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Label-free DNA hybridization and denaturation detection by means of field-effect nanoplate SOI capacitors functionalized with gold nanoparticles

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Abstract

A new approach for a label-free electrical detection of DNA hybridization and denaturation using an array of individually addressable field-effect nanoplate SOI (silicon-on-insulator) capacitors functionalized with gold nanoparticles is presented. By using a constant-capacitance measuring setup in a differential mode, signal changes of ~110 mV and ~70 mV have been registered after the DNA hybridization and denaturation events, respectively.

1. Introduction

Broad interest in DNA (deoxyribonucleic acid) chips and their growing use in genetics, medicine, environmental monitoring, food and drug industry is connected with their ability to perform massive parallel sequence analysis of nucleic acid samples. Most of the DNA-hybridization detection techniques require labelling of either the probe or target single-stranded DNA (ssDNA) with fluorescent, enzymatic or radiochemical markers and have been proven to be time-consuming, complicated and expensive. Recently, considerable research efforts have been invested towards the label-free direct electrical detection of charged macromolecules [1-4] as well as charged nanoobjects [5, 6] using field-effect devices (FEDs). In this work, we present a novel platform for a label-free electrical detection of DNA hybridization and denaturation using field-effect electrolyte-insulator-silicon-on-insulator (EISOI) nanoplate capacitors functionalized with gold nanoparticles. The proposed device detects the charge changes of the Au-nanoparticle/DNA hybrids induced by the hybridization or denaturation event. In previous experiments, the SOI technology has been used for the fabrication of (bio-)chemically sensitive field-effect transistors [7], nanowire-transistor array [8], and silicon thin-film resistors [9].

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2. Experimental

The nanoplate capacitive sensors were fabricated from an SOI wafer (SOITEC, France) with a 360 nm thick top Si layer (p-Si <100>, boron doped, ρ= 1-10 Ωcm) on a 400 nm thick buried SiO_2 layer (BOX). For the fabrication of the field-effect capacitors, first a high quality thermal silicon oxide layer (30 nm) was grown on the top Si layer. Then, the top SiO_2 layer was structured by optical lithography and wet etching with an ammonium fluoride etching mixture. For isolation of individual nanoplate capacitors, the top Si layer was anisotropically etched in 12.5% tetramethylammoniumhydroxid at 90 °C using the structured top SiO_2 layer as a mask [10]. In the next step, the top SiO_2 layer was structured again by optical lithography and etched to open contact windows to the top Si layer. As contact layer, a 300 nm Al was deposited on front of the top Si layer followed by lift-off and annealing processes. Finally the wafer was cut to 12 mm x 12 mm chips. Each chip consists of four electrically isolated nanoplate capacitive field-effect sensors.

For ssDNA immobilization, the sensor surface was first silanized in a mixture of 5% MPTES (3-mercaptopropyl trimethoxysilane) and toluol, then functionalized with 5-8 nm Au nanoparticles [11]. A scanning electron microscopy micrograph of an EISOI sensor surface functionalized with Au nanoparticles is shown in Fig. 1. The thiol-modified 20 base pair ssDNA probe molecules were immobilized on the surface of the Au nanoparticles. Fig. 2 shows the schematic of the EISOI chip for the DNA-hybridization/denaturation detection. In this study, Sensor 4 was immobilized with the probe ssDNA (5'THI-ATGAACACTGCATGTAGTCA-3') perfectly matched to the target ssDNA sequence (5'-TGACTACATGCAGTGTTCAT-3'), while Sensor 2 and Sensor 3 were immobilized with the fully mismatched probe ssDNA (5'THI-TACTTGTGATGTACATCAGT-3'); Sensor 1 was used for the pH control.

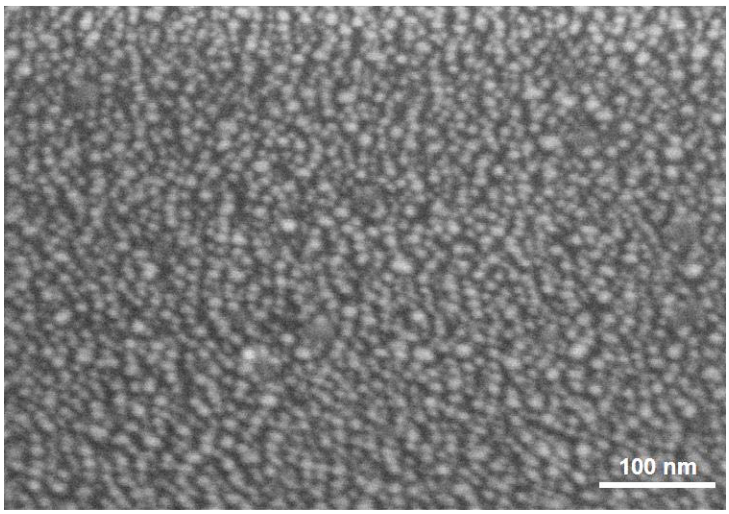

Fig. 1. Scanning electron microscopy micrograph of an EISOI sensor surface functionalized with Au nanoparticles.

