



A new method for selective functionalization of silicon nanowire sensors and Bayesian inversion for its parameters

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ABSTRACT

In this work, a modification procedure for the functionalization of silicon nanowire (SiNW) is applied in biological field effect transistor (BioFET) system. The proposed method precedes the silanization reaction in a manner that the only SiNW and not its SiO₂ substrate is functionalized by (3-Aminopropyl) triethoxysilane (APTES) initiators. This method has an effective role in increasing the sensitivity of BioFET sensors and can be applied in commercial ones. Furthermore, we introduce an efficient computational technique to estimate unknown sensor parameters. To that end, Bayesian inversion is used to determine the number of PSA target molecules bound to the receptors in both selective and nonselective SiNWs. The approach is coupled with the Poisson-Boltzmann-drift-diffusion (PBDD) equations to provide a comprehensive system to model all biosensor interactions.

1. Introduction

Semiconductor-based materials are of substantial importance in biotechnology and especially in fabricating label-free biosensors, in nanofluidic separation systems, and in lab-on-chip technologies. The arrival of mechanical, chemical and electrochemical approaches coupled with these nano- and micro-scale technologies has been a milestone for the functionalization and selective activation of surfaces (Rohde et al., 2006).

One suitable method to electrically detect biomolecules is implementing biologically activated nanowires embedded in field-effect transistors (FETs). These sensors offer several benefits for the detection of biological species because (i) nanowires constitute the conducting channel in a FET system, (ii) they are generally embedded on the surface of the SiO₂ substrate (i.e. in direct association with the reagent solution), and (iii) the cross-section of the nanowires together with large surface area provides excellent sensitivity toward minuscule changes in the surrounding environment. Charged target biomolecules can specifically bind to the receptors linked to the surface of the BioFET and change FET conduction (Allen et al., 2007). One of these examples is immobilizing antibodies which can interact with a specific target as the recognition elements on the SiNW. The difference between the FET

current before and after the interaction of the target with the antibodies is the basis for the identification and detection of the target molecules in the system (Kim et al., 2007; Tran et al., 2015). It should be noted that even though the SiNW has no direct interaction with the molecules, it can be modified with specific recognition elements that have high affinity to target molecules (Kim et al., 2007).

Oxidized silicon has been widely investigated and used for immobilization of different biomolecules. Hydroxylation (OH groups) leads to siloxane linkages (Si–O–Si) using a variety of silane molecules. The silicon surface preparation for the silanization reaction requires some cleaning steps to remove surface contaminants such as using HF buffer, piranha solution, and oxygen plasma (OP). The most common, commercially routine, and inexpensive reactant for silanization reaction includes APTES reaction. Since the reaction has been widely investigated in the biosensor field, many efforts have been directed to perform silanization reactions spatially selective on the SiNW. Up to now, the most common procedures provided for the silanization reaction includes ligands that cover the entire homogeneous surface in the conventional nonselective functionalization methods and in this way, the number of analytes which bind to the SiNW sensing surface decreases significantly (Masood et al., 2010; Zafar et al., 2018).

In general, one of the main impediments in this regard is the

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unwanted conjugation of the probe molecules to the silicon substrate which causes a drastic decrease in sensitivity and selectivity of the sensor (Masood et al., 2010). Thus, selective functionalization of only the SiNW, i.e., heterogeneous functionalization, is a challenging task for researchers in the BioFET field. To do so, many attempts have been performed to find a simple, cost-effective, and reliable method (Rani et al., 2018).

Renault and colleagues have used micro-contact-printing method to stabilize proteins selectively on the surface of silicon (Renault et al., 2002). In spite of the high performance and the availability of commercial devices for micro-contact printing, this method is only suitable for functionalization at the microscale and of larger scales and cannot be used at the nanometer scale.

In (Bunimovich et al., 2004), the authors reported a method for the spatially selective biofunctionalization of silicon micro- and nanostructures. In their method, an electroactive monolayer of hydroquinone was formed on the surface of H-terminated silicon working electrodes via an olefin reaction with UV-generated surface radicals. They extended the approach to the selective biopassivation of 100 nm wide, 50 nm high nanowires and suggest the method can be used for spatially selective immobilization of proteins (Bunimovich et al., 2004), but this method is based on electrochemical reactions using electro-active materials, and this may lead to some limitations for the desired reaction. In another study (Masood et al., 2010), the authors used an alkylation reaction in the presence of UV light for the specification of the surface of silicon (111) in the presence of surface (100). Since surface reactivity of (111) is greater than (100), the intensity of the UV light is set so that only the surface (111) is involved in an alkylated reaction. Terminal amine groups on the functional monolayer surfaces were used for conjugation of biotin *n*-hydroxysuccinimide ester to selectively functionalize SiNW(111) in the presence of Si(100) as the substrate. But it should be noted that the conditions of such a reaction are complicated, i.e., all steps of the reaction must be carried out under vacuum conditions and also the reactant solutions must be degassed continuously to reduce the possibility of surface oxidation during the functionalization process. In overall, this process is time-consuming and costly.

