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Ultrasensitive Detection of Oligonucleotides: Single-Walled Carbon Nanotube Transistor Assembled by DNA Block Copolymer

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Biosensors, which harness the unique specific binding properties of biomaterials such as proteis, are increasingly recognized as a powerful tool for chemical sensing. In this context, the detection of nucleic acids DNA and RNA will take on ever more importance for screening as the fields of genomics and diagnostics advance. The same properties that allow molecular recognition–strong, specific non-covalent interactions–also enable bottom-up assembly of sensing architectures. Here, we take advantage of such interactions for the self-assembly of field-effect transistors from semiconducting single-walled carbon nanotubes selectively dispersed by DNA-block-copolymers and anchored to the electrodes through DNA hybridization. These transistors can sensitively detect the hybridization of complementary target DNA strands through transduction of the chemical recognition event into electrical doping, achieving an analyte sensitivity of 10 fM. Such ultra-sensitive electrical-based detection removes the need for DNA amplification and offers a new route to nucleic acids diagnostics.

Keywords: 10 fM Limit of Detection, Biosensors, Ambipolar FETs, CNT Devices.

1. INTRODUCTION

Biosensors hold great interest and potential in a range of practical applications and fundamental research fields spanning materials science, electrochemistry, device physics, biology and diagnostics.[1, 2] Recent advances in nanotechnology have led to powerful "bottom-up" paradigms for sensing biomolecules, allowing the construction of complex, multi-component sensing platforms for spectroscopic or electronic detection. The most common motifs involve antigen-antibody or ligand-receptor interactions, which can be detected through specific molecular binding and subsequent signal transduction through biological pathways, whereas nucleic acid sensing relies on fundamentally different molecular mechanisms.[3–6] First and foremost, DNA or RNA chains inherently encode information in their sequence of nucleobases, meaning they can recognize exact "complementary sequence" via base-pairing (i.e., in DNA Adenine pairs with Thymine, Cytosine pairs with Guanine and vice versa; in RNA the same rules apply but Thymine is replaced by Uracil). Due to the significant non-covalent interactions between complementary bases, the pairing of two fully matched nucleotide sequences to form the famous double helix is spontaneous, significantly lowering the Gibbs free energy. Indeed, the resulting double-stranded nucleic acids are very stable and can only be dissociated at elevated temperature or in the presence of denaturing agents. Though the target DNA/RNA sequences in samples of interest are generally present in very low quantity, biological machinery such as enzymes can be easily harnessed to amplify the analyte content more than a million-fold with the polymerase chain reaction. A final unique characteristic of nucleic acids for biosensing lies in their chemical

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structure: they are linear biopolymers with each unit consisting of sugar, nucleobase and phosphordiester. The phosphates along the DNA and RNA chains carry substantial negative charge. The strong effects of these charges on electronic sensors can complicate detection strategies but potentially offer simple routes to high sensitivity.[7, 8]

In the quest to reduce nucleic acid detection thresholds, tremendous efforts have been made in fluorimetric and electronic sensing. For instance, catalytic fluorogenic reactions using organic dyes have been developed to detect DNA with 10 pM and even 10 fM ultra-low limits of detection with single-base mismatch sensitivity.[9, 10] Electronic sensing is more appealing for practical applications, though, both for the greater intrinsic sensitivity of current-based detection and easier integration into full devices. One such approach used amphiphilic DNA non-covalently immobilized along Si nanowire electronic channels, with electronic detection of base-pairing with short complementary oligonucleotides.[11] Such "bottom-up" self-assembly of devices is promising and has motivated a search for further materials systems suited to electronic DNA sensing. Parallel developments in materials science have seen the creation of new classes of electronic devices built on supramolecular assembles, such as gold nanoparticles,[12, 13] silicon nanowires,[14] carbon nanotubes,[15, 16] and polymers.[17] A particularly relevant materials system for DNA detection is the use of a polymer-DNA conjugate to disperse single-walled carbon nanotubes (SWNT).[18] These hybrids could be deposited on surfaces to bridge two electrodes and build functioning field-effect transitions (FETs) in high yield (98%), taking advantage of the self-assembly properties of the DNA component. However, no further explorations of the potential applications of this device system were explored.

