Rapid pathogen detection using an organic field effect transistor

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A pentacene field effect transistor (FET) for the detection of DNA from pathogenic organisms is fabricated. The pentacene FET is an excellent candidate for disposable sensor applications because of its low-cost fabrication process and fast detection. A viral (λ-phage) genomic DNA was chosen as a model organism and its presence was successfully detected by probe DNA hybridisation on the pentacene layer. The process produced a dramatic change in the channel current and field-effect mobility of the devices. This result demonstrates the feasibility of our device as a disposable sensor for DNA hybridisation and can lead to the development of a biosensor for rapid pathogen detection.

1. Introduction: In the post-human-genome sequencing era, the detection and quantification of DNA are of great importance to many applications, such as medical diagnostics, forensic investigation, genotyping and pathogen detection [1–7]. In particular, detection of pathogen, such as bacteria, virus, fungi and so on, is of utmost importance primarily for health and safety reasons and involves isolation of DNA from pathogen cells, followed by traditional methods of DNA detection. These traditional methods mainly focus on chromophore or radioactive labelling; optical detection using fluorochrome-tagged oligonucleotides [8, 9] or polymerase chain reaction-based amplification of the target DNA [10]. However, these methods have limitations because of the complications in sample preparation as well as the necessary usage of complex and expensive optical, electrochemical or thermocycling systems, along with specialised analysis. Compared with these techniques, a pathogen sensor using the organic field effect transistor (OFET) is an excellent candidate for the application as disposable sensors owing to their potentially low cost and convenient fabrication process and quicker detection [11, 12]. Moreover, it is easier to integrate OFETs with biological systems because of the biocompatibility and flexibility of an organic semiconductor material. For these reasons, we have fabricated pentacene FETs in the present work for the detection of pathogen. Pentacene was the choice for the organic semiconductor material because of its excellent electrical properties and ease in immobilisation of DNA over it.

The DNA molecules were immobilised on the hydrophobic pentacene surface by physical adsorption and hydrophobic interactions. These molecules also have negatively charged phosphate groups on their backbone, which affects the electrical performance of the pentacene FETs. When the DNA molecules are immobilised on the pentacene surface, the negative charge of DNA molecules attract holes from the channel region, thereby increasing the scattering of holes, whereas holes move down from the source to the drain electrode. We expected the lifetime of hole (τ_{cp}) to decrease as the scattering increases. This effect decreases field effect mobility (μ_{FET}), which can be calculated as per (1):

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

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.

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

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

where W is the width of the channel, L is the length of the channel, C_{PVP} is the capacitance per unit area of the poly(4-vinylphenol) (PVP) gate insulator, V_{GS} is the gate voltage and V_{TH} is the threshold voltage.

The bacteriophage lambda virus (λ-phage) was used as the model organism in this study. The λ-phage DNA was hybridised with probe DNA on the pentacene surface. Such adsorption attracts holes from the channel region, causing a change in the resultant channel current (I_{DS}) and field-effect mobility (μ_{FET}). The magnitude of this change was significantly different for λ-phage DNA molecules, thereby allowing us to sense the pathogen.

2. Experimental: For this purpose, top-contact pentacene FETs were fabricated on the glass substrate. The glass substrates were cleaned ultrasonically with sodium dodecyl sulphate (detergent), acetone, isopropanol and de-ionised (DI) water for 2 min each, respectively. After the cleaning procedure, the glass substrates were transferred to a high-vacuum chamber with the pressure under 5×10^{-6} Torr. An 80 nm-thick Al gate electrode was deposited through a shadow mask by thermal evaporation. The gate insulator, poly(4-vinylphenol) (PVP) was deposited over the Al gate electrode to a thickness of 480 nm by spin coating and then baked at 100°C for 10 min and 200°C for 1 h in a conventional oven. 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 solvent. The accumulation p-type pentacene active layer was patterned through the shadow mask by thermal evaporation at a rate of 0.1 Å/s to a thickness of 70 nm at a high vacuum of 5×10^{-6} Torr. Subsequently, the source and drain electrodes, and a 100 nm-thick Au layer were deposited through the shadow mask using thermal evaporation. The pentacene FETs obtained thereby had a channel length (L) and width (W) of 100 and 1000 μm, respectively (Fig. 1*c*). The overall size of the device was 1 × 2 mm.