For the DNA hybridization, the target complementary DNA (cDNA) solution was pipetted only onto the surface of Sensor 3 and Sensor 4, followed by incubating the sensors at room temperature for about 30 min and washing to remove the non-hybridized cDNA. On the other hand, Sensor 2 with immobilized fully mismatched ssDNA was not exposed to the hybridization solution and was used as reference sensor. In order to achieve high sensor signal, in this study, the sensor output signal after the hybridization was measured in a low ionic-strength solution (0.2 mM PBS, pH 7.5).

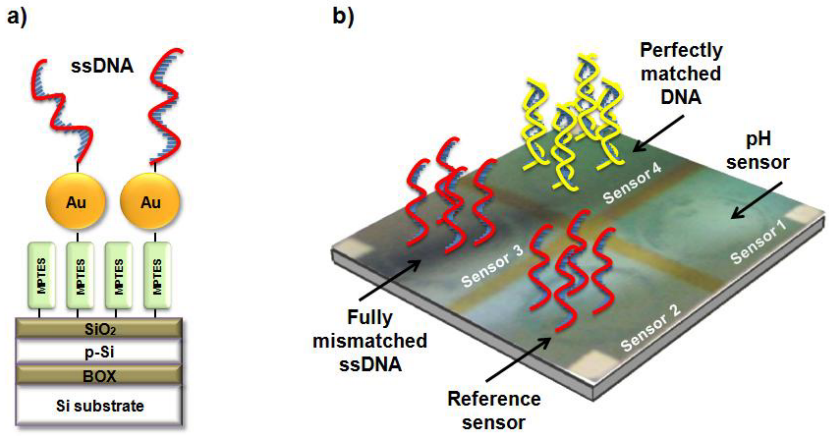

Fig. 2. Schematics of an EISOI sensor functionalized with Au nanoparticle/DNA hybrids (a) and a 4-channel EISOI sensor chip (b).

3. Results and discussion

The bare SiO_2-gate pH-sensitive EISOI sensors as well as EISOI structures modified with Au-nanoparticel/DNA hybrids have been characterized by means of the constant-capacitance (ConCap) method [12] using an impedance analyzer (Zahner Elektrik). In the ConCap mode, the capacitance of the EISOI sensor at the working point is kept constant by using a feedback-control circuit, which allows a direct dynamic monitoring of potential changes at the gate isolator/electrolyte interface caused from local pH changes or adsorption and binding of charged macromolecules. For operation, a DC polarization voltage is applied via a conventional Ag/AgCl reference electrode

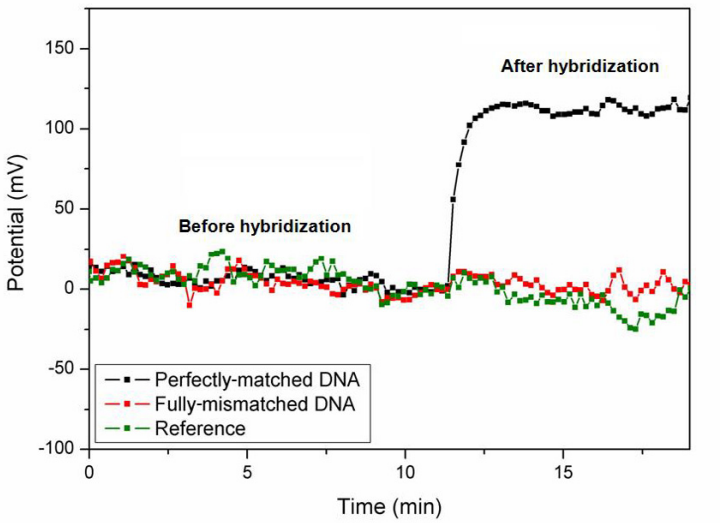

Fig. 3. DNA-hybridization detection by means of the EISOI sensor in constant-capacitance setup.

to set the working point of the EISOI sensors, and a small AC voltage (20 mV, 30 Hz) is applied to the system in order to determine the capacitance of the EISOI sensors. For the measurements, the EISOI chip was mounted into a home-made measuring cell and sealed by an O-ring. The contact area of each sensor with the solution was about 0.126 cm^2. The developed sensor chip and measuring setup allow addressable biasing and electrical readout of multiple nanoplate EISOI sensors in the array.

Fig. 3 demonstrates an example of DNA-hybridization detection by means of the EISOI sensor chip in the constant-capacitance mode. The potential change of ~110 mV has been registered after the DNA hybridization for the sensor immobilized with perfectly matched ssDNA, while practically no signal changes have been observed for the sensors with fully mismatched ssDNA. The high DNA-hybridization signal has been achieved via immobilization of ssDNA probe molecules on the Au nanoparticles as well as by using low ionic-strength solutions for the measurement of the signal changes induced by the DNA hybridization. The denaturation signal was ~70 mV. The obtained results demonstrate the potential of the nanoplate EISOI capacitors as a possible new basic structural element for the development of field-effect based DNA chips as well as for multi-parameter sensing using the same transducer principle [13].

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