Here, we further develop SWNT-FETs as a highly sensitive DNA detection platform. Crucial elements in the system design are selective dispersion of semiconducting SWNTs using an amphiphilic DNA-block-copolymer (DBC) and solution-based self-assembly through DNA base-pairing. In this system the DNA serves two distinct purposes, enabling interaction with the complementary DNA (cDNA) both for anchoring to the Au source and drain electrodes and for detection of low-concentration DNA targets. The DBC used here is composed of poly(9,9-di-*n*-octylfluorenyl-2,7-diyl) (PFO) covalently connected to a 22-mer single-stranded (ss) oligodeoxyribonucleotide (i.e., DNA block), and it was used to disperse high-pressure CO conversion (HiPCO) SWNTs.[18–21] We previously demonstrated that the hydrophobic PFO segment preferably interacts with the SWNT sidewall, leading to the selective dispersion of particular semiconducting SWNT species.[18] The dispersed SWNTs in water due to the PFO segments (hPFO) are interactions between the DNA blocks of hPFO and mixed self-assembled monolayers (SAMs) of thiolated cDNA and mercaptohexanol formed on the Au surfaces, leading to the formation of a FET channel between the two electrodes. Crucially, the single-stranded DNA (ssDNA) segments of the DBCs far from the electrodes are unable to participate in the bridging and remain available for hybridization with cDNA. Indeed, we observe that the hole current of SWNT-FETs significantly increases upon exposure to a low-concentration solution of cDNA, while exposure to non-complementary DNA (ncDNA) solution exhibits the opposite effect. This self-assembled system thus enables high-sensitivity electrical detection of DNA, and we anticipate that these results will spur the development of advanced DNA diagnostics.

2. EXPERIMENTAL DETAILS

2.1. Materials

The DBC and hPFO dispersion were prepared in accordance with our previous report,[18] using a 22mer oligodeoxyribonucleotide sequence of 5′-CCT CGC TCT GCT AAT CCT GTT A-3′ and a PFO block of molecular weight 6,000 g/mol. We used 5′-thoilated cDNA for fabrication of DNA functionalized electrodes and pristine cDNA as the target sequence for detection. The cDNA (target) sequence was 5′-TAA CAG GAT TAG CAG AGC GAG G-3′ and the ncDNA (control) sequence was 5′-CGT ACA CGT AGC ATG GAT TAG C-3′. DNA was purchased from Biomers (Germany) and other common chemicals such as salts and solvents were purchased from Sigma-Aldrich (The Netherlands). All chemicals were used as delivered without further purification.

2.2. Fabrication of SWNT-FETs for Electrical Detection of DNA

The SWNT-FETs using hPFO were fabricated on a highly *n*-doped (0.007 Ω cm) silicon wafer covered with a 500 nm silicon oxide dielectric layer. A 350 nm thick electron beam sensitive resist (ZEP-520, Zeon Co.) was spin-coated and subsequently baked at 180 °C for 90 s on a hot plate. The resist was exposed to 30 keV electrons (nominal dose of 100 μC/cm^2), then exposed structures were subsequently developed in *n*-Amyl-acetate. Using an e-gun evaporator, 5 nm of Ti and 35 nm of Au were deposited at $P = 10^{-7}$ mbar vacuum for the source and drain electrodes. Liftoff was done in hot PRS300 (70 °C) and rinsed with ultrapure water (18 MΩ). The channel length between the source and the drain was ∼150 nm. The prepared substrate was cleaned under O_2 plasma for 90 s prior to the hPFO immobilization protocol. The substrate was submerged in 1 mM mercaptohexanol solution for 1 min, then rinsed with ultrapure water (4 × 1.0 mL). Using adhesive DNA chambers (Bio-Rad, USA), an immobilization well was then positioned around each electrode set (a set contains 34 source-drain electrode pairs). Keeping the humidity high in the chambers using wet tissue papers, we added 10 μL of immobilization solution consisting of 4 μM thiol-modified cDNA in 1× phosphate

