

# “Bio-functionalization study of Memristive-Biosensors for Early Detection of Prostate Cancer”

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**Abstract**—Silicon nanowires are reported for their application in bio sensing area and their potential in the detection of various biomolecules. In the present work, freestanding two-terminal Schottky-barrier silicon nanowire arrays exhibiting memristive behavior are fabricated to obtain Memristive-Biosensors. Scanning electron microscopy reveal details on the morphology of the fabricated structures. The memristive devices are functionalized with anti-free-Prostate Specific Antigen (PSA) antibody by two strategies: a) direct passive adsorption on the device surface, and b) bio-affinity approach using Biotin-Streptavidin combination. The electrical behavior of the so-obtained Memristive-Biosensors is examined dealing with the two systems of bio-functionalization. The presence of biomolecules linked to the surface of the nanostructures is detected by a voltage gap appearing in the memristive electrical characteristics. The system shows the potential for applications in molecular diagnostics especially due to possibilities for detection in the femto molar ranges that allow early detection of the cancer disease.

**Keywords**—Biosensor; Memristive behavior; Silicon nanowires; Bio-functionalization; PSA

## INTRODUCTION

Semiconductor nanowires and semiconducting carbon nanotubes are reported as promising building blocks for biosensors, enabling direct electrical detection of biomolecules and fast, low-cost analysis of biological processes [1-6]. Thanks to the high surface-to-volume ratio that the nanowire structures present, their electrical properties are strongly influenced by minor perturbations [7, 8]. Furthermore, the nanowire structures exhibit tunable electron transport properties and the charge accumulation or depletion takes place in the bulk of the structure. Consequently, these nanostructures present qualities not available in larger scale devices and can potentially enable label-free sensing via direct electrical readout when the nanostructures are used as a semiconducting channels of field-effect transistors [9] for example for cancer markers [3] or DNA [10] detection.

Studies on memristive behaviour have attracted both physicists and researchers from other fields for their precise molecular response. In 1971, Leon Chua presented the fourth fundamental circuit element named as memristor linking electric charge and magnetic flux [11]. In 2008 Strukov et al. [12] introduced the first physical implementation of a memristor consisting of a two-layer thin film of TiO<sub>2</sub>, one of them doped with oxygen vacancies, sandwiched between platinum contacts.

The model can be considered as a simple analytical example that memristance arises naturally in nanoscale systems in which solid-state electronic and ionic transport are coupled under an external bias voltage. Pronounced hysteresis in electrical characteristics is a unique and important signature of memory devices [13]. The hysteresis appearing in the current-to-voltage characteristics has been attributed to a wide range of possible phenomena, including molecular redox events, and metal filament formation and destruction. Memristive devices have already been used in many applications, since memristors enable new possibilities for computation and non-volatile memory storage as for example the construction of memristor-based digital logic circuits [14], applications regarding artificial synapses [15-17], Resistive RAM (ReRAM) memories [18] and Generic Memristive Structure (GMS) for 3-D FPGA applications have been proposed [19]. Moreover, memristive silicon nanowire devices functionalized with antibody films are recently introduced as Memristive-Biosensors for bio sensing purposes taking advantage of their memristive electrical characteristics [20, 21]. More specifically, the hysteresis loop of the current-to-voltage characteristic curve of the devices provides a new approach in bio-detection, sensing the presence of biomarkers and achieving a low-cost label-free detection of biological processes in dry conditions. Furthermore, in another recent application, memristive silicon nanowire devices were used as pH sensors demonstrating the possibility of dry sensing of pH via the detection of a different amount of hydrogen ions of a salt solution deposited over the memristive pH sensor before

sample excitation [22]. In the present work we focus instead on two different bio-functionalization techniques to fabricate optimized Memristive-Biosensors for Prostate Specific Antigen early detection.

## MATERIALS AND METHODS

### *Fabrication of Memristive-Biosensors*

Memristive silicon nanowires are fabricated through a top-down fabrication process performed using commercially available (100) oriented Silicon-On-Insulator (SOI) wafer with low boron concentration ( $N_A \approx 10^{15}$  atoms/cm<sup>3</sup>). The substrate is first coated with PMMA (poly (methyl methacrylate)), and Electron-Beam Lithography is applied to pattern the first mask for metal contacts. A 25nm-thick layer of Nickel is then evaporated onto the device. A lift-off process using acetone as solvent follows, leading to the definition of Nickel contact regions. Subsequently Nickel silicidation is performed in order to form NiSi contact pads for electrical characterization. Nickel silicidation is obtained by annealing performed by successive exposure for 20 minutes to forming gas at 200°C, 300°C and 400°C, respectively.