In (Seol et al., 2012), the “localized joule heating method” was used for selective functionalization of SiNW. In this method, PMMA polymer stabilized on silicon nanowire evaporates by applying a potential of 7 V, while the polymer remains on the substrate, thus allowing the materials to be stabilized only on SiNW without the interference of immobilization of the material on the substrate (Kim et al., 2007). This method, in spite of the success of the specific modification of SiNW, is not specifically capable of modifying the SiNWs in commercial scales, because the PMMA polymer used in this process is expensive and the use of this material has no economic justification.

Dip-pen nanolithography invented by Chad Mirkin has the ability of patterning biomaterials such as proteins and DNA at the nanoscale, but this method can be used only in laboratory scales and cannot be applied in mass production (Lee et al., 2016). Ingebrandt and colleagues used an advanced microspotting system for selective functionalization of biomaterial on the SiNW surfaces (Rani et al., 2018), but the resolution of this kind of functionalization is confined to the microscale and in this method, some nonselective immobilization is predictable.

In (Veerbeek et al., 2018) the thermal hydrosilylation of unsaturated carbon-carbon bonds onto H-terminated silicon used to selectively functionalize the SiNWs with a monolayer of 1,8-nonadiyne and enhanced the selectivity towards the Si-H regions by introducing an extra HF treatment after the 1,8-nonadiyne monolayer formation. This step (partly) removed the monolayer from the SiO₂ regions, whereas the Si-C bonds at the silicon areas remained intact (Veerbeek and Huskens, 2018). This method needs to be controlled precisely because HF is very corrosive and the SiNW surface may be influenced so that the corrosion and the change in the structure of the SiNWs may affect the sensitivity and reproducibility of the sensor. Moreover, the use of HF of large scales has the destructive effects on the environment.

In this study, the selective functionalization of SiNW surface was performed by just the modification of the silanization conditions such as the oxide thickness of SiNW, APTES solvent, the water content in the environment, and temperature. As a matter of the fact, the common silanization reaction was performed on the SiNW surface in the presence of a silicon substrate. This method needs no extra technical conditions apart from routine laboratory ones used in the silanization reaction.

In the presented method, APTES is used as the initiator of the silanization reaction on the SiNW surface. The procedure, i.e., the silanization reaction condition including oxygen plasma (OP), APTES concentration, solvent, and silanization reaction temperature are modified so that only the SiNW will be functionalized without any special or additional equipment. To that end, the APTES parents just react with the SiNW surface rather than the silicon substrate surface. After that, the silanization reaction precedes on the SiNWs vertically without substrate interference in the reaction. We also fabricated the selective device and compare its performance with the nonselective SiNW sensor.

The Poisson-Boltzmann equation is a well-established continuum model to describe the electrostatic interaction of the molecules and the effect of charged biomolecules on the transducer. It can be coupled additionally to the drift-diffusion equations to monitor the sensor electrical conductance (Baumgartner et al., 2013). The effect of the selective functionalization on the BioFET sensitivity will be investigated by comparison of the simulated data and the experimental ones. Furthermore, Bayesian inference is an efficient technique to determine various sensor parameters. The determination of the number of bound PSA molecules to the receptors is essential for reliable device simulation. However, this information cannot be easily obtained with experimental techniques. Therefore, we use the Bayesian inversion to estimate the probe-target density for different PSA concentrations.

2. Fabrication process

2.1. Materials and devices

APTES, Toluene, Acetone, Glutaraldehyde (GA), and streptavidin-coated gold nanoparticles (AuNPs) were purchased from Sigma-Aldrich. Monoclonal anti-prostate specific antigen-antibody (ab10185) and PSA protein (ab167932) were purchased from Abcam (Toronto, Canada). The general equipment used included ultrasonic device and oxygen plasma system for the cleaning and oxidation of silicon chip surfaces respectively. FESEM image and energy-dispersive X-ray spectroscopy (EDS) mapping data were obtained using Tescan MIRA SEM system which features a high brightness Schottky emitter to achieve high-resolution and low-noise imaging. Dropsens STAT 400, a portable potentiostat was used for real-time current measurements.

Figure S1 shows the SiNW on the silicon substrate for selective functionalization of SiNW. Seven arrays of 20 SiNWs on a SiO₂ substrate were used in this study. The SiNW dimensions are a length (L) of 8 μm, a width (W) of 50 nm, and a height (t) of 100 nm. As shown in the schematic diagram of the nanowire field-effect transistor used in this work, we used a rectangular cross-section, which is also called a nanobelt.

2.2. Selective functionalization of SiNWs

In this work, SiNWs were selectively functionalized by APTES as the initiators. Then, the modified chip was functionalized with GA for the surface activation for reaction with (1) AuNPs to characterize the SiNWs surfaces or (2) PSA antibodies to be used for the detection of PSA protein. Fig. 1 shows the detailed steps performed for (a) the selective functionalization of SiNWs using silanization reaction, (b) surface functionalization using AuNPs for characterization examination, and (b) surface functionalization using PSA Ab for PSA detection and sensitivity increase investigation. All steps in SiNWs functionalization are