buffer (1.0 M KH_2PO_4/K_2HPO_4, pH = 7.2) to the center of each electrode sets. After 3 hours of incubation each well was gently rinsed with 1× phosphate buffer (4 × 40 μL) and with 0.1× phosphate buffer (2 × 40 μL). Excess solution was carefully removed with filter paper (Whatman, USA) to yield a sparse monolayer of immobilized cDNA on the Au surface. For hybridization of the electrode-bridging SWNT dispersion, each well was filled with hPFO (5 μL of hPFO stock) in the presence of hybridization buffer (10 μL, 250 mM NaCl and 10 mM $MgAc_2$) and left in a high-humidity chamber for 13 h. After removal of excess solution, each well was rinsed with rinsing buffer (4 times, 10 μL, 250 mM NaCl and 5 mM $MgAc_2$) and ultrapure water (twice, 15 μL) before drying under gentle N_2 stream.

2.3. Electronic Measurements Upon DNA Analyte Treatment

To introduce cDNA targets to the unoccupied ssDNA segments along the SWNT-FETs, 1 μL of cDNA (10 fM) in hybridization buffer was deposited to a device following same hybridization and rinsing procedures above. Similarly, a solution of 1 μL of ncDNA (10 fM) in hybridization buffer was deposited to a separate device set to control for sequence-specific DNA detection. Current–voltage (I–V) measurements were performed in

