Supplementary Information

Reconfigurable Carbon Nanotube Multiplexed Sensing Devices

Xinzhao Xu,[†] Pierrick Clement,[†] Johnas Eklöf- Österberg,[§] Nancy Kelley-Loughnane,[||] Kasper Moth-Poulsen,[§] Jorge L. Chávez,[||] Matteo Palma [†]

† School of Biological and Chemical Sciences, Institute of Bioengineering, and Materials Research Institute, Queen Mary University of London, Mile End Road, London, E1 4NS, UK

§ Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Gothenburg, 412 96, Sweden

|| Air Force Research Laboratory, 711th Human Performance Wing, Wright-Patterson Air Force Base, Dayton, OH 45433, USA

1. Materials and Methods

2-[Methoxy(polyethyleneoxy)6-9propyl]trimethoxysilane (PEG-silane) was purchased from Fluorochem. Ltd. Steroid free serum was purchased from MP Biomedicals, Inc. Dulbecco's phosphate buffered saline (DPBS) was purchased from Thermo Scientific. (7,6) enriched SWCNTs were purchased from Sigma Aldrich. All other analytical grade chemicals were purchased from Sigma Aldrich.

All DNAs were obtained from IDT. Cortisol binding aptamer, Neuropeptide Y (NPY) binding aptamer and dehydroepiandrosterone sulfate (DHEAS) binding aptamer were already functionalized with an azide group on the 5' terminal position (via N-hydroxysuccinimide ester reaction). The final sequences of the three aptamers are:

Cortisol aptamer: 5'-azide-GGA ATG GAT CCA CAT CCA TGG ATG GGC AAT GCG GGG TGG AGA ATG GTT GCC GCA CTT CGG CTT CAC TGC AGA CTT GAC GAA GCT T-3'

NPY aptamer: 5'-azide-AGC AGC ACA GAG GTC AGA TGC AAA CCA CAG CCT GAG TGG TTA GCG TAT GTC ATT TAC GGA CCT ATG CGT GCT ACC GTG AA-3'

DHEAS aptamer: 5'-azide-CTG CTC TCG GGA CGT GGA TTT TCC GCA TAC GAA GTT GTC CCG AG-3'

Single stranded DNA used to wrap (7,6) enriched SWCNTs was modified with an amine group on the 3' terminal position. The final sequence is 5'-GTT GTT GTT G-amine-3' ($(GTT)_3$G-amine). This sequence was employed for the wrapping of the nanotubes as it was shown to be able to wrap different chiralities of SWCNTs[1] and worked efficiently for the dispersion of the enriched (7,6) chirality SWCNTs employed in this study.

The sequence of the DNA complementary to cortisol aptamer is 5'-AAG CTT CGT CAA GTC TGC AGT GAA GCC GAA GTG CGG CAA CCA TTC TCC ACC CCG CAT TGC CCA TCC ATG GAT GTG GAT CCA TTC C-3'.

For the non-complementary control experiment, the sequence of the non-complementary DNA shown in Figure S6 is 5'-GAT TCA GCA ATT AAG CTC TAA GCG ATC CGC AAC ACT GAC CTC TTA TCA AAA GGA GCA ATT AAA GGT ACT CTC TAA TCC TGA CGG G -3'.

For analyte detection, the Tris-HCl buffer was composed of 50 mM Tris-HCl, 100 mM NaCl, 5 mM $MgCl_2$ and was adjusted to pH = 7.4 at 25 ℃. 0.1 M phosphate buffer was prepared with 0.1 M Na_2HPO_4 solution and adjusted to pH = 9 with 0.1 M NaH_2PO_4 solution at 25 ℃.

2. Substrate Fabrication

The electron beam (e-beam) lift-off resist (MCC NANO Copolymer EL6) was bought from Microlithography Chemicals Corp. The top layer ebeam resist (ARP 6200.13:Anisol 1:2), was bought from Allresist GmBH. The photo lift-off resist (LOR3A) and the protective resist (MCC NANO Copolymer EL10) was bought from Microlithography Chemicals Corp and the photoresist (S1813) was bought from Microresist GmBH.

The nanosized features were fabricated using electron beam lithography (EBL). A 4 inch p-doped (10-20 mΩ cm) silicon wafer (100), with a 400 nm thick grown silicon dioxide was used as a substrate. This was spin-coated with a 100 nm thick lift-off resist at 6000 rpm for 60 s and baked at 160 °C. Subsequently, a second ebeam resist was spin-coated at 600 rpm for 60 s and baked at 160 °C for 5 min.An e-beam writer (EBL-JEOL JBX 9300 FS) system set to use an accelerating voltage of 100 kV, a current of 2 nA and an electron dose of 160 μC/cm^2 was used to expose the substrate according to a predesigned pattern. The pattern was developed for 20 s in *o*-xylene and rinsed in 2-propanol. This was followed by a second development in a mixture of 7 parts deionized water and 93 parts 2-propanol, rinsing in 2-propanol and dried in N_2. Residues were removed in O_2 plasma for 10 s (50 w, 250 mTorr, 15 sccm) using a Dry etch RIE-Plasma-Therm-Oxygen. The entire sample was coated with 5 nm of chromium using an electron beam evaporator (Lesker PVD 225). The sample was then covered with 40 nm of gold. A lift off in acetone was performed overnight, followed by rinsing in first acetone, then 2-propanol, finally deionized water and dried

