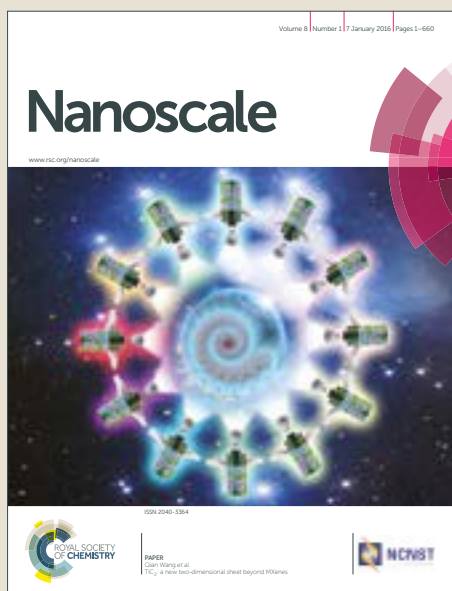


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Memristive Effect as a Novelty in Drug Monitoring

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KEYWORDS: Memristive effect, ultra-sensitive drug monitoring, in-serum detection, aptamer regeneration, Si-nanowires

ABSTRACT

Nanoscale devices exhibiting memristive properties show great potential in plethora of applications. In this work memristive nanowires are presented for the first time as ideal candidates for absolutely novel, ultrasensitive, highly specific and selective drug-biosensors, also paving the way for real-time monitoring applications, in coupling with the restoration properties DNA-aptamers. The hysteretic properties exhibited by the hereby-presented special nanodevices, modified via surface treatments, is leveraged along the complete cycle consisting of DNA-aptamers immobilization, target binding, DNA-aptamers regeneration for successful and effective detection of Tenofovir, an antiviral drug for HIV treatment, in buffer as well as in non-diluted human serum. This, results in an ultrasensitive, label-free monitoring of the therapeutic compound with a limit of detection of 3.09 pM in buffer and 1.38 nM in full serum, respectively. These LODs demonstrate 10 times higher sensitivity for the in-buffer drug detection, and twice better performance for drug sensing in-full human serum, ever obtained. The selectivity of the memristive biosensor for Tenofovir detection was verified through both positive and negative controls in full human serum. In addition, the DNA-aptamer regeneration character is portrayed for the first time through a memristive effect, and scanning electron microscopy throws more light to the binding mechanism efficiency through the variation of the nanodevice surface properties at the nanoscale.

The results presented in this work demonstrate that the coupling of memristive effect and aptamers regeneration provides the best ever realized nano-bio-sensor for drugs detection also in full human serum.

INTRODUCTION

Therapeutic drug monitoring (TDM) is a modern therapeutic approach that proposes to monitor the concentration of drug compounds over time in patient's circulating system in order to maintain drug concentrations in plasma or blood within a targeted therapeutic range. This aims to the individualization of the drug dosage regime for optimal efficacy and safety of the treatment¹. This results in a chemical treatment based on patients' characteristics (genetic profile, age, race, gender, environmental agent etc.), as well as on drug characteristics including pharmacodynamics, pharmacokinetics, drug-drug interactions, adverse effects etc². As opposed to traditional monitoring technologies usually proposed for TDM, Point-of-Care (PoC) devices will provide a cutting-edge for economical and user-friendly monitoring new systems directly available to patients too, which would help them to continuously monitor drug concentrations and administrations whenever is required, also at home.

Despite the body of research available about drugs detection, innovative ultrasensitive biosensing assays with improved performance in terms of reliability, sensitivity and detection limit, and the miniaturization capability are still needed, especially targeting few nM concentration-ranges.

DNA-aptamers are synthetic DNA receptors developed via an in-vitro selection technique (SELEX) to bind specifically and selectively to a particular target. Aptamers are chemically highly stable and retain most of their functionality even after multiple regeneration steps³. In contrast to antibodies, such a highly repeatable and easy regeneration of aptamers allows for continuous monitoring even upon decreasing target concentrations. These benefits make them ideal candidates for novel biosensors design in continuous diagnostics. In addition, structures like semiconductor nanofabricated wires manifest great potential for the realization of extremely miniaturized bioassays⁴⁻⁷. Thanks to their tunable electron transport properties and the fact that their electrical response is strongly influenced by even minor perturbations, as a consequence of their high surface-to-volume ratio obtained when designed at the nanoscale, such semiconductor wires offer the possibility for a direct and highly sensitive electrical readout⁸.