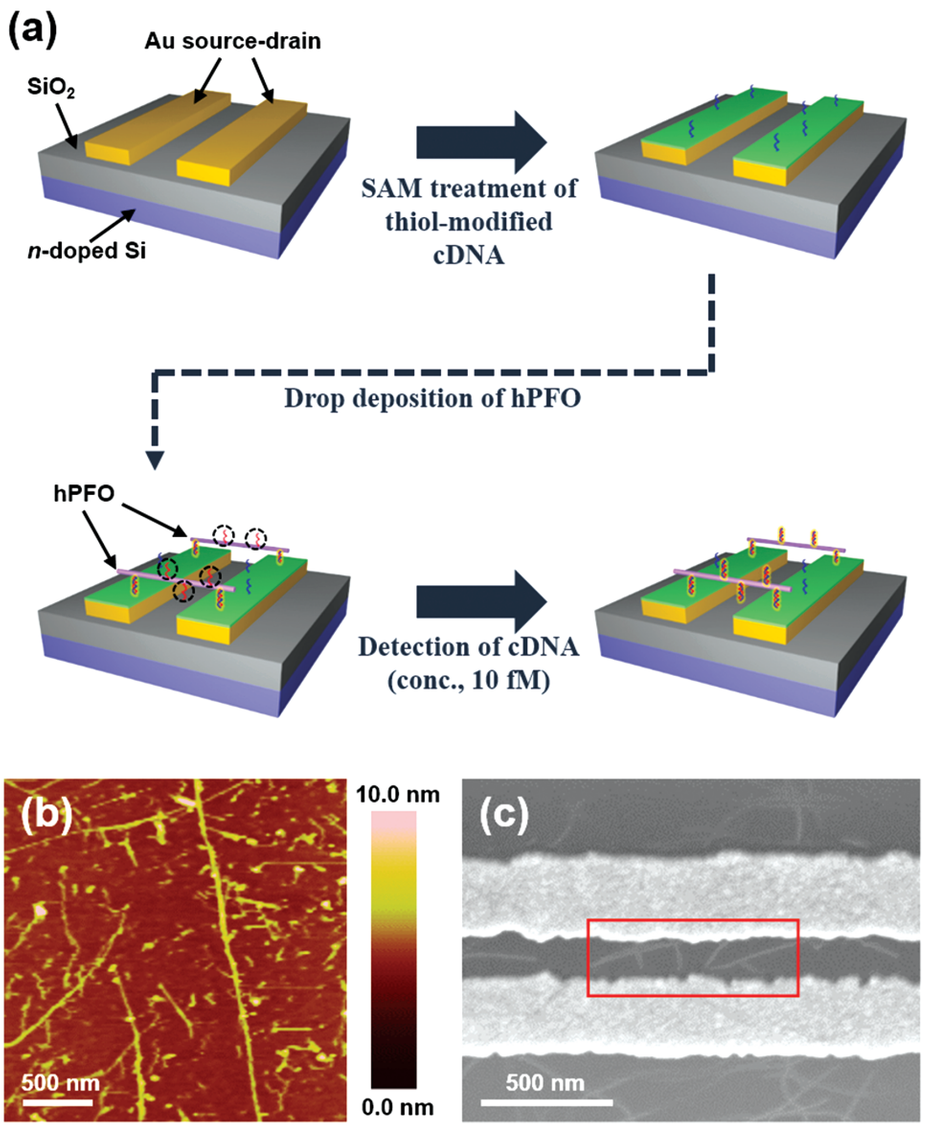

Figure 1. (a) Schematic illustration of preparation and use of a SWNT-FET for detecting DNA. (b) AFM topography image of hPFO on mica. (c) SEM image of the Au source and drain electrodes and the hPFO channel on the silicon wafer prepared as illustrated in (a). Red box indicates the locations of hPFO bridging between the source and drain electrodes.

a home-built probe station using a Keithley 4200 Semiconductor Analyzer Characterization System. The probe station was pressurized at $10^{-6}\sim10^{-7}$ mbar before measurements. To make electrical contact with the back gate electrode, the silicon oxide dielectric layer was scratched off and covered with silver paste.

3. RESULTS AND DISCUSSION

The SWNT-FET devices used in this work were prepared as illustrated schematically in Figure 1(a). The patterned Au source and drain electrodes were prepared on the highly doped *n*-type silicon wafer with a thermally grown silicon oxide dielectric layer via electron beam lithography. The prepared Au electrodes were subsequently treated with thiol-modified cDNA to form a SAM through Au–S bonding.[22–24] The dispersion (hPFO) containing particular semiconducting SWNT species, with (n, m) indices of (7, 5), (7, 6), (8, 6) and (8, 7),[18] was then drop-deposited and immobilized onto the Au electrodes (see Fig. 1(b) for AFM image of hPFO on mica) via DNA base-pairing between the complementary DNA of hPFO and the SAM/Au. Bridging the Au electrodes with hPFO resulted in the formation of FET channels as shown in Figure 1(c). The unoccupied DNA segments of the bridging hPFO after surface immobilization, about 10 SWNTs across the parallel source-drain Au electrodes were observed in the SEM images, could be additionally hybridized with cDNA in the analyte solution, consequently affecting FET operation.

The output characteristics of the SWNT-FET device before and after deposition of cDNA solution are shown in Figure 2. The pristine SWNT-FET device exhibits clear ambipolar behavior (see Figs. 2(a–b)), consistent with previous work hPFO-based FETs.[18–21, 25] Interestingly, exposure to the cDNA solution changes to the device characteristic to *p*-type characteristics. In particular, the hole current almost doubled from ~5.1 μA to ~11.7 μA at a fixed gate voltage (V_G) of −50 V and a source-drain voltage (V_{SD}) of −1.5 V. Likewise, the electron current in the *n*-type region was significantly suppressed from ~3.4 μA to ~1.2 μA at $V_G = +50$ V and $V_{SD} = +1.5$ V (see Fig. 2). A similar sensitivity to the presence of cDNA was observed in the device transfer characteristics, as shown in Figure 3. The ambipolar behavior of the pristine device (blue and green curves) is best reflected in the similar transfer curves for both *p*- and *n*-type regions. Following deposition of the cDNA solution, the transfer curve in the