in N_2. 60 s O_2-plasma was used to clean the nano-features. The larger features on the wafer was fabricated using a laser writer (Heidelberg Instruments DWL 2000) equipped with a 405 nm diode laser. First a photo lift-off resist was spin-coated at 3000 rpm for 45 s and baked at 190 °C for 5 min. A photo resist was then spin-caoted at 3000 rpm and baked at 100 °C for 2 min. The sample was then exposed according to a predesigned pattern. The wafer was then developed in MF319 for 45 s, followed by rinsing in a bath of deionized water and drying in N_2. Finally, 60 s of O_2-plasma was used to clean the sample. A protective resist was spin-coated at 1000 rpm for 60 s and baked at 160 °C for 5 min prior to dizing the sample in 10 × 10 mm chips using a dicing saw (Loadpoint Microace 3+). These chips in total were equipped with gold electrodes. Each chip contained 2 sets containing 10 electrode pairs each with a gap of 300 nm and 2 sets containing 10 electrode pairs with a gap of 400 nm. The electrode pairs in each set was separated with 50 μm. A distance of 800 μm between a set of gaps to any contact pad assured that any short circuit during deposition was evaded. After the fabrication of electrodes, a PEG-silane monolayer was formed on the surface of the as prepared substrate. The substrate was incubated overnight into a solution of 10 μL PEG-silane containing 20 mL of anhydrous toluene and 50 μL acetic acid (99%).

3. Preparation of CNT-aptamer Hybrids

1.0 mg (1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl N-succinimidyl carbonate (BCN-NHS) was dissolved in 42 μL dimethyl sulfoxide (DMSO), then diluted with 225 μL phosphate buffer (0.1 M, pH 9). 12 μL of 25 mg/mL of $(GTT)_3$G-amine was added to the solution, and additional water (*MilliQ*) was added to get the total volume of 300 μL. After overnight incubation at room temperature, ethanol precipitation was used to remove free BCN-NHS. 30 μL NaCl (3 M) and 825 μL ethanol (95%) were added to the mixture, kept in the freezer for 2 h and then centrifuged at 13

Krpm (*Eppendorf 5415C*) for 30 mins. The precipitation was washed with 70% cold ethanol (-20 ℃). After being dried, the modified DNA ($(GTT)_3$G-BCN) was re-dissolved in 0.1 M NaCl. Subsequently, 3 KDa Amicon filter (*Millipore*) was used to further remove free BCN-NHS by centrifugation at 13K rpm for 10 mins three times. The final solution was diluted with 0.1 M NaCl and the volume was kept to 300 µL.

To demonstrate the successful modification of $(GTT)_3$G-amine with BCN group, we analysed $(GTT)_3$G-BCN and $(GTT)_3$G-amine samples with HPLC (High Performance Liquid Chromatography, Agilent LC 1100, XBridge Column Reversed-Phase 2.5 µm, 4.6 mm *x* 50 mm). As shown in Figure S1, HPLC analysis of the BCN-ssDNA compared to the amine-ssDNA shows a shift in the retention time of the more hydrophobic BCN-ssDNA, suggesting the successful BCN functionalization of the DNA following the BCN-NHS chemistry on the amine-terminated DNA.

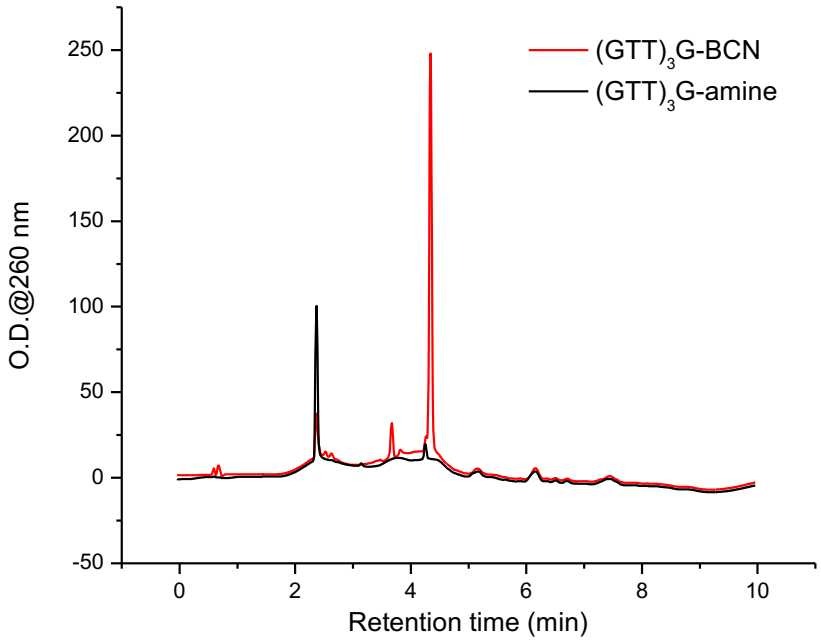

Figure S 1: HPLC analysis of the chemical modification of $(GTT)_3$G-amine to $(GTT)_3$G-BCN

0.2 mg (7,6) enriched SWCNTs were dispersed into the as prepared $(GTT)_3$G-BCN solution (300 μL) by sonication (*Sonics, VC130*) for 30 mins. The mixture was centrifuged at 13K rpm for 30 mins to remove unwrapped CNTs.

For the functionalization of CNT with aptamers, typically, 6 μL of as prepared CNT solution was mixed with 2 μL aptamer solution (100 μM) and 12 μL DPBS was added to the mixture. Then, the mixture was incubated at 37 ℃ overnight. After the reaction, the mixture was dialysed against water using Slide-A-Lyzer™ MINI Dialysis Devices with a 20 kDa cut-off (purchased from Thermo Scientific) overnight to remove free aptamers.

