Reconfigurable Carbon Nanotube Multiplexed Sensing Devices

*Xinzhao Xu,[†, ‡] Pierrick Clément, [†,‡, *] 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

Here we report on the fabrication of reconfigurable and solution processable nanoscale biosensors with multi-sensing capability, based on single walled carbon nanotubes (SWCNTs). Distinct DNA-wrapped (hence water-soluble) CNTs were immobilized from solution onto different pre-patterned electrodes on the same chip, via a low-cost dielectrophoresis (DEP) methodology. The CNTs were functionalized with specific, and different, aptamer sequences that were employed as selective recognition elements for biomarkers indicative of stress and neuro-trauma conditions. Multiplexed detection of three different biomarkers was successfully performed, and real-time detection was achieved in serum down to physiologically relevant concentrations of 50 nM, 10 nM

and 500 pM for cortisol, dehydroepiandrosterone-sulfate (DHEAS), and neuropeptide Y (NPY), respectively. Additionally, the fabricated nanoscale devices were shown to be reconfigurable and reusable via a simple cleaning procedure. The general applicability of the strategy presented, and the facile device fabrication from aqueous solution, hold great potential for the development of the next generation of low power consumption portable diagnostic assays for the simultaneous monitoring of different health parameters.

Keywords: multiplexed sensing, solution-processable, single-walled carbon nanotubes, biosensor, aptamer, biomarkers

The uncovering of biomarkers holds great potential in the early detection of disease and physiological dysfunction;[1-4] in this context miniaturized/portable sensing apparatuses can allow for continuous functionality in diagnostic or treatment.[5] Developing a platform for achieving this is of importance to both fundamental biology and practical point of care and home diagnosis, where low-cost processability and multipurpose analysis capability are among the most sort-out features that sensing devices would need to possess.

Different detection methods have been employed so far, from the use of enzymes, to nanoparticles, nanopores, as well as electrochemical and mechanical strategies.[6-13] Notably, biosensing platforms that allow for the simultaneous detection of several types of biotargets on a single platform have been fabricated;[14-18] while these results are promising, challenges still remain in terms of fabrication and power costs, as well as biochip size, mainly due to the top-down fabrication methods employed.

In this regard, electrical detection methodologies based on nanomaterials can offer unique advantages, such as simplicity, low-cost fabrication, and label-free real-time electrical detection

in a non-destructive manner,[19, 20] as well as the ability to be effectively merged with miniaturized hardware.[21-25] In particular, there has been great interest in the use of one-dimensional nanostructured materials,[25-28] and SWCNTs emerged as strong candidates.[29-36] It has been demonstrated that target biomolecules in close proximity to SWCNTs can alter their electronic properties via various mechanisms;[37-45] additionally, the use of SWCNTs ensures appropriate size compatibility with biological analytes.[19, 34]

The use of nucleic acid aptamers as the sensing moieties on SWCNT electrical platforms has emerged of particular interest due to: i) aptamers' high affinity and specificity (comparable with those of antibodies); ii) little or no batch-to-batch variation in their production (unlike antibodies); and iii) the easiness in their design and engineering.[46-56] Nevertheless, the fabrication of these sensing platforms is still costly and time-consuming, typically involving numerous fabrication steps, from chemical vapor deposition of the CNTs, to lithographic patterning. Moreover, and most importantly, the SWCNT-aptamer biosensing devices so far presented do not allow for multi-sensing capability nor low-cost processability (ideally from solution).

Here we present a strategy for the facile fabrication of reconfigurable and solution processable nanoscale multiplexed biosensors, based on SWCNTs. DNA-wrapped (hence water-soluble) SWCNTs[57] functionalized with specific nucleotide sequences were employed as selective recognition elements. Distinct SWCNT-aptamer hybrids were then immobilized on the same chip from solution onto pre-patterned electrodes via dielectrophoresis (DEP). This allowed us to fabricate a multisensing platform for the simultaneous electrical detection of different biomarkers. As a proof-of-concept, we employed our devices for both the selective detection of ss-DNA (i.e. hybridization events) and, most notably, the label-free multiplexed sensing of cortisol,[58, 59] neuropeptide Y (NPY),[60, 61] and dehydroepiandrosterone-sulfate (DHEAS),[62, 63] due to the roles of

these biomarkers in various physiological processes such as energy metabolism, blood pressure regulation, cognitive function, post-traumatic stress disorder and traumatic brain injury.[58, 60] We demonstrate the real-time detection of these hormones at their physiological relevant concentrations, from pM to μM; additionally, we show how the platform developed is reconfigurable and reusable via a simple cleaning procedure.

We wrapped single-chirality (7,6) enriched semiconducting SWCNTs with single stranded DNA (ss-DNA) containing a bicyclononyne (BCN) functionality [see the Supporting information (SI) and Figure S1]. This allowed us to then tether azide-terminated aptamers to the DNA-wrapped CNTs, via a simple copper-free cycloaddition, directly in solution and without altering the electronic properties of the nanotubes by covalent attachment.[64] Notably, the reaction of different azide-terminated aptamers to separate solutions of BCN-DNA wrapped CNTs permits the preparation of distinct aptamer-functionalized SWCNTs solutions. We employed this strategy to produce three different solutions of SWCNTs, each functionalized with a distinct aptamer selective to a specific biomarker, namely cortisol, NPY, and DHEAS: the schematic in Figure 1 outlines this approach (see also the SI).

