Label-free nucleic acid biosensors using carbon nanotubes

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Single-wall carbon nanotubes (CNTs) belong to a unique class of one-dimensional nanostrcutures due to their all-carbon structure coupled with their very high aspect (at least 1000:1) and surface-to-volume ratios. Unlike bulk wires, charge variations on the surface of CNTs cause distinct changes in their electronic properties. These aspects make CNTs promising components for electrical biosensors. [1] Electrical biodetection methods are continuously being sought for, in order to miniaturize device footprint to obtain sensors in a compact and portable format. Furthermore, bulky optical reading instruments that are required with current technologies can be avoided. Here we demonstrate a highly sensitive biosensor for the detection of nucleic acids. It is based on the use of CNT field-effect devices and is capable of detecting as low as 2000 copies of deoxyribonucleic acid (DNA) molecules in a 30 microlitre sample droplet.

Label-free methods for the detection of DNA have been demonstrated in many configurations, the majority of which are based on field-effect or electrochemical detection. Although the use of a label is avoided in these sensors, the limit of detection is of the same order of magnitude as with optical detection techniques. This implies that a pre-amplification step using polymerase chain reaction (PCR) is necessary. Our CNT-DNA-biosensors show a detection limit in the attomolar range, which is two orders of magnitude lower than that demonstrated with silicon nanowires and five orders of magnitude better than previously reported carbon nanotube sensors.

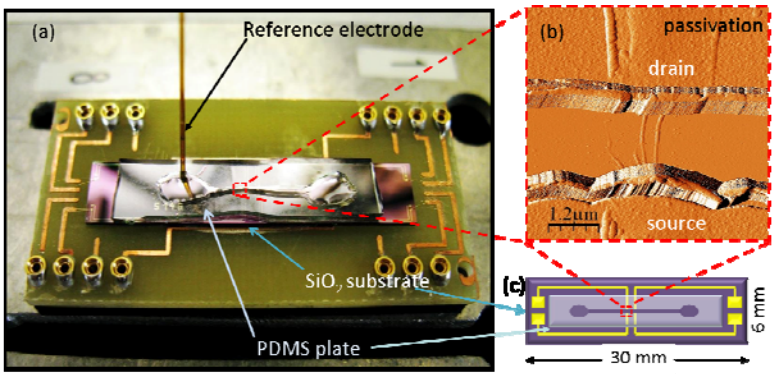

Figure 1: DNA biosensor based on CNTs. (a) Photograph showing the chip carrier with the sensor chip covered with a polydimethylsiloxane (PDMS) microchannel containing a test solution. (b) AFM image of the sensor area showing CNTs trapped between two electrodes. (c) Schematic illustration of the sensor chip showing lead electrodes, the electrode gap and the microchannel.

Figure 1 shows an overview of the assembled CNT field-effect device for the sensor trials. Electrode gaps made of platinum were prepared on 4” wafers using photolithography. Individual chips were cut out of this wafer. CNTs were trapped in the electrode gaps from an aqueous dispersion using AC dielectrophoresis. Subsequently the electrode regions were passivated with silicon oxide in order to ensure that only the nanotubes are in contact with the solution. The liquid is delivered to the sensor chip through a microchannel that is fixed on the chip. An Ag/AgCl reference electrode acts as a gate in order to measure the electrical characteristics in a liquid-gated or electrochemically gated configuration.

In order to specifically detect target DNA sequences through hybridization, a complementary probe sequence needs to be immobilized on the nanotube surface. We have performed this using an electrochemical approach in two steps. Firstly, the nanotubes were coated with a layer of (poly) aminobenzoicacid through electropolymerization. This results in a surface filled with carboxyl groups. The carboxyl groups are utilized to attach the probe DNA in a second step through carbodiimide chemistry. [2] Furthermore, the carboxyl groups serve as a protective coating to minimize direct non-specific binding of DNA on to the highly hydrophobic nanotube surface. A key advantage of this functionalization approach is that the probe DNA is exclusively attached in a site-specific manner on to the nanotube surface, which is key to attain high sensitivity.

The sensing trials were performed at varying concentrations of 24-base pair target DNA in 10 mM potassium phosphate buffer containing 0.1 M NaCl. The complex impedance (with magnitude and phase components) of the contacted nanotube constitutes the sensor response, which is measured in a frequency range of 200 Hz to 2 MHz. The frequency response is measured at varying liquid gate voltages to characterize the field-effect behavior. [3] The use of high-frequency detection ensures very low noise. This coupled with the stable Ag/AgCl reference electrode provides for a good stability and the same sensor can be used for repetitive sensor trials.