4. AFM images and their corresponding height profiles

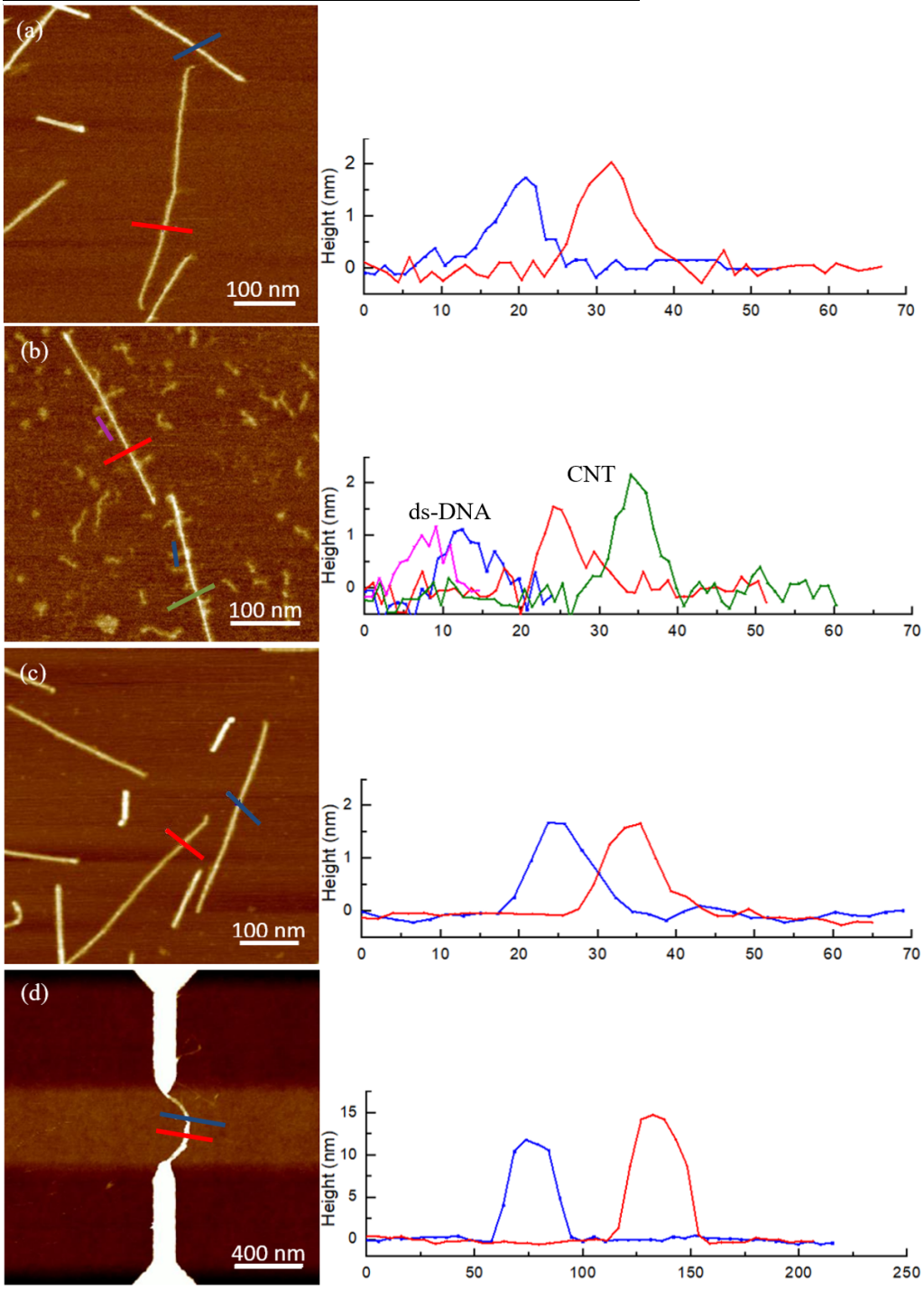

Figure S 2: AFM images and their corresponding height profiles of (a) CNT-aptamer hybrids, and (b) CNT-aptamer hybridized with complementary DNA. Free dsDNA not bound to the SWCNTs can also be found in the vicinity of the nanotubes; this can be differentiated from the dsDNA tethered to the SWCNTs because of both its distance from the nanotube and its conformation: the

dsDNA linked to the tubes clearly protrudes at a 90º angle with respect to the nanotube (additionally, it is reasonable to expect only minimal non-specific adsorption of dsDNA on the SWCNTs, due to electrostatic repulsion with the DNA wrapping the nanotubes). AFM images and their corresponding height profiles of control experiment (c) with non-complementary DNA, and (d) of CNT-aptamer hybrids immobilized between electrodes: a height up to 15 nm, indicating the presence of up to 7 CNTs in the device.

5. Dielectrophoresis

Dielectrophoresis (DEP) is performed applying a voltage between a pair of electrodes after having cast the aptamer-functionalized CNT solution. By tuning the voltage applied, the time of application, and the concertation of the solution we can control the immobilization between one to few tens of CNTs bridging the patterned electrodes. We have optimized these parameters by checking via AFM the amount of CNTs immobilized; once the DEP parameters were optimized, we could assemble aptamer-functionalized CNTs on the electrode pairs with a bridging yield up to 95%. The frequency of the generator was switched onto typically $V_{p\text{-}p}$ = 3V at f = 400 KHz; a drop of CNT-aptamer hybrids solution (5 μL, ~100 ng/mL) was cast to the chip with a pipette. After a delay of typically 30 seconds, the substrate was washed with water and blown gently with Nitrogen gas. The devises are usable with more than one CNT bridging the electrode. The immobilization of 1 CNT with this method is also possible, but at lower yield (ca. 20%).