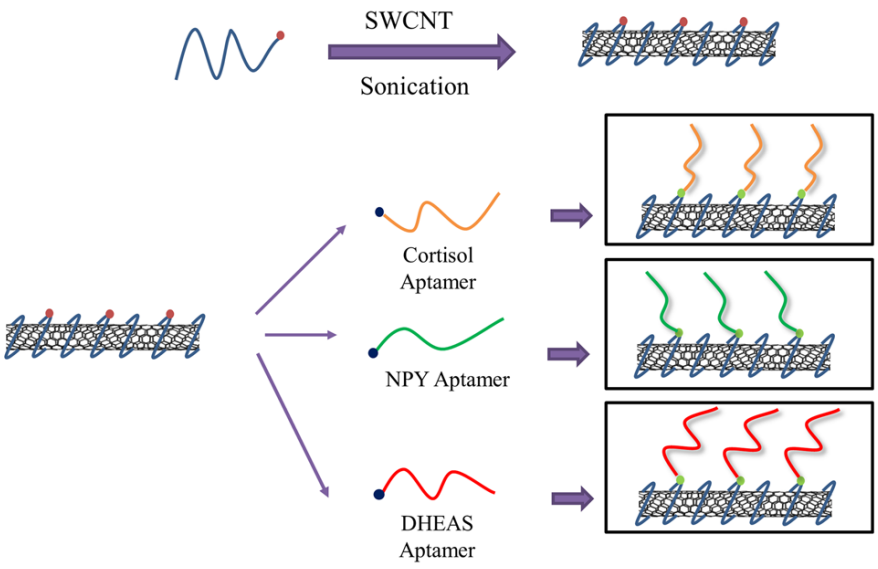

Figure 1. DNA-wrapping of the SWCNTs and tethering of different aptamers: cortisol (orange), NPY (green) and DHEAS (red) binding aptamers

In order to verify the successful functionalization of DNA wrapped SWCNTs with the employed aptamers, we hybridized cortisol aptamer-functionalized nanotubes with complementary ss-DNA directly in solution, then cast these on muscovite mica substrates and imaged them with Atomic Force Microscopy (AFM). Figure 2 shows representative AFM images of the aptamer-functionalized SWCNTs before and after hybridization with the complementary strands. The double stranded (ds) DNA portion protruding out of the nanotubes is clearly visible in the samples that were exposed to the aptamer's complementary sequence (free dsDNA not bound to the SWCNTs can also be found in the vicinity of the nanotubes: see also Figure S2a-c). This demonstrates that the functionalization strategy was successful: the nucleic acid aptamers are present on the SWCNTs and are accessible to other biomolecules, ss-DNA in this case, an important feature for the subsequent use of these hybrids as selective recognition elements in a device. From the analysis of AFM images of multiple nanotubes in different samples, we determined that each SWCNT exhibited on average 4 ± 2 aptamers per 100 nm, and that these are available for hybridization. Additionally, it is reasonable to assume that the tens of nm distance between the aptamers on each nanotube will prevent potential detrimental crowding effects on the biosensing properties of these hybrids once immobilized in a device.

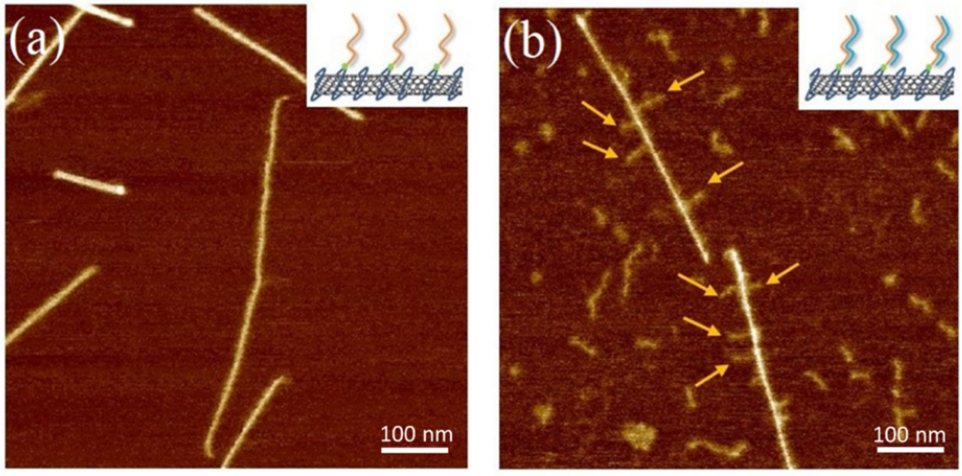

Figure 2. AFM images, and cartoon insets, of aptamer-functionalized SWCNTs (a) without and (b) with hybridized ss-DNA sequences. The yellow arrows show the hybridized aptamers along the nanotubes. Z-scales = 2.5 nm.

