Robust Microfluidic Integrated Electrolyte-Gated Organic Field-Effect Transistor Sensors for Rapid, In Situ and Label-Free Monitoring of DNA Hybridization

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Electrolyte-gated organic field-effect transistors (EGOFETs) are subject to intense research for biosensing in fluids. The ability of these devices to quantify a variety of chemical and biological molecules sensitively and selectively, but ex situ, has been widely demonstrated. However, continuous monitoring of analyte-receptor interactions in real time by EGOFETs, in high demand for practical applications, has rarely been explored. Here, an EGOFET array integrated with a microfluidic device, for real-time detection of the hybridization of DNA is presented. The integrated devices exhibit highly reproducible electrical performance in the array and can operate under different electrical stresses over >1h with 85–96% figures of merit retention. The utility of the devices is demonstrated to detect the hybridization of a complementary target DNA in $10 \times 10^{-3}$ M phosphate-buffered saline (1X PBS) selectively with a temporal resolution of <1s in a flow of analyte with no incubation step. The detection time is <30s and the relative standard deviation of the sensing reproducibility is <15% under the target concentration of $100 \times 10^{-9}$ M.

1. Introduction

There are tremendous opportunities for reliable portable systems that are capable of onsite environmental monitoring, disease diagnosis, or prognosis and this is catalyzing extensive research into novel bioelectronic devices. Miniaturized biosensors to deliver point-of-need testing devices are under investigation based on a variety of detection modalities.[1] One approach is to use printed electronic platforms based on organic field-effect transistors (OFETs).[2] The electrical output of these OFETs is sensitive to subtle changes in surface potential, and by tailoring the interfaces in these devices with a specific biorecognition element, selective label-free identification, and multiparametric quantification of a target analyte is realizable.[3,4] The miniaturization and integration of OFETs to other microsystems on various substrates is straightforward.[5] However, conventional OFETs are limited for application as in situ biosensors because it is challenging to operate these devices at a bias <1V, that is consistent with electrical operation in an aqueous, biological medium.

Electrolyte-Gated Organic Field-Effect Transistors (EGOFETs) have been applied as label-free biosensors.[6] These devices are particularly interesting as the electrolyte in the gate terminal from the source-drain-channel layer,[7] and the biological medium, the electrolyte, can be an integral part of the device. Upon biasing the gate and drain terminals (source grounded), the electrolyte acts as the gate capacitor of the transistor by formation of Electrical Double Layers (EDLs). Generally, full modulation of the drain current ($I_{DS}$) from off to on-state is achievable in these devices with application of $V_{GS} \leq 1$ V.[8] EGOFT sensors have been reported to detect various molecules and ions with good selectivity and high sensitivity.[6,8,9,10] Commonly, the selectivity is obtained by incorporating a biorecognition element at the semiconductor or gate terminal interface with the electrolyte.[6] This layer of receptors interacts with the target analyte to perturb the electronic properties of the device to achieve sensitivities within the relevant range for detection of biological molecules.[12,11] Most of these early EGOFET biosensors used a droplet gated configuration, where a droplet of electrolyte is placed on the organic semiconductor (OSC) and the gate contact is immersed into the droplet. Biosensing could be performed after incubation of the analyte on the sensitive layer of the device for 30 min up to 1 h and a cleaning step to remove nonspecifically adsorbed molecules.[6,14] More recently EGOFET devices have been integrated

DOI: 10.1002/aelm.202200142

Adv. Electron. Mater. 2022, 8, 2200142 © 2022 The Authors. Advanced Electronic Materials published by Wiley-VCH GmbH
with fluidic handling systems to automate the incubation and cleaning step before ex situ single-spot sensing. While some of these studies demonstrated real-time sensing, including the quantification of changes in solute concentration over time and the transport rate of molecules at the solid-liquid interface during crystallization, none have explored the continuous monitoring of biomolecule recognition (i.e., binding of carbohydrates, lipids, nucleic acids, and proteins).