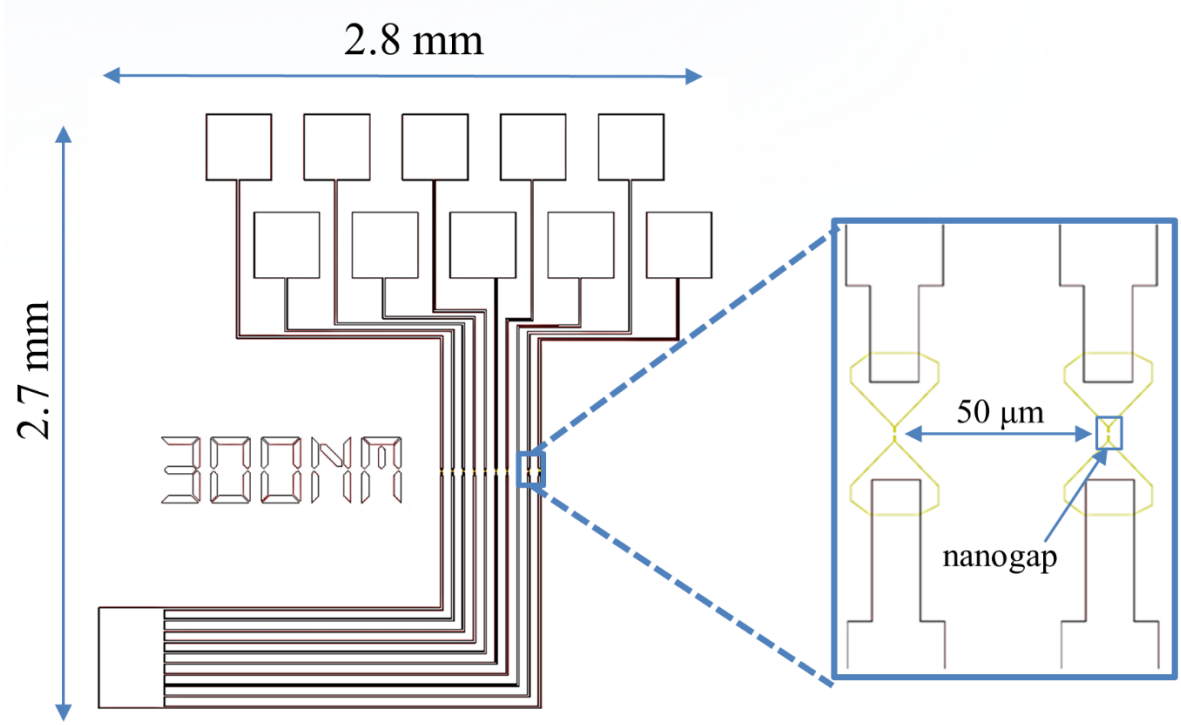

Figure S3: Scheme of the electrodes pattern used to immobilize the aptamer-functionalized CNT

6. Characterization

6.1 AFM measurement

Topography analysis of the electrodes were imaged with a Bruker Dimension Icon atomic force microscope (AFM) with ScanAsyst Air tips. The sample solution of CNT-aptamer hybrids was deposited onto a piece of freshly cleaved mica, which was pre-treated with 1 M $MgSO_4$ solution to enhance DNA adsorption, rinsed with water and dried before AFM measurement. These samples were imaged with a Bruker Dimension Icon atomic force microscope with ScanAsyst Air tips.

6.2 Electrical measurement

Electrical measurements were performed using a probe station (*PS-100, Lakeshore*) equipped with a semiconducting parameter analyser (*Keithley, 4200SCS*) at room temperature. Gate bias

sweeping mode (-6V to 6V) was used to record the source-drain current (I_{sd}) versus gate bias (V_g) data. For real time measurements, 100 mV source-drain bias and -2 V gate bias were applied across the devices whereas different solutions were cast on the substrate.

6.3 DNA hybridization and denaturation

40 µL complementary DNA (diluted in DPBS, 1 µM) was cast on the devices. Then the devices were incubated in water bath at 50 ℃ for 2 h and cooled down to room temperature. The devices were withdrawn from the solution, washed with water and dried with Nitrogen gas for electrical characterization.

After hybridization, the devices were immersed in a 50% formamide/water solution at 30 ℃ for 4 h to denature the double stranded DNA. Then the devices were removed from the solution, washed with water and dried with Nitrogen gas for further electrical characterization.

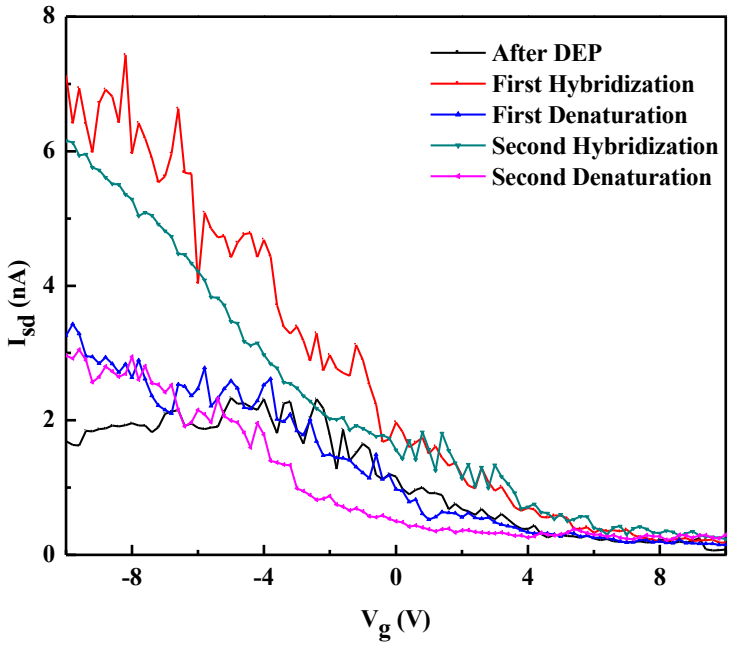

Figure S 4: Electrical response of the SWCNT-aptamer field effect transistor, before (black) and after DNA hybridization (red) and DNA denaturation (blue), and an additional cycle of DNA hybridization (green) and DNA denaturation (purple); V_{sd} = 100 mV