To employ the so formed SWCNT-aptamer hybrids in electrical biosensing devices we: i) patterned metal electrode pairs on doped silicon wafers via electron beam lithography (see Figure S3), ii) cast the solutions on the so fabricated substrates, and iii) immobilized the nanotubes between the electrodes via DEP. The assembly between electrodes is induced by an applied AC voltage bias: Figure 3 shows the schematic of the strategy utilised, as well as a representative AFM image of aptamer-functionalized SWCNTs aligned between two pre-patterned electrodes (see also the SI and Figure S2d).

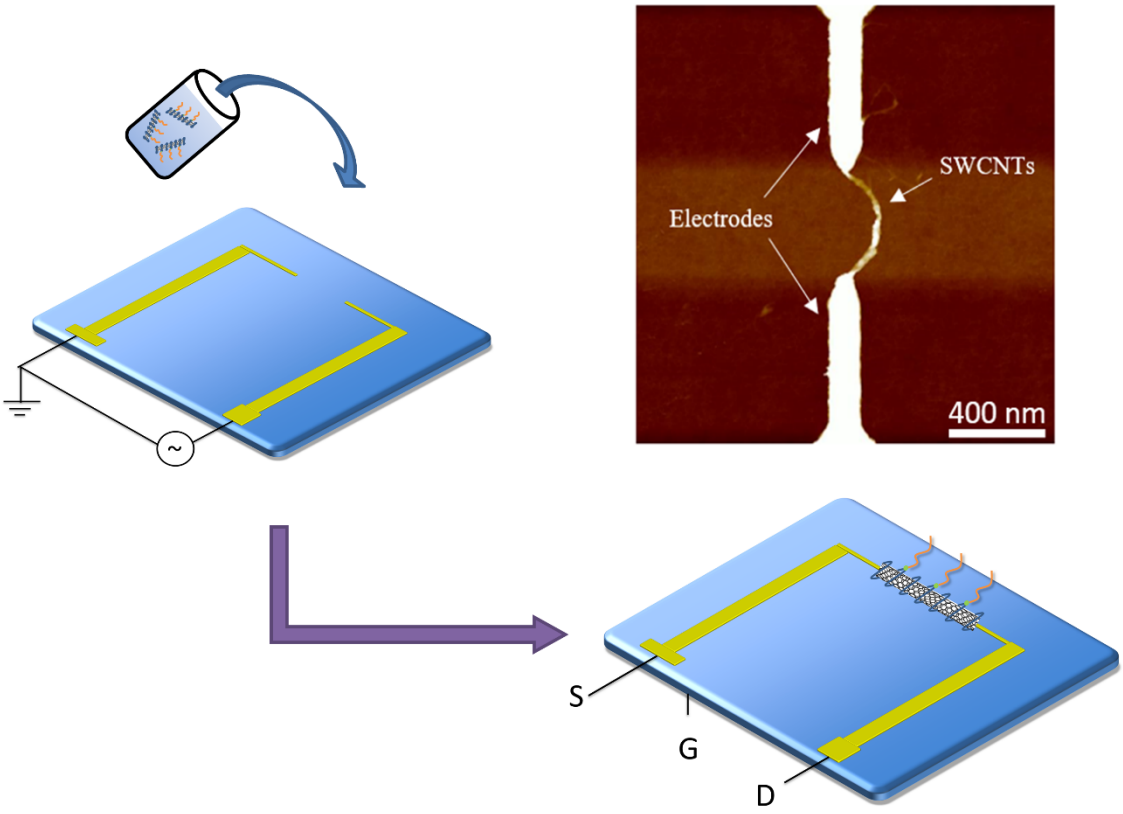

Figure 3. DEP of SWCNT-aptamer hybrids with the corresponding AFM picture. S, D and G indicate respectively the source, drain and gate (electrodes) Z-scales = 10 nm.

To confirm the accessibility of the nucleotide recognition element within the SWCNT-aptamer hybrids immobilized in the device configuration, we performed *in-situ* hybridization experiments exposing the chip to the cortisol aptamer's complementary ss-DNA, and recording the electrical response of the device when the ds-DNA (double-stranded) was formed. Figure S4 shows a representative electrical response of the SWCNT-aptamer field effect transistor (FET), before and after DNA hybridization. The change in source-drain current indicates the occurred recognition of the complementary ss-DNA by the aptamer in the SWCNT-based devices. The observed shift of V_{TH} (taken as the *abscissa*-intercept of the line tangent to the steepest part of the drain current versus gate voltage curve) points to a potential scattering mechanism occurring upon the rearrangement of the aptamer's conformation due to DNA hybridization.[65]

When the CNTs in the devices were not functionalized with the aptamer, we did not record any change in current upon addition of the complementary ss-DNA (see Figure S5). Moreover, the exposure of the devices to non-complementary DNA did not induce any significant electrical response of the devices (see Figure S6), demonstrating the selectivity of the DNA hybridization detection. By exposing the chip to formamide, we were able to denature the ds-DNA,[66] without affecting the electrical properties of the SWCNT-aptamer hybrids, that indeed showed comparable current responses to the initial stage: this further proves the reconfigurable nature of the platform, via a simple cleaning procedure (see Figure S4 and Figure S6).