In this work, a sensing platform based on an array of EGOFETs integrated with a microfluidic cell is demonstrated for real-time detection of the hybridization of DNA under a flowing electrolyte with <1 s temporal resolution. The array configuration was adopted because sensor platforms typically require parallel sensing to rapidly quantify multiple analytes in a single, precious biological sample, and to increase detection reliability for an individual analyte by using multiple replicates of individual devices on a single substrate. The selectivity of the individual EGOFET in the array toward the target DNA (ssDNA-t) was achieved by functionalizing the gate electrode with a self-assembled monolayer (SAM) of single-strand capture DNA (ssDNA-Cap). The device exhibits excellent and reproducible electrical performance and can operate under different electrical stresses over >1 h with 85% to 96% figures of merit retention. Hybridization of the complementary DNA (19 bases, 100 × 10^{-9} m) was detected in a continuous flow of analyte with no incubation step. The response of the device reaches ~80% of its maximum in <1 s and is almost saturated after 25 s with robust statistical reproducibility. Excellent selectivity of the device was verified by clearly discriminating ssDNA-t from a noncomplementary DNA (ssDNA-nc) at a comparable concentration.

2. Results and Discussion

2.1. Design and Fabrication of the Microfluidic-Integrated EGOFET Biosensor

The microfluidic integrated EGOFET biosensor was fabricated by combining techniques of polymer microfabrication and fast prototyping (Figure 1). First, an array of 16 interdigitated source-drain electrodes were deposited on a polyethylene naphthalate (PEN) substrate via direct laser photolithography (Figure 1a). Then, the substrate was spin-coated with poly(diketopyrrolo-pyrrole-dithiophene-thienothiophene) (PDPPDTT) (Figure 1a-II.a).[18] PDPPDTT was selected as it has previously been established as a high performance material for use in EGOFETs.[18] Then a double-sided tape (DST) with four laser cut flow channels (Figure 1a-IV.b). Holes were cut at the edges of the gate electrode array using a CO2 laser and stainless-steel tubes inserted to act as inlets and outlets for the electrolyte. The gate electrodes were cleaned using UV/O3 and a SAM was deposited on this electrode from solution (Figure 1a-III.b). The complete device was assembled by affixing the functionalized gate onto the source-drain substrate using the DST with the defined fluidic channels (Figure 1a-IV.b). Top view photographs of the electrode substrates and the assembled device are presented in Figure 1b–d respectively.

The gate electrode was chosen for integration of the biorecognition layer as functionalization at the surface of the OSC may result in a degradation of the electronic performance of the EGOFET.[19] Some solvent-free techniques have been developed to functionalize the OSC interface, which results in less significant impact on the device figures of merit.[20,21] However, these biorecognition layers may not remain stable during the experiment due to the weaker electrostatic or Van de Waals forces binding to the OSC and the shear force applied by the electrolyte during flow can remove the recognition layer. Initially, the gate electrode was treated with a dilute solution (1 μM) of the thiol terminated ssDNA-Cap (Figures S1 and S2, Supporting Information), followed by a dilute solution of 6-mercaptohexanol (MCH) solution (1 mM) in 1× PBS (Figure S1, Supporting Information) to minimize nonspecific interactions and reduce the concentration of any physisorbed ssDNA-Cap molecules.[22,23] The water contact angle of a control gate after incubation in 1× PBS was 49° ± 1°. For a gate electrode covered with ssDNA-Cap diluted in the same 1× PBS solution it was 10° lower than the control (39.1° ± 0.3°) (Figure 1c and Table S1, Supporting Information), consistent with effective immobilization of ssDNA-Cap on the gate. Subsequent incubation of the functionalized gate in MCH diluted in 1× PBS improves single-strand DNA (ssDNA) SAM orientation and fills the remaining free space on the gate.[24,25,26]

2.2. Device Performance Uniformity and Long-Term Operational Stability

Current-voltage/time curves of the devices were recorded in flowing 1× PBS at 210 μL min^{-1} in the fluidic channel (Figure 2a,b and Figures S3 and S4a, Supporting Information), and a typical I_Ds versus V_GS is plotted in Figure 2b.