6.4 Multiplexed detection in buffer

Cortisol and DHEAS were dissolved in methanol, and then diluted in the Tris-HCl buffer to prepare cortisol and DHEAS solutions with different concentrations from 10 nM to 1 μM. NPY was dissolved in water and diluted with the Tris-HCl buffer to prepare NPY solutions with different concentrations from 100 pM to 1 μM.

For multiplexed detection experiments, three different solutions were used, namely, 100 nM cortisol solution in the Tris-HCl buffer, 100 nM cortisol solution and 50 nM NPY in the Tris-HCl buffer, and 100 nM cortisol solution, 50 nM NPY and 100 nM DHEAS in the Tris-HCl buffer. For each detection, the devices were immersed in the solution, incubated for 0.5 h, and finally washed with water and dried with Nitrogen gas for electrical characterization. Subsequently, after each detection and in order to regenerate the biosensors, the substrates were immersed in a Tris-HCl buffer solution with 8 M of Urea. After 1h, the devices were washed with water and dried with Nitrogen gas for characterization.

6.5 Analytes detection in serum

Steroid-free serum was diluted with the Tris-HCl buffer by 10-fold. Different analytes concentrations were diluted with serum (from 100 pM to 2 μM). A drop of 2 μL diluted serum was cast onto the substrate and then 100 mV source-drain bias and -2 V gate bias were applied for the baseline. For each analyte detection in serum, 50 seconds were recorded (as baseline) and then 2 μL of the analyte dissolved in serum was added to the previous drop.

7. Control experiment with non-functionalized device

We prepared a device in which CNTs were not been functionalized with aptamers, i.e. $(GTT)_3G$-amine wrapped CNTs were directly immobilised between electrodes via DEP. The current showed no significant change after exposure of this device to complementary DNA (Figure S 5).

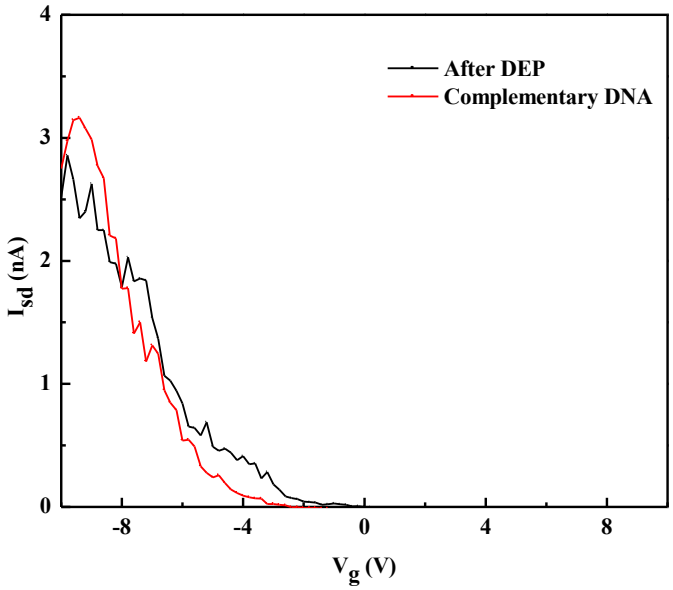

Figure S 5: I_{sd} vs V_g characterization of the non-functionalized device before and after exposure to the ss-DNA, V_{sd} = 100 mV

8. Control experiments with non-complementary and complementary DNAs

To confirm that the change in current comes from DNA hybridization with the complementary DNA, we exposed devices functionalized with cortisol aptamers to non-complementary DNA (5'-GAT TCA GCA ATT AAG CTC TAA GCG ATC CGC AAC ACT GAC CTC TTA TCA AAA GGA GCA ATT AAA GGT ACT CTC TAA TCC TGA CGG G -3'): no obvious change in current was observed. However, subsequent exposure to the complementary DNA induced an increase in current. After denaturation, the current decreased to the initial stage (Figure S 6).

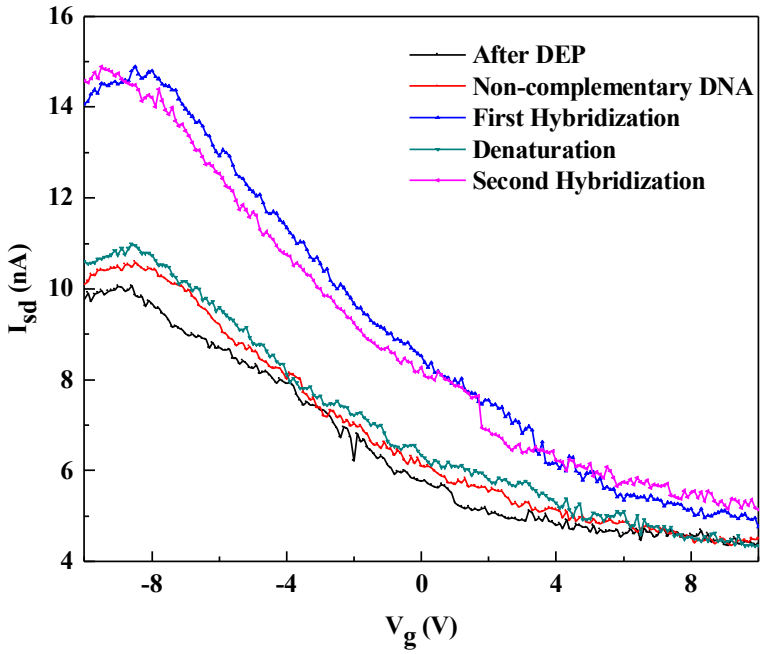

Figure S 6: I_{sd} vs V_g characterization of the device functionalized with cortisol aptamer before (black) and after exposure to non-complementary DNA (red), after exposure to complementary DNA (blue), after DNA denaturation (green) and a second hybridization with the complementary DNA (purple); V_{sd} = 100 mV