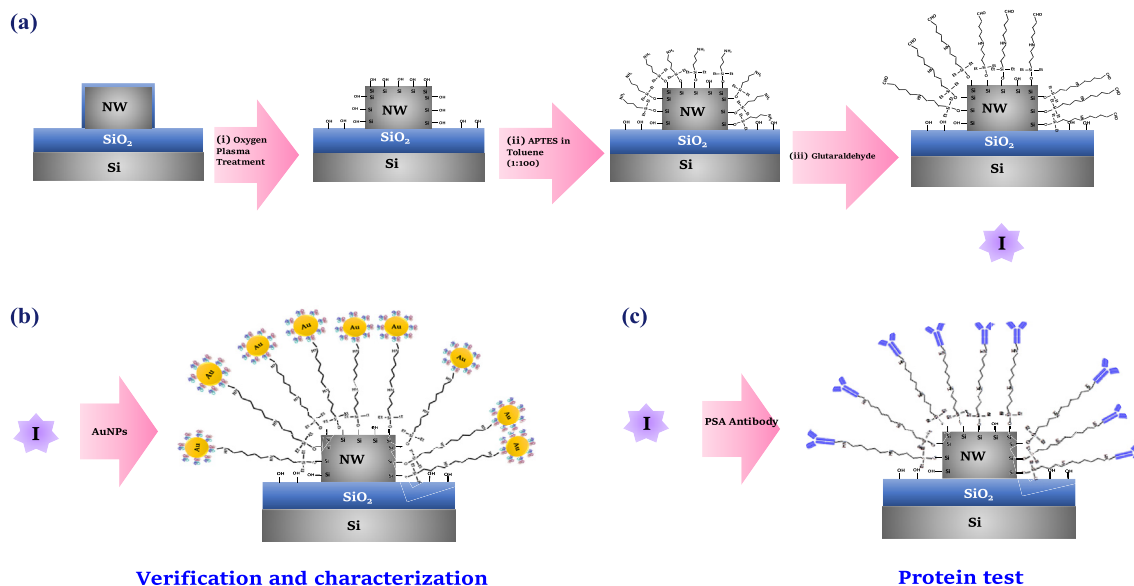


Fig. 1. Schematic of selective functionalization of SiNWs in detailed steps. (a) The selective functionalization of SiNWs using silanization reaction: (i) etching of silicon surfaces for surface oxide elimination, (ii) silanization of oxidized silicon surfaces with APTES, and (iii) Surface activation using GA (b) SiNW surface functionalization using AuNPs for characterization examination, and (c) SiNW surface functionalization using PSA antibody for PSA detection and sensitivity increase investigation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

described in detail in the following:

- **Etching of silicon surfaces for surface oxide elimination:** The p-type SiNW samples were first cleaned using acetone and acetonitrile by low power sonication for 5 min. Afterwards, SiNW samples were treated with OP (50 W, 2 min) by placing the chips on a microscope slide facing upwards to obtain a silicon surface with OH-termination. The treated chips were immediately used after the OP treatment in order to prevent surface degradation (Fig. 1a(i)).
- **Silanization of oxidized silicon surfaces with APTES:** In order to convert surface silanol groups (SiOH) to amines (NH₂), the chip was immersed in APTES: Toluene (1:100) solution placed in a desiccator. The desiccator atmosphere was kept at low nitrogen overpressure for 20 min. Then, the temperature was increased up to 110 °C for 10 min (Fig. 1a(ii)).
- **Surface activation using GA:** After silanization, SiNW was placed in 2.5% GA in PBS for 45 min, then washed with deionized water, and dried using N₂ gas. In this process, aldehyde groups from GA react with the amino groups to form the linker between the APTES and desired compound (Fig. 1a(iii)).
- **AuNPs immobilization on modified SiNW surface:** After Surface activation using GA, the SiNW was placed in 10% streptavidin coated AuNPs in PBS buffer for 1-h, then a washing step was performed using PBS buffer and the modified Si chip was kept in PBS for characterization examination (Fig. 1b).
- **The protein test examination:** After examination of the possibility of selective functionalization of SiNWs using AuNPs, in the following, a new Si chip was functionalized with PSA antibodies. The sensitivity of the selective and nonselective modified SiNWs was investigated by measurement of the current in the presence of PSA biomolecules and by comparison of results with the simulated data. The procedure was explained in detail by Lieber (Patolsky et al., 2006). In summary, A and B chips were functionalized with APTES using the selective (using OP + Toluene as APTES solvent (100:1)) and the non-selective method (using OP + Ethanol as APTES solvent (100:1)), respectively. After the reaction of 2.5% GA, the SiNWs of both A and B Chips were immersed in (10 μg/ml antibodies in 10 mM phosphate buffer solution, pH 8.4, containing 4 mM sodium cyanoborohydride) with silanized SiNWs, and the chips were

washed with 10 mM phosphate buffer at pH 8 (Fig. 1c). The unreacted aldehyde surface groups were passivated by drawing a solution of ethanolamine (100 mM ethanolamine in 10 mM phosphate buffer, pH 8.4) in the presence of 4 mM cyanoborohydride into the channel and allow to stand for 1–2 h. Finally, the surface washed using a continuous flow of 10 mM phosphate buffer, pH 8.4, through the channel for 10 min.