The device characteristics confirm the effective establishment of a stable EDL in these devices and a dominant field-effect charge transport under flowing electrolyte conditions. Three different substrates were characterized (total of 36 individual EGOFET devices) to comprehensively study the reliability of the fabricated sensing platform. Performance reproducibility, repeatability, and long-term operational stability were chosen as the parameters for operational reliability, as these are fundamental criteria a system has to fulfill before realistic applications can be considered. In OFETs, these are investigated by extracting device figures of merit from current-voltage characteristics using standard metal-oxide-field-effect-transistor approximated equations.[27,28] These equations can be applied to the developed device, as $\sqrt{I_{DS}}$ scales linearly with the gate bias (Figure 2b). Hence, the electrical parameters were extracted from the tested devices by linearly fitting Equation (1) to $\sqrt{I_{DS}}$ versus $V_{GS}$ and calculating the slope of log ($I_{DS}$) versus $V_{GS}$ at very low $V_{GS}$ for the subthreshold swing (SS) as shown in Figure 2b.

$$\sqrt{I_{DS}} = \sqrt{\frac{\mu C_i W}{2L}} (V_{GS} - V_T)$$

(1)
$W, L, C_i, \mu$, and $V_{th}$ are the channel width and length, the EDLs areal capacitance, the field-effect mobility, and the threshold voltage, respectively. The distribution of the extracted values, namely in $V_{th}$, normalized transconductance ($\mu \times C_i$), and on-to-off drain current ratio ($I_{on}/I_{off}$), are displayed in Figure 2c. The recorded $I_{DS}$ lies between 2.9 and 5.2 $\mu$A at a $V_{GS}$ of –0.9 V (Table S2, Supporting Information). The mean value was 3.9 $\mu$A with a relative standard deviation of 13%, calculated from 36 devices over three substrates (12 devices/substrate were characterized). On a single substrate, the estimated relative error on the $I_{DS}$ was lower and the standard deviation was reduced to 8% (Figure S4 and Table S3, Supporting Information). The variation in the extracted $\mu \times C_i$ values was larger at $\approx 10\%$ over one substrate and $17\%$ between substrates. The variation in the device figures of merit, particular between substrates may be due to the manual fabrication and assembly process of the device that could potentially be reduced by more automated processes.

The operational stability of the device under a continuous flow of the electrolyte (210 $\mu$L min$^{-1}$) was investigated by recording the variation in the $I_{DS}$ and gate currents ($I_{GS}$) under a constant $V_{GS}$ and $V_{DS}$ (source grounded) as a function of time. The output $I_{DS}$ was normalized against the maximum value and the percentage change (Figure 3a) calculated. The normalized $I_{DS}$, starting at $\approx 35\%$ of its maximum value, gradually increased to reach a maximum after 100 s and then remained stable for over 5000 s. A logarithmic plot is presented in the upper panel.
of the inset of Figure 3a showing a drift in the $I_{DS}$ of about 4% (between 500 to 5000 s). This trend, an increase of $I_{DS}$ over bias stressing, has been observed in an EGOFET in which the OSC was protected by SAM of cationic/anionic molecules. Such bias stress instability could originate from charge detrapping from the OSC and polarization or ion drift in the EDL. It should be emphasized that the current retention during 1h continuous operation observed in the developed device is significantly higher than that of previously reported EGOFETs ($\approx 96\%$ vs 60% to $\approx 90\%$) despite the flow of the electrolyte and the higher gate bias ($V_{GS} = -1.2 \text{ V}$). The improved operational stability may come from the more ordered morphology and the interdigitation of the alkyl side chains reducing water diffusion and ion doping. Similarly, the initial $I_{GS}$ rapidly decreased to reach 96% of its minimum value after 100 s (lower panel of the inset of Figure 3a). The simultaneous variation of the $I_{DS}$ and $I_{GS}$ can be correlated with the time taken to establish the EDL at the interfaces, and it required about 100 s to reach the maximum capacitance. The EDL capacitance reached a steady state after this period and the $I_{DS}$ plateaued with a small drift on prolonged measurement. For PDPPDTT-based EGOFET, when static or flowing water (Figure S5, Supporting Information) is employed as the electrolyte it required respectively only 1–2 s and 80 s for the current ($I_{DS}$, $I_{GS}$) to reach steady-state operation. In the experiment using a higher ionic strength ($1 \times$ PBS) and flowing electrolyte the establishment of stable operation required $\geq 100$ s.