In order to use the devices for multipurpose analysis, we assembled SWCNT-aptamer hybrids exhibiting distinct bio-recognition elements at different locations on the same chip. By separately addressing distinct electrodes pairs it is possible to immobilize, via DEP, *n* aptamer-functionalized SWCNTs on *n* different electrode pairs. The organisation of distinct SWCNT-aptamer hybrids from solution to surfaces in parallel 2D device configurations on the same chip can then allow for

the fabrication of multifunctional, high-throughput bio-electronic devices with parallel multi-purpose sensing capability (see Figure 4a). The electronic devices prepared in this way should indeed withstand and respond to various environmental changes on the same substrates, depending on the different aptamers employed: upon recognition of an analyte, the specific aptamer will undergo a structural rearrangement[67, 68] and induce a change in the electrical response (resistance) of the CNT embedded in the device.

To demonstrate the multi-sensing capability of the devices, we immobilized three distinct SWCNT-aptamer hybrids on separate electrode pairs, on the same chip: this step is marked as "after DEP" in the curves shown in Figure 4b. In particular, we used aptamers targeting the aforementioned biomarkers indicative of stress and neuro-trauma conditions, i.e. cortisol, NPY and DHEAS. We performed subsequent detection experiments on the same chip, employing different solutions containing either one, two, or all three biomarkers. Upon selective binding of a metabolite to the specific aptamer tethered to the SWCNTs we observed a reduction in the current response only for the corresponding device on the chip, without any crosstalk between the different devices selective to the other analytes, nor any false-positive signals (see Figure 4b and Figure S7). Notably, 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[69] ("cleaning" in Figure 4b): this allowed us to perform multiple detection tests on the same chip, and successfully demonstrate the multiplexed electrical detection of the three biomarkers of interest.