Meanwhile, memory effects widely appear in nature⁹⁻¹¹, as well as in electronic-based devices where a pronounced hysteresis in the electrical characteristics is a unique fingerprint of the memristive phenomena^{12,13}. Theoretically conceived and presented for the first time by L. Chua in 1971¹⁴⁻¹⁶ and physically implemented in 2008 by HP laboratory¹⁷, nanoscale devices exhibiting memristive behavior can be nowadays fabricated by various materials^{18,19} and show immense potential in plethora of applications. Such nonlinear dynamical memory devices present growing interest also for computation, logic operations²⁰⁻²², as well as, for storage applications²³. At the same time, implementations in biophysics and temporal dynamics have drawn considerable attention. Nevertheless, so far,

the main focus is in the field of bio-inspired computation and mainly concentrated on neuronal systems^{24–28}.

Memristive aptasensors have been proposed for cancer biomarkers detection and experimented as a proof of concept, and results are published by Tzouvadaki and Jolly et al²⁹. Moreover, we hereby present a new work that opens to a completely new class of Memristive Aptasensors for continuous monitoring of therapeutic compounds. DNA-aptamer regeneration coupled with memristive effect is demonstrated for the effective drug detection in human serum by the benefit of this kind of memory effect when DNA-aptamers are anchored on the surface of a nanoscale silicon device. The present work actually bridges for the first time ever-reported the memristive effect and the hysteretic electrical properties expressed by nanoscale devices with the DNA-aptamer nature and physical functions. Moreover, successful label-free screening of Tenofovir (TFV), proposed as drug model in this case of study, is further achieved considering as starting point this highly controllable DNA-aptamer regeneration character. This ultrasensitive label-free screening of the drug is initially shown in buffer and then efficaciously demonstrated in full human serum. This approach, based on such unique and definitely innovative nanoscale devices, holds great promise for continuous monitoring of drugs in real-time, and may have high impact in the medical practice especially in the field of personalized medicine, which still lacks of analytical methods for the continuous monitoring of therapeutic compounds. Furthermore, the suggested approach may pave the way for potential new integrations and multiplexing aspects in diagnostics and personalized medicine.

EXPERIMENTAL

Materials

Amino group terminated TFV-aptamers (5'-Aptamer-C6 Amino-3') were commercially provided by BasePair Biotechnologies (Pearland, Houston, USA). Human serum, magnesium chloride (MgCl_2), DMSO and PBS (10 mM; pH-7.4) were all purchased from Sigma-Aldrich (Switzerland). PBS buffer were always filtered through 0.2 μm filters before use. Sodium hydroxide solution (NaOH) were purchased from Sigma Aldrich (Switzerland) and diluted to the concentration of 1 M. Silane (3-Glycidyloxypropyl) trimethoxysilane (GPTES) were purchased from Sigma-Aldrich (Switzerland) and used as received. TFV and enzalutamide powders were purchased from Medchemtronica (Stockholm, Sweden) and were dissolved in dimethyl sulfoxide (DMSO) to the concentration of 5 mM as stock solution, and then they were diluted to the concentration of 100 μM and, subsequently, diluted in sample solutions (buffer or undiluted human serum) to the required concentrations. All other reagents were of analytical grade. All aqueous solutions were prepared using 18.2 M Ω cm ultrapure water with a Pyrogard filter (Millipore, Feltham, UK).

Memristive Nano- Biosensors Fabrication

The nano-biosensor suggested combines top-down nanofabrication approach for the realization of the memristive wires, as well as, bottom-up strategies for the acquisition of the final memristive Nano-Bio-Sensor.

Silicon nanowire devices are fabricated through a top-down fabrication process performed on (100) oriented Silicon-On-Insulator wafers with low boron concentration ($N_A \approx 10^{15}$ atoms/cm³). The nanofabrication process can be summarized in two e-beam lithography operations. The first combined with Ni evaporation, liftoff and annealing processes for obtaining NiSi contact pads for electrical characterization, and, the second for the nanowires definition, that are then etched through repeated Deep Reactive Ion Etching cycles (DRIE) of crystalline silicon. Schottky-barrier junctions are formed between the Si and NiSi terminals. With this process, vertically stacked, suspended, silicon nanowire (Si-NW) arrays anchored between two NiSi pillars are achieved.

A gas phase silanization (CVD) was performed on the surface of silicon nanowires in cleanroom, in order to form an absolutely homogenous layer of GPTES molecules. To this aim, bare silicon nanowires were activated, firstly, by oxygen plasma treatment for 15 minutes (Harrick Oxygen-Plasma, 200 mTorr, 29 W). This bombardment of the surface with high-energy oxygen ions formed a layer of hydroxyl group on the surface (hydroxylation). Then, activated nanowires were placed in a desiccator in normal vacuum conditions along with 500 μ L of the silane-solution for 2 hours. Chemical reaction between hydroxyl groups and methoxy groups on silane molecules in gas phase formed durable and homogenous binding (covalent bonds) of GPTES across the nanowire surfaces⁴⁸.