The probe DNA (50-mer Poly A for the target as 50-mer Poly T or 20-mer 5′-GCA-AGT-ATC-GTT-TCC-ACC-GT-3′ for the target as λ-phage DNA) was first immobilised by pipetting a 1 μl drop of DI water containing the DNA onto the pentacene FETs channel and then air-drying for 60 min. Subsequently, 1 ml of DI water was dropped slowly onto the pentacene FET channel area by slanting

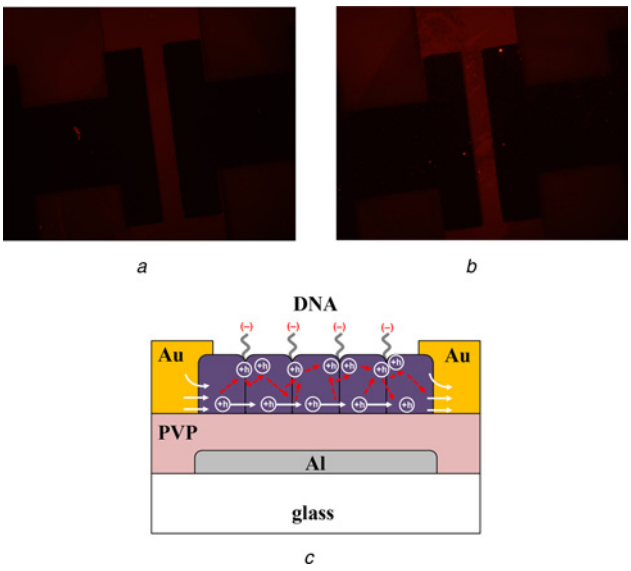

Figure 1 *Immobilisation of DNA on pentacene FETs*
a Fluorescence images of pentacene FET without DNA while labelled with EtBr
b Pentacene FET with EtBr-labelled ds-DNA
c Schematics of the DNA-sensing mechanism on the pentacene FET

the FET device for thorough washing of the surface. The devices were then air-dried for 30 min and characterised at room temperature in ambient air using a Keithley 236 meter. To validate hybridisation of target DNA on the devices, pentacene FETs having immobilised probe DNA were used for immobilisation of complementary target DNA (λ-phage DNA as target for the 20-mer probe or 50-mer poly T as target for poly A). Before such application, the BstII-digested λ-phage DNA was preheated at 100°C for 30 min to separate its strands and 1 μl of this sample was applied on the probe-immobilised FET surface. The devices were air-dried for 60 min, washed again with DI water and then characterised using a Keithley 236 meter upon air-drying for 30 min. The meter was interfaced with a computer using GPIB connection and LabVIEW software.

3. Results and discussion: The immobilisation of DNA on pentacene FET was confirmed through fluorescence microscopy. The images of fluorochrome labelled DNA were obtained with a fluorescence microscope (Olympus BX50, Japan) using excitation and emission wavelengths as 510–490 and 590 nm, respectively. Ethidium bromide (EtBr) was used as fluorochrome to intercalate the DNA. Fig. 1*a* shows the fluorescence image of a control device, in which the pentacene channel was labelled with EtBr without DNA immobilisation. As expected, the control device did not show fluorescence on exposure. Another device was immobilised with ds-DNA (Poly A + T) with the usual hybridisation procedure and further interacted with EtBr. As EtBr is an intercalating dye and binds only to the ds-DNA, therefore the device with immobilised ds-DNA (Poly A + T) showed red fluorescence (Fig. 1*b*), which confirms the immobilisation and hybridisation of DNA on a pentacene FET. The sensing mechanism of DNA on the pentacene FETs is shown in Fig. 1*c*. In this study, we found a dramatic difference in the channel current (I_{DS}) and changes in the field effect mobility (μ_{FET}) pattern on exposure to either 50-mer, 200 pmol ss-DNA or ds-DNA on the same pentacene FET. Fig. 2 shows the difference in the sensor output and transfer characteristics from the original pentacene FETs (without immobilised DNA) compared to the pentacene FETs with immobilised probe DNA (ss-DNA) or target DNA (ds-DNA after hybridisation). After immobilising ds-DNA, the channel current (I_{DS}) of the device