- **The real-time detection of PSA protein:** Regarding measurements in a liquid environment, the silicon chips (Fig. S2a) were wire bonded on PCB (Alfa Madar Sanat, Iran) and encapsulated using PDMS and a biocompatible epoxy glue (U300 80Z, EPO-TEK, USA) (Fig. S2b). The recording was performed on a wafer station designed for the chip connection to the reader device (Dropsens, Metrohm, Switzerland) (Fig. S2c). In order to operate in a liquid environment, the gate voltage was applied via a small, liquid-junction Ag/AgCl electrode (Super Dry-Ref (SDR2), WPI, Germany) and the position of this electrode with respect to the SiNW array was fixed on top of the station. We should note that signals from biomolecular reactions at surfaces (PSA protein and antibody interaction) are obtained by potentiometric DC. For DC readout, we should note that the molecules have been detected according to a re-distribution of ions near the liquid-solid interface or the biomolecules intrinsic charge. By functionalization of SiNWs with biomolecular receptors, the absorption of charged target biomolecules gives rise to depletion or accumulation of charge carriers inside the nanowires and therefore in a change of the drain-source current of the transistor. This is due to the fact that the changes in the surface charge density shift the flat-band voltage of the transistor after the protein binding process (Vu et al., 2010). In this work, we will monitor these effects by recording the SiNWs real-time current after PSA protein addition to the environment (Fig. S2d). The I-t diagram was recorded for two concentration of 1 ng/ml and 100 ng/ml of PSA protein in both A and B chips.

3. Fabrication results

3.1. Selective functionalization characterization

The Si chips with functionalized AuNPs were used to characterize

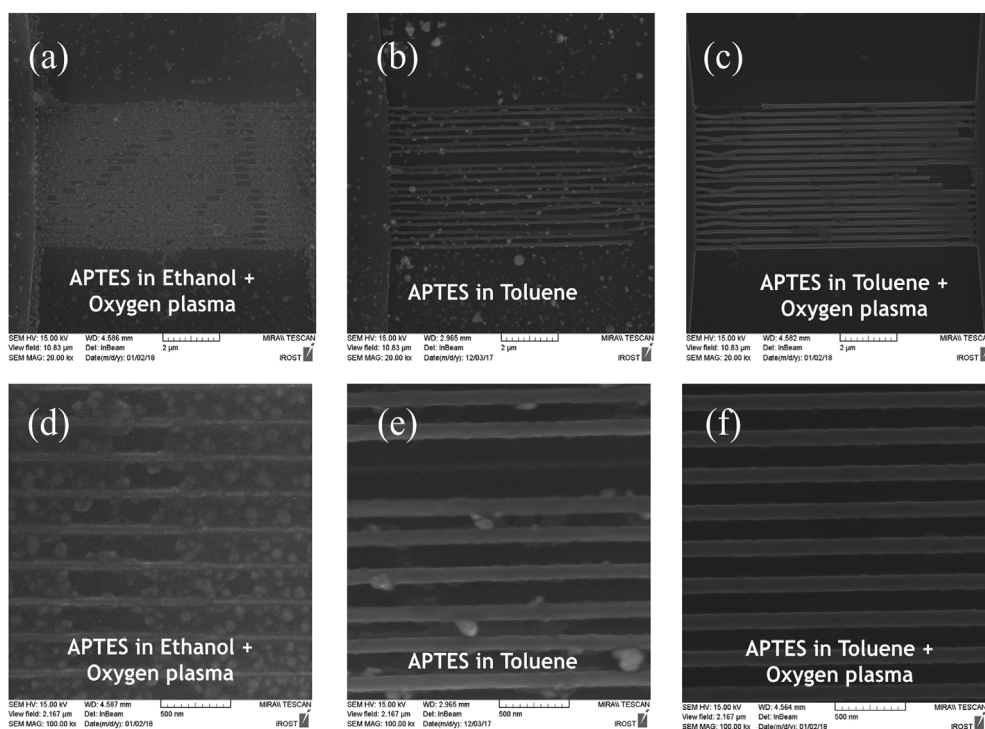


Fig. 2. FESEM image of SiNWs functionalized with AuNPs using APTES as crosslinker in different condition: (a,d) APTES in Ethanol and OP treatment, (b,e) APTES in Toluene, and (c,f) APTES in toluene and OP treatment. (a,b,c) The scale bar is 2 μm . (d,e,f) The scale bar is 500 nm.

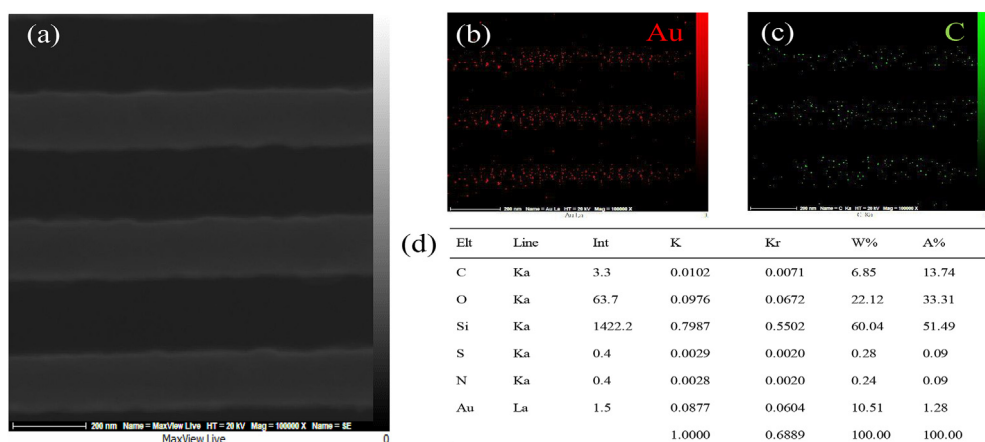


Fig. 3. (a) FESEM image of SiNWs functionalized with AuNPs using APTES in toluene and OP treatment. The scale bar is 200 nm. (b) The corresponding elemental mapping of constituent elements including Au and (c) C. (d) EDS analysis of the mentioned SiNWs.

