# Hybrid Organic/Inorganic Nanomaterials for Biochemical Sensing


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**Abstract** In this paper, different nanostructured semiconductors with advanced properties are explored for the realization of both optical and electrical biosensors for DNA detection. A hybrid sensor constituted by graphene oxide (GO) covalently grafted on a porous silicon (PSi) matrix is realized. A peptide nucleic acid (PNA) probe, able to recognize its complementary DNA (c-DNA) sequence, is immobilized on the surface of PSi/GO device for label-free optical sensing. Electrical sensing of DNA is also demonstrated using a Zinc Oxide Nanowires (ZnONWs) sensor functionalized with PNA probe; the I–V characteristic of the device depends on the c-DNA concentration under analysis.

**Keywords** Nanomaterial · Semiconductor · PNA probe · Bioconjugation · Biochemical sensing

## 1 Introduction

Biosensors are analytical devices constituted by a biological element, generally defined as probe, immobilized on a transduction system, converting a molecular interaction in a measurable output signal. In the last decades, the growing prevalence of diseases and the increasing demand for their rapid and reliable detection fueled the biosensors market. Biosensors industry includes two categories of bioanalytical devices: traditional practices in clinical laboratories, requiring trained personnel and characterized by a long and expensive samples processing; cheap, portable and easy-to-use point-of-care (POC) devices for analyses in both clinical and nonclinical ambients. The successful commercialization of POC glucose tests (e.g. FreeStyle Lite®, Abbott Inc.) favored the development of other innovative portable diagnostic

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devices that contribute, today, to approximately 57% of overall biosensors market [1]. On the other hand, the recent advances in nanotechnology are helping to address challenges in conventional biosensing such as high sensitivity, selectivity, minimal sample volume, low costs, fast detection. Nanomaterials can offer advanced properties compared to the corresponding bulk materials, such as unique physico-chemical features and a very high specific surface area that assures an increase of sensitivity for the detection of target analytes.

In this work, different nanostructured platforms were explored for the realization of both optical and electrical portable biosensors. Silicon and its related materials (i.e. silicon oxide, silicon nitrides and porous silicon (PSi)), the semiconductors mainly used in the microelectronic industry, were extensively exploited in biosensors fabrication [2]. Other nanostructured oxides, such as Graphene Oxide (GO) and Zinc Oxide Nanowires (ZnONWs) were also investigated as sensing platforms due to their peculiar properties [3–6].

The devices were obtained by merging conventional microelectronic techniques with liquid synthesis processes and surface modification strategies. Since a valid surface functionalization strategy is crucial in the development of biosensors in order to correctly immobilize the bioprobes on the material surfaces, several chemical processes were explored in this work. In particular, strategies based on silane (e.g. APTES, APDMES) grafting and hydrosilylation were used as treatments to passivate and/or functionalize nanostructured surfaces with biomolecules such as DNA or peptide nucleic acid (PNA) [7, 8].

## 2 Experimental

### *2.1 Chemicals*

Hydrofluoric acid (HF), undecylenic acid (UDA), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), MES hydrate, Dimethyl sulfoxide (DMSO), tert-Butyloxycarbonyl-NH-PEG-Amine (BOC-NH-PEG-$NH_2$), trifluoroacetic acid (TFA), chloroform, tetrahydrofuran, (3-Aminopropyl)triethoxysilane (APTES), toluene, bis(sulfosuccinimidyl)suberate ($BS^3$) were purchased from Sigma Aldrich (St. Louis, MO, USA), Graphene oxide (GO) nanosheets were purchased from Biotool.com (Houston, TX, USA) as a batch of 2 mg/ml in water with a nominal size sheets between 50 and 200 nm.