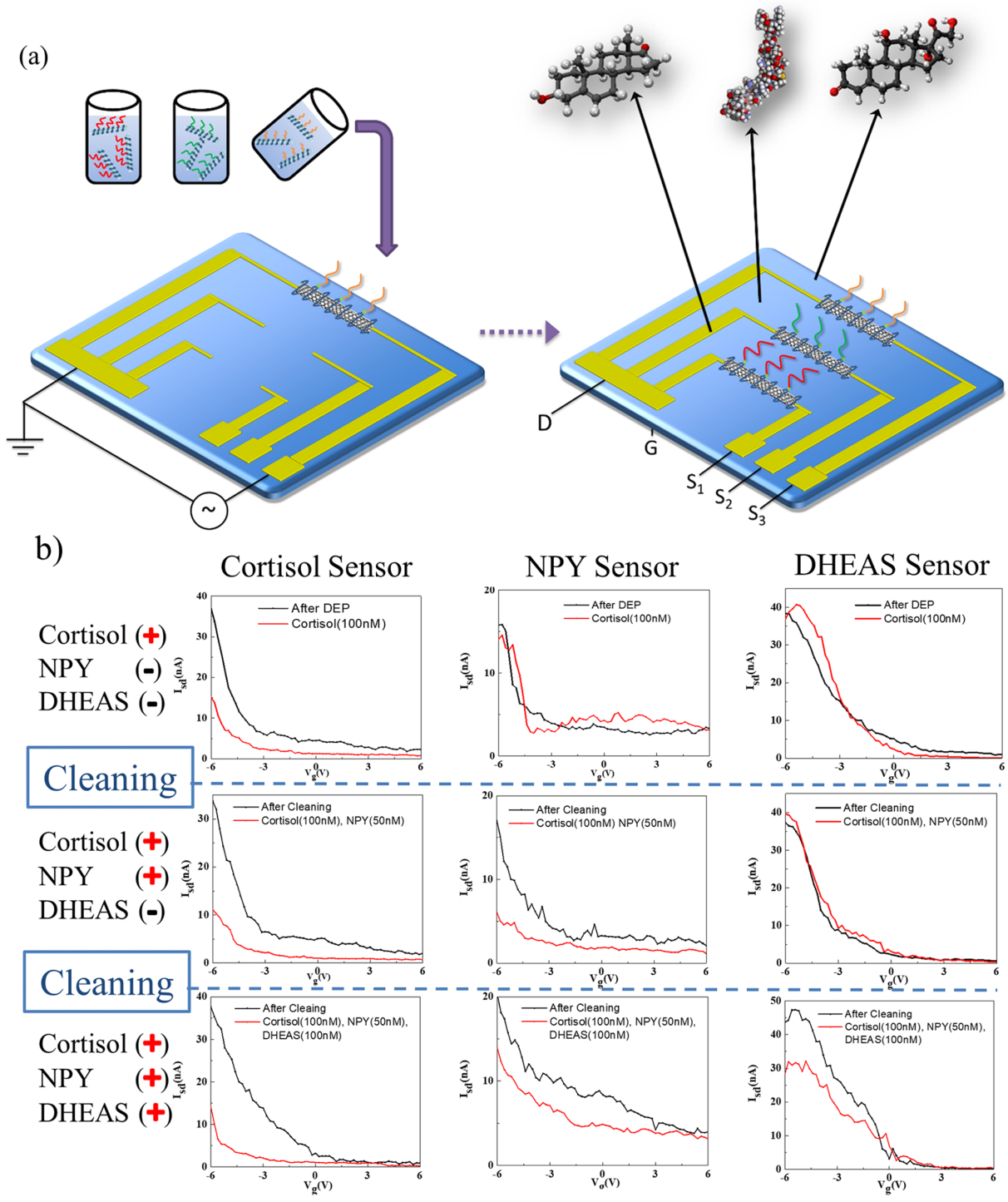

Figure 4. (a) Schematic of the DEP strategy employed for the fabrication of multi-sensing devices: a drop of the chosen SWNCT-aptamer solution is cast on the chip mainly over one electrode pair

, a voltage is then applied only across this electrode pair in order to direct the assembly of SWCNT-aptamer hybrids only across this pair of electrodes, and not the others (see also the SI); (b) multiplexed sensing: electrical responses of the different biosensors on the same chip (V_{sd} =100 mV): the + sign indicates the addition/presence of the analyte of interest; the "cleaning step" indicates the addition of 8M of urea in order to regenerate the sensor after each detection; "after cleaning" indicates the measurements performed after this step.

We further investigated the real-time detection of cortisol, DHEAS, and NPY, at their relevant physiological concentrations[58, 60, 62] in serum, i.e. from 1 μM to 100 pM. The devices were first immersed in serum; subsequently, different concentrations of each biomarker were added to the devices at different time intervals. The electrical response increased with the change in concentration; we detected in real-time concentrations of cortisol, DHEAS and NPY down to ca 50 nM, 10 nM and 500 pM respectively: see Figure 5 and the calibration curves shown in Figure S8. The drop in I_{sd}-V_g is likely due to a screening charge in the CNTs induced by the change in conformation of the aptamer upon analyte binding, as previously observed for ss-DNA folding in CNT-DNA devices.[30, 44, 70] Control samples, where the nanotubes in the deivces were not functionalized with any aptamer, did not exhibit any change in their electrical reponse upon the additon of the aformentioned biomarkers (see Figure S9). This further confirms the selectivity and biosensing nature of the devices presented here.

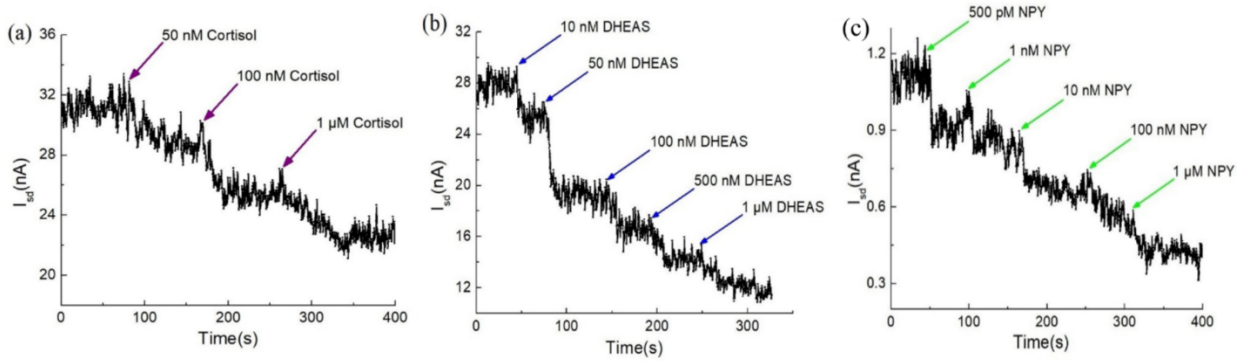

Figure 5. Real time detection of (a) cortisol (from 50 nM to 1 µM), (b) DHEAS (from 10 nM to 1 µM) and (c) NPY (from 500 pM to 1 µM) at various concentrations, in serum (V_{sd} = 100 mV, V_g = –2V)

To further demonstrate the selectivity of the fabricated multiplexed platform also for real-time measurements, we tested the DHEAS-sensitive sensor with a molecule possessing similar molar mass and chemical structure to DHEAS (Sodium deoxycholate, SDC).[62] As shown in Figure S10a, SDC was added at a concentration of 1 µM and no changes were observed in the current of the DHEAS-sensitive sensor. The sample was then cleaned with DI water and exposed to a 1 µM DHEAS solution. A sharp decrease in the conductance was at this point observed as expected (Figure S10b), in line with the results shown in Figure 5b. Additionally, the source-drain versus gate voltage measurements further confirmed the high selectivity of the platform (see Figure S10c).