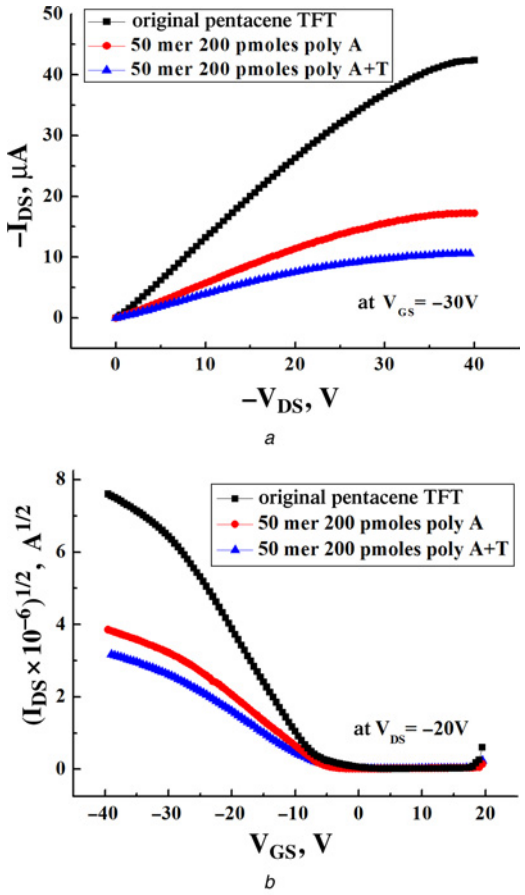

Figure 2 *Performance of the pentacene FETs with DNA immobilised on pentacene surface*
a Output characteristics of three pentacene FETs (original, ss-DNA, ds-DNA)
b Transfer characteristics of three pentacene FETs (original, ss-DNA, ds-DNA)

reduced approximately to 87.67% compared to that of the original device (at $V_{DS} = -30$ V, $V_{GS} = -30$ V) which was more than that for ss-DNA (80.35%). In addition, the field effect mobility (μ_{FET}) reduced from 2.286 cm^2/Vs to 0.473 and 0.334 cm^2/Vs for ss-DNA and ds-DNA, respectively. The reduction in the channel current (I_{DS}) and field effect mobility (μ_{FET}) following DNA hybridisation was dramatic and in contradiction with previous reports [13, 14], wherein the researchers have demonstrated an increase in channel current (I_{DS}) because of hybridisation of DNA on the pentacene surface. Such difference could be owing to the use of a buffer in these reports compared to double-distilled water that we used in our present work in order to avoid the effect of charges carried by buffer components. It can be safely regarded as more authentic to use plain distilled water to determine the change in channel current than by using buffer components that carries charge in itself. Furthermore, the reduction in channel current and μ_{FET} because of DNA hybridisation have been demonstrated previously by our group [15], wherein we reported our feasibility study for sensing DNA molecules through hybridisation on the pentacene surface. The DNA should ideally attract holes after attachment to pentacene and that shall decrease the lifetime of these holes from the channel region, thereby reducing the channel current level and field effect mobility.

Keeping up with this trend, the ds-DNA reduced the channel current (I_{DS}) and field effect mobility (μ_{FET}) even to a greater extent when compared with that by the ss-DNA, since the ds-DNA carry more net negative charge. This enabled the direct electrical detection of the target DNA through the measurement

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of channel current (I_{DS}) and field effect mobility (μ_{FET}) for the pentacene FETs. The net difference in the channel current (I_{DS}) and field effect mobility (μ_{FET}) on the pentacene FETs because of the single- or double-stranded DNA was the basis for the analysis of the pathogenic DNA through probe hybridisation.