A layer of 50nm Hydrogen Silsesquioxane (HSQ), negative tone resist for Electron-Beam Lithography, is spin-coated on top of the wafer and patterned into lines using Electron-Beam Lithography process followed by repeated Deep Reactive Ion Etching cycles to obtain vertically-stacked, free-standing silicon nanowires anchored between two NiSi pillars.

#### *Method A: Passive Adsorption*

The fabricated memristive silicon nanowires are first incubated in a piranha solution (H<sub>2</sub>O<sub>2</sub>: H<sub>2</sub>SO<sub>4</sub> in ratio 3:1) for 20 min in order to clean the sample surfaces from any organic residues as well as to generate more surface hydroxyl-terminating groups to enable chemical attachment of the biomolecules. Subsequently, different substrates of silicon nanowire arrays are functionalized by exposure to antibody solution. For this, the nanostructures are separately incubated overnight at room temperature in the dark in 1mL solution of 50µg/mL anti-free PSA Ab (Abcam, Ab10187) for the study of direct linkage bio-functionalization.

#### *Method B: Affinity Approach*

In parallel nanowire structures originating from the same fabrication process and the same SOI wafer, are separately incubated for 4 h at room temperature in dark, in 1mL solution consisting of a mixture of 200 µL/mL Biotin-BSA and 100 µL/mL Streptavidin in filtered PBS (filtered PBS is used for dilution and rinsing).

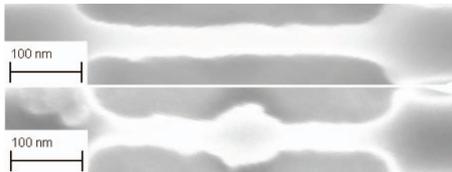


Fig. 1. Top view SEM images of the nanofabricated structures before bio-functionalization (Memristive device) on top and after the bio-functionalization process with Passive Adsorption (Memristive-Biosensor) on the bottom.

After the incubation, all the substrates are gently washed twice with 1.5 mL of PBS, and once with MilliQ water and then are left for 50sec to dry in air at room temperature, semi-covered, before the electrical measurements.

The substrates bio-modified with the Biotin-Streptavidin complex are subjected to a further bio-modification by overnight incubation at room temperature and in the dark in a 0.5mL solution of biotinylated anti-free PSA Ab, at a concentration of 50 µg/mL (Abcam, Ab182031). In both cases the so-obtained devices are then gently washed with 1.5 mL of PBS and then with MilliQ water and are left for 50sec to dry in air at room temperature semi-covered, before the new electrical measurements.

## RESULTS AND DISCUSSION

### *A. Morphological analysis*

Morphological analysis of the nanofabricated structures is carried out using a Scanning Electron Microscopic MERLIN, from Zeiss, directly after the nanofabrication process and also after bio-functionalization. The accelerating voltage used for the SEM imaging is equal to 3kV and 2kV respectively.

A 2 nm layer of Osmium tetroxide (OsO<sub>4</sub>) is sputtered onto the bio-functionalized devices in order to avoid the protein charging effect and to provide the needed contrast to images. Fig.1. shows a top view of nanofabricated structures of initial mean width of 38 nm and length of 410 nm after the fabrication process and after bio-functionalization.

Due to Deep Reactive Ion Etching process the width along the structures is not perfectly homogenous. However this is an advantage for our detection aim. In fact, the surface roughness finally enhances the binding of the proteins on the device by increasing the potential binding area and overall enables the bio-functionalization process. Fig.2. presents a tilted view of nanofabricated structures of initial mean width of 90 nm and length of 980 nm. The stage holder is tilted at 35.9° and 35.4° in order to clearly visualize the nanostructures. An increase in the device diameter is demonstrated by Fig.1. and Fig.2. due to the presence of the adsorbed layer of antibodies on the nanowire surface. Accumulated data considering several measures along the structure on different devices depict a mean increase of  $20 \text{ nm} \pm 4 \text{ nm}$  at the initial mean width of the structures, which is compatible with a single layer of antibodies expected with sizes close to 14 nm.