The aptamer was developed for specific interaction with TFV. These aptamers were functionalized with amino group at their 3' terminal to be used as a handle for immobilization. In order to activate the aptamers they were diluted to the final concentration of 2 μ M in 10 mM PBS solution and 1 mM MgCl₂, then they were unfoloed by heating them at 90 °C for 5 minutes and let them to cool down to room temperature (approximately 30 minutes). The silane-coated nanowires (described above) were incubated in this solution of 2 μ M TFV-aptamer for 30 minutes. This interval assures the complete forming of sensing surface by covalent binding between amino groups of aptamers and glycidyl groups of GPTES. After that, nanowire chips were washed with PBS and distilled water to remove the any unbound aptamers and salts. At the end, chips were dried by nitrogen gas, and stored at room temperature.

A series of denaturation, pH shocking and refolding was used for surface regeneration (unbound the target from aptamer and restore the aptamers to their folded and functional

form they had before the conformational change): Nanowires were excessively washed with deionized water to remove any drug or salts; then, there were incubated in NaCl 2M solution at 85°C for 10 minutes in order to denature the aptamers and, thus, unbound target from aptamer; further immersed in NaOH 1M solution to give a pH shock to them to eliminate any remaining drug-aptamer binding, followed by deionized-water rinsing; finally inserted in PBS-MgCl₂ 1mM solution at 85°C for 2 minutes and let to cool down for 15 minutes. Last step caused any denatured or collapsed aptamer to be refolded, and thus sensing surface was functional for a new detection.

Electrical and Morphological Analysis

The electrical characteristics of the nanofabricated memristive structures are acquired using a probe station and contact probes configuration in the semi-logarithmic scale using a Keithley 6430 semiconductor characterization system from in a two terminal configuration by double sweeping the source to drain voltage between -2.4 V and +2.4 V. The responsive speed for the measurement of each nanodevice is in the range of 1,5 - 2 minutes. These measurements allow the observation of the changing hysteresis properties of the memristive biosensors as a function of the surface treatment that leads to a charge variation. Electrical characterization performed on wires after the nanofabrication process indicates a hysteretic loop at zero voltage for the forward and the backward regimes of the current. In these devices, the memory effect depends on the charge carrier rearrangement at the nano-scale due to external perturbations, such as an applied voltage bias. The hysteresis modification after the surface treatment is studied in terms of voltage difference calculated between the forward and backward current minima of the electrical characteristic curves. All of the measurements were carried out at room temperature, in dry conditions under controlled relative humidity (rH%). It is important to highlight that throughout the present work a stable value for the rH% is maintained to assure that the observed hysteresis variations are directly corresponding only to the drug binding reaction. In order to ensure a proper control, the rH% was continuously monitored in this work. More specifically, the monitoring was performed using feedback from a Rotronic HC2-C04 Thermo-Hygrometer tool that provides an accurate control of rH% of the measurement environment.

In addition to the electrical validation, morphological analysis of the memristive biosensors is carried out using a Scanning Electron Microscopic MERLIN, from Zeiss. The imaging is performed for 1.7 kV (Fig.1 main), 15 kV and stage at 34.9° (inset image Fig.1) and 1.2 kV (Fig. 3). Due to the top-down nanofabrication process used, the width along the structures is not perfectly homogeneous. However, this can increase the freely available area for DNA aptamer binding, which increases DNA aptamer loading and hence better performance of the biosensors.

RESULTS AND DISCUSSION

Coupling electrical hysteresis and DNA-aptamer regeneration nature

The regeneration properties of the DNA-aptamers are directly reflected on the biosensors hysteretic electrical response, and expressed through a voltage difference appearing in the semi-logarithmic current to voltage characteristics. The electrical properties of these nanostructures strongly depend on the local environment surrounding the freestanding nanowire, and on the charges' change on the surface of the wire. These charges induce a field effect surrounding the conductive channel in the wire of the memristive device that significantly changes the inside carriers' concentration and consequently affect the whole conductance. Therefore, the hysteresis observed on the device conductance is modified when charged substances are bound to the surface, and the current minima for the forward and the backward regimes appear shifted to different voltage values as shown in Figure.1.