EGOFET biosensors are multiparametric sensors and more than one output parameter (e.g., $I_{DS} \times C_i$, $V_{th}$) can be affected by chemical and biological events. It is vital to understand the variation in these parameters with device operation to establish if they can be used to detect aqueous analytes. Therefore, multiple $I_{DS}$ versus $V_{GS}$ curves, spaced with 1 min gate bias stress ($V_{DS} = -0.7 \text{ V}$, $V_{GS} = -1.2 \text{ V}$), were measured. The evolution of the normalized $I_{DS}$ versus $V_{GS}$ (at $V_{GS} = -1.2 \text{ V}$), $V_{th}$ and $\mu \times C_i$ are depicted in Figure 3b,c. The drift of $I_{DS}$ toward a higher value ($\approx 4\%$) seen in the previous continuous current monitoring experiment (Figure 3a, $V_{GS} = -1.2 \text{ V}$) was also observed in these bias stress measurements (right panel of Figure 3c). The drift in $I_{DS}$ at the maximum $V_{GS}$ ($V_{GS} = -1.2 \text{ V}$) was larger at $\approx 13\%$ over 1 h of operation. This is relatively stable than previously reported results on EGOFET stability over bias stressing ($\approx 13\%$ vs $>15\%$ up to 25%). For the other figures of merit, it was found that they also drift by $\approx 15\%$ when cycled continually for an hour of operation. $V_{th}$ and $\mu \times C_i$ changed by $\approx 14\%$ and $\approx 10\%$, respectively (middle and right panel of Figure 3c). These changes in the figures of merit of the devices were minor in the time scale of the experiment (Figure 3b,c) and it is evident that the device is robust and reliable for $>1$ h continuous operation in flowing electrolyte condition.

![Figure 2. Biosensing device gated with phosphate-buffered saline solution (0.01 m phosphate buffer, 0.0027 m potassium chloride, and 0.137 m sodium chloride in deionized water, pH 7.4, at 25 °C) at a flow rate of 210 µL min$^{-1}$: a) schematic representation of the characterization setup, b) typical transfer characteristic of the device and c) distribution of electrical figures of merit extracted from the transfer characteristics ($V_{DS} = -0.7 \text{ V}$).](image-url)
2.3. Rapid and In Situ Monitoring of DNA Hybridization

The platform was used for real-time detection of the hybridization of DNA (Figure 4). Two analytes were investigated, a fully complementary target ssDNA (ssDNA-t) to the ssDNA-Cap immobilized on the device gate electrodes and a noncomplementary ssDNA (ssDNA-nc). The ssDNA-nc was used as a negative control to evaluate the selectivity of the device and the chemical structures of the ssDNA-Cap, ssDNA-t, and ssDNA-nc can be found in the Supporting Information (Figure S2, Supporting Information). The sensing experiments were performed using the characterization setup depicted in Figure 4a.

The evolution of $\Delta I_{DS}$ was measured as a function of time while the electrolyte was switched from 1× PBS to a dilute ssDNA-t ($100 \times 10^{-9}$ M) or ssDNA-nc ($100 \times 10^{-9}$ M) solution in 1× PBS. A blank measurement switching from 1× PBS to 1× PBS was also carried out. The measured currents were normalized ($N_{\Delta I_{DS}}$) to the current obtained before the change ($I_{DS(\text{PBS})}$) using Equation (2):

$$N_{\Delta I_{DS}} = \frac{I_{DS(\text{PBS})} - I_{DS(\text{PBS})}}{I_{DS(\text{PBS})}} \cdot 100\%$$

