layer as the poly A was already immobilized on this surface. In this way, direct electrical detection of the DNA hybridization was possible through the measurement of I_{DS} for the pentacene TFTs. The net difference in the I_{DS} (ΔI_{DS}) on the pentacene TFTs due to the single or double stranded DNA was the basis for the analysis of the DNA hybridization. Moreover, the detection was highly reproducible (less than 5% variation of electrical properties of unhybridized devices from 10 different batches) making this method a potential candidate for highly selective DNA hybridization sensor.

4. Conclusions

The single and double stranded DNA was immobilized on the surface of the flexible pentacene TFT layer, producing a change in the performance of the TFTs. Such change was attributable to the negative charges on the DNA molecules having the ability to attract holes from the channel region. The electrical characteristic of the pentacene TFTs with the ds-DNA immobilization gives a lower current output and field-effect mobility as it carries more net negative charge compared with ss-DNA. Therefore, we propose that a "label-free" detection technique for DNA hybridization with high sensitivity and selectivity is possible by direct electrical detection on OTFTs. The flexible and disposable substrate used for fabricating these devices shall give more leverage in realizing portable and disposable DNA sensor having applications in molecular biology laboratories, medical diagnostics, forensic investigations, genotyping, etc.

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