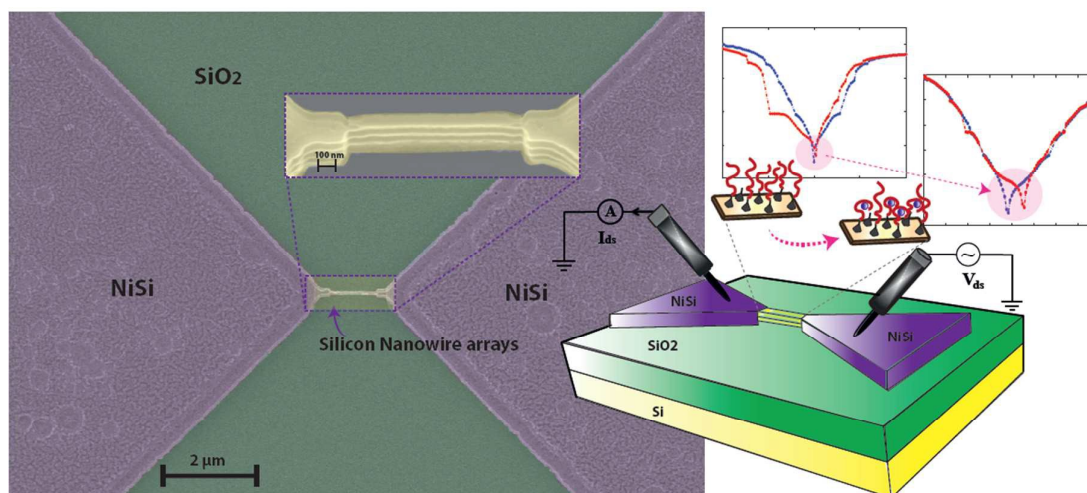


Figure 1. Schematic representation illustrating the memristive aptasensor and SEM micrograph depicting the Si-NW arrays anchored between the NiSi pads, which serve as electrical contacts of the freestanding memristive nano-device. Schottky-barrier junctions are formed between the Si and NiSi terminals. The position of the current minima for the forward and the backward regimes changes after the surface treatment introducing a voltage difference in the semi-logarithmic current to voltage characteristics.

The whole sensing cycle consisting of aptamers immobilization, target-drug binding, aptamer regeneration, and target-drug re-binding is efficiently, and, for the first time illustrated through the variations of the electrical hysteresis of the memristive nanostructures.

The average hysteretic response of nine independent the memristive nanodevices to the consecutive surface treatment steps performed is featured in Figure 2. All measurements are performed in air under controlled and stable rH%. It is worth to note that after

treatment with DNA-aptamers the electrical response of the nanodevices indicates a voltage difference of 116 ± 34 mV. Furthermore, as hypothesized, following the nanodevices exposure to the target drug solution of 100 nM, an increase of the voltage difference occurs as an aftereffect of the drug binding, reaching the value of 156 ± 24 mV indicating an increase of 34.5 %. This change is a consequence of the modification in the charge density at the surface of the device due to the introduction of the negatively charged drug molecules.

However, the most promising outcome undoubtedly lays on the electrical response after the regeneration of aptamers that follows the drug binding. Interestingly, it is demonstrated that the voltage difference decreases after the regeneration process to the value of 120 ± 15 mV, and, actually returns at the level that corresponds to the value obtained initially just after the aptamer immobilization on the surface. In order to verify the nano-biosensor reliability in repeated measures and the capability for further capturing and detecting the target molecules after the aptamers regeneration as well as the biosensors response to a different concentration of the sensing target the very same nano-devices are anew exposed to the drug solution, but, this time, a higher concentration of $1\mu\text{M}$ is introduced. Higher concentration of reagent implies higher charge density and, as expected, the uptake of higher concentration of the drug results in a more pronounced modification of the voltage-gap. In this case a 100 % increased signal is acquired and the voltage difference indicates the value of 240 ± 23 mV that is twice the signal exhibited by DNA-aptamers. It is worth to mention that the percentage of the signal difference for the hysteresis modification between the two different drug concentrations is estimated around 53.8 %. Finally, a further regeneration step is carried out to illustrate the nano-biosensors repeated-regeneration character. It is indeed demonstrated that the voltage difference of 121 ± 39 mV registered after this further regeneration process also coincides with the value achieved initially for the aptamer immobilization on the surface that is also the same to the one obtained after the first regeneration.