Switching from 1× PBS to $100 \times 10^{-9}$ M of ssDNA-t diluted in 1× PBS lead to a large change in the $N_{\Delta I_{DS}}$. The measured current decreased sharply and then started to saturate (Figure S6, Supporting Information), the change in $N_{\Delta I_{DS}}$ was 92% in $\approx 25$ s (black curve in Figure 4b). In comparison the blank response and the ssDNA-nc, negative control had a very small impact on $N_{\Delta I_{DS}}$ (blue and red curves in Figure 4b) with ≤1% fluctuation over time. Switching back from $100 \times 10^{-9}$ M of ssDNA-t to 1× PBS results in a minor decrease in $N_{\Delta I_{DS}}$ reaching a plateau that remains stable. In contrast, switching back from 1× PBS or ssDNA-nc ($100 \times 10^{-9}$ M) to 1× PBS has no noticeable impact on $N_{\Delta I_{DS}}$. The device can clearly discriminate ssDNA-t from a ssDNA-nc at a comparable concentration. It is worth mentioning that the $N_{\Delta I_{DS}}$ behavior during ssDNA-t flow is similar to the response of the organic transistor-based DNA sensor reported by Bao and co-workers.[33] However, instead of EGOFET, the authors used a conventional OFET in bottom-gate top-contact architecture, the recognition layer was at the OSC/electrolyte interface, the target ssDNA is 15 bases (compared to 19 bases in this work) and the electrolyte was a sodium acetate buffer solution (ABS). At a target concentration of $100 \times 10^{-9}$ M, the fabricated device displayed a greater $N_{\Delta I_{DS}}$ increase over a shorter time in comparison to the reported conventional OFET sensor in the detection of a target ssDNA.[33]

To understand the changes in $N_{\Delta I_{DS}}$ seen during in operando DNA detection, the transfer characteristics were recorded before and after the experiment. This provided information on...
the device figures of merit that are responsible for the change in the measured $N\Delta I_{DS}$.\(^{[34]}\) Figure 4c shows typical transfer characteristics from three separate devices measured before and after hybridization of ssDNA-t ($100 \times 10^{-9}$ m) with the capturing probe (ssDNA-Cap) on the gate terminal. The black curves were recorded with $1 \times$ PBS flowing over the fluidic channel. The blue and red curves were measured after switching the flow to a solution of $100 \times 10^{-9}$ m ssDNA-t. The blue curve corresponds to measurement taken during the elution of the ssDNA-t solution and the red curve after the electrolyte solution was flushed from the system. The current drops from an initial value of $3.9 \pm 0.8$ to $1.5 \pm 0.5$ $\mu$A (Table S4, Supporting Information). These results echo the decrease in the current (increases of $N\Delta I_{DS}$ signal) seen during the in operando measurement of DNA hybridization. A shift in $V_{th}$ toward a more negative gate bias was also observed (from $-0.433 \pm 0.004$ to $-0.505 \pm 0.03$ V). The large negative shift of $V_{th}$ on hybridization of the two ssDNA strands is the predominant sensing mechanism. This shift of $V_{th}$ to more negative gate bias can be induced by the redistribution of dipoles at the gate surface to increase its work function due to DNA hybridization. It is noteworthy that the measured blue and red curves are almost identical, indicating that the hybridization of the two ssDNA strands is irreversible under the experimental conditions (i.e., 2 min of flowing $1 \times$ PBS). This is consistent with the observation of Bao and coworkers: rinsing a OFET DNA sensor with ABS for 60 s after DNA hybridization has only a minor impact on device response as hybridization is irreversible under these conditions.\(^{[33]}\)

3. Conclusion
A label-free biosensor consisting of an array of EGOFT devices integrated with a multichannel microfluidic platform was developed on a flexible substrate. The devices showed reproducible electrical performance and can operate under different electrical stresses over >1 h with 85% to 96% figures of merit.