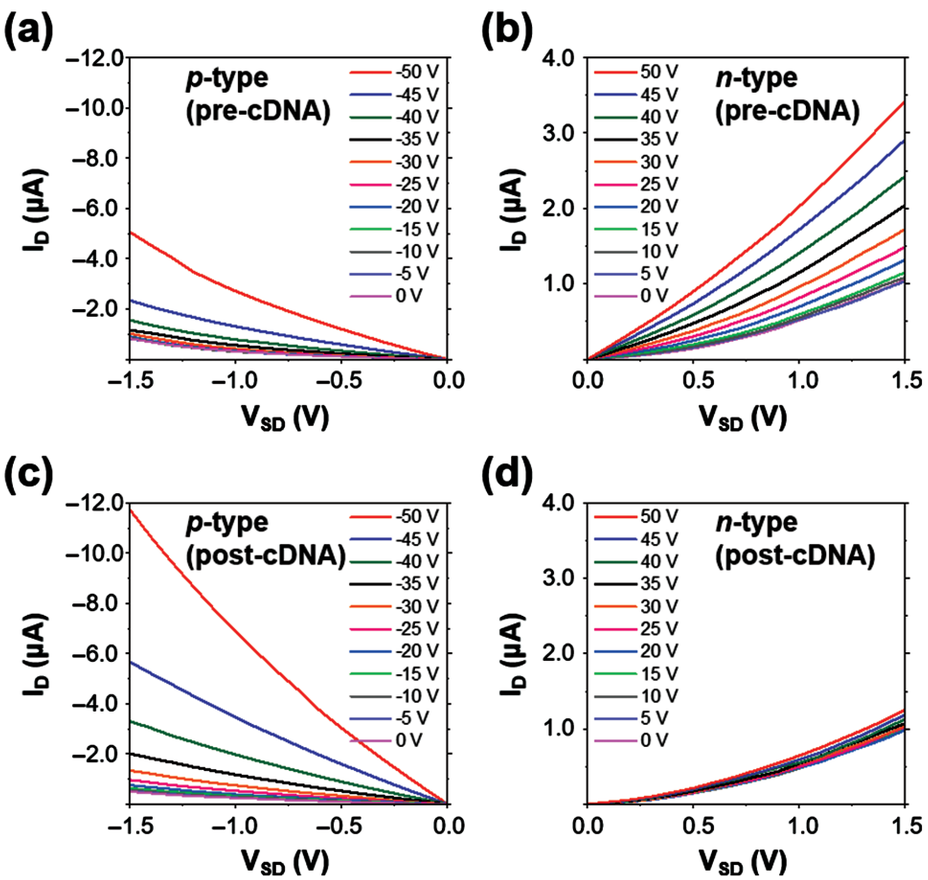

Figure 2. Output characteristics of a representative SWNT-FET device (a, b) pre- and (c, d) post-cDNA solution treatment in both (a, c) *p*-type and (b, d) *n*-type regions.

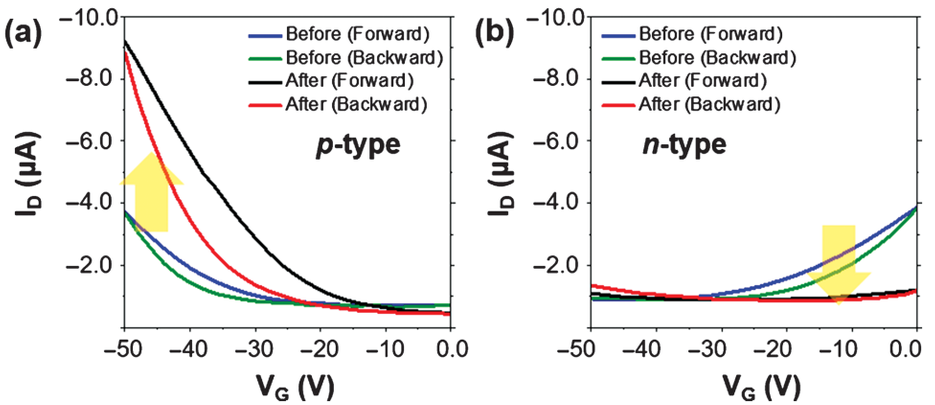

Figure 3. Transfer characteristics of SWNT-FET device from Figure 2, before (blue and green lines) and after (black and red lines) cDNA solution treatment, in both (a) *p*-type and (b) *n*-type regions. The arrows indicate the trends of current enhancement and vanishment in *p*-type and *n*-type regions, respectively, upon the treatment of cDNA.

n-type region could only be weakly discerned while current in the *p*-type region was significantly enhanced. These results imply that hybridization with the target cDNA solution exerts a similar effect to doping with *p*-type dopants. Such dopants typically increase FET hole currents, while the dopant-induced density of states (DOS) within the bandgap efficiently suppresses ambipolar behavior, leading to unipolar *p*-type operation.[26] DNA hybridization thus enables electrical detection through *p*-doping effects in hPFO-based SWNT-FETs, even at cDNA concentration as low as 10 fM. We note that the strong increase in hysteresis observed in the *p*-type region upon cDNA hybridization is also related to the induced DOS within the bandgap, which is anticipated to be partially localized and represent trap states.[27,28] It is worth noting that the enhancement of hole current according to the deposition of the cDNA solution is accompanied with a considerable reduction in threshold voltage in the *p*-type region, which can be another criterion for detecting the target molecules.[29]