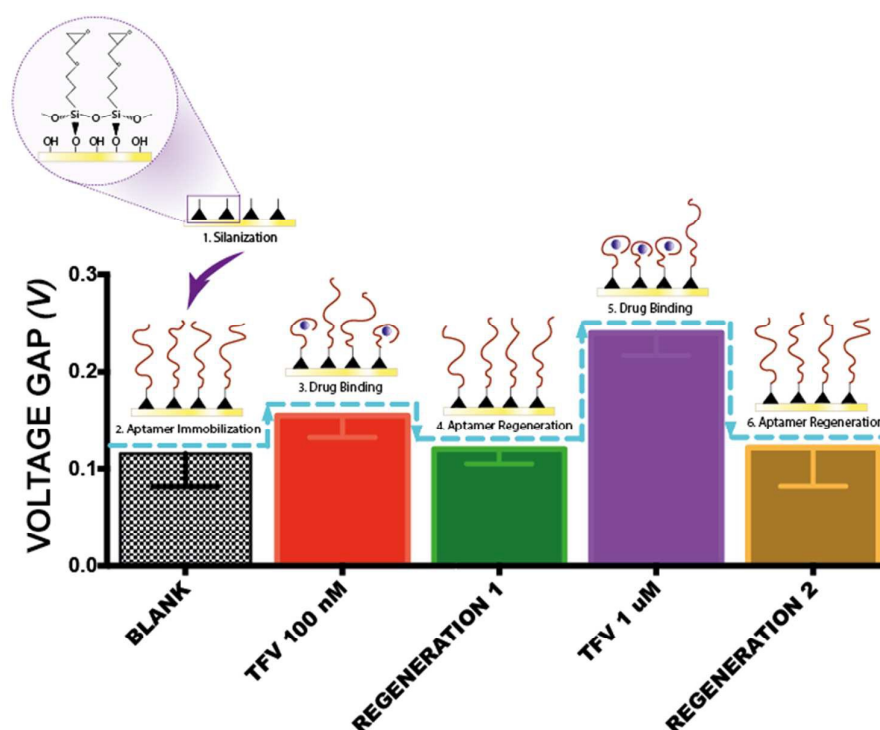


Figure 2. DNA-aptamer immobilization, target molecule binding and DNA-aptamer regeneration cycle, illustrated through the electrical hysteresis variations.

That means the biosensors is very reproducibly down to the original value of the voltage-gap after any regeneration process and, therefore, ready for a new measure of the target molecule concentration. The reproducibility of the device after the regeneration, here presented as the percentage differences between the hysteresis modification signals obtained after the initial aptamer immobilization and the two regeneration procedures applied, is around 3.4 % and 4.3 % for these two regenerations, respectively. Those percentages can be considered negligible indeed, also comparing with measurement errors usually obtained in detection on drug binding. Overall, it is worth highlighting that all these findings indicate a clear evidence that DNA-aptamer natural characteristics to binding to drugs and related functions may indeed be efficiently transduced by using the memristive phenomena and its electrical hysteretic properties as well, which constitute here a premiere case of evidence.

Effective, Label-free, drug screening

Having demonstrated the direct and highly efficient response of the nano-biosensor prototype to accurately follow the various steps of DNA-aptamer binding-regeneration cycle, we further leverage this holistic approach for the label-free, ultrasensitive drug detection, considering TFV, an antiviral drug for HIV treatment, as a model of drugs for this case study.

The average voltage difference with increasing TFV concentration is presented in Figure.3.

Solutions of TFV are prepared in the concentration range from 100 aM to 1 μ M. The detection is then performed for drug concentrations belonging within and slightly below the clinical range, opening to the possibility for future applications with also diluted serum as well as, assuring economy of reagents, thus, requiring minimum amount of clinical samples. To better show the performance of the proposed new biosensors, first, the TFV detection is proposed in Phosphate Buffer Saline (PBS) solutions as a proof of concept. Second the effective drug detection in human serum is proposed in this paper too. All measurements are performed in air under controlled and stable ambient conditions (see supplementary information). In both cases, the successive uptakes of negative charge at the nano-device surface leads to an increasing trend of the voltage gap that follows the increasing concentration of the detection target drug.

The average value obtained by nine independent nano-sensors indicates a voltage gap of 29 ± 6 mV is first obtained for a concentration of 100 aM in buffer solution. An increasing trend of this parameter of the hysteresis is then depicted following the dose increase, as demonstrated in Figure.3a till the value of 193 ± 51 mV for 100 nM, the highest concentration implemented for the case of buffer solution. In human serum, a hysteresis modification of 69 ± 38 mV is initially indicated for a concentration of 100 fM and reaches finally the value 295 ± 61 mV for 1 μ M as well shown in Figure.3b. At the end of the complete dose response cycle in the human serum a regeneration step is performed. Then the regenerated memristive aptasensor is anew exposed to an intermediate TFV concentration of 1 nM, so-called as “positive control” in order to further validate the consistency and effectiveness of the drug-detection methodology. It is depicted that indeed the signal obtained for the hysteresis voltage gap depicting the value of 97 ± 31 mV indicating a signal that corresponds within the defined-dose response curve and to the value foreseen by the previously recorded dose-response. This is a very important result that clearly demonstrates the efficiency of the proposed method and its applicability for continuous monitoring of therapeutic compounds as well. Moreover, the regeneration capability of memristive aptasensor is also demonstrated in real sample environment thanks to the regeneration step followed by the intermediate drug detection. This aspect is presented with more detail in supporting information Figure S3. Furthermore, the implementation of a negative control drug, enzalutamide (a widely used anti-prostate cancer drug) at a concentration also equal to 1nM is directly performed following the positive control step. Indicatively, the 89 ± 35 mV voltage gap measured for the negative control actually coincides to the value obtained for the positive control bringing no significant hysteresis modification (<9 % difference) with respect to the value obtained just before with the implementation of the 1 nM concentration for the specific drug. Thus, it can be concluded that no drug binding occurs for the negative control case, exhibiting the specificity and selectivity of the methodology applied. The obtained calibration curves shown in Figure 3 demonstrating the dose response for the specific target achieved, follow indeed a typical polynomial fitting equation of third-order $y = \text{Intercept} + \alpha x^3 + \beta x^2 + \gamma x$, with a root-mean-square value (**R**-Square) of 0.986, Residual Sum of Squares (RSS) of