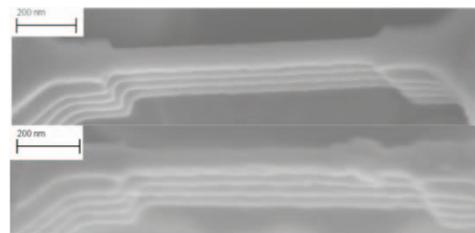


Fig. 2. Tilted SEM images acquired from the nanofabricated structures before bio-functionalization (Memristive device) on top and after the bio-functionalization process with Affinity Approach (Memristive-Biosensor) on the bottom.

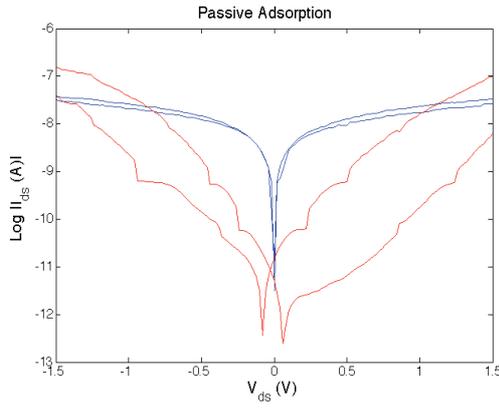


Fig. 3. Semi-logarithmic current to voltage characteristics exhibited by a Memristive-Biosensor. Electrical characteristics for bare silicon nanowire-Reference measurement- (Blue) and for the same nanowire after the bio-functionalization process with direct passive adsorption of anti-PSA (Red).

In some cases a more significant increase of the structures' width of  $47 \text{ nm} \pm 6.7 \text{ nm}$  is observed with respect to the device initial mean width, due to protein aggregation. The final Ab-layer size is expected to be larger than that of a monolayer since the value registered on the functionalized wires needs to be reduced due to the layer of  $\text{OsO}_4$  sputtered onto the bio-functionalized nanowires. Furthermore, issues such as protein aggregation may also explain the deviation from ideal monolayer coating on the nanowire surface.

#### B. Electrical measurements

The electrical characteristics of the Memristive-Biosensors of initial mean width of  $38 \text{ nm}$  and length of  $410 \text{ nm}$  are obtained by measuring the current-to-voltage characteristics of the device structures in air on dried samples using a Cascade Microtech Probe Station and a Hewlett-Packard 4165A Precision Semiconductor Parameter Analyzer.

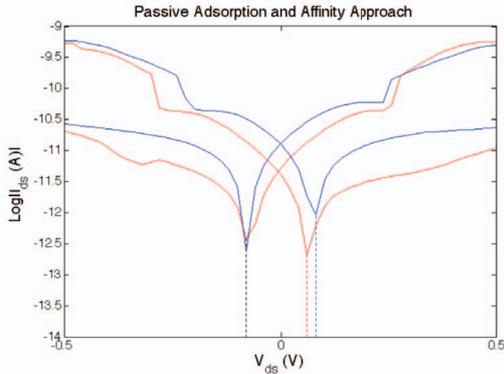


Fig.5. Semi-logarithmic current to voltage characteristics exhibited by Memristive-Biosensors with different bio-modified surface: Direct adsorption of anti-PSA on the nanowire surface -Passive Adsorption- (Red) presenting a Voltage gap of  $0.14 \text{ V}$  and Streptavidin-Biotin combination+anti-PSA-Affinity Approach- of a Voltage gap of  $0.16 \text{ V}$  (Blue).

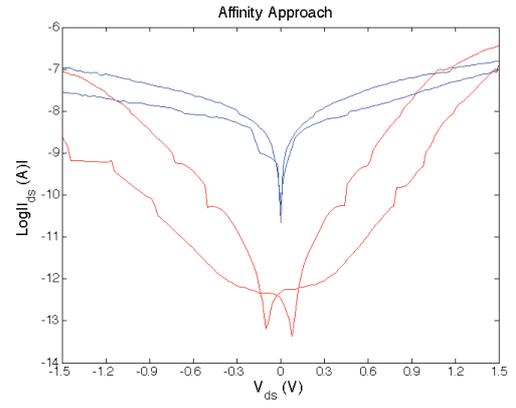


Fig. 4. Semi-logarithmic current to voltage characteristics exhibited by a Memristive-Biosensor. Electrical characteristics for bare silicon nanowire-Reference measurement- (Blue) and for the same nanowire after the bio-functionalization process with Streptavidin-Biotin combination + anti-PSA (Red).