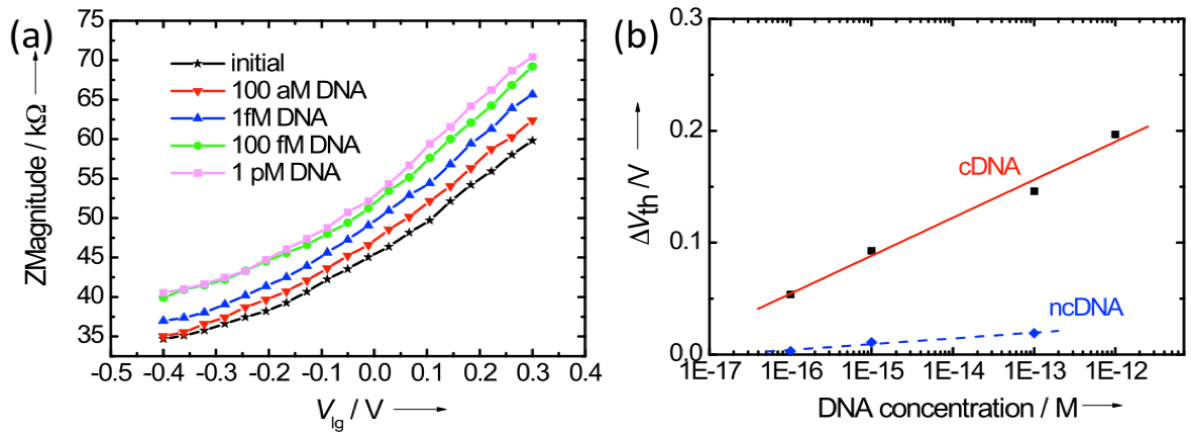

Figure 2: Sensivity of CNT-DNA-sensors. (a) Gate dependence of resistance (magnitude of impedance) at a frequency of 1 kHz in buffer solution (initial) and for varying concentrations of complementary target DNA (cDNA) in buffer. The threshold voltage shifts to more negative voltages as the cDNA concentration increases. (b) Calibration plot showing the shift in threshold voltage as a function of DNA concentration for both complementary and non-complementary (ncDNA) sequences. It is apparent that the response for cDNA is linear over a broad concentration range and is distinct from that of ncDNA. A detection limit of 100 aM can be extracted from this calibration curve.

Figure 2(a) presents the sensor response (at one frequency) of the CNT-DNA-sensors to varying concentrations of DNA. The CNT bundles incorporated in the contacted bundles exhibit a quasi-metallic behaviour, whereby the resistance (ZMagnitude) can be modulated as a function of the liquid gate voltage. Upon binding of the complementary DNA sequence on to the probe DNA on the CNT surface, the transfer characteristics are shifted towards negative voltages. This can be explained by considering that the binding of the complementary target leads to an increase in surface charge density. This results in a shift in the gate voltage characteristics analogous to the working principle of an ion-sensitive field-effect transistor (ISFET).

It is also apparent from fig.2 that the shift in the gate characteristics increases with increasing concentration of complementary DNA (cDNA). We have also tested the sensor response with non-complementary DNA (ncDNA) sequences. These two responses are compared in the calibration curve in fig. 2(b). The sensor response for the ncDNA is negligible (much less than 20 mV, which is the maximum drift in our sensors) in comparison to the cDNA, signifying a high degree of selectivity to the target sequence of interest. Furthermore, a concentration as low as 100 aM could be unambiguously detected using this sensor. This corresponds to less than 2000 DNA molecules in our 30 microlitre sample droplet. This is the lowest detection limit reported until now for the direct label-free detection of DNA.

The capability to detect such trace amounts of DNA motivates the evaluation of this strategy in realistic biological samples. As a first step in this direction, we have investigated the sensor response in a heterogeneous mixture of three different ncDNA sequences (each at 3 fM) and one target cDNA sequence (at 200 aM). The threshold shift of the sensor response with and without the target sequence is shown in fig.3. It is apparent that cDNA at a concentration of 200 aM and comprising just 2% of the heterogeneous sample was able to generate a significant threshold shift of around 65 mV. These measurements signify the unique combination of high selectivity and ultralow detection limit of our sensors and soars hope for the direct detection of low quantitites of DNA without the use of PCR in realistic samples.

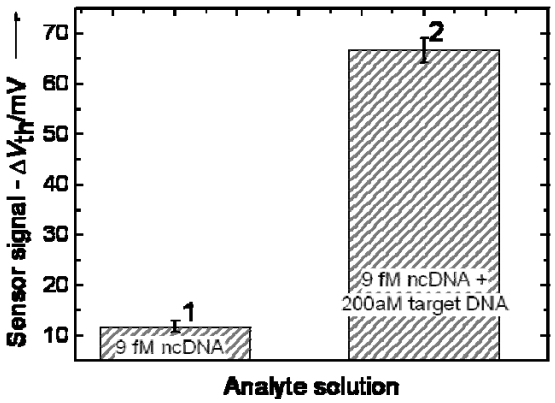

Figure 3: Attomolar target DNA differentiation in a heterogeneous DNA mixture: Sensor signal (threshold shift) for a heterogeneous mixture of three different DNA sequences (each at 3 fM, total concentration: 9fM) without (1) and with (2) addition of 200 fM complementary DNA. The cDNA amounts to just 2% of the total DNA in the mixture. The CNT-DNA-sensor is capable of differentiating this small amount from the non-complementary background present at a much higher concentration of 9fM. This signifies a very high selectivity coupled to an ultralow detection limit.

References:

[1] *Balasubramanian, K., and M. Burghard.* J. Mater. Chem. **18**, 3071-3083 (2008).

[2] *Kurkina, T., A. Vlandas, A. Ahmad, K. Kern, K. Balasubramanian.* Angew. Chem. Intl. Ed. **50**, 3710-3714 (2011).

[3] *Vlandas, A., T. Kurkina, A. Ahmad, K. Kern, K. Balasubramanian.* Anal. Chem. **82**, 6090-6097 (2010).

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