4.36×10^{-6} , Intercept of 49 ± 6 mV and coefficients $\alpha = 2.35041 \times 10^{-4} \pm 1.285 \times 10^{-4}$, $\beta = 0.00272 \pm 3.589 \times 10^{-4}$, $\gamma = 0.0091 \pm 0.0027$ for buffer and a **R-Square** of 0.99, RSS of 0.19, Intercept of 112 ± 12 mV and coefficient $\alpha = 9.39543 \times 10^{-4} \pm 3.42 \times 10^{-4}$, $\beta = 0.0081 \pm 0.0016$, $\gamma = 0.0291 \pm 0.003$ for human serum. A very low limit of detection (LOD) of 3.09 pM for PBS buffer and of 1.38 nM for human serum are calculated from 9 independent samples following the method reported by Armbruster et al.³⁰ (supplementary material). The hereby demonstrated LODs are compared to the state of the art, and they are highlighted as the best ever obtained, to the best of our knowledge, so far reported in literature for drug detection in general, and for TFV in particular (Table 1).

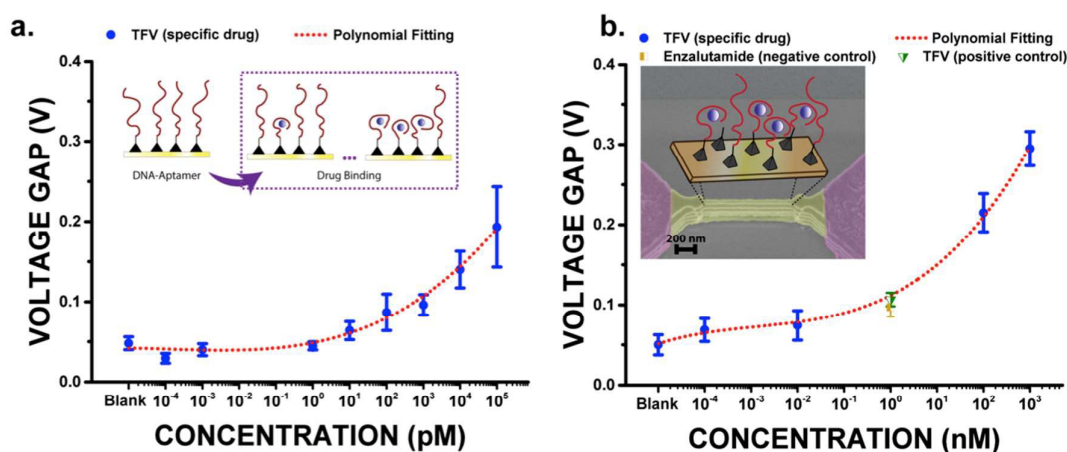


Figure 3. Analytical performance and effective drug detection through the electrical hysteresis variations in buffer (a) and in full human serum (b).

The LOD presented here demonstrates 10 times higher sensitivity for the in-buffer drug detection with respect to the literature, and the table 1 shows twice better performance for drug sensing in human serum, ever obtained. The best case reported so far in human serum is mentioned in the work of Radhapyari et al.³¹ that reached 0.035 nM but in 10 times-diluted human serum, while we reached 1.38 nM in un-diluted one, which is away much better. Thus, it is worth to point out here that we worked with non-diluted human serum, further indicating the higher sensitivity and efficiency of our suggested methodology with respect to the best ever presented in literature.

Table 1. State of the art list of reported drug detection to date

Target drug	Method*	Surface	LOD (nM)		Linear Range (nM)	Ref.	Comments
			Buffer	Bio-matrix			
6-mercaptopurine	Voltam	Graphite/Polypyrrole/MWCNT	-	80	200 - 100000	32	Diluted human urine sample
Gleevec	Conduc	Si-nanowire	-	-	Up to 100	33	-
Paracetamol	Voltam	MWCNT*	2.9	-	5 – 1000	34	-
Penicillin	Voltam	Boron-doped diamond	-	320	400 - 100000	35	Human urine sample
Tamoxifen	Voltam	Enzyme/Polyaniline/Pt	0.2	-	27 - 297	36	-
Clenbuteral	Voltam	Benzedithiol-GNPs*	-	43.96	100 - 800	37	Diluted rat urine sample
Chloramphenicol	Ampero	Cds NPs/GNPs	0.14	-	0.15 – 2.94	38	-
Artesunate	Ampero	Gr-Polyaniline Nanocomposite	0.031	0.035	0.13 - 1	31	Highly diluted human serum
TFV	BSI	Glass chip	2.5	-	Up to 20	39	-
TFV	LC-MS	-	-	680	1360 to 350000	40	Human urine
TFV	Voltam	HMDE*	450	870	Up to 17000	41	Diluted and precipitated human plasma
TFV	LC-UV	-	-	10.4	35 to 3480	42	-
TFV	LC-MS	-	-	7	35 to 3480	42	-
TFV	Memristive	Si-nanowire arrays	0.0031	1.38	0.001 to 1000	Present work	Full human serum

