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Supporting information

Electrostatic gating in carbon nanotube aptasensors

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1. Liquid gated electrical properties of CNT FETs fabricated on a polyimide substrate

The electrical properties of four CNT FET devices on a single polyimide substrate in a liquid PBS buffer are shown in Fig. S1. A fixed bias V_{ds} = 100 mV is applied between the source and the drain and the liquid gate voltage V_{lg} between the source and the Ag/AgCl gate electrode is swept in each direction to produce the transfer curves shown. All four devices have the same device structures with channel lengths of 40 µm and widths of 100 µm. The exposed CNT film areas are 10 µm by 100 µm. All four devices behave similarly, with weak hysteresis as shown in Fig. S1: the CNT film shows p-type semiconductor properties with V_{th} varying between 0.4 – 0.47 V. The on/off current ratios are ~ 5 × 10³ and the hole mobilities are ~ 4 cm²/V·s



Fig. S1. The electrical properties of four devices on a single polyimide substrate using 99% IsoNanotube-S CNTs measured in aqueous buffer using liquid gating (V_{ds} = 100 mV).

One of the problems with CNTs in device applications can be the tube to tube variation in electronic response.¹ However, by using network films form high quality initial CNTs, the average properties of the film are consistent across the device substrate. The performance and reproducibility of the CNT FETs on flexible substrates show that they are suitable for aptasensor device applications. In these liquid gated CNT FETs it is the electric double layer (EDL), which forms upon application of the gate bias that then acts as the gate dielectric. Typically the EDL is only a few nanometers thick. FETs operated in back gated configuration typically use a few hundred nanometer thickness of SiO₂ as the dielectric. The capacitance of the EDL is typically 10 nF/m^{2,3} across the lateral direction which is higher than 1.4 nF/ m in SiO₂ (100 nm thickness), a higher field dependency is achieved in liquid gating. Without requirements of deposited dielectrics on substrates, using the EDL as gate dielectrics has shown high electrical performances, Fig. S1.

2. Non-specific response in resistive sensor mode

To investigate the specificity of the signals from aptamer-K⁺ binding, we have measured the K⁺ response of pristine CNT FETs (no aptamers) and CNT FET functionalized with 3-point mismatched aptamers in resistive measurement mode, in Fig. S2. The 120 μ L volume of 2 mM concentration Tris-HCl buffer was initially added before starting the measurement. Further conductance measurements are taken 10 mins after the addition of 10 μ L K⁺ solution. Fig. S2 shows no response to K⁺ ions over the concentration range 1 pM–1.1 μ M. Fig. S2 (b) shows that the device functionalized mismatched aptamer exhibits only a weak response to K⁺.



Fig. S2. The response to K⁺ ions and buffer for the pristine CNT FET (a) and the 3-point mismatched aptamer functionalized CNT FET (b), both using *I-V* resistance measurements in 2 mM tris-HCl buffer.

3. Reproducible measurements in real time sensing mode

3.1 Experimental method for two-step functionalisation

To immobilise aptamers on the CNT surface, we used a two-step functionalisation method that ensures any hydrolysed PBASE molecules are reconverted to the reactive ester immediately before the coupling step, similar to our previous publication.⁴ First of all, the CNT FETs are functionalised by 1-pyrenebutanoic acid, succinimidyl ester (PBASE) by submerging the entire devices in a 1 mM PBASE methanol solution for an hour.^{5,6} Then the excess PBASE is washed off three times in clean methanol, followed by a 5 second dip in DI water before drying with N₂. The potassium aptamers are initially denatured at 70 °C in an oven for 5 minutes before gradually cooling down to room temperature. The aptamers are diluted to 1 µM concentration using 20 mM tris-HCl buffer mixed together with 1mg/ml N- (3-dimethylaminopropyl)N-ethylcarbodiimidehydrochloride/ N-hydroxysuccinimide (EDC/NHS) coupling solution. The CNT FETs are exposed to aptamer/NHS/EDC solution for an hour. After functionalisation, the excess aptamers are washed off by rinsing in 20 mM tris-HCl buffer three times followed by rinsing in DI water before drying in clean N₂.