Figure 4. Monitoring of ssDNA-t hybridization by EGOFT device at a flow rate of 210 $\mu$L min$^{-1}$: a) schematic representation of the characterization setup, b) In situ monitoring of DNA, arrows indicate the switching of the flow to a different analyte (switching from $1 \times$ PBS to ssDNA-t, or ssDNA-nc or $1 \times$ PBS, and switching back to $1 \times$ PBS). The insert is the zoom of the evolution of $N\Delta I_{DS}$ when ssDNA-nc or $1 \times$ PBS was the analyte ($V_{DS} = -0.7$ V, $V_{GS} = -0.9$ V). c) Typical transfer curves from three different devices. The black curves were recorded just before real-time detection of $100 \times 10^{-9}$ m ssDNA-t. The blue and red were measured straight after the saturation of the sensor response during real-time detection of ssDNA. The electrolyte for the black, blue, and red curves was $1 \times$ PBS, $100 \times 10^{-9}$ m of ssDNA-t and $1 \times$ PBS, respectively ($V_{DS} = -0.7$ V).
meric retention. The device utility for real-time monitoring was demonstrated by detecting the hybridization of DNA in situ and operando at <1 s temporal resolution under flowing electrolyte condition with good reproducibility and selectivity. Compared to previous EGOFET biosensors, the developed platform does not require incubation and cleaning steps to remove nonspecifically adsorbed molecules. Moreover, detection is performed in high ionic strength solution and in situ, whereas previous work reports on ex situ singlet-spot detection and mostly in water. The detection time was <30 s and the relative standard deviation of the sensing reproducibility was <15% under the target concentration of 100 × 10^{-9} \text{M}. The sensing relies on a shift in device $V_{th}$ due to the change in gate electrode work function upon DNA hybridization. The design of the device allows for the detection of multiple analytes if specific receptors to the molecules of interest can be immobilized on individual gate electrodes in the array for disease diagnostics, food, and homeland safety, and environmental monitoring.

4. Experimental Section

Materials: Teijin DuPont film Co. Ltd. provided 125 µm thick poly(ethylene 2,6-naphthalate) (PEN) foil and Platifix CS2325NA4 adhesive. Silicon wafers and Shipley 1805 photoresists were obtained from the Manchester Centre for Mesoscale and Nanotechnology. Diketoypyrrrolopyrrole-alt-3,5-(2,5-di(thien-2-yl)thieno[3,2-b]thiophene] (PDPPDTT, Mw 399 066 and Mn <8 m) was obtained from OMIC. Polyethylene terephthalate (PET) and stainless-steel tubes (5 mm with a land safety, and environmental monitoring.

Fabrication of the Microfluidic-Integrated EGOFET Device: An array of Au (40 nm) interdigitated source/drain electrodes were patterned by a Shipley 1805 photoresist direct laser lithography process on a polyethylene naphthalate substrate (PEN). The interdigit distance was 60 µm and the perimeter of the electrodes was 16 000 µm. The same procedure was employed to define an array of gate electrodes onto a second PEN substrate and this was stuck onto a 3 mm thick PMMA sheet with DST (280 µm thick) purchased from Cole-Parmer Instrument Company Ltd. DNA (capture, target, and probe), 1× PBS (0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride in deionized water, pH 7.4, at 25°C), DL-dithiothreitol (DTT), 1,2-dichlorobenzene, dimethyl sulfoxide, and all others solvents were purchased from Sigma–Aldrich.