Voltam: voltammetry; Conduc: conductance; Ampero: amperometry; BSI: back scattering interferometry; LC: liquid chromatography; MS: mass spectroscopy; UV: ultra violet; MWCNT: multiwalled carbon nanotubes; GNP: gold nanoparticle; HMDE: hanging mercury drop electrode

Surface Characterization

The alterations of the surface morphology of the device upon the consecutive treatment steps, is a further proof of the applied bio-modification methodology efficiency. It is worth to mention that the surface analysis is performed immediately after each modification step and no other intermediate step or treatment is applied to the devices than the treatment with the molecules of interest, leading to the heuristic conclusion that the morphology of the surface is clearly changed due to the reagents of interest that are indeed present on the nanostructure.

Of course, the variations of the nanodevices mean width measured after the different bio-modification steps throw light on the effective binding of the related molecules. Data are analyzed considering several measures along the structure on different devices depict a mean increase of 3.25 ± 1.22 nm at the initial mean width of the structures for measurement performed upon the immobilization of DNA-aptamers on the silanized surface while the mean width of 82.78 ± 1.08 nm is registered initially, just after silanization. This increase is compatible with the thickness of a layer of DNA-aptamer as registered immobilized on Au surface with sizes in the ranges of 2.6 - 4.5 nm or 4.8 - 4.9 nm in salt and in aqueous solution, respectively⁴³. In literature, the aptamer height is also indicated with a value of 4-5 nm for an aptamer hairpin loops immobilized on DNA crossover tiles⁴⁴ and of 4.15 nm for DNA-aptamers bound on the surface of Si-NWs⁴⁵. Then, according to literature, the binding of large molecules such as proteins overall increases the height of the biological assay that is a well-expected consequence of the presence of the large bio-molecules, like for example proteases or proteins⁴⁴⁻⁴⁷. Nevertheless, it is important to mention that an opposite effect has been reported as well especially when up-taken molecules are very small like for example adenosine⁴³. This phenomenon is due to a change in aptamer conformation during the small molecules binding. Following this trend, we expect the device mean width appears anew reduced upon the drug binding for the case of TFV: and in fact, we have measured a value back to only 84.08 ± 2.36 nm has been registered after the TFV uptake. The average difference after the drug binding of 1.95 ± 2.42 nm corresponds to the aptamer bending mainly due to folding effect that follows the drug binding, taking into consideration mean value of 86.03 ± 0.57 nm obtained after the aptamer immobilization. This value adheres in good approximation to the value reported in literature for the DNA-aptamer conformational changes after the drug binding^{46, 51}. Namely, a comparison between the initial heights for the unfolded DNA-aptamers of 4.8 nm and the height for the folded aptamer of 3.8 nm in the presence of adenosine, a small molecule at the size range of drugs, have been reported⁴³. The case of unbounded, unfolded but largely-collapsed aptamers due to the present of a salt solution is as well mentioned, and finally results to an average aptamer height of 2.6 nm⁴³. Those findings finally give an aptamer height difference at the range of 1 nm to 2.2 nm⁴³ after the exposure to the small molecules in salt containing solution.

In the present study a combination of these phenomena occurs as well, a fact that is also demonstrated by the average mean width difference value upon the exposure of the device to the drug solution, that indeed arises within the before-mentioned range. Upon the device exposure to the drug solution, part of the DNA-aptamers is folded after successfully binding with the target drug. However, not all DNA-aptamers finally bind efficiently with the TFV, and, as a consequence, those aptamers remain unfolded or collapse due to the presence of salts and positive substances that are included in the buffer or the serum solution. This phenomenon is well reflected through the standard error hereby presented. Namely, the value of average difference varies from the height of full standing aptamer on the silanized surface, to the height of folded aptamers due to the drug binding that is the favorable behavior of the system, and finally in some cases to a completely collapsed system on the surface.