the functionalization process. Fig. 2(a–f) show a FESEM image of SiNWs in AuNPs conjugated states in different conditions in which the brighter parts are related to AuNPs while the darker parts indicate silicon. As shown in Fig. 2 (a,d), in the case of using ethanol as APTES solvent, non-selective attachment of AuNPs have been observed neither in the presence or absence of OP. In fact, using ethanol as the solvent (or any solvent that increases the possibility of APTES hydroxylation reaction) will cause AuNPs attachment on both SiNW and SiO_2 substrate and also some aggregation of nanoparticles. In terms of use of anhydrous toluene as solvent (or any solvent that decreases the possibility of APTES hydroxylation reaction) non-specific conjugation of nanoparticles to the substrate can be observed again (Fig. 2 (b,e)), however, the particles were less aggregated. On the other hand, using toluene and OP together resulted in selective functionalization of SiNWs (Fig. 2 (c,f)). In the explanation of this observation, it can be said the OP application will cause complete elimination of oxide layer on SiNW that is much thinner

than the substrate, while the substrate still has a significant thickness of silicon oxides. Because the more reactivity of Si in comparison with SiO_2 in the case of OP activation, the surface reactivity of SiNWs is significantly more than its substrate (Alam et al., 2014). In this way primary cores of APTES will be formed on SiNWs surface preferentially.

After the formation of primary cores of APTES on the SiNW, the silanization reaction will continue based on the water content in the environment with different mechanisms (Ghorbanpour and Falamaki, 2014). In 96% ethanol, in the presence of significant water from the surrounding environment, hydrolysis of ethoxy groups precedes siloxane bond formation and a high extent of lateral covalent connections occurs between them, so they other spread a Si–O–Si network mainly in a 2D space and eventually a horizontal network will be formed (Fig. S3a) (Zhu et al., 2011). On the other hand, in toluene solvent, the APTES molecules in the organic phase are partly hydrolyzed because of the minute water presence in the organic phase. Some of the molecules

probably reach through the solvent to react with the surface silanol groups of the SiNWs leading to a covalent bond (Fig. S3b). Some can be involved in the electrostatic reaction to form a weak 3D network between residual APTES molecules and original APTES molecules. In fact, the rare amount of water impurity in the organic liquid phase precedes the vertical growth of the original APTES molecules that cause a relatively thick layer on the SiNW (Ghorbanpour and Falamaki, 2014).

The elemental mapping via EDS in a scanning electron microscope (Fig. 3(a-d)) was used to investigate the elemental distribution of SiNWs after the selective functionalization. Fig. 3b and c show the spatial distributions of Au (red) and C (green) from a SiNW oriented in the SiNWs zone axis. The elemental distribution matches the geometry shown in the schematic and the SEM image, and confirms that the AuNPs are primarily localized on the SiNW facets.

For the transfer characteristics of the devices, the current is measured as a function of the applied gate voltage V_g (Fig. S4). The high surface-to-volume ratio of nanowires leads to a strong influence of the surface modification on the electrical characteristics of the nanowires based FETs. Fig. S4 shows the curves for functionalization steps of SiNWs with AuNPs in the non-selective method (using ethanol and OP) and the selective method (using Toluene and OP), respectively. In both diagrams, the APTES layer shifted the curve of a p-type SiNW toward positive V_g (Chu et al., 2013).

For the case of AuNPs immobilization, the shifts in the threshold voltage after modification of subsequent molecule is a positive shift in the threshold due to negatively charged AuNPs located right on top of SiNWs. However, this positive shift is significantly more in selective method compared to nonselective immobilization. This observation can be attributed to the thickness increase of the AuNPs layer on SiNWs surface via the vertical growth of APTES molecules occurred on the selective functionalization process (Römhildt and und Nanotechnik, 2014).

As a summary of this part of the study work, the silanization reaction was modulated by the modification of OP treatment condition and the amount of water involved in the APTES reaction, so that AuNPs were immobilized only on the SiNWs surface. The results show that selective functionalization of all SiNW can be performed by tuning only these parameters.

3.2. Covalent attachment of biomolecules on SiNWs

The proposed functionalization method was performed for antibody immobilization to investigate the **biomaterial selective functionalization and sensitivity enhancement possibilities**. Fig. 4 (a) shows an SEM image after the immobilization of PSA antibodies on the SiNWs. The antibodies are selectively immobilized on the SiNW surface as we observed in the case of the AuNPs ones. The data analysis of the EDX

mapping of the SEM image (Fig. 4b-e) shows that the C and N amount on the SiNWs is significantly higher than on the SiO₂ substrates that represent the preferential and spatially selective immobilization of antibodies on the SiNW surfaces.