9. Control experiment of multi-sensing capability of the devices with cortisol and NPY

Here we demonstrate the multi-sensing capability of the device with cortisol and NPY sensor on the same chip. Each distinct nanoscale device on the chip could be reversed to its initial state by removing the metabolite bound to the aptamer via the addition of a urea solution (see Figure S7).

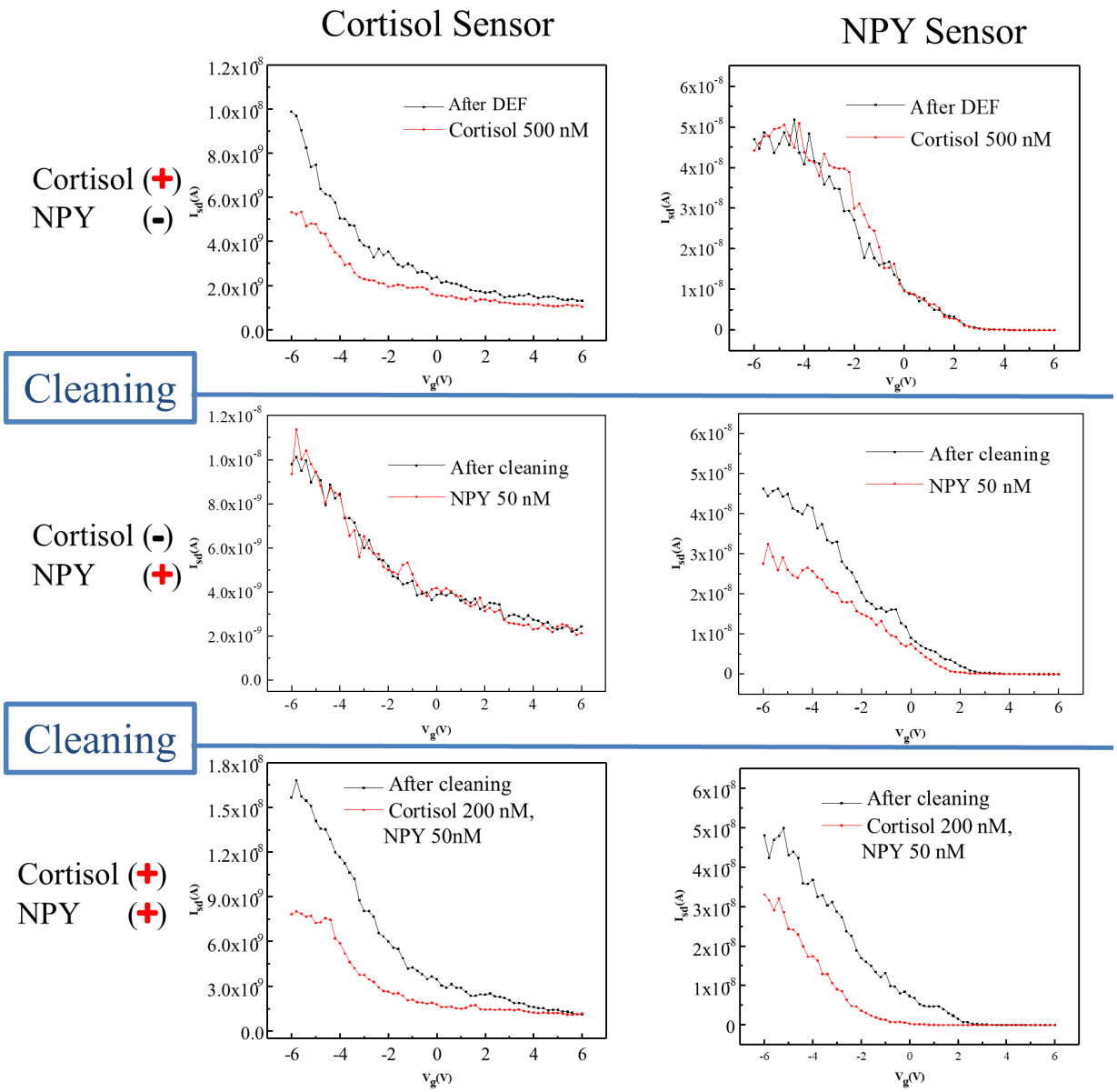

Figure S 7: Electrical responses of Cortisol and NPY biosensors on the same chip (V_{sd} =100 mV): the + sign indicates the addition/presence of the analyte of interest and the cleaning step indicates the addition of 8M of urea