### *2.2 Fabrication of the PNA-Based PSi/GO Optical Sensor*

PNA-based PSi/GO hybrid device was realized following the scheme reported in Fig. 1. PSi structure was fabricated by electrochemical etching of n-type crys-

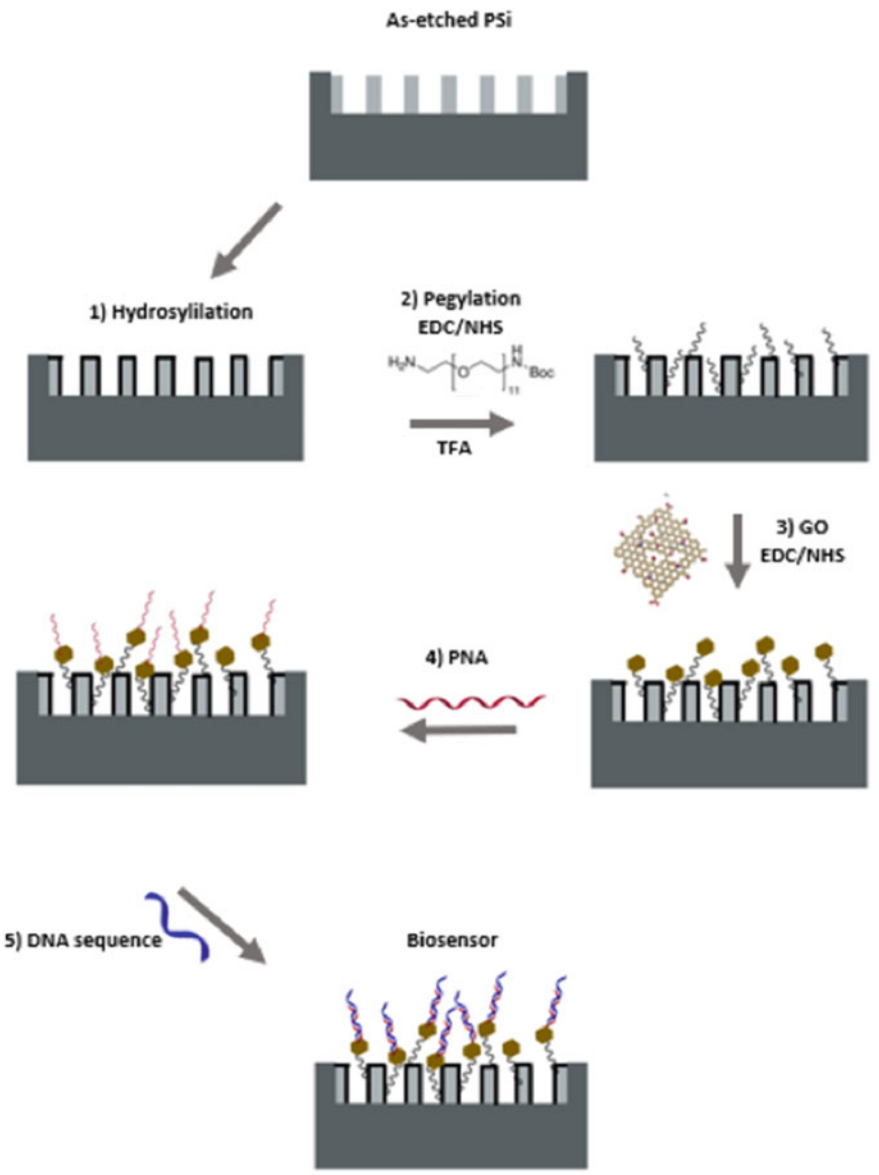


**Fig. 1** Fabrication process of the PNA-based PSi/GO biosensor

talline silicon (0.01–0.02 Ω cm resistivity, ⟨100⟩ orientation, 500 μm thickness) in HF (5% in weight)/ethanol solution at room temperature. A current density of 20 mA $cm^{-2}$ for 90 s was applied to obtain a single layer of PSi with 61% in porosity ($n_{PSi} = 1.83$ at $\lambda = 1.2$ μm), thickness L of 2.1 μm and pore dimension included between 50 and 250 nm. The as-etched PSi was placed in a Schlenk tube containing deoxygenate neat UDA (99% v/v). The reaction was conducted at 110 °C for 18 h in argon. The hydrosilylated-PSi was extensively washed in chloroform and tetrahydrofuran [9]. The carboxyl acid groups of UDA were activated by EDC/NHS (0.005 M in 0.1 M MES buffer) for 90 min at RT. Afterwards, a solution containing BOC-NH-PEG-$NH_2$ (0.004 M, overnight at 4 °C) was used to completely recover the PSi chip. A solution of TFA (95% v/v, 90 min, RT) was used to deprotect the amino group of the PEG molecule. The PSi chip was washed with deionized water. The GO was activated by EDC/NHS and added to the PSi chip, to allow the covalent bond. The GO-PSi device was exposed to 100 μM of PNA (COOH-KKTCGTGGCTCGGGNHCO$CH_3$) in presence of EDC/NHS and washed in deionized water. Complementary (5′-AGGAGAGCACCGAGCCCCTGAG-3′) and non-complementary (5′-CCTTTTTTTTTT-3′) DNA sequence were incubated on the chip for two hours. The sample was gently rinsed in deionized water to remove the unhybridized target.