The reproducibility is one of the important aspects of immunosensors. The reproducibility of the thickness of the immobilized antibody layers on the SiNWs can affect the sensor reproducibility via Debye length (Shoorideh and Chui, 2014). To examine the immobilized antibody thickness, five different arrays of SiNW were selected and the functionalization procedure was performed separately. The images of functionalized arrays are shown in Fig. S5. The relative standard deviation (RSD) of the thickness of the SiNWs was calculated to be 1.43. This amount is satisfactory and shows that this sensor has the potential of high reproducibility.

4. Experimental and simulation results

The effect of the selective functionalization on the conductivity can be studied for different PSA concentrations. The PBDD system is an efficient model to monitor the electrostatic and electrochemical behavior of the sensor. The model used by the authors to simulate the electrical current and sensitivity of nanowire PSA (Baumgartner et al., 2013) and troponin (Khodadadian et al., 2017) sensitive sensors. Bayesian inversion (Dashti and Stuart, 2016) is an efficient computational technique to estimate different physical and chemical parameters of the sensors that cannot be measured directly or need significant experimental efforts. In (Khodadadian et al., 1904), the method was used to estimate unknown parameters of PSA sensors, e.g., molecule surface charge, doping concentration, mobilities, and probe-target density. In Appendix we review both computational techniques and here we apply them to the SiNW sensors.

The nanowire is characterized by $W = 50$ nm, $t = 100$ nm and $L = 8$ μ m and it is coated by an oxide layer with 2 nm, the height of oxide layer is 200 nm, and the distance between the nanowires is 600 nm. In the device fabrication, the doping concentration is $C_{dop} = 1 \times 10^{15}$ cm⁻³ and the thermal voltage is $U_T = 26$ mV. Fig. S6 shows the mesh generation of the cross-section of one-nanowire with APTES. Here the distribution of molecule on a selective (left) and non-selective (right) nanowires are shown as well.

First, we show the current as a function of time (until equilibrium) at $V_g = -1$ V for two different PSA concentrations, i.e., 1 ng/mL as well as 100 ng/mL in selective and nonselective devices. Fig. S7 shows the current. Due to adding the target molecules at a pressure (in the laboratory), the currents reach a (temporary) peak and then their equilibrium value. The significant difference between bulk current (current without PSA molecule) and the current after adding the target molecules shows the high sensitivity of the sensor. From now on, the

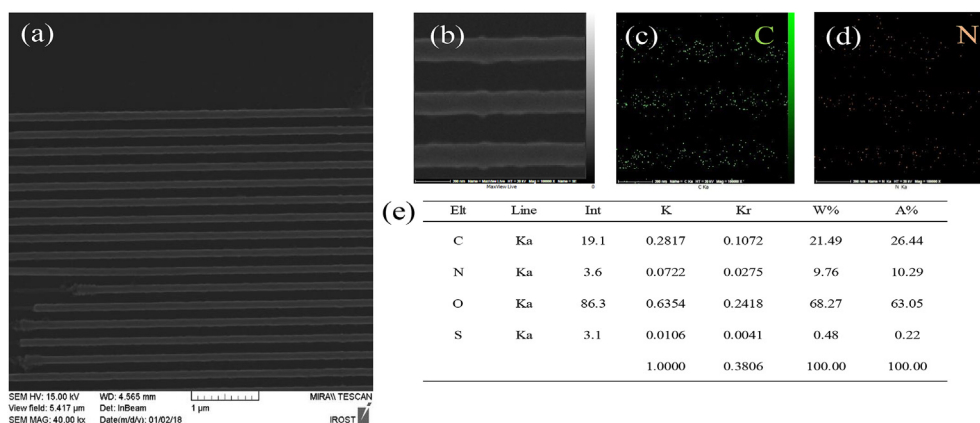
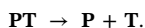
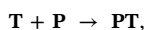


Figure 4. (a,b) FESEM image of SiNWs functionalized with PSA antibody using APTES in toluene and OP treatment. In (a) the scale bar is 1 μ m and in (b) the scale bar is 200 nm. (c,d) The corresponding elemental mapping of constituent elements including (c) Au and (d) C. (e) EDS analysis of the mentioned SiNWs.

experimental currents in equilibrium will be considered.

In order to estimate the measurement error, we measured the current for both concentrations 52 times. Fig. S8 shows the experimental results at $V_g = -1$ V where for the selective devices the current variation is less than the nonselective sensor. These results enable us to estimate variance σ^2 of the measurement error in (6). The standard deviation of the measurement error at 1 ng/mL is $\sigma = 1.06 \times 10^{-8}$ A and $\sigma = 5.89 \times 10^{-9}$ A for nonselective and selective nanowires, respectively, while at 100 ng/mL for these sensors $\sigma = 3.04 \times 10^{-8}$ A and $\sigma = 1.97 \times 10^{-8}$ A has been achieved.

In (Khodadadian et al., 1904), for PSA sensitive sensors, we determined influential parameters such as molecule surface charge, doping density, and diffusion coefficients. The binding and unbinding process of the target molecules to receptors is modeled as the reaction process



The equation explains association and dissociation of the probe-target complex at the surface, T is the target-molecule (here PSA molecule) concentration, and P is the probe-molecule concentration.