Further, to derive the correlation between device response and DNA concentration, the output and transfer characteristics of the devices were measured with varying concentrations of the ss-DNA on the pentacene surface (Fig. 3). As DNA concentration was increased, the channel current (I_{DS}) also decreased and so a decrease in the field effect mobility (μ_{FET}) was observed ranging from 2.286 cm^2/Vs to 1.323, 1.081 and 0.473 cm^2/Vs for 50, 100 and 200 pmol ss-DNA, respectively. Such a reduction in the channel current (I_{DS}) and field effect mobility (μ_{FET}) was owing to the increase in the immobilised DNA concentration on the pentacene surface, which collectively attracts more holes from the channel region. The sensitivity of our device was found to be 0.282 μA/pmol and 0.00836 $cm^2\ V^{-1}\ s^{-1}$/pmol of ss-DNA with respect to reduction in channel current and field effect mobility, respectively. These results indicate the possibility of dynamic response from devices having low concentrations of hybridised target DNA.

Finally, as a proof of concept, we attempted to detect a model organism bacteriophage lambda through hybridisation of its genomic DNA over a probe-immobilised pentacene FET device. For this purpose, a 20-mer ss-probe 5′-GCA-AGT-ATC-GTT-TCC-ACC-GT-3′ was immobilised on a pentacene surface and was characterised (Fig. 4). Subsequently, the λ-phage DNA digest (with restriction enzyme BstII) was preheated at 100°C for 30 min to separate its strands and 1 μl of this sample (containing

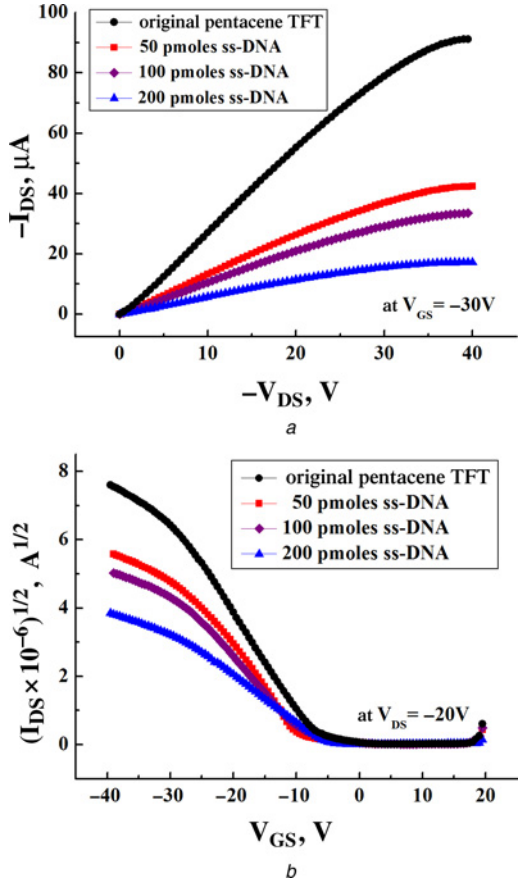

Figure 3 *Pentacene FETs with 50, 100 and 200 pmoles DNA immobilised on pentacene surface*
a Output characteristics
b Transfer characteristics