Bio-Functionalization of Gate Electrodes and Assembly of the Device: The 16 gate electrodes were functionalized with a SAM of thiol-terminated single-stranded DNA and 6-mercaptopentanol (MCH). This was conducted by taking a 100 × 10^{-6} m solution of [ThiC6] TTA GTT CTC CAG CTA TCT T diluted in 1× PBS and mixing it with DL-dithiothreitol (15 mg mL^{-1}). This solution was left to stand for 10 min at room temperature to form thiol-terminated single strand capturing DNAs (ssDNA-Cap) via the reduction of the disulfide bonds. The obtained solution was desalted with an Illustra Microspin G25 column (following Sigma–Aldrich protocol) and diluted in 1× PBS to produce a 1 × 10^{-6} m ssDNA-Cap solution. At the same time, a 1 × 10^{-11} m solution of 6-mercaptopentanol in a mixture of dimethyl sulfoxide (DMSO) with 1× PBS (9:1 ratio) was prepared. The gate electrodes were covered with the prepared solution containing 1 × 10^{-6} m of ssDNA-Cap for 1 h. The gate was then flushed three times with 1× PBS to remove nonbonded ssDNA-Cap and subsequently covered with the solution of 6-mercaptopentanol for 1 h. The gate electrode was then further flushed (×3) with 1× PBS and blown dry under a stream of nitrogen. Note: before the incubations, the gate electrode was subject to 10 min under UV/O3 to remove any organic contaminants. The 6-mercaptopentanol was used to minimize any nonspecific binding of substances onto the gate electrode. The device was completed by sealing the bottom cover with the top cover so that the area of bio-functionalized gate electrodes was inside the flow cells and in front of its contact IDE. Note that assembly was carried out under a binocular microscope by hand.

Measurement of the Device Electrical Performance: The output and transfer characteristics of the EGOFETs were measured under continuous flow (210 µl min^{-1}) of 1× PBS through the microfluidic cell using a semiconductor parameter analyzer (Agilent B1500A). For the transfer characteristic, $I_{DS}$ was recorded as a function of $V_{CS}$ (swept from −0.05 to −0.9 V in steps of 0.001 V in forward and reverse direction) at a constant $V_{GS} (−0.7 V)$. For the output characteristic, $I_{DS}$ was measured at constant $V_{CS} (−0.9 V)$ while sweeping the $V_{DS}$ from 0 V to −0.7 V with an increment of 0.001 V. For the measurement of device stability, $I_{DS}$ was measured as a function of time at a constant $V_{CS} (−1.2 V)$ and $V_{DS} (−0.7 V)$ while 1× PBS flowed through the flow cell at 210 µl min^{-1}. The bias stress measurement consists of measuring several transfer characteristics with a gate bias stress of 1 min ($V_{DS} = −0.7 V$ and $V_{CS} = −1.2 V$) between two consecutive transfer curves.

Electrical Measurement of the Interaction Between ssDNA: Two syringe pumps were equipped with two 12 ml syringes. One syringe was filled with 1× PBS and the other with a solution of either a target ssDNA- (Bttn AAG ATA CCT CGA CAA CTA A) or a noncomplementary ssDNA-nc (Bttn AGT CTT TCA GTT GCT TCA C) diluted in 1× PBS or 1× PBS. The syringes were coupled to a three-way stopcock. First, 1× PBS was pumped into the flow cell at a rate of 210 µl min^{-1} and the transfer curves were measured three times to confirm that the current was stable. Then, $I_{DS}$ was recorded over time at a constant $V_{CS} (−0.7 V)$ and $V_{CS} (−0.9 V)$ while 1× PBS flowed through the channel. When the current was stable, the valve of the three-way stopcock was switched to inject the solution of the ssDNA (target or noncomplementary strand) diluted in 1× PBS or 1× PBS into the flow cell and the current recorded. After equilibrium between the bulk concentration and the corresponding surface coverage the valve of the three-way stopcock was switched back to inject 1× PBS. The transfer characteristic of the EGOFET was recorded after the transient measurement using the diluted ssDNA solution and 1× PBS after 2 min.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The authors acknowledge the financial support of Cambridge Display Technology (CDT) Limited (registered number 2672530)
and the Engineering and Physical Sciences Research Council (Grant number: EP/K03099X/1). The authors thank the Manchester Centre for Mesoscience and Nanotechnology for access to the cleanroom equipment and materials. The authors thank DuPont and D. J. Tate for supplying PEN substrate and PDPPDTT polymer, respectively.

Conflict of Interest
The authors declare no conflict of interest.

Data Availability Statement
The data that support the findings of this study are available in the supplementary material of this article.

Keywords
biosensors, DNA, donor–acceptor conjugated polymers, electrolyte-gated organic field-effect transistor (EGOFET), microfluidic

Received: February 8, 2022
Revised: April 21, 2022
Published online: May 31, 2022

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