3.2 Reproducibility of aptasensors from real time measurement

To demonstrate the reproducibility of aptasensors, we measured the real time response to potassium ions over the same concentration range (100 pM to 1.1 μ M) from three independent devices. The normalised current response ($\Delta I/I_0$) is taken from real time measurements, and plot against the ion concentration as shown in Fig. S3. These three aptasensors behave similarly and all show linear response to the logarithmic ion concentration.



Fig. S3. The dependence of K⁺ concentration on the normalised current response $\Delta I/I_0$ based on real time measurement from potassium aptamer functionalised CNT FETs, measured in 2 mM tris-HCl buffer. Error bars in Fig. 7b of the main text are are from these triplicate measurements.

4. Non-specific response from non-tethered aptamers

The functionalisation of tethered aptamers on CNT surfaces is essential for making reliable aptasensors. Functionalisation is carried out by linking amine groups of the aptamers onto the N-hydroxysuccinimide groups on PBASE via nucleophilic substitution,⁵ as shown in Fig. 2 (b) (main text). If the tethering is not successful, aptamers may instead non-specifically adsorb to CNT surfaces via π -stacking interactions.^{4,7} The mode of attachment can be also distinguished by the transfer characteristics, as shown in Fig. S4 (a). Non-specifically adsorbed aptamers result in an increased on/off current ratio, without an obvious shift in the threshold voltage. However, when the aptamers are tethered via the linker (Fig. 3 of main text), a positively shifted threshold voltage and increased current were observed. Importantly, when the aptamers are not specifically tethered, the sensor shows no clear response to potassium ions, as demonstrated in both real time measurement (Fig. S4 (b)) and resistive measurement (Fig. S4 (c)).



Fig. S4. The potassium ion response (electrolyte gated in 2 mM tris-HCl) when the aptamer tethering is not successful. In this case, the PBASE had hydrolysed and the two-step functionalization procedure (above) was not used to regenerate the reactive ester form: (a) Transfer characteristics of a pristine CNT FET and CNT FET functionalised by potassium aptamers without successful coupling, resulting in non-specific adsorption: $V_{ds} = 200$ mV. (b) Corresponding real time sensing measurement for a CNT FET with non-specifically adsorbed aptamers. (c) Diode sensing measurement for a CNT FET with non-specifically adsorbed aptamers.

5. Bending flexible substrates

Our aptasensors are fabricated using flexible polyimide substrates. We also tested the conduction of a CNT FET aptasensor when it is flat, compared with the conduction after bending around a 1.5 mm radius tube by 57° for 1 minute, as shown in Fig. S5. The resistance of the aptasensor is only increased by 1% after bending.



Fig. S5. The electronic response of a CNT FET aptasensor upon bending: I-V characteristics of a flat CNT FET functionalised with potassium aptamers (squares), and the same device after being bent at 57° around a 1.5 mm radius tube for 1 minute (circles).

6. Regeneration of potassium aptasensors

To test the regeneration of the potassium aptamers, the aptasensor after response to potassium (until current reaches saturation) is submerged in DI water overnight, and then washed three times in clean DI water before drying with N₂. This device is then retested for K⁺ response in the real time mode, as shown in Fig. S6, compared with the original K⁺ response before the regeneration, and the K⁺ response from a bare CNT FET control sample. Compared with the original sensor response, the regenerated aptamer device shows no clear response to K⁺, instead showing a similar lack of response to K⁺ as the pristine CNT device. This experiment shows that further investigation is required to identify a suitable regeneration method.



Fig. S6. The K⁺ response from the potassium aptamers, potassium aptamers after regeneration, and a pristine CNT FET: $V_{lg} = 0$ and $V_{ds} = 100$ mV in 2 mM tris-HCl.

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