The measurements are carried out at room temperature in controlled humidity environment. For sensing measurement, a drain to source voltage sweep is performed forward and backward in the range  $-2.4 \text{ V}$  to  $+2.4 \text{ V}$ . The back-gate potential  $V_{BG}$  is kept grounded during the acquisition. A reference measurement is first applied on the fabricated structures just after the nano-fabrication process. Then follows is the electrical monitoring of the Memristive-Biosensors functionalized with antibodies for the direct comparison of the two bio-functionalization approaches, as shown in Fig.3. and Fig.4. respectively.

For bare nanofabricated wires, the current to voltage characteristics indicate pinched hysteresis loop at zero voltage. In these devices, the memory effect depends on the carrier rearrangement at the nanoscale due to external perturbation. However, the pinched hysteresis appears shifted from zero voltage to different voltage values when biological substances are present on the device surface. Moreover, this shift is different in the forward branch of the curve with respect to the backward one. Namely, a voltage gap is created in the semi-logarithmic current to voltage curve after the nanowire bio-modification as a further memory effect on the voltage scan across the Memristive-Biosensor as also reported in [21].

The presence of biological substances around the freestanding nanowire contributes to extra charges surrounding the device and the net contribution of their charged residues acts by creating an electrical field surrounding the channel of the memristive device, resulting in an effect equivalent to that obtained in case of nanostructures without any bio-functionalization but fabricated with an all-around silicon gate [20]. The all-around gate contributes to this particular conductivity of these structures, which includes the presence of a voltage shift in the electrical characteristics. It can be observed that though narrow, the voltage gap is still distinctly present for the direct antibody adsorption in Fig.3. This result indicates that the method of direct attachment demands high concentration of reagents for successful sensing output with the memristive detection methodology.

Considering the electrical characteristics originating from the implementation of the bio-affinity based functionalization methodology that takes advantage of the extremely strong Biotin-Streptavidin binding, the voltage gap is clearly noticed in Fig.4. A comparison of the Voltage gap obtained for the different bio-functionalization methods under study is shown in Fig.5. Data of 16 devices for each bio-functionalization method indicate an average voltage gap of  $0.1407 \text{ V} \pm 0.0046 \text{ V}$  for the direct adsorption method and of  $0.1582 \text{ V} \pm 0.0051 \text{ V}$  for the affinity method respectively, a small yet statistically significant difference regarding the values of the voltage gap obtained. It is worth mentioning that these results are obtained for the same concentration of antibodies, thus highlighting the economy of reagents required for miniaturized bio-assays.

All the more, as there exists a difference in the voltage gap, both before and after the antibody adsorption, the final read-out corresponds to the antibody adsorbed on the devices and not due to the signal arising from biotin-streptavidin molecules only. In addition, although the antibody is biotinylated, the size of biotin is several folds smaller than the size of the antibody itself, thus making the contribution of the labeled biotin to the final read-out negligible.

These measurements suggest that with the bio-affinity based functionalization methodology it is possible to detect, through the memristive behavior, biological substances at a lower concentration than achievable via direct adsorption only. Thus, the bio-affinity based functionalization method holds a better promise for the measurement of low concentration of biological substances, especially required for clinical readings of cancer markers in the early stages of the disease.

#### CONCLUSIONS

In the current work, two different bio-functionalization techniques are applied using PSA-specific antibodies to obtain biosensors arrays that exhibit memristive electrical characteristics (called Memristive-Biosensors). The voltage gap appearing in the semi-logarithmic current-to-voltage curve after the nanowire bio-modification leads to a bio-detection method for anti-free PSA Ab. Furthermore, the results obtained for both methods of functionalization under study indicate that the implementation of the bio-affinity-based functionalization methodology leads to a slightly larger voltage gap for the same concentration of antibodies and is thus more suitable for applications with lower concentration of target cancer markers.

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