10. Calibration curves of the real-time detection of three analytes

Based on the data of the real-time detection, we calculated the calibration curves for three analytes (see Figure S8). In the real time detection experiment (shown in Figure 5 of the manuscript), we added different concentrations of analytes, which induced significant decreases in current, reaching plateaus for each addition of analyte. The initial current is recorded, and the average value of the data is taken as the baseline value (I_0). Similarly, we can calculate the average current of each plateau (I) and this is done for each detection/concentration. Thus, we can calculate the sensitivity (S) of the sensor (to each concentration of analytes), according to Equation (1):

$$S = \frac{|I - I_0|}{I_0} \times 100\% \quad (1)$$

The data was plotted and fitted by using equation (2):

$$y = a \times ln(x - b) + c \quad (2)$$

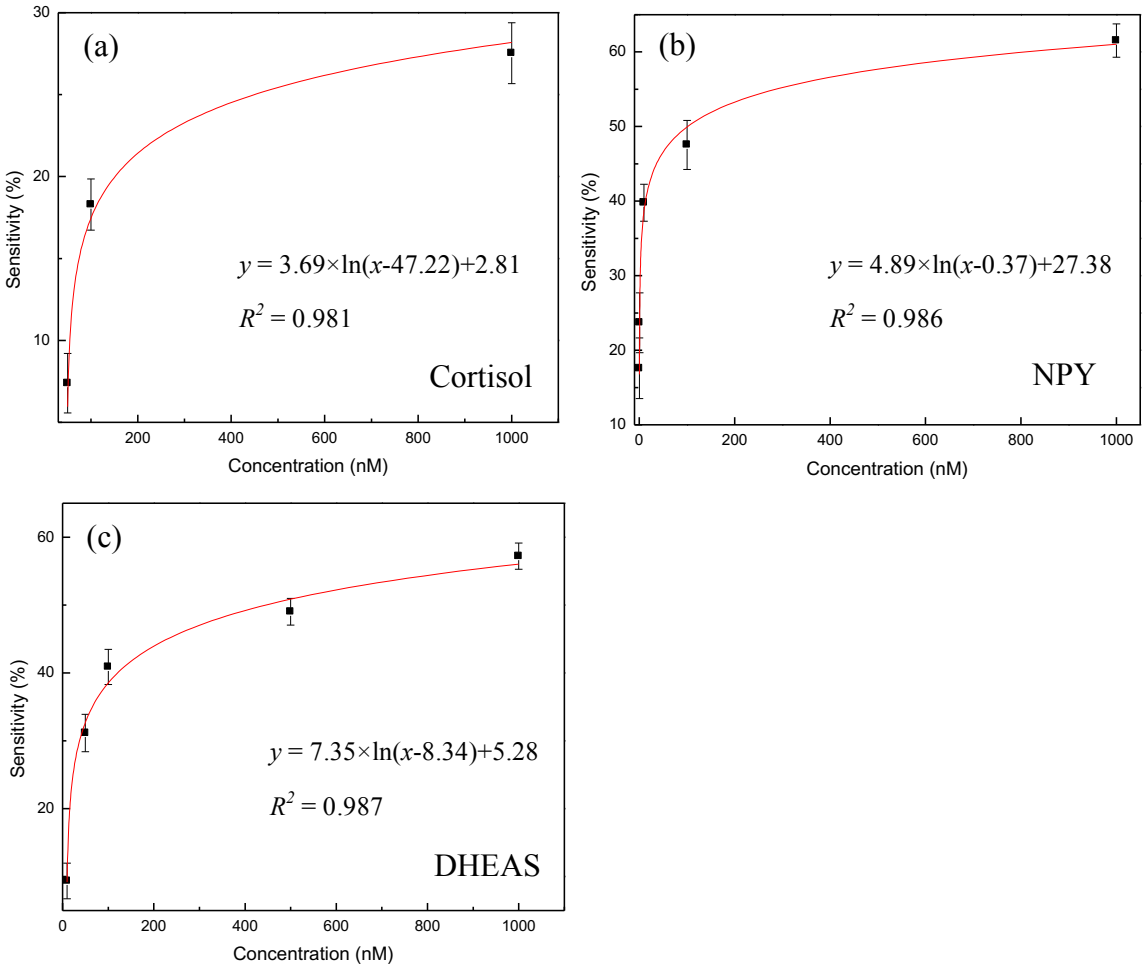

Figure S 8: Calibration curves of the real-time detection of three analytes (a) Cortisol (b) NPY (c) DHEAS

11. Control experiments of detection of DHEAS with non-functionalized device in serum

To confirm that the change in current originates from the aptamers binding to the analytes, we performed real time detection of DHEAS on a non-functionalized CNT device. Initially, the device was covered with a drop of serum solution, then DHEAS solutions with different concentrations were added. We noticed that there was no significant change in current after adding different concentrations of DHEAS (Figure S 9).