## 2.3 Spectroscopic Reflectometry

The reflectivity of PSi/GO device was measured at normal incidence by means of a Y optical reflection probe (Avantes), connected to a white light source and to an optical spectrum analyzer (Ando, AQ6315B).

## 2.4 Fabrication of the PNA-Based ZnONWs Electrical Sensor

The ZnONWs electrical sensor was realized using the fabrication process described in the following. A uniform ZnO seed layer, 150 nm thick, was deposited on a thermally oxidized silicon substrate using a radio frequency (RF) magnetron sputtering equipment with a 99.999% pure ceramic ZnO target. ZnONWs were then growth on the sputtered ZnO thin film by hydrothermal synthesis, immersing the substrate in a solution obtained by dissolving 0.5 M of hexamethylenetetramine ($C_6H_{12}N_4$) and the $Zn^{2+}$ salt ($Zn(NO_3)_2$), which acts as a precursor, in deionized water. The hydrothermal synthesis was performed at a temperature of 90 °C for 4 h. Gold interdigitated electrodes were deposited on the ZnONWs through an iron shadow mask. The device was finally treated by rapid thermal annealing at 400 °C for 5 min. The ZnONWs surface was functionalized in order to immobilize PNA

probe, by applying the silane chemistry. Briefly, naturally hydrolyzed ZnONWs were treated with a solution of 5% APTES in toluene anhydrous for 30 min at room temperature, cured on heater at 100 °C for 10 min. Amino-modified ZnONWs were then treated by cross-linker $BS^3$ 1.7 mM in PBS 1X pH 7.4 at 4 °C for 3 h. The sulfo-NHS-terminated NWs were then incubated at 4 °C overnight with 300 μM PNA dispersed in deionized water. Increasing concentrations (25–200 μM) of complementary DNA were drop-deposited on the PNA-modified ZnONWs sensor for biorecognition monitoring.

### *2.5 Electrical Characterization*

The I−V characteristics of the ZnONWs biosensor were acquired using a Source Meter Agilent B2902A. Samples were accommodated on the holder of a probe station and electrodes connected by two XYZ micromanipulators (Micromanipulator, 450/360MT-6 and 550/360MT-6 series) provided of micrometric tips.

## 3 Results and Discussion

PSi/GO sensor is a nanostructured hybrid device characterized by a large specific surface area useful for an efficient sensing. Moreover, the presence of GO makes the device COOH-terminated and allows the covalent immobilization of biomolecules containing $–NH_2$ groups. The probe used for the biosensor realization is a peptide nucleic acid (PNA), a nucleic acid with a peptide backbone that does not contain any charges. The employment of PNA instead of DNA as a probe for complementary-DNA (c-DNA) detection, enables a high hybridization efficiency due to its non-ionic backbone. The interaction between PNA and c-DNA at different concentrations was monitored by label-free optical measurements based on the reflectance spectrum analysis (Fig. 2). Red-shift variations were registered at increasing c-DNA concentrations; the phenomenon is due to an increase of the average refractive index of the device.