Now we use the Metropolis-Hastings (MH) algorithm to estimate the PT-density of selective and nonselective nanowires. For a better investigation, we choose a high concentration (100 ng/mL) and a low concentration (1 ng/mL) which enables us to study the effect of target concentration on the PT-complex more efficiently. Fig. 5 illustrates the posterior and prior distribution (the proposed values) and the proposal distribution (values obtained from the algorithm) of the PT for both sensors and concentrations. Here, the mean value of 1 ng/mL PSA concentration for selective nanowire is 2.82×10^{10} molecules/cm² and 2.36×10^{10} molecules/cm² is determined for nonselective sensor. At 100 ng/mL, also 9.75×10^{10} molecules/cm² and 9.07×10^{10} molecules/cm² are the estimated densities for selective and nonselective SiNW sensors.

The obtained results allow us to predict the binding process more realistically.

For different gate voltages varying between $V_g = 0.25$ V and $V_g = -6$ V we simulated the electrical behavior and estimated the conductivity. After estimating the PT-density for high and low target concentrations we validated the simulations with the measured data for the functionalization of SiNW with APTES using nonselective and selective methods. Fig. 6 shows the experimental and simulation (using the PBDD system and Bayesian inference) currents, where for all gate voltages an excellent agreement between measurement and simulations was achieved. The currents are shown for subthreshold and linear regimes, where the sensitivity in the subthreshold region is more than linear. As it is shown the current difference of 1 ng/ml and 100 ng/ml PSA protein in selective mode is obviously higher than the nonselective mode. All of the results lead us to conclude that the biosensor sensitivity will be improved significantly using the selective functionalization method. Furthermore, the Bayesian estimation of the parameters (e.g., PT-density) makes the simulations much more precise and therefore there is an excellent agreement between the measurement and simulation data.

4.1. Analytical application

Figure S9 shows the calibration curve of PSA measurement for two selective and nonselective functionalization method. As we already discussed, the sensitivity of selective diagram is about 3 times more than the nonselective one. The sensitivity of the bioFET, calculated from the slopes of the calibration plots, was 0.0016 and 0.0005 for two selective and nonselective functionalization methods, respectively. The obtained detection limit for the selective method was 90 pg/mL, whereas 0.7 ng/mL was estimated for the nonselective method based on 3 times the standard deviation of the blank (Sb) per slope of the calibration curve. The experimental data shows a very good agreement