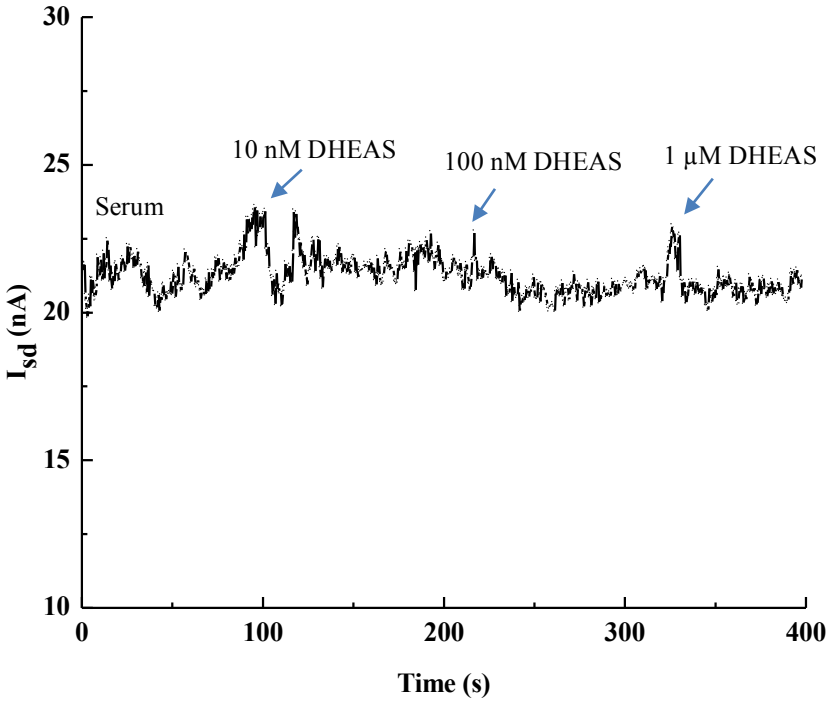

Figure S 9: Real time response of a non-functionalized CNT-device upon addition of DHEAS at different concentrations (from 10 nM to 1 µM)

12. Control experiments with Sodium deoxycholate (SDC) and DHEAS

To demonstrate the selectivity of the devices, we performed real time detection of SDC and DHEAS on the same device, a DHEAS-sensitive CNT-aptamer sensor. As shown in Figure S10a, there was no obvious change in current after adding 1 µM SDC to the device. Subsequently, adding 1 µM DHEAS to the device induced a sharp decrease in current (Figure S10b). I_{sd} vs V_g characterization showed a similar result (Figure S 10c).

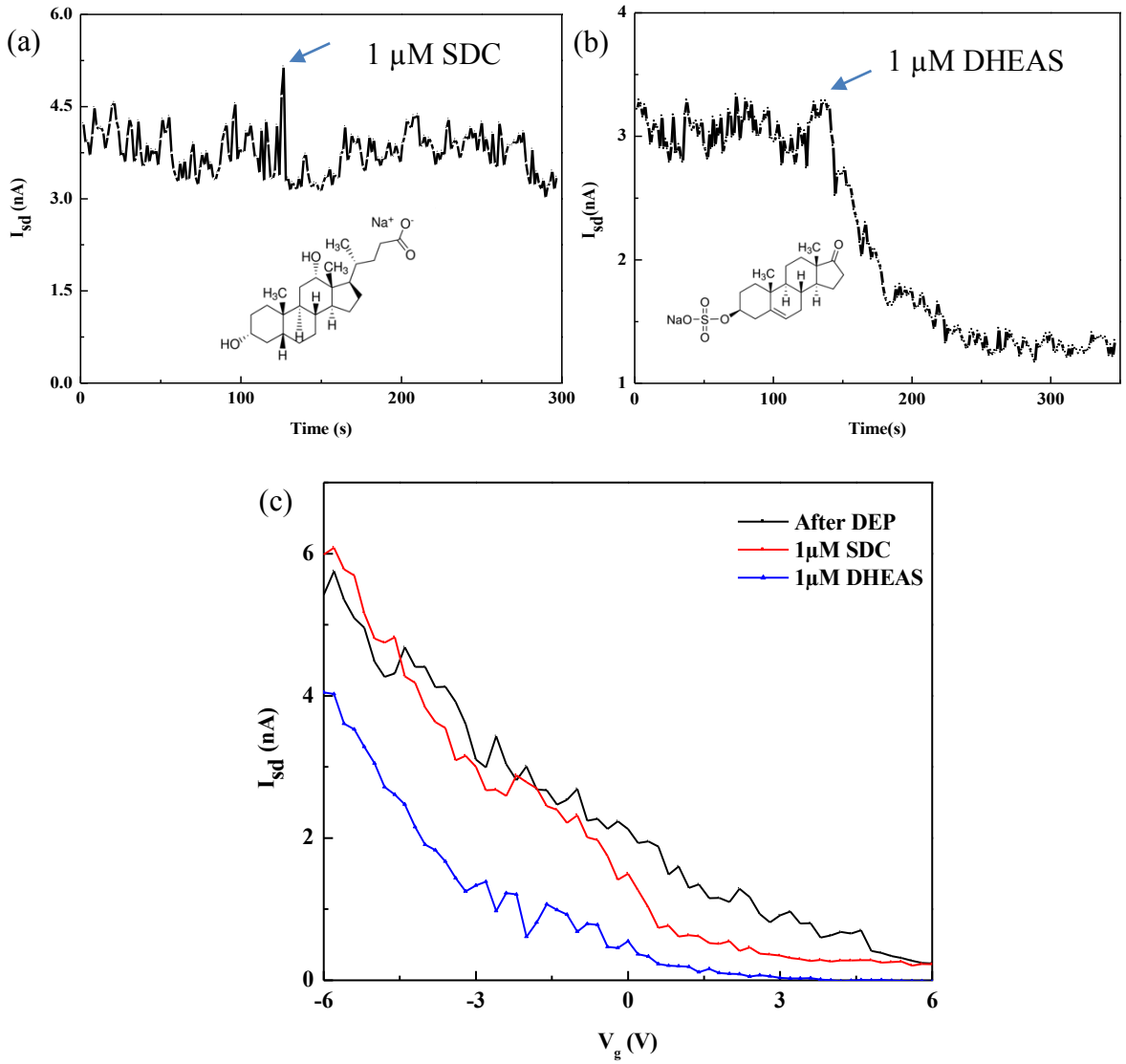

Figure S 10: Real time detection of (a) 1 µM SDC and (b) 1 µM DHEAS on the same device and (c) I_{sd} vs V_g characterization of the device before detection and after SDC and DHEAS

References

[1] Tu, X.; Manohar, S.; Jagota, A.; Zheng, M. *Nature* **2009,** 460, 250-253