ZnO nanostructures (i.e. nanoribbons, nanorods, nanowires) are interesting transducer materials for biochemical sensing applications due to their large surface over volume ration and very reactive surface. The main technologies used for fabrication of ZnO nano-objects are Vapor–Liquid–Solid growth (VLS), Metal Organic Chemical Vapor Deposition (MOCVD) and High Pressure Pulsed Laser Deposition (HP-PLD), requiring complex-equipment [10]. ZnONWs, realized in this work, were obtained by an alternative approach based on the hydrothermal synthesis. Figure 3a shows a photograph of the ZnONWs sensor; in the inset, a SEM image of ZnONWs is reported. ZnONWs appeared as columns perpendicular to the substrate, with a diameter of about 200 nm. Empty spaces of some hundreds of nanometers were observed between adjacent columns of ZnONWs. The current over voltage profile

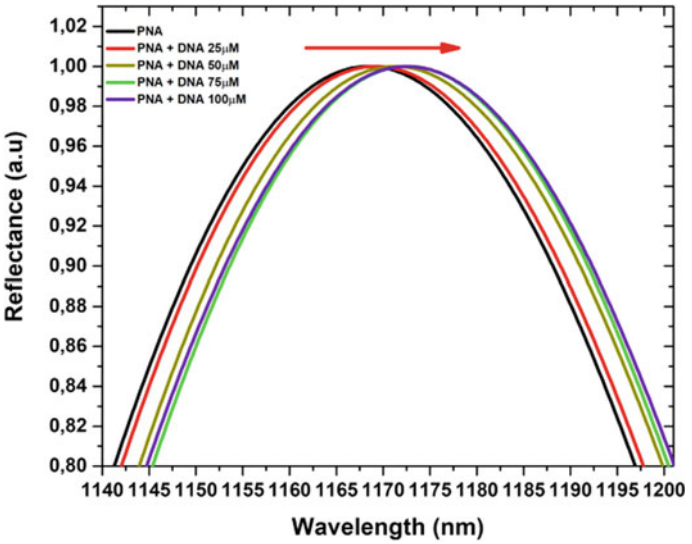


**Fig. 2** Reflectance spectra of PNA-based PSi/GO biosensor after interaction with increasing concentration of c-DNA

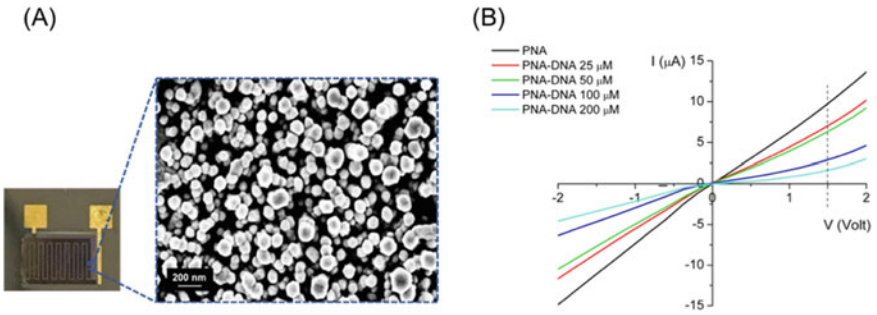


**Fig. 3** **a** A photograph of the ZnONWs electrical sensor; in the inset, a SEM image of ZnONWs grown by hydrothermal synthesis. **b** Current over voltage profiles of ZnONWs sensor functionalized with PNA probe, before and after interaction with increasing concentrations of complementary DNA

of the electrical biosensor (i.e. ZnONWs sensor functionalized with PNA probe) was acquired after the exposure to increasing concentrations of c-DNA in the range included between 25 and 200 μM. The results reported in Fig. 3b shown that the presence of c-DNA induced a linear increase of the sensor resistance at 1.5 V.

## 4 Conclusion

A key revolution in the development of portable and efficient biosensors is the integration of structures at the nanoscale. Due to their large specific surface area and enhanced physico-chemical properties compared to related bulk materials, nanostructured sensing materials opened new horizons for biosensing. The biomolecules immobilization on the nanostructured surfaces can be achieved using proper functionalization procedures that guarantee the biological activity of biomolecule. In this work, optical and electrical sensors realized using nanostructured semiconductors are described. The devices were functionalized with PNA, a nucleic acid able to recognize c-DNA with high hybridization efficiency due to its non-ionic backbone. The detection of c-DNA concentrations included between 25 and 200 $\mu$M was demonstrated.

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