In conclusion, we presented a novel solution-processable method of general applicability for the fabrication of label-free nanoscale biosensing devices, that permits the real-time and simultaneous detection of multiple analytes on the same chip. We assembled hybrids of SWCNTs and aptamers from solution to surfaces in nanoscale device configurations, where the nanotubes could act as the transducer elements, and the aptamers as the recognition components, of an electrical biosensing platform. As proof of concept, we demonstrated the selective recognition of different biomarkers

indicative of stress and neuro-trauma conditions, at various physiologically relevant concentrations, from pM to μM. The devices exhibited high selectivity and sensitivity, as well as multiplexing ability thanks to the immobilization of CNT-aptamer hybrids with distinct bio-recognition elements on the same nanoscale chip via a DEP-based strategy; this grants low cost processability and low power consumption. Additionally, the devices are reconfigurable and reusable via a simple cleaning procedure. To the best of our knowledge, these results represent the first example of solution-processable and reconfigurable nanoscale multiplexing sensing devices based on the use of carbon nanostructures. By and large, the general applicability of the strategy developed, and the solution processability of the nanoscale multiplexing biosensing devices we fabricated, hold great potential for the development of the next generation of portable, point of care and home diagnostic assays for the continuous and simultaneous monitoring of different health parameters.

ASSOCIATED CONTENT

Supporting information available: Materials and methods, substrate fabrication, preparation of CNT-aptamer hybrids, dielectrophoresis, additional characterizations, control experiments.

AUTHOR INFORMATION

Corresponding Author

*E-mails : p.clement@qmul.ac.uk; m.palma@qmul.ac.uk

Author Contributions

‡These authors contributed equally.

ACKNOWLEDGMENT

We gratefully acknowledge financial support from the Air Force Office of Scientific Research under award FA9550-16-1-0345, and the Engineering and Physical Sciences Research Council under Award EP/ M029506/1. X.X. is financially supported by the China Scholarship Council.

REFERENCES

1. Etzioni, R.; Urban, N.; Ramsey, S.; McIntosh, M.; Schwartz, S.; Reid, B.; Radich, J.; Anderson, G.; Hartwell, L. *Nature Reviews Cancer* **2003,** 3, (4), 243-252.

2. Pepe, M. S.; Etzioni, R.; Feng, Z.; Potter, J. D.; Thompson, M. L.; Thornquist, M.; Winget, M.; Yasui, Y. *Journal of the National Cancer Institute* **2001,** 93, (14), 1054-1061.

3. Chen, X.; Ba, Y.; Ma, L.; Cai, X.; Yin, Y.; Wang, K.; Guo, J.; Zhang, Y.; Chen, J.; Guo, X. *Cell research* **2008,** 18, (10), 997-1006.

4. DeKosky, S. T.; Marek, K. *Science* **2003,** 302, (5646), 830-834.

5. Kim, D.-H.; Lu, N.; Ma, R.; Kim, Y.-S.; Kim, R.-H.; Wang, S.; Wu, J.; Won, S. M.; Tao, H.; Islam, A. *science* **2011,** 333, (6044), 838-843.

6. Landry, M. P.; Ando, H.; Chen, A. Y.; Cao, J.; Kottadiel, V. I.; Chio, L.; Yang, D.; Dong, J.; Lu, T. K.; Strano, M. S. *Nature nanotechnology* **2017,** 12, (4), 368-377.

7. Diehl, K. L.; Anslyn, E. V. *Chemical Society Reviews* **2013,** 42, (22), 8596-8611.

8. Saha, K. K.; Drndic, M.; Nikolic, B. K. *Nano letters* **2011,** 12, (1), 50-55.

9. Arlett, J.; Myers, E.; Roukes, M. *Nature nanotechnology* **2011,** 6, (4), 203-215.

10. Wei, F.; Patel, P.; Liao, W.; Chaudhry, K.; Zhang, L.; Arellano-Garcia, M.; Hu, S.; Elashoff, D.; Zhou, H.; Shukla, S. *Clinical Cancer Research* **2009,** 15, (13), 4446-4452.

11. Zangar, R. C.; Daly, D. S.; White, A. M. *Expert review of proteomics* **2006,** 3, (1), 37-44.

12. Drummond, T. G.; Hill, M. G.; Barton, J. K. *Nature biotechnology* **2003,** 21, (10), 1192.

13. Fritz, J.; Baller, M.; Lang, H.; Rothuizen, H.; Vettiger, P.; Meyer, E.; Güntherodt, H.-J.; Gerber, C.; Gimzewski, J. *Science* **2000,** 288, (5464), 316-318.

14. Vo-Dinh, T.; Griffin, G.; Stokes, D. L.; Wintenberg, A. *Sensors and Actuators B: Chemical* **2003,** 90, (1-3), 104-111.

15. Shafiee, H.; Asghar, W.; Inci, F.; Yuksekkaya, M.; Jahangir, M.; Zhang, M. H.; Durmus, N. G.; Gurkan, U. A.; Kuritzkes, D. R.; Demirci, U. *Sci Rep* **2015,** 5, 8719.