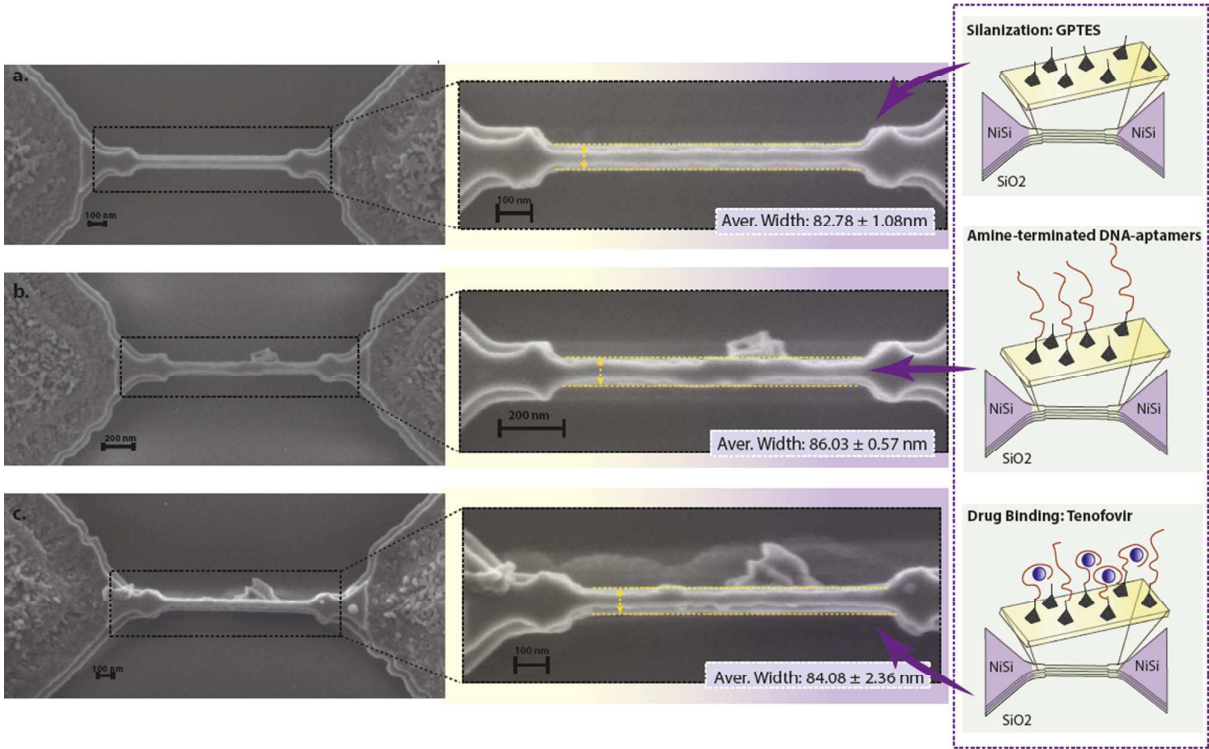


Figure 4. SEM micrograph depicting the nanodevices anchored between the NiSi pads, which serve as electrical contacts of the freestanding nanostructures. The nanodevices are imaged directly after the silanization process (a), after the DNA-aptamer immobilization on the surface (b) as well as after exposure to TFV solution of 10 μM . The mean width of the nanodevices changes accordingly, following the conditions introduced by each surface treatment.

CONCLUSIONS

In the present work, a novel application of the memristive effect is presented for the first time, for detection of therapeutic compounds, through the coupling with the DNA-aptamer properties.

The hysteretic electrical characteristics exhibited by nanofabricated silicon nanowire-arrays, arising via surface treatment, are leveraged for successfully performing complete cycles of detection comprising DNA-aptamers immobilization, target binding, DNA-aptamers regeneration. A successful label-free screening of TFV, used here, as a model drug and case of study of any therapeutic compound, is hereby demonstrated by achieving the best ever obtained LOD of 1.38 nM in drug monitoring applications in full human serum.

This novel approach holds great promise for the real-time continuous monitoring of therapeutic compounds that is a very important perspective of the suggested nano-biosensor in the medical practices. Especially, in the field of personalized medicine, which still lacks of analytical methods for the continuous monitoring of low traces of drug species and therapeutic compounds.

Moreover, this paradigm can be expanded to detect a wide range of different molecules by accordingly choosing the corresponding aptamer-probes, and offer the potential for integration in more complex platforms for multiplexing configurations.

The present work, as a holistic approach, is of great significance for the field of label-free electrochemical nano-biosensors, boosting breakthrough applications in personalized medicine.

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Author contributions

I.T and N.A contributed equally. S. C leded the research and conceived some experiments with I.T and N.A. I.T and N.A performed all the fabrications, surface treatments, data acquisition, data analysis, and SEM imaging. I.T, N.A and S. C perform comparison with the

literature. I.T and N.A have prepared the manuscript. S. C gave suggestions on the manuscript. I.T and N.A, S. C, and G.d.M have revised the manuscript.

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GRAPHICAL ABSTRACT