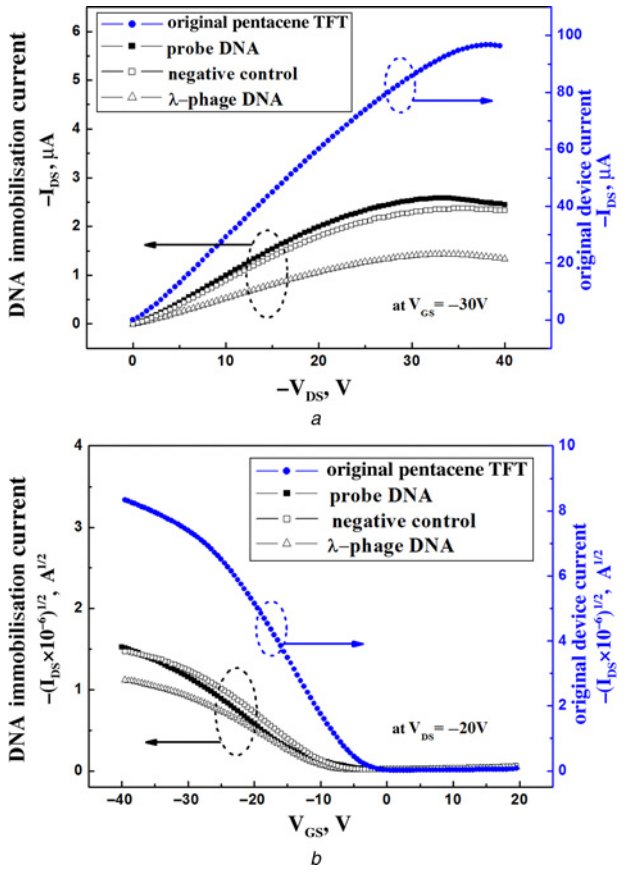

Figure 4 *Performance of the pentacene FETs with single-stranded 20-mer probe immobilised on pentacene surface and on hybridisation of λ-phage DNA*
a Output characteristics of three pentacene FETs (original, probe-DNA, hybridised *E. coli* DNA as negative control, hybridised λ-phage DNA)
b Transfer characteristics of three pentacene FETs (original, probe-DNA, hybridised *E. coli* DNA as negative control, hybridised λ-phage DNA)

1 μg DNA) was applied on the probe-immobilised FET surface. Restriction digestion of λ-phage DNA ensured five smaller fragments of ds-DNA for ease in separating the strands and subsequent hybridisation. The devices were air dried, washed with DI water and used in further characterisation as before. As seen from Fig. 4, the device showed significant change in electrical properties in response to complementary DNA from λ-phage. After hybridisation of λ-phage DNA, the channel current (I_{DS}) of the device reduced approximately to 44.88% compared to devices with only probe DNA (at $V_{DS} = -30$ V, $V_{GS} = -30$ V). In addition, the field effect mobility (μ_{FET}) also reduced approximately to 40.67%. On the other hand, during a control experiment, the pentacene FET device did not show significant change in electrical properties when the same concentration of bacterium *Escherichia coli* (*E. coli*) DNA was applied over a 20-mer probe 5′-GCA-AGT-ATC-GTT-TCC-ACC-GT-3′ hybridised device. In this device, *E. coli* DNA did not immobilise on the pentacene channel layer as the 20-mer probe was already immobilised on this surface. In addition, as expected, *E. coli* DNA did not hybridise to 20-mer probe as it was specifically designed against its target from λ-phage. This result indicates the possibility of selective response from devices having specific probe DNA molecules immobilised on the pentacene. Moreover, the response time for these sensors was about 65 min, which mainly included drying time for ds-DNA on the pentacene surface following DNA hybridisation. The sensor was stable up to 30 days when stored at room temperature in a non-humid condition. The sensor having ss-DNA immobilised produced up to 95% response as compared to the original device when stored under such condition. These results suggest a feasible

DNA hybridisation sensor with high sensitivity, selectivity and relatively shorter response time when compared with existing methods involving DNA hybridisation.

4. Conclusion: Therefore, in conclusion, the single-stranded λ-phage probe DNA was immobilised on the surface of the pentacene layer and hybridised with its double-stranded target DNA, thereby producing a significant change in the performance of the pentacene FETs. It was attributable to the net negative charges on the DNA molecules having the ability to attract holes from the channel region. The electrical characterisation of the pentacene FETs with the immobilised ds-DNA produced a lower current output (I_{DS}) and field effect mobility (μ_{FET}) since the ds-DNA carry more net negative charge. It was possible to selectively quantify the target DNA from the organism. With these results, we propose a 'label-free' detection technique for pathogen with high sensitivity and selectivity. This method shall enable the possibility for a portable and disposable pathogen sensor and can also find use in diverse applications such as medical diagnostics, forensic investigations, genotyping as well as combinatorial synthesis.

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