16. Kingsmore, S. F. *Nature reviews. Drug discovery* **2006,** 5, (4), 310.

17. Ahn, S. R.; An, J. H.; Song, H. S.; Park, J. W.; Lee, S. H.; Kim, J. H.; Jang, J.; Park, T. H. *ACS nano* **2016,** 10, (8), 7287-7296.

18. Gao, W.; Emaminejad, S.; Nyein, H. Y. Y.; Challa, S.; Chen, K.; Peck, A.; Fahad, H. M.; Ota, H.; Shiraki, H.; Kiriya, D. *Nature* **2016,** 529, (7587), 509-514.

19. Guo, X. *Adv Mater* **2013,** 25, (25), 3397-408.

20. Rosi, N. L.; Mirkin, C. A. *Chemical Reviews* **2005,** 105, (4), 1547-1562.

21. Xu, M.; Luo, X.; Davis, J. J. *Biosens Bioelectron* **2013,** 39, (1), 21-5.

22. Bansal, A. K.; Hou, S.; Kulyk, O.; Bowman, E. M.; Samuel, I. D. *Advanced Materials* **2015,** 27, (46), 7638-7644.

23. Luo, X.; Davis, J. J. *Chemical Society Reviews* **2013,** 42, (13), 5944-5962.

24. Zhu, R.; Azzarelli, J. M.; Swager, T. M. *Angewandte Chemie* **2016,** 128, (33), 9814-9818.

25. Fennell, J. F.; Liu, S. F.; Azzarelli, J. M.; Weis, J. G.; Rochat, S.; Mirica, K. A.; Ravnsbæk, J. B.; Swager, T. M. *Angewandte Chemie International Edition* **2016,** 55, (4), 1266-1281.

26. Zhang, A.; Lieber, C. M. *Chemical reviews* **2015,** 116, (1), 215-257.

27. Chiesa, M.; Cardenas, P. P.; Otón, F.; Martinez, J.; Mas-Torrent, M.; Garcia, F.; Alonso, J. C.; Rovira, C.; Garcia, R. *Nano letters* **2012,** 12, (3), 1275-1281.

28. Zheng, G.; Patolsky, F.; Cui, Y.; Wang, W. U.; Lieber, C. M. *Nat Biotech* **2005,** 23, (10), 1294-1301.

29. Choi, Y.; Moody, I. S.; Sims, P. C.; Hunt, S. R.; Corso, B. L.; Perez, I.; Weiss, G. A.; Collins, P. G. *Science* **2012,** 335, (6066), 319-324.

30. So, H.-M.; Won, K.; Kim, Y. H.; Kim, B.-K.; Ryu, B. H.; Na, P. S.; Kim, H.; Lee, J.-O. *Journal of the American Chemical Society* **2005,** 127, (34), 11906-11907.

31. Kauffman, D. R.; Star, A. *Chemical Society Reviews* **2008,** 37, (6), 1197-1206.

32. Rosenstein, J. K.; Lemay, S. G.; Shepard, K. L. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* **2015,** 7, (4), 475-493.

33. Sorgenfrei, S.; Chiu, C.-y.; Gonzalez Jr, R. L.; Yu, Y.-J.; Kim, P.; Nuckolls, C.; Shepard, K. L. *Nature nanotechnology* **2011,** 6, (2), 126-132.

34. Schnorr, J. M.; Swager, T. M. *Chemistry of Materials* **2010**.

35. Wang, X.; Gao, L.; Liang, B.; Li, X.; Guo, X. *Journal of Materials Chemistry B* **2015,** 3, (26), 5150-5154.

36. Antonucci, A.; Kupis-Rozmysłowicz, J.; Boghossian, A. A. *ACS Applied Materials & Interfaces* **2017,** 9, (13), 11321-11331.

37. Weizmann, Y.; Chenoweth, D. M.; Swager, T. M. *Journal of the American Chemical Society* **2011,** 133, (10), 3238.

38. Sims, P. C.; Moody, I. S.; Choi, Y.; Dong, C.; Iftikhar, M.; Corso, B. L.; Gul, O. T.; Collins, P. G.; Weiss, G. A. *Journal of the American Chemical Society* **2013,** 135, (21), 7861-7868.

39. Pugliese, K. M.; Gul, O. T.; Choi, Y.; Olsen, T. J.; Sims, P. C.; Collins, P. G.; Weiss, G. A. *Journal of the American Chemical Society* **2015,** 137, (30), 9587-9594.

40. Allen, B. L.; Kichambare, P. D.; Star, A. *Advanced Materials* **2007,** 19, (11), 1439-1451.

41. Kim, S. N.; Rusling, J. F.; Papadimitrakopoulos, F. *Advanced materials* **2007,** 19, (20), 3214-3228.

42. Snow, E. S.; Perkins, F.; Robinson, J. A. *Chemical Society Reviews* **2006,** 35, (9), 790-798.

43. Salehi-Khojin, A.; Khalili-Araghi, F.; Kuroda, M. A.; Lin, K. Y.; Leburton, J.-P.; Masel, R. I. *Acs Nano* **2010,** 5, (1), 153-158.