To confirm the reproducibility and reliability of DNA detection, thirty four SWNT-FET devices were examined and statistically analyzed for each analyte solution (cDNA or ncDNA). Figure 4 shows the averaged, normalized change of hole current ratio at fixed $V_G = -50$ V and $V_{SD} = -1.5$ V following analyte deposition. Concentrations of both solutions were identical (10 fM). As indicated in Figure 4, the hole-current ratio corresponding to the cDNA specimen solution primarily ranged from 1.5 to 2.0. However, in case of the ncDNA solution the hole-current ratio was below 1.0 for every device measured, indicating that the presence of ncDNA actually leads to a decrease in the field-effect current. These results suggest that ncDNA undergoes some non-specific interaction with the FET such as physical adsorption, rather than DNA base-pairing. Since ncDNA at such a low concentration is not able to participate in DNA hybridization with hPFO, the ncDNA segments remaining in the FET device following the hybridization procedure can be considered as local defects rather than dopants. Such defects can induce a high density of deep trap states, causing a decrease in the field-effect current. Thus, we find that cDNA and ncDNA have opposite effects on SWNT-FET operation, the first as a dopant and the latter as a defect. This strong difference enables high reliability and sensitivity in the sequence-specific detection of cDNA.

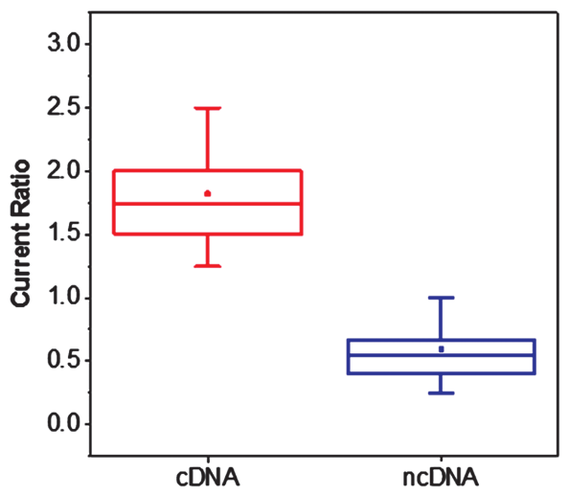

Figure 4. Statistical distribution of hole current ratios corresponding to treatment with cDNA and ncDNA analyte solutions.

4. CONCLUSION

We have successfully demonstrated a highly sensitive DNA detector using SWNT-FETs, which were fabricated via selective dispersion of particular semiconducting SWNTs with amphiphilic DBC and subsequent immobilization on cDNA-labelled Au electrodes. The unoccupied DNA blocks of the bridging hPFO after immobilization present an opportunity for hybridization

with additional cDNA, resulting in a distinctive change in FET performance. In particular, the resulting *p*-doping yields a significant increase in hole current and larger hysteresis, whereas the electron current in the *n*-type region is efficiently suppressed. In contrast, the ncDNA segments act as defect sites in the FET channel and accelerate degradation of the devices overall. Such opposite behaviors of cDNA and ncDNA on the operation and performance of SWNT-FETs consequently lead to high reliability and sensitivity in the detection of DNA, of which similar working mechanisms were also well-demonstrated with other carbon nanotube-based FETs.[30, 31] We note that a single drop (1 μL) of DNA analytes (10 fM) contains merely six thousands molecules (10 zeptomoles; 1×10^{-21} mol), yet this SWNT-FET-based system is able to rival the cutting-edge detection limits previously achieved using silicon nanowire FETs (10 pM) and fluorogenic reactions (10 pM and 10 fM). We thus anticipate that this solution-processible detection system will contribute to the development of advanced DNA sensors which can be applied to varied number of DNA sequences and ultrasensitive diagnostics without the need for DNA amplification.

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