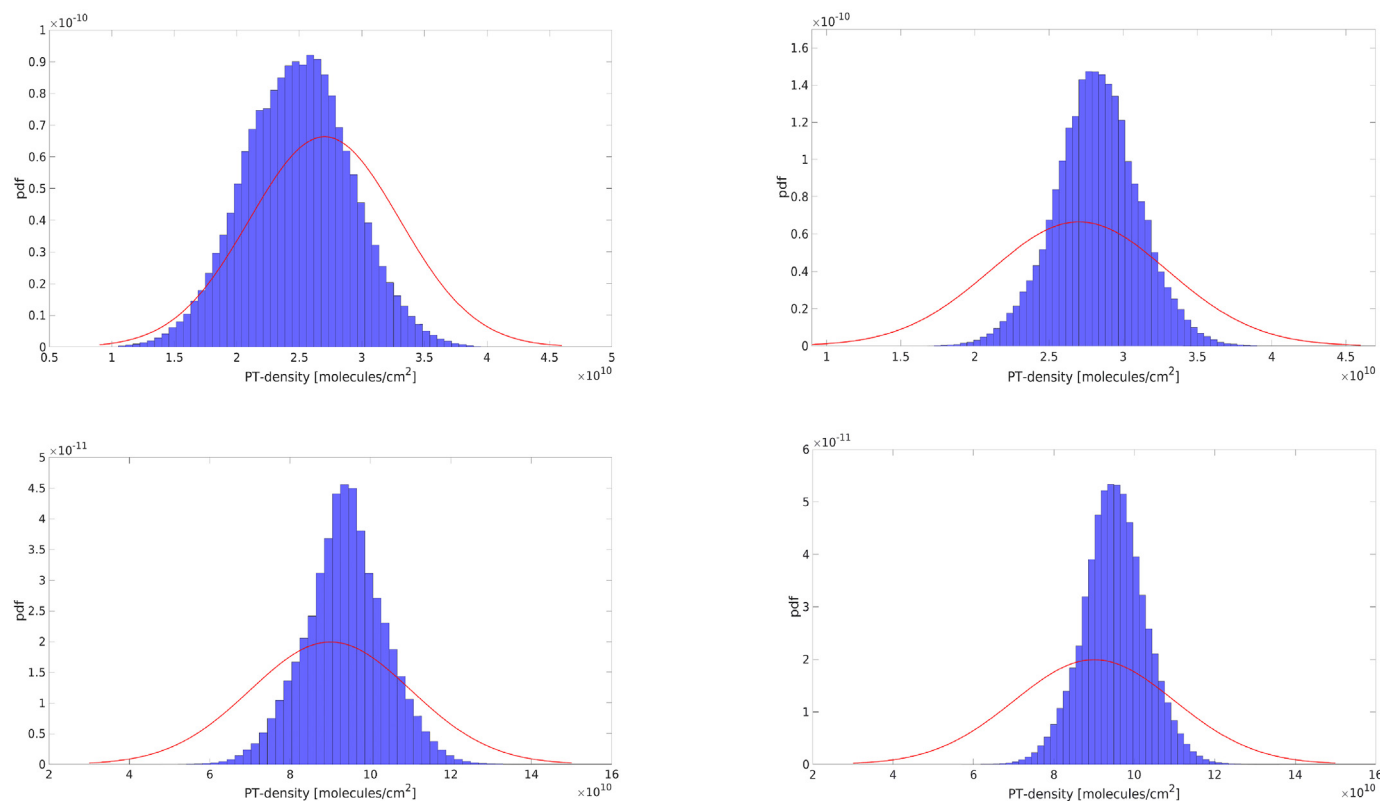


Fig. 5. The posterior (histogram) and prior (red line) distribution of PT-complex for nonselective (left) and selective (right) functionalization at 1 ng/mL (top) and 100 ng/mL (bottom) PSA concentration. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

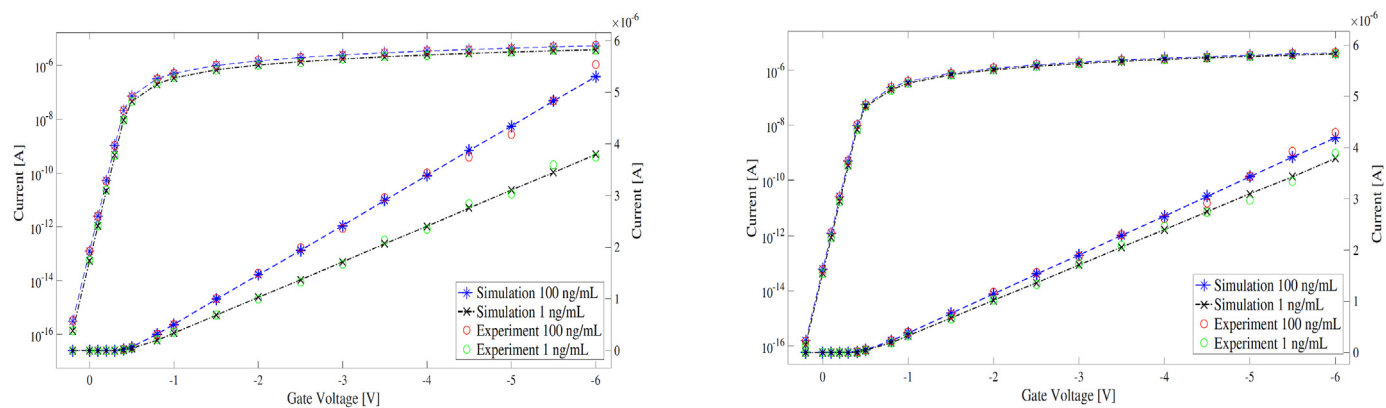


Fig. 6. The experimental and simulation currents in the linear and subthreshold regions for selective (left) and nonselective (right) nanowires at different PSA concentrations.

with the simulations.

The repeatability of the bioFETs was evaluated from the RSD of three different measurements of each standard solution. The RSD value was less than 1.9% and demonstrates that the proposed bioFET is reliable for PSA measurement.

The sensor reproducibility was investigated by application of five bioFETs for 25 ng/mL PSA measurements conducted by five different individuals. The obtained RSD for assays is calculated to be 1.22%, which shows the high reproducibility of the designed bioFET.

4.2. Serum specimen analysis

Because of the use of a PSA-specific antibody, the high specificity of antigen/antibody affinity allowed us to quantify PSA without interference from other substrates in real samples. For verification of the suitability of the assay for detection of PSA in serum samples, different concentrations of PSA proteins in the range of 1 to 100 ng/mL were spiked to healthy female serum. The measurement was performed after each sample dilution (1:2). The results that are presented in Table S1 indicate a very good agreement between the spiked and obtained values of PSA in serum samples. The recovery values are close to 100%, confirming that the performance of the bioFET has not been affected by the nature of the serum samples. Reproducibility of this bioFET is comparable to that found in standard solutions, therefore confirms the suitability of the developed approach for real sample analyses.

In the next step, for real PSA sample validation of the proposed bioFET, a commercially available ELISA kit response was compared to the bioFET (Table S2). The RSD between 1.06% to 2.25% displays a satisfactory consistency between the two analytical methods.

5. Conclusions

We describe a modification method to selectively functionalize SiNWs utilizing silanization reaction. This method can be especially valuable for the biofunctionalization of SiNWs in biosensor structures. In this work, we used this functionalization method in bioFET sensor for selective functionalization of SiNWs with PSA antibody. Our simulations and experimental results demonstrate that the sensor sensitivity is 3 times more than the bioFET designed based on nonselective functionalization. Furthermore, this sensor showed acceptable sensitivity, selectivity, stability, as well as reproducibility, and proved suitable for direct measurement of the target biomarker in human serum samples. In a future study, we will use this procedure for the immobilization of other biomaterials such as aptamers in biosensor devices.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bios.2019.111527>.

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