44. Bouilly, D.; Hon, J.; Daly, N. S.; Trocchia, S.; Vernick, S.; Yu, J.; Warren, S.; Wu, Y.; Gonzalez, R. L.; Shepard, K. L.; Nuckolls, C. *Nano Letters* **2016,** 16, (7), 4679-4685.

45. Wang, F.; Swager, T. M. *Journal of the American Chemical Society* **2011,** 133, (29), 11181-11193.

46. Hamaguchi, N.; Ellington, A.; Stanton, M. *Analytical biochemistry* **2001,** 294, (2), 126-131.

47. Shangguan, D.; Meng, L.; Cao, Z. C.; Xiao, Z.; Fang, X.; Li, Y.; Cardona, D.; Witek, R. P.; Liu, C.; Tan, W. *Analytical Chemistry* **2008,** 80, (3), 721-728.

48. Hermann, T.; Patel, D. J. *Science* **2000,** 287, (5454), 820-825.

49. Liu, J.; Cao, Z.; Lu, Y. *Chemical reviews* **2009,** 109, (5), 1948.

50. Stojanovic, M. N.; Kolpashchikov, D. M. *Journal of the American Chemical Society* **2004,** 126, (30), 9266-9270.

51. Harvey, J. D.; Jena, P. V.; Baker, H. A.; Zerze, G. H.; Williams, R. M.; Galassi, T. V.; Roxbury, D.; Mittal, J.; Heller, D. A. *Nature Biomedical Engineering* **2017,** 1, (4), 0041.

52. Maehashi, K.; Katsura, T.; Kerman, K.; Takamura, Y.; Matsumoto, K.; Tamiya, E. *Analytical Chemistry* **2007,** 79, (2), 782-787.

53. So, H. M.; Park, D. W.; Jeon, E. K.; Kim, Y. H.; Kim, B. S.; Lee, C. K.; Choi, S. Y.; Kim, S. C.; Chang, H.; Lee, J. O. *Small* **2008,** 4, (2), 197-201.

54. Khosravi, F.; Loeian, S. M.; Panchapakesan, B. *Biosensors* **2017,** 7, (2), 17.

55. Ordinario, D. D.; Burke, A. M.; Phan, L.; Jocson, J. M.; Wang, H.; Dickson, M. N.; Gorodetsky, A. A. *Anal Chem* **2014,** 86, (17), 8628-33.

56. Liu, S.; Zhang, X.; Luo, W.; Wang, Z.; Guo, X.; Steigerwald, M. L.; Fang, X. *Angewandte Chemie International Edition* **2011,** 50, (11), 2496-2502.

57. Zheng, M.; Jagota, A.; Semke, E. D.; Diner, B. A.; McLean, R. S.; Lustig, S. R.; Richardson, R. E.; Tassi, N. G. *Nature materials* **2003,** 2, (5), 338-342.

58. Gatti, R.; Antonelli, G.; Prearo, M.; Spinella, P.; Cappellin, E.; Elio, F. *Clinical biochemistry* **2009,** 42, (12), 1205-1217.

59. Martin, J. A.; Chávez, J. L.; Chushak, Y.; Chapleau, R. R.; Hagen, J.; Kelley-Loughnane, N. *Analytical and bioanalytical chemistry* **2014,** 406, (19), 4637-4647.

60. Andrews, J. A.; Neises, K. D. *Journal of neurochemistry* **2012,** 120, (1), 26-36.

61. Mendonsa, S. D.; Bowser, M. T. *Journal of the American Chemical Society* **2005,** 127, (26), 9382-9383.

62. Lapchak, P. A.; Chapman, D. F.; Nunez, S. Y.; Zivin, J. A. *Stroke* **2000,** 31, (8), 1953-1957.

63. Yang, K.-A.; Pei, R.; Stefanovic, D.; Stojanovic, M. N. *Journal of the American Chemical Society* **2012,** 134, (3), 1642-1647.

64. Park, H.; Zhao, J.; Lu, J. P. *Nano Letters* **2006,** 6, (5), 916-919.

65. Martínez, M. T.; Tseng, Y.-C.; González, M. n.; Bokor, J. *The Journal of Physical Chemistry C* **2012,** 116, (42), 22579-22586.

66. Blake, R.; Delcourt, S. G. *Nucleic acids research* **1996,** 24, (11), 2095-2103.

67. Baker, B. R.; Lai, R. Y.; Wood, M. S.; Doctor, E. H.; Heeger, A. J.; Plaxco, K. W. *Journal of the American Chemical Society* **2006,** 128, (10), 3138-3139.

68. Lai, R. Y.; Plaxco, K. W.; Heeger, A. J. *Analytical chemistry* **2007,** 79, (1), 229-233.

69. Idili, A.; Ricci, F.; Vallée-Bélisle, A. *Nucleic Acids Research* **2017**.

70. Heller, I.; Janssens, A. M.; Männik, J.; Minot, E. D.; Lemay, S. G.; Dekker, C. *Nano Letters* **2008,** 8, (2), 